MEASUREMENT OF ACTIVE BIOMASS IN ACTIVATED SLUDGE MIXED LIQUOR

by

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I, GERHARD LOUIS CRONJÉ, hereby declare that this thesis is my own work and it has not been submitted for a degree at another University.

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SYNOPSIS

BACKGROUND

Over the past two decades significant advances have been made in the areas of engineering (design) and technology (implementation and operation) of the single sludge activated sludge system. Activated sludge systems have been successfully designed and implemented at full-scale for the biological removal of carbon (C), nitrogen (N) and phosphorus (P). This implementation has been aided by the development of a suite of steady state design models (e.g. WRC, 1984; Wentzel *et al.*, 1990; Maurer and Gujer, 1994) and kinetic simulation models (e.g. Dold *et al.*, 1980, 1991; Van Haandel *et al.*, 1981; Henze *et al.*, 1987; Wentzel *et al.*, 1992; Henze *et al.*, 1995). These models constitute a common conceptualization of the processes acting in the bioreactor of the activated sludge system, based on the understanding of the interactions between the mixed liquor components and the influent wastewater. Fundamental to the steady state design and kinetic simulation models for activated sludge systems is the parameter heterotrophic active biomass (X_{BH}, mgAVSS/*l* as measured per the VSS test, or Z_{BH}, mgCOD/*l* as measured per the COD test). This mixed liquor organic suspended solids component mediates the biodegradation processes of COD removal and denitrification (and associated processes). In addition, in the models all the relevant specific process rates associated with the heterotrophic active biomass are expressed in terms of it. More recently, with the proliferation of kinetic simulation computer programmes that invariably include active biomass concentrations as parameters (e.g. Biowin, Simba, GPX, UCTOLD, UCTPHO), the active mass parameters and the use of specific rates in terms of them, have become much more widely accepted. However, X_H exists only hypothetically within the structure of the design procedures and kinetic models. Although indirect evidence does provide support for this parameter (by consistence between observations and predictions over a wide range of conditions, e.g. Dold *et al.*, 1980, 1991; Alexander *et al.*, 1980; Van Haandel *et al.*, 1981; Warner *et al.*, 1986), due to the lack of suitable experimental techniques, it has not been directly measured experimentally and compared to the hypothetical model values. This deficiency casts a measure of uncertainty on the entire framework within which the models have been developed and is a major weakness in the models, namely the lack of *independent* quantification of the active biomass, specifically X_H.

Parallel to the developments in the engineering and technology of the activated sludge system, significant advances have been made in the microbiological and biochemical areas of activated sludge. In particular, the development of a number of new analytical techniques to study microorganisms *in situ*, e.g. ATP analysis (Nelson and Lawrence, 1980), DNA analysis (Liebeskind and Dohmann, 1994), quinone profiling (Hu *et al.*, 1998), microautoradiography (Nielsen *et al.*, 1998) and using florescent probes for ribosomal RNA (Wagner *et al.*, 1994; Wat. Sci. Technol., 1998), has shown promise of providing quantitative information. While the microbiological and biochemical knowledge and developments have made a considerable contribution to the understanding of the biological nutrient removal activated sludge system, the full potential of these developments have yet to be realised for the system.

It remains for the results that these techniques provide to be integrated with the design and kinetic modelling theory. The consequence of this is that the engineering and technology (modelling) paradigm has largely worked independently of the microbiological and biochemical paradigm. One area that can form a starting point to build bridges between the two paradigm sets, is measurement of heterotrophic active biomass. Additionally, this will address the main area of deficiency in the models identified above.

This research project investigates measurement of heterotrophic active biomass within the engineering and technology (modelling) paradigm. If this parameter can be successfully quantified within this paradigm and agreement obtained between the measurements and the theoretical modelling values, this will provide the basis for future comparison with the quantitative data arising from the new measurement techniques within the microbiological and biochemical paradigm. This will establish a common link between the two paradigm sets.

BATCH TEST METHOD TO QUANTIFY HETEROTROPHIC ACTIVE BIOMASS

Within The engineering and technology paradigm, Kappeler and Gujer (1992) describe a simple batch test to quantify heterotrophic active biomass in activated sludge mixed liquor; a small quantity of mixed liquor is mixed with centrifuged wastewater and the oxygen utilization rate (OUR) response is monitored with time. From the observed exponential increase in the OUR, the initial OUR in the batch test can be determined, which can be used to derive an estimate for the heterotrophic active biomass concentration. Wentzel *et al.* (1995) and Mbewe *et al.* (1995) modified and extended this method for application to aid the characterization of municipal wastewaters through the quantification of the wastewater heterotrophic active biomass, readily biodegradable COD (RBCOD) and unbiodegradable soluble COD (USCOD). Mbewe *et al.* (1995) found that the RBCOD and USCOD measured in the batch test correlate closely to that measured via conventional methods. However, they were not able to evaluate the results for wastewater heterotrophic active biomass, since no conventional tests were available.

Recently, Ubisi *et al.*, (1997a,b) describe the development of a simple batch test to quantify the ordinary heterotrophic organism (OHO) active biomass concentration in an activated sludge system. Wentzel *et al.* (1998) evaluated this batch test method by drawing mixed liquor samples from well defined laboratory-scale anoxic/aerobic activated sludge systems operated at 12 and 20 days sludge age and comparing the results from the batch tests with theoretical values for OHO active biomass concentrations from steady state design (WRC, 1984) and kinetic simulation (Dold *et al.*, 1991) models. From the comparison they concluded that the results obtained were both encouraging and perplexing. With the parent system at 12d sludge age, the agreement between measured and theoretical values was remarkably good. However, with the parent system at 20d sludge age, the agreement was poor, with the theoretical values being about 2 times those measured.

Wentzel *et al.* (1998) could provide no explanation for this inconsistency, but concluded that the results do indicate that the batch test method may prove to be a valuable tool that can be used to provide greater insight into the behaviour of the aerobic and anoxic/aerobic activated sludge systems.

RESEARCH OBJECTIVES

The batch test method above developed by Ubisi *et al.* (1997a,b) has shown considerable promise as an *independent* means to quantify the hypothesized concentration of heterotrophic active biomass present in an activated sludge system within the engineering and technology paradigm. However, due to the inconsistency of the batch test results the uncertainty around the active biomass concept largely remained. ***The principle aim of this investigation was to evaluate the batch test method as a reliable means of quantifying the concentration of heterotrophic active biomass present in an activated sludge system.*** To achieve this aim, three primary objectives for this research project have been identified.

1. To establish whether the inconsistent batch test results could be related to the batch test procedure itself, or whether the inconsistencies were the result of incorrect mathematical formulations within the framework of the steady state design and kinetic simulation models.

2. Having established the reasons for the inconsistent batch test results, to develop a modified batch test procedure that would eliminate the identified sources of error.

3. In addressing aims 1 and 2 above, it was found that the maximum specific growth rate of the heterotrophs in the continuously fed system was low, and this caused the estimate for heterotrophic active biomass concentration to be unduly sensitive to the analytical procedure. Accordingly, the influence of increased heterotroph maximum specific growth rates was investigated. This also provided the opportunity to evaluate the reliability of the modified batch test procedure to quantify the heterotrophic active biomass in activated sludge systems subjected to dissimilar operating conditions.

RESEARCH APPROACH

The research approach adopted was twofold:

During the first part of this research project the research approach adopted was to operate and monitor a well-defined and controlled continuously fed anoxic/aerobic activated sludge system identical to that of Ubisi *et al.*, (1997a,b). This parent system provided the mixed liquor samples for measuring the heterotrophic active biomass to address objective (1) above. In addition, to address objective (2), this parent system also provided the mixed liquor samples required for evaluating the modified batch test method.

During the latter part of this research project the research approach adopted was to operate and monitor a well-defined and controlled intermittently fed fill and draw (IFFD) activated sludge system. This parent system provided the mixed liquor samples for measuring the heterotrophic active biomass to address objective (3) above.

EVALUATION OF THE BATCH TEST METHOD

Parent system

To determine if the inconsistent batch test results were related to the sludge age of the system, a well defined and controlled parent laboratory-scale nitrification / denitrification activated sludge system identical to that of Ubisi *et al.* (1997a,b) was operated, but at a different sludge age of 10 days. The system layout constituted a Modified Ludzack-Ettinger (MLE) configuration and consisted of an anoxic reactor (25% of the total system volume), an aerobic reactor (75% of the total system volume) and a secondary settling tank, all in series with an underflow recycle (s-recycle) of 1:1 and a-recycle of 2:1 both with respect to the influent flow. The anoxic and aerobic reactors were completely mixed and the aerobic reactor was aerated.

The influent was raw (unsettled) wastewater from the Mitchell's Plain Treatment Plant in Cape Town (South Africa). This sewage is primarily domestic, with a small (< 25%) industrial component. The sewage was collected in batches, stored in 400*l* stainless steel tanks at 4°C and served as feed for both the parent system and batch tests (see below) for 10 to 14 days. For the parent system, the wastewater was drawn daily from the storage tanks after thorough mixing and diluted with tap water to give influent feed total COD ~ 500 mgCOD/*l*. The system received a daily influent flow of 20 *l*/d. System operation procedures detailed by Ekama *et al.* (1986) were followed: Daily monitoring included influent COD, TKN; all reactors nitrite + nitrate; aerobic reactor TSS, VSS, COD and TKN; effluent COD, TKN, nitrate + nitrite (Standard Methods, 1985).

The parent system was operated for 323 days and received 23 batches of unsettled municipal wastewater from Mitchell's Plain, Cape Town. Each wastewater batch was accepted as a steady state period, and the results for each batch were averaged (after statistical analysis for outliers). From the averaged data the following were calculated:

- System COD and TKN mass balances.
- Influent wastewater unbiodegradable soluble and unbiodegradable particulate COD fractions ($f_{S,us}$ and $f_{S,up}$ respectively).
- Mixed liquor COD/VSS and TKN/VSS ratios (f_{CV}, and f_N respectively).
- The heterotrophic active biomass fraction of the mixed liquor organic suspended solids (f_{av}).
- The theoretical heterotrophic active biomass concentration in the steady state system bioreactor.

From the results on the parent system:

- N mass balances were variable, but generally fell in the range 90 to 110%. Sewage batches that gave mass balances falling outside this range were rejected for further analysis. On this basis, sewage batches rejected were 10, 17 and 21. For these sewage batches, batch tests were only conducted during sewage batch 21 – data for this sewage batch was analysed, but it was noted that the data should be rejected.

- Generally COD mass balances were poor, with 8 out of 19 sewage batches giving mass balances < 90%. The mixed liquor organic solids were determined by three independent tests – VSS, COD and TKN. Mean ratios for these measurements gave COD/VSS = 1.48 mgCOD/mgVSS (sample standard deviation = 0.04) and TKN/VSS = 0.100 mgN/mgVSS (sample standard deviation = 0.004). These values equal the accepted standard values of 1.48 mgCOD/mgVSS and 0.10 mgN/mgVSS respectively (WRC, 1984). Consequently, it was accepted that the error in the COD mass balance did not lie in the measurement of the mixed liquor organic solids, the parameter of importance in the measurement of heterotrophic active biomass. Accordingly, the lower limit for the COD mass balance was set at 80%. On this basis, sewage batches 6a and 9 were rejected for further analysis. No batch tests were conducted during these sewage batches.

- The wastewater mean unbiodegradable soluble COD fraction ($f_{S,us}$) was determined to be 0.085 (sample standard deviation = 0.008), while the mean unbiodegradable particulate COD fraction ($f_{S,up}$) was determined to be 0.103 (sample standard deviation = 0.033). These values conform closely to those observed by Ubisi *et al.* (1997a,b) for the same Mitchell's Plain wastewater, $f_{S,us}$ = 0.09 and $f_{S,up}$ = 0.12 respectively.

Batch tests

To determine if batch tests conducted in accordance with the procedures of Ubisi *et al.* (1997a,b) would yield the same inconsistent results, the batch test method was evaluated by applying the batch test to mixed liquor samples drawn from the well defined and controlled parent laboratory-scale nitrification / denitrification activated sludge system, identical to that of Ubisi *et al.* (1997a,b), but operated at a different sludge age of 10 days.

In the batch test procedure detailed by Ubisi *et al.* (1997a,b), a 3ℓ volume of diluted wastewater is preheated to 20°C and placed in a continually stirred batch reactor - called the *wastewater only* reactor. In a separate batch reactor a defined volume of mixed liquor (varying from 100 ml to 400 ml) drawn from the parent laboratory-scale activated sludge system is added to the required volume of the same diluted wastewater to make up a combined volume of 3 ℓ inside the parallel reactor - called the *wastewater and mixed liquor* reactor. However, to maintain consistency between the parallel tests it was decided to use the same volume of diluted wastewater in each of the separate batch reactors.

Accordingly, the same volume of wastewater required in the wastewater and mixed liquor batch reactor was added in the wastewater only batch reactor and the 3 ℓ was made up by adding filtered effluent from the parent system. By making this minor adjustment, it was now possible to directly compare the OUR responses from the parallel batch tests, since the wastewater added to each batch reactor contained the same concentration of heterotrophic organisms.

The wastewater was diluted to the same COD concentration (± 500 mgCOD/ℓ) as that fed to the parent system. A sufficient volume of diluted wastewater (± 6 ℓ) served as wastewater source for both batch tests.

Wastewater only batch tests

The wastewater only batch tests were conducted using a mixture of wastewater and filtered effluent from the parent system. The volume of filtered effluent added equaled the volume of mixed liquor harvested from the parent system and added in the parallel batch test. The required volume of diluted wastewater was carefully measured, preheated to 20°C and placed in a continually stirred batch reactor maintained at a constant temperature of 20°C. Immediately after the diluted wastewater was placed in the batch reactor, the filtered effluent was added, giving a combined volume of 3 ℓ for the mixture. A sample was drawn to obtain the initial total COD concentration (Standard Methods, 1985). The OUR in the batch reactor was measured using an automated technique (Randall *et al.*, 1991). In operating the batch test, the surface of the wastewater was covered by small plastic balls to limit surface exchange of oxygen. The pH was controlled to 7.5 (± 0.2). Because of the low OUR values, during an aeration cycle the walls of the reactor were regularly brushed to prevent particulate matter adhering to them. At regular intervals, samples were drawn from the reactor, immediately filtered, poisoned and stored for subsequent nitrate and nitrite analysis. The duration of the batch tests varied between 7 and 9 hours. At the end of the batch tests, the contents of the batch reactor were homogenized in a liquidiser, a sample drawn and the total COD concentration measured.

Wastewater and mixed liquor batch tests

For the batch tests with a mixture of wastewater and mixed liquor, a sample of mixed liquor was harvested from the aerobic reactor of the parent system and a defined volume (variously 100, 200, 300 or 400 mℓ) placed in the batch reactor. The batch reactor volume was made up to 3ℓ with the same diluted unsettled municipal wastewater used in the wastewater only batch tests, also preheated to 20°C (see above). The batch test procedure detailed above was then followed.

From the batch test data the following was calculated:

- COD recovery(%).

- Heterotrophic active biomass [$X_H = Z_{BH}$ in COD units (mgCOD/*l*)] at the start of the batch test: For the wastewater only batch tests, this gives Z_{BH} in the wastewater; for the wastewater + mixed liquor batch tests, this gives the Z_{BH} due to both the wastewater and mixed liquor.

- The difference between Z_{BH} in the two types of batch tests, gives the Z_{BH} due to the addition of mixed liquor – this represents the measured Z_{BH} in the parent system mixed liquor.

In total 23 parallel batch tests were conducted. From the batch tests, the following was concluded:

- The correlation coefficients (R^2) in linear regression of the *l*n$OUR_{H(t)}$-time plots for the wastewater (WW) only batch tests were generally very good. For the vast majority of the batch tests $R^2 > 0.95$, which indicates that the observed responses conform to the hypothesized behaviour.

- For the WW only batch tests the mean %COD recovery of 93% is relatively good. However, 9 out of 23 batch tests yielded %COD recoveries which were marginally less than 90%. The cause for these low % COD recoveries was traced to inadequate sampling for measurement of the final total COD concentration in the batch test. When this deficiency was rectified, acceptable COD mass balances > 90% were obtained. Since the source of the error lay in the measurement of COD and not OUR, the low % COD recoveries do not impact the batch test analysis as this relies on OUR measurements.

- No nitrification was observed in the batch tests with wastewater only, indicating the inactivity of autotrophic organisms in the wastewater.

- For the WW only batch tests, the wastewater heterotrophic active biomass ranged from 1.3 to 7.4 % of the total wastewater COD, with a mean of 3.6 % and a sample standard deviation of 2.0 %. This mean compares favourably with the values measured by Ubisi *et al.* (1997a,b) and Mbewe *et al.* (1995) of 4.5 % and 6.1 % respectively, for the same wastewater type.

- For the batch tests conducted with a mixture of wastewater and mixed liquor (WW+ML), the correlation coefficients (R^2) were generally lower than the R^2 values obtained in the wastewater only batch tests. However the R^2 values were generally still acceptable, with the vast majority of the R^2 values > 0.90.

- The flocculation filtration procedure (using alum flocculation and filtration through a glass fibre filter) effectively removed all active biomass from the wastewater. This was demonstrated by no measurable OUR being observed in separate aerobic batch tests, conducted on the flocculated filtered wastewater only.

- In general, the modified batch tests yielded good %COD recoveries with only 2 out of 20 batch tests rejected on the basis of %COD recovery > 110%. The mean %COD recovery for all the batch tests was 100.5% with sample standard deviation of 7.3%. The good %COD recoveries lend credibility to the measurements and the modified batch test procedure.

- Excluding the rejected batch test results, good agreement existed between the theoretical heterotrophic active biomass concentration (Z_{BH}) and the Z_{BH} values measured in the modified batch tests. The mean Z_{BH} value (1587 mgCOD/ℓ) measured in the batch tests compared remarkably closely with the theoretical steady state design value of 1567 mgCOD/ℓ calculated for the parent system.

- Despite the remarkable agreement between the theoretical Z_{BH} and the mean of the measured Z_{BH} values, the individually measured Z_{BH} values were prone to significant variation. This was attributed mainly to the sensitivity of the measured heterotrophic active biomass values to the low values measured for the slopes of the $\ell n(OUR_H)$-time plots. Even a small change in the slope of the $\ell n(OUR_H)$-time plot resulted in a marked variation of the measured Z_{BH} values. The slope of the $\ell n(OUR_H)$-time plot was low because the maximum specific growth rates for the heterotrophs were low (mean K_{MP} and $\mu_H = 0.84$/d for both parameters).

EXTENSION OF THE MODIFIED BATCH TEST INVESTIGATION

In evaluating the modified batch test procedure above, it was found that the low values for the maximum specific growth rates of the heterotrophs drawn from the continuously fed parent system caused undue sensitivity in the analytical procedure. If the maximum specific growth rates of the heterotrophs could be increased, this sensitivity could be correspondingly decreased. Accordingly, the effect of increased heterotroph maximum specific growth rate was investigated. From the literature, operating activated sludge systems in an intermittently fed fill and draw (IFFD) mode induces high heterotroph maximum specific growth rates (Ekama *et al.*, 1986). Thus, it was decided to change the operation of the continuously fed system above to IFFD. This would also provide the opportunity to evaluate the capacity of the modified batch test method to provide a reliable estimate for heterotrophic active biomass concentrations in activated sludge systems operated under significantly different conditions. Accordingly, a well-defined and controlled laboratory-scale IFFD parent system was operated at the same sludge age of 10 days and bioreactor volume of 10ℓ as the continuously fed system and received the same COD mass load per day. This parent system provided the mixed liquor samples required for measuring heterotrophic active biomass.

Parent system

The system consisted of a single aerobic reactor of 10 *l* volume. The reactor was completely mixed and was aerated. The dissolved oxygen (DO) concentration was measured by means of a DO probe immersed in the solution, which also measured the OUR, using an automated technique (Randall *et al.*, 1991).

In accordance with the steady state design model, the same quantity of heterotrophic active biomass present in a continuously fed system will be established in an IFFD system if the sludge age and bioreactor volume are the same and the same mass of biodegradable COD is introduced daily with the same wastewater. For the continuously fed parent system the influent COD concentration was controlled at ± 500 mgCOD/*l* and was introduced at a constant rate of 20 *l*/d. To achieve the same daily COD loading (10 000 mgCOD/d), the raw wastewater was diluted to ± 1000 mgCOD/*l*, and fed twice daily batch wise to give a total daily feed volume of 10 *l*/d. To evaluate the OUR response for different COD loading patterns, the volumes of the wastewater fed twice daily were varied from 5*l* : 5*l* to 2.5*l* : 7.5*l* during the latter part of the investigation. In addition, the raw wastewater COD measured for sewage batch 28 was significantly less than 1000 mgCOD/*l*, resulting in an increased daily feed volume of 12.5 *l* to attain the required daily COD mass of 10 000 mgCOD/d.

The same sewage was used to feed the parent IFFD system and was collected in batches from the Mitchell's Plain Treatment Works. The sewage batches were changed every ± 2 weeks to prevent degradation under storage. To attain steady state conditions, the system was operated for 3 sewage batches before testing was commenced. System operation procedures detailed by Ekama *et al.* (1986) were followed: Daily monitoring included influent COD, TKN; reactor nitrite + nitrate, TSS, VSS, COD and TKN; effluent COD, TKN, nitrate + nitrite (Standard Methods, 1985).

The parent system was operated for 59 days and received 5 batches of unsettled municipal wastewater from Mitchell's Plain, Cape Town. Each wastewater batch was accepted as a steady state period, and the results for each batch were averaged (after statistical analysis for outliers). From the averaged data the following were calculated:

- System COD and TKN mass balances.
- Influent wastewater unbiodegradable soluble and unbiodegradable particulate COD fractions ($f_{S,us}$ and $f_{S,up}$ respectively).
- Mixed liquor COD/VSS and TKN/VSS ratios (f_{CV}, and f_N respectively).
- The heterotrophic active biomass fraction of the mixed liquor organic suspended solids (f_{av}).
- The theoretical heterotrophic active biomass concentration in the steady state system.

From the results on the parent IFFD system:

- Compared to the variable N mass balances achieved for the continuously fed nitrification/denitrification parent system, the N mass balances for all the sewage batches tested during this investigation showed remarkable consistency. The N mass balances for the four sewage batches tested were all in the range 96 % – 102 %.

- The COD mass balances calculated for the four sewage batches tested were all in the range 98 % – 108 %. Together with the good N mass balance results, the good COD mass balances lend credibility to the reliability of the measurements. No sewage batches were rejected for further analysis.

- Measurement of the mixed liquor VSS, COD and TKN gave the following ratios; COD/VSS = 1.48 mgCOD/mgVSS (sample standard deviation = 0.03) and TKN/VSS = 0.095 mgN/mgVSS (sample standard deviation = 0.002). These values correspond closely to the accepted standard values of 1.48 mgCOD/mgVSS and 0.10 mgN/mgVSS respectively (WRC, 1984), lending further credibility to the reliability of the measurements.

- The wastewater mean unbiodegradable soluble COD fraction ($f_{S,us}$) was determined to be 0.073 (sample standard deviation = 0.004). This value is slightly lower than the value of $f_{S,us}$ = 0.085 obtained for the same wastewater in the continuously fed system, and that observed by Ubisi *et al.* (1997a,b), $f_{S,us}$ = 0.09 for the same Mitchell's Plain wastewater.

- The wastewater mean unbiodegradable particulate COD fraction ($f_{S,up}$) was determined to be 0.1822 (sample standard deviation = 0.019). This mean is significantly higher than the value of $f_{S,up}$ = 0.103 obtained for the same wastewater in the continuously fed system, and that observed by Ubisi *et al.* (1997a,b), $f_{S,up}$ = 0.12 for the same Mitchell's Plain wastewater.

- Because the parent IFFD system received the same daily mass of COD and was operated at the same sludge age and bioreactor volume as the continuously fed system, it was expected that the two systems would produce equal concentrations of volatile suspended solids (VSS). However, the IFFD system produced VSS concentrations that were consistently higher than the continuously fed system despite receiving the same COD load daily and having the same sludge age and system volume. In terms of the steady state design model, the high VSS concentration reflects in a consistently high wastewater unbiodegradable particulate COD fraction ($f_{S,up}$), which in turn gives a low mixed liquor heterotrophic active biomass fraction (f_{av}). Previous research indicates that it is unlikely that the Mitchell's Plain wastewater had a consistently high $f_{S,up}$ fraction. One possibility is that slowly biodegradable COD remains unbiodegraded in the IFFD system, and therefore adds to the unbiodegradable particulates. If this possibility is true, then the theoretical estimate for heterotrophic active biomass concentration in the parent system mixed

liquor will be acceptable. However, the comparison between the theoretical and measured heterotrophic active biomass concentration in the modified batch tests suggests an under-prediction of the "true" theoretical heterotrophic active biomass in the parent system.

- It was thus concluded that the steady state design model does not accurately reflect the behavioural response emanating from the cyclic feed / starve conditions present in the IFFD system.

- The observed daily OUR response in the parent IFFD system deviated from the typical IFFD system OUR response pattern (Still *et al.*, 1996). The expected OUR plateau associated with RBCOD utilization, followed by a well-defined precipitous drop in OUR, were not evident from the observed OUR responses. Instead a high initial OUR with a subsequent progressive decrease in OUR was observed.

- It was concluded that the deviation was mainly the result of continually decreasing growth rates of the organisms when subjected to cyclic feed-starve conditions. The nitrate generation profile, recorded during the period when the system was feeding, indicated that the nitrification rate gradually leveled off after an initial high rate of nitrification. The decreasing nitrification rate, in particular, had a significant impact on the shape of the OUR profile. Previous research projects on similar systems subjected to high COD loading rates (Dold *et al.*,1980 and Dold *et al.*,1991), indicate that the heterotrophic organisms may exhibit a similar decreasing rate of RBCOD utilization, but during this investigation no explicit substantiation of this concept could be detected.

- Although the intracellular storage of RBCOD could have been a contributing factor to the progressive decrease in OUR, this possibility was discarded through the interpretation of similar results in terms of the heterotrophic metabolism concept of the UCT kinetic simulation model.

- It was thus concluded that the IFFD system induced a behavioural response that could not be interpreted in terms of the current activated sludge modelling theory.

From the modified batch tests conducted on the mixed liquor samples drawn from the parent IFFD system, the following was concluded:

- The shape of OUR response curve recorded during each of the modified batch tests, did not conform to the expected OUR profile described by Wentzel *et al.* (1995) and that observed for the batch tests conducted on the continuously fed parent system mixed liquor. In contrast to the expected exponential increase in OUR, a relatively high initial OUR was established, followed by a progressive decrease in OUR during the first period of the batch test. To a large extent the shape of OUR response recorded during the first period of each batch test correlated well with the unexpected daily OUR profiles observed for the parent IFFD system after feeding. The consistency of occurrence indicated that the deviation of the observed response from the expected behaviour was replicated in the batch tests.

- In contrast to the parent system observations, where the deviation in OUR response could be attributed principally to a continually decreasing nitrification rate, the nitrate generation profiles recorded during the batch tests revealed only a moderate impact of the nitrification oxygen demand on the observed OUR response.

- Batch tests conducted with filtered effluent demonstrated that the high initial OUR levels observed in the batch tests were not related to intracellular COD storage in the parent IFFD system, and subsequent utilization of this stored COD in the batch tests.

- Through a process of elimination it was thus concluded that the shape of OUR response during the initial stages of the batch tests could only be the result of progressively decreasing substrate utilization/growth rates of the heterotrophic active biomass.

- When disregarding the initial batch test OUR data, the analysis of the subsequent exponentially increasing OUR data revealed that the IFFD mixed liquor heterotroph maximum specific growth rate (μ_H) values (1.35/d) were only slightly higher than the values μ_H calculated for the continuously fed system (0.84/d). However, the mean μ_H value of 1.35/d was significantly lower than the 4.3 /d obtained via an earlier investigation by Dold *et al.* (1991) for mixed liquor samples harvested from a similar IFFD system. That the μ_H for the heterotrophs was not significantly increased by the IFFD operation negated one of the principle objectives for this part of the investigation, namely to investigate the effect of increased μ_H on the batch test procedure.

- In general, the variable %COD recoveries achieved in the batch tests appear to reflect the uncertainty surrounding the OUR responses observed in the modified batch tests conducted on the IFFD system mixed liquor.

It is therefore apparent that the modified batch test in its present format is unable to provide reliable estimations of the heterotrophic active biomass present in a parent system subjected to cyclic feed/starve conditions, such as the IFFD system. It appears that the IFFD system induced a behaviour in the mixed liquor that cannot be accommodated within the current activated sludge system modelling theory.

CONCLUSIONS

The good correlation that exists between the theoretical and measured values of Z_{BH}, indicates that the method can be successfully applied as a reliable means to quantify the heterotrophic active biomass present in continuously fed anoxic/aerobic activated sludge systems. For continuously fed anoxic/aerobic activated sludge systems, the results appear to justify the incorporation of the simplified mathematical formulations derived from the UCT kinetic simulation model. The results also underline the importance of incorporating the exponential approach to nitrification in the interpretation of the batch test results.

From the extended investigation, it is apparent that the modified batch test in its present format is unable to provide reliable estimations of the heterotrophic active biomass present in a parent system subjected to cyclic feed/starve conditions, such as the IFFD system. It appears that this type of system produces organisms with behavioural responses that can not be readily accommodated within the current activated sludge modelling theory, used to interpret the batch test results. This suggests that the current modelling theory requires review for application to these types of systems.

Furthermore, in systems that include biological excess phosphorus removal the batch test method will not be effective. In these systems the heterotrophic organisms mediating the phosphorus removal are present also and the batch test will not be able to distinguish between the two groups of heterotrophic organisms. In addition, experimental evidence from such systems indicates that there are behaviours in these systems that are not recognised in the models (Ekama and Wentzel, 1997). For example, for the same wastewater there is an inconsistency in the sludge production between aerobic and anoxic/aerobic systems on the one hand and anaerobic/anoxic/aerobic systems on the other; also, the sludge production in anaerobic/anoxic/aerobic systems has been found to be linked to filamentous bulking. In the current models both these observations have to be taken into account by using different influent unbiodegradable particulate fractions ($f_{S,up}$) which markedly affects the heterotrophic active biomass as a fraction of the mixed liquor organic solids (f_{av}), clearly an unacceptable solution.

Despite the deficiencies of the batch test method to accommodate the behavioural responses observed in the systems mentioned above, the results do indicate that the batch test method will (and already has) prove to be a valuable tool that can be used to provide greater insight into the behaviour of the various activated sludge systems. The quantification of heterotrophic active biomass within the engineering and technology (modelling) paradigm, will establish a possible common link with the information arising from the new analytical techniques developed within the microbiology and biochemistry paradigm, thereby facilitating future comparisons and integration of the two paradigm sets.

RECOMMENDATIONS

From this investigation the following recommendations can be made:

- Although the modifications to the batch test method constitute a significant advance, the modified batch test was only evaluated with mixed liquor drawn from a parent system at 10d sludge age. The modified batch test method needs to be tested on mixed liquor drawn from parent systems at different sludge ages, say at 20d where the heterotrophic active biomass fraction of the mixed liquor is significantly different.

- Quantifying heterotrophic active biomass within the engineering and technology (modelling) paradigm provides the ideal platform for cross-linking and overlap with the microbiological and biochemistry paradigm. In particular, the latest developments in the *in situ* analytical techniques can definitely contribute to a better understanding of the activated sludge processes and may even facilitate the integration of these techniques into the models. The exploration of ways to integrate the different paradigms should receive serious attention.

8.1 CLOSURE

Due to their convenience as a tool to aid the research, design and operation of activated sludge systems, the design and kinetic simulation models have achieved widespread acceptance and have had a significant impact on the approach to design, operation and control of the activated sludge system, and on research into its' behaviour. However this acceptance should not inhibit critical evaluation of the principles on which these models are based; the models will always need to be used with great circumspection. The results obtained should be interpreted in terms of experience of real systems; the models should not be regarded as a substitute for knowledge and experience. The limitations of the models need to be comprehensively understood and taken into account in their application.

Parallel to the developments in the field of engineering and technology, significant advances have been made in the microbiological and biochemical areas of activated sludge. These advances have been driven by the development of new analytical techniques to allow microbial communities to be studied *in situ* in the activated sludge environment. However, there has been little cross-linking between the engineering and technology and the microbiological and biochemical paradigms. In particular the microbiological and biochemical information has not been integrated into the engineering and technology paradigm, to enable improved design and optimization. One area that can form a starting point to build bridges between the two paradigm sets is active biomass. It is hoped that this project will initiate the development of links and overlap between the two paradigm sets.

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LIST OF SYMBOLS

AA	Anoxic-aerobic
ATP	Adenosine triphosphate
b_A	Autotroph specific death rate (/d)
BA	Biological activity
BEPR	Biological excess phosphorus removal
b_{HT}^*	Specific endogenous mass loss rate at temperature T, endogenous respiration theory (/d)
b_{H20}^*	Specific endogenous mass loss rate at 20°C, endogenous respiration theory (/d)
b_{HT}	Specific endogenous mass loss rate at temperature T, death regeneration theory (/d)
b_{H20}	Specific endogenous mass loss rate at 20°C, death regeneration theory (/d)
BNR	Biological nutrient removal
C	Carbon
°C	Degrees Celsius
Ca	Calcium
CO_2	Carbon dioxide
COD	Chemical Oxygen Demand
COD_{FE}	Total unfiltered effluent COD concentration (mgCOD/ℓ)
COD_{END}	Total unfiltered COD concentration at end of test (mgCOD/ℓ)
COD_{ML}	Total unfiltered mixed liquor COD concentration (mgCOD/ℓ)
COD_{START}	Total unfiltered COD concentration at start of test (mgCOD/ℓ)
COD_{WW}	Total unfiltered wastewater (influent) COD concentration (mgCOD/ℓ)
$COD_{t=O}$	Total unfiltered COD concentration at start of test (t=O) (mgCOD/ℓ)
$COD_{t=T}$	Total unfiltered COD concentration at end of test (t=T) (mgCOD/ℓ)
d	Day
DNA	Deoxyribonucleic acid
DO	Dissolved Oxygen (mgO/ℓ)
DSVI	Diluted sludge volume index (mℓ/gTSS)
e^-	Electron
e.g.	For example
f^*	Endogenous residue fraction, endogenous respiration theory
f_E^*	Endogenous residue fraction, endogenous respiration theory
FISH	Fluorescent *in situ* hybridization
F-RBCOD	Fermentable readily biodegradable COD (mgCOD/ℓ)
FSA	Free and saline ammonia (mgN/ℓ)
$f_{S,up}$	Unbiodegradable particulate fraction of the influent (mgCOD/mgCOD)
$f_{S,us}$	Unbiodegradable soluble fraction of the influent (mgCOD/mgCOD)
f_N	Nitrogen to COD ratio of mixed liquor (mgN/mgCOD)
f_{av}	Active fraction of the volatile suspended solids (mgAVSS/mgVSS)
f_{cv}	COD to VSS ratio of the mixed liquor (mgCOD/mgVSS)

h	Hour
H	Hydrogen
H^+	Hydrogen ion
H_2O	Water
i.e.	That is
IAWQ	International Association of Water Quality
IFFD	Intermittently fed fill and draw
ISS	Inorganic suspendded solids (mgISS/ℓ)
K_{SH}	Heterotrophic half saturation rate on RBCOD (mgCOD/ℓ)
K_{SP}	Heterotrophic half saturation rate on SBCOD (mgCOD/ℓ)
K_{MP}	Heterotrophic maximum specific growth rate on SBCOD (/d)
ℓ	Litre
LR	Loading rate
Mg	Magnesium
MLE	Modified Ludzack-Ettinger
ML	Mixed liquor
MLOSS	Mixed liquor organic suspended solids
MLSS	Mixed liquor total suspended solids concentration (mgTSS/ℓ)
MLVSS	Mixed liquor volatile suspended solids concentration (mgVSS/ℓ)
MNc	Mass of nitrate generated(mgN/d)
MNne	Mass of nitrate in the effluent (mgN/ℓ)
MNnw	Mass of nitrate in the waste sludge (mgN/ℓ)
MNte	Mass of effluent TKN (mgN/ℓ)
MNti	Mass of influent TKN (mgN/ℓ)
MNw	Mass of TKN in the waste sludge (mgN/ℓ)
MOc	Mass of carbonaceous oxygen demand (mgO/d)
MOd	Equivalent mass of oxygen demand for denitrification (mgO/d)
MOn	Mass of oxygen demand for nitrification (mgO/d)
MOt	Mass of total oxygen demand (mgO/d)
MX_{BA}	Mass of autotrophic active biomass (mgVSS)
MX_{BH}	Mass of heterotrophic active biomass (mgVSS)
MX_E	Mass of endogenous material (mgVSS)
MX_I	Mass of inert material (mgVSS)
MX_V	Mass of volatile suspended solids (mgVSS)
NaCl	Sodium chloride
N	Nitrogen
N_2	Nitrogen gas
NH_3	Ammonia (mgN)
NH_4^+	Ammonium (mgN)
Nne	Nitrate concentration in the effluent (mgN/ℓ)
Nte	TKN concentration in the effluent (mgN/ℓ)
No.	Number
NO_2^-	Nitrite (mgN)
NO_3^-	Nitrate (mgN)
$\Delta NO_3/\Delta t$	nitrification rate (mgN/ℓ/h)

O_2	Oxygen
OHO	Ordinary heterotrophic organism
OUR	Oxygen utilization rate (mgO/ℓ/h)
$OUR_{H(t)}$	Heterotrophic active biomass oxygen utilization rate at time t (mgO/ℓ/h)
$OUR_{M(t)}$	OUR measured at time t (mgO/ℓ/h)
$OUR_{N(t)}$	Autotrophic oxygen utilization rate at time t (mgO/ℓ/h)
OUR_{SBCOD}	Oxygen utilization rate due to SBCOD utilization (mgO/ℓ/h)
ρ	Process rate
P	Phosphorus
PAO	Phosphate accumulating organism
polyP	Polyphosphate
PVC	Poly vynil chloride
Q or Qi	influent flow (ℓ/d)
RBCOD;S_{bsi}	Readily biodegradable COD (mgCOD/ℓ)
rDNA	Ribosomal deoxyribonucleic acid
R_S	Sludge age (d)
RNA	Ribonucleic acid
s	Second
SBCOD;S_{bpi}	Slowly biodegradable COD (mgCOD/ℓ)
SCFA	Short Chain fatty acids
SLR	Sludge loading rate (mgCOD/mgVSS)
SSD	Sample standard deviation
S_{ads}	Adsorbed slowly biodegradable COD concentration (mgCOD/ℓ)
S_b	Biodegradable COD concentration (mgCOD/ℓ)
S_{bi}	Biodegradable COD concentration in the influent (mgCOD/ℓ)
S_{bp}	Biodegradable COD concentration that is particulate (mgCOD/ℓ)
S_{bpi}	Particulate biodegradable COD concentration in the influent (mgCOD/ℓ)
S_{bs}	Biodegradable COD concentration that is soluble (mgCOD/ℓ)
S_{bsi}	Soluble biodegradable COD concentration in the influent (mgCOD/ℓ)
S_{NO}	Oxidized nitrogen concentration (mgN/ℓ)
$S_{NO(0)}$	Initial oxidized nitrogen concentration (mgN/ℓ)
$S_{NO(t)}$	Oxidized nitrogen concentration at time t (mgN/ℓ)
SO_4	Sulphate
S_{te}	Filtered effluent COD concentration (mgCOD/ℓ)
S_{ti}	Unfiltered influent COD concentration (mgCOD/ℓ)
S_{ui}	Unbiodegradable COD concentration in the influent (mgCOD/ℓ)
S_{up}	Unbiodegradable particulate COD concentration (mgCOD/ℓ)
S_{upi}	Unbiodegradable particulate COD conc. in the influent (mgCOD/ℓ)
S_{us}	Unbiodegradable soluble COD concentration (mgCOD/ℓ)
S_{use}	Unbiodegradable soluble COD conc. in the effluent (mgCOD/ℓ)
S_{usi}	Unbiodegradable soluble COD conc. in the influent (mgCOD/ℓ)
t	Time
TDS	Total dissolved solids (mgTDS/ℓ)

TIS	Total inorganic solids (mgTIS/ℓ)
TKN	Total Kjedahl Nitrogen (mgN/ℓ)
TS	Total solids (mgTS/ℓ)
TSS	Total suspended solids (mgTSS/ℓ)
UCT	University of Cape Town
USCOD	Unbiodegradable soluble COD (mgCOD/ℓ)
μ_A	Autotrophic maximum specific growth rate (/d)
μ_H	Heterotrophic maximum specific growth rate on RBCOD (/d)
μm	Micrometer
VSS	Volatile suspended solids (mgVSS/ℓ)
V	System volume (ℓ)
V_p	Volume of reactor (ℓ)
V_{ML}	Volume of mixed liquor added to batch test (ℓ)
V_{WW}	Volume of wastewater added to batch test (ℓ)
WRC	Water Research Commission
WW	Wastewater
X_{BA}	Autotrophic active biomass concentration (mgVSS/ℓ)
X_{BH}	Heterotrophic active biomass concentration (mgVSS/ℓ)
X_E	Endogenous material concentration (mgVSS/ℓ)
X_I	Inert material concentration (mgVSS/ℓ)
X_V	Volatile suspended solids concentration (mgVSS/ℓ)
$X_V(PS)$	Mixed liquor VSS concentration in parent system (mgVSS/ℓ)
Y_{ZH}	Heterotrophic active biomass yield, COD units (mgCOD/mgCOD)
Y_H^*	Heterotrophic active biomass yield, VSS units (mgVSS/mgCOD)
Y_{ZA}	Autotrophic biomass yield (mgCOD/mgN)
Z_{BH}	Heterotrophic active biomass concentration (mgCOD/ℓ)
$Z_{BH(o)}$	Heterotrophic active biomass concentration at the start of the batch test (mgCOD/ℓ)
$Z_{BH(o)}(ML)$	Heterotrophic active biomass due to added mixed liquor (mgCOD/ℓ)
$Z_{BH(o)}(WW)$	Heterotrophic active biomass in original wastewater (mgCOD/ℓ)
$Z_{BH(o)}(WW)_D$	Heterotrophic active biomass due to wastewater at the start of batch test with wastewater + mixed liquor (mgCOD/ℓ)

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

To comply with more stringent effluent legislation, over the past two decades significant advances have been made in the areas of engineering (design) and technology (implementation and operation) of the single sludge activated sludge system. Activated sludge systems have been successfully designed and implemented at full-scale to progressively include the biological removal of carbon (C), nitrogen (N) and phosphorus (P). This implementation has been aided by the development of a suite of steady state design models (e.g. WRC, 1984; Wentzel *et al.*, 1990; Maurer and Gujer, 1994) and kinetic simulation models (e.g. Dold *et al.*, 1980, 1991; Van Haandel *et al.*, 1981; Henze *et al.*, 1987; Wentzel *et al.*, 1992; Henze *et al.*, 1995).

These models are based on a common conceptualization of the processes acting in the system. In terms of this group of models, in the bioreactor of the non-nitrifying aerobic activated sludge system, the mixed liquor organic (volatile) suspended solids (MLOSS) is made up of three components; (1) ordinary heterotrophic organism (OHO) active biomass, (2) endogenous residue and (3) inert material. In the nitrifying aerobic and anoxic/aerobic activated sludge systems, a fourth component is included; (4) autotrophic organism (AO) active biomass. All four MLOSS components settle out in the secondary settling tank and are returned to the bioreactor via the underflow recycle; these components leave the system via the waste flow. If an anaerobic reactor is included to stimulate biological excess phosphorus removal (BEPR), additionally (5) phosphate accumulating organism (PAO) active biomass and this organism group's endogenous residue will contribute to the MLOSS (Wentzel *et al.*, 1992; Henze *et al.*, 1995). The active biomass components of the MLOSS mediate the relevant biological processes deemed to be of importance; OHO's mediate COD removal and denitrification, AO's mediate nitrification and PAO's mediate BEPR and COD removal.

Historically the MLOSS has been measured as a lumped parameter, via the VSS or COD test (Standard Methods, 1985). Specific rates for the biological processes (e.g. denitrification; oxygen utilization) often were (and still are) expressed in terms of this lumped parameter. However, from the above, only parts of the MLOSS are active biomasses, and only these parts mediate the relevant biological processes, e.g. OHO's for COD removal and denitrification. Accordingly, the specific rates for the relevant (and associated) biological processes should be expressed in terms of the appropriate active biomass concentration to allow a meaningful comparison of rates measured in different systems. More recently, with the proliferation of kinetic simulation computer programmes that invariably include active biomass concentrations as parameters (e.g. Biowin, Simba, GPX, UCTOLD, UCTPHO), these parameters and the use of specific rates in terms of them, have become much more widely accepted.

However, this acceptance has not been driven by sound scientific proof of the active biomass concept, but rather by the convenience of the computer programmes. It must be remembered that active biomass exists only hypothetically within the structure of the design procedures and kinetic models. Although indirect evidence does provide some support for the active biomass parameters (by consistency between observations and predictions over a wide range of conditions, e.g. Dold *et al.*, 1980, 1991; Alexander *et al.*, 1980; Van Haandel *et al.*, 1981; Warner *et al.*, 1986), these have not been directly measured experimentally and compared to the hypothetical model values. This deficiency casts a measure of uncertainty on the entire framework within which the models have been developed and is a major weakness in the models. The problem in measurement has been the lack of suitable experimental techniques.

Recently a simple batch test procedure has been developed to quantify OHO active biomass concentration (Kappeler and Gujer, 1992; Wentzel *et al.*, 1995; Mbewe *et al.*, 1995). This batch test method was modified by Wentzel *et al.* (1998) and used to quantify the OHO active biomass concentration of mixed liquor samples drawn from a well-defined parent laboratory-scale anoxic/aerobic activated sludge system operated at 12 and 20d sludge age. The measured OHO active biomass concentrations were in close agreement with those calculated theoretically for the parent system at 12d sludge age, but were about ½ the theoretical values for the system at 20d sludge age. While the good correspondence at 12d sludge age provides substantive direct evidence supporting both the models and the experimental method, reasons for the poor correspondence at 20d sludge age need to be found. Wentzel *et al.* were not able to provide an explanation for this inconsistency, so the uncertainty around the active biomass concept largely remains.

Parallel to the developments in the engineering and technology of the activated sludge system, significant advances have been made in the microbiological and biochemical areas of activated sludge. As researchers in these fields have moved away from pure culture work to the activated sludge environment, a number of new analytical techniques have been developed to study microorganisms *in situ*, e.g. ATP analysis (Nelson and Lawrence, 1980), DNA analysis (Liebeskind and Dohmann, 1994), quinone profiling (Hu *et al.*, 1998), microautoradiography (Nielsen *et al.*, 1998), using florescent probes for ribosomal RNA (Wagner *et al.*, 1994; Wat. Sci. Technol., 1998).

While the microbiological and biochemical knowledge and developments have made a considerable contribution to the understanding of the biological nutrient removal activated sludge system, the full potential of these developments have yet to be realised for the system. It remains for the results that these techniques provide to be integrated with the design and kinetic modelling theory. The consequence of this is that the engineering and technology (modelling) paradigm has largely worked independently of the microbiological and biochemical paradigm. To facilitate links and overlap between the two paradigm sets, the new developments in the microbiological and biochemical analytical techniques can be implemented to address the deficiency in the engineering and technology paradigm of the active biomass concept.

One area that can form a starting point to build bridges between the two paradigm sets, is the measurement of heterotrophic active biomass. ***This research project investigates the measurement of heterotrophic active biomass within the engineering and technology (modelling) paradigm.*** If this parameter can be successfully quantified within this paradigm and agreement obtained between the measurements and the theoretical modelling values, this will provide the basis for future comparison with the quantative data arising from the new measurement techniques within the microbiological and biochemical paradigm. This will establish a common link between the two paradigm sets.

1.2 SIGNIFICANCE OF THE HETEROTROPHIC ORGANISM GROUP

Since the investigation of the heterotrophic active biomass within the engineering and technology (modelling) paradigm forms the basis of this research project, it is important to determine the significance of this organism group within the modelling conceptual framework of the various organism groups and compounds that comprise an activated sludge system. In this section this conceptual framework will be outlined briefly, to provide an overview of the current modelling understanding of the processes that operate in the biological reactor, how these processes relate to the influent wastewater and give rise to the various mixed liquor components of which the active heterotrophic organisms form an integral part.

1.2.1 Wastewater characterization

The activated sludge system comprises a biological reactor and a secondary settling tank. Irrespective of whether or not biological N and/or P removal are included, many different biological and physical processes take place in the biological reactor, and the physical process of sedimentation takes place in the secondary settling tank. These processes form the basis for subdividing the influent wastewater, carbon (C), nitrogen (N) and phosphorus (P) materials into subfractions (see Fig 1.1). On entry of the influent into the biological reactor, the particulate materials, which include both settleable and suspended (non-settleable or colloidal), organic and inorganic materials, are enmeshed (a biologically mediated flocculation) and become part of the activated sludge mixed liquor. The soluble materials, both organic and inorganic, remain in solution. In the biological reactor, the bacteria present will act on the biologically utilizable material, termed *biodegradable*, whether organic or inorganic, soluble or particulate, and transform these to other compounds or products, either gaseous, soluble or particulate: The gaseous products escape to the atmosphere, the particulate products become (or remain) part of the mixed liquor solids and the soluble products become (or remain) dissolved in solution. The non-biologically utilizable material, termed *unbiodegradable*, will not be transformed and will remain in either the soluble or particulate form. Following from the processes in the bioreactor, the first major division of the influent is based on whether the material is *biodegradable* or *unbiodegradable*, see Fig. 1.1.

After biological treatment the flow passes from the biological reactor to the secondary settling tank. In the secondary settling tank, the particulate materials making up the mixed liquor (whether organic or inorganic, biodegradable or unbiodegradable) settle out and are returned to the biological reactor. The particulate components of the mixed liquor entering the settling tank are thus retained in the system. All the soluble components of the mixed liquor (whether organic or inorganic, biodegradable or unbiodegradable) cannot settle out and escape with the effluent, see Fig. 1.1.

The settling behaviour in the secondary settling tank therefore forms the basis for subdividing the influent *unbiodegradable* material into subfractions: The influent unbiodegradable material passes unmodified through the biological reactor to the secondary settling tank; ideally all the particulate (and colloidal) material settles out in the secondary settling tank and these constituents are therefore termed *unbiodegradable particulate*, the soluble constituents cannot settle out so that these constituents are termed *unbiodegradable soluble*, see Fig. 1.1. With regard to the influent *biodegradable* material, because a substantial amount of this material has been biologically transformed in the biological reactor preceding the secondary settling tank, it cannot be subdivided into subfractions based on its behaviour in the secondary settling tank; subdivision of the biodegradable material is based on the rates of transformation/utilization by the bacteria in the biological reactor (see below).

From the above, in terms of the conceptualization of the activated sludge system behaviour, the wastewater constituents are characterized (1) biologically, i.e. as biodegradable (biologically utilizable) or unbiodegradable (non-biologically utilizable) material, and (2) physically, i.e. as soluble or particulate material. Therefore, for the models based on fundamentals of behaviour, it is necessary to divide the influent constituents into at least three components:

- Biodegradable
- Unbiodegradable soluble
- Unbiodegradable particulate.

This general wastewater characterization structure (see Fig. 1.2) conforms to the biological degradation and physical solid/liquid separation processes that take place in the activated sludge system.

From the description above:

(1) The unbiodegradable soluble component of the influent passes unmodified through the bioreactor to the secondary settling tank and leaves the system via the secondary settling tank overflow; this influent component has no influence on the mixed liquor solids, but does influence the effluent quality.

(2) The unbiodegradable particulate component of the influent, on entry into the bioreactor is enmeshed in the mixed liquor mass and will settle out in the secondary settling tank to be retained in the system; thus this influent component

has a direct influence on the mixed liquor solids in the reactor, but no influence on the effluent quality. The unbiodegradable particulate component leaves the system with the mixed liquor wasted to maintain the sludge age.

(3) The biodegradable component of the influent is transformed by biological action in the bioreactor to other compounds or products, either gaseous, soluble or particulate. The particulate compounds/products will settle out in the secondary settling tank to be retained in the system and will therefore influence the mixed liquor solids in the reactor; the soluble components will not settle out in the secondary settling tank and so will pass out in the effluent while the gaseous components will escape to the atmosphere - the latter two components will not, therefore, influence the mixed liquor solids.

Thus, the mixed liquor solids present in the bioreactor will be comprised of components arising directly from the influent and from biological transformations of the influent. This conceptualized characterization of the influent components in the activated sludge system can be applied to any material in the influent, organic or inorganic. Of particular interest to this discussion is the organic carbonaceous material. The carbonaceous material usually is quantified by means of the chemical oxygen demand (COD) test, which measures the electron, or equivalently energy, donating capacity of organic material, so that in effect the conceptualization of the fate of the influent is applied to the electrons (e^-) in the carbonaceous material.

1.2.2 Organism groups

As noted above, the biodegradable components of the influent wastewater are biologically transformed in the bioreactor to other components or products, either gaseous, soluble or particulate. These biological transformations are mediated by a complex mixture of microorganisms and in the bioreactor of the activated sludge system a wide diversity of organism species have been identified.

However, in both the steady state design (e.g. WRC, 1984) and dynamic kinetic simulation (e.g. Dold *et al.*, 1980 and Dold *et al.*, 1991) models it has been recognized that including a complete description of every organism species would be impractical. Instead microorganisms that fulfill a particular function in the activated sludge system are grouped together as a single entity which has been called a "surrogate" organism. This surrogate organism is assigned a set of unique characteristics that reflect the behaviour of the group, but may not reflect the characteristics of any individual organism or species of organisms in the group. Thus, for modelling of activated sludge systems the "organizational level" (Odum, 1971) that is followed is the mass behaviour of a population or group of selected organisms; these populations or groups are identified and selected based on identifiable functions.

The principle organism groups, their functions and the zones in which these functions are performed are summarized in Table 1.1. ***This research project considers only the aerobic and anoxic/aerobic activated sludge systems.***

Thus, two organism groups need to be taken into account; (i) heterotrophic organisms unable to accumulate polyP, termed heterotrophs and (ii) autotrophic organisms mediating nitrification, termed autotrophs or nitrifiers. The biological transformation mediated by these two organism groups are described below.

1.2.3 Heterotrophic organisms

The heterotrophic organisms obtain both energy and carbon from complex organic compounds. This is a diverse group of organisms, which, given sufficient time and appropriate environmental conditions, will utilize every type of organic material. The group is ubiquitous and in any given situation those members of the group that derive maximum benefit from the specific organic material and environmental conditions will develop. As the organic source and environmental conditions change, so associated changes in the heterotrophic organism species take place.

Table 1.1: Principle organism groups included in models for activated sludge systems, their functions and the zones in which these functions are performed.

ORGANISM	BIOLOGICAL PROCESS	ZONE
1. Ordinary heterotrophs (unable to accumulate polyP)	• COD removal (organic degradation; DO uptake)	Aerobic
	• Ammonification ($organicN \rightarrow NH_4^+$)	Aerobic
	• Denitrification (organic degradation; $NO_3^- \rightarrow NO_2^- \rightarrow N_2$)	Anoxic
	• Fermentation (F-RBCOD→SCFA)	Anaerobic
2. PolyP heterotrophs (accumulate polyP)	• P release (SCFA uptake; PHA storage)	Anaerobic
	• P release (SCFA uptake; PHA storage)	Anoxic
	• P uptake (PHA degradation; denitrification?)	Anoxic
	• P uptake (PHA degradation; DO uptake)	Aerobic
3. Autotrophs (nitrifiers)	• Nitrification ($NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$; DO uptake)	Aerobic

As noted above, for the purpose of modelling the activated sludge system the heterotrophic organism species are grouped together as a "surrogate" which is assigned a unique set of characteristics reflecting the behaviour of the group. This group of organisms mediates the processes of COD removal and denitrification. Conceptually, in the models this "surrogate" heterotroph is subject to two main biological processes; (i) synthesis or growth and (ii) endogenous mass loss/death.

(i) Synthesis/growth

For the heterotrophic organisms, the organic material in the influent serves two functions: (a) it is the supply of materials which are transformed into new cell materials, and (b) it is the supply of energy to effect these transformations. In terms of the models, on entry into the bioreactor some of the influent organic materials are transformed (via a number of biochemical pathways, collectively called anabolism) to new heterotroph cell material.

Accepting the general formulation for protoplasm as $C_5H_7O_2N$ (McCarthy 1964), with glucose as an example substrate the synthesis reaction can be summarized as:

$$5\ C_6H_{12}O_6 + 6\ NH_3 \rightarrow 6\ C_5H_7O_2N + 18\ H_2O \qquad (1.1)$$

This synthesis/growth process requires energy. The energy is obtained as follows: Some of the organic molecules are split by the heterotrophs to give hydrogen ions, electrons and carbon dioxide. For example, consider the organic molecule glucose:

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6\ CO_2 + 24\ H^+ + 24\ e^- \qquad (1.2)$$

Because it releases electrons the organic molecule is termed an electron donor and on yielding the electrons, the molecule is said to be oxidized. The electrons (and protons) are captured by the heterotrophs, and transferred via an internal sequential set of oxidation reduction (redox) reactions eventually to a molecule which can accept them; this molecule is called the terminal electron acceptor. In this series of redox reactions, free energy is released (i.e. energy that is available to do work) which is captured by the organism (collectively this process is called catabolism). The energy captured by the organism principally is used by the organism for the transformation reactions that synthesize new cell mass, Eq. (1.1). From bioenergetics it is possible to determine the amount of glucose that must be oxidized to synthesize one mole of new cell mass (WRC, 1996).

In the oxidation of substrate, if oxygen is present (aerobic conditions) the terminal electron acceptor is oxygen (O_2) which is reduced to water (H_2O), i.e.

$$O_2 + 4\ H^+ + 4\ e^- \rightarrow 2\ H_2O \qquad (1.3)$$

If oxygen is absent, but nitrate (or nitrite) is present (anoxic conditions), the nitrate (NO_3^-) (or nitrite, NO_2^-) serves as terminal electron acceptor and is reduced to nitrogen gas (N_2), giving rise to the denitrification process:

$$NO_3^- + 12\ H^+ + 10\ e^- \rightarrow N_2 + 6\ H_2O \qquad (1.4)$$

Thus, of the original biodegradable organic material present, a part is oxidized to yield free energy. This free energy is utilized by the heterotrophs to "reorganize" the remaining organic material into new cell mass, the free energy being lost as heat in the re-organization. Noting that the heterotrophic organisms use electron transfer (redox) reactions to generate the free energy, the transformation of the biodegradable organic material can best be traced by monitoring the transformations in electrons. This has the advantage, *inter alia*, that, due to the indestructibility and hence conservation of electrons, electron mass balances can be conducted. Accordingly, of the electrons present in the biodegradable organic material (measured via the chemical oxygen demand (COD) test, see WRC, 1984), a fraction are transformed into new heterotrophic cell mass, and the remainder pass to the terminal electron acceptor, oxygen if aerobic and nitrate if anoxic.

Now, the ratio of electrons captured in new cell mass to the electrons present in the biodegradable substrate is termed the yield (Y_{ZH}), i.e.

$$Y_{ZH} = \frac{e^- \text{ in synthesized material}}{e^- \text{ in biodegradable substrate}} \qquad (1.5a)$$

Accepting the COD test as a measure of the electrons, then

$$Y_{ZH} = \frac{\text{COD synthesized material}}{\text{COD biodegradable substrate}} \qquad (1.5b)$$

And, from a mass balance on electrons (COD):

COD of biodegradable material = COD of synthesized material + e^- to electron acceptor.

For activated sludge systems with the wide diversity of biodegradable organics in the influent and heterotrophs in the bioreactor, it has been found that the yield can be accepted to be approximately constant, at Y_{ZH} = 0.666 mgCOD/mgCOD (Dold *et al.*, 1980; 1991). In other words, for every biodegradable organic COD utilized, a constant fraction will be transformed to heterotrophic active biomass, and the balance of the e^- will pass to the electron acceptor.

A question that needs to be addressed is how much of the influent biodegradable material will be transformed in the bioreactor, within the residence time in the system. This is a problem of kinetics or rates. In considering the kinetics of biodegradable organic

material transformation, it is subdivided into two components, readily biodegradable (soluble) COD (S_{bsi}) and slowly biodegradable (particulate) COD (S_{bpi}), see Fig. 1.3. This division is based on observed biological responses of activated sludge mixed liquor to domestic wastewater (Dold *et al.*, 1980; Van Haandel *et al.*, 1981), that is, the division is a biokinetic one: Under dynamic loading of activated sludge (short sludge age cyclic loading, plugflow reactors, batch tests) two distinct rates of utilization of domestic wastewater biodegradable COD substrate were apparent with either oxygen (Dold *et al.*, 1980; Ekama *et al.*, 1986) or nitrate (Van Haandel *et al.*, 1981; Ekama *et al.*, 1986) as electron acceptor (aerobic or anoxic conditions respectively). A fraction (called readily biodegradable COD, RBCOD) was taken up rapidly by the sludge and metabolized, giving rise to a high oxygen or nitrate utilization rate respectively. The other fraction (called slowly biodegradable COD, SBCOD) was taken up much more slowly and metabolized, giving rise to oxygen or nitrate utilization rates about 1/10 of the rate with RBCOD. To explain these observations, the RBCOD was hypothesized to consist of simple *soluble* molecules that can be absorbed readily by the organism and metabolized for energy and cell synthesis, whereas the SBCOD was assumed to be made up *of particulate / colloidal / complex* organic molecules that require extra cellular adsorption and enzymatic breakdown (hydrolysis) prior to absorption and utilization.

The hypothesized difference in molecule size between RBCOD and SBCOD has been used to classify the RBCOD as a biodegradable soluble COD and the SBCOD as a biodegradable particulate COD. Since the RBCOD is soluble, it is exposed to biological treatment only as long as the liquid remains in the reactor, i.e. for the hydraulic retention time which is relatively short (~ 6 – 24h). However, the rate of RBCOD utilization is high and for sludge ages greater than about 3 days the concentration of RBCOD in the effluent is negligible even though the retention time is relatively short. Accordingly, for most completely aerobic and anoxic/aerobic systems it can be safely assumed that all the RBCOD will be utilized in the system. For the SBCOD, the extracellular breakdown (hydrolysis) is slow and forms the limiting rate in the utilization of SBCOD. Although the rate of SBCOD utilization is relatively slow, the SBCOD does not appear in the effluent. This is because on entry of the influent into the bioreactor, the SBCOD becomes enmeshed in the mixed liquor, settles out in the secondary settling tank and is retained in the system. Therefore, the particulate biodegradable organics (SBCOD) are exposed to biological treatment for as long as the solid (settleable) material is retained in the system, i.e. for the sludge age.

Thus, even though the utilization of the SBCOD is around 10 times slower than that for the RBCOD, because the sludge age in most activated sludge systems is usually more than 10 times longer than the hydraulic retention time, the SBCOD is completely utilized also. From simulation studies using dynamic kinetic models (Dold *et al.*, 1991) all the SBCOD is completely utilized for sludge ages greater than about 2 to 3 days and temperatures greater than about 20°C (5 to 6 days at 14°C). Accordingly, for most activated sludge systems it is sufficient to assume all the SBCOD will be utilized in the system.

Thus, for activated sludge systems at 20°C for sludge ages greater than about 3 days all the biodegradable organic material in the influent, whether soluble or particulate, will be transformed in the bioreactor by biological action mediated by the heterotrophic active biomass. The products of this transformation are:

(1) Gaseous - carbon dioxide (only considered if the fate of the carbon is traced) and nitrogen, produced if nitrate acts as terminal electron acceptor, Eq. (1.4), i.e. anoxic conditions.

(2) Soluble - water, produced when oxygen acts as terminal electron acceptor, Eq. (1.3), i.e. aerobic conditions.

(3) Particulate - new heterotrophic active biomass.

The particulate heterotrophic active biomass will settle out in the secondary settling to be retained in the system and thus will be another component of the mixed liquor solids. Since the influent SBCOD is virtually totally utilized for systems with sludge ages > 3d at 20°C, the SBCOD will not be a significant component of the mixed liquor solids, and hence can be neglected.

(ii) Endogenous mass loss/death

The endogenous mass loss phenomenon is the loss of active organism mass with time. The phenomenon can be observed as a reduction in organism active mass when the organism population is aerated with no substrate added, for example, in the aerobic digestion of mixed liquor. The phenomenon also manifests itself as a decrease in specific organism yield with increase in sludge age. In the models, two conceptual approaches have been followed to describe this phenomenon; endogenous respiration and death-regeneration.

Endogenous respiration

This approach is the simpler and is followed in the steady state design models. In the approach, active organism mass is lost at a constant specific rate. Of the active organism mass that is lost, a part (about 80%) is oxidized to provide energy for maintenance of the active mass remaining and balance (about 20%) remains as a particulate unbiodegradable organic fraction accumulating in the system, and is called endogenous residue. The oxidation of the biodegradable part of the active mass lost gives rise directly to an endogenous oxygen consumption under aerobic conditions, and indirectly to a nitrate demand (denitrification) under anoxic conditions. Whether the active mass oxidized is some "deep" storage material or from the death of individual organisms is of little practical importance in the models, but conceptually death of individual organisms would seem more likely; on organism death the biodegradable part of the organism is oxidized by those remaining for maintenance energy, and the unbiodegradable part accumulates as a particulate endogenous residue. The simplicity of this approach allows analytical solutions to be readily determined.

Death-regeneration

This approach is followed in the dynamic kinetic simulation models. In the death-regeneration approach an attempt is made to separate out the processes which take place during the organism's "death phase". Disappearance of live active mass is hypothesized to be due to the net effect of death (natural or predation) and regeneration of organisms: On death the cell material is released through lysis; a part is particulate unbiodegradable endogenous residue (about 8%); the remaining part (about 92%) is biodegradable and adds to the slowly biodegradable COD (SBCOD) which passes through the path of adsorption, hydrolysis and synthesis of new cell mass (i.e. regeneration) described above. The synthesis of new cell mass gives rise to an associated aerobic oxygen or anoxic nitrate demand. Thus, in death-regeneration the oxygen or nitrate demand arises in fact from the energy requirements for resynthesis of organism active mass (regeneration) from the SBCOD liberated from organism death. The main implication of this approach is that "maintenance energy" per se (the oxygen / nitrate requirement for maintenance) is considered to be so small that it can be lumped with, and completely swamped by, the oxygen / nitrate demand for resynthesis of new cell mass from the lysed biodegradable substrate.

Comparing the two approaches, for the mixed cultures present in the activated sludge system, predation is likely to be a significant cause for death of organisms, "liberating" substrate for synthesis of new cell mass (predator and others). Thus, conceptually the death-regeneration would appear superior. However, provided all the biodegradable COD has been depleted and a terminal electron acceptor (oxygen or nitrate) is continually available, with the appropriate selection of constants the two approaches give the same nett result, i.e. same loss of heterotrophic active biomass, utilization of oxygen / nitrate and generation of endogenous residue (Dold *et. al*, 1980, 1991). If a terminal electron acceptor is not available, then the endogenous-respiration approach is deficient and the death-regeneration approach preferable. Also, the two approaches do deviate slightly when significant biodegradable COD is present, in for example, batch tests.

Irrespective of whether the endogenous respiration or the death-regeneration approach is followed, in both a part of the organism active mass that is lost is unbiodegradable particulate which will settle out in the secondary settling to accumulate in the system as endogenous residue. Thus, the endogenous residue will be another component of the mixed liquor solids.

1.2.4 Autotrophic organisms

In the activated sludge system the autotrophic organisms of importance are the nitrifiers. These organisms mediate the process of nitrification, whereby free and saline ammonia is oxidized to nitrite and nitrate. It is generally accepted that the nitrification is due to two specific groups of autotrophic bacteria, the Ammonia oxidizers and Nitrite oxidizers. Since these organisms are autotrophs, they obtain the carbon required to form cellular material from carbon dioxide. Their energy requirements are obtained by oxidizing ammonia to nitrite and nitrate.

Ammonia oxidizers utilizes ammonium (NH_4^+) as electron donor and oxygen as electron acceptor; the ammonium is oxidized to nitrite and the oxygen reduced to water, i.e.

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + H_2O + 2\ H^+ \qquad (1.6)$$

Nitrite oxidizers uses nitrite as electron donor and oxygen as terminal electron acceptor; the nitrite is oxidized to nitrate and the oxygen reduced, i.e.

$$NO_2^- + 0.5\ O_2 \rightarrow NO_3^- \qquad (1.7)$$

The nitrifiers can only execute these two reactions if oxygen is present, and are thus obligate aerobes.

Thus, the full nitrification process takes place in two steps. However, the rate of conversion of ammonium to nitrite by Ammonia oxidizers is very much slower than the rate of conversion of the nitrite to nitrate by Nitrite oxidizers; as fast as the nitrite is formed it is nitrified to nitrate. The rate limiting step in the nitrification sequence, therefore, is that due to the Ammonia oxidizers - only the kinetics of this organism needs to be considered.

As a consequence, in the models the two stage nitrification process is approximated as a single stage process with the ammonium being converted directly to nitrate, i.e.

$$NH_4^+ + 2\ O_2 \rightarrow NO_3^- + H_2O + 2\ H^+ \qquad (1.8)$$

This step is modelled as being mediated by a single nitrifying organism group, whose kinetics and characteristics closely approximate the Ammonia oxidizers (since this organism group mediates the rate limiting step).

Part of the energy released in the single nitrification process is used for synthesis of new nitrifier organism active mass (growth), i.e. accepting $C_5H_7O_2N$ as a generalized formulation for nitrifier protoplasm (Mc Carthy, 1964), then:

$$5\ CO_2 + NH_4^+ + 2\ H_2O \rightarrow C_5H_7O_2N + 5\ O_2 + H^+ \qquad (1.9)$$

Recognizing that ammonium serves as the "substrate" for the nitrifiers, the specific yield is expressed in terms of the ammonium utilized, i.e.

$$Y_{ZH} = \frac{\text{COD of nitrifiers formed}}{NH_4^+ \text{ - N utilized}} \qquad (1.10)$$

The nitrifier active biomass that is formed in the activated sludge system is important in considering the mixed liquor solids because it is particulate, will settle in the secondary settling tank and so be retained in the system. The nitrifier active biomass, therefore, will be one component making up the mixed liquor solids.

As with the heterotroph active biomass, the nitrifier active biomass also is subject to endogenous mass loss/death. Although conceptually for consistency, this endogenous mass loss/death should result in the generation of an endogenous residue, compared to the other mixed liquor fractions the relative amount on endogenous residue, generated by the autotrophs is so small that it is of little practical importance and can be neglected.

1.2.5 Activated sludge mixed liquor organic components

From the discussion above, the mixed liquor in the bioreactor of the activated sludge system is comprised of a number of organic components (see Fig. 1.4):

(1) Inert material - derived from the unbiodegradable particulate organics present in the influent.

(2) Heterotroph active biomass - synthesized in the bioreactor from the biodegradable organics present in the influent.

(3) Endogenous residue - generated from the unbiodegradable portion of the heterotrophic (and autotrophic) active biomass that is lost in the endogenous respiration/death-regeneration process.

(4) Autotrophic active biomass - synthesized in the bioreactor in the nitrification of ammonia to nitrate.

All four mixed liquor organic solids components settle out in the secondary settling tank and are returned to the bioreactor via the underflow recycle; these components leave the activated sludge system via the waste flow.

For activated sludge systems receiving "normal" municipal wastewaters (influent TKN/COD , 0.12 mgN/mgCOD) the autotrophic active biomass component of the mixed liquor organic solids is very small compared to the other three components (< 2% of the total) – the substrate source for the autotrophs (NH_4^+) is very much less than that for the heterotrophs (COD) and their specific yield is very much lower (autotrophs Y_{ZA} = 0.15 mgCOD/mgN; heterotrophs Y_{ZH} = 0.666 mgCOD/mgCOD, Dold *et al.*, 1980, 1991). Accordingly, in considering the components making up the mixed liquor organic solids, with very little error the autotrophic active biomass can be neglected, so that the number of organic components reduces to three; (i) inert, (ii) endogenous residue and (iii) heterotrophic active biomass. (If the nitrification process itself is being considered, then the autotroph active biomass is of fundamental importance and must be taken into account).

Accepting that the autotroph active biomass can be neglected, a schematic diagram showing the origin of the mixed liquor organic fractions is shown in Fig. 1.5. For the three mixed liquor organic solids components, two, the inert material and endogenous residue, are inactive and do not participate in any of the biological processes.

The heterotrophic active biomass component, on the other hand, mediates the biodegradation processes of COD removal and denitrification. Thus, the rates for these processes are directly related to the amount of heterotrophic active biomass present. Thus, the heterotrophic active biomass is a central crucial parameter within the conceptual framework of models for the activated sludge system. However, as noted above, this parameter remains largely hypothetical. Independent measurement of this parameter is of fundamental importance, to provide greater surety in the models. This research project addresses this issue.

1.3 BATCH TEST METHOD TO QUANTIFY HETEROTROPHIC ACTIVE BIOMASS

Kappeler and Gujer (1992) describe a simple batch test to quantify heterotrophic active biomass in activated sludge mixed liquor; a small quantity of mixed liquor is mixed with centrifuged wastewater and the oxygen utilization rate (OUR) response is monitored with time. From the observed exponential increase in the OUR, the initial OUR in the batch test can be determined, which can be used to derive an estimate for the heterotrophic active biomass concentration. However, they did not evaluate the proposed method. Wentzel *et al.* (1995) and Mbewe *et al.* (1995) modified and extended this method for application to the characterization of municipal wastewaters: The batch test was conducted on unsettled municipal wastewater *without* the addition of activated sludge mixed liquor. From the OUR-time response and a flocculated-filtered COD measurement at the end of the test, the wastewater heterotrophic active biomass, readily biodegradable COD (RBCOD) and unbiodegradable soluble COD (USCOD) could be determined. Mbewe et al. (1995) found that the RBCOD and USCOD measured in the batch test correlate closely to that measured via conventional methods. However, they were not able to evaluate the results for wastewater heterotrophic active biomass, since no conventional tests were available. They did note that measurements appeared to reflect operational changes at the wastewater treatment plant where the wastewater was collected. At the treatment plant, due to operational problems with sludge handling unit processes, on occasion waste activated sludge mixed liquor was discharged into the sewer at a point upstream of where the wastewater was collected; the batch test method could correctly detect the increase in heterotrophic active biomass during these periods.

Ubisi *et al.* (1997a,b) describe the development of a simple batch test to quantify the OHO active biomass concentration in an activated sludge system. In this test a small sample of mixed liquor is drawn from the activated sludge system and mixed with raw wastewater in a batch reactor where the oxygen utilization rate (OUR) and nitrate and nitrite concentrations are monitored with time. In parallel, a similar batch test is conducted on the raw wastewater without mixed liquor addition. From analysis of the OUR and nitrate and nitrite responses of the two parallel tests, the mixed liquor OHO active biomass concentration can be quantified. Wentzel *et al.* (1998) evaluated this batch test method by drawing mixed liquor samples from a well defined laboratory-scale anoxic/aerobic activated sludge system operated at 12 and 20 days sludge age.

They compared the results from the batch tests with theoretical values for OHO active biomass concentrations from steady state design (WRC, 1984) and kinetic simulation (Dold *et al.*, 1991) models. From the comparison they concluded that the results obtained were both encouraging and perplexing. With the parent system at 12d sludge age, the agreement between measured and theoretical values was remarkably good. However, with the parent system at 20d sludge age, the agreement was poor, with the theoretical values being about 2 times those measured. Wentzel *et al.* could provide no explanation for this inconsistency, but concluded that the results do indicate that the batch test method may prove to be a valuable tool that can be used to provide greater insight into the behaviour of the aerobic and anoxic/aerobic activated sludge systems.

In this research project, the batch test method of Ubisi *et al.* (1997a,b) will be further investigated, to attempt to identify the cause(s) for the inconsistency noted by Wentzel *et al.* (1998) and propose modifications to the procedure to overcome these.

1.4 RESEARCH OBJECTIVES

The development of the batch test procedure above constitutes a significant advance in attempting to resolve a major weakness within the activated sludge steady state design and kinetic simulation models, namely the lack of *independent* quantification of the active biomass. The batch test method developed by Ubisi *et al.* (1997a,b) has shown considerable promise as an independent means to quantify the hypothesized concentration of heterotrophic active biomass present in an activated sludge system. However, the inconsistency of the batch test results has not resolved the uncertainty around the active biomass concept.

The principle aim of this investigation is to evaluate the batch test method as a reliable means of quantifying the concentration of heterotrophic active biomass present in an activated sludge system. To achieve this aim, three primary objectives for this research project have been identified. These are listed below, together with the specific tasks to be completed to address the objectives:

(1) To establish whether the inconsistent batch test results could be related to the batch test procedure itself, or whether the inconsistencies were the result of incorrect mathematical formulations within the framework of the steady state design and kinetic simulation models. The specific tasks are:

- Operate and maintain a parent laboratory-scale anoxic/aerobic activated sludge system, identical to that of Ubisi *et al.* (1997a,b), which would have the same mixed liquor characteristics.

- Perform batch tests identical in procedure to those conducted by Ubisi *et al.* (1997a,b), using mixed liquor samples drawn from the parent laboratory-scale anoxic/aerobic activated sludge system.

- Comparing the batch test results with those of Ubisi *et al.* and the theoretically predicted values, identify deficiencies in the understanding and analysis of the batch test method, in particular regarding the techniques used to separate the heterotrophic organisms' behavioural response from that of other organisms and compounds present in the total activated sludge mass.

(2) Having established the reasons for the inconsistent batch test results, to develop a modified batch test procedure that would eliminate the identified sources of error. The specific tasks are:

- Apply the modified batch test procedure to quantify the heterotrophic active biomass present in the same parent laboratory-scale anoxic/aerobic activated sludge system described in (1) above.

- Evaluate the reliability of the modified batch test results by comparing the measured heterotrophic active biomass concentrations with the theoretical values predicted via the steady state design and kinetic simulation models. Good correspondence between the theoretical and measured values would provide substantive direct evidence supporting both the models and the modified experimental method.

(3) In addressing the tasks above, it was found that the maximum specific growth rate measured for the heterotrophs was low. In analysis of the batch test data, this caused the estimate for heterotroph active biomass concentration to be overly sensitive to small variations in fitting best fit lines to the measured data. It was concluded that this sensitivity could be reduced if the heterotroph maximum specific growth rate could be increased. Accordingly, the effect of increased heterotroph maximum specific growth rate was investigated. From the literature, operating systems as intermittently fed fill and draw (IFFD) induce high heterotroph maximum specific growth rates (Ekama *et al.*, 1986). Thus, it was decided to change the operation of the continuously fed system above to IFFD. This would also provide the opportunity to evaluate the applicability of the modified batch test procedure to provide a reliable quantification of the heterotrophic active biomass present in other activated sludge systems, subjected to dissimilar operating conditions. As this constituted an independent investigation, the specific tasks are:

- Operate and maintain a laboratory-scale activated sludge system that would theoretically generate the same mass of active heterotrophic organisms, but would stimulate an appreciable increase in heterotrophic organism maximum specific growth rate. Accordingly, for this investigation an intermittently fed fill and draw (IFFD) system will be maintained and operated at the same sludge age and receiving the same daily mass of COD as the system described in (1) above.

- Apply the modified batch test procedure to quantify the heterotrophic active biomass in the parent system, using mixed liquor samples harvested from the IFFD system.

- Comparing the modified batch test results with the theoretically predicted values, evaluate the reliability of the modified batch test procedure and its application to activated sludge systems exhibiting a wide range of heterotrophic organism behaviour.

1.5 RESEARCH APPROACH

The research approach adopted was twofold:

During the first part of this research project the research approach adopted was to operate and monitor a well-defined and controlled continuously fed anoxic/aerobic activated sludge system (Chapter 2). This parent system provided the mixed liquor samples for measuring the heterotrophic active biomass to address objective (1) above (Chapters 3 and 4). In addition, to address objective (2), this parent system also provided the mixed liquor samples required for evaluating the modified batch test method (Chapter 5).

During the latter part of this research project the research approach adopted was to operate and monitor a well-defined and controlled intermittently fed fill and draw (IFFD) activated sludge system (Chapter 6). This parent system provided the mixed liquor samples for measuring the heterotrophic active biomass to address objective (3) above (Chapter 7).

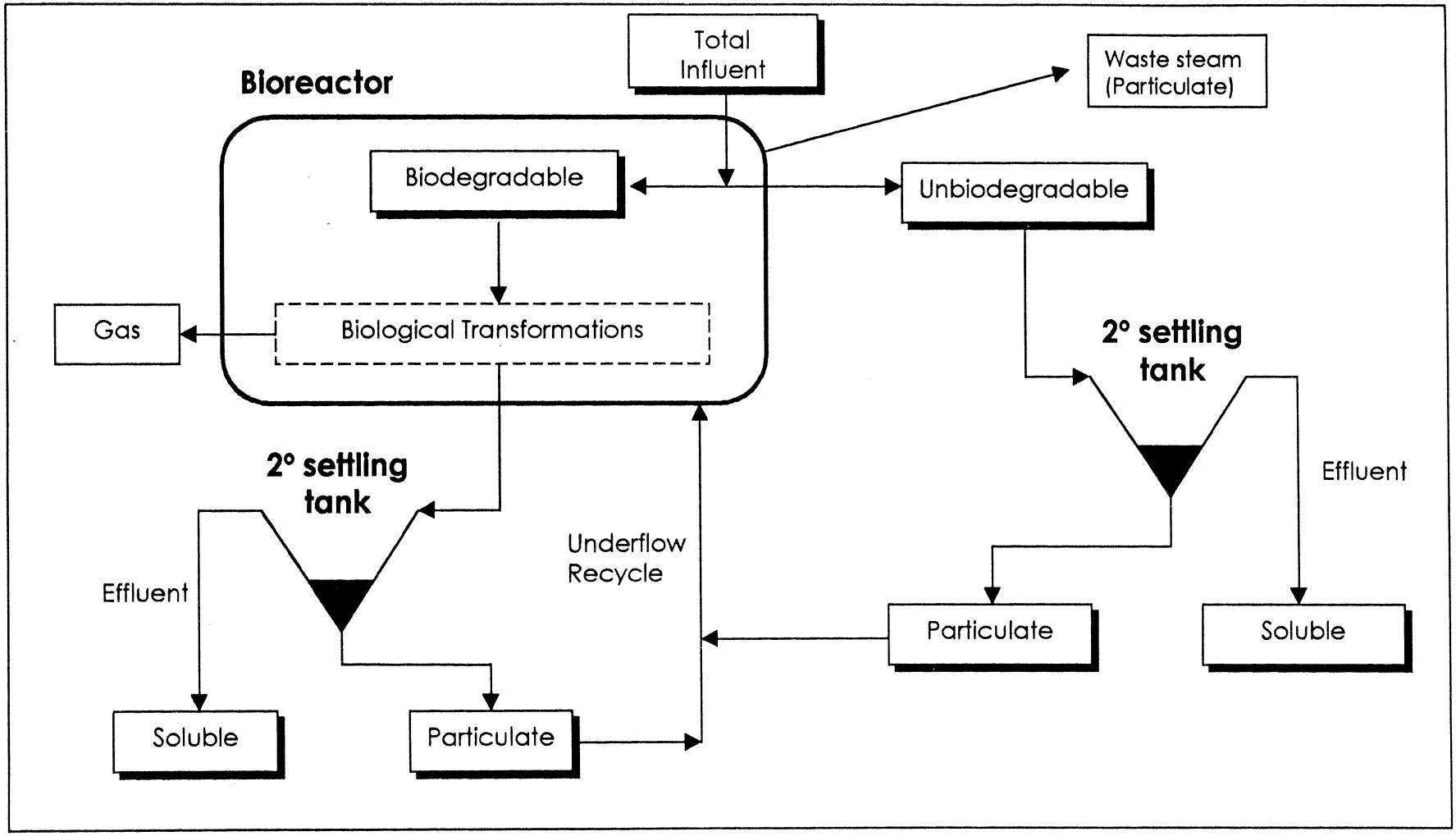


Figure 1.1: Division of influent wastewater carbon (C), nitrogen (N) and phosphorus (P) materials according to the biological and physical processes in the activated sludge system.

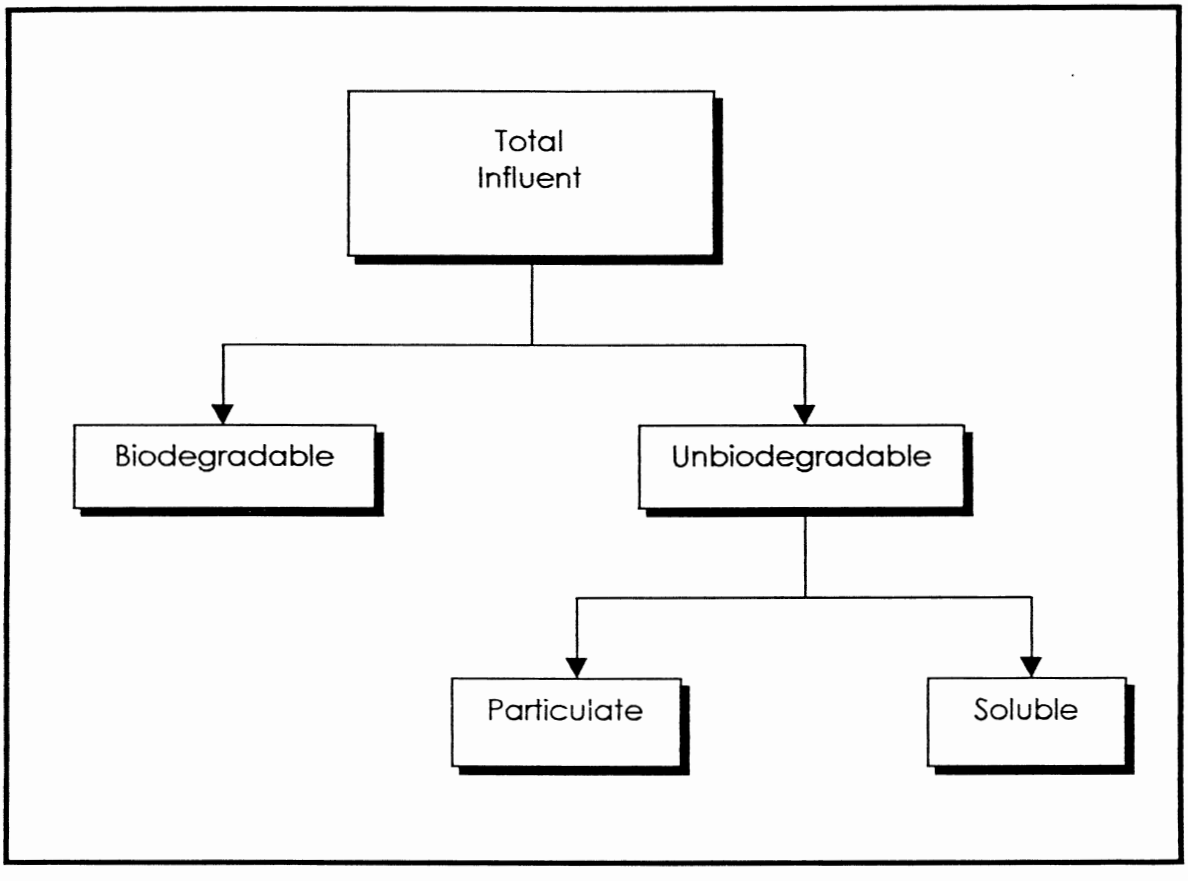


Figure 1.2: The general wastewater characterization structure for carbon (C), nitrogen (N) and phosphorus (P).

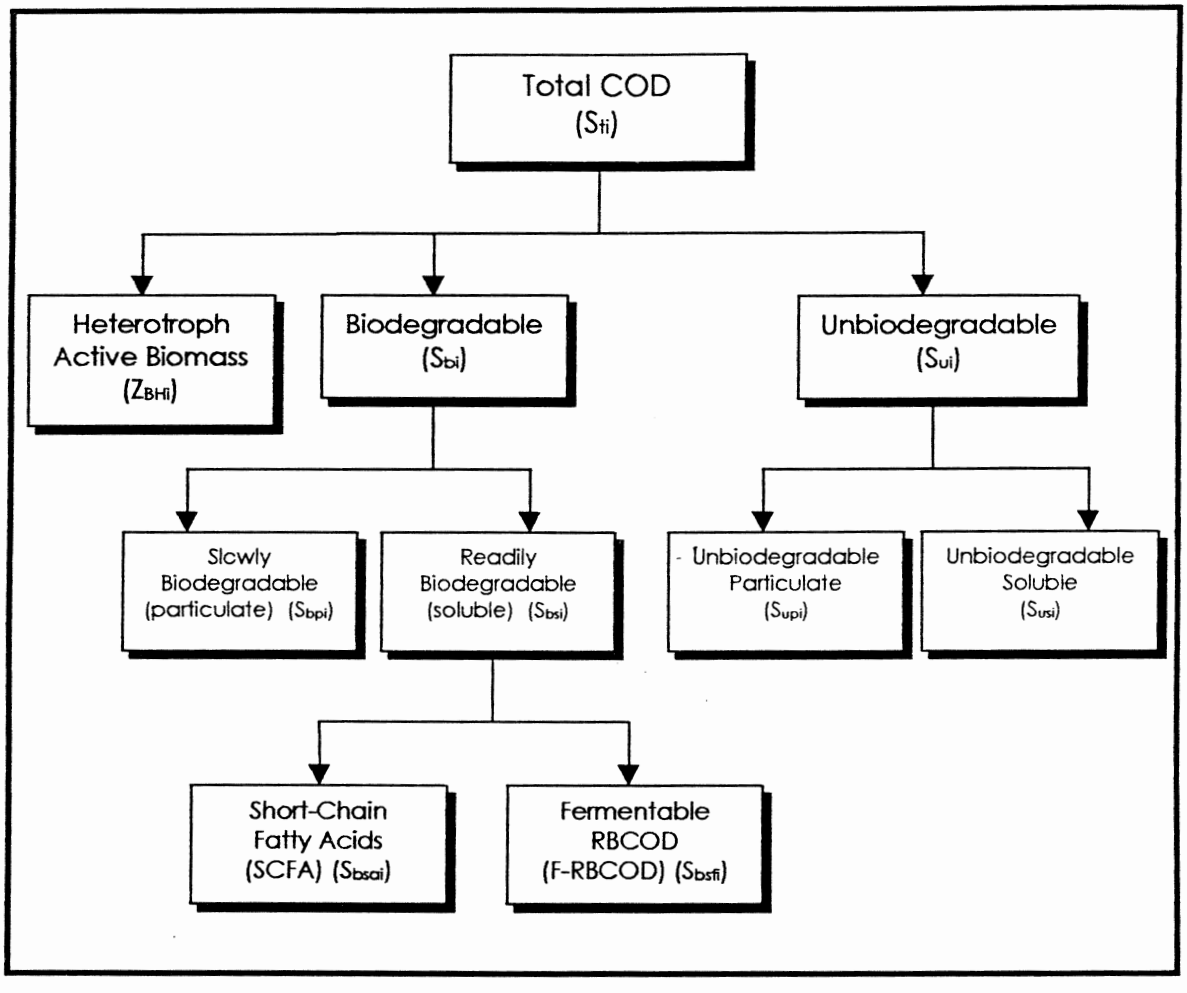


Figure 1.3: The division of the influent COD into its different constituents.

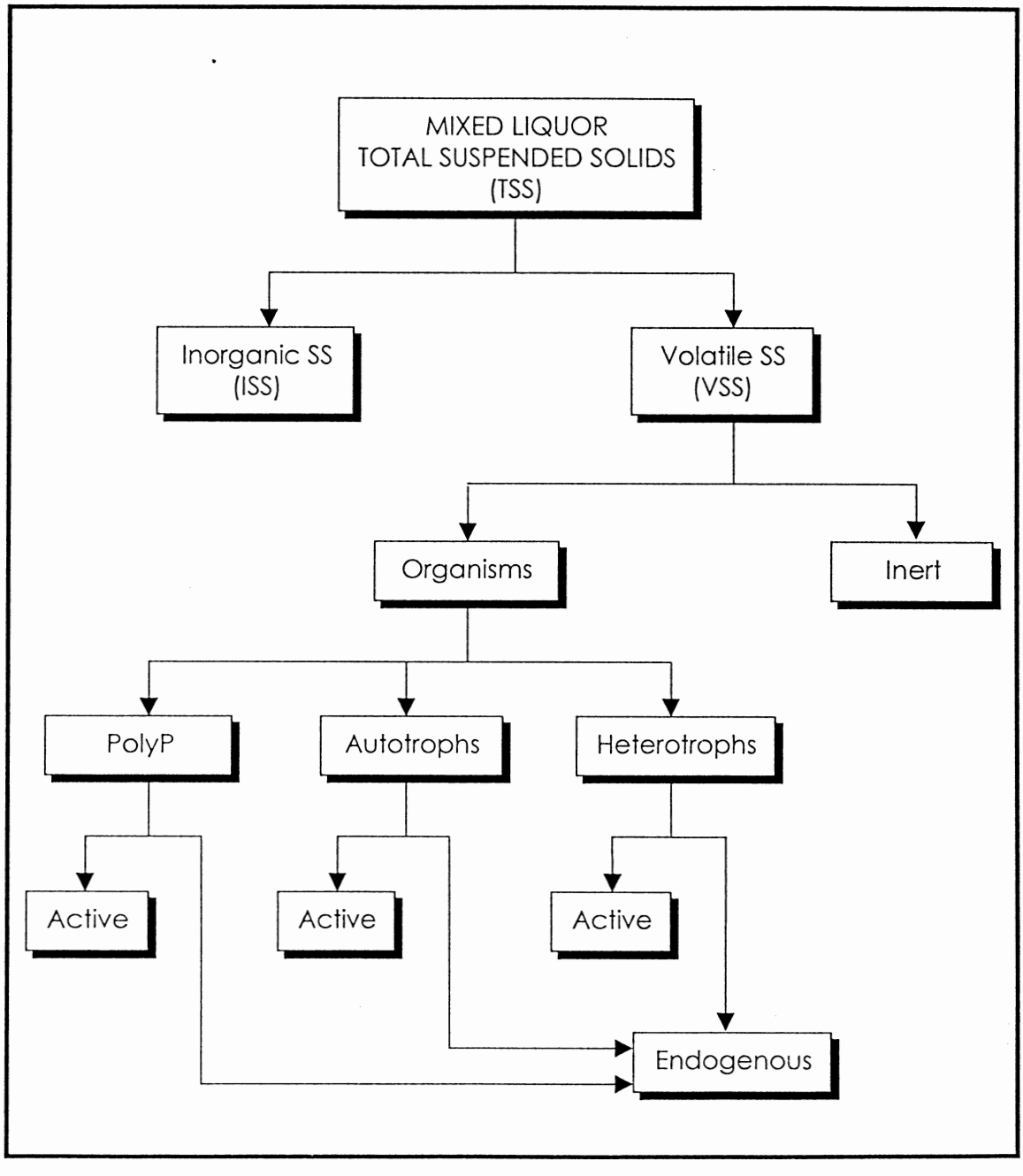


Figure 1.4: The organic and inorganic components of the mixed liquor in the bioreactor.

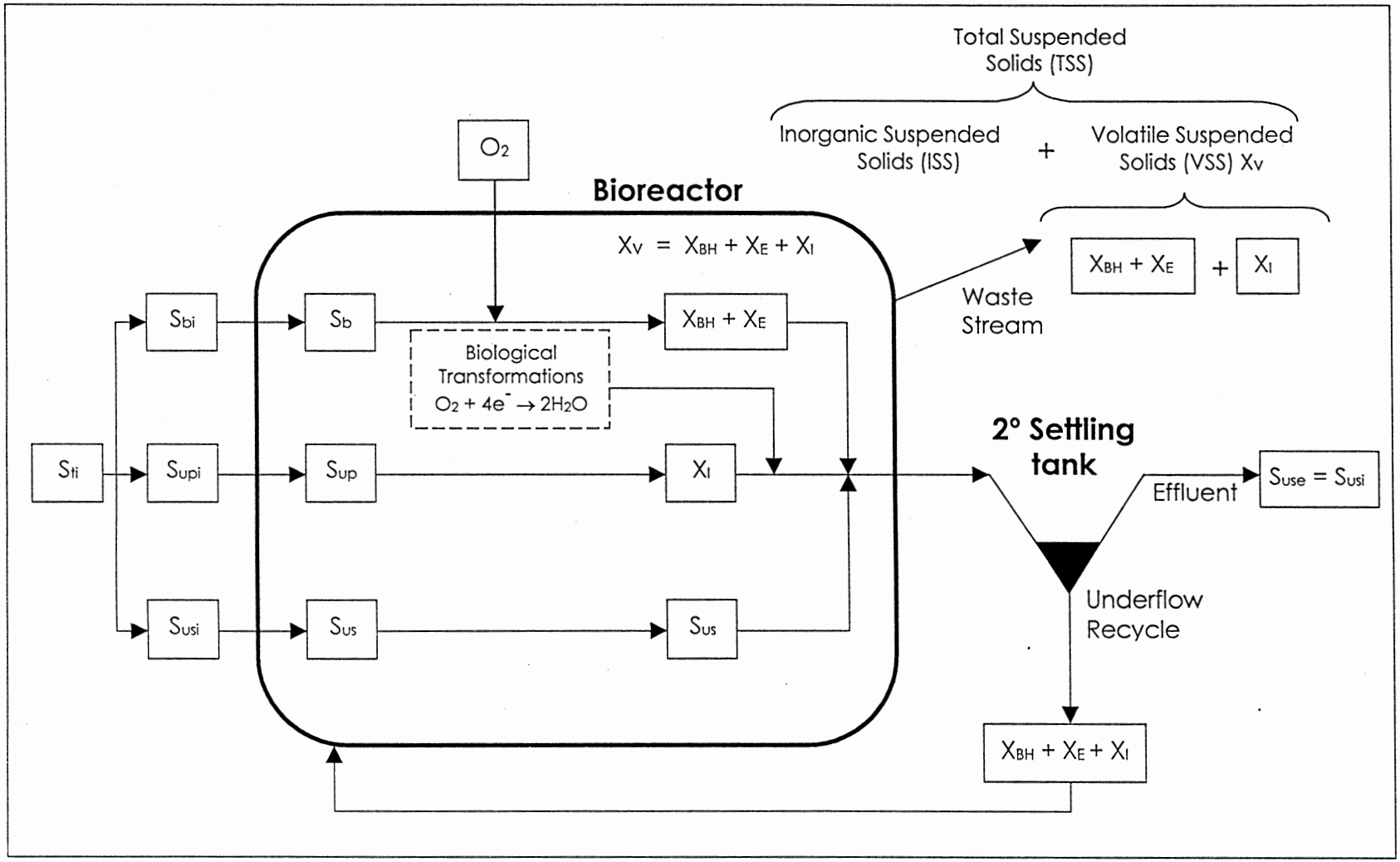


Figure 1.5: Schematic representation of the transformations of the influent COD fractions within the activated sludge system.

CHAPTER 2

THE PARENT LABORATORY-SCALE NITRIFICATION / DENITRIFICATION ACTIVATED SLUDGE SYSTEM

2.1 INTRODUCTION

As described in Chapter 1, the results from the batch tests conducted by Ubisi *et al.* (1997a,b) on samples harvested from a nitrification / denitrification laboratory-scale activated sludge system operated at 12 days sludge age, showed remarkably good agreement between the measured and theoretical heterotrophic active biomass concentration. In contrast, the results from batch tests conducted on a similar system operated at 20 days sludge age showed poor agreement, with theoretical values being approximately 2 times those measured.

To investigate this inconsistency, one of the objectives of this research project was to operate and maintain a parent laboratory-scale nitrification / denitrification activated sludge system identical to that of Ubisi *et al.* (1997a,b) which would have the same mixed liquor characteristics. However, to determine if the inconsistency was related to the sludge age of the system it was decided to operate the system at a different sludge age. Accordingly, for this research project a well defined and controlled parent laboratory-scale system was operated at steady state at a sludge age of 10 days. The system was monitored closely and provided the mixed liquor samples required for measuring heterotrophic active biomass (see Chapter 3 and 4). This chapter describes the configuration and operation of the parent laboratory-scale system, and details the response of the system.

2.2 SYSTEM LAYOUT

A schematic layout of the system is shown in Figs 2.1 and 2.2. The system layout constituted a Modified Ludzack-Ettinger (MLE) configuration and consisted of an anoxic reactor (25% of the total system volume), an aerobic reactor (75% of the total system volume) and a secondary settling tank, all in series with an underflow recycle (s-recycle) from the settling tank to the anoxic reactor of 1:1 and from the aerobic reactor to the anoxic reactor (a-recycle) of 2:1. All the recycle ratios are given with respect to the influent flow.

The contents of the anoxic and aerobic reactors were completely mixed by means of independent stirring. The aerobic reactor was aerated by passing low pressure air through a small bore Perspex tube which terminated at the bottom of the tank. The secondary settling tank was an inclined tube at 60° to the horizontal fitted with an intermittent slow

stirring (1,33 rpm) wiper blade. Pumping of influent feed and recycle flows was by means of a multiple channel peristaltic pump, with flow rate controlled by timers switching the pump on and off (for further details, see Burke *et al.*, 1986). The physical construction of the system is shown in Fig. 2.1.

The system configuration was identical to that described by Ubisi *et al.* (1997a,b). In their investigation they found that the inclusion of an unaerated zone (anoxic reactor) was essential to prevent the proliferation of the filamentous organism *Sphaerotilus natans* which caused severe sludge bulking in the completely aerobic laboratory-scale activated sludge system.

2.3 WASTEWATER COLLECTION AND STORAGE

The influent for the parent laboratory-scale activated sludge system was raw (unsettled) wastewater from the Mitchell's Plain Treatment Plant in Cape Town (South Africa). This sewage is primarily domestic, with a small (< 25%) industrial component. The sewage was collected in batches from the head of the works, after the course screens, but before the fine screens, grit removal and primary sedimentation tanks. The sewage batch was brought to the laboratory and stored in 400 *l* stainless steel tanks in a cold room at 4°C for 10 to 14 days, then discarded and a new batch of sewage collected; experience has shown that storage of sewage for periods longer than 3 weeks leads to hydrogen sulfide build-up in the tanks and a change in the characteristics of the sewage. For each new batch of sewage, immediately after storage in the cold room a COD test was done to determine the COD concentration required for subsequent dilution (see below) (COD of the undiluted sewage ranged from 1 000 to 1 500 mgCOD/*l*).

2.4 FEED PREPARATION

The total COD concentration for the feed to the parent laboratory-scale activated sludge system was set at 500 ± 50 mgCOD/*l*. Knowing the total COD concentration of the sewage batch collected, volumes of sewage and tap water to dilute the sewage to the required concentration (500 mgCOD/*l*) could be calculated. Daily, the contents of the storage tanks were thoroughly mixed and a volume of sewage was then drawn from the tank: The sewage was drawn from a tap at the bottom of the tank, passed through a 1 mm sieve into a graduated 20 *l* plastic bucket. Then the appropriate volume of tap water was added to dilute the sewage to the COD concentration (500 mgCOD/*l*) required in the tests. To maintain the pH in the aerobic reactor at ± 7.5, the alkalinity of the influent was increased by 200 mg/*l* (as $CaCO_3$): A buffer solution was made up in a separate container by dissolving 67.2 g sodium bicarbonate ($NaHCO_3$) into 1 *l* of distilled water. By adding 100 ml of the buffer solution to the 20 *l* diluted sewage an increase in alkalinity of 200 mg/*l* (as $CaCO_3$) was achieved.

To maintain a minimum level of nitrification in the system, the TKN/COD ratio of the diluted sewage was controlled to have a minimum TKN/COD ratio of 0.08. The TKN/COD ratio of sewage batch No. 6 was found to be below 0.08 and the influent TKN was supplemented by adding a small volume of ammonia solution ($NH_4C\ell$) of known concentration to the influent to achieve a TKN/COD ratio of 0.08 (i.e. 40 mgN/ℓ TKN per 500 mgCOD/ℓ).

After thorough mixing, samples were drawn for influent analysis.

2.5 FEEDING THE SYSTEM

After thoroughly mixing the diluted sewage above, the feed (20 ℓ) for the parent activated sludge system over the next 24h was drawn and placed in an upright PVC bucket which had a stirrer driven at 10rpm, to keep the contents in the bucket completely mixed and limit settling of particulate matter. The feed bucket was placed in a large chest refrigerator at a temperature of 4 – 8°C to minimize biological degradation of the sewage. The diluted sewage was pumped at a constant rate from the storage bucket to the parent activated sludge system over the 24h period. The feed bucket was cleaned daily with boiling water and two sets of influent feed tubes were used. One set was used for feeding and the spare influent feed tubes were washed with boiling water and stored until the following day when the two sets of tubes were swopped. This practice prevents the growth of the filamentous organism *S. natans* in the feed pipes and minimizes the build up of other organisms in the feed pipes.

The calibration of the feed pump was checked daily through ensuring that the feed bucket was empty at exactly the same time (say 11h00) every day. The feeding rate was increased or decreased by adjusting the pumping frequency.

2.6 SYSTEM MAINTENANCE AND OPERATION

The general maintenance and operational procedures set out in detail by Burke *et al.* (1986) and Clayton *et al.* (1989) were followed.

The volume of the mixed liquor in the anoxic reactor was maintained at 2.5ℓ and that in the anoxic reactor at 7.5ℓ (total volume 10ℓ) by controlling the outlet water level in the reactors. The sludge age (R_s) was controlled hydraulically at 10 days, by wasting daily from the aerobic reactor 1ℓ of mixed liquor (including any samples drawn for analysis). When conducting batch tests, the volume of mixed liquor drawn from the reactors usually exceeded the amount to be wasted daily (depending on the volume of mixed liquor used in the batch test): To avoid over wasting, the effluent used in diluting the mixed liquor when conducting the DSVI test was decanted, and the mixed liquor rediluted to the correct volume with effluent and the appropriate volume required to avoid over wasting returned to the system. The system was operated in a temperature controlled room, kept constant at 20°C. In the bioreactor, pH was controlled at 7.5 (±0.2).

Also, the dissolved oxygen (DO) concentration was controlled to between 2.0 and 4.5 mgO/ℓ, by using an automated DO meter (Randall *et al.*, 1991); this meter also recorded the oxygen utilization rate (OUR) automatically (see below). The DO meter and probe were recalibrated ***at least*** once a week.

2.7 SAMPLING AND MEASUREMENTS

Daily, the following samples were drawn for analysis:

- ***Influent***: Before the influent was poured into the feed bucket, the feed was thoroughly mixed and a 200 mℓ sample was taken in a glass jar and stored at 4°C for analysis the following day. Experience showed that inaccuracies were encountered when the 5 mℓ or 10 mℓ volumes required for TKN and COD tests were pipetted from the refrigerated 200 mℓ sample the following day. To avoid this, the influent sample was warmed to 20°C just prior to pipetting the samples for COD and TKN testing.

- ***Reactors***: Three 50 mℓ samples were drawn from the aerobic reactor and two from the anoxic reactor whilst the system was still feeding on the previous day's feed. When the mixed liquor samples were drawn from the reactors, care was taken not to disrupt the steady state behaviour of the system. The following sampling sequence was adopted:

  - First the two 50 mℓ nitrite/nitrate samples were drawn from the anoxic and aerobic reactors, respectively. **Immediately** after the samples were drawn, they were filtered separately through 0.45 µm filter paper. The filtrates of the two samples were stabilized by adding 2 drops of a mercuric chloride solution (8.6 g $HgC\ell_2/\ell$, Standard Methods, 1985) to each sample and were stored at 4°C for not more than 7 days before conducting the nitrite/nitrate analysis. (Prior to sewage batch No. 4, the 50 mℓ mixed liquor samples were not filtered immediately and were left standing for ± 30 minutes. This incorrect sampling procedure allowed the organisms to proceed with the denitrification process and resulted in distorted nitrite/nitrate results).

  - After storing the nitrite/nitrate samples, the internal surfaces of the two reactors were thoroughly brushed to dislodge sludge deposits from the tank floors and to re-introduce wall deposits above and below the water surface back into the mixed liquor.

  - After brushing, the system was allowed to operate normally for ± 30 minutes to allow sludge particles to be distributed evenly throughout the system. As a result of the aeration process, some sludge particles were splashed out of solution and deposited above the surface level of the aerobic reactor wall. When these

particles were brushed back into the mixed liquor solution, it increased the sludge concentration in the aerobic reactor in relation to the anoxic reactor.

- After the 30 minutes had elapsed, two 50 mℓ samples were drawn from the anoxic and aerobic reactors respectively for TSS, VSS and ISS testing (see 9. below). An additional 50 mℓ sample was drawn from the aerobic reactor, diluted 1:10 with distilled water and homogenized in a liquidizer for mixed liquor COD and TKN testing (see 4. below).

- Lastly a 500 mℓ mixed liquor sample was drawn from the aerobic reactor to perform the DSVI test (see 7. below).

- ***Effluent***: After thorough mixing a one litre sample was drawn from the effluent bucket.

The following analyses were performed on the samples daily, and is shown in Table 2.1:

1. COD and TKN (Standard Methods, 1985) on the unfiltered influent sample.
2. COD and TKN on the unfiltered effluent sample.
3. COD and TKN on filtered effluent sample (filtered through 0.45 μm filter paper).
4. COD and TKN on the aerobic reactor mixed liquor unfiltered samples.
5. Oxygen utilization rate (OUR) in the aerobic reactor (see below).
6. pH of the aerobic reactor.
7. DSVI (Ekama and Marais, 1984) on aerobic reactor mixed liquor.
8. Nitrate and nitrite concentration (Technicon Auto Analyser) on effluent, aerobic and anoxic reactors, all samples filtered through 0.45 μm filter paper.
9. Total suspended solids (TSS), organic/volatile suspended solids (VSS) and inorganic suspended solids (ISS) on aerobic and anoxic reactor mixed liquor (Standard Methods, 1985).

Previous experience showed that varying analytical results were obtained during the first two days after the introduction of a new sewage batch. These variations were ascribed to the phenomenon that the active organisms require a certain period to adapt to the changed sewage characteristics of a new sewage batch. As a result no testing (apart from the influent) was done during the first two days after the introduction of a new sewage batch.

The oxygen utilization rate (OUR) in the aerobic reactor was measured continually by using an automated technique (Randall *et al.*, 1991): A dissolved oxygen (DO) probe (YSI) from an automatic DO meter/OUR logger (Hi Tech Microsystems) was immersed in the mixed liquor. The low and high DO set point of the meter were 2.0 and 4.5 mgO/ℓ respectively: When the DO reached ± 4.5mgO/ℓ the air switched off automatically and the decrease in DO with time was monitored; when the DO reached ± 2.0 mgO/ℓ, the air was switched on again automatically and the cycle repeated. Automatically, for each cycle the slope of the DO-time data during the air off period was determined by linear regression; this gives the OUR, which was stored by the meter (together with regression

analysis and time data). The OUR results were down-loaded from the DO meter to a PC early the following day whilst the system was still feeding on the previous day's feed. OUR results with a regression correlation coefficient less than 0.99 ($R^2 = 0.99$) were rejected, and a mean OUR determined from the remaining data, the mean being used as the OUR for the day. The number of OUR readings ranged between 120 and 150 per day.

Table 2.1: Daily tests conducted on parent steady state system.

Test	Influent	Anoxic reactor	Aerobic reactor	Effluent
COD	♦		♦	♦○
TKN	♦		♦	♦○
Nitrate		○	○	○
Nitrite		○	○	○
OUR			□	
TSS		×	×	
VSS		×	×	
ISS		×	×	
pH		□	□	
DSVI			□	

♦ Unfiltered sample
○ Sample filtered through 0.45 μm filter paper
□ Direct measurement taken
× Centrifuge pellet

2.8 PARENT SYSTEM CONDITIONS

As noted earlier, the parent laboratory-scale activated sludge system was operated to supply mixed liquor samples for the batch tests, to measure heterotrophic active biomass. This required that the conditions present in the parent system be precisely defined. The details of the parent system have been described above.

The sewage used to feed the system was collected in batches from the Mitchell's Plain Treatment Works and was changed every ± 2 weeks to prevent degradation under storage. In total 23 batches of sewage were fed to the parent system. These sewage batches and the dates they were used as feed are listed in Table 2.2. Also indicated are the sewage batches during which mixed liquor samples were drawn from the system for batch tests. As described in Chapters 4 and 5, the batch test method used by Ubisi *et al.* (1997a,b) was subsequently modified and in Table 2.2 reference is also given to the periods when the unmodified (Ubisi) batch tests and modified batch tests were

performed. During the annual Christmas recess period (15 December 1999 to 14 January 2000) no testing was done on the parent system. However, the system was operated and maintained during this period by the technical staff of the Water Research Laboratory.

Table 2.2: Details of the Parent laboratory-scale activated sludge system sewage batch number, sewage feed dates, days of operation, batch tests conducted and type of batch test.

Sewage Batch No.	Date of tests (1999/2000)	Days of operation (d)	Batch Test	Type of Batch Test
1	1 Jun – 4 Jun	day 1 – 4	No	Unmodified
2	5 Jun – 18 Jun	day 5 – 18	No	
3	19 Jun – 6 Jul	day 19 – 36	No	
4	7 Jul – 24 Jul	day 37 – 54	No	
5	25 Jul – 6 Aug	day 55 – 67	No	
6	7 Aug – 27 Aug	day 68 – 88	No	
7	28 Aug – 13 Sept	day 89 – 105	No	
8	14 Sept – 27 Sept	day 106 – 119	No	
9	28 Sept – 11 Oct	day 120 – 133	No	
10	12 Oct – 26 Oct	day 134 – 148	No	
11	27 Oct – 9 Nov	day 149 – 162	Yes	
12	10 Nov – 19 Nov	day 163 – 172	Yes	
13	20 Nov – 3 Dec	day 173 – 186	Yes	
14	4 Dec – 14 Dec	day 187 – 197	Yes	
15	15 Dec – 30 Dec	day 198 – 213	No	
16	31 Dec – 14 Jan	day 214 – 228	No	
17	15 Jan – 28 Jan	day 229 – 242	No	
18	29 Jan – 12 Feb	day 243 – 257	Yes	
19	13 Feb – 25 Feb	day 258 – 270	Yes	
20	26 Feb – 9 Mar	day 271 – 283	Yes	
21	10 Mar – 24 Mar	day 284 – 298	Yes	Modified
22	25 Mar – 4 Apr	day 299 – 309	Yes	
23	5 Apr – 18 Apr	day 310 – 323	Yes	

2.9 RESULTS

2.9.1 Steady state periods

Daily results for the parent activated sludge system are listed in Appendix C, Tables C3 and C4. Each wastewater batch was accepted as a steady state period. The daily data for each wastewater batch were analysed statistically to determine outliers; data lying outside the 95% confidence interval (i.e. data lying outside the range mean ± 2* sample standard deviation) were rejected (these data are shown marked in the appropriate tables). Excluding the rejected data, for each wastewater batch (steady state period) the daily data were averaged and the sample standard deviations calculated. The averages, sample standard deviations and number of data for the different wastewater batches are listed in Table 2.3 (a and b).

2.9.2 COD and N mass balances

The reliability of the experimental measurements was checked by means of COD and N mass balances on the steady state periods (Ekama *et al.*, 1986): A brief description of the method for calculating the N and COD mass balances for the parent nitrification–denitrification system, using the averaged data from sewage batch 4 as an example, is given below. The description is followed by an analysis of the results obtained for the various wastewater batches.

Nitrogen (N) mass balance: Calculation method

In the N mass balance, the influent TKN mass must be accounted for by the sum of the mass of TKN and nitrate in the effluent, mass of nitrate denitrified to nitrogen gas in the anoxic reactor and the mass of TKN abstracted through the waste sludge:

$$\begin{matrix}\text{mass of} \\ \text{influent TKN}\end{matrix} = \begin{matrix}\text{mass of} \\ \text{effluent TKN}\end{matrix} + \begin{matrix}\text{mass of} \\ \text{effluent } NO_3\end{matrix} + \begin{matrix}\text{mass of N} \\ \text{denitrified}\end{matrix} + \begin{matrix}\text{mass of} \\ \text{TKN wasted}\end{matrix}$$

$$MNti = MNte + MNne + MNd + MNw \quad (mg\ N/d) \qquad (2.1)$$

The mass of nitrate denitrified (MNd) was found from a NO_3 balance around each reactor in the system including the settling tank. In this investigation, denitrification (indicated by a loss in nitrate across the reactor/settler) occurred only in the anoxic reactor, with the exception of sewage batch 17 where a small amount of denitrification was also observed in the settling tank. This was taken into account in the N mass balance for this sewage batch. For the anoxic reactor:

$$MNd = NO_3 \text{ mass in} - NO_3 \text{ mass out}$$

$$MNd = [NO_{3(AE)} \cdot Qi \cdot 2 + NO_{3(FE)} \cdot Qi \cdot s] - [NO_{3(AN)} \cdot Qi \cdot (1 + a + s)]$$

Where,

Qi = influent flow rate (20ℓ/d)
a = a –recycle ratio = 2
s = s –recycle ratio = 1
$NO_{3(AE)}$ = NO_3 concentration in the aerobic reactor *(ie. 19.0 mgN/ℓ for batch 4)*
$NO_{3(FE)}$ = NO_3 concentration of the filtered effluent *(ie. 21.8 mg N/ℓ for batch 4)*
$NO_{3(AN)}$ = NO_3 concentration in the anoxic reactor *(ie. 10.6 mgN/ℓ for batch 4)*

for sewage batch No. 4: MNd = [(19,0•20•2) + (21.8•20•1)] – [(10.6•20•(1+2+1)]
MNd = 348 mgN/d

The mass of effluent TKN (MNte) was taken as the unfiltered TKN concentration of the effluent (Nte) • Qi :

for sewage batch No. 4: MNte = Nte• Qi = 7.2• 20 = 144 mgN/d

The mass of effluent NO_3 (MNne) was calculated as $NO_{3\,(FE)}$• Qi :

for sewage batch No. 4: MNne = 21.8• 20 = 436 mgN/d

The mass of TKN in the waste sludge (MNw) was found by subtracting the **filtered** effluent TKN (already taken into account) from the measured mixed liquor TKN concentration (N_{ML}) and multiplying this value by the waste flow (Qw):

for sewage batch No. 4: MNw = $(N_{ML} - N_{FE})$ • Qw = (226 – 5.2) • 1.0 = 220.8 mgN/d

The influent TKN mass (MNti) was taken as the influent TKN concentration (Nti) • Qi :

for sewage batch No. 4: MNti = 62• 20 = 1240 mgN/d

By substituting the masses calculated above into Eq (2.1), the **N mass balance** is given by

Nbal (%) = 100 • (MNte + MNne + MNd + MNw) / MNti (2.2)
for sewage batch No. 4: Nbal (%) = 100• (144+436+348+220,8) / 1240
Nbal (%) = 92.6%

The data can be considered acceptable if the N mass balance falls in the range 90 – 110%.

Nitrogen (N) mass balance: Analysis of results

The results of the N mass balances calculated for each sewage batch are listed in Table 2.4 and are shown graphically in Fig. 2.3. The results can be commented on as follows.

- N Mass balances were not calculated for sewage batches 1, 2 and 3 due to the distorted nitrate concentrations that emanated from the incorrect sampling procedure, as described under section 2.7.

- During sewage batch 6 the influent TKN was supplemented by adding the ammonia solution to the diluted wastewater, as described in section 2.4. The addition of ammonia to the influent had a distinct influence on the system response and therefore sewage batch 6 was subdivided into two steady state periods: sewage batch 6a *without* ammonia addition and sewage batch 6b *with* the ammonia addition.

- In general, the N mass balances for the sewage batches showed considerable variability.

- For sewage batch 17 the N mass balance was marginally < 90% (89%).

- For sewage batches 10, 19, 21 and 22 the N mass balances were > 110%. No batch tests were conducted during sewage batch 10. However, batch tests were conducted during sewage batches 19, 21 and 22 and possible causes for the high N mass balances were sought. From Fig 2.3, the N in the waste sludge and effluent TKN was not significantly different in these sewage batches compared with those where acceptable N mass balances were obtained. Accordingly, the cause for the high N mass balances would appear to lie in either the nitrate or influent TKN measurements.

- A particularly poor N mass balance (>120%) was obtained for sewage batch 21. The nitrate concentrations measured for sewage batch 21 were notably high compared to the influent TKN (see Table 2.3(a)). However, batch tests conducted during this period showed that the nitrate concentrations measured at the start of the batch tests correlated well with the high nitrate concentrations in the aerobic reactor. Thus, the problem did not appear to lie in the measurement of nitrate. The other possible source of error is inaccurate measurement of the influent TKN concentration which could have an influence of 10 – 15% on the N mass balance if an error of 5 mgN/ℓ is made in the measurement of the influent TKN value. Comparing the influent TKN/COD ratios for the sewage batches with those recorded by fellow researchers for the same sewage during the same period in the UCT laboratory (Söteman, 2000) it appears that the influent TKN values were similar for sewage batches 1 to 18, 20, 21 and 23, but were under measured for sewage batches 19 and 22. Accordingly, N mass balances were calculated using Söteman's TKN/COD ratio's for these sewage batches and yielded 104% and 108% respectively. The influent TKN/COD ratios calculated by Söteman are listed for the various sewage batches in Appendix C, Table C5. Thus, for these sewage batches the influent TKN/COD ratios from Söteman were accepted. With these changes, only sewage batch 21 has N mass balance > 110%. Since no assignable cause could be identified for the high N mass balance this sewage batch should be rejected for further analysis.

COD mass balance: Calculation method

In the COD mass balance, the influent COD mass must be accounted for by the sum of the masses of effluent COD, carbonaceous oxygen demand, the equivalent oxygen demand of denitrification and COD mass of the waste sludge:

mass of influent COD	=	mass of effluent COD	+	mass of carbonaceous oxygen demand	+	equivalent mass of oxygen demand for denitrification	+	mass of COD wasted

$$MSti = MSte + MOc + MOd + MXsvw \quad (mgCOD/d) \qquad (2.3)$$

The mass of effluent COD (MSte) was taken as the unfiltered COD concentration of the effluent (Ste) • Qi:

for sewage batch No. 4: MSte = 74• 20 = 1480 mgCOD/d

From the direct measurement of the OUR in the aerobic reactor, the total oxygen demand (MOt) can be calculated. However, the total oxygen demand is the sum of the carbonaceous oxygen demand (MOc) and the oxygen demand for nitrification (MOn):

$$24 \cdot OUR \cdot V_{AE} = MOt = MOc + MOn \quad (mgO/d) \qquad (2.4)$$

where,

V_{AE} = volume of the aerobic reactor (7.5ℓ)
OUR = mean oxygen utilization rate measured in the aerobic reactor (mgO/ℓ/h)

Therefore, to be able to calculate MOc an estimate of MOn is essential. The oxygen demand for nitrification is related to the mass of nitrate generated (MNc)

$$MOn = 4.57 \cdot MNc \quad (mgO/d) \qquad (2.5)$$

where,

4.57 = mass of oxygen utilized per mgNH_4-N nitrified.

Provided the N mass balance is acceptable, the mass of nitrate generated is found from the sum of the nitrate mass denitrified (MNd) and the nitrate mass in the effluent (MNne):

$$MNc = MNd + MNne \quad (mgN/d) \qquad (2.6)$$

Both MNd and MNne were determined from the N mass balance calculations above:

for sewage batch No. 4: MNc = 348 + 436 = 784 mgN/d

The carbonaceous oxygen demand (MOc) was calculated by substituting Eq (2.5) into Eq (2.4) and solving for MOc:

$$MOc = (24 \cdot OUR \cdot V_{AE}) - (4.57 \cdot MNc) \quad (mgO/d) \qquad (2.7)$$

for sewage batch No. 4: $MOc = (24 \cdot 31.79 \cdot 7,5) - (4.57 \cdot 784)$
$MOc = 2139.3\ mgO/d$

The equivalent mass of oxygen demand for denitrification (MOd) was calculated from:

$$MOd = 2.86 \cdot MNd \quad (mgO/d) \qquad (2.8)$$

Where 2.86 = oxygen equivalent of nitrate as electron acceptor.

for sewage batch No. 4: $MOd = 2.86 \cdot 348$
$MOd = 995.3\ mgO/d$

The mass of COD in the waste sludge (MX_{SVW}) was found by subtracting the **filtered** effluent COD (COD_{ML}– already taken into account) from the measured mixed liquor COD concentration (COD_{ML}) and multiplying this value by the waste flow (Qw):

$$MX_{SVW} = (COD_{ML} - COD_{FE}) \cdot Qw \quad (mgCOD/d) \qquad (2.9)$$

for sewage batch No. 4: $MX_{SVW} = (3294 - 37) \cdot 1.0$
$MX_{SVW} = 3257\ mgCOD/d$

The mass of influent COD (MSti) was taken as the influent COD concentration (Sti) • Qi.

for sewage batch No. 4: $MSti = 491 \cdot 20 = 9820\ mgCOD/d$

By substituting the masses calculated above into Eq. (2.3), the **COD mass balance** is given by:

$$CODbal(\%) = 100 \cdot (MSte + MOc + MOd + MXSVW)/MSti \qquad (2.10)$$

for sewage batch No. 4: $CODbal(\%) = 100*(1480 + 2139,3 + 995,3 + 3257)/9820$
$CODbal(\%) = 80,2\%$

As with the N mass balance, the COD mass balance should also fall within the range 90 – 110% for the experimental data to be acceptable.

Table 2.3 (a): Parent system steady state data; for each sewage batch (steady state period, see Table 2.2) the data have been averaged and the means, sample standard deviations (SSD) and number of tests are listed.

Sew. Batch No.	TKN (mgN/l)									NITRATES (mgN/l)								
	INFLUENT			UNFILT. EFFLUENT			MIXED LIQUOR			ANOXIC			AEROBIC			EFFLUENT		
	Mean	SSD	No. of tests	Mean	SSD	No. of tests	Mean	SSD	No. of tests	Mean	SSD	No. of tests	Mean	SSD	No. of tests	Mean	SSD	No. of tests
1	51	1	3	5,7	0,3	2	202	22	3	5,7	1.0	4	6,8	1,5	4	12,3	1,7	4
2	48	1	6	5,2	1,2	7	206	14	7	1,9	1,3	10	7,2	1,7	10	8,7	2,1	10
3	45	2	10	4,6	1,1	9	220	5	10	0,9	0,5	10	8,4	0,9	10	8,9	1,1	10
4	62	2	11	7,2	3,0	10	226	25	11	10,6	0,5	5	19,0	2,4	12	21,8	2,5	11
5	62	4	8	5,5	1,4	9	245	8	8	8,5	2,1	9	17,4	1,0	8	19,0	1,3	9
6a	30	2	5	3,9	0,4	4	247	20	5	0,5	0,3	5	3,2	0,5	5	3,0	0,9	5
6b	55	1	6	3,3	1,9	6	227	7	7	10,6	2,0	7	18,0	2,5	7	20,8	2,1	7
7	53	1	8	5,2	1,4	7	231	9	8	10,6	1,6	8	17,9	1,7	7	19,1	1,9	8
8	64	5	2	5,4	2,3	3	262	6	3	10,8	7,5	3	21,8	9,7	3	23,8	5,3	3
9	63	3	5	5,4	0,8	4	246	14	5	12,9	3,4	6	24,0	4,5	6	27,4	2,7	6
10	51	6	5	5,7	2,7	5	262	28	4	5,0	2,5	6	14,1	3,9	6	15,5	3,5	6
11	46	1	8	3,7	0,2	7	248	9	8	1,1	0,7	8	6,7	1,3	9	8,1	1,5	9
12	47	2	6	4,0	0,9	6	248	33	5	2,2	1,5	6	7,8	2,9	6	10,2	2,4	6
13	47	1	7	4,1	0,3	7	243	6	8	3,7	0,5	8	9,8	0,8	8	11,3	0,9	8
14	48	1	4	3,7	0,4	4	226	7	4	5,5	1,2	4	11,7	0,8	4	13,2	0,5	4
17	60	2	10	6,1	0,6	10	198	7	8	19,7	1,6	10	29,0	2,5	10	28,5	2,5	10
18	44	4	8	5,3	0,5	8	230	7	8	4,1	0,9	7	10,6	1,7	9	11,5	1,2	8
19	46	1	9	4,3	0,4	8	231	7	8	5,5	2,6	9	13,8	2,8	9	15,4	2,6	9
20	39	1	6	5,2	0,5	8	219	4	7	1,8	2,1	5	7,1	3,9	7	9,2	2,4	8
21	50	3	8	4,0	0,9	8	250	8	7	10,3	2,1	9	20,6	2,0	9	22,9	2,4	9
22	45	2	6	5,0	0,6	6	219	11	6	8,1	1,1	7	15,7	1,2	7	18,1	0,8	6
23	51	5	7	6,2	1,9	7	216	26	7	7,8	1,1	7	14,9	0,9	7	16,7	1,1	7

Table 2.3(b): Parent system steady state data; for each sewage batch (steady state period, see Table 2.2) the data have been averaged and the means, sample stadard deviations (SSD) and number of tests are listed.

Sew. Batch No.	COD (mgCOD/l)									OUR (mgO/l/h)			VSS (mgVSS/l)		
	INFLUENT			UNFILT. EFFLUENT			MIXED LIQUOR								
	Mean	SSD	No. of tests	Mean	SSD	No. of tests	Mean	SSD	No. of tests	Mean	SSD	No. of tests	Mean	SSD	No. of tests
1	488	16	4	49	12	4	2333	170	4	26,5	3,8	4	1752	69	4
2	503	31	10	53	10	9	2879	532	10	29,2	3,0	9	2011	305	10
3	501	21	10	70	15	10	3358	164	10	29,0	2,0	8	2350	110	10
4	491	20	12	74	14	12	3294	209	11	31,8	3,7	12	2273	80	10
5	497	11	8	83	30	9	3522	114	9	34,8	3,5	8	2444	66	9
6a	526	16	5	44	19	5	3678	107	5	23,0	0,8	5	2481	73	5
6b	547	69	7	78	23	6	3679	176	7	34,6	1,4	7	2386	68	7
7	473	27	8	75	25	8	3443	232	8	33,3	1,3	8	2319	62	8
8	507	12	2	71	35	3	3771	154	3	38,4	0,0	2	2541	69	3
9	540	43	6	81	23	6	3458	210	6	38,1	0,1	2	2407	138	6
10	457	22	6	62	16	6	3831	366	6	32,7	0,0	1	2545	251	6
11	488	29	9	52	12	9	3471	230	9	33,5	0,7	3	2419	111	9
12	500	42	6	43	9	6	3372	121	6	33,5	1,9	6	2293	70	6
13	540	25	8	59	14	7	3681	148	8	34,8	0,7	7	2469	83	8
14	517	25	4	61	12	4	3403	185	4	37,4	2,1	4	2227	163	4
17	489	19	10	89	4	8	2916	134	10	42,8	3,8	10	2016	73	9
18	526	37	9	73	10	9	3502	118	9	36,1	1,7	8	2387	59	8
19	498	22	8	60	7	9	3513	103	9	35,4	1,2	8	2400	90	8
20	500	10	8	83	8	8	3418	109	8	30,3	1,4	8	2202	84	8
21	510	39	9	69	7	8	3664	92	7	39,4	1,6	8	2457	74	8
22	493	28	7	79	17	7	3427	170	7	35,2	2,5	7	2138	135	7
23	530	30	6	80	15	7	3116	263	7	34,1	1,3	7	2043	145	7

Table 2.4: Steady state N and COD mass balances, wastewater fractions and mixed liquor parameters for parent system. Data calculated from data in Table 2.3. (* indicate batches rejected as outliers at 95% confidence interval).

Sewage Batch No.	MASS BALANCE (%)		WASTEWATER FRACTIONS		MIXED LIQUOR	
	N	COD	Unbio. Soluble ($f_{S,us}$) (mgCOD/mgCOD)	Unbio. Particulate ($f_{S,up}$) (mgCOD/mgCOD)	COD/VSS (f_{CV}) (mgCOD/mgVSS)	TKN/VSS (f_N) (mgN/mgVSS)
1			0.0820	-0.0007*	1.33*	0.115*
2			0.0795	0.0408	1.43	0.102
3			0.0878	0.1164	1.43	0.094
4	93	80	0.0754	0.1049	1.45	0.099
5	91	90	0.0744	0.1346	1.44	0.100
6a	90	77	0.0532*	0.1050	1.48	0.100
6b	90	82	0.0786	0.0784	1.54	0.095
7	91	92	0.0867	0.1380	1.48	0.100
8	104	89	0.0888	0.1503	1.48	0.103
9	109	79	0.1259*	0.1076	1.44	0.102
10	113	95	0.1225*	0.2221*	1.51	0.103
11	90	94	0.0861	0.1434	1.43	0.103
12	92	87	0.0740	0.0999	1.47	0.108
13	93	88	0.0907	0.1053	1.49	0.098
14	90	93	0.0909	0.0777	1.53	0.100
17	89	97	0.0879	0.0573	1.45	0.098
18	101	93	0.0932	0.1027	1.47	0.096
19	113	90	0.0884	0.1300	1.46	0.096
20	106	90	0.0920	0.0882	1.55	0.099
21	124	90	0.0961	0.1330	1.49	0.102
22	113	92	0.1217*	0.0934	1.60*	0.102
23	96	83	0.0925	0.0312	1.53	0.106
MEAN			0.0853	0.1029	1.48	0.100
Std. Deviation			0.0079	0.0333	0.04	.0.004

COD mass balances: Analysis of results

The results of the COD mass balances calculated for each sewage batch are listed in Table 2.4 and are shown graphically in Fig. 2.4. The results can be commented on as follows:

- Errors in the N mass balance are carried through to the COD mass balance via the oxygen demand for nitrification and that recovered by denitrification. For this reason COD mass balances were not calculated for sewage batches 1, 2 and 3 due to the distorted nitrate concentrations that emanated from the incorrect sampling procedure described under 2.7.

- In general, the COD mass balances were poor; only 11 out of 19 sewage batches (5, 7, 10, 11, 14, 17, 18, 19, 20, 21 and 22) gave mass balances > 90%.

- However, the COD mass balances showed less variation compared to the N mass balances and the COD mass balances compared well with those obtained by Ubisi *et al.* (1997a,b).

- A possible cause for the poor COD mass balance could have been the fact that the influent feed bucket was not covered with a floating plastic disc to minimize air entrapment from the atmosphere into the feed. As an experiment, the floating cover was used during sewage batch 9 to investigate if it would improve the COD mass balance. However, sewage batch 9 yielded the second worst COD mass balance of 79%, possibly due to attached growth forming on the disc, and the floating disc was discarded. Subsequently, sewage batch 10 yielded a COD mass balance of 95% which indicated that the absence of the floating disc did not affect the COD mass balance adversely.

- Investigations showed that the COD measurement techniques was accurate (checked with standard potassium hydrogen phthalate, Standard Methods, 1985); the measured COD data could be accepted.

- Thus, the problem would appear to lie in measurement of the OUR or mixed liquor organic solids. Of particular importance to this investigation is the measurement of the mixed liquor organic solids: For the batch tests, the solids measurements are used to estimate the theoretical heterotrophic active biomass active fraction (see Chapter 3).

- Three independent measurements were made on the mixed liquor organic solids, volatile suspended solids (VSS), COD and TKN. To check the reliability of these measurements, the ratios of COD/VSS and TKN/VSS for the parent system mixed liquor were calculated for each sewage batch, see Table 2.4. Statistical plots for these ratio's were constructed, see Figs. 2.5 and 2.6 respectively. (See Appendix F for interpretation of statistical plots). From the statistical plots it is evident that the data are normally distributed; this indicates that no single factor has had a dominating influence on the measurements. The means for COD/VSS and TKN/VSS were 1.48 mgCOD/mgVSS and 0.100 mgN/mgVSS respectively, with sample standard

deviations 0.04 and 0.004 respectively. These values correspond to the accepted standard values of 1.48 mgCOD/mgVSS and 0.10 mgN/mgVSS respectively (WRC, 1984).

- Accordingly, it can be accepted that the errors in the COD mass balances do not lie in the measurement of the mixed liquor organic solids. For this reason, the lower limit for the COD mass balance was set at 80%. With this limit, sewage batches 6a and 9 were rejected for further analysis. This does not impact on the batch test analysis since no batch tests were conducted during these sewage batches .

Batches rejected for further analysis:

From the analysis of the COD mass balances only sewage batches 6a and 9 were rejected for further analysis. This does not impact on the batch test data analysis since batch tests were not conducted during this period.

From the analysis of the N mass balances, sewage batches which yielded balances outside the 90 % - 110 % range should be rejected for further analysis. These were sewage batches 10, 17, 19, 20, 21 and 22. No assignable cause for the poor N mass balances for sewage batches 10 and 17 could be identified and these were rejected for further analysis. However, analysis indicated that the cause for the poor N mass balances for sewage batches 19 and 22 could be related to the inaccurate measurement of the influent TKN value. Influent TKN values from a parallel study gave acceptable N mass balances for sewage batches 19 and 22. However, the N mass balance for sewage batch 21 remained > 110% and this sewage batch should be rejected for further analysis. The poor N mass balances may not impact negatively on the characteristics of the mixed liquor organic compounds that form the basis of this investigation. This was illustrated through the fact that the mean TKN/VSS ratio of the mixed liquor compares remarkably well with the accepted standard value of 0,10 mgN/mgVSS.

The COD mass balances analysis indicated that sewage batches 6a and 9 should be rejected for further analysis.

Batch test were conducted on the rejected sewage batch 21. The batch test data collected on this sewage batch will be included where appropriate, and will be analysed, but it will be noted that the data should be rejected and the data will not be used to draw conclusions. This will be done to determine the influence of poor mass balances on the test and analytical procedures.

2.9.3 Determination of unbiodegradable soluble and particulate fractions

The unbiodegradable soluble and particulate fractions of the influent COD ($f_{S,us}$ and $f_{S,up}$ respectively) were determined using the methods of Ekama *et al.* (1986).

Unbiodegradable soluble COD fractions ($f_{S,us}$)

According to Ekama *et al.* (1986), the unbiodegradable soluble COD is given by the COD of the filtered effluent of the activated sludge system. Hence:

$$f_{S,us} = S_{te} / S_{ti} \qquad (2.11)$$

where

S_{te} = filtered effluent COD concentration (mgCOD/ℓ)
S_{ti} = unfiltered influent COD concentration (mgCOD/ℓ)

For the average data on each sewage batch (Table 2.3), the $f_{S,us}$ was calculated using Eq.(2.11), and the values are listed in Table 2.4. The $f_{S,us}$ data from all the sewage batches were analysed using a statistical plot, see Figure 2.7. (For interpretation of the statistical plot, see Appendix F). Four outliers were identified, $f_{S,us}$ = 0.0532, 0.1217, 0.1225 and 0.1259, see Figure 2.7. Rejecting these points, the data are shown plotted in Figure 2.8. The data are normally distributed, giving for Mitchell's Plain raw wastewater a mean $f_{S,us}$ = 0.085 and sample standard deviation of 0.008. This value compares favourably with that observed by Ubisi *et al.* (1997a,b), $f_{S,us}$ = 0,09 for Mitchell's Plain wastewater.

Unbiodegradable particulate COD fractions ($f_{S,up}$):

Following the method of Ekama *et al.* (1986), the mixed liquor volatile suspended solids (MLVSS) was used to determine $f_{S,up}$. From the average data on each sewage batch (Table 2.3) and the calculated $f_{S,us}$ (Table 2.4), the $f_{S,up}$ was calculated using the following equation (Ekama *et al.*, 1986):

$$MX_V = \frac{MS_{ti}(1 - f_{S,us} - f_{S,up})\, Y_H^* R_S}{(1 + b_H^* R_S)} (1 + f^* b_H^* R_S) + f_{S,up}\, MS_{ti}\, R_S / f_{cv} \qquad (2.12)$$

MX_V = total volatile solids mass (mgVSS)
= $X_V \cdot V_P$
X_V = MLVSS concentration (mgVSS/ℓ)
= measured value (Table 2.3)
V_P = system volume
= 10ℓ
Y_H^* = heterotrophic active biomass yield (VSS units)
= 0.45 mgVSS/mgCOD
R_S = sludge age (d)
= 10 d
b_H^* = net specific endogenous mass loss rate
= 0.24 / d at 24°C
f^* = endogenous residue fraction
= 0.20

f_{cv} = COD/VSS ratio of mixed liquor (mgCOD/mgVSS)
= 1.48 mgCOD/mgVSS
MS_{ti} = total influent COD mass fed per day (mgCOD/d)
= Qi • Sti
Qi = influent flow rate
= 20ℓ / d
S_{ti} = influent COD concentration (mgCOD/ℓ)
= measured value (Table 2.3)

For each batch of wastewater tested, successive values for $f_{S,up}$ were substituted into Eq. (2.12); the $f_{S,up}$ value that gave a theoretical MLVSS mass (MX_V) equal to the measured value was accepted. The $f_{S,up}$ values for the different wastewater batches are listed in Table 2.4.

The $f_{S,up}$ data for all the wastewater batches were analyzed using a statistical plot, see Fig. 2.9. Two outliers were identified, $f_{S,up}$ = -0.0007, and 0.2221, see Figure 2.9 (The low value of –0.007 reflects the establishment of VSS during the start-up period, sewage batch 1). Rejecting these points, the data are shown plotted in Figure 2.10. The data are normally distributed, giving for Mitchell's Plain raw wastewater a mean $f_{S,up}$ = 0.103 with sample standard deviation of 0.033. This mean is in close agreement with the value observed by Ubisi *et al.* (1997a,b), $f_{S,up}$ = 0,12 for Mitchell's Plain wastewater and the value recommended for the design of N removal activated sludge systems receiving South African unsettled municipal wastewaters $f_{S,up}$ = 0.13 (WRC, 1984). This indicates the data obtained for $f_{S,up}$ are acceptable.

2.9.4 Sludge settleability

Although not explicitly part of this research project, the sludge settleability of the system was monitored daily by means of the Diluted Sludge Volume Index (DSVI). According to the hypothesis of Casey *et al.* (1994a,b) which explains the proliferation of anoxic/aerobic (AA) group of filaments, the nitrate/nitrite concentration in the primary anoxic reactor preceding the aerobic reactor is of fundamental importance: If the nitrate/nitrite concentration is high, the growth of AA filaments is stimulated and *vice versa*.

In Appendix C, graphical representations are given of the daily variation of the prominent compounds measured during this investigation. In Fig C5, Appendix C, the primary anoxic reactor nitrate concentration is shown plotted on the same graph as the DSVI versus day of operation. During the first part of this investigation (prior to the Christmas recess – day 193), the DSVI and the anoxic reactor nitrate concentration behaviour tends to conform to the hypothesis of Casey *et al.* : There is a general trend for the anoxic nitrate concentration and DSVI to increase or decrease concomitantly, and for the DSVI to be high when the anoxic nitrate concentration is high, and *vice versa*. From Fig C5 it is evident that the interruption during the Christmas recess (day 193 – 229) disrupted this trend, but the trend was re-established soon after the system recovered.

2.9.5 Operational problems

Occasional pipeline blockages, caused by lumps of sludge which settled in the outlet pipelines of the two reactors, resulted in minor sludge losses from overflowing reactors. Minor sludge losses occurred during sewage batch 3 (day 30), batch 6 (day 79), batch 7 (day 98), batch 8 (day 109) and batch 9 (day 128 and 133).

During the day following a sludge spillage, the reactors were drained and the mixed liquor was screened through the 1 mm sieve to remove sludge lumps. All pipelines were removed, cleaned and replaced and no testing was done on the system for that day. The volume of sludge wasted for that day was also decreased to account for the lost sludge.

During sewage batch 10, sludge losses were particularly severe (days 141 and 142). This probably accounts for the high N mass balance for this batch, see section 2.9.2. To prevent pipeline blockages and consequent sludge losses, towards the end of sewage batch 10 a surgical needle, connected to a low pressure air supply, was inserted into the outlet pipe of the aerobic reactor, as shown on Figure 2.1. The air bubbles mildly agitated the sludge particles in the rising pipeline and prevented heavy sludge lumps from settling at the bottom of the pipe. No sludge overflows were experienced after this measure was introduced.

On day 284 (sewage batch 21) major sludge losses occurred due to a broken silicone tube on the peristaltic pump. To account for the lost sludge, no sludge was wasted from the system on days 285 – 287. No testing was done during this period either.

When the parent laboratory-scale system was receiving sewage batch No. 17 (day 234 – 242), the laboratory's air-conditioning system failed and resulted in higher ambient temperatures (22°C to 29°C) than the prescribed 20°C. From the data in Table 2.3 (a and b) it would appear that the mixed liquor organic suspended solids (MLOSS) concentration measured with the COD, VSS and TKN tests were all lower compared to the sewage batches immediately preceding and following. In contrast, the OUR for sewage batch 17 was higher. Although this will cause the theoretical active fraction of the MLOSS to be higher, it would not impact on the batch test data analysis since batch tests were not conducted during this period.

2.10 CLOSURE

The parent system was operated for 323 days and received 23 batches of unsettled municipal wastewater from Mitchell's Plain, Cape Town. From the results obtained from the system:

- N mass balances were variable, but generally fell in the range 90 to 110%. Sewage batches that gave mass balances falling outside this range were 10, 17, 19, 21 and 22. However, comparing the influent TKN/COD ratio's with data recorded by others on the same sewage during the same period (Söteman, 2000), revealed that the poor N mass balances for sewage batches 19 and 22 could be attributed to inaccuracies in the measurement of the influent TKN value and not necessarily measurement of the mixed liquor organic solids. Accordingly, for these sewage batches the influent TKN/COD ratios from Söteman were accepted. Thus, sewage batches rejected were 10, 17 and 21. For these sewage batches, batch tests were only conducted during sewage batch 21 – data for this sewage batch will be analysed, but it will be noted that the data should be rejected.

- Generally COD mass balances were poor, with 8 out of 19 sewage batches giving mass balances < 90%. The mixed liquor organic solids were determined by three independent tests – VSS, COD and TKN. Mean ratios for these measurements gave COD/VSS = 1.48 mgCOD/mgVSS (sample standard deviation = 0.04) and TKN/VSS = 0.100 mgN/mgVSS (sample standard deviation = 0.004). These values equal the accepted standard values of 1.48 mgCOD/mgVSS and 0.10 mgN/mgVSS respectively (WRC, 1984). Consequently, it was accepted that the error in the COD mass balance did not lie in the measurement of the mixed liquor organic solids, the parameter of importance in the measurement of heterotrophic active biomass investigation. Accordingly, the lower limit for the COD mass balance was set at 80%. On this basis, sewage batches 6a and 9 were rejected for further analysis. No batch tests were conducted during these sewage batches.

- The wastewater mean unbiodegradable soluble COD fraction ($f_{S,us}$) was determined to be 0.085 (sample standard deviation = 0.008), while the mean unbiodegradable particulate COD fraction ($f_{S,up}$) was determined to be 0.103 (sample standard deviation = 0.033). These values conform closely to those observed by Ubisi *et al.* (1997a,b) for Mitchell's Plain wastewater, $f_{S,us} = 0.09$ and $f_{S,up} = 0.12$ respectively.

- Minor sludge losses occurred during sewage batches 3, 6, 7, 8, 9 and 10, but did not impact significantly on the steady state behaviour of the parent system, nor on batch test data analysis since no batch tests were conducted during these periods.

- Operational problems were experienced when the parent system was receiving sewage batch No. 17; the laboratory's air-conditioning system failed, resulting in ambient temperatures in excess of 20°C. This influenced the steady state behaviour of the system (decreased sludge production), but did not impact on the batch test data analysis since batch tests were not conducted during this period.

Figure 2.1: Physical construction of parent laboratory-scale Modified Ludzack ttinger (MLE) anoxic/aerobic activated sludge system.

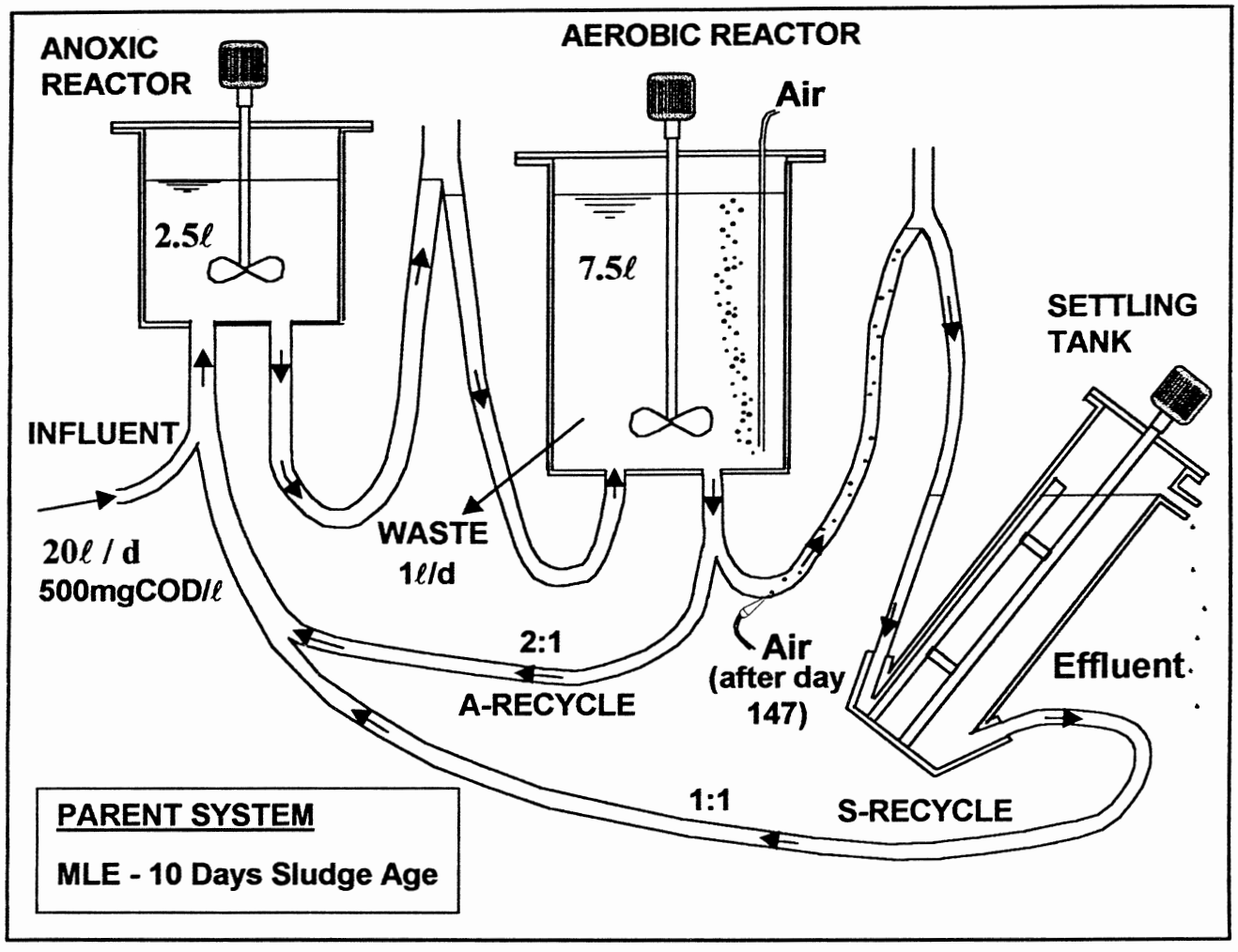
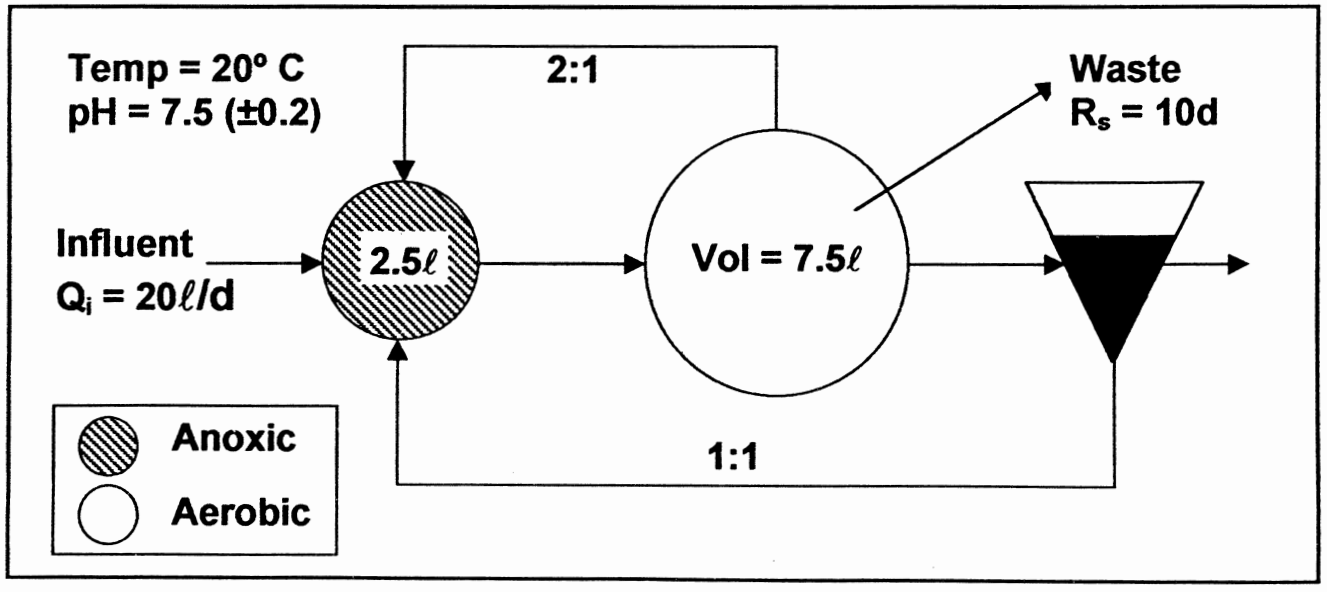


Figure 2.2: Schematic layout and operational data for parent laboratory-scale Modified Ludzack-Ettinger (MLE) anoxic/aerobic activated sludge system.



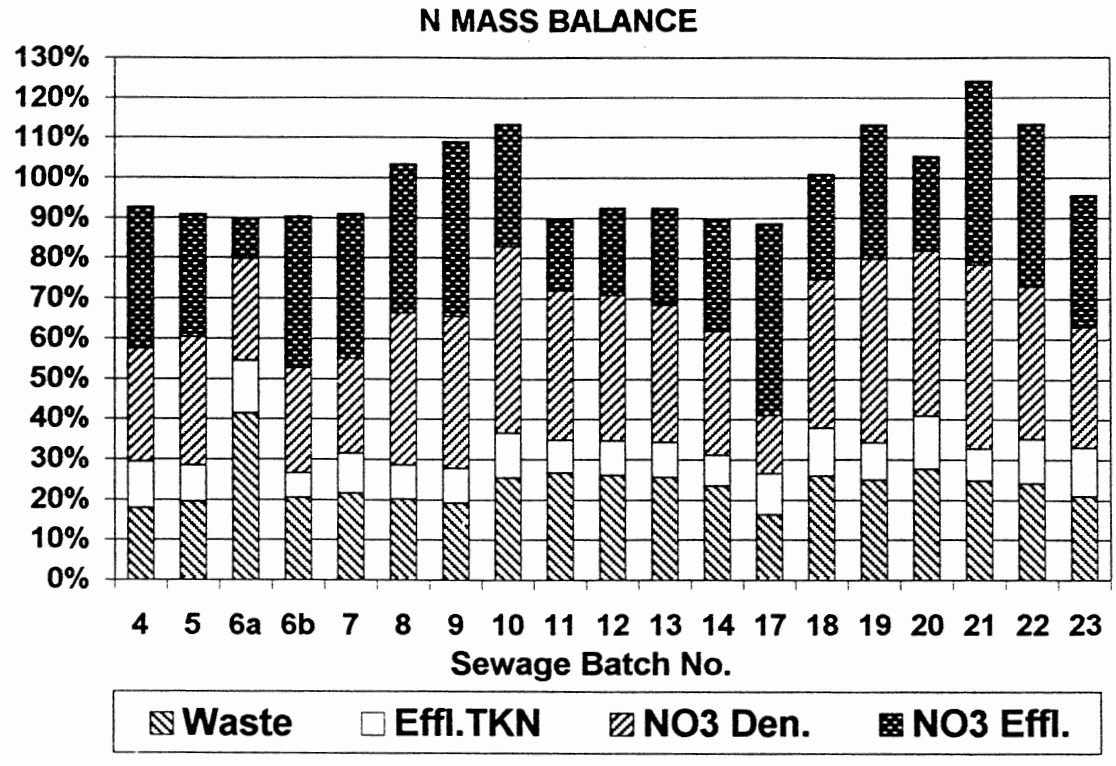


Figure 2.3: Graphical representation of the percentage Nitrogen (N) mass balance for the various wastewater batches. Percentages are also shown for : Nitrogen for sludge production (waste) , Effluent TKN , effluent nitrate and nitrate for denitrification.

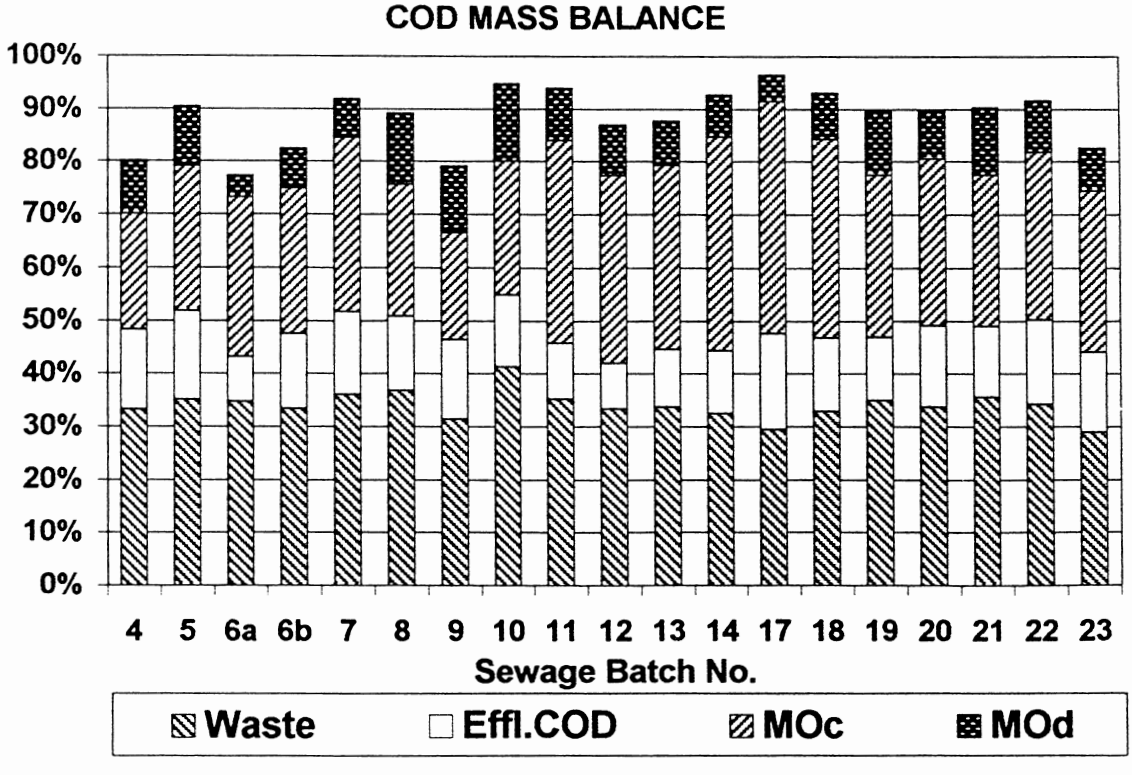


Figure 2.4: Graphical representation of the percentage COD mass balance for the various wastewater batches. Percentages are also shown for : Waste sludge COD , unfiltered effluent COD, carbonaceous oxygen demand and oxygen demand for nitrification.

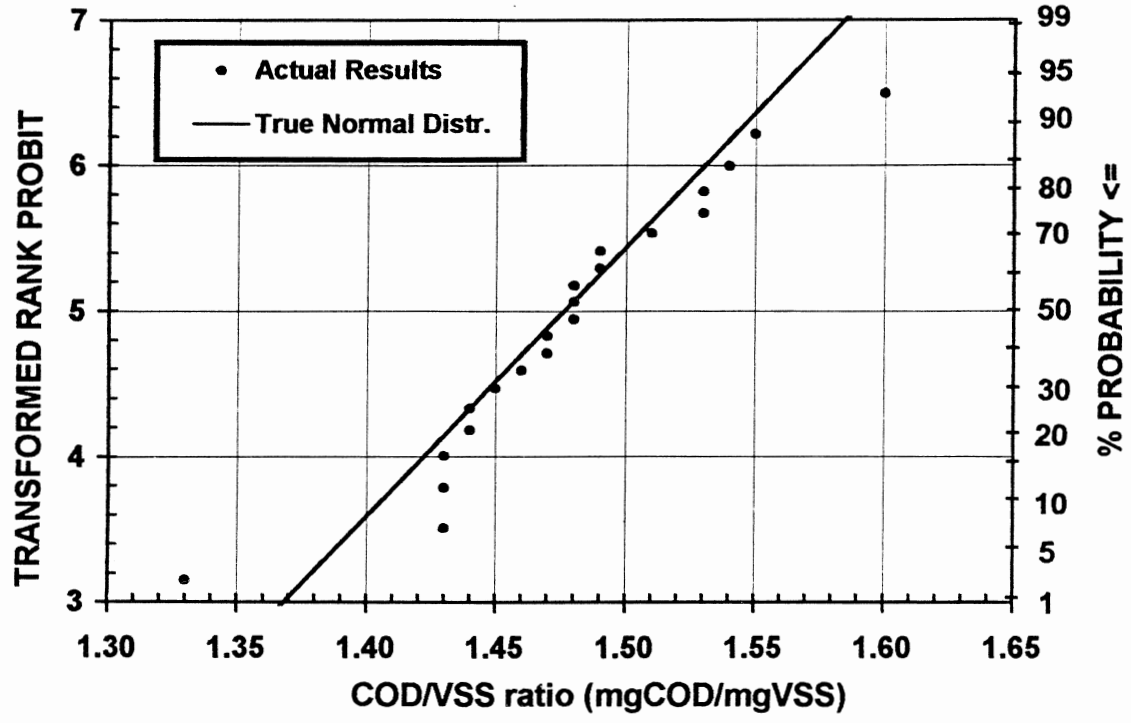


Figure 2.5 : Statistical plot for COD/VSS ratio for the parent laboratory-scale activated sludge system mixed liquor.

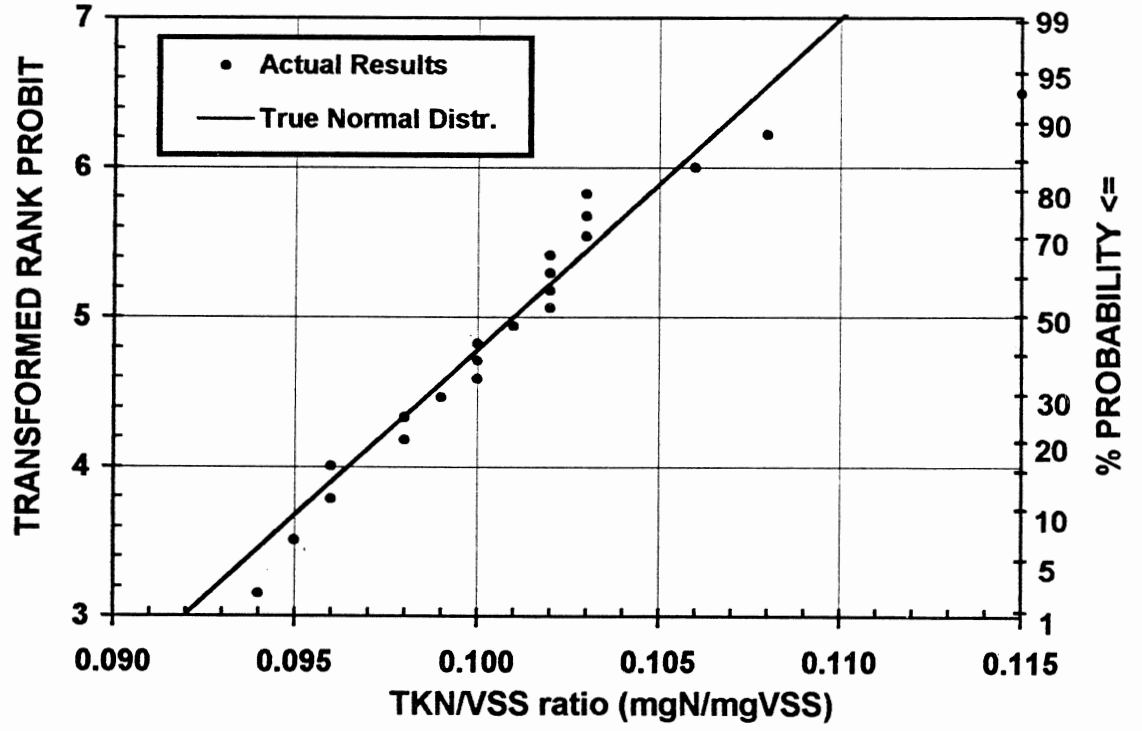


Figure 2.6 : Statistical plot for TKN/VSS ratio for the parent laboratory-scale activated sludge system mixed liquor.

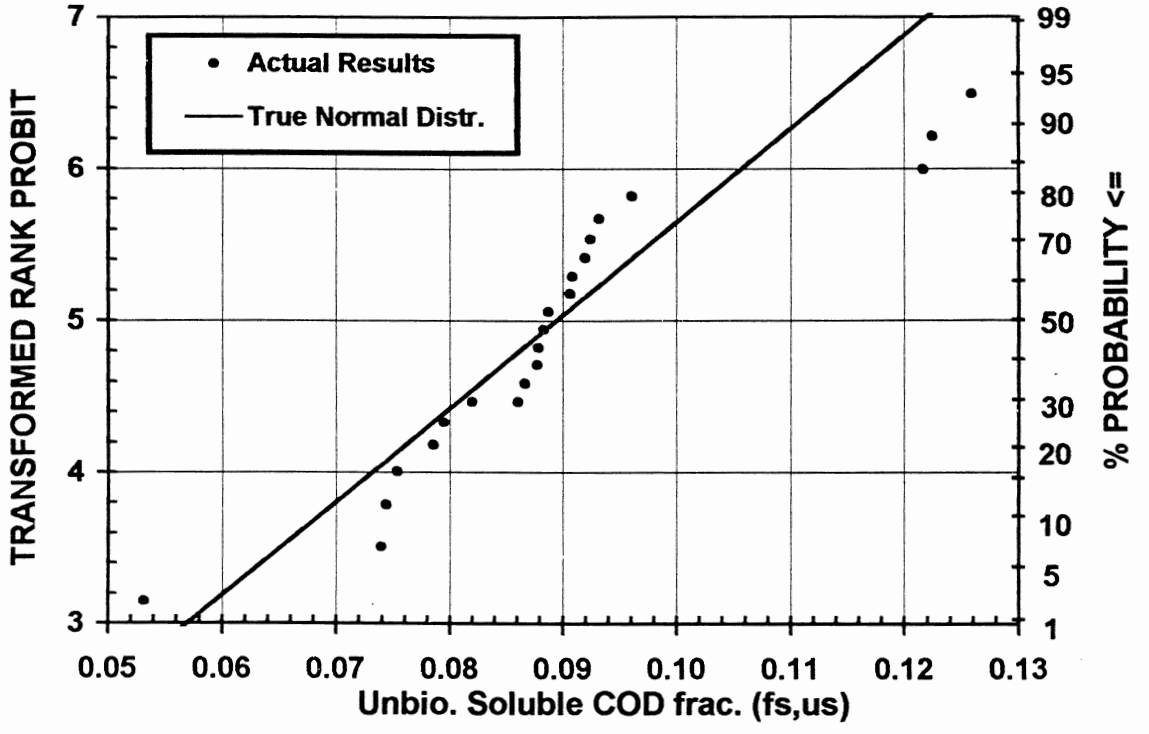


Figure 2.7 : Statistical plot for unbiodegradable soluble COD fractions ($f_{s,us}$) for the parent laboratory-scale activated sludge system fed with municipal wastewater from Mitchell's Plain (Cape Town, South Africa).

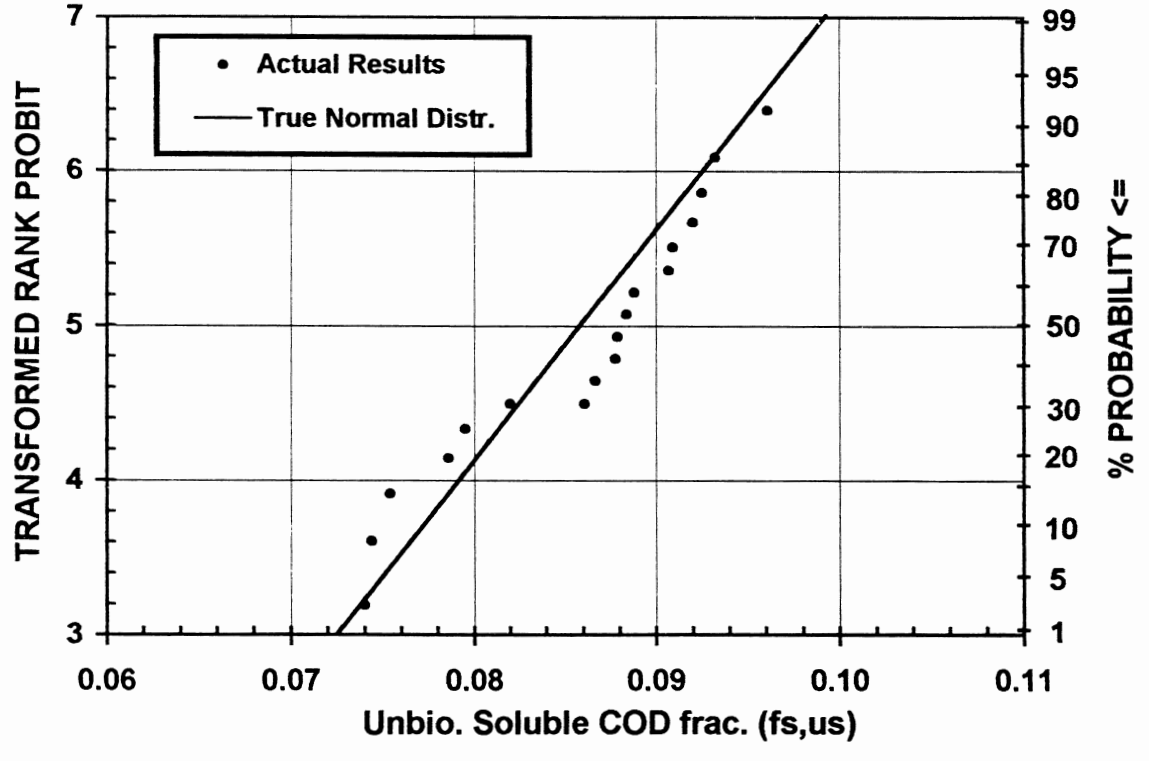


Figure 2.8 : Statistical plot for unbiodegradable soluble COD fractions ($f_{s,us}$) for the parent laboratory-scale activated sludge system fed with municipal wastewater from Mitchell's Plain (Cape Town, South Africa) - rejected outliers omitted.

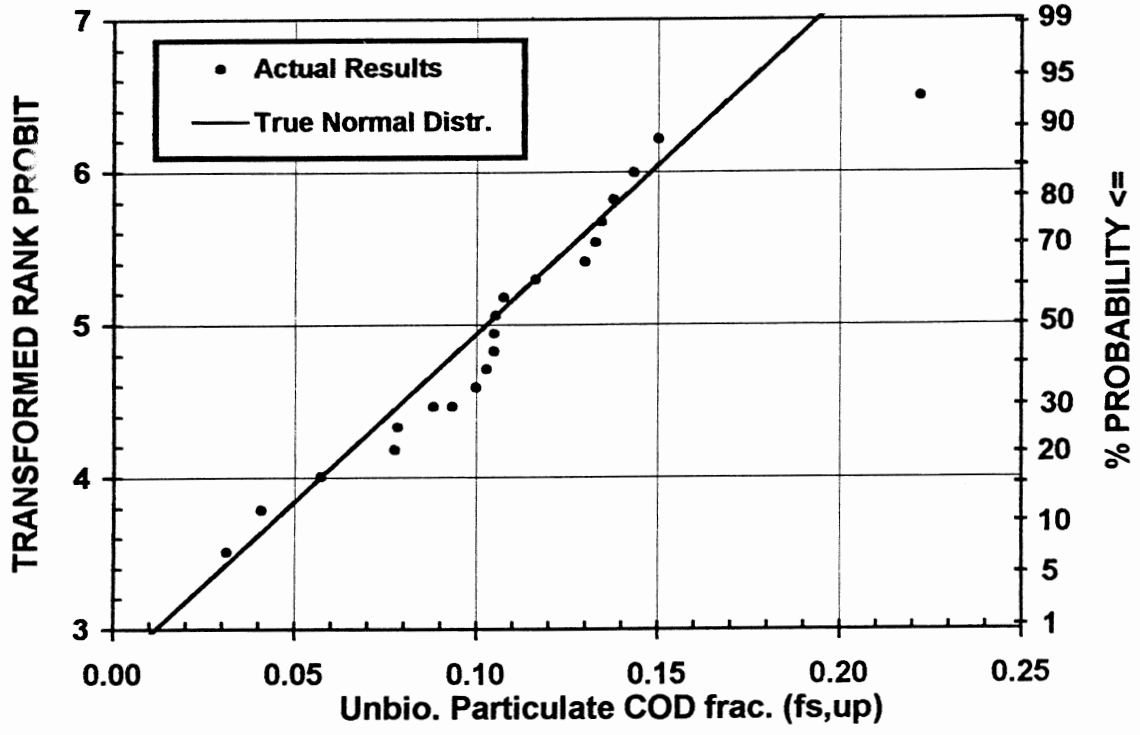


Figure 2.9 : Statistical plot for unbiodegradable particulate COD fractions ($f_{s,up}$) for the parent laboratory-scale activated sludge system fed with municipal wastewater from Mitchell's Plain (Cape Town, South Africa).

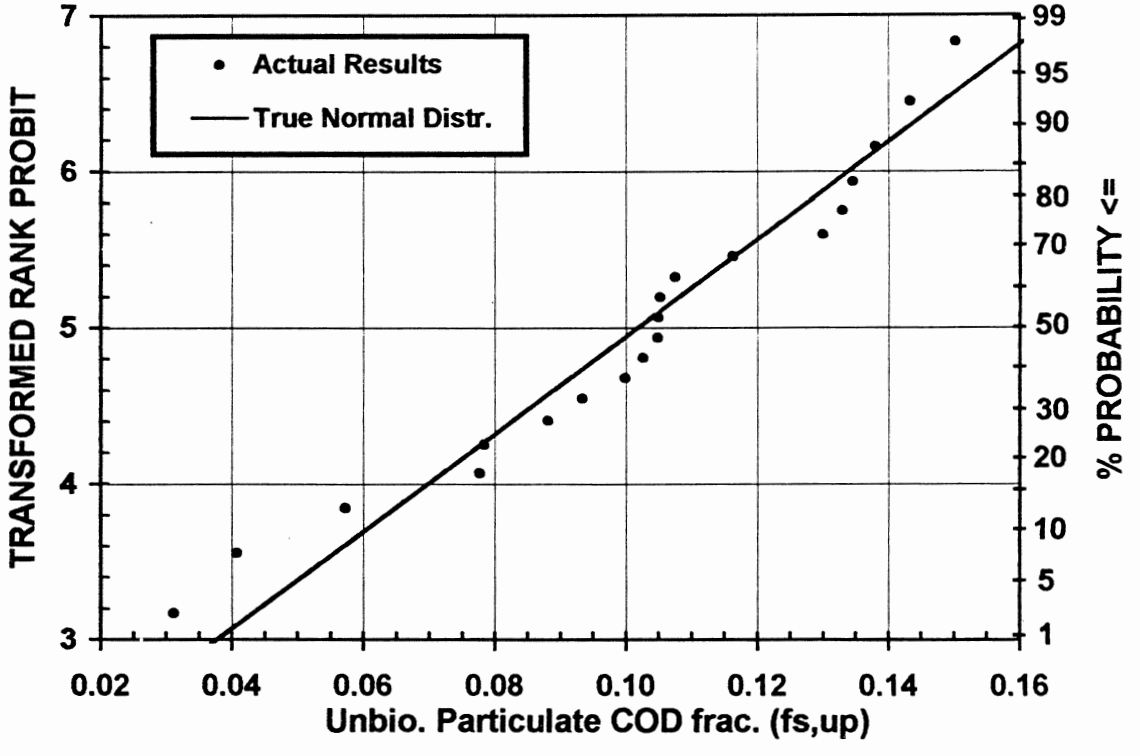


Figure 2.10: Statistical plot for unbiodegradable particulate COD fractions ($f_{s,up}$) for the parent laboratory-scale activated sludge system fed with municipal wastewater from Mitchell's Plain (Cape Town, South Africa) - rejected outliers omitted.

CHAPTER 3

BATCH TEST PROCEDURE TO QUANTIFY HETEROTROPHIC ACTIVE BIOMASS

3.1 INTRODUCTION

The batch test procedure developed by Kappeler and Gujer (1992) presented a means of quantifying the ordinary heterotrophic organism (OHO) active biomass concentration through monitoring the organisms' OUR response with time in a batch reactor. This procedure was extended by Wentzel *et al.* (1995) and Mbewe *et al.* (1995) to quantify the RBCOD concentration and the OHO active biomass concentration in the wastewater obtained from the Mitchell's Plain Treatment Plant. Ubisi *et al.* (1997a,b) modified this method to develop a batch test procedure to determine the OHO active biomass concentration in laboratory-scale nitrification/denitrification (ND) activated sludge systems, and evaluated the method for mixed liquor from laboratory-scale ND activated sludge systems operated at sludge ages of 12 and 20 days respectively. The theoretical OHO active biomass concentrations predicted by the current steady state design and kinetic simulation models for the two activated sludge systems were calculated and compared to the measured values obtained through the batch tests. The comparisons showed that for the 12 day sludge age system, good agreement existed between the measured and theoretical values. For the 20 days sludge age system, however, the values compared poorly, with the theoretical values being approximately 2 times those measured. These contrasting results raised uncertainty regarding the reliability of the results obtained from the batch test. One of the objectives of this investigation was to perform batch tests identical in procedure to those conducted by Ubisi *et al.* (1997a,b), using mixed liquor samples drawn from a well defined and controlled parent laboratory-scale nitrification/denitrification activated sludge system operated at 10 days sludge age (see Chapter 2). In this chapter the experimental procedure and the interpretation of data obtained from the batch tests will be described in more detail. The results from the batch tests will be evaluated in Chapter 4.

3.2 EXPERIMENTAL PROCEDURE

3.2.1 Experimental approach

The application of the batch test to quantify the OHO active biomass concentration hinges around the capacity of the heterotrophic organisms to utilize oxygen as final electron acceptor in the process of aerobic degradation of substrate (COD) present in the wastewater. In the aerobic batch test environment the active organisms present in the mixed liquor sample drawn from the parent system are mixed with a much larger volume of wastewater and thus are surrounded by a high concentration of substrate in the form of readily biodegradable COD (RBCOD) and slowly biodegradable COD (SBCOD).

Under these conditions the organisms utilize the substrate for the synthesis of new cell mass and consume oxygen in the growth process. Within the framework of current kinetic simulation models (e.g. UCTOLD, Dold *et al.*, 1991; IAWQ ASM No. 1, Henze *et al.*, 1987) the oxygen utilization rate (OUR) is directly related, *inter alia*, to the OHO active biomass concentration. Therefore, through monitoring the OUR for the duration of the batch test, and interpreting the data in terms of the kinetic simulation models, the OUR response can serve as a means to quantify the OHO active biomass concentration present at the start of the test. In analysis of the batch test data, due consideration must be taken of the OUR for nitrification and this must be subtracted from the total OUR. Ordinary heterotrophic organisms are also present in the wastewater; these are quantified in a separate batch test on wastewater only, performed in parallel with the mixed liquor plus wastewater batch test. The twin batch reactor setup is illustrated in Fig. 3.1. The difference in heterotrophic active biomass concentration between the batch tests with and those without mixed liquor addition gives the heterotrophic active biomass due to the mixed liquor addition. Ubisi *et al.* (1997a,b) describe the batch test procedures in detail, but this is repeated below for elucidation purposes.

3.2.2 Wastewater preparation

In the batch test procedure detailed by Ubisi *et al.* (1997a,b), a 3 *l* volume of diluted wastewater was preheated to 20°C and placed in a continually stirred batch reactor - called the wastewater only reactor. In a separate batch reactor a defined volume of mixed liquor (varying from 100 ml to 400 ml) drawn from the parent laboratory-scale activated sludge system was added to the required volume of the same diluted wastewater to make up a combined volume of 3 *l* inside the parallel reactor - called the wastewater and mixed liquor reactor. This procedure was initially adopted for this investigation - the first four batch tests, conducted during sewage batches 11 and 12 (Chapter 2) were conducted in accordance with this procedure. However, to maintain consistency between the parallel tests it was decided to use the same volume of diluted wastewater in each of the separate batch reactors. Accordingly, from sewage batch 13 the same volume of wastewater required in the wastewater and mixed liquor batch reactor was added in the wastewater only batch reactor and the 3 *l* was made up by adding filtered effluent from the parent system. This minor volumetric modification is illustrated in Fig. 3.1 where the same volume of diluted wastewater (2,8 *l*) was introduced in each reactor. In batch reactor 1, a 0,2 *l* sample of mixed liquor was added and in batch reactor 2 0,2 *l* of filtered effluent (containing no organisms) was added to make up a combined volume of 3 *l* in each reactor. By making the reasonable assumption that the wastewater added to each batch reactor contained the same concentration of heterotrophic organisms, which theoretically should have the same OUR, it was now possible to compare directly the OUR responses from the parallel batch tests. This aspect is described in more detail in Chapter 4.

Each of the parallel batch tests conducted during this investigation was preceded by drawing off unsettled wastewater from the storage tanks following to the same procedure described for feed preparation in Chapter 2. The wastewater was diluted to approximately the same COD concentration (± 500 mgCOD/*l*) as that fed to the parent

system. A sufficient volume of diluted wastewater (± 6 ℓ) was measured off and placed in a separate container which served as wastewater source for both batch test procedures. All containers used were thoroughly cleaned with boiling water beforehand to ensure that no external organisms could enter the batch reactors accidentally.

3.2.3 Wastewater only batch Tests

As described above, the mixed liquor only batch test were conducted using a mixture of wastewater and filtered effluent from the parent system.

Filtered effluent

A sufficient volume of effluent was drawn from the parent system's effluent bucket and filtered through 0,45 µm filter paper. The volume of filtered effluent added equaled the volume of mixed liquor harvested from the parent system and added in the batch test conducted in the parallel reactor. The required volume of filtrate (e.g. 0,2 ℓ in Fig. 3.1) was carefully measured in a clean measuring cylinder.

Wastewater

After the contents of the 6 ℓ container (see 3.2.2 above) were thoroughly mixed, the required volume of diluted wastewater (e.g. 2,8 ℓ in Fig. 3.1) was carefully measured, preheated to 20°C and placed in a continually stirred batch reactor maintained at a constant temperature of 20°C.

Immediately after the diluted wastewater was placed in the batch reactor, the filtered effluent was added, giving a combined volume of 3 ℓ for the mixture. A sample was drawn to obtain the initial total COD concentration (Standard Methods, 1985). The reactor was aerated by passing low pressure air through a small bore Perspex tube which terminated at the reactor bottom (see Fig. 3.1). A dissolved oxygen (DO) probe (YSI) from an automatic DO meter / OUR logger was immersed in the solution. The oxygen supply and the OUR in the batch reactor was measured using an automated technique (Randall *et al.*, 1991). The DO was raised to ± 6 mgO/ℓ, the air was switched off automatically and the decrease in DO with time was monitored; when the DO reached ± 4 mgO/ℓ, the air was switched on again automatically and the cycle repeated (the exact values for the high and low DO set points were varied depending on the organisms' OUR - if the OUR was low, the high and low DO set points were moved closer together and *vise versa*). Automatically, for each cycle the slope of the DO-time data during the air off period was determined by linear regression; this gives the OUR, which was stored by the meter (together with regression analyses and the time data). OUR data with linear correlation coefficient < 0.95 were rejected for analysis. A typical OUR (mgO/ℓ.h) versus time (h) response is shown plotted in Fig. 3.2. During the first period of the batch test (<5h) the OUR exhibits an exponential increase due to heterotrophic active biomass growth on both RBCOD and SBCOD added with the wastewater.

After ± 5 h the OUR drops precipitously due to the depletion of the wastewater RBCOD, and the subsequent OUR reflects heterotrophic active biomass growth on SBCOD only. As the first period is the important part for subsequent analysis, the batch tests were generally continued for approximately 2 hours after the precipitous drop in OUR was observed. The duration of the batch tests varied between 7 and 9 hours. In operating the batch test, the surface of the wastewater was covered by small plastic balls to limit surface exchange of oxygen. As a result of the intermittent aeration process, particulate matter (including organisms) was frequently splashed out of solution and deposited on the batch reactor walls, above the water surface. Because these organisms would cease to contribute to the OUR measured in the solution, the walls of the reactor were regularly brushed (during every aeration cycle) to return the organisms back into the solution. At regular intervals, samples were drawn from the reactor, immediately filtered through 0,45 µm filter paper, 2 – 3 drops of $HgC\ell_2$ were added to the filtrate which was stored for subsequent nitrate and nitrite analysis. At the end of the batch tests, the contents of the batch reactor was homogenized in a liquidiser, a sample drawn and the total COD concentration measured. The OUR results were downloaded from the DO meter to a PC.

3.2.4 Wastewater and mixed liquor batch tests

For the batch tests with a mixture of wastewater and mixed liquor, a sample of mixed liquor was harvested from the aerobic reactor of the parent system and a defined volume (variously 100, 200, 300 or 400 mℓ) placed in the batch reactor. The batch reactor volume was made up to 3ℓ with the same diluted unsettled municipal wastewater used in the wastewater only batch tests, also preheated to 20°C (see above). The batch test procedure detailed above was then followed.

3.3 DATA INTERPRETATION

The data from the batch tests can be interpreted in terms of either the UCT (Dold *et al.*, 1980; 1991) or the IAWQ (Henze *et al.*, 1987) kinetic simulation models. For this investigation the UCT model was selected to define the processes and compounds responsible for the OUR-time responses observed in the batch tests. The batch tests conducted on 8 December 1999 (08–12, wastewater batch no. 14) are used here to illustrate the calculation procedures. As noted above, two types of batch tests were conducted, one type with wastewater only and the other type with wastewater and mixed liquor.

3.3.1 Wastewater only batch tests

The data for the batch tests are set out in detail in Appendix A1. As an example, the OUR (mgO/ℓ/h) versus time (h) response for the batch test with 0,2 ℓ filtered effluent added to 2,8 ℓ diluted wastewater (Batch test no. W8, Table 3.1) is shown plotted in Fig 3.2. Because nitrate and nitrite was present in the filtered effluent, small concentrations of nitrate and nitrite were detected in this series of batch tests.

The low concentrations of nitrate and nitrite remained unchanged for the duration of the batch tests indicating the absence of nitrification, that is, no autotrophic biomass was present in the wastewater. (Should nitrification take place, it can be taken into account by following the procedures set out in section 3.3.2 below.)

Referring to the OUR time plot (Fig 3.2), the profile conforms to that described by Wentzel *et al.* (1995): During the first period of the batch test (<5.0h) the OUR exhibits an exponential increase due to heterotrophic active biomass growth on both RBCOD and SBCOD. After ± 5.0h, the OUR drops precipitously due to the depletion of the wastewater RBCOD. For the remainder of the batch test, the OUR exhibits an inverted S pattern typical of saturation kinetics, due to SBCOD utilization.

In terms of the UCT model the following information can be obtained from the batch tests:

- COD recovery (%)
- Wastewater heterotrophic active biomass, $Z_{BH(O)}$ (mgCOD/ℓ)

Wentzel *et al.* (1995) and Mbewe *et al.* (1995) describe the derivation of equations to quantify the above two parameters in terms of the UCT model. The derivations provide a logical insight into the mathematical modeling of the processes involved in the batch test and thus will be repeated here to clarify the interpretation of the OUR-time response.

COD recovery

The acceptability of the data from the batch test can be evaluated by doing a COD mass balance, as follows:

$$\% \text{ COD recovery} = \frac{COD_{t=T} + \int_{t=0}^{t=T} OUR \cdot dt}{COD_{t=0}} \cdot 100 \qquad (3.1)$$

where

t	=	time (h)
$COD_{t=T}$	=	total unfiltered COD concentration at end of test (t=T) (mgCOD/ℓ)
$COD_{t=0}$	=	total unfiltered COD concentration at start of test (t=0) (mgCOD/ℓ)
OUR	=	oxygen utilization rate (mgO/ℓ/h)
$\int_{t=0}^{t=T} OUR \cdot dt$	=	integral (area) under the OUR versus time plot between start and end of test (mgO/ℓ)
	=	oxygen concentration consumed over the test

Table 3.1 : Matrix representation of the UCT model (Dold *et al.*, 1991), simplified for conditions present in the batch test.

COMPOUND i → j ↓ PROCESS	1 Z_{BH}	2 Z_E	3 Z_I	4 S_{ads}	5 S_{enm}	6 S_{bs}	7 S_{us}	8 O	PROCESS RATE, ρ_j
1 Aerobic growth of Z_{BH} on S_{bs}	1					$-1/Y_{ZH}$		$-\frac{1-Y_{ZH}}{Y_{ZH}}$	$\mu\left[\frac{S_{bs}}{K_{SH}+S_{bs}}\right]Z_{BH}$
2 Aerobic growth of Z_{BH} on S_{ads}	1			$-1/Y_{ZH}$				$-\frac{1-Y_{ZH}}{Y_{ZH}}$	$K_{MP}\left[\frac{(S_{ads}/Z_{BH})}{K_{SP}+(S_{ads}/Z_{BH})}\right]Z_{BH}$
3 Death of Z_{BH}	-1	f_E			$1-f_E$				$b_H Z_{BH}$
4 Adsorption of S_{enm}				1	-1				$K_A S_{enm} Z_{BH}(f_{MA} - S_{abs}/Z_{BH})$
Biological (active) heterotrophic mass M (COD) L^{-3}	Endogenous mass M (COD) L^{-3}	Inert mass M (COD) L^{-3}	Adsorbed slowly biodegradable substrate M (COD) L^{-3}	Enmeshed slowly biodegradable substrate M (COD) L^{-3}	Readily biodegradable (soluble) substrate M (COD) L^{-3}	Unbiodegradable soluble substrate M (COD) L^{-3}	Oxygen M (-COD) L^{-3}		

Stoichiometric constants
Y_{ZH} = Heterotroph yield
f_E = Endogenous residue
f_{MA} = Max. ratio S_{ads}/Z_{BH}

Kinetic constants
$\hat{\mu}_H$ = Heterotroph max. specific growth rate on S_{bs}
K_{SH} = Heterotroph 1/2 saturation on S_{bs}
K_{MP} = Heterotroph max. specific growth rate on S_{ads}
K_{SP} = Heterotroph 1/2 sat. on S_{ads}
b_H = Heterotroph specific death rate
K_A = S_{enm} specific adsorption rate

In Eq (3.1), since in this series of batch tests no nitrification was observed, the measured OUR at time t ($OUR_{M(t)}$) is due to heterotrophic organism growth only and equals $OUR_{H(t)}$. Integrating the area under the measured OUR time profile and substituting into Eq (3.1), the COD recoveries for the different batch tests with wastewater only were calculated. Using the data in Fig 3.2 as an example, $COD_{t=0}$ = 449.7 mgCOD/ℓ; $COD_{t=T}$ = 402.7 mgCOD/ℓ and $\int_{t=0}^{t=T} OUR \cdot dt$ = 49.7 mgO/ℓ, then:

$$\% \text{ COD recovery} = \frac{402.7 + 49.7}{449.7} \cdot 100 = 100.6\%$$

COD recoveries between 90 – 110% indicate that the test results are acceptable, and these should generally be obtained in most batch tests without undue difficulty (see Chapter 4).

Wastewater heterotroph active biomass, $Z_{BH(O)}$

In applying the UCT model to the wastewater only batch test, Wentzel *et al.*, (1995) noted that the model can be simplified by recognising that specific conditions prevail in the batch test, namely:

- Aerobic conditions - denitrification processes need not be included
- No nitrification - nitrification processes need not be included
- Excess ammonia present - nitrate as an n-source for growth need not be considered
  - transformations from organic to ammonia nitrogen need not be included.

The simplified UCT model is presented in Table 1 of Wentzel *et al.*, (1995), and duplicated in Table 3.1. From the simplified UCT model, the rate of growth of heterotrophic biomass (dZ_{BH}/dt) is given by:

$$\frac{dZ_{BH}}{dt} = \text{growth on RBCOD} + \text{growth on SBCOD} - \text{death}$$

$$\frac{dZ_{BH}}{dt} = \mu_H \frac{S_{bs}}{K_{SH} + S_{bs}} \cdot Z_{BH} + K_{MP} \frac{S_{ads}/Z_{BH}}{K_{SP} + S_{ads}/Z_{BH}} \cdot Z_{BH} - b_H \cdot Z_{BH} \quad (3.2)$$

where

Z_{BH} = heterotroph active biomass concentration (mgCOD/ℓ)
S_{bs} = RBCOD concentration (mgCOD/ℓ)
K_{SH} = half saturation constant for RBCOD
= 5 mgCOD/ℓ
S_{ads} = absorbed SBCOD concentration (mgCOD/ℓ)
K_{SP} = half saturation constant for SBCOD
= 0,027 mgCOD/mgCOD

It can be accepted that during the initial stages of the batch test (before RBCOD is depleted and the OUR drops precipitously) $S_{bs} >> K_{SH}$ and $S_{ads} / Z_{BH} >> K_{SP}$, and therefore,

$$\frac{dZ_{BH}}{dt} = (\mu_H + K_{MP} - b_H) Z_{BH} \quad (3.3)$$

Integrating Eq (3.3) and solving yields the active organism concentration at time t [$Z_{BH(t)}$, mgCOD/ℓ] in terms of the initial active organism concentration [$Z_{BH(0)}$, mgCOD/ℓ], time (t, in h) and the net specific growth rate ($\mu_H + K_{MP} - b_H$) viz;

$$Z_{BH(t)} = Z_{BH(0)}\, e^{(\mu_H + K_{MP} - b_H)\, t/24} \quad (3.4)$$

The OUR at time t (OUR_t, mgO/ℓ) is a function of $Z_{BH(t)}$, the net specific growth rate and heterotroph yield coefficient, Y_{ZH} = 0,666 mgCOD/mgCOD:

$$OUR_{(t)} = \frac{1-Y_{ZH}}{Y_{ZH}} (\mu_H + K_{MP})\, Z_{BH(t)}/24 \quad (3.5)$$

Substituting Eq (3.4) for $Z_{BH(t)}$ in Eq (3.5) and taking natural logs yields

$$\ell n\, OUR_{(t)} = \ell n \left[\frac{1-Y_{ZH}}{Y_{ZH}} (\mu_H + K_{MP})\, Z_{BH(0)}/24\right] + (\mu_H + K_{MP} - b_H)\, t\,/24 \quad (3.6)$$

which is a straight line with,

$$\text{slope} = (\mu_H + K_{MP} - b_H)\,/24 \quad (3.7)$$

$$\text{y-intercept} = \ell n(OUR_{(t=0)}) = \ell n \left[\frac{1-Y_{ZH}}{Y_{ZH}} (\mu_H + K_{MP})\, Z_{BH(0)}/24\right] \quad (3.8)$$

To the determine heterotrophic active biomass at the start of the batch test ($Z_{BH(0)}$), the OUR values for the data up to the precipitous drop in OUR were plotted ℓn OUR versus time (h) (for example, the ℓn OUR from the data in Fig 3.2 are shown plotted in Fig 3.3),

and linear regression applied to determine the y-intercept, slope and correlation coefficient. These are listed in Table 3.2 and from the slopes and y-intercepts, $Z_{BH(0)}$ can be determined from Eqs. (3.7) and (3.8) i.e. (Wentzel *et al.*, 1995):

$$Z_{BH(0)} = \frac{(e^{y\text{-intercept}}) \cdot 24}{\frac{1-Y_{ZH}}{Y_{ZH}} \cdot (\text{slope} \cdot 24 + b_H)} \quad (\text{mgCOD}/\ell) \qquad (3.9)$$

where

$Z_{BH(0)}$ = heterotrophic active biomass concentration at the start of the batch test (mgCOD/ℓ batch reactor)
Y_{ZH} = heterotrophic active biomass yield, COD units (mgCOD/mgCOD)
= 0,666 mgCOD/mgCOD (Dold *et al.*, 1980, 1991; Wentzel *et al.*, 1995)
b_{HT} = heterotroph specific death rate at temperature T (/d)
= $b_{H20}\ 1.029^{(T-20)}$
b_{H20} = heterotrophic specific death rate at 20°C
= 0.62/d (death/regeneration theory, Dold *et al.*, 1980; Wentzel *et al.*, 1995).

Accepting Eq (3.9) and substituting into the equation the regression data listed in Table 3.2 for the batch test, the value for $Z_{BH(0)}$ for the batch test with wastewater (WW) only was calculated and is shown in Table 3.2 as a concentration and percentage of the total wastewater COD: As reported by Mbewe *et al.* (1995), Wentzel *et al.*, (1995) and Ubisi *et al.*, (1997a,b) for Mitchell's Plain wastewater, heterotrophic active biomass was found to be present at low concentrations, 3.5 – 5% of total COD.

Table 3.2: Batch test W8 with wastewater (WW) only: COD recovery, regression data from ℓn OUR versus time plot and heterotrophic active biomass at the start of the batch test [$Z_{BH(O)}$(WW)].

BATCH TEST: WASTEWATER (WW) ONLY										
Sew. Batch no.	Batch test no.	Batch test date	Volume (ℓ)		COD Recov. (%)	Regression			$Z_{BH(0)}$ WW	
			WW	FE		Y-intcpt	Slope	R^2	Concentration (mgCOD/ℓ)	Fraction of Total COD (%)
14	W8	08-12	2.8	0.2	100.6	0.9877	0.342	0.9624	14.556	3.26

3.3.2 Wastewater + mixed liquor batch tests

The data for the batch tests are set out in detail in Appendix A2. As an example, the OUR (mgO/ℓ·h) versus time (h) response for a batch test with 200 mℓ mixed liquor added to 2.8 ℓ diluted wastewater (Batch test no. M8, Table 3.3) is shown plotted in Fig 3.4. Referring to Fig 3.4, the general shape of the OUR time profile is the same as that for wastewater only (see Fig 3.2). However, due to the larger concentration of heterotrophic active biomass present (added with the mixed liquor) the OURs are higher and the time to the precipitous drop in OUR shorter. Further, since mixed liquor was drawn from a

nitrifying activated sludge system and added to the batch test, nitrification can be expected and indeed was oberved, see Fig 3.5. The OUR due to this nitrification must be taken into account in deriving estimates for % COD recovery and $Z_{BH(0)}$, since both these parameters are determined from the OUR for heterotrophs only. This can be done by noting that the measured OUR at any time t ($OUR_{M(t)}$) is made up of the OUR due to heterotrophic growth ($OUR_{H(t)}$) and due to nitrification ($OUR_{N(t)}$), i.e.

$$OUR_{M(t)} = OUR_{H(t)} + OUR_{N(t)} \qquad (mgO/\ell/h) \qquad (3.10)$$

[Note that for the batch tests with wastewater only, no nitrification was observed, i.e. in Eq (3.10) $OUR_{N(t)} = 0$ and $OUR_{M(t)} = OUR_{H(t)}$]. Rearranging Eq (3.10):

$$OUR_{H(t)} = OUR_{M(t)} - OUR_{N(t)} \qquad (mgO/\ell/h) \qquad (3.11)$$

Accordingly, to determine $OUR_{H(t)}$, an estimate for $OUR_{N(t)}$ is required. The $OUR_{N(t)}$ can be readily determined from the nitrate concentration-time profile (see Fig 3.5). For the batch tests with nitrification, ammonia – N is available in excess and nitrification proceeds at the maximum rate. Further, since the yield and maximum specific growth rate of the autotrophs are relatively low, Wentzel *et al.* (1995) and Mbewe *et al.* (1995) assumed the nitrification rate to be constant within the time scale of the batch test – this assumption was confirmed by the linearity of the nitrate concentration-time profiles. Accepting a constant nitrification rate, the slope of a "best-fit" linear line to the nitrate (mgN/ℓ) time (h) profile is the nitrification rate ($\Delta NO_3^-/\Delta t$, mgN/ℓ/h), and the $OUR_{N(t)}$ is given by:

$$OUR_{N(t)} = 4.57 \cdot \Delta NO_3^-/\Delta t \qquad (mgO/\ell/h) \qquad (3.12)$$

Linear regression was applied to the batch test nitrate time profile to determine the y-intercept, slope and correlation co-efficient, see Table 3.3. From the slope, the OUR_N for the batch test was calculated using Eq (3.12) and is shown in Table 3.3. The OUR_N is assumed constant over the batch test (see above) so that $OUR_N = OUR_{N(t)}$, and hence $OUR_{H(t)}$ can be calculated via Eq. (3.11), i.e. from each measured OUR value ($OUR_{M(t)}$), a constant nitrification OUR value (OUR_N, Table 3.2) is subtracted to give the OUR due to the heterotrophs only ($OUR_{H(t)}$).

Table 3.3: Batch test with mixture of wastewater (WW) and mixed liquor (ML) drawn from parent system: Regression data from nitrate versus time plot, nitrification rates ($\Delta NO_3^-/\Delta t$) and OUR for nitrification (OUR_N).

BATCH TEST: WASTEWATER (WW) + MIXED LIQUOR (ML)							
Sewage Batch no.	Batch test no.	Batch test date	Regression : Nitrate vs time			$\Delta NO_3^-/\Delta t$	OUR_N
			Y-intercept	Slope	R^2	(mgN/ℓ.h)	(mgO/ℓ.h)
14	M8	08-12	0.8281	0.1257	0.9756	0.1257	0.5744

Having determined the $OUR_{H(t)}$ for the batch test, the % COD recovery was calculated using Eq. (3.1) and is shown in Table 3.4. COD recoveries ranging from 90 110% provide support for the reliability of the measurements.

To determine the heterotrophic active biomass present at the start of the batch tests, the $OUR_{H(t)}$ (i.e. $OUR_{M(t)}$ - OUR_N) up to the precipitous drop in OUR were plotted ℓn $OUR_{H(t)}$ versus time (h), for example see Fig. 3.6. Linear regression was used to determine the y-intercept, slope and correlation coefficient of the ℓn $OUR_{H(t)}$ versus time plot, see Table 3.4. From the y-intercept and slope data, the value for $Z_{BH(0)}$ (ML + WW) [the heterotrophic active biomass present at the start of the batch tests, due to that added with the mixed liquor (ML) and that present in the original wastewater (WW)] was calculated using Eq. (3.9) and is also given in Table 3.3.

Table 3.4: Batch test with mixture of wastewater (WW) and mixed liquor (ML) drawn from parent laboratory-scale system: Volumes added, COD recovery, regression data from ℓn OUR_H versus time plot and heterotrophic active biomass present in the batch test ($Z_{BH(O)}$) due to mixed liquor + wastewater (ML+WW), wastewater (WW) only and mixed liquor (ML). Also shown is the theoretical heterotrophic active biomass due to the mixed liquor addition (from Eq 3.22, section 3.4.1 below).

BATCH TEST: WASTEWATER (WW) + MIXED LIQUOR (ML)											
Sew. Batch no.	Batch Test No	Volume (ℓ)		COD Recov (%)	Regression			$Z_{BH(0)}$ (mgCOD/ℓ)			
								Measured			Theoretical ML
		ML	WW		Y-intcpt	Slope	R^2	ML+WW	WW	ML	
14	M8	0.2	2.8	93.2	1.654	0.2568	0.9787	37	15	22	112

3.3.3 Determining heterotrophic active biomass concentrations

In the batch tests with both wastewater (WW) and mixed liquor (ML) present, heterotrophic active biomass at the start of the test [$Z_{BH(0)}$], is due to the heterotrophic active biomass present in the original wastewater (WW) and that added with the mixed liquor (ML). Because in this investigation the same volume of wastewater was used in both batch tests, the $Z_{BH(0)}$ due to the wastewater [$Z_{BH(0)}$ (WW)] may be subtracted directly from the $Z_{BH(0)}$ due to both the wastewater and mixed liquor [$Z_{BH(0)}$ (WW + ML)] to determine $Z_{BH(0)}$ due to the mixed liquor only [$Z_{BH(0)}$ (ML)]:

$$Z_{BH(0)}\,(ML) = Z_{BH(0)}\,(ML + WW) - Z_{BH(0)}\,(WW) \qquad (3.13)$$

Using Eq. (3.13), the *measured* heterotrophic active biomass concentration in the total batch test volumes (WW + ML) due to the added mixed liquor [$Z_{BH(0)}$ (ML)] was calculated and is given in Table 3.4.

Because in the batch test method employed by Ubisi *et al.* (1997a,b), 3ℓ of wastewater was used in the wastewater *only* batch test, while the volume of wastewater added to the wastewater + mixed liquor batch test was always less than 3ℓ, the actual *concentration* of "wastewater" active heterotroph biomass [$Z_{BH(0)}$ (WW)] would be lower in the latter batch test. The dilution in $Z_{BH(0)}$ (WW) caused by the addition of mixed liquor to the batch tests with wastewater and mixed liquor was taken into account as follows:

$$Z_{BH(0)}(WW)_D = Z_{BH(0)}(WW) \cdot V_{WW}/(V_{ML} + V_{WW}) \quad (3.14)$$

Where

$Z_{BH(0)}(WW)_D$ = heterotrophic active biomass due to wastewater at start of batch test with WW + ML, taking due account of dilution (mgCOD/ℓ)

$Z_{BH(0)}(WW)$ = heterotrophic active biomass present at start of the (3ℓ) wastewater only batch test, (mgCOD/ℓ)

V_{WW} = volume of wastewater added to the WW + ML batch test (ℓ)

V_{ML} = volume of mixed liquor added to the WW + ML batch test (ℓ)

Now, the *measured* heterotrophic active biomass concentration in the total batch test volumes (WW + ML) due to the added mixed liquor [$Z_{BH(0)}$ (ML)] could be calculated:

$$Z_{BH(0)}(ML) = Z_{BH(0)}(ML + WW) - Z_{BH(0)}(WW)_D \quad (3.15)$$

As noted earlier this procedure was initially adopted for this investigation - the first four batch tests (see batch tests M1 - M4 in Appendix A2) were conducted in accordance with this procedure. Taking due account of dilution, Eq. (3.15) was used in the calculation of the measured mixed liquor $Z_{BH(0)}$ (ML) values for these four batch tests only.

3.3.4 Determining maximum specific growth rates

From the analysis of an $OUR_{H(t)}$ (mgO/ℓ/h) versus time (h) response obtained in the batch test, valuable information pertaining to the heterotrophic organisms' growth rates can also be determined. Wentzel *et al.* (1995) and Mbewe *et al.* (1995) concluded that the $OUR_{H(t)}$ before the precipitous drop could be separated into the OUR contribution due to RBCOD degradation and the OUR contribution due to SBCOD degradation (see Fig. 3.2). This is equivalent to separating the overall growth rate ($\mu_H + K_{MP}$) into its μ_H and K_{MP} components. The derived equation for K_{MP} – heterotroph maximum specific growth rate on SBCOD and μ_H – heterotroph maximum specific growth rate on RBCOD will be repeated here to illustrate the calculation procedures.

In terms of the UCT model, growth of heterotrophic organisms on RBCOD and SBCOD is independent (see Table 3.1). The OUR's (mgO/ℓ/h) associated with these two growth processes are given by Eq. (3.5) which can be separated to give:

$$(1) \quad OUR_{RBCOD(t)} \cdot 24 = \frac{1-Y_{ZH}}{Y_{ZH}} \cdot \mu_H \cdot Z_{BH(0)} \cdot e^{(\mu_H + K_{MP} - b_H)\, t/24} \qquad (3.16)$$

$$(2) \quad OUR_{SBCOD(t)} \cdot 24 = \frac{1-Y_{ZH}}{Y_{ZH}} \cdot K_{MP} \cdot Z_{BH(0)} \cdot e^{(\mu_H + K_{MP} - b_H)\, t/24} \qquad (3.17)$$

Heterotroph maximum specific growth rate on SBCOD, K_{MP}

Up to the precipitous decrease, the OUR is the sum of both the RBCOD and SBCOD utilization [Eq. (3.16) + Eq. (3.17)]. Once the RBCOD is depleted, which causes the precipitous OUR decrease, the OUR is that for SBCOD only [Eq. (3.17)]. If the precipitous decrease occurs at t = s hours at which time the OUR is $OUR_{SBCOD(t = s)}$, then from Eq. (3.17) K_{MP} is given by

$$K_{MP} = \frac{OUR_{SBCOD(t = s)} \cdot 24}{\frac{1-Y_{ZH}}{Y_{ZH}} \cdot Z_{BH(0)} \cdot e^{(\mu_H + K_{MP} - b_H) \cdot (t = s)/24}} \qquad (3.18)$$

where

$OUR_{SBCOD(t = s)}$	=	OUR due to SBCOD only, i.e. observed OUR immediately following the precipitous drop in OUR (mgO/ℓ/h).
(t = s)	=	time immediately following the precipitous drop in OUR(h)
$(\mu_H + K_{MP} - b_H)/24$	=	slope of ℓn $OUR_{(t)}$ versus time (h) plot.

Using the data in Fig 3.6 and 3.7 as an example, $Z_{BH(0)}$ = 37 mgCOD/ℓ; time s = 4,5h ; $OUR_{SBCOD\,(t = 4,5h)}$ = 9,6 mgO/ℓ/h; slope of ℓn $OUR_{(t)}$ vs time plot = 0,257 (Fig 3.7):

$$K_{MP} = \frac{9,6 \cdot 24}{\frac{1 - 0,666}{0,666} \cdot 37 \cdot e^{0,257 \cdot 4,5}} = 3,9/d$$

Heterotroph maximum specific growth rate on RBCOD, μ_H

For the UCT model (see Table 3.1), the value for μ_H can be calculated from the value for K_{MP} derived above and the slope of the ℓn $OUR_{H(t)}$ versus time plot, as follows

$$\mu_H = \text{slope} \cdot 24 - K_{MP} + b_H \qquad (3.19)$$

For the example,

$$\mu_H = 0,257 \cdot 24 - 3,9 + 0,62 = 2,9/d.$$

The maximum specific growth rate data for both types of batch tests are listed in detail in Appendix A1 and A2.

3.4 COMPARISON WITH PARENT SYSTEM

This section deals with the calculation of the theoretical heterotrophic active biomass concentration in the parent system [Z_{BH}(theor.)]. The calculated Z_{BH}(theor.) contained in the mixed liquor sample harvested from the parent system and added in the batch test will ultimately be compared with the heterotrophic active biomass concentration measured at the start of each batch test [$Z_{BH(O)}$(ML)], as described in section 3.3 above.

3.4.1 Theoretical heterotrophic active biomass concentration in the parent system

The parent laboratory-scale system and its operation have been described in detail in Chapter 2. To ensure steady state, the parent system was operated for more than 15 sludge ages before the batch tests were commenced, during sewage batch no. 11 (see Table 2.2). Detailed data on the parent system have been furnished in Chapter 2. To illustrate the comparison with the batch test results, the data from sewage batch no. 14 will be used; the averaged data for this sewage batch are listed in Table 3.5 together with the average data from the preceding two sewage batches (no. 12 and 13).

Table 3.5: Steady state results for parent laboratory-scale anoxic/aerobic sludge system. For each of the wastewater batches tested, the daily results have been averaged and the averages are listed with sample standard deviations in brackets.

PARENT ANOXIC/AEROBIC STEADY STATE SYSTEM													
WW Batch	No of Tests	COD (mg/ℓ)		TKN (mg/ℓ)		Nitrate (mgN/ℓ)			Aerobic OUR	Mixed liquor (mg/ℓ)			pH
		Inf	Eff	Inf	Eff	Anoxic	Aerobic	Eff	mgO/ℓ/h	VSS	COD	TKN	
12	6	500 (42)	43 (9)	47 (2)	4.0 (0.9)	2.2 (1.5)	7.8 (2.9)	10.2 (2.4)	33.5 (1.9)	2293 (70)	3372 (121)	248 (33)	7.53
13	8	540 (25)	59 (14)	47 (1)	4.1 (0.3)	3.7 (0.5)	9.8 (0.8)	11.3 (0.9)	34.8 (0.7)	2469 (83)	3681 (148)	243 (6)	7.57
14	4	517 (25)	61 (12)	48 (1)	3.7 (0.4)	5.5 (1.2)	11.7 (0.8)	13.2 (0.5)	37.4 (2.1)	2227 (163)	3403 (185)	226 (7)	7.61

Following the procedures described by Ekama *et al.* (1986), and set out in Chapter 2, the following were determined: The influent wastewater unbiodegradable soluble and particulate COD fractions ($f_{S,us}$ and $f_{S,up}$ respectively); system COD and N mass balances; the COD and TKN to VSS ratios of the mixed liquor (f_{cv} and f_N respectively). These are listed in Table 3.6. Referring to Table 3.6, reasonable COD and N mass balances were obtained; thus, the steady state data for the parent system can be considered acceptable.

Table 3.6: Steady state COD and N mass balances, wastewater fractions and mixed liquor parameters for parent laboratory-scale anoxic/aerobic activated sludge system. Data calculated from data Table 3.5, either directly or using the steady state (SS) design (WRC, 1984) or kinetic simulation (sim.) models (Dold *et al.*, 1991).

PARENT ANOXIC/AEROBIC STEADY STATE SYSTEM										
WW Batch	No. of tests	Mass Balance (%)		Wastewater fractions			Mixed liquor			
		COD	N	Unbiod Soluble COD ($f_{S,us}$)	Unbio. Particulate COD ($f_{S,up}$)		COD/VSS ratio (f_{cv}) (mgCOD/ mgVSS)	TKN/VSS ratio (f_n) (mgN / mgVSS)	Active Fraction (f_{av})	
					SS Design	Kinetic Sim.			SS Design	Kinetic Sim.
12	6	87	92	0.0740	0.0999	0.0790	1.47	0.108	0.4798	0.4906
13	8	88	93	0.0907	0.1053	0.0855	1.49	0.098	0.4618	0.4722
14	4	93	90	0.0909	0.0777	0.0560	1.53	0.100	0.4949	0.5063

From the parent system steady state data, the theoretical heterotrophic active biomass of the mixed liquor drawn from the parent system and added to the batch tests was calculated. To do this, two approaches could be followed – either the steady state design or the kinetic simulation models could be used. In the kinetic simulation models (Dold *et al.*, 1980, 1991; Henze *et al.*, 1987), at 20°C (the temperature for all experiments) for sludge ages > about 3 days, virtually all the influent biodegradable COD is depleted. Certainly this will be the case for the parent system at 10d sludge age with the small anoxic mass fraction (25%) present. Under these conditions the steady design equations (WRC, 1984) and the kinetic simulation models should give near identical values for heterotrophic active biomass, provided equivalent values for the constants are used; to verify this, both the steady state and kinetic approaches were used.

Steady state model: From WRC (1984), the heterotrophic active biomass fraction of the mixed liquor volatile suspended solids (VSS) (f_{av}) can be determined from:

$$f_{av} = MX_{BH}/MX_V$$

$$= MX_{BH}/(MX_{BH} + MX_E + MX_I + MX_{BA}) \quad (3.20)$$

where

MX_V = mass of volatile suspended solids = $V \cdot X_V$, VSS units (mg VSS)
MX_{BH} = mass of heterotrophic active biomass = $V \cdot X_{BH}$, VSS units (mgVSS)
MX_E = mass of endogenous material = $V \cdot X_E$, VSS units (mgVSS)
MX_I = mass of inert material = $V \cdot X_I$, VSS units (mgVSS)
MX_{BA} = mass of autotrophic active biomass = $V \cdot X_{BA}$, VSS units (mgVSS)
V = system volume (ℓ)
X_{BH} = heterotrophic active biomass concentration, VSS units (mgVSS/ℓ)
X_E = endogenous material concentration, VSS units (mgVSS/ℓ)
X_I = inert material concentration, VSS units (mgVSS/ℓ)
X_V = volatile suspended solids concentration, VSS units (mg VSS/ℓ)

In Eq (3.20), for activated sludge systems receiving "normal" municipal wastewaters (influent TKN/COD ratio < 0.12 mgN/mgCOD) the autotrophic active biomass (MX_{BA}) component of the mixed liquor organic suspended solids is very small compared to the other three components (<2% of the total for the parent system here). Thus, with very little error, the autotrophic active biomass can be neglected when calculating the mixed liquor VSS. Accordingly, from WRC (1984), substituting in Eq (3.20) for $MX_{BA} = 0$ and $MX_V = (MX_{BH} + MX_E + MX_I)$:

$$\frac{1}{f_{av}} = 1 + f_E^* b_{HT}^* R_S + \frac{f_{S,up}(1 + b_{HT}^* R_S)}{f_{cv} Y_H^* (1 - f_{S,us} - f_{S,up})} \qquad (3.21)$$

where

f_E^* = fraction of heterotrophic active biomass that is endogenous residue
= 0.2 (endogenous respiration theory, Dold *et al.*, 1980).
b_{HT}^* = specific endogenous mass loss rate at temperature T (/d)
= $b_{H20}^* 1.029^{(T-20)}$
b_{H20}^* = specific endogenous mass loss rate at 20°C
= 0.24/d at 20°C (endogenous respiration theory, Dold *et al.*, 1980)
R_S = system sludge age (d)
= 10 d
$f_{S,up}$ = fraction of influent substrate that is unbiodegradable particulate
$f_{S,us}$ = fraction of influent substrate that is unbiodegradable soluble
f_{cv} = COD to VSS ratio of mixed liquor organic suspended solids (mgCOD/mgVSS)
Y_H^* = heterotrophic active biomass yield, VSS units (mgVSS/mgCOD)
= 0.45 mgVSS/mgCOD (WRC, 1984)

Using Eq (3.21) and the data in Table 3.5, values for f_{av} were calculated and are listed in Table 3.6.

Kinetic simulation: For the kinetic simulation models, using the UCTOLD computer programme (Dold *et al.*, 1991), values for f_{av} were determined from simulations of the parent system steady state periods – for each steady state period the values for the influent wastewater unbiodegradable particulate COD fraction ($f_{S,up}$) were adjusted until the simulated and measured reactor mixed liquor organic suspended solids concentration (COD units) were near equal and the f_{av} calculated from the simulated results: These values for $f_{S,up}$ and f_{av} are also listed in Table 3.6. Comparing the values for f_{av} from the steady state design procedure and the kinetic simulations, near identical values were obtained for each wastewater batch. The small differences arise principally because (i) with the steady state design models the $f_{S,up}$ and f_{av} were determined from the measured mixed liquor organic suspended solids (MLOSS) VSS concentration whereas with the kinetic simulation models these were determined from the MLOSS COD concentration, and (ii) the kinetic simulation models include autotrophic active biomass and non-utilized slowly biodegradable COD in the MLOSS whereas these are ignored in the steady state design models.

Due to their near equality, the f_{av} from either the steady state design or the kinetic simulation models could be used, but the steady state design model is probably preferable for the simpler more direct analytical calculation procedure.

Now, knowing f_{av} and the concentration of the mixed liquor VSS that was drawn from the parent system [X_V(PS)] to be added to the batch tests (available from the averaged steady state VSS concentration measured in the parent system, Table 3.5), the *theoretical* heterotrophic active biomass concentration in the batch reactor due to the added mixed liquor [$X_{BH}(theor)_{BT}$, COD units] is given by:

$$Z_{BH}(theor)_{BT} = [X_V(PS) \cdot f_{av} \cdot f_{cv} \cdot V_{ML}] / (V_{ML}+V_{WW}) \qquad (3.22)$$

where

$Z_{BH}(theor)_{BT}$ = theoretical heterotrophic active biomass concentration in batch test reactor due to added mixed liquor, COD units (mgCOD/ℓ batch reactor)

X_V(PS) = mixed liqour VSS concentration measured in parent system, from Table 3.5 (mgVSS/ℓ)

V_{ML} = volume of mixed liquor from parent system added to batch test (ℓ)

V_{WW} = volume of wastewater added to batch test (ℓ).

As an illustration, for batch test M8 the *theoretical* heterotrophic active biomass concentration in the batch reactor due to the addition of the mixed liquor sample drawn from the parent system (value given in Table 3.4) was calculated as follows :

The average VSS concentration for the parent system during sewage batch 14 is given in Table 3.4 as $X_V(PS) = 2227$ mgVSS/ℓ. From Table 3.5 for sewage batch 14, $f_{av} = 0.4949$; $f_{cv} = 1.53$ mgCOD/mgVSS and the volumes used in batch test M8, $V_{ML} = 0.2\ell$; $V_{WW} = 2.8\ell$ substituted into Eq 3.22 :

$$Z_{BH}(theor)_{BT} = \frac{(2227 \cdot 0.4949 \cdot 1.53 \cdot 0.2\ell)}{(0.2\ell + 2.8\ell)} = 112 \text{ mgCOD}/\ell$$

Note that in Eq (3.22) the parent system mixed liquor organic suspended solids are expressed in VSS units [X_V(PS)], whereas the heterotrophic active biomass is expressed in COD units. This is done because conventionally the mixed liquor organic suspended solids in activated sludge systems are measured via the VSS test, whereas the kinetic models used to develop the batch test are in terms of the COD parameter. However, the two units of measure are directly related through the COD/VSS ratio of the mixed liquor organic suspended solids (f_{cv}) which was measured, see Table 3.6. If a value for f_{cv} is not available from measurement, the standard value of 1.48 mgCOD/mgVSS (WRC, 1984) can be accepted.

The comparison between the measured and theoretical mixed liquor $Z_{BH(0)}$(ML) values obtained from Table 3.4 can be illustrated graphically in Fig 3.8 where the values are plotted against each other. The results from a series of batch tests plotted in this fashion can give a good indication of the agreement that exists between the measured and theoretical values. Ubisi *et al.* (1997a,b) used these graphical comparisons to illustrate two contradictory trends that transpired from their batch test results: In Fig 3.9 the results from a series of batch tests performed on mixed liquor samples harvested from a laboratory-scale nitrification/denitrification activated sludge system operated at 12 days' sludge age, revealed a trend which underlined the good agreement that existed between the measured and theoretical results. In Fig 3.10, however, the results from batch tests performed on mixed liquor samples drawn from an identical system, but operated at a sludge age of 20 days, revealed a trend which illustrated poor agreement between the measured and theoretical values. Ubisi *et al.* were not able to provide an explanation for this inconsistency, which cast a measure of uncertainty around the reliability of the batch test procedure to provide substantiated support for the active heterotrophic biomass concept.

3.5 CLOSURE

In this chapter a review is given of the batch test method developed by Ubisi *et al.* (1997a,b) to quantify the heterotrophic active biomass concentration present in a nitrification/denitrification activated sludge system. The experimental procedures together with the interpretation of experimental data with respect to the concepts of the UCT kinetic simulation model have been described in detail. Ubisi *et al.* observed that in some instances the experimental results showed remarkable agreement with theoretically predicted responses. Thus, the batch test method has shown promise as a means to substantiate the hypothesized concentration of active heterotrophic biomass present in the above-mentioned activated sludge system and would therefore appear to validate the mathematical formulations contained within the simulation model.

However, the batch test method has also produced results which are inconsistent with the theoretical models. One of the primary objectives of this investigation was to establish whether the inconsistencies could be related to the batch test procedure itself or, more perplexing, whether the inconsistencies were the result of incorrect mathematical formulations within the framework of the simulation model. These aspects will be dealt with in more detail in Chapter 4.

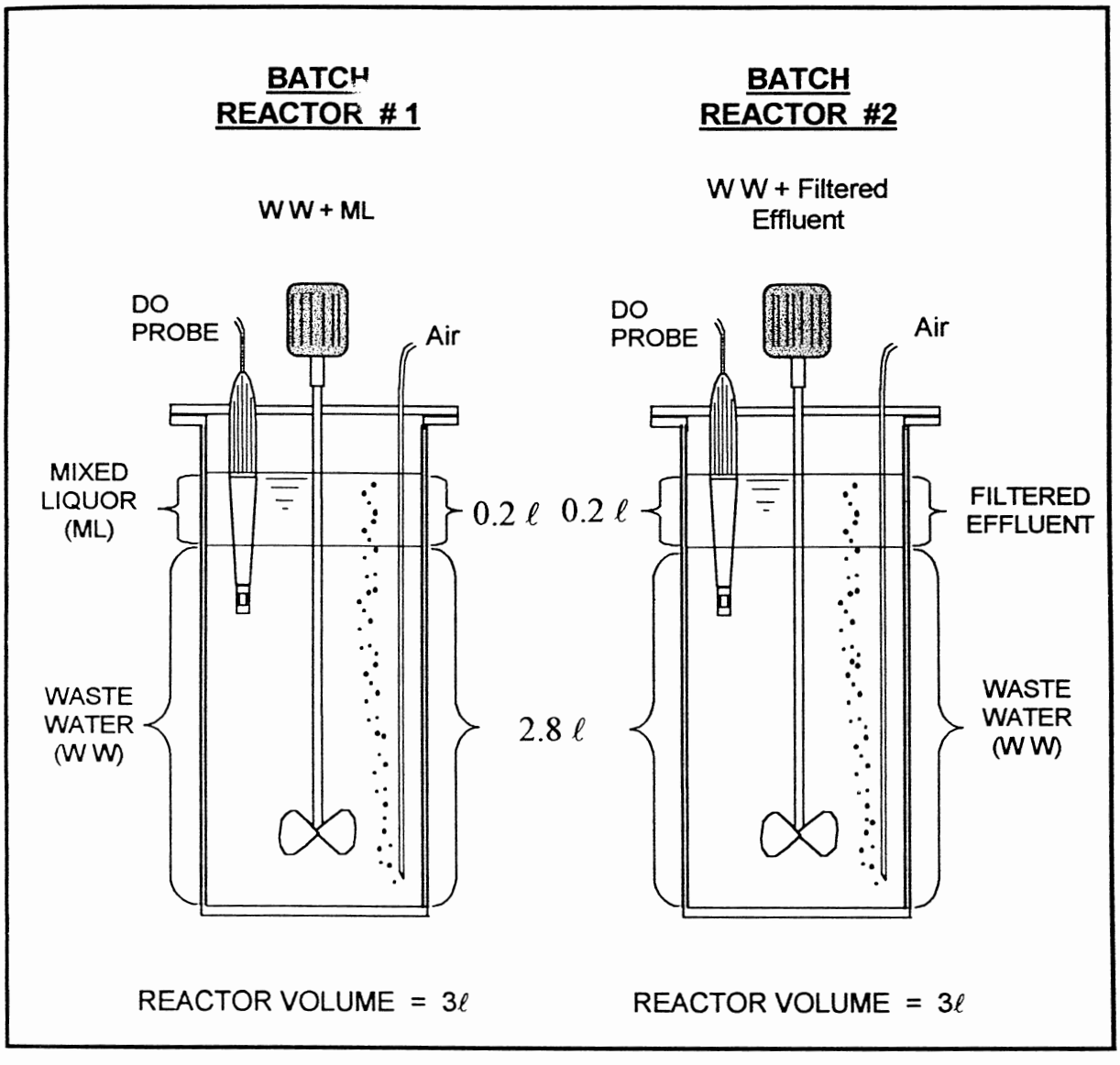


Figure 3.1 : Parallel batch reactor arrangement showing batch reactor no. 1 with mixed liquor added to wastewater (ML+WW) batch test, and batch reactor no. 2 with filtered effluent added to wastewater (WW) only batch test.

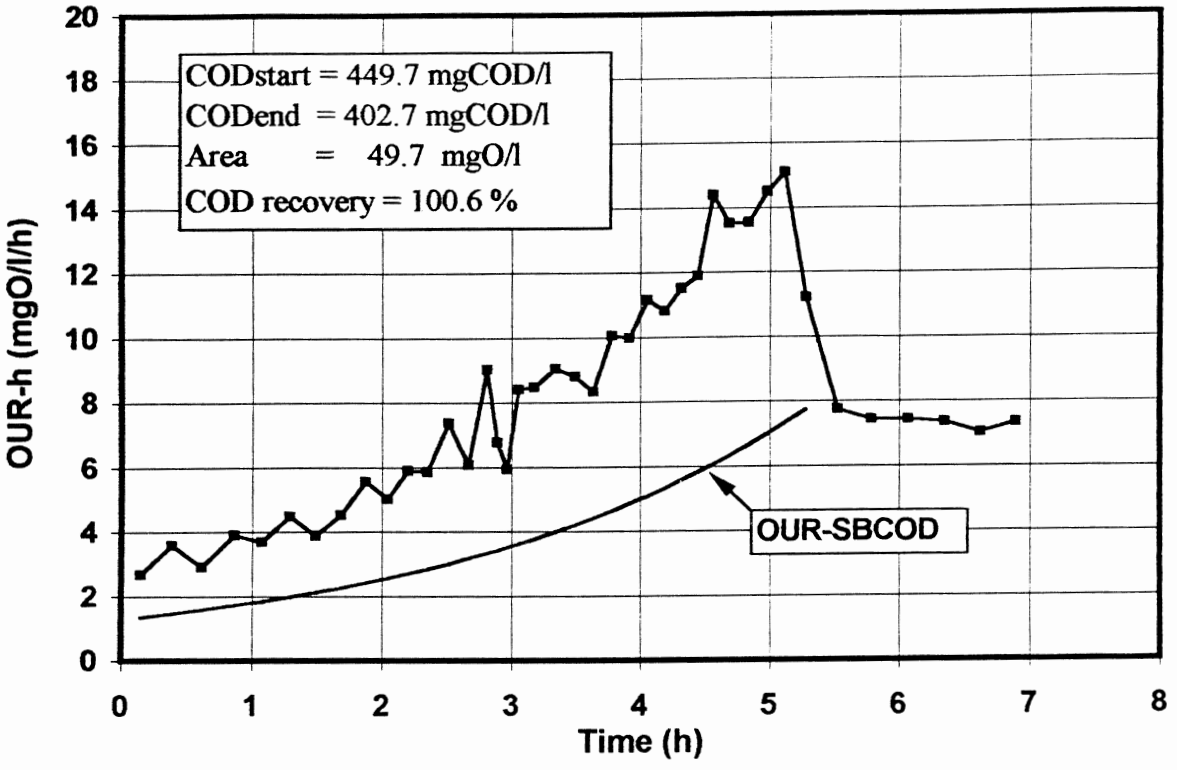


Figure 3.2 : Oxygen utilization rate (OUR) response with time for wastewater (2.8ℓ) plus filtered effluent (0.2ℓ) aerobic batch test no.W8, 08-12 , sewage batch no.14. Raw municipal wastewater from Mitchell's Plain (Cape Town, South Africa)

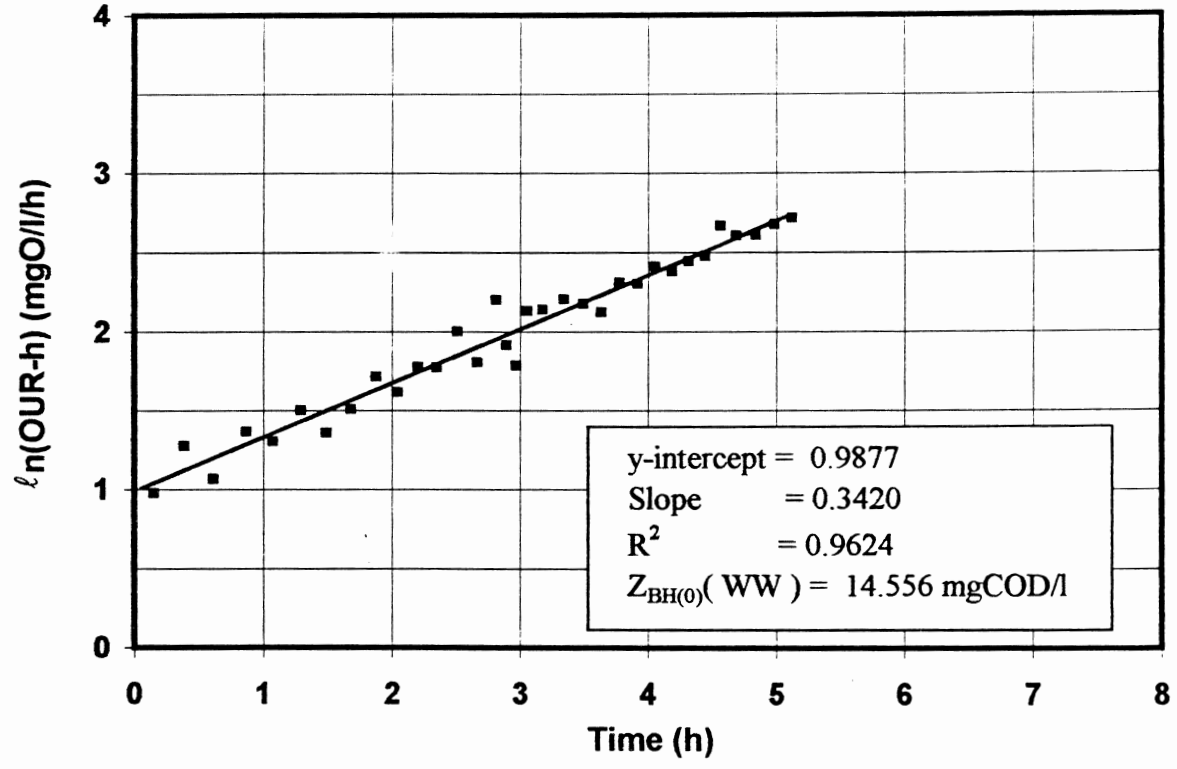


Figure 3.3 : ℓn oxygen utilization rate (ℓn OUR) versus time for OUR data in Fig. 3.2, up to the precipitous drop in OUR. Batch test no.W8, 08-12 , sewage batch no.14.

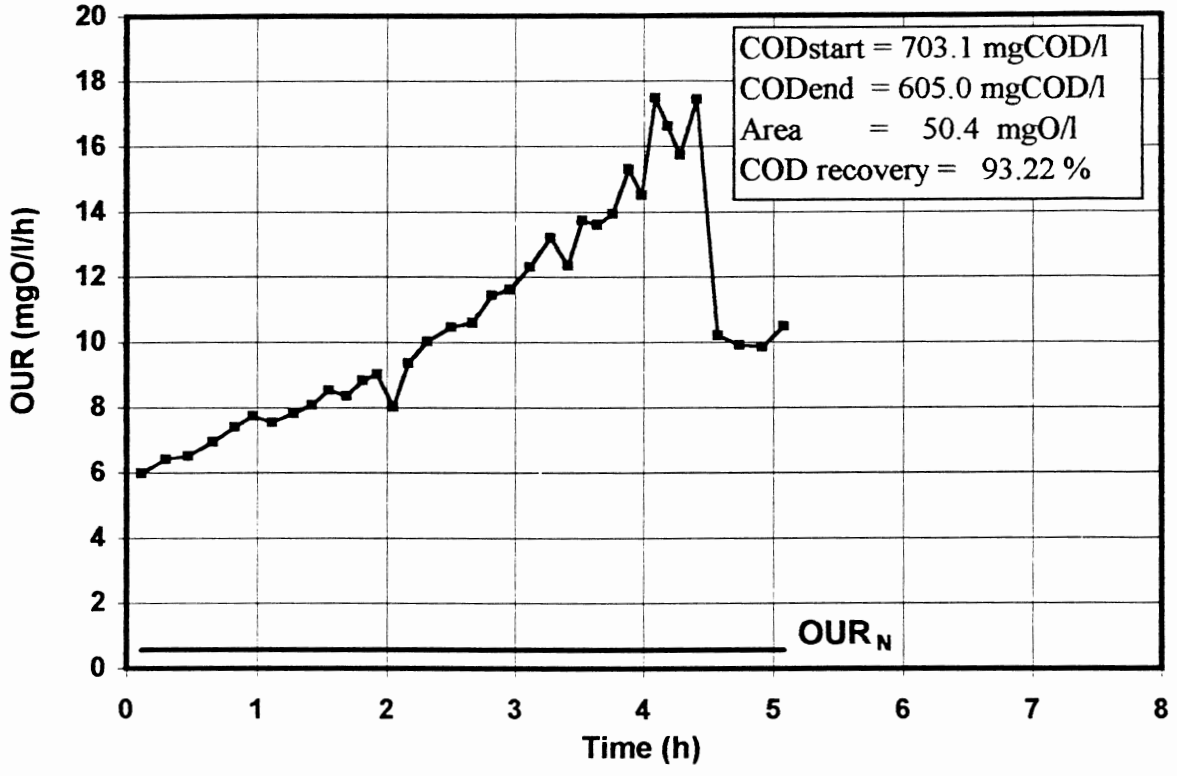


Figure 3.4 : Oxygen utilization rate (OUR) response with time for aerobic batch test on a mixture of wastewater (2.8ℓ) and mixed liquor (0.2ℓ) drawn from the aerobic reactor of the parent system. Batch test no. M8, 08-12 , sewage batch no.14. Raw municipal wastewater from Mitchell's Plain (Cape Town, South Africa)

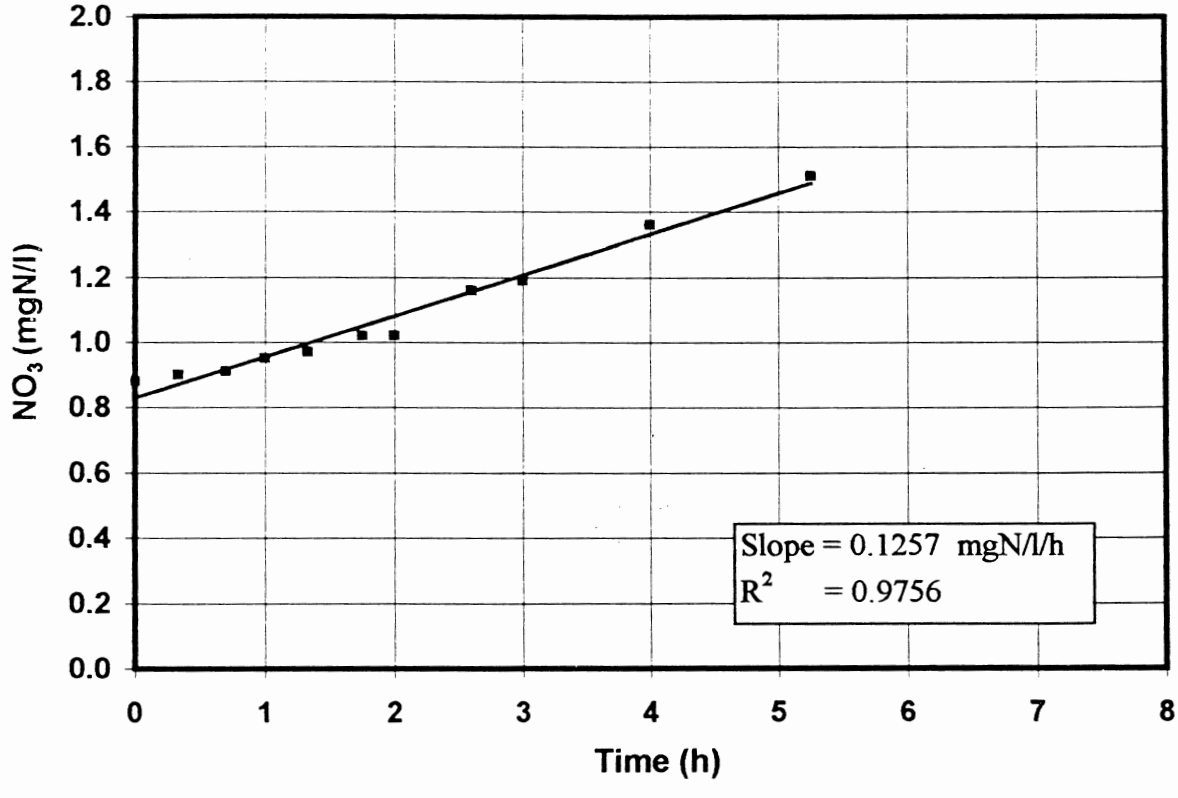


Figure 3.5 : Nitrate variation with time for aerobic batch test in Fig 3.4. The constant OUR for nitrification , calculated from OUR_N = 4.57 * (ΔNO3 / Δt) , is shown in Fig 3.4.

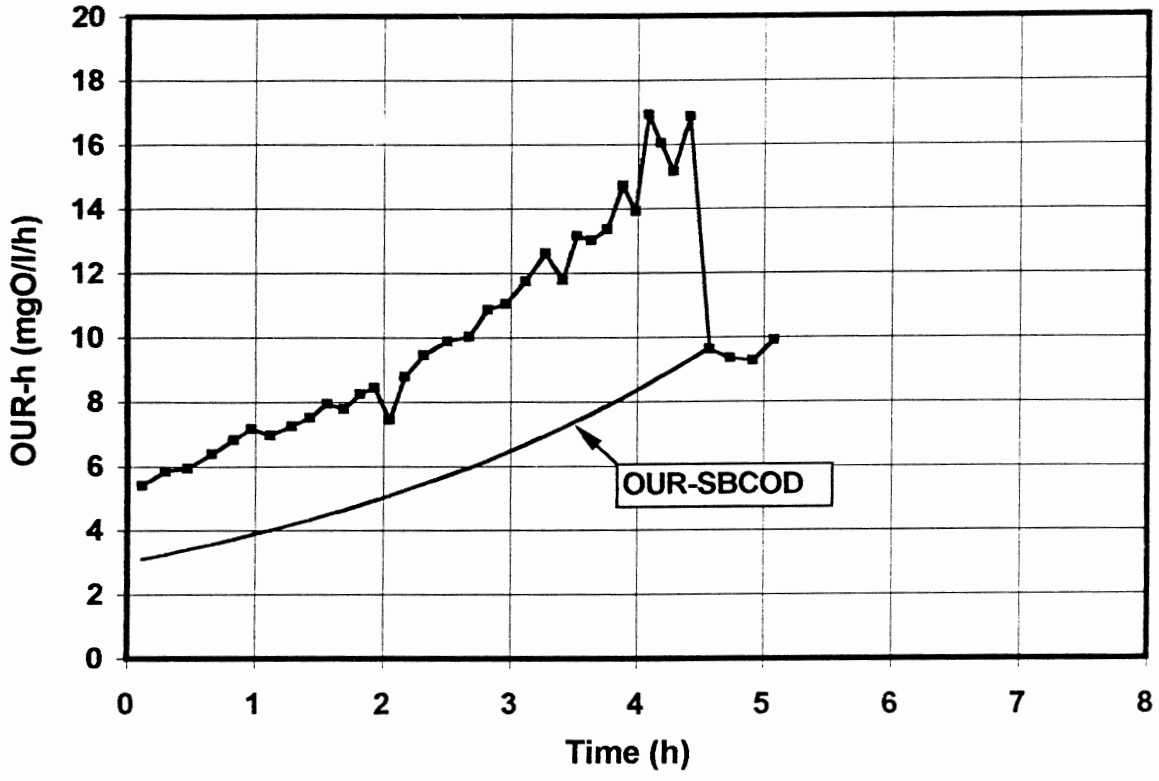


Figure 3.6 : Oxygen utilization rate due to heterotrophic active biomass (OUR-h) versus time for aerobic batch test no. M8, 08-12 , sewage batch no.14. The OUR due to nitrification (OUR_N) subtracted from the measured OUR data in Fig 3.4.

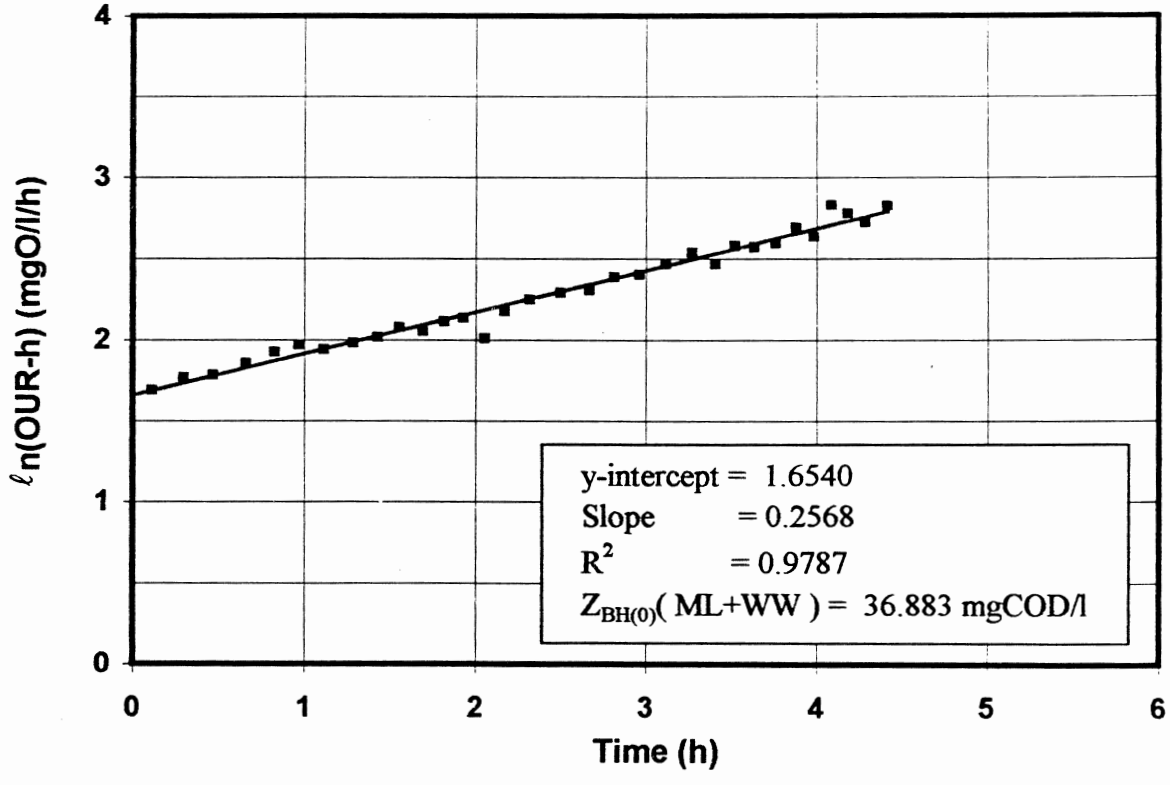


Figure 3.7 : ℓn oxygen utilization rate due to heterotrophic active biomass (ℓn OUR-h) versus time for OUR-h data in Fig. 3.6, up to the precipitous drop in OUR. Batch test no. M8, 08-12 , sewage batch no.14.

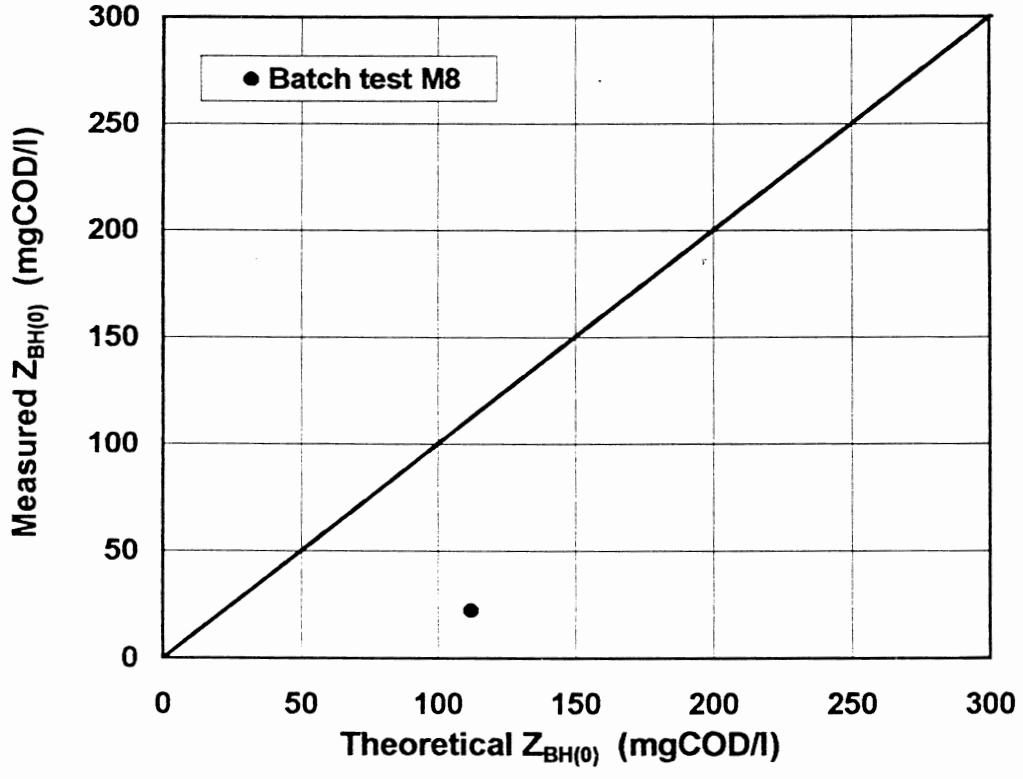


Figure 3.8 : Measured versus theoretical heterotrophic active biomass concentration ($Z_{BH(0}$ in the ML+WW batch test M8 due to addition of mixed liquor (0.2ℓ)drawn fro the parent laboratory-scale activated sludge system (see Table 3.3).

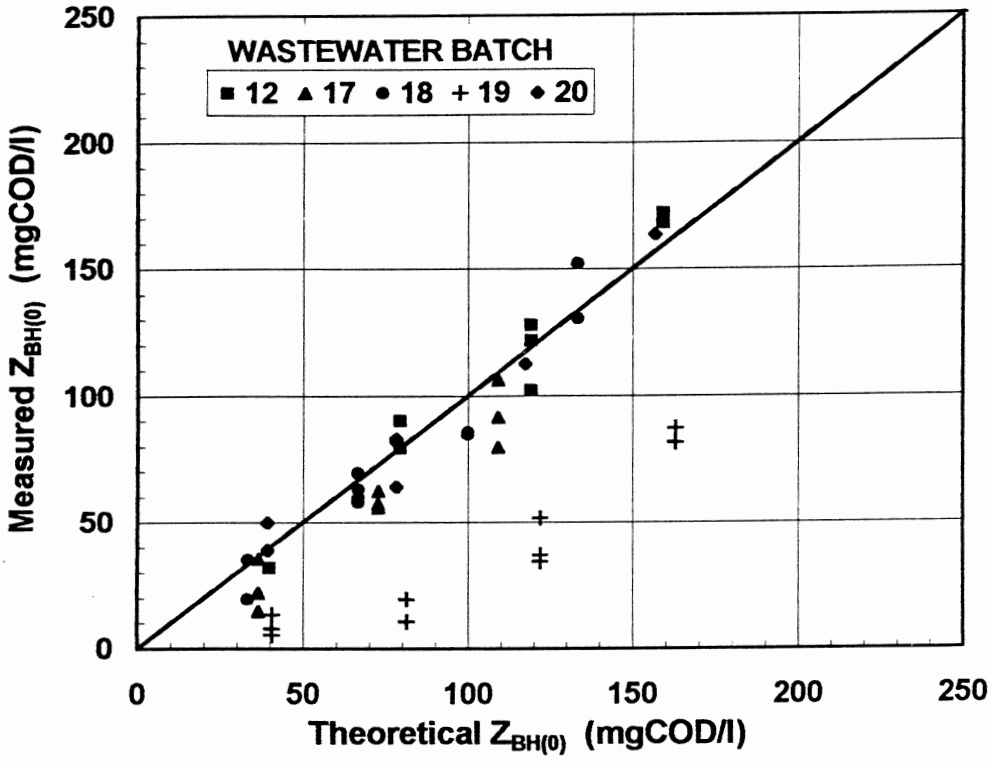


Figure 3.9 : Comparison by Ubisi *et al.* (1997a,b) for measured versus theoretical mixed liquor active heterotrophic biomass $Z_{BH(0)}$(ML) for various wastewater batches with parent laboratory-scale system operated at 12 days' sludge age.

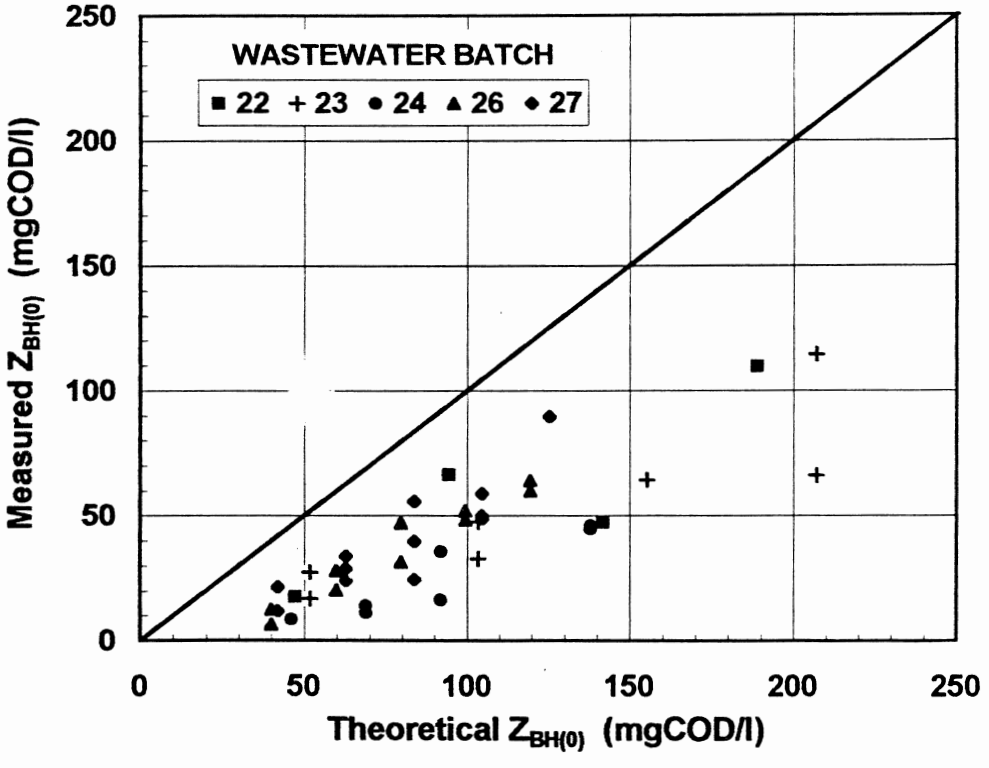


Figure 3.10 : Comparison by Ubisi *et al.* (1997a,b) for measured versus theoretical mixed liquor active heterotrophic biomass $Z_{BH(0)}$(ML) for various wastewater batches with parent laboratory-scale system operated at 20 days' sludge age.

CHAPTER 4

EVALUATION OF THE BATCH TEST METHOD TO QUANTIFY HETEROTROPHIC ACTIVE BIOMASS

4.1 INTRODUCTION

In Chapter 3 a detailed description is given of the batch test procedure developed by Ubisi *et al.*(1997a,b) to quantify the heterotrophic active biomass concentration present in a nitrification/denitrification (ND) activated sludge system. Ubisi et al. evaluated the batch test procedure by comparing theoretical values for heterotrophic active biomass predicted by the UCT steady state design and kinetic simulation models with values measured on two separate laboratory-scale ND systems operated at 12 and 20 day sludge ages respectively. In the case of the batch tests performed on the system operated at a 12 day sludge age, good agreement existed between the theoretical values and the values measured in the batch tests. Conversely the results from the batch tests performed on the system operated at 20 day sludge age exhibited poor agreement between the theoretical and measured values. Ubisi *et al.* were unable to identify any specific cause for these contradicting results and the uncertainty in the heterotrophic active biomass parameter largely remained.

The promise that the batch test method holds as a means of quantifying the heterotrophic active biomass stimulated an investigation to establish possible causes for the contradicting results. Some possibilities were:

- Deficiencies existed in the understanding and analysis of the batch test data, in particular the techniques used to separate the heterotrophic organisms' behavioral responses from other organisms and compounds in the total activated sludge mass.
- The mathematical expressions formulated for the batch test analysis could have been inadequate to simulate the organisms' behaviour under batch test conditions.
- The characteristics of the parent system activated sludge differed from the theoretical representations at different sludge ages. This possibility would suggest that the steady state design and kinetic simulation models could yield distorted predictions of the activated sludge composition at different sludge ages with a resultant limitation to the scope of applicability.
- The parent laboratory-scale system at 20 days sludge age operated by Ubisi *et al.* exhibited behaviour that deviated from the "normal" expected behaviour.

The point of departure for this investigation was to repeat a series of batch tests in accordance with the procedures followed by Ubisi *et al.*, as detailed in Chapter 3. This would establish whether the results of Ubisi *et al.* are reproducible, and whether their good correlation at 12 days sludge age was fortuitous. This chapter details the outcome of these batch tests and their comparison with the results obtained by Ubisi *et al.* In identifying possible causes for inconsistent results, the data would be rigorously analyzed to identify any procedural or conceptual deficiencies.

4.2 EXPERIMENTAL METHOD

4.2.1 Experimental approach

The experimental approach set out in Chapter 3 was as followed:

A well defined and controlled parent laboratory-scale nitrification/denitrification activated sludge system was operated at 10 days sludge age and continually monitored, see Chapter 2. Mixed liquor samples were harvested regularly from the parent system in various quantities and combined with unsettled municipal wastewater in a batch reactor under aerobic conditions. The oxygen utilization rate (OUR) response in each batch test was monitored with time and used to derive estimates for heterotrophic active biomass concentration. A parallel separate batch test was conducted on the wastewater, without mixed liquor addition, to quantify the heterotrophic active biomass concentration present in the wastewater itself. The difference in heterotrophic active biomass concentration between the batch tests with and without mixed liquor addition gives the heterotrophic active biomass concentration due to the mixed liquor addition only. The heterotrophic active biomass measurements were evaluated by comparing the results from the batch tests with the results calculated using the steady state design model (WRC, 1984).

4.2.2 Parent system

The parent laboratory-scale system and its operation have been described in detail in Chapter 2. To ensure steady state, the parent system was operated for more than 15 sludge ages (150 days) before the batch tests were commenced. A total number of 23 parallel batch tests were conducted during this investigation. The sewage was fed to the parent system in batches and the sewage batches was changed every ± 2 weeks to prevent degradation under storage. Each sewage batch constituted a steady state period and batch tests were conducted during 7 of these sewage batches. The sewage batches during which batch tests were conducted are set out in Table 4.1.

Table 4.1: The sewage batch number and the dates it was used as feed for the parent system. Also, the number of batch tests conducted with wastewater (WW) only and wastewater + mixed liquor (WW+ML) during each sewage batch.

Sewage batch no.	Dates (1999/2000)	Number of batch tests	
		WW only	WW + ML
11	27 Oct – 9 Nov	3	3
12	10 Nov – 19 Nov	1	1
13	20 Nov – 3 Dec	3	3
14	4 Dec – 14 Dec	1	1
18	29 Jan – 12 Feb	4	4
19	13 Feb – 25 Feb	5	5
20	26 Feb – 9 Mar	6	6
TOTAL		23	23

4.2.3 Batch tests

The batch test procedure set out in Chapter 3 was followed. In conducting the batch tests, the following modifications and observations were made:

- In the batch test method employed by Ubisi *et al.* (1997a,b) a 3ℓ volume of wastewater was used in the wastewater (WW) only batch test. This procedure was initially adopted for this investigation and was applied to the first four wastewater only batch tests (see batch tests W1–W4 in Table 4.4). As described in Chapter 3, section 3.2.2, it was decided to use the same volume of wastewater in both batch reactors and thus it could be assumed that each batch reactor contained the same mass of heterotrophic active organisms originating from the wastewater itself. This modification enabled a direct comparison between the wastewater (WW) only OUR response and the wastewater + mixed liquor (WW+ML) OUR response on the basis that the (WW+ML) batch test OUR response values in *excess* of the OUR response values measured in the parallel WW only batch test, would constitute the OUR response induced by the organisms present in the mixed liquor sample only. Hence, from batch test W5 (sewage batch 13) onwards, the same volume of wastewater required in the respective parallel WW+ML batch test (Table 4.6), was used in the WW only batch test. The remainder of the 3ℓ volume was supplemented by adding the required volume of filtered effluent, obtained from the system, as described in Chapter 3.

- To establish an estimate for the *wastewater* heterotrophic active biomass [$Z_{BH(0)}$(WW)] present at the start of each WW+ML batch test, Ubisi *et al.* used an *average* value calculated from a number of individual WW only batch tests conducted during a particular sewage batch period. When calculating the heterotrophic active biomass due to the mixed liquor addition only [$Z_{BH(0)}$(ML)], this *average* $Z_{BH(0)}$(WW) was subtracted from the total heterotrophic active biomass $Z_{BH(0)}$(WW+ML) measured in each WW+ML batch test conducted during that particular sewage batch. During this investigation it was observed that the $Z_{BH(0)}$(WW) value, measured for successive WW only batch tests conducted during a particular sewage batch period, appeared to increase towards the end of the ± 2 week period. This increase would indicate some growth of wastewater heterotrophic organisms during storage in the cold room at 4°C. To use an average $Z_{BH(0)}$(WW) value in the calculation of the $Z_{BH(0)}$(ML) value from each individual WW+ML batch test, would most likely overestimate the value of $Z_{BH(0)}$(WW) during the earlier part of a particular sewage batch and underestimate the value towards the latter part of the same sewage batch. To avoid distorted values for $Z_{BH(0)}$(ML), for this investigation the $Z_{BH(0)}$(WW) measured in each WW only batch test was applied directly in the calculation of $Z_{BH(0)}$(ML) measured in the parallel WW+ML batch test, conducted on the same day.

4.3. DATA ANALYSIS AND RESULTS

4.3.1 Parent system

As described earlier, the sewage that was fed to the parent system was changed every ± 2 weeks to prevent degradation under storage and to minimize changes in the nature of the wastewater. Although the intention was to keep the wastewater feed as consistent as possible throughout the investigation period, invariably the wastewater characteristics changed from one sewage batch to the next, resulting in different behavioral responses from the parent system for each sewage batch. Each sewage batch therefore constituted a steady state period during which the characteristics of the wastewater governed the parent system behaviour. For this reason, the approach adopted was to analyse each sewage batch separately to determine the various parent system parameters, as set out in Chapters 2 and 3. During the 7 sewage batch periods (Table 4.1) when batch tests were conducted for this investigation, the various parent system parameters were monitored and the averaged data (from Table 2.3.(a) and (b), Chapter 2) are listed in Table 4.2.

Table 4.2: Steady state results for parent laboratory-scale anoxic/aerobic activated sludge system receiving sewage batches during which batch tests were performed. For each sewage batch tested, the daily results have been averaged and the averages are listed with sample standard deviations in brackets.

PARENT ANOXIC/AEROBIC STEADY STATE SYSTEM													
WW Batch	No of Tests	COD (mg/ℓ)		TKN (mg/ℓ)		Nitrate			OUR mgO/l/h	Mixed liquor (mg/ℓ)			pH
		Inf	Eff	Inf	Eff	Anoxic	Aerobic	Eff		VSS	COD	TKN	
11	9	488 (29)	52 (12)	46 (1)	3.7 (0.2)	1.1 (0.7)	6.7 (1.3)	8.1 (1.5)	33.5 (0.7)	2419 (111)	3471 (230)	248 (9)	7.59 (0.09)
12	6	500 (42)	43 (9)	47 (2)	4.0 (0.9)	2.2 (1.5)	7.8 (2.9)	10.2 (2.4)	33.5 (1.9)	2293 (70)	3372 (121)	248 (33)	7.53 (0.03)
13	8	540 (25)	59 (14)	47 (1)	4.1 (0.3)	3.7 (0.5)	9.8 (0.8)	11.3 (0.9)	34.8 (0.7)	2469 (83)	3681 (148)	243 (6)	7.57 (0.03)
14	4	517 (25)	61 (12)	48 (1)	3.7 (0.4)	5.5 (1.2)	11.7 (0.8)	13.2 (0.5)	37.4 (2.1)	2227 (163)	3403 (185)	226 (7)	7.61 (0.06)
18	9	526 (37)	73 (10)	44 (4)	5.3 (0.5)	4.1 (0.9)	10.6 (1.7)	11.5 (1.2)	36.1 (1.7)	2387 (59)	3502 (118)	230 (7)	7.52 (0.06)
19	9	498 (22)	60 (7)	46 (1)	4.3 (0.4)	5.5 (2.6)	13.8 (2.8)	15.4 (2.6)	35.4 (1.2)	2400 (90)	3513 (103)	231 (7)	7.48 (0.05)
20	9	500 (10)	83 (8)	39 (1)	5.2 (0.5)	1.8 (2.1)	7.1 (3.9)	9.2 (2.4)	30.3 (1.4)	2202 (84)	3418 (109)	219 (4)	7.64 (0.13)

In accordance with the steady state design and kinetic simulation models, the heterotrophic active biomass performs an integral role in the behaviour of the activated sludge system. The theoretical mass of active heterotrophic organisms would invariably fluctuate as the wastewater characteristics change from one sewage batch to the next. As the heterotrophic active biomass forms the focal point of this investigation, it was imperative to monitor the fluctuation of key system parameters between sewage batches. To formulate a theoretical estimate for the heterotrophic

active biomass concentration present in the parent system during each sewage batch period, the following were determined from the data in Table 4.2:

- Influent wastewater unbiodegradable soluble and particulate COD fractions ($f_{S,us}$ and $f_{S,up}$, respectively); system COD and N mass balances (Ekama *et al*; 1986) and the COD and TKN to VSS ratios for the mixed liquor (f_{cv} and f_N, respectively). The calculation procedures are set out in detail in Chapter 2 and the results for each sewage batch are listed in Table 4.3.

- Following the procedure in Chapter 3, Section 3.4.1, the heterotrophic active biomass fractions of the mixed liquor organic suspended solids (f_{av}) were determined for each sewage batch using the steady state design model (Eq 3.21) and the UCTOLD kinetic simulation computer programme, see Table 4.3 below. Reasonably close agreement was obtained between the steady state and kinetically simulated predictions for f_{av} obtained for each sewage batch tested. The kinetically simulated predictions were persistently higher than the values for f_{av} estimated via the steady state approach (average difference = 2.2%). As noted in Chapter 3, section 3.4.1, the differences arise principally because (i) with the steady state design models, $f_{S,up}$ and f_{av} were determined from the measured mixed liquor VSS concentration, whereas with the kinetic simulation model, these were determined from the measured mixed liquor COD concentration, and (ii) the kinetic simulation models include autotrophic active biomass and non-utilized slowly biodegradable COD in the mixed liquor whereas these are not included in the steady state design model. Due to their near equality, the f_{av} from either the steady state design or kinetic simulation models could be used, but because of the simpler more direct analytical calculation procedure, the f_{av} values form the steady state design model were used.

Table 4.3 : Steady state COD and N mass balances, wastewater fractions and mixed liquor parameters for parent laboratory-scale anoxic/aerobic activated sludge system. Data calculated from data Table 4.2, either directly or using the steady state (SS) design (WRC, 1984) or kinetic simulation (sim.) models (Dold *et al.*, 1991).

PARENT ANOXIC/AEROBIC STEADY STATE SYSTEM										
WW Batch	No. of tests	Mass Balance (%)		Wastewater fractions			Mixed liquor			
		COD	N	Unbiod Soluble COD ($f_{S,us}$)	Unbio. Particulate COD ($f_{S,up}$)		COD/VSS ratio (mgN/mg VSS) (f_{cv})	TKN/VSS ratio (mgN/mg VSS) (f_n)	Active Fraction (f_{av})	
					SS Design	Kinetic Sim.			SS Design	Kinetic Sim.
11	9	94	90	0.0861	0.1434	0.1250	1.43	0.103	0.4244	0.4336
12	6	87	92	0.0740	0.0999	0.0790	1.47	0.108	0.4798	0.4906
13	8	88	93	0.0907	0.1053	0.0855	1.49	0.098	0.4618	0.4722
14	4	93	90	0.0909	0.0777	0.0560	1.53	0.100	0.4949	0.5063
18	9	93	101	0.0932	0.1027	0.0820	1.47	0.096	0.4732	0.4842
19	9	90	113	0.0884	0.1300	0.1110	1.46	0.096	0.4341	0.4436
20	9	90	106	0.0920	0.0882	0.0697	1.55	0.099	0.4696	0.4790

Having determined the active fraction (f_{av}) of the mixed liquor VSS concentration, the theoretical heterotrophic active biomass concentration present in the parent system can be calculated from the measured VSS values (Table 4.2) for each sewage batch. When applied to the batch tests, the theoretical heterotrophic active biomass concentration [$Z_{BH}(theor)_{BT}$] contained in the mixed liquor sample that was drawn from the parent system and added to the batch tests conducted during a particular sewage batch period, was calculated using Eq (3.22) (Chapter 3, section 3.4.1). The theoretical concentrations (Theoretical ML) calculated in this fashion for each WW+ML batch test are given in Table 4.6.

4.3.2 Batch tests

From Chapter 3, two types of batch tests were conducted, one type with wastewater only and the other type with wastewater and a sample of mixed liquor harvested from the parent laboratory-scale system.

4.3.2.1 *Wastewater only batch tests*

Twenty-three batch tests with wastewater only were conducted during seven sewage batch periods; sewage batches 11 to 14 and batches 18 to 20. The OUR versus time plots for these batch tests are set out in detail in Appendix A1 and the summarized results are given in Table 4.5 below. The procedures set out in Chapter 3, sections 3.3.1 and 3.3.4, were followed to determine the following:

- Because nitrate and nitrite were present in the filtered effluent added to batch tests W5 – W23, small concentrations of nitrate and nitrite were detected in this series of batch tests. The low concentrations remained unchanged for the duration of the batch tests, indicating the absence of nitrification and the inactivity of autotrophic biomass in the wastewater.

- %COD recoveries for the batch tests were calculated using Eq (3.1), see Table A1, Appendix A1. The summarized %COD recoveries are shown in Table 4.4 below. In general, it is accepted that %COD recoveries in the range 90% - 110% lend credibility to the reliability of the measurements and to the batch test procedure itself. A statistical plot of the %COD recovery for all the wastewater only batch tests was constructed; see Fig 4.1. From the statistical plot, the data are normally distributed indicating no single factor in the test had a dominating influence; the mean %COD recovery for the wastewater only batch tests was determined to be 93% with sample standard deviation of 8%. Although 9 of 23 batch tests yielded %COD recoveries $< 90\%$, most of these were only marginally below 90 %, with only 3 of 23 %COD recoveries below 85%.

- Although a number of batch test yielded %COD recoveries $< 90\%$, with the implication that these should be rejected for further analysis, in some instances the poor COD recoveries could be attributed to the method of harvesting the samples from the batch reactor at the end of each batch test. For batch tests W1 to W4 the samples were drawn using a 50 ml pipette and stabilized by adding two drops of mercuric chloride, before the COD tests were conducted. During each batch test a

small accumulation of particulate matter in the corners at the bottom of the batch reactor was observed. Through sampling by pipette it was impossible to obtain a representative sample containing the particulate matter at the bottom of the reactor (which contribute significantly towards the total COD). From batch test W5 onward, a sampling method was employed at the end of the batch test where the reactor was thoroughly brushed to dislodge any sludge deposits; the reactor was continuously aerated to increase the turbulence that kept all particulate matter in suspension; a 500 ml sample was siphoned into a separate container; the sample was then homogenized in a blender before the COD tests were conducted. A significant improvement in the %COD recovery was achieved through this sampling method, with batch tests W5 – W8 yielding an averaged %COD recovery of 102%. As an experiment, sampling by pipette was performed again at the end of batch test W12 which yielded the lowest %COD recovery of 75%.

- In spite of the fact that the %COD recovery is a function of the batch test method and should not be specific to each batch of wastewater tested, observations to the contrary suggested that compounds contained in the wastewater might have an influence on the %COD recovery. When conducting batch tests W9 – W13, a considerable amount of foam emanated from the solution during each aeration period and a distinct "soapy" fragrance was emitted. The %COD recoveries for these batch test were notably < 90% which suggests that possibly detergents present in the wastewater influence the results from COD tests. The observed OUR responses for these batch tests, however, show good correlation with the hypothesized response and the calculated average heterotrophic active biomass is in close agreement with the average value observed for all batch tests. This indicate that the detergents may not have a significant impact on the heterotrophic organisms' behaviour.

- The OUR values for the data up to the precipitous drop in OUR were plotted ℓnOUR versus time; linear regression was applied to the ℓnOUR-time data to determine the y-intercept, slope and correlation coefficient; the plots with linear regression data are given in Appendix A1. From the slopes and y-intercepts the wastewater heterotrophic active biomass concentrations at the start of the batch tests [$Z_{BH(0)}$(WW)] were determined using Eq.(3.9); see Appendix A1, Table A1. These values represent the heterotrophic active biomass concentration present in the wastewater and the results from all the batch tests are listed in Table 4.4 below.

- In general, the correlation coefficients (R^2) for the linear regression on the ℓnOUR–time data (see Appendix A1, Table A1) were very good; for the vast majority of the batch tests $R^2 > 0.93$, which indicates that the observed response conforms to the hypothesized behaviour. Only two batch tests yielded correlation coefficients < 0.90. These were batch tests W22 and W23 with $R^2 = 0.75$ and $R^2 = 0.84$ respectively. Although the results from these two batch tests should be rejected for further analysis, the poor R^2 values resulted from single data points that deviated significantly from the rest of the data points. From a visual observation, the majority of the data points conforms to the trend attained through linear regression. For this reason the results from above two batch tests would be included in further analysis.

Table 4.4 : Summary of batch test results with wastewater (WW) only : Batch test numbers, dates of batch tests, volumes added, COD recoveries, maximum specific growth rates, heterotrophic active biomass present in the batch test ($Z_{BH(0)}$) due to wastewater only (WW) and expressed as a fraction of the total COD of the wastewater at the start of the test (from Table 4.5).

BATCH TESTS : WASTEWATER (WW) ONLY									
Sew. Batch no.	Batch test no.	Date of test	Volume (ℓ)		COD recov. (%)	Max. specific growth rates (/d)		Measured $Z_{BH(0)}$ (mgCOD/ℓ)	
			FE	WW		K_{MP}	μ_H	Concentration (mgCOD/ℓ)	Fraction of total COD (%)
11	W1	29-10	-	3.00	92	3.0	4.8	19 *	3.7
	W2	03-11	-	3.00	91	3.5	5.3	14 *	2.8
	W3	05-11	-	3.00	88	3.6	4.3	28 *	6.0
12	W4	15-11	-	3.00	89	3.2	5.8	9 *	2.1
13	W5	30-11	0.20	2.80	109	3.9	4.3	30	7.4
	W6	01-12	0.10	2.90	101	4.5	4.6	27	6.0
	W7	03-12	0.25	2.75	99	4.4	4.7	31	7.5
14	W8	08-12	0.20	2.80	101	4.2	4.7	15	3.3
18	W9	07-02	0.20	2.80	84	3.4	4.1	16	3.4
	W10	08-02	0.25	2.75	86	2.1	7.0	6	1.4
	W11	09-02	0.30	2.70	83	3.1	4.0	18	4.0
	W12	10-02	0.15	2.85	75	3.3	3.2	30	7.1
19	W13	15-02	0.30	2.70	86	4.3	5.3	6	1.5
	W14	16-02	0.35	2.65	87	4.4	5.4	7	1.6
	W15	17-02	0.40	2.60	88	3.2	4.3	14	3.2
	W16	22-02	0.30	2.70	102	3.5	5.6	11	2.4
	W17	23-02	0.35	2.65	98	3.2	6.9	17	3.9
20	W18	29-02	0.25	2.75	93	3.9	5.8	6	1.3
	W19	01-03	0.20	2.80	96	3.5	6.3	6	1.3
	W20	02-03	0.15	2.85	100	3.6	6.1	6	1.4
	W21	06-03	0.30	2.70	96	2.7	4.9	13	2.9
	W22	07-03	0.35	2.65	97	2.2	5.5	19	4.6
	W23	08-03	0 40	2.60	102	2.0	6.6	17	4.1

* Original batch test method ; when compared to WW+ML batch tests, dilution must be taken into account (see Table 4.6) :

$$Z_{BH(0)}(WW)_D = Z_{BH(0)}(WW) \cdot V_{WW} / (V_{ML} + V_{WW})$$

- For the purpose of comparison with previous research work, for each WW only batch test the measured heterotrophic active biomass was expressed as a percentage of the total wastewater COD concentration, see Table 4.4. The respective % fraction of the total COD was calculated from the $Z_{BH(0)}(WW)$ and COD concentrations measured at the start of each test.

- For batch tests W1 – W4 the % fraction of total COD could be calculated directly. For the remainder of the batch tests, however, the total COD measured at the start of each batch test contained the COD contributions from both the wastewater itself and the filtered effluent that was added to the wastewater.

To obtain an estimate of the total COD concentration of the wastewater prior to adding the filtered effluent (initial COD_{WW}), due account was taken of the dilution with filtered effluent, as follows:

$$\text{initial } COD_{WW} = \frac{[(V_{WW} + V_{FE}) \cdot COD_{START}] - [V_{FE} \cdot COD_{FE}]}{V_{WW}} \qquad (4.1)$$

where

initial COD_{WW}	=	Initial COD of the wastewater, prior to dilution with filtered effluent (mgCOD/ℓ)
COD_{START}	=	Total (diluted) COD measured at the start of the batch test (mgCOD/ℓ)
COD_{FE}	=	Filtered effluent COD measured for the parent system on the same day that the batch test was performed - from Table C3, Appendix C (mgCOD/ℓ)
V_{WW}	=	Volume of wastewater used in batch test (ℓ)
V_{FE}	=	Volume of filtered effluent used in batch test (ℓ)

From the $Z_{BH(0)}(WW)$ values measured at the start of each batch test, an estimate for the initial heterotrophic active biomass mass present in the wastewater (prior to dilution with filtered effluent) could be obtained as follows:

$$\text{initial } Z_{BH}(WW) = Z_{BH(0)}(WW) \cdot \frac{(V_{WW} + V_{FE})}{V_{WW}} \qquad (4.2)$$

Where

initial $Z_{BH}(WW)$	=	wastewater heterotrophic active biomass concentration prior to dilution with filtered effluent in batch test (mgCOD/ℓ)
$Z_{BH(0)}(WW)$	=	measured heterotrophic active biomass concentration at the start of the wastewater only batch test (mgCOD/ℓ)
V_{WW}	=	Volume of wastewater used in batch test (ℓ)
V_{FE}	=	Volume of filtered effluent used in batch test (ℓ)

As an illustration, the calculated initial wastewater COD and heterotrophic active biomass concentrations together with the % fraction of the total COD calculated for each batch test is given in Table 4.5 below.

Table 4.5: Wastewater heterotrophic active biomass concentration (Z_{BH}) expressed as a percentage of the total wastewater COD for wastewater only batch tests W1 - W23, taking due consideration of dilution: Batch test numbers; measured $Z_{BH(0)}$; measured (diluted) COD at start of each batch test; volumes added, COD of filtered effluent from parent system (from Table C3, Appendix C); calculated initial COD_{WW} prior to dilution (Eq 4.1); calculated initial Z_{BH} in wastewater (Eq 4.2) and % fraction of total wastewater COD.

Sew. Batch No.	Batch Test No.	Measured (mgCOD/ℓ)		WW Vol. (V_{WW}) (ℓ)	Added FE* vol. (V_{FE}) (ℓ)	COD of added FE* (COD_{FE}) (mgCOD/ℓ)	Initial COD_{WW} (Sti) (mgCOD/ℓ)	Initial Z_{BH} in WW ($Z_{BH(WW)}$) (mgCOD/ℓ)	Z_{BH} % of Sti
		$Z_{BH(0)}$	COD start						
11	W1	19.062	512	3.00	-	-	512	19.062	3.72
	W2	13.929	494	3.00	-	-	494	13.929	2.82
	W3	27.914	467	3.00	-	-	467	27.914	5.98
12	W4	8.946	428	3.00	-	-	428	8.946	2.09
13	W5	29.641	401	2.80	0.2	29	428	31.758	7.43
	W6	26.749	450	2.90	0.10	47	464	27.671	5.96
	W7	30.940	417	2.75	0.25	49	450	33.753	7.49
14	W8	14.556	450	2.80	0.20	49	479	15.596	3.26
18	W9	15.611	459	2.80	0.20	37	489	16.726	3.42
	W10	6.300	467	2.75	0.25	57	504	6.873	1.36
	W11	18.341	461	2.70	0.30	39	508	20.379	4.01
	W12	30.424	429	2.85	0.15	39	450	32.025	7.12
19	W13	6.191	421	2.70	0.30	41	463	6.879	1.49
	W14	6.709	429	2.65	0.35	53	479	7.595	1.59
	W15	13.526	435	2.60	0.40	53	494	15.607	3.16
	W16	10.668	449	2.70	0.30	29	496	11.853	2.39
	W17	16.758	430	2.65	0.35	41	481	18.971	3.94
20	W18	5.470	447	2.75	0.25	47	483	6.076	1.26
	W19	6.049	459	2.80	0.20	47	488	6.481	1.33
	W20	6.165	455	2.85	0.15	51	476	6.489	1.36
	W21	12.735	444	2.70	0.30	47	488	14.150	2.90
	W22	19.193	423	2.65	0.35	47	473	21.728	4.60
	W23	16.907	414	2.60	0.40	45	471	19.508	4.14

FE* - filtered effluent from parent system

- A statistical plot of the calculated wastewater heterotrophic active biomass as a percentage of the total wastewater COD for the different WW only batch tests is given in Fig. 4.2. For the wastewater tested (Mitchell's Plain, Cape Town), the heterotrophic active biomass ranged from 1.3 to 7.4 % of the total COD, with a mean of 3.6 % and sample standard deviation of 2.0 %.

- This mean agrees reasonably closely with the values measured by Ubisi *et al.* (1997a,b) and Mbewe *et al.* (1995) of 4.5 % and 6.1 %, respectively for the same wastewater. From the statistical plot (Fig 4.2) it is evident that there is a clear deviation from the true normal distribution with a number of batch tests (6) yielding values between 1 and 2 %. This underlines that fact that a relatively low heterotrophic active biomass concentration for Mitchell's Plain wastewater is as expected; this wastewater is primarily of domestic origin with a small industrial component (< 20 %) and the sewers are anaerobic and have a relatively short retention time (4-6 hours).

- As noted above (section 4.2.3), the heterotrophic active biomass concentration in the wastewater tended to increase as the storage time for the wastewater increased (Table 4.5). This indicated some growth of heterotrophic active biomass under sewage storage. Although the relative increase is significant and was taken into account in analysis of the batch test data, the absolute increase is small and will not affect the response of the parent system significantly.

4.3.2.2 *Wastewater and mixed liquor batch tests*

Twenty-three batch tests with wastewater and mixed liquor (WW+ML) were conducted in parallel with the wastewater only batch tests during the seven sewage batch periods; 11 to 14 and 18 to 20. The OUR versus time plots for these batch tests are set out in detail in Appendix A2. The procedures set out in Chapter 3, section 3.3.2 and 3.3.3, were followed to determine the following:

- Since the mixed liquor was drawn from a nitrifying activated sludge system and added to the batch test, nitrification was expected and was indeed observed. The nitrite concentrations observed were negligible. Using Eq (3.12), the OUR for nitrification (OUR_N) was determined from the nitrate concentration-time profiles given in Appendix A2. Linear regression was applied to the profiles to determine the slope and correlation coefficient (R^2). Ubisi *et al.*(1997a,b) observed two linear rates of nitrification from the nitrate concentration - time profiles; an initial slower linear rate that was maintained approximately until the precipitous drop in OUR occurred, followed by a faster linear rate that remained until the end of the batch test. The two district linear rates suggested that the presence of RBCOD in the solution may have inhibited nitrification. The two linear nitrification rates, observed by Ubisi *et al*, were indeed observed during this investigation, but was only notably evident in three of the batch tests; M11, M14 and M15, and to a lesser extent in one batch test, M3. The rare occurrence of this trend can be attributed to the fact that the batch tests conducted during this investigation were not continued for a long period after the precipitous drop in OUR occurred. Hence for this investigation, the nitrification rate was accepted to remain constant for the duration of all the batch test (including M11, M14 and M15) and was approximated by a "best fit" straight line through the nitrate concentration - time data points. As expected, the nitrification rates were closely linked to the volume of mixed liquor added to the batch test; the more mixed liquor added (with more nitrifier organism mass), the higher the observed nitrification rate.

- For all the batch tests, the OUR for heterotrophic growth ($OUR_{H(t)}$) was determined by subtracting the appropriate (constant) nitrification OUR (OUR_N) from the measured OUR (Eq 3.11).

- Having determined $OUR_{H(t)}$ for the batch tests, the %COD recoveries were calculated using Eq (3.1) and the results are listed in Table A2, Appendix A2. The %COD recovery for each batch test is summarized in Table 4.6 below. In general, better COD recoveries were achieved compared with the wastewater only batch tests ; all the WW+ML batch tests yielded %COD recoveries in the range 90 - 110 %. A statistical plot of %COD recovery for all the WW+ML batch tests was constructed; see Fig 4.3. From the statistical plot the data are normally distributed indicating that no single factor dominated the response; the mean %COD recovery was 98.0 % with sample standard deviation 4.6 %. The good %COD recoveries lend credibility to the reliability of the measurements and to the batch test procedure.

- For each batch test, the $OUR_{H(t)}$ values up to the precipitous drop in OUR were plotted $\ell n\ OUR_H$ versus time; plots are given in Appendix A2. Linear regression was applied to the ℓnOUR-time data to determine the y-intercept, slope and correlation coefficient (R^2); these are listed in Table A2, Appendix A2. The correlation coefficients (R^2) were reasonable, with the majority of values > 0.9, but were generally lower than the R^2 values achieved for the wastewater only batch tests. In general, lower R^2 values were observed with increasing volumes of mixed liquor added to the batch tests. When a large volume of mixed liquor was added in the batch test, RBCOD was consumed more rapidly and the precipitous drop in OUR was reached sooner than when a small volume of mixed liquor was added. As a result, less data were recorded before the precipitous drop in OUR and, accordingly, less data were available for the regression analysis of the ℓnOUR-time plot, resulting in lower R^2 values. It is also important to note that no data points were omitted from the ℓnOUR-time plots. In some instances single data points deviated visibly from the distinct linear trend displayed by the rest of the data points, resulting in low R^2 values. For the sake of completeness, however, these data points were not omitted from the analysis.

- From the y-intercepts and slopes, the heterotrophic active biomass concentration due to the mixed liquor addition, and that present in the wastewater [$Z_{BH(0)}$(WW+ML)] at the start of each WW+ML batch test was calculated using Eq (3.9); see Table A2, Appendix A2.

- The measured heterotrophic active biomass concentration in each batch test due to the added mixed liquor $Z_{BH(0)}$(ML) was calculated by subtracting the heterotrophic active biomass concentration present in the wastewater [$Z_{BH(0)}$(WW)], measured in the parallel WW only batch test and taking due account of the dilution (Eq 3.14) for batch tests W1 – W4, from the total measured heterotrophic active biomass concentration [$Z_{BH(0)}$(WW+ML)]. This is illustrated in the summary of all batch tests given in Table 4.6 below.

Table 4.6 : Summary of results from batch tests with a mixture of wastewater (WW) and mixed liquor (ML) drawn from parent laboratory-scale system : Batch test numbers, dates of batch tests, volumes added, COD recoveries, maximum specific growth rates, heterotrophic active biomass present in the batch test ($Z_{BH(0)}$) due to mixed liquor + wastewater (ML+WW), wastewater only (WW) taking due account of dilution for batch tests W1-W4, and mixed liquor. Also shown are the values for the theoretical heterotrophic active biomass due to the mixed liquor addition.

BATCH TESTS : WASTEWATER (WW) + MIXED LIQUOR (ML)											
Sew. Batch no.	Batch test no.	Date of test	Volume (ℓ)		COD recov (%)	Max. specific growth rates (/d)		$Z_{BH(0)}$ (mgCOD/ℓ)			
								Measured			Theor.
			ML	WW		K_{MP}	μ_H	ML+WW	WW	ML	ML
11	M1	29-10	0.10	2.90	97	3.2	4.6	26	18	8	49
	M2	03-11	0.15	2.85	99	3.7	4.1	29	13	16	74
	M3	05-11	0.30	2.70	95	3.3	1.6	101	25	76	147
12	M4	15-11	0.30	2.70	100	2.8	1.8	83	8	75	162
13	M5	30-11	0.20	2.80	102	3.4	2.0	90	30	60	113
	M6	01-12	0.10	2.90	90	5.1	4.2	33	27	7	57
	M7	03-12	0.25	2.75	91	3.7	3.0	72	31	41	142
14	M8	08-12	0.20	2.80	93	3.9	2.9	37	15	22	113
18	M9	07-02	0.20	2.80	103	4.1	3.6	30	16	14	110
	M10	08-02	0.25	2.75	106	3.2	2.2	53	6	46	138
	M11	09-02	0.30	2.70	101	3.6	2.6	57	18	38	166
	M12	10-02	0.15	2.85	100	3.7	3.1	43	30	12	83
19	M13	15-02	0.30	2.70	103	3.4	3.0	37	6	31	153
	M14	16-02	0.35	2.65	95	2.9	1.8	85	7	79	178
	M15	17-02	0.40	2.60	99	3.0	1.5	102	14	89	203
	M16	22-02	0.30	2.70	94	2.7	2.0	71	11	61	153
	M17	23-02	0.35	2.65	99	2.7	2.5	76	17	59	178
20	M18	29-02	0.25	2.75	94	3.1	2.7	38	6	33	134
	M19	01-03	0.20	2.80	97	3.2	3.5	28	6	22	107
	M20	02-03	0.15	2.85	91	2.4	3.4	29	6	23	80
	M21	06-03	0.30	2.70	97	1.4	1.6	132	13	119	161
	M22	07-03	0.35	2.65	100	2.4	3.1	79	19	60	187
	M23	08-03	0.40	2.60	108	2.2	2.2	129	17	112	214

4.3.3 Comparison between measured and theoretical heterotrophic active mass

In Table 4.6 the *measured* heterotrophic active biomass concentration at the start of each WW+ML batch test is compared with the *theoretical* heterotrophic biomass concentration contained in the appropriate mixed liquor sample drawn from the parent system, predicted via the steady state design model.

To illustrate the comparison, the measured versus theoretical mixed liquor heterotrophic active biomass data for all the sewage batches are shown plotted in Fig 4.4. From Fig 4.4 it is evident that very poor correspondence between the measured and theoretical mixed liquor active biomass was obtained. The measured values were consistently lower and the comparison shows remarkable resemblance with the comparison obtained by Ubisi *et al* (1997a,b) for batch tests conducted on a parent system operated at 20 days' sludge age (see Fig 3.10). The comparison is furthermore almost identical to the comparison obtained by Ubisi *et al.* for batch tests conducted on a parent system operated at 12 days' sludge age; during the period when the parent system in question received sewage batch no. 19 (see Fig 3.9).

These results, obtained from batch tests conducted on independent parent systems, clearly demonstrate that the batch test method (in its present format) cannot serve as a reliable means of measuring the heterotrophic active biomass present in activated sludge systems. However, the undoubted promise that the batch test concept holds, ***stimulated a thorough scrutiny of the present batch test method and the mathematical formulations applied in the analysis of recorded data.***

4.4 EXTENDED EVALUATION OF BATCH TEST RESULTS

The poor correlation between the theoretically predicted values and the measured batch test values obtained in this investigation substantiated the perception from the work by Ubisi *et al.* (1997a,b), that the batch test method in its present format was unable to yield reliable estimations of the heterotrophic active biomass present in activated sludge systems. The poor correlations obtained in the various investigations could be due to deficiencies in either the concepts on which the theoretical models are based, or the batch test method itself. With regard to the theoretical models, these were used to calculate the theoretical values for heterotrophic active biomass concentrations. In evaluating whether the models give theoretical heterotrophic active biomass concentrations that are in error, it was noted that:

- On examining the measured versus theoretical values for the three independent systems operated at 10, 12 and 20 day sludge ages, it is clear that a remarkable similarity exists between the measured and theoretical results; although the measured values are lower, they are consistently only about half the theoretical values.

- The theoretical models have been applied to successfully predict the behaviour of a wide variety of system configurations operated under a range of conditions (e.g. Dold *et al.*, 1991).

The consistent (though erroneous) correlation between the batch test measured and theoretical heterotrophic active biomass concentrations obtained in the different investigations, and the reliable application of the models indicated that most likely the deficiency lay in the batch test method, either in the method itself or in the techniques applied in analysing the batch test data. Thus, it was concluded that the batch test method should be evaluated in more detail, to investigate possible causes for the deviation of measured heterotrophic active biomass from the theoretically predicted values.

4.4.1 Differentiation between wastewater and mixed liquor heterotrophic organisms

From an examination of Fig 4.4, showing the comparison between the theoretical and measured heterotrophic active biomass, it was noted that the theoretical values were approximately 4 – 6 times the measured values when small samples of mixed liquor was used in the WW+ML batch tests. In contrast, when larger samples of mixed liquor was used, the theoretical values were approximately 2 times the measured values. From this observation it was proposed that the heterotrophic biomass present in the wastewater [$Z_{BH}(WW)$] added to the WW+ML batch tests was dominating the OUR response, and that as the concentration of mixed liquor heterotrophic active organisms was increased (by adding larger mixed liquor samples) this dominance diminished. It was postulated further that the characteristic behaviour of the wastewater heterotrophic organisms differed substantially from the behaviour of the mixed liquor heterotrophic organisms, so the behavioural response of the mixed liquor heterotrophic active organisms could not be reliably abstracted from the observed behaviour using the procedures above. Accordingly, these proposals were investigated further.

4.4.2 Evaluation of measured maximum specific growth rates

The proposal that the characteristic behaviour of the wastewater heterotrophic organisms is significantly different from the mixed liquor organisms' behaviour, appeared to be substantiated when comparing the heterotrophic active organism maximum specific growth rate on RBCOD (μ_H), measured in the WW only batch tests, with the μ_H measured in the WW+ML batch tests.

As described in Chapter 3, section 3.3.4, the values for K_{MP} (maximum specific growth rate on SBCOD) and μ_H can be calculated from the $OUR_{H(t)}$ response using Eq (3.18) and Eq (3.19) respectively. Accordingly, the growth rates K_{MP} and μ_H were calculated for all the wastewater only batch tests and are listed in Table 4.4 above. Similarly, K_{MP} and μ_H were calculated for all the wastewater plus mixed liquor batch tests and are listed in Table 4.6 above.

- In terms of K_{MP}, the values for the two types of batch tests did not differ significantly at the 95% confidence interval: The wastewater only batch tests gave a mean K_{MP} of 3.4/d with sample standard deviation of 0.7/d, while the wastewater plus mixed liquor batch tests gave a mean K_{MP} of 3.2/d with sample standard deviation of 0.8/d. Both values are, however, significantly higher than the value accepted for the UCT kinetic model (K_{MP} = 1.35/d).

- A comparison between the μ_H values measured in the WW only batch tests and the μ_H values measured in the WW+ML batch tests is shown plotted in Fig 4.5; significantly different mean values and trends are evident. The μ_H values measured in the WW+ML batch tests displayed a clear decreasing trend with increased volumes of mixed liquor added to the batch test and gave a mean maximum specific growth rate of 2.7/d, with sample standard deviation of 0.9/d. In contrast, the μ_H values measured in all the WW only batch tests remained fairly constant. From a statistical plot of the μ_H values measured in the WW only batch

tests (Fig 4.6), the μ_H values were normally distributed (i.e. no single factor had a dominating influence on the WW only μ_H values), giving a higher mean growth rate of 5.2/d and sample standard deviation of 1.0/d.

- That the mixed liquor (ML) heterotrophic organisms exhibited a considerably lower growth rate on RBCOD, should have far-reaching implications on the interpretation of the OUR responses recorded in the WW+ML batch tests. When a small volume of mixed liquor (say 0,1ℓ) is added to the wastewater, the mass of heterotrophic organisms added more or less equals the mass of wastewater heterotrophic organisms. However, the ML heterotrophic organisms' low growth rate is dominated by the wastewater organisms' high growth rate and this would be reflected in the OUR response.

- This effect was indeed observed where the $OUR_{H(t)}$ response was used to determine the value of μ_H. From Fig 4.5 it is evident that the WW+ML batch tests conducted with 0.1ℓ mixed liquor samples yielded almost exactly the same value for μ_H as the parallel batch tests conducted with wastewater only.

- In the analysis of the $OUR_{H(t)}$ response to calculate the mass of heterotrophic active organisms present in the WW+ML batch test, no differentiation is made between organisms that exhibit higher or lower growth rates; the $OUR_{H(t)}$ response is merely indicative of <u>single</u> "composite" population of heterotrophic organisms exhibiting a single "composite" growth rate. As described in Chapter 3, section 3.3.1, the observed growth rate dictates the slope of the straight line fitted to the $\ell nOUR_{H(t)}$-time values plotted in Fig 3.7. In turn, the measured slope of the $\ell nOUR_{H(t)}$-time plot impacts on the measurement of the heterotrophic active biomass, given by Eq (3.9):

$$Z_{BH(0)} = \frac{(e^{\text{y-intercept}}) \cdot 24}{\frac{1-Y_{ZH}}{Y_{ZH}} \cdot (\text{slope} \cdot 24 + b_H)} \quad \text{(mgCOD/l)}$$

It is clear that a lower overall growth rate ($K_{MP} + \mu_H$) would give a lower value for the slope in the $\ell nOUR_{H(t)}$-time plot. A lower value for the slope would increase the measured $Z_{BH(0)}$ value in Eq (3.9). Conversely, a higher overall growth rate would result in a decrease in $Z_{BH(0)}$, given that the same y-intercept is observed in both instances.

- With the mixture of WW+ML exhibiting approximately the same "composite" growth rate (and therefore the same slope for the ℓnOUR- plot) as that observed in the WW only batch test, the capacity of the batch test analysis to detect a significant mass of ML heterotrophic active organisms, is limited to the difference in y-intercepts (Eq (3.9)). With small samples of mixed liquor added to the WW+ML batch tests, the observed differences between the y-intercept values recorded for the WW+ML batch tests and that recorded for the WW only batch tests were small, giving $Z_{BH(0)}$ (ML+WW) values only marginally greater than the $Z_{BH(0)}$ (WW) values. This explains the poor correlation between the theoretical

and measured active biomass values (Fig 4.4) when small samples of mixed liquor were used in the WW+ML batch tests.

- When large samples of mixed liquor (say 0.4ℓ) were added in the WW+ML batch test, the mass of ML heterotrophic organisms was sufficient to reduce the "composite" growth rate to a value of approximately 2.0/d (see Fig 4.5). Although the impact on the $OUR_{H(t)}$ response was significant enough to detect a measured ML active heterotrophic biomass of about half the theoretical value (Fig 4.4), it can be assumed that the influence of the high growth rate exhibited by the wastewater heterotrophic organisms was appreciable in determining the $OUR_{H(t)}$ response.

4.4.3 Analytical separation of $OUR_{H(t)}$ responses

To portray the difference between OUR responses obtained from batch tests with wastewater and mixed liquor (WW+ML) and wastewater (WW) only batch tests, the OUR responses were plotted on the same graph. The measured $OUR_{H(t)}$–time responses were plotted up to the time when the precipitous drop in OUR occurred in each of the separate WW+ML and WW only batch test. As an illustration, the $OUR_{H(t)}$-time responses for the wastewater only batch test W8 and the WW+ML batch test M8 (From Fig 3.2 and Fig 3.6, respectively), are shown plotted in Fig 4.7.

Because the same volume of wastewater and final batch test volume were used in the parallel batch tests (excluding batch tests M1 – M4 and W1 – W4), it would be reasonable to assume that each of the parallel batch reactors contained the same concentration of wastewater heterotrophic active organisms. On this basis it would be plausible to accept that the *difference* in OUR values between the WW+ML OUR response and the WW only OUR response would represent the OUR response due to the mixed liquor (ML) heterotrophic active organisms. Because the OUR data points were recorded in two separate batch tests, the time increments between data points did not correspond; a direct subtraction was therefore not possible. To overcome this difficulty, the following procedure was followed:

(i) Exponential regression was applied to each data set to obtain the "best fit" exponential expression that closely approximated the OUR response measured in each batch test. Fig 4.7 shows the "best fit" exponential expressions and correlation coefficients (R^2) obtained for the WW+ML OUR response and the WW only OUR response.

(ii) Time intervals were appropriately chosen (0.1h in Fig 4.7) and the "approximated" values for the WW+ML and WW only OUR responses were calculated using the exponential expressions shown in Fig 4.7.

(iii) For each time interval, the "approximated" value for the ML only OUR response was calculated by subtracting the "approximated" WW only OUR value from the "approximated" WW+ML OUR value.

(iv) The series of "approximated" ML only values, calculated in this fashion, is shown in Fig 4.7 and represents an approximated ML only $OUR_{H(t)}$ response.

From Fig 4.7 the postulated low growth rate of the ML heterotrophic organisms reflects in the approximated ML only OUR response. Considering that the heterotrophic organism population present in the wastewater and the population present in the mixed liquor sample have evolved from completely different environments, the marked difference in growth rate is expected. Wentzel *et al* (1995) and Mbewe *et al* (1995) hypothesized that the two populations would exhibit different kinetic characteristics: The heterotrophic organism population that develops in the activated sludge system is likely to differ appreciably from that which develops in the wastewater, since the conditions present in the wastewater (high COD, low active mass) differ significantly from those in the activated sludge system (low COD, high active biomass).

- In Fig 4.8, the approximated ML $OUR_{H(t)}$ values were plotted $\ell nOUR_{H(t)}$ versus time. Linear regression was applied to the ℓnOUR-time data to determine the y-intercept, slope and correlation coefficient. From the slope and y-intercept, the heterotrophic active biomass at the start of the batch test, due to the mixed liquor addition only [$Z_{BH(0)}(ML)^*$], was calculated using Eq (3.9). For this specific batch test (M8) the $Z_{BH(0)}(ML)^*$ value = 45mgCOD/ℓ, calculated via the analytical separation procedure described above, was more than double the value obtained from the original batch test analytical procedure; $Z_{BH(0)}(ML)$ = 22 mgCOD/ℓ (Table 4.6). Although the $Z_{BH(0)}(ML)^*$ was still significantly smaller than the theoretical value of 113mgCOD/ℓ, the analytical separation technique provided promising results and accordingly was applied to all the batch tests and detailed profiles obtained via this technique are given in Appendix A3.

- From the analytically separated profiles (Appendix A3) and the analytical technique above, the approximated heterotrophic active biomass concentrations due to the mixed liquor addition only [$Z_{BH(0)}(ML)^*$] were calculated for all batch tests (excluding batch tests M1 – M4) and the results are listed in Table A3, Appendix A3. The approximated $Z_{BH(0)}(ML)^*$ values versus the theoretical values are shown plotted in Fig 4.9. Most of the analytically separated values were approximately double the values measured for the ML heterotrophic active biomass using the original batch test analytical procedure, but were still significantly less than the theoretical values. When larger samples of mixed liquor were used in the WW+ML batch tests, the analytically separated values were also approximately double the values measured in the original batch tests, with the difference that the analytically separated values now compared favourably with the theoretical values.

At this point it is important to note that the analytical separation technique may not be fundamentally satisfactory for direct analytical purposes, but served as a useful tool to illustrate the fact that the heterotrophic organism populations that had evolved from two different environments, displayed significantly different growth rates and OUR responses. The analytical separation technique also exposed the deficiency of the present batch test method as a reliable means to quantify the true mass of heterotrophic active organisms present in the batch test.

It was concluded that a new approach was necessary to resolve the difficulty that the wastewater heterotrophic organisms mask the OUR response of the heterotrophic organisms added with the mixed liquor. The development of a modified batch test procedure to physically eliminate the OUR contribution of the wastewater heterotrophic organisms, is described in more detail in Chapter 5.

4.5 EVALUATION OF NITRIFICATION ANALYSIS

In the present batch test method developed by Ubisi *et al.* (1997a,b), the nitrification rate is estimated by fitting a straight line to the recorded nitrate concentration - time data, as described in Chapter 3, section 3.3.2. The slope of the "best fit" straight line gives a constant nitrification rate ($\Delta NO_3^-/\Delta t$), from which the constant oxygen demand due to nitrification (OUR_N) is calculated using Eq (3.12).

$$(OUR_N) \quad = \quad 4{,}57 \cdot \text{slope} \qquad (\text{mgO}/\ell/\text{h})$$

The linear interpretation of the nitrate concentration - time data was ascribed to the relatively low yield and maximum specific growth rate of the nitrifiers [Wentzel *et al* (1995) and Mbewe *et al* (1995)], and appeared to be justified within the time scale of the batch tests. The linearity of the nitrate concentration-time data up to the point where the precipitous drop in OUR occurred, was confirmed during the initial batch tests conducted by Ubisi *et al.* After Ubisi *et al.* extended the nitrate sampling period, to include the period subsequent to the precipitous drop in OUR, a number of the nitrate concentration-time plots did not exhibit a single linear profile. Instead, these plots indicated that two linear nitrification rates could be identified, one linear rate being operative up to the precipitous in OUR and a second faster linear rate being operative from the precipitous drop in OUR to the end of the batch test.

During this investigation the two distinct linear rates of nitrification were indeed observed during some of the batch tests conducted with wastewater and mixed liquor, but this was limited to three batch tests (M11, M14 and M15 - see Appendix A2). The infrequent observation of the two linear nitrification rates could have been ascribed to the short duration of batch tests conducted during this investigation - most batch tests were discontinued within two hours after the precipitous drop in OUR occurred. However, the faster second rate of nitrification would induce a significantly higher OUR_N, which could have a substantial impact on the measurement of the heterotrophic active biomass present in the batch test. The possibility that the adopted (single) linear interpretation of the nitrate concentration-time data could have introduced error into the approximations of the actual nitrification rate, stimulated an investigation into the nitrification approach.

4.5.1 Recent developments in nitrification kinetics

In investigating and evaluating approaches for determining the maximum specific growth rate of nitrifiers, Antoniou *et al* (1990) and Sözen *et al* (1996) observed that during batch tests conducted on domestic sewage, the oxidized nitrogen concentration (Nitrate plus nitrite) displayed a distinct exponential increase with time. Antoniou *et al.* (1990) derived the following basic exponential equation to describe the nitrification kinetics observed under batch test conditions:

$$S_{NO}(t) = \frac{\mu_A X_A(0)}{Y_A(\mu_A - b_A)} e^{(\mu_A - b_A)t} - \frac{\mu_A X_A(0)}{Y_A(\mu_A - b_A)} + S_{NO}(0) \quad (4.3)$$

where

$S_{NO}(t)$ = oxidized nitrogen concentration at time t
$X_A(0)$ = autotrophic biomass concentration at time zero
μ_A = maximum specific growth rate
b_A = specific decay rate
Y_A = overall yield coefficient of autotrophic biomass
$S_{NO}(0)$ = initial oxidized nitrogen concentration at time zero

Antoniou *et al.* argued that, "with the exception of an initial start up time, the sum of the second and third terms in the right hand side of Eq (4.3) will be negligible, since they are constant and of opposite sign, while the first term increases exponentially". Consequently, with the exception of a limited period during the initial stages of the batch test when the second and third terms in Eq (4.3) have an influence on the nitrification rate, Antoniou *et al.* accepted that the nitrification rate would be dominated by the first term in Eq (4.3), i.e.

$$S_{NO}(t) = \frac{\mu_A X_A(0)}{Y_A(\mu_A - b_A)} e^{(\mu_A - b_A)t} \quad (4.4)$$

With the aim to simplify the analysis, Antoniou *et al.* took the natural logs in Eq (4.4) to obtain a linear expression:

$$\ell n(S_{NO}) = \ell n \left[\frac{\mu_A X_A(0)}{Y_A(\mu_A - b_A)} \right] + (\mu_A - b_A)t \quad (4.5)$$

In terms of the linear interpretation, the maximum specific growth rate in nitrification (μ_A) can be estimated by determining the slope [($\mu_A - b_A$) in Eq 4.5] from a $\ell n(S_{NO})$ - time profile straight line fit and making a reasonable estimate of b_A.

Sözen *et al.* (1996), however, dismissed the existence of an initial "start up" time and developed a non-linear "curve fitting" approach to provide a more reliable assessment of the ($\mu_A - b_A$) value. Sözen *et al.* observed an excellent agreement between the theoretical S_{NO} profile [Eq (4.3)] and the measured S_{NO} response for the entire duration of a number of similar batch tests. The observed behaviour during the initial stages of the batch tests contested the assumption by Antoniou *et al.* that a slower initial rate of nitrification could be attributed to a period of adaptation when the nitrifier organisms, harvested from a parent activated sludge system, acclimatized to the batch reactor environment.

Sözen *et al.* argued that the adaptation period is not likely, since the batch tests were started with biomass fully acclimatized and sustained in a continuously fed, batch fill and draw parent system operated at a sludge age of 10 days and fed the same wastewater used in the batch tests. Sözen *et al.* concluded that the initial biomass concentration, X_A, significantly affected the experimental results and therefore the dismissal of the second and third terms in Eq 4.3 could not be justified. Both Antoniou *et al.* and Sözen *et al.* observed a deviation from the expected S_{NO}-time profile towards the latter stages of batch tests conducted over long periods of time (4 – 7 days), where lower S_{NO} values indicated lower nitrification rates. This occurrence was attributed to substrate limitation resulting from the depletion of ammonia nitrogen, and possibly the inhibitory or toxic effects that some of the wastewater compounds have on nitrification.

In summarizing, it is evident that both Antoniou *et al.* and Sözen *et al.* observed a distinct exponential increase in the oxidized nitrogen concentration with time for batch tests conducted with a mixture of domestic sewage and mixed liquor harvested from a nitrifying activated sludge system. Although the two research groups disputed the existence of an initial "start up" period and the interpretation of results recorded during this period to obtain a reliable value of μ_A, both groups were in concurrence that a deviation from the true exponential trend, due to substrate limitation or toxin accumulation, can be expected towards the latter stages of batch tests conducted over a long period of time.

4.5.2 Application to this investigation

A closer inspection of the nitrate concentration-time data recorded during the wastewater plus mixed liquor (WW+ML) batch tests conducted during this investigation, revealed that the observed trend was more an exponential increase than a linear increase. To ascertain whether the observed exponential increase in nitrate concentration would result in a significant increase in the oxygen demand for nitrification, which would ultimately impact on the measurement of heterotrophic active biomass, it was decided to apply non-linear regression to the observed data for each batch test to obtain the most appropriate exponential expression describing the observed increase in nitrate concentration with time. A typical example of the exponential curve obtained in this way is shown in Fig 4.10 for batch test M16, conducted with a 0,3ℓ mixed liquor sample during sewage batch 19. In establishing the most appropriate exponential expression, due consideration was given to the following:

- The nitrite concentrations observed were negligible. It was deemed acceptable, therefore, to consider the measured nitrate concentrations to be of the same value as the oxidized nitrogen concentration (S_{NO}) defined by Eq (4.3).

- It was observed for a number of batch tests that the initial rate of nitrification was visibly lower compared to the trend observed for the majority of the data points. This observation would appear to substantiate the existence of the initial "start up" period postulated by Antonio *et al.* (1990). In the instances where the presumed "start up" period was evident, the inconsistent data points were disregarded in the establishment of a "best fit" curve (see Fig 4.10).

- During the latter stages of several batch tests the recorded data showed a tendency to deviate from the exponential trend observed during the greater part of the batch test. The lower rates of nitrification observed towards the end of the batch tests were attributed to substrate limitation and/or toxin accumulation which inhibited the nitrification capacity of the nitrifiers. Where necessary, these lower data points were disregarded in the construction of the "best fit" exponential curve, as shown in Fig 4.10. In general, the lower rates of nitrification occurred subsequent to the precipitous drop in OUR and the exclusion of the data points in question did result in a closer approximation of the observed nitrification rates during the important period preceding the precipitous drop in OUR.

- Because a measure of uncertainty existed in determining accurate estimates for the initial nitrate concentration (S_{NO}) and autotrophic biomass concentration ($X_A(0)$) at the start of each batch test, a general exponential expression was used to approximate the measured nitrate concentrations. For example, the general expression obtained in Fig 4.10 was

$$S_{NO} = 2{,}0059\ e^{0{,}1059t}$$

  In the general expression, no provision is made for the second and third terms contained in Eq (4.3). However, although the general exponential expression may not be mathematically correct, it provided an accurate approximation of the measured values. For the purpose of estimating the nitrification oxygen demand based on the observed increase in nitrate concentrations, the general exponential expression was accepted to be satisfactory.

4.5.3 Influence on the measurement of heterotrophic active biomass

To illustrate the influence of increasing nitrification rates (as opposed to the constant nitrification rate assumed in the present batch test method) on the measurement of heterotrophic active biomass, the results from WW+ML batch test M18 will be re-evaluated. To aid with the interpretation of the calculation procedure, the values of key parameters derived from this specific batch test are listed in Table 4.7:

- From the measured nitrate concentrations (Fig 4.11) the most appropriate general exponential expression was obtained through non-linear regression:

$$S_{NO} = 0{,}9340\ e^{0{,}1502t} \qquad (mgN/\ell) \qquad (4.6)$$

- Because the nitrification rate increases exponentially it would induce an exponential increase in the oxygen demand due to nitrification (OUR_N). To estimate the oxygen demand due to the increasing nitrification rate, approximated nitrate concentrations were sequentially calculated for a series of time intervals corresponding to the time intervals observed in the WW+ML batch test M18. At each recorded point in time (column 1, Table 4.7) the approximated nitrate concentration (column 2, Table 4.7) was calculated, using Eq (4.6).

- To obtain the nitrification rate (column 3) for each time, the nitrate concentration calculated at the preceding time was subtracted from the relevant calculated nitrate concentration and the increase in nitrate concentration (ΔNO_3) was divided by the duration of the time interval (Δt) between data points.

- The OUR_N at each time (column 4) was calculated from Eq (3.12) as being 4,57 • nitrification rate (column 3).

- Subtracting the calculated OUR_N from the OUR measured in the batch test (OUR_M) gives an estimate of the OUR due to the heterotrophic active biomass (OUR_H) only. In table 4.7: (column 5 - column 4) = column 6.

Table 4.7 : Determination of the OUR due to heterotrophic active biomass (OUR_H) from an approximated increasing OUR due to nitrification (OUR_N). Key parameters calculated for the first 10 data intervals of WW+ML batch test M18 , 29-02 , conducted with a 0.25ℓ mixed liquor sample.

1 Time (h)	2 Approximate NO_3 conc. (mgN/l)	3 Nitrification rate $\Delta NO_3 / \Delta t$ (mgN/l/h)	4 OUR_N (mgO/l/h)	5 ML + WW OUR_M (mgO/l/h)	6 ML + WW OUR_H (mgO/l/h)
0.12	0.95	0.145	0.66	5.98	5.32
0.33	0.98	0.145	0.66	6.14	5.48
0.51	1.01	0.149	0.68	6.52	5.84
0.71	1.04	0.154	0.70	6.09	5.39
0.88	1.07	0.158	0.72	6.12	5.40
1.08	1.10	0.162	0.74	6.83	6.09
1.25	1.13	0.167	0.76	7.04	6.28
1.43	1.16	0.172	0.78	7.05	6.27
1.60	1.19	0.176	0.81	9.21	8.40
1.77	1.22	0.181	0.83	8.36	7.53

In Fig 4.12 the measured OUR response (OUR_M) is shown plotted with the approximated OUR_N response calculated in Table 4.7 (column 4). The OUR_H response, calculated in Table 4.7 (column 6), is shown plotted in Fig 4.13 as the ML+WW data series. An approximated OUR_H response due to the mixed liquor addition only (ML only) was obtained in Fig 4.13 by analytically separating the "best fit" ML+WW and WW only OUR responses, in accordance with the analytical separation technique described in section 4.4.3.

In Fig 4.14 the approximate ML only OUR_H data is shown plotted $\ell n OUR_H$ with time. Linear regression was applied to the $\ell n OUR_H$-time data to determine the y-intercept, slope and correlation coefficient. From the slope and y-intercept, the heterotrophic active biomass at the start of the batch test, due to the mixed liquor addition only [$Z_{BH(0)}$(ML)*], was calculated using Eq (3.9).

- For this specific batch test (M18) the $Z_{BH(0)}(ML)^*$ value = 80mgCOD/ℓ, calculated via the analytical separation procedure and assuming an exponential increase in the nitrate concentration described above, was almost 50% more than the value obtained via the analytical separation procedure [$Z_{BH(0)}(ML)^*$ = 57mgCOD/ℓ - see Appendix A3], assuming a linear increase in the nitrate concentration and hence a constant OUR_N.

- The reason for the increased measurement of the heterotrophic active biomass is demonstrated in the shape of the OUR_N curve shown in Fig 4.12. The linear interpretation of the nitrate concentration data gives a constant nitrification rate and thus a constant OUR_N, of 1.09 mgO/ℓ/h (4.57 • nitrification rate in Appendix A3). From Fig 4.12 it is evident that the exponential approach to nitrification produced an initial OUR_N less than the 1.09 mgCOD/ℓ and the continuous increase in the nitrification rate resulted in an OUR_N that is slightly more than the 1.09 mgCOD/ℓ when the precipitous drop in OUR occurs.

- When the OUR_N values are subtracted from the measured OUR values in Fig 4.12 the ML+WW OUR_H response is obtained in Fig 4.13. Compared to the OUR_H response obtained via analytical separation (Appendix A3) the initial ML+WW OUR_H values are now slightly higher, while the OUR_H values obtained towards the latter stages of the batch test exhibit a small decrease in value. These changes are replicated in the $\ell nOUR_H$ values and linear fit shown in Fig 4.14. The adjustments cause the y-intercept to be fractionally higher (1.4566 compared to 1.3545 for the corresponding analytically separated data in Appendix A3) and a small decline in the slope (0.0813 compared to 0.1105 in Appendix A3). However, the impact on the measurement of the heterotrophic active biomass calculated from Eq (3.9) is substantial:

$$Z_{BH(0)}(ML)^* = \frac{(e^{\text{y-intercept}}) \cdot 24}{\frac{1-Y_{ZH}}{Y_{ZH}} \cdot (\text{slope} \cdot 24 + b_H)}$$

- It is clear that a higher value for the y-intercept, coupled with a lower value for the slope, would result in an increase in the value of $Z_{BH(0)}(ML)^*$. It is also evident that the small magnitude of the slope exhibited by the ML only response, makes the mathematical formulation of $Z_{BH(0)}(ML)^*$ in Eq (3.9) sensitive to even small changes in the slope of the $\ell nOUR_H$ linear fit, hence the remarkable increase in $Z_{BH(0)}(ML)^*$.

The modified analytical separation procedure described above was used to produce similar profiles for all the batch tests and these profiles are given in Appendix A4. $Z_{BH(0)}(ML)^*$ values were calculated for all the batch tests (excluding batch tests M1 – M4) and the results are listed in Table A4, Appendix A4. The approximated $Z_{BH(0)}(ML)^*$ values versus the theoretical values are shown plotted in Fig 4.15. From Fig 4.15 it is noticeable that when larger mixed liquor samples were used in the batch tests (> 0,25 ℓ), the correspondence between theoretical and measured values improved significantly compared with the correspondence observed in Fig 4.9 for the analytically separated values, assuming a constant nitrification rate.

The fact that the measured $Z_{BH(0)}(ML)^*$ values, calculated on the basis of an exponential increase in the oxygen demand for nitrification (OUR_N), were now comparing favourably with the theoretical values in Fig 4.15, suggested that the simplified linear approach to nitrification employed in the present batch test method, is incorrect and that mathematical interpretation of the nitrate concentration-time data should be modified. From the observations of Antoniou *et al.* (1990) and Sözen *et al.* (1996), it was concluded that the exponential approach to nitrification offered the most reliable approximation of the oxygen utilization rate due to nitrification and that this concept should be incorporated into the development of a modified batch test procedure, described in more detail in Chapter 5.

4.6 CONCLUSIONS

From the batch tests conducted in accordance with the procedures employed by Ubisi *et al.* (1997a,b), the following can be concluded:

- The correlation coefficients (R^2) in linear regression of the $ln OUR_{H(t)}$-time plots for the wastewater (WW) only batch tests were generally very good. For the vast majority of the batch tests $R^2 > 0.95$, which indicates that the observed responses conform to the hypothesized behaviour.

- For the WW only batch tests the mean %COD recovery of 93% is good. However, that 9 out of 23 batch tests yielded %COD recoveries which were marginally less than 90%, to some extent compromised the reliability of the data. The cause for these low % COD recoveries was traced to inadequate sampling for measurement of the final total COD concentration in the batch test. When this deficiency was rectified, acceptable COD mass balances > 90% were obtained. Since the source of the error lay in the measurement of COD and not OUR, the low % COD recoveries do not impact the batch test analysis as this relies on OUR measurements.

- No nitrification was observed in the batch tests with wastewater only, indicating the inactivity of autotrophic organisms in the wastewater.

- For the WW only batch tests, the wastewater heterotrophic active biomass ranged from 1.3 to 7.4 % of the total wastewater COD, with a mean of 3.6 % and a sample standard deviation of 2.0 %. This mean compares favourably with the values measured by Ubisi *et al.* (1997a,b) and Mbewe *et al.* (1995) of 4.5 % and 6.1 % respectively, for the same wastewater type.

- For the batch tests conducted with a mixture of wastewater and mixed liquor (WW+ML), the correlation coefficients (R^2) were generally lower than the R^2 values obtained in the wastewater only batch tests. However, the R^2 values were generally still acceptable, with the vast majority of the R^2 values > 0.90.

- All the WW+ML batch tests yielded %COD recoveries in the range 90 – 110 % with a mean of 98.0 % and a sample standard deviation of 4.6 %. The good %COD recoveries lend credibility to the reliability of the results.

- For the WW+ML batch tests, nitrification was observed since the mixed liquor added to the batch tests was drawn from a nitrifying activated sludge system.

- With the parent system operated at 10 days sludge age, the heterotrophic active biomass measured in the batch tests showed poor correlation with the theoretically predicted values. The measured values were consistently lower than the theoretical values and the comparison shows remarkable similarity to the comparisons obtained by Ubisi *et al.* for batch tests conducted on parent systems operated at sludge ages of 12 and 20 days.

- The similarity in the results obtained from batch tests conducted on the three independent parent systems, operated at sludge ages of 10, 12 and 20 days, coupled with the successful application of the models to a wide variety of systems, indicated that in all likelihood the steady state design and kinetic simulation models gave reasonable theoretical predictions, and that limitations within the batch test method compromised the accuracy of the measured values.

From an evaluation of the batch test procedure and data interpretation, the following can be concluded:

- The heterotroph maximum specific growth rate on RBCOD (μ_H), exhibited by the wastewater heterotrophic organisms was markedly higher than the μ_H observed in the WW+ML batch tests. Furthermore, the μ_H values calculated in the WW+ML batch tests revealed a distinct decreasing trend with increased volumes of mixed liquor added to the batch test. This observation suggests that the heterotrophic organisms present in the mixed liquor sample exhibit such a low growth rate, that the wastewater organisms totally dominate the OUR response for batch tests conducted with small samples of mixed liquor. The fact that the interpretation of the batch test data only makes provision for the analysis of a single "composite" OUR response, significantly limits the batch test's capacity to accurately detect the presence of the mixed liquor heterotrophic active biomass.

- Through analytically separating the ML only OUR response from the ML+WW OUR response, it was verified that the ML heterotrophic organisms displayed significantly lower OUR response values. The approximated heterotrophic active biomass values, calculated from the analytically separated ML only OUR responses, showed an improved comparison with the theoretical values.

- Acknowledging the observations of Antoniou *et al.* (1990) and Sözen *et al.* (1996) that nitrification follows an exponentially increasing trend, as opposed to the linear approach adopted in the present interpretation, has a notable effect on the outcome of the batch test results. If the batch test results are interpreted according to the analytical separation concept and the exponential nitrification approach is incorporated to estimate the oxygen utilization rate due to nitrification, the measured heterotrophic active biomass values compare favourably with the theoretical values in instances where larger volumes of mixed liquor (> 0.25 *l*) were used in the batch tests.

- Where smaller volumes of mixed liquor were used, the dominating growth rate of the wastewater heterotrophic organisms still sufficiently masks the mixed liquor organisms' OUR response to prevent accurate measurements of the mixed liquor heterotrophic active biomass. For this reason the measured heterotrophic active biomass values still compare poorly with the theoretical values where smaller volumes of mixed liquor were used.

It was concluded that modifications to the batch test procedure were necessary, to take account of the dominating influence of the wastewater heterotrophic organisms on the OUR response. One possibility would be to remove the wastewater heterotrophic organisms from the batch test, so that the OUR response reflects only that due to the mixed liquor. This proposal is investigated in Chapter 5. Furthermore in the interpretation of the batch test data provision for the exponential increase in nitrification would need to be included.

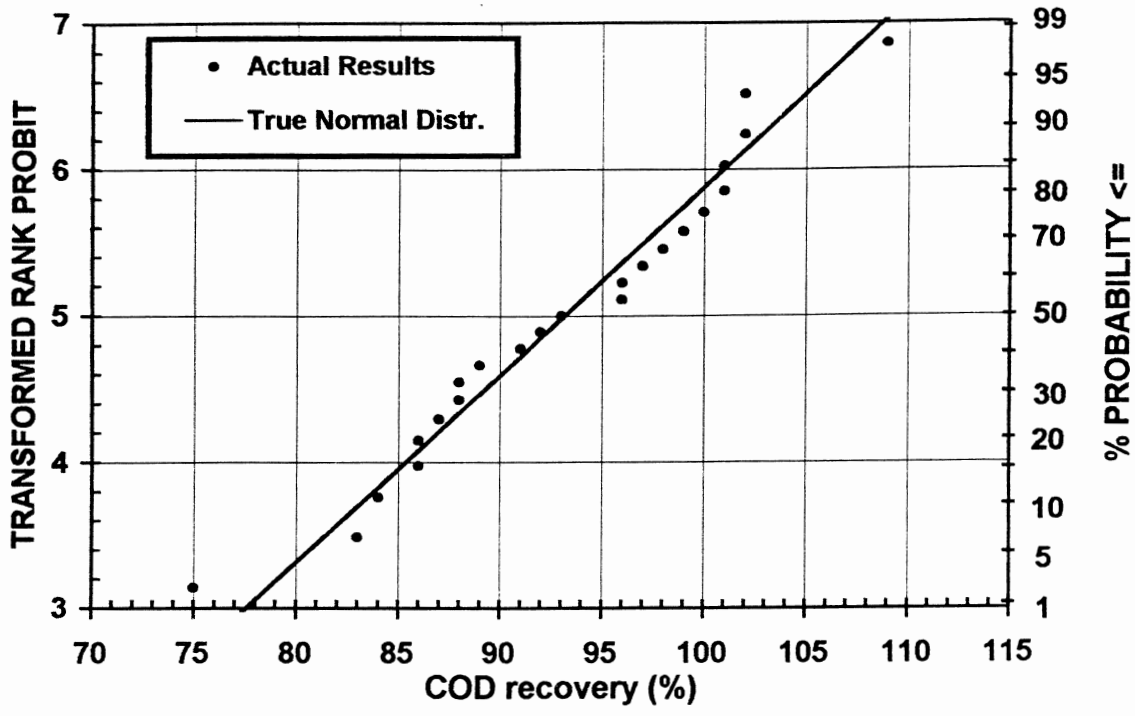


Figure 4.1 : Statistical plot of %COD recovery for all the wastewater only batch tests

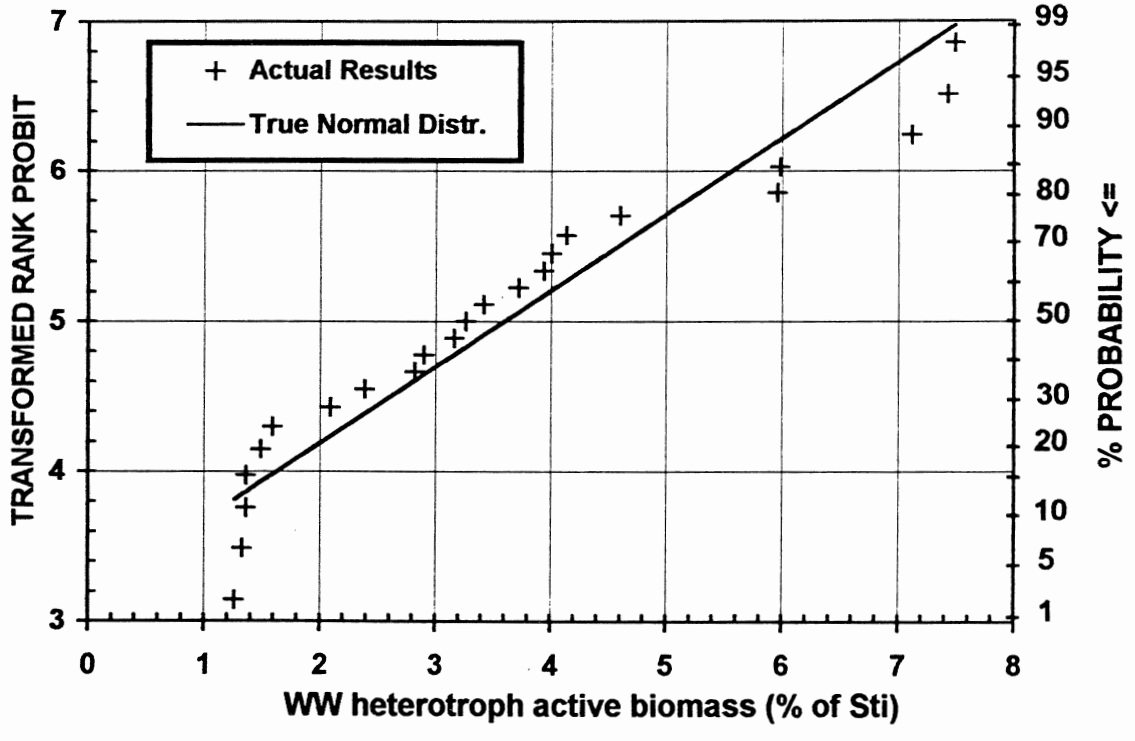


Figure 4.2 : Statistical plot of the measured heterotrophic active biomass for all the wastewater only batch tests.

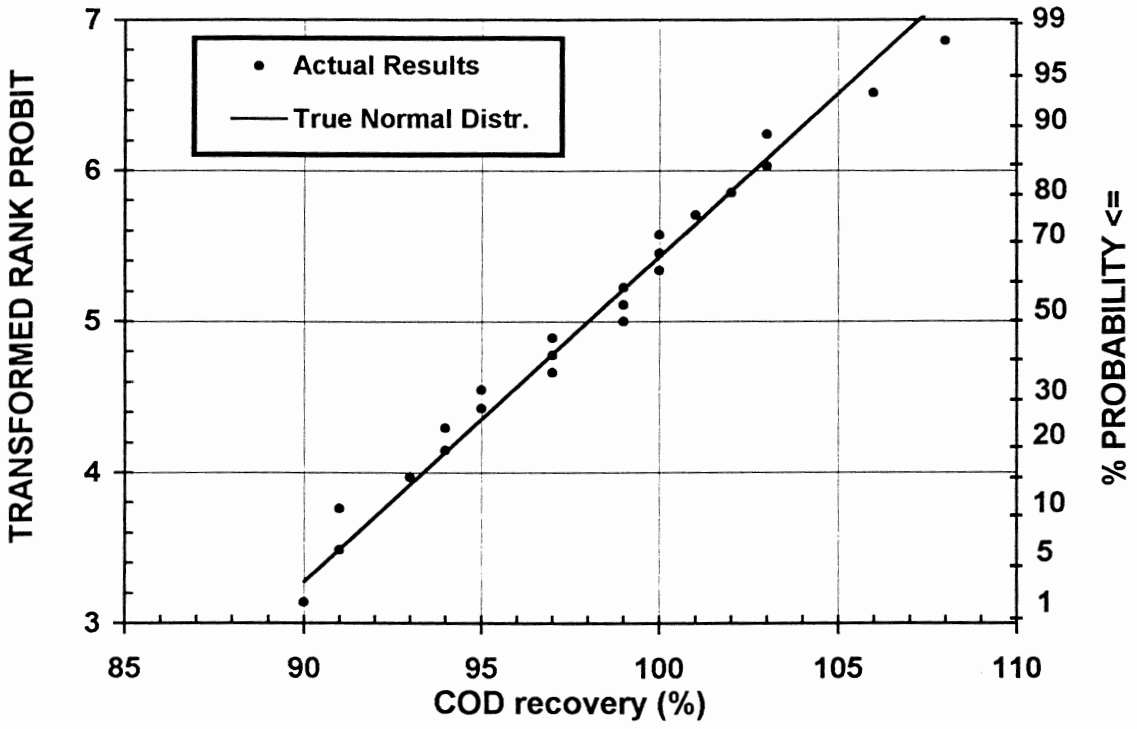


Figure 4.3 : Statistical plot of %COD recovery for all the wastewater and mixed liquor batch tests

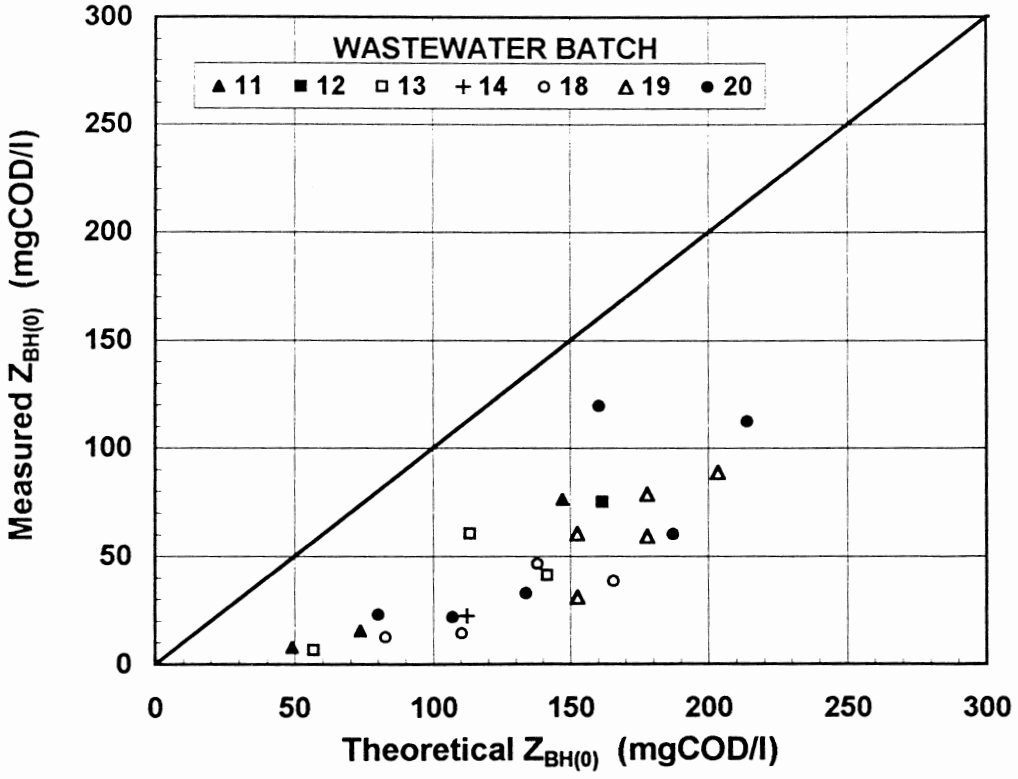


Figure 4.4 : Graph of measured versus theoretical active biomass $Z_{BH(0)}$(ML) for the various wastewater batches with the parent laboratory-scale system operated at 10 days sludge age.

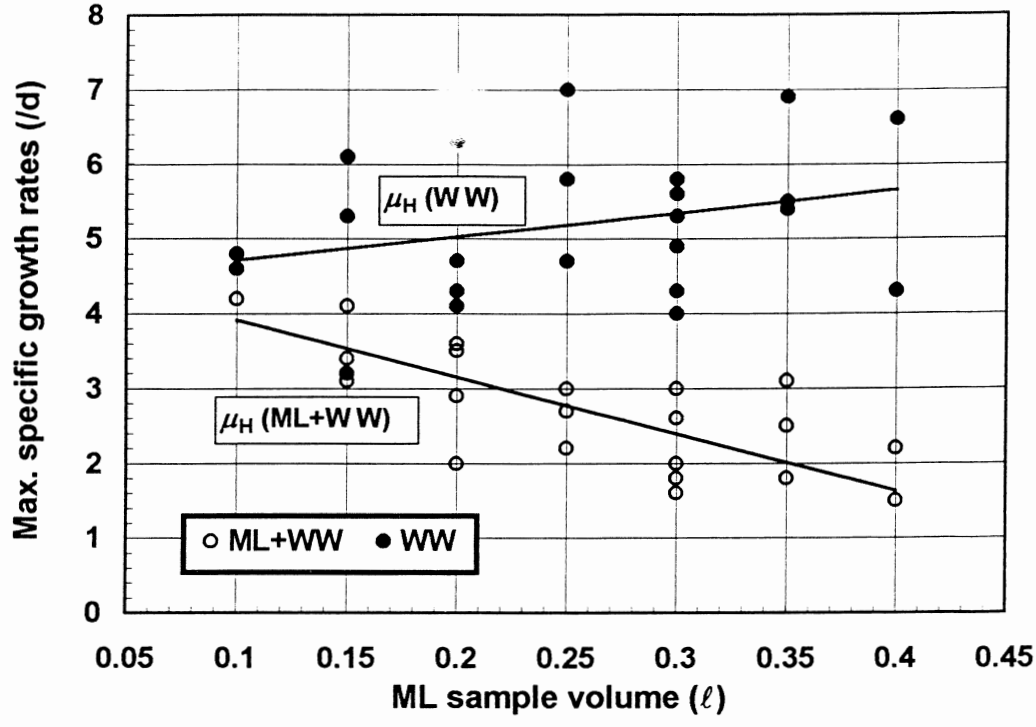


Figure 4.5 : Graph of the mixed liquor sample volume versus the measured heterotroph max. specific growth rate on RBCOD (μ_H), measured in all the wastewater plus mixed liquor (WW+ML) and wastewater (WW)only batch tests.

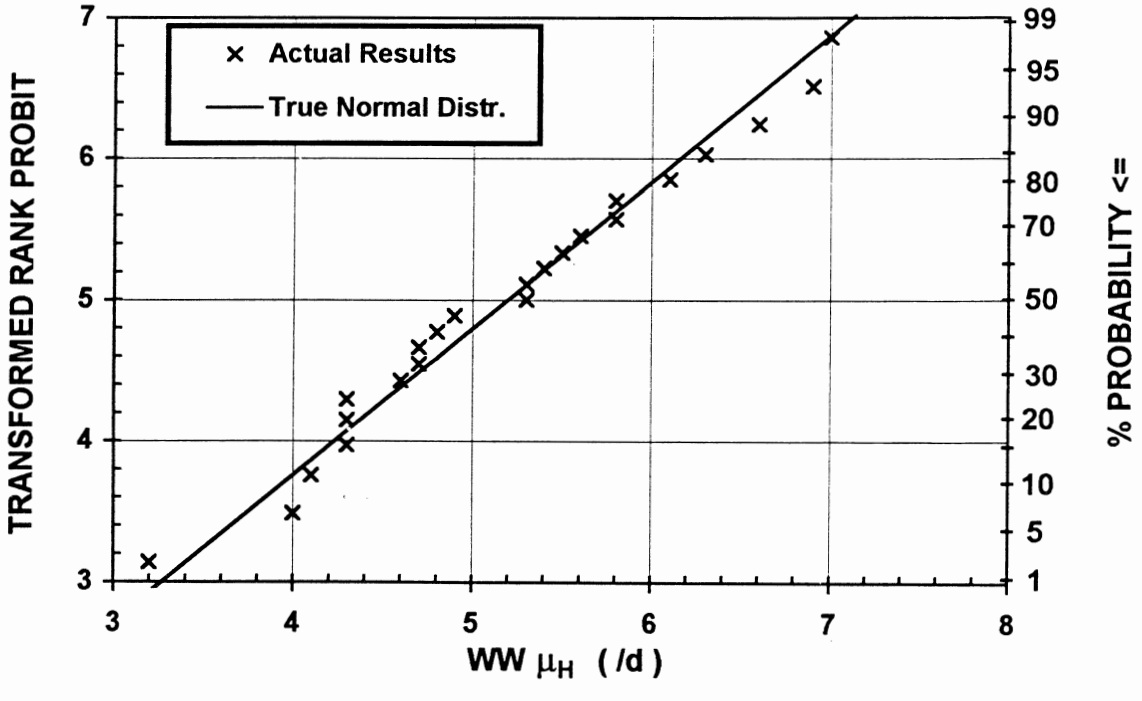


Figure 4.6 : Statistical plot of the heterotroph maximum specific growth rate on RBCOD , μ_H , measured in all the wastewater only batch tests.

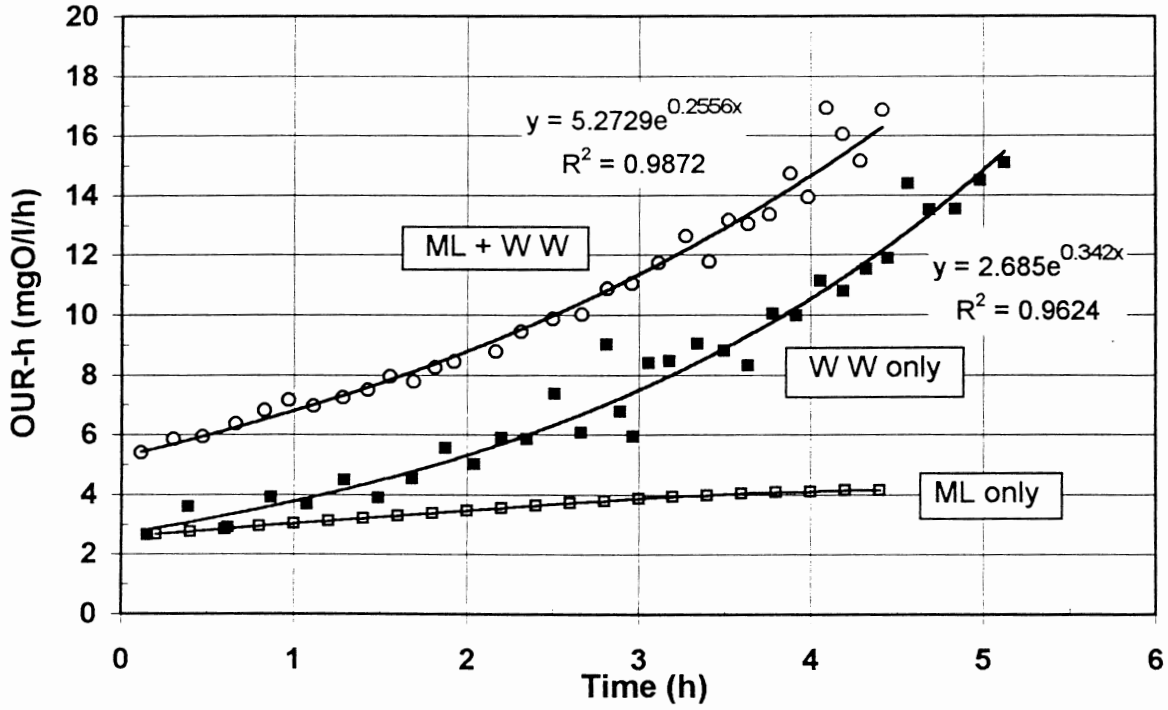


Figure 4.7 : $OUR_{H(t)}$ - time responses for wastewater (WW) only batch test W8 and wastewater plus mixed liquor (WW+ML) batch test M8 , 08-12 , using 0.2ℓ mixed liquor sample. Approximated OUR response due to mixed liquor addition only (ML only) obtained through analytically separating the"best fit" WW+ML and WW only OUR responses.

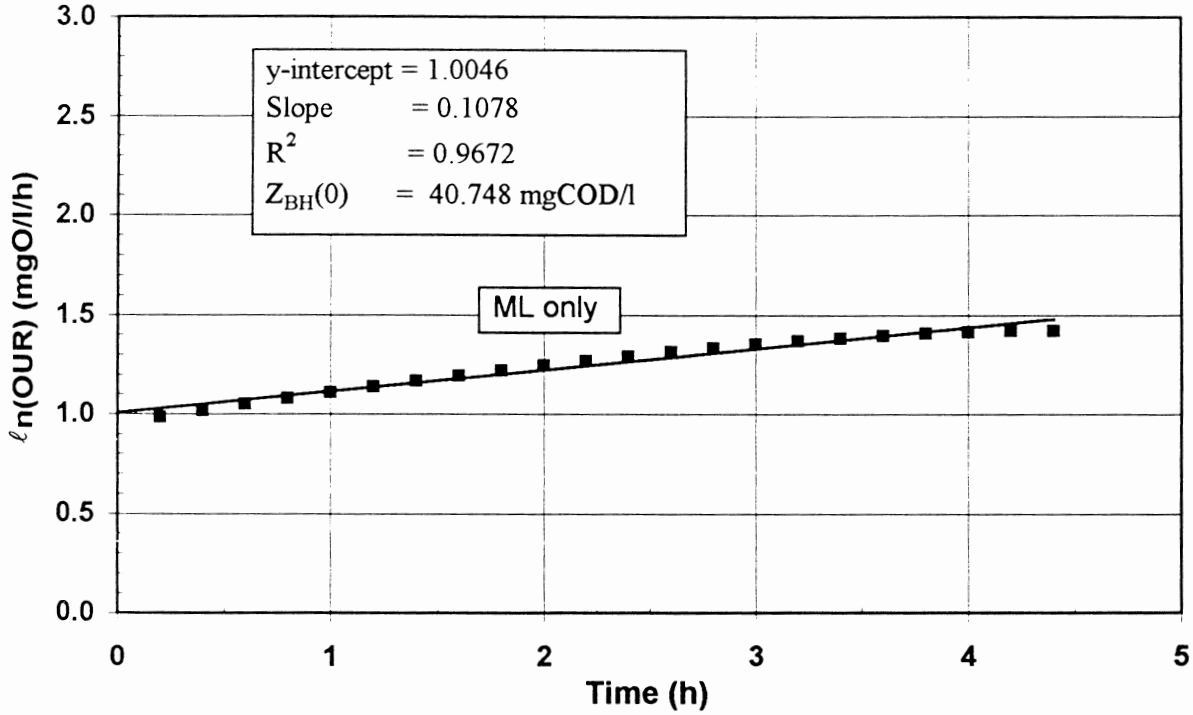


Figure 4.8 : $\ell nOUR_{H(t)}$ versus time for approximated mixed liquor only (ML only) OUR data in Fig. 4.7. Data from batch tests W8 and M8 , 08-12 , 0.2ℓ mixed liquor sample.

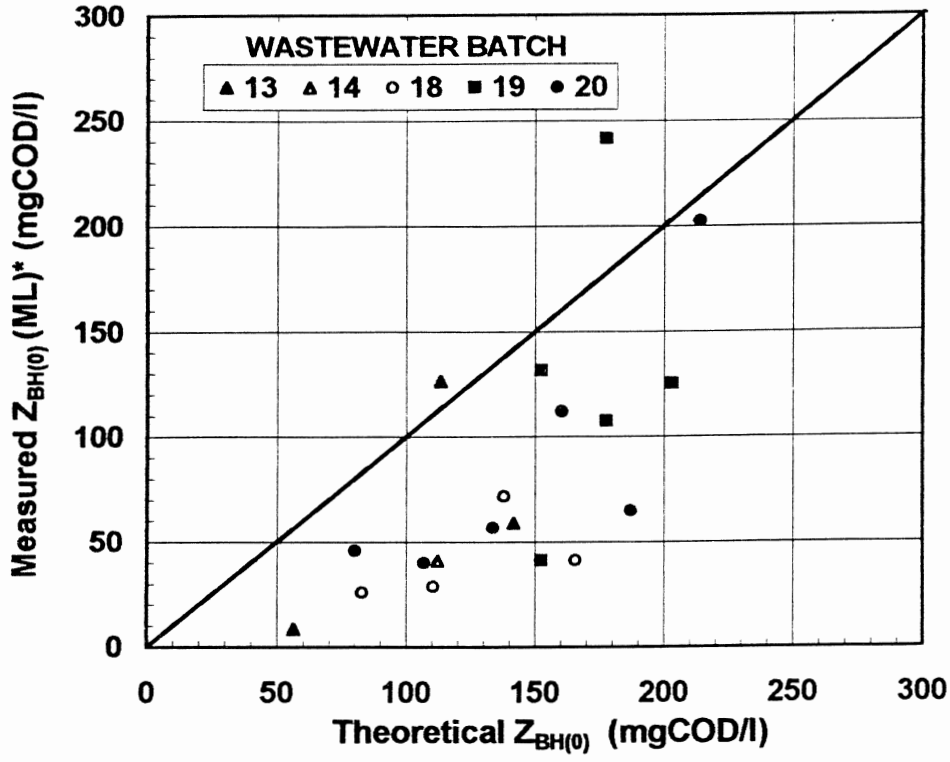


Figure 4.9 : Comparison between the theoretical and *approximated* mixed liquor heterotrophic active biomass $Z_{BH(0)}(ML)^*$, determined through analytical separation of WW+ML and WW only OUR responses recorded during WW+ML and WW only batch tests.

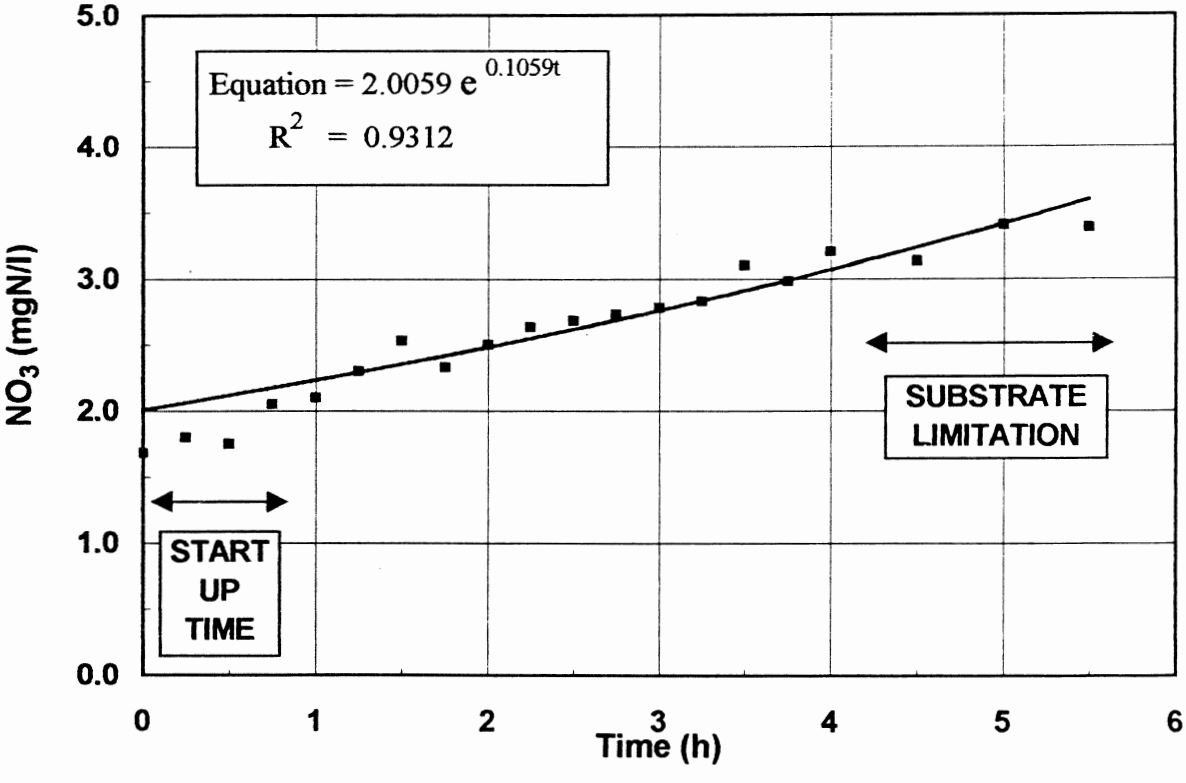


Figure 4.10 : Measured nitrate concentration versus time plot for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M16 , 22-02 , wastewater batch no.19 , showing best fit exponential expression and disregarded data.

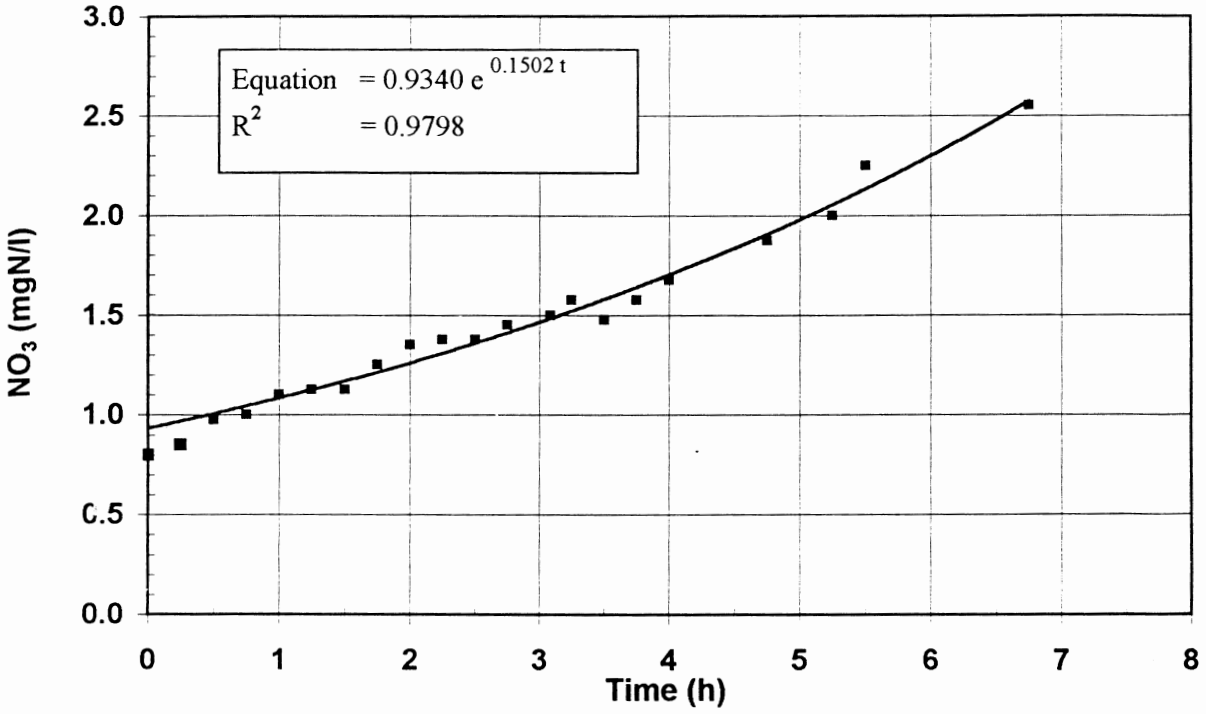


Figure 4.11 : Measured nitrate concentration versus time plot for wastewater (2.75ℓ) plus mixed liquor (0.25ℓ) batch test M18, 29-02, wastewater batch no.20, showing best fit exponential expression.

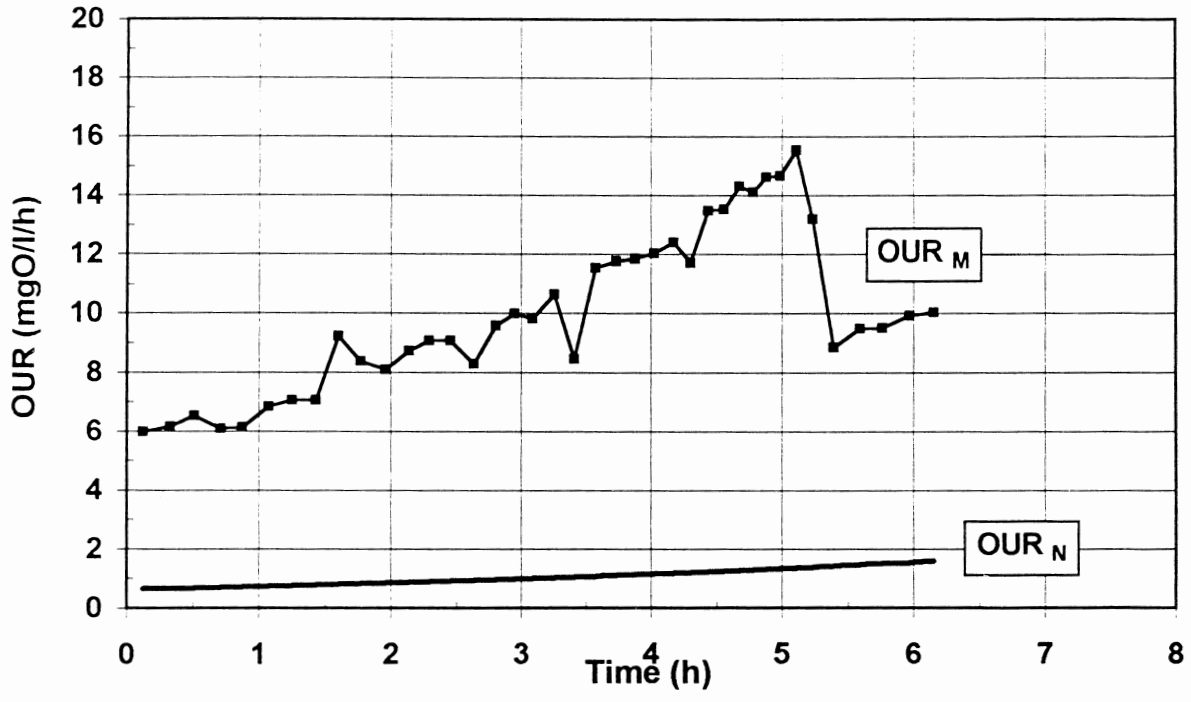


Figure 4.12 : Oxygen utilization rate (OUR) response versus time for wastewater plus mixed liquor batch test M18, 29-02, wastewater batch no.20. Also shown is the OUR due to nitrification, calculated from the exponential curve shown on Fig. 4.11.

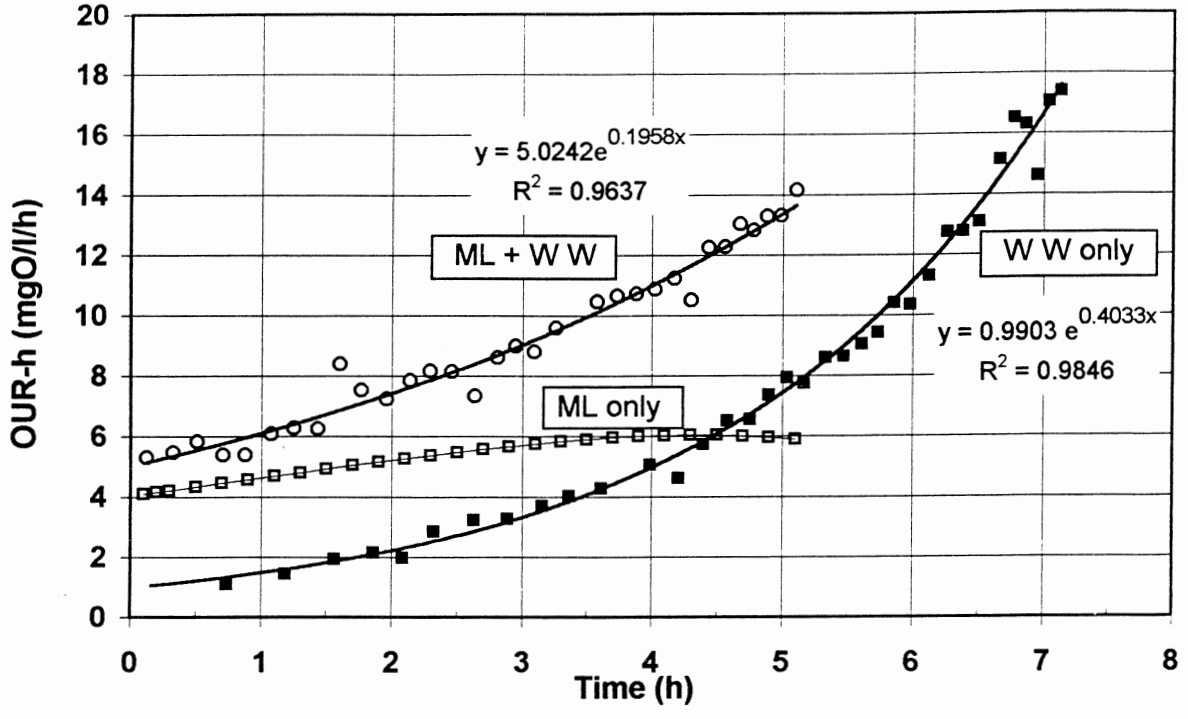


Figure 4.13 : $OUR_{H(t)}$ - time responses for WW only batch test W18 and WW+ML batch test M18, 29-02, assuming an ***exponential*** increase in nitrification for the construction of the WW+ML OUR response. Approximated ML only OUR response obtained via analytically separating the "best fit" WW+ML and WW only OUR responses.

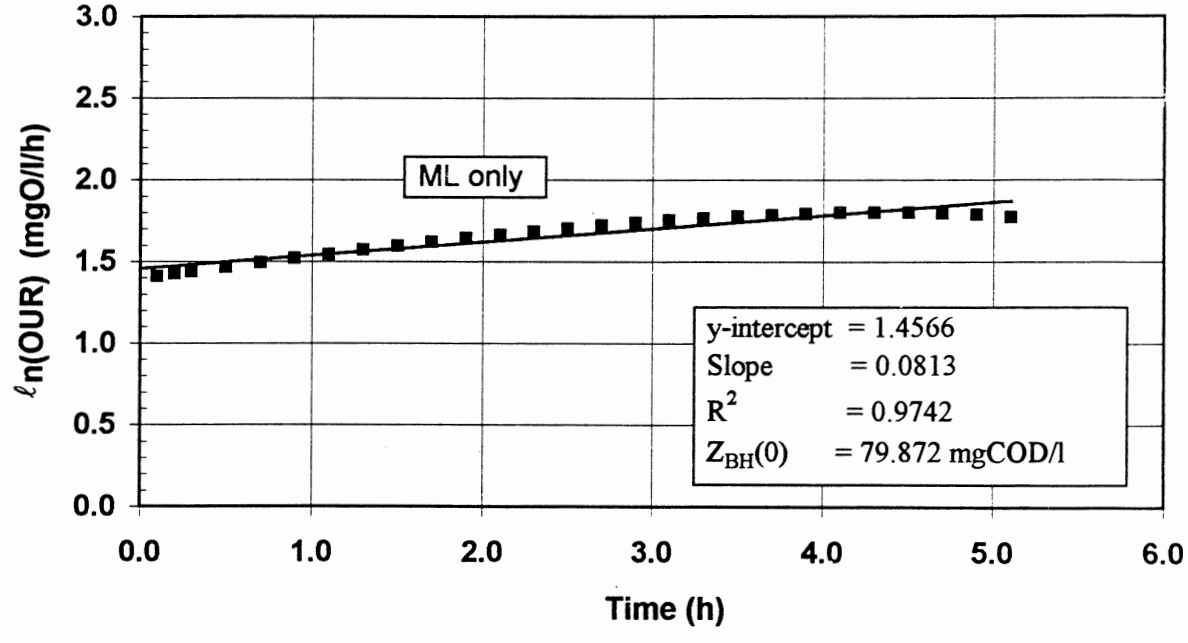


Figure 4.14 : $\ell nOUR_{H(t)}$ versus time for the approximated mixed liqour only (ML only) OUR data in Fig 4.13, assuming an ***exponential*** increase in nitrification.

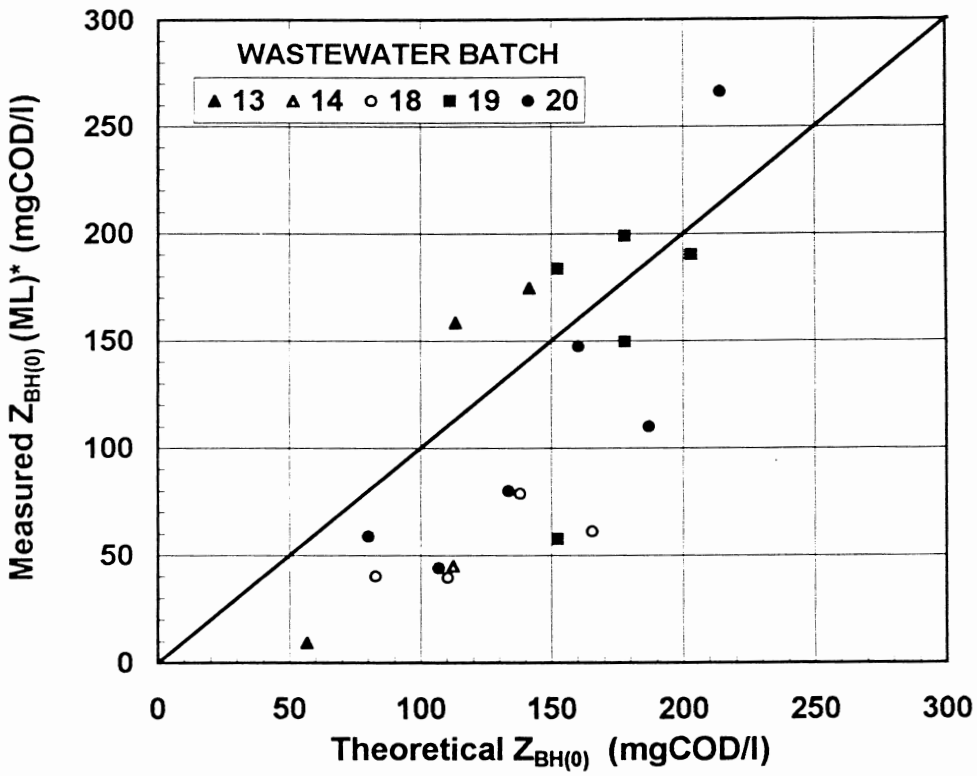


Figure 4.15 : Comparison between the theoretical and *approximated* mixed liquor heterotrophic active biomass $Z_{BH(0)}$(ML)* , determined through analytical separation *and* taking due account of exponential increase in nitrification rate.

CHAPTER 5

DEVELOPMENT AND EVALUATION OF A MODIFIED BATCH TEST PROCEDURE TO QUANTIFY HETEROTROPHIC ACTIVE BIOMASS

5.1 INTRODUCTION

In Chapter 4 it was concluded that the batch test procedure developed by Ubisi *et al.* (1997a,b) proved to be inadequate as a reliable means to quantify the heterotrophic active biomass present in activated sludge systems. The poor correlation between the active biomass measured in the batch tests and the theoretical active biomass predicted via the steady state design model was attributed to mainly two factors:

(i) It was observed that the ordinary heterotrophic organisms (OHO) present in the wastewater exhibited a growth rate that was much faster than the growth rate exhibited by the heterotrophic organisms present in the mixed liquor of the parent laboratory-scale activated sludge system. In examining the OUR responses of the batch tests conducted with a mixture of wastewater and mixed liquor, it was observed that the wastewater OHO active mass partially masked the OUR response of the OHO active mass from the mixed liquor. Accordingly, a potential source of error in the batch test arises when subtracting the wastewater OHO active biomass concentration (determined from the wastewater only batch test OUR) from that for the mixed liquor and wastewater OHO active biomass concentration, to derive the mixed liquor OHO active biomass concentration.

(ii) The premise that nitrification in the batch test with wastewater and mixed liquor could be associated with a linear increase in the nitrate concentration with time, proved to be unduly simplified. It was observed that the generation of nitrate in the batch reactor was better represented by an exponential increase than a linear increase with time. Thus, in the present batch test concept when the oxygen demand due to nitrification (OUR_N), with a constant value in terms of the linear approach, is subtracted from the measured OUR response to obtain the OUR response due to the heterotrophic active biomass (OUR_H), another potential source of error is introduced.

To eliminate these two possible sources of error, the following modifications to the batch test procedure and the interpretation of results were proposed:

- To physically remove the OHO active biomass from the wastewater, the raw wastewater used in the batch test would be pre-filtered.

- In the calculation of the oxygen demand due to nitrification (OUR_N), it would be assumed that the generation of nitrate follows an exponentially increasing trend.

In this chapter the modifications to the experimental procedure, the interpretation of the data recorded and the evaluation of the results obtained from the modified batch tests will be described in detail.

5.2 EXPERIMENTAL PROCEDURE

5.2.1 Experimental approach

The experimental approach is similar to the approach described in Chapter 3, with the exception that filtered wastewater is used as substrate source. Having removed the OHO active biomass from the wastewater through filtration, the need to operate a wastewater only batch test in parallel to the mixed liquor and wastewater batch test is no longer necessary. The oxygen utilization rate (OUR) response observed in a single filtered wastewater and mixed liquor batch test can be considered as the OUR response emanating from the mixed liquor active biomass addition only, requiring no correction for the OUR contribution from the active biomass in the wastewater. The accuracy of the OHO active biomass measurement is enhanced due to the fact that an estimate of the wastewater OHO active biomass is no longer required. However, due consideration must be taken of the OUR for nitrification and this must be subtracted from the total OUR when the results are analysed. The fact that direct results for measurement of the mixed liquor OHO active biomass at the start of the batch test can be obtained in a single batch reactor, simplifies the batch test procedure. Having assembled a parallel reactor configuration in the UCT laboratory for this investigation, presented the opportunity to conduct two separate batch tests, using mixed liquor samples of different volume, simultaneously.

5.2.2 Wastewater preparation

The preparation of the wastewater for the modified batch test incorporated flocculating and filtrating the raw wastewater. Because these processes showed the tendency to be rather time consuming, the wastewater preparation was performed the day before the batch test was conducted. The raw wastewater was drawn from the storage tanks according to the same procedure described for the parent system feed preparation in Chapter 2. The wastewater was diluted to approximately the same COD concentration (± 500 mgCOD/ℓ) as that fed to the parent system. A sufficient volume of diluted wastewater (± 7 ℓ) was measured off and placed in a separate container which served as wastewater source for the two separate batch tests to be performed during the next day.

Flocculation / settling

To expedite the filtration process, the diluted wastewater was subjected to flocculation prior to filtration; 10 mℓ of stock aluminium sulfate [$A\ell(SO_4) \cdot 15\ H_2O$, stock at 50g/ℓ]

were added per ℓ wastewater, the mixture was stirred rapidly (~200rpm) for 2 minutes (rapid mix phase) and then slowly (~1rpm) for 30 minutes (flocculation and settling phase). The flocculation and settling phase was conducted in custom-built settling cylinders of 3 ℓ capacity each. The settling cylinders (110 mm dia.) were equipped with magnetic stirring arms regulated to achieve the slow rotational speed of 1 rpm. During the course of this investigation it was found that enhanced settling was achieved when the stirring was discontinued after the 30 minute period and the contents of the cylinder was allowed to settle (without stirring) for a further 30 minutes period. Optimum flocculation was achieved with the pH in the range 4,5 – 6,5.

Filtration

The clear supernatant that developed in the settling cylinders was drawn off and filtered through a glass fibre filter (Whatman's GF/C). Although filtering through a 0,45 µm filter is the accepted requirement to remove almost all the particulate material in the wastewater, this procedure proved to be unduly laborious, considering that ± 6 ℓ of wastewater had to be filtered at a time. ***Aerobic batch tests on the flocculation filtered wastewater only indicated that the procedure (using a glass fibre filter) effectively removed all active biomass from the wastewater - no measurable OUR was observed.***

The filtered wastewater was stored overnight in the cold room at 4°C. All containers used were thoroughly cleaned with boiling water beforehand to ensure that no external organisms could enter the batch reactors accidentally.

5.2.3 Filtered wastewater and mixed liquor batch tests

As described above, the modified batch tests were conducted using a mixture of filtered wastewater and mixed liquor drawn from the parent system. The single batch reactor configuration is illustrated in Fig. 5.1.

After retrieving the stored filtered wastewater from the cold room, the required volume of filtered wastewater (i.e. 2,8 ℓ in Fig. 5.1) was carefully measured off, preheated to 20°C in a warm water bath and placed in the continually stirred batch reactor maintained at a constant temperature of 20°C. To account for the reduction in pH during the flocculation phase, the pH of the flocculation filtered wastewater was raised to pH ~ 7.5 prior to the commencement of each batch test. This was done by adding the required amount of the sodium bicarbonate ($NaHCO_3$) solution (see Chapter 2, section 2.6) to the wastewater. The required volume of mixed liquor (0,2 ℓ in Fig 5.1) was harvested from the aerobic reactor of the parent system and added to the filtered wastewater, giving a combined volume of 3 ℓ for the mixture in the batch reactor. The volume of mixed liquor added was varied between 100 ml and 400 ml for all the modified batch tests conducted. Immediately after the mixed liquor was added to the filtered wastewater, a sample was drawn to obtain the initial total COD concentration (Standard Methods, 1985).

The reactor was aerated by passing low pressure air through a small bore Perspex tube which terminated at the bottom of the reactor (see Fig 5.1). A dissolved oxygen (DO) probe (YSI) from an automatic DO meter/OUR logger was immersed in the solution.
The oxygen supply and OUR response in the batch test was measured using an automated technique – described in detail in Chapter 3, section 3.2.3. In operating the batch test, the water surface was covered by small plastic balls to limit surface exchange of oxygen. As a result of the intermittent aeration process, particulate matter (including organisms) was frequently splashed out of solution and deposited on the batch reactor walls, above the water surface. The walls of the reactor were regularly brushed to return the organisms back into the solution.

The batch tests were generally continued for approximately 2 hours after the precipitous drop in OUR occurred. Because the mixed liquor heterotrophic organisms exhibited such a low growth rate, some of the batch tests conducted on small samples of mixed liquor continued for more than 15 hours before the precipitous drop in OUR was observed. These batch tests were left to continue for 24 hours before the tests were terminated. In general, however, the duration of the batch tests varied between 5 and 14 hours, depending on the volume of mixed liquor added.

At intervals samples were drawn from the reactor, immediately filtered through 0,45 μm filter paper, 2 – 3 drops of $HgC\ell_2$ (8.6 g/ℓ) added to the filtrate and the filtrate stored for subsequent nitrate and nitrite analysis. At the end of the batch tests, the contents of the batch reactor were homogenised in a liquidiser, a sample drawn and the total COD concentration measured. The OUR results were downloaded from the DO meter to a PC.

5.3 DATA ANALYSIS AND RESULTS

5.3.1 Parent system

A well defined and controlled parent laboratory-scale nitrification/denitrification activated sludge system was operated at 10 days' sludge age and was continually monitored - see Chapter 2. As described earlier, the sewage was fed to the parent system in batches which lasted for approximately 2 weeks. Each sewage batch constituted a steady state period. A total number of 20 modified batch tests were conducted and, due to the fact that two batch tests could be conducted per day, the 20 batch tests could be concluded during 3 sewage batch periods. The sewage batches during which batch tests were conducted are set out in Table 5.1. An important consequence of having two batch reactors is that it required only 11 days of testing to complete 20 modified batch tests compared to the 20 testing days that would have been required previously to perform 20 (unmodified) batch tests using the parallel reactor, WW only and WW+ML batch test procedure.

Table 5.1: The sewage batch number and the dates it was used as feed for the parent system together with the number of modified batch tests with flocculation filtered wastewater and mixed liquor conducted during each sewage batch.

Sewage batch no.	Dates (2000)	Number of batch tests
21	10 Mar – 24 Mar	7
22	25 Mar – 4 Apr	7
23	5 Apr – 18 Apr	6
TOTAL		20

Although each sewage batch period constituted a steady state period, the wastewater characteristics invariably fluctuated from one sewage batch to the next. As the activated sludge population dynamics are directly related to the wastewater characteristics, the parent system heterotrophic active biomass concentration would exhibit a corresponding fluctuation between different sewage batches. To formulate a theoretical estimate for the heterotrophic active biomass concentration present in the parent system during each sewage batch period, certain key compounds and parameters were monitored on a daily basis (see Chapters 2 and 4). Detailed data on the parent system are given in Appendix C, Tables C3 and C4. For each sewage batch tested, the daily results have been averaged and the average values are listed in Table 5.2 below.

Table 5.2: Steady state results for parent laboratory-scale anoxic/aerobic sludge system receiving sewage batches during which *modified* batch tests were performed. Averages are listed with sample standard deviations in brackets.

PARENT ANOXIC/AEROBIC STEADY STATE SYSTEM													
WW Batch	No of Tests	COD (mg/ℓ)		TKN (mg/ℓ)		Nitrate (mgN/ℓ)			OUR mgO/l/h	Mixed liquor (mg/ℓ)			pH
		Inf	Eff	Inf	Eff	Anoxic	Aerobic	Eff		VSS	COD	TKN	
21	9	510 (39)	69 (7)	50 (3)	4.0 (0.9)	10.3 (2.1)	20.6 (2.0)	22.9 (2.4)	39.4 (1.6)	2457 (74)	3664 (92)	250 (8)	7.53 (0.08)
22	7	493 (28)	79 (17)	45 (2)	5.0 (0.6)	8.1 (1.1)	15.7 (1.2)	18.1 (0.8)	35.2 (2.5)	2138 (135)	3427 (170)	219 (11)	7.46 (0.04)
23	7	530 (30)	80 (15)	51 (5)	6.2 (1.9)	7.8 (1.1)	14.9 (0.9)	16.7 (1.1)	34.1 (1.3)	2043 (145)	3116 (263)	216 (26)	7.52 (0.08)

Using the average values in Table 5.2, for each sewage batch the following were determined :

- Influent wastewater unbiodegradable soluble and particulate COD fractions ($f_{S,us}$ and $f_{S,up}$, respectively); system COD and N mass balances (Ekama *et al.* 1986) and the COD and TKN to VSS ratios for the mixed liquor (f_{cv} and f_N, respectively). The calculation procedures are set out in detail in Chapter 2 and the results for each sewage batch are listed in Table 5.3.

- The heterotrophic active biomass fractions of the mixed liquor organic suspended solids (f_{av}) were determined for each sewage batch using the steady state design model (Eq. 3.21) and the UCTOLD kinetic simulation computer programme, see Chapter 3, section 3.4.1. The results are listed in Table 5.3; close agreement was obtained between the steady state and kinetically simulated predictions for f_{av}. For the reasons set out in Chapter 3, section 3.4.1, the kinetically simulated values for f_{av} were persistently higher (mean difference = 2,3%). Because of the simpler, more direct analytical calculation procedure, the f_{av} values from the steady state design model were used.

Table 5.3 : Steady state COD and N mass balances, wastewater fractions and mixed liquor parameters for parent laboratory-scale anoxic/aerobic activated sludge system. Data calculated from data Table 5.2, either directly or using the steady state (SS) design (WRC, 1984) or kinetic simulation (sim.) models (Dold *et al.*, 1991).

PARENT ANOXIC/AEROBIC STEADY STATE SYSTEM										
WW Batch	No. of tests	Mass Balance (%)		Wastewater fractions			Mixed liquor			
		COD	N	Unbiod Soluble COD ($f_{S,us}$)	Unbio. Particulate COD ($f_{S,up}$)		COD/VSS ratio (mgN/mg VSS) (f_{cv})	TKN/VSS ratio (mgN/mg VSS) (f_n)	Active Fraction (f_{av})	
					SS Design	Kinetic Sim.			SS Design	Kinetic Sim.
21	9	90	124	0.0961	0.1330	0.1127	1.49	0.102	0.4203	0.4305
22	7	92	113	0.1217	0.0934	0.0736	1.60	0.102	0.4424	0.4525
23	7	83	96	0.0925	0.0312	0.0084	1.53	0.106	0.5841	0.5978

Examination of the f_{av} values calculated for the three sewage batch periods revealed that a higher mixed liquor active fraction was obtained for sewage batch 23; f_{av} = 0.5841 compared to the average f_{av} = 0.4314 calculated for the two preceding sewage batch periods. The high f_{av} value is inversely related to the low wastewater unbiodegradable particulate fraction ($f_{S,up}$) calculated for sewage batch 23. The f_{av} values were calculated from Eq (3.21):

$$\frac{1}{f_{av}} = 1 + f_E\, b_{HT}\, R_S + \frac{f_{S,up}\,(1 + b_{HT}\, R_S)}{f_{cv}\, Y_H\,(1 - f_{S,us} - f_{S,up})}$$

In turn, the low $f_{S,up}$ = 0.0312 is the result of the low average VSS concentration observed for sewage batch 23 (VSS = 2043 mgVSS/ℓ in Table 5.2). The relationship between $f_{S,up}$ and the VSS concentration (X_v) is given by Eq (2.12):

$$X_v = \frac{S_{ti}\,(1 - f_{S,up} - f_{S,us}) Y_H\, R_S}{(1 + b_{HT}\, R_S)}\,(1 + f_E\, b_{HT}\, R_S) + f_{S,up}\, S_{ti}\, R_S / f_{cv}$$

The low VSS concentrations recorded for sewage batch 23 would suggest that the parent system's behavioural response during this period deviated significantly from the steady state behaviour and that the batch test results obtained, should be rejected for further analysis. This observation is supported by the low COD mass balance (83 %) that was obtained when the parent system was receiving sewage batch 23, see Chapter 2. However, the cause for the low VSS concentration was attributed to the following changes to the sampling technique, which should be taken into account when considering whether to reject sewage batch 23:

- As a result of the aeration in the aerobic reactor of the parent system, some sludge particles were splashed out of solution and deposited above the water surface level on the reactor wall. In accordance with the sampling procedures adopted for this investigation, the reactors were usually thoroughly brushed to re-introduce wall deposits back into the mixed liquor to obtain a representative sample of the organic suspended solids present in the parent system.

- However, during the evening when the system was left unattended, the sludge particles deposited on the reactor wall were unable to participate in the biological processes occurring in the mixed liquor solution. As sewage batch 23 was anticipated to be the last period during which the modified batch tests would be conducted, it was decided to experiment with the sampling techniques.

- To determine if the expulsion of sludge particles from the solution would have a significant effect on the measurement of the active biomass, the mixed liquor samples for the batch tests were drawn before brushing the reactor. In addition, on the 3 days that the batch tests were conducted (10, 11, 12-04-2000) the reactor was not brushed before the parent system's VSS samples were drawn. This is clearly reflected in the lower VSS data recorded for these 3 days - see Table C4, Appendix C.

- The fact that the wall deposits were not reintroduced into solution and that a considerable volume of mixed liquor was harvested for the batch test (i.e. 0,65 ℓ on 12-04-2000) before samples were drawn for the VSS analysis, contributed largely to the low VSS concentrations observed during sewage batch 23 and is reflected in the distorted active fraction (f_{av}) obtained.

- Thus, the unconditional rejection of the batch test results obtained during sewage batch 23 would seem unreasonable, since the rejection would be based on known distortions in the VSS data. The low VSS values were also instrumental in yielding a low COD mass balance for sewage batch 23. From Fig 2.4 (Chapter 2) it is clear that the percentage COD contribution of the waste sludge (29 % in terms of total COD recovery) is the lowest of all the sewage batches tested and is 5 % lower than the average percentage waste sludge COD contribution of 34 %.

- Thus, the batch test data collected during sewage batch test 23 will be included where appropriate, and will be analyzed, but it would be noted in the comparison with the theoretical active biomass present in the parent system that the calculated f_{av} value might not provide a true reflection of the actual theoretical active biomass.

Now, knowing f_{av} and the concentration of the mixed liquor VSS (available from the averaged steady state VSS concentration in the parent system, Table 5.2) the *theoretical* heterotrophic active biomass concentration in the parent system [X_{BH}(theor), COD units] was derived from Eq. (3.20):

$$X_{BH}(\text{theor}) = f_{av}\, X_v\, f_{cv} \qquad (\text{mgCOD}/\ell) \qquad (5.1)$$

where,

X_{BH}(theor) = theoretical heterotrophic active biomass concentration in the parent system, COD units (mgCOD/ℓ)
f_{av} = heterotrophic active biomass fraction of the mixed liquor volatile suspended solids (mgVSS / mgVSS) - Table 5.3.
X_v = volatile suspended solids concentration, VSS units, from Table 5.2 (mgVSS/ℓ)
f_{cv} = COD to VSS ratio of mixed liquor organic suspended solids from Table 5.3 (mgCOD / mgVSS)

Accordingly, the theoretical heterotrophic active biomass concentrations in the parent system were calculated for each sewage batch and the values are given in Table 5.5 (X_{BH} - Theoretical ML). Based on these values the *theoretical* heterotrophic active biomass concentration in the batch reactor, due to the addition of the mixed liquor samples drawn from the parent system, was calculated as follows:

$$Z_{BH}\,(\text{theor})_{BT} = X_{BH}\,(\text{theor}) \cdot V_{ML} / (V_{ML} + V_{WW}) \qquad (\text{mgCOD}/\ell) \qquad (5.2)$$

where,

Z_{BH} (theor) $_{BT}$ = theoretical heterotrophic active biomass concentration in batch reactor due to added mixed liquor, COD units (mgCOD/ℓ batch reactor)
X_{BH} (theor) = theoretical heterotrophic active biomass concentration in the parent system from Eq (5.1) (mgCOD/ℓ)
V_{ML} = volume of parent system mixed liquor added to the batch test (ℓ)
V_{WW} = volume of filtered wastewater added to the batch test (ℓ)

Using the equations above, the theoretical heterotrophic active biomass concentration in the batch reactor was calculated for each modified batch test and the values are listed in the right hand column of Table 5.5 (Z_{BH} - Theoretical Batch test).

5.3.2 Modified batch tests

For this investigation the data from the batch tests were interpreted in terms of the UCT (Dold *et al.* 1980; 1991) kinetic simulation model to define the processes and compounds responsible for the OUR-time responses. Twenty batch tests with filtered wastewater and mixed liquor were conducted during 3 sewage batch periods 21 to 23. The OUR versus time plots are set out in detail in Appendix B. As an example, the OUR (mg/O/ℓ/h, Fig 5.2) and nitrate concentration (mgN/ℓ, Fig 5.3) versus time responses for the modified batch test F1 conducted on 14 March 2000 (14–03, sewage batch no 21) with a mixture of filtered wastewater (2,8 ℓ) and mixed liquor (0,2 ℓ) are used here to briefly explain the calculation procedures.

Oxygen utilization rate due to nitrification (OUR_N)

As explained earlier, the major difference between the modified batch test and the batch test method developed by Ubisi *et al.* (1997a,b) lies in the removal of the wastewater heterotrophic organisms from the batch test. However, an equally significant modification was incorporated into the analysis of the nitrate concentration - time profiles to establish the oxygen utilization rate due to nitrification (OUR_N). For the modified batch tests it was accepted that the nitrate concentration - time profile follows an exponentially increasing trend, as opposed to the linear increase in the nitrate concentration followed in the analysis of Ubisi *et al.* A detailed description of the exponential approach to nitrification is given in Chapter 4, section 4.5, but to illustrate its application, a brief description of the approach to determine the OUR_N and ultimately the oxygen utilization rate due to the heterotrophic active biomass (OUR_H), is given here. To aid with the interpretation of the calculation procedure, the values of key parameters calculated for batch test F1 are listed in Table 5.4.

- From the measured nitrate concentration – time profile (Fig 5.3) the most appropriate general exponential expression was obtained through non-linear regression:

  $$S_{NO} = 1{,}6892\ e^{0{,}1217\,t} \qquad (mgN/\ell) \qquad (5.3)$$

  From Fig 5.3 it is evident that the general exponential expression [Eq (5.3)] yielded a good representation of the observed nitrate concentration data with time, which is reflected in the good correlation coefficient (R^2) of 0,9572. In fact, for virtually all the modified batch tests the exponential expressions yielded good approximations of the observed data, with only 2 of 20 batch test yielding $R^2 < 0{,}9$.

- Because the nitrification rate increases exponentially it would induce an exponential increase in the OUR_N. To estimate the increasing OUR_N values, approximated nitrate concentrations were sequentially calculated for a series of time intervals corresponding to the time intervals observed in the OUR response of modified batch test F1 (Fig 5.2). At each recorded point in time (column 1, Table 5.4) the approximated nitrate concentration (column 2, Table 5.4) was calculated, using Eq (5.3).

- To obtain the nitrification rate (column 3) at each point in time, the nitrate concentration calculated at the preceding point in time was subtracted from the calculated nitrate concentration in question and the increase in nitrate concentration (ΔNO_3) was divided by the duration of the time interval (Δt) between data points.

- The OUR_N at each point in time (column 4) was calculated from Eq (3.12) as being 4,5 • nitrification rate (column 3).

- Subtracting the calculated OUR_N from the OUR measured in the batch test (OUR_M) gives an estimate of the OUR due to the heterotrophic active biomass (OUR_H). In Table 5.4: (column 5 - column 4) = column 6.

Table 5.4 : Determination of the OUR due to heterotrophic active biomass (OUR_H) from an exponentially increasing OUR due to nitrification (OUR_N). Key parameters calculated for modified batch test F1 , 14-03, conducted with a 0.2 ℓ mixed liquor sample.

1 Time (h)	2 Approximate NO_3 conc. (mgN/l)	3 Nitrification rate $\Delta NO_3 / \Delta t$ (mgN/l/h)	4 OUR_N (mgO/l/h)	5 ML + WW OUR_M (mgO/l/h)	6 ML + WW OUR_H (mgO/l/h)
0,09	1,71	0,209	0,96	7,01	6,05
0,19	1,73	0,209	0,96	5,50	4,54
0,34	1,76	0,212	0,97	6,30	5,33
0,49	1,79	0,216	1,01	6,31	5,32
0,66	1,83	0,221	1,03	6,01	5,00
0,85	1,87	0,225	1,05	5,88	4,85
1,00	1,91	0,230	1,07	6,25	5,20
1,18	1,95	0,235	1,10	6,31	5,24
1,36	1,99	0,240	1,12	6,18	5,08
1,53	2,03	0,245	1,14	6,21	5,09

In Fig 5.2 the measured OUR response (OUR_M) is shown plotted with the approximated OUR_N response calculated from Table 5.4 (column 4). The OUR_H response, calculated from Table 5.4 (column 6), is shown plotted in Fig 5.4.

Referring to the OUR_H – time plot (Fig 5.4), the profile conforms to the profile described by Wentzel *et al.* (1995) and to the OUR profiles described in Chapters 3 and 4. During the first period of the batch test (< 6.0 h) the OUR exhibits an exponential increase due to heterotrophic active mass growth. However, the observed OUR_H values are considerably lower than the OUR_H values recorded for the ML+WW batch tests described in Chapters 3 and 4. In addition, the exponential increase in OUR_H is markedly slower.

To an extent, these differences can be attributed to the reduced slowly biodegradable COD concentration in the filtered wastewater, but more significantly the differences arise as a result of the low overall growth rate exhibited by the mixed liquor heterotrophic active biomass. The absence of the wastewater heterotrophic organisms clearly illustrates the low overall growth rate for the mixed liquor heterotrophic organisms, postulated in Chapter 4, section 4.4.2.

Having determined the $OUR_{H(t)}$ response for the batch test (Fig 5.4) the following information can be obtained from the batch tests:

- COD recovery (%)
- Heterotrophic active biomass at the start of the batch test ($Z_{BH(0)}$)
- Maximum specific growth rates, K_{MP} and μ_H.

The calculation procedures are basically the same as described in Chapter 3, but will be briefly repeated here for elucidation purposes:

COD recovery

The acceptability of the data from the batch test can be evaluated by doing a COD mass balance, as defined by Eq (3.1):

$$\% \text{ COD recovery} = \frac{COD_{t=T} + \int_{t=0}^{t=T} OUR_{H(t)} \cdot dt}{COD_{t=0}} \cdot 100$$

%COD recoveries ranging from 90 – 100 % provide support for the reliability of the measurements. The %COD recoveries for all the batch tests were calculated using Eq (3.1) and the results are listed in Table B1, Appendix B. The %COD recovery for each modified batch test is summarized in Table 5.5 below.

- In general, good %COD recoveries were achieved with only 2 batch tests (F2 and F4) yielding %COD recoveries > 110%. Although the results from these batch tests should be rejected for further analysis, they would be included where appropriate for completeness, but will be appropriately marked. The %COD recovery for batch test F15 was marginally < 90 % (89.1 %); however, statistical analysis indicated that this COD mass balance arose from random effects and accordingly this batch test data were not rejected for further analysis. A statistical plot of the %COD recovery for all the modified batch tests was constructed; see Fig 5.6. From Fig 5.6 the mean %COD recovery was 100.5 % with sample standard deviation of 7.3 %. The good %COD recoveries lend credibility to the reliability of the measurements and the modified batch test procedure.

Determining the heterotrophic active biomass concentration

For each batch test, the recorded $OUR_{H(t)}$ data up to the precipitous drop in OUR were plotted ℓn (OUR) versus time. For example, the $OUR_{H(t)}$ data in Fig 5.4 are shown plotted ℓn ($OUR_{H(t)}$) in Fig 5.5. Linear regression was applied to the ℓn ($OUR_{H(t)}$) data to determine the y-intercept, slope and correlation coefficient (R^2).

- The R^2 values obtained in all the modified batch tests are listed in Table B1, Appendix B. The R^2 values obtained for the modified batch test were generally poor, despite the fact that from a visual inspection the correlation appeared to be good. The mean R^2 value was 0,69 and the poor statistical correlation can be attributed to the exceptionally flat slopes obtained from the ℓn(OUR) – time straight line fits.

From the y-intercepts and slopes, the heterotrophic active biomass concentration at the start of each batch test was calculated using Eq (3.9):

$$Z_{BH(0)} = \frac{(e^{\text{y-intercept}}) \cdot 24}{\frac{1-Y_{ZH}}{Y_{ZH}} \cdot (\text{slope} \cdot 24 + b_H)} \quad (\text{mgCOD}/\ell)$$

The heterotrophic active biomass values measured at the start of each modified batch test are listed in Table B1, Appendix B. The results are summarized in Table 5.5.

- The low growth rate of the mixed liquor heterotrophic organisms resulted in extremely flat slopes measured from the ℓn($OUR_{H(t)}$) plots. The low slope values made the mathematical formulation in Eq (3.9) very sensitive to even the smallest changes in the measured slope. For this reason, in some instances data points which were clearly isolated from the tre observed for the vast majority of data points recorded during a batch test, were omitted from the analysis as they contributed to small distortions in the slope of the "best fit" straight line. In Fig 5.4, as an example, 3 data points (1st, 11th and 19th point) were omitted from the data set when calculating the ℓn(OUR_H) values in Fig 5.5.

The projected heterotrophic active biomass concentrations in the parent system were calculated for each modified batch test:

$$Z_{BH(ML)} = Z_{BH(0)BT} \cdot (V_{ML} + V_{WW}) / V_{ML} \quad (\text{mgCOD}/\ell) \qquad (5.4)$$

where,

$Z_{BH(ML)}$	=	projected heterotrophic active biomass concentration in the parent system, COD units (mgCOD/ℓ)
$Z_{BH(0)BT}$	=	heterotrophic active biomass measured at the start of each batch test (mgCOD/ℓ)
V_{ML}	=	volume of parent system mixed liquor added to the batch test (ℓ)
V_{WW}	=	volume of filtered wastewater added to the batch test (ℓ)

The projected mixed liquor heterotrophic active biomass concentrations in the parent system were calculated using Eq (5.4) and the values are listed in Table 5.5 (Z_{BH} - Measured ML).

Determining the maximum specific growth rates

From the analysis of an $OUR_{H(t)}$ versus time plot, the maximum specific growth rates on slowly biodegradable COD (K_{MP}) and readily biodegradable COD (μ_H) can be calculated. Although a significant fraction of the slowly biodegradable COD (SBCOD) is associated with the particulate matter that was removed through flocculating and filtering the wastewater, it has to be assumed that the filtered wastewater will contain some "soluble" SBCOD. Thus, up to the precipitous drop in OUR_H (Fig 5.4), the OUR is the sum of both the SBCOD and RBCOD utilization. When the precipitous drop in OUR_H occurs, the RBCOD is depleted and the OUR is that for SBCOD only.

If the precipitous drop in OUR_H occurs at t = s hours at which time the OUR is $OUR_{SBCOD(t=s)}$, the maximum specific growth rate on SBCOD (K_{MP}) can be calculated via Eq (3.18):

$$K_{MP} = \frac{OUR_{SBCOD(t=s)} \cdot 24}{\frac{1-Y_{ZH}}{Y_{ZH}} \cdot Z_{BH(0)} \cdot e^{(\mu_H + K_{MP} - b_H) \cdot (t=s)/24}}$$

The overall growth rate ($\mu_H + K_{MP} - b_H$) in Eq (3.18) is given by the slope of the $\ell n(OUR_H)$ plot in Fig 5.5. For the UCT model the value for the maximum specific growth rate on RBCOD (μ_H) can be calculated from the value for K_{MP} derived above and the slope of the $\ell n(OUR_H)$ versus time plot, as given by Eq (3.19):

$$\mu_H = \text{slope} \cdot 24 - K_{MP} + b_H$$

For each batch test, the values for K_{MP} and μ_H were calculated using Eq (3.18) and Eq (3.19) respectively. The results are given in Table B1, Appendix B, and are summarized in Table 5.5 below.

- From Table 5.5 it is evident the K_{MP} values are of the same magnitude as the μ_H values. Statistical plots for μ_H and K_{MP} were constructed, see Figs 5.10 and 5.11 respectively. From the plots, the *mean* K_{MP} and μ_H values calculated for all the batch tests came to exactly the same value of 0.84 / d for both parameters. The K_{MP} and μ_H values were significantly lower than the growth rates measured in the ML+WW batch tests evaluated in Chapter 4. These observations provide valuable support for the postulation that the wastewater heterotrophic active biomass exhibited a much faster overall growth rate which dominated the OUR response of the ML+WW batch tests; see Chapter 4, section 4.4.2.

Table 5.5 : Results from modified batch tests with a mixture of filtered wastewater (WW) and mixed liquor (ML) : Batch test numbers, dates of batch tests, volumes added, COD recoveries, max. specific growth rates, *measured* heterotrophic active biomass present the start of the batch test ($Z_{BH(0)}$) and the projected parent system mixed liquor (ML) active biomass. Also shown are the *theoretical* parent system mixed liquor (ML) heterotrophic active biomass and the projected active biomass present the start of the batch test.

MODIFIED BATCH TESTS : FILTERED WASTEWATER (WW) + MIXED LIQUOR (ML)										
Sew. Batch no.	Batch test no.	Date of test	Volume (ℓ)		COD recov. (%)	Max. specific growth rates (/d)		$Z_{BH(0)}$ (mgCOD/ℓ)			
								Measured		Theoretical	
			ML	WW		K_{MP}	μ_H	Batch test	ML	ML	Batch test
21	F1	14-03	0.20	2.80	104.5	1.15	0.74	121	1821	1540	103
	F2	15-03a	0.10	2.90	114.2	1.06	0.74	81	2418	1540	51
	F3	15-03b	0.15	2.85	102.7	0.84	1.28	50	1009	1540	77
	F4	20-03a	0.35	2.65	115.0	0.67	0.47	276	2369	1540	180
	F5	20-03b	0.40	2.60	95.0	0.90	0.91	184	1386	1540	205
	F6	21-03a	0.25	2.75	93.8	1.50	0.89	90	1077	1540	128
	F7	21-03b	0.30	2.70	100.0	0.94	0.70	161	1613	1540	154
22	F8	28-03	0.35	2.65	94.3	0.68	0.67	210	1800	1516	177
	F9	29-03a	0.30	2.70	95.0	0.65	0.82	170	1700	1516	152
	F10	29-03b	0.40	2.60	106.6	0.66	0.60	239	1791	1516	202
	F11	30-03a	0.20	2.80	96.3	0.41	0.66	156	2345	1516	101
	F12	30-03b	0.25	2.75	100.0	0.57	0.77	148	1780	1516	126
	F13	04-04a	0.10	2.90	100.8	1.03	1.07	29	857	1516	51
	F14	04-04b	0.15	2.85	105.7	0.74	0.89	62	1248	1516	76
23	F15	10-04a	0.20	2.80	89.1	0.83	1.10	86	1292	1820	121
	F16	10-04b	0.25	2.75	94.0	0.79	0.73	116	1396	1820	152
	F17	11-04a	0.10	2.90	90.2	0.72	1.03	52	1549	1820	61
	F18	11-04b	0.15	2.85	108.4	0.91	0.86	69	1370	1820	91
	F19	12-04a	0.30	2.70	99.9	1.14	0.98	111	1106	1820	182
	F20	12-04b	0.35	2.65	104.3	0.54	0.79	212	1815	1820	212

5.4 COMPARISON BETWEEN MEASURED AND THEORETICAL ACTIVE BIOMASS

In Table 5.5 the *measured* heterotrophic active biomass concentration at the start of each modified batch test is compared with the *theoretical* heterotrophic active biomass concentration contained in the mixed liquor sample drawn from the parent system, predicted via the steady state design model. To illustrate the comparison, the measured versus theoretical mixed liquor heterotrophic active biomass data for all the modified batch tests are shown plotted in Fig 5.7. In Fig 5.7 the comparative data for the batch test results rejected on the basis of poor %COD recovery (batch tests F2 and F4) are also shown. Both rejected batch tests yielded %COD recoveries > 110 % and it is clear from Fig 5.7 that for these two batch tests poor agreement was obtained between the theoretical and measured results.

The comparisons for batch tests conducted during sewage batch 23 also show poor agreement between the measured and theoretical results; the poor correlation seems to be accentuated with increasing volumes of mixed liquor used in the batch tests. The fact that the measured values were consistently lower indicate that the high theoretical active fraction (f_{av}) calculated in section 5.3.1 for sewage batch 23, may indeed constitute an over-prediction of the "true" heterotrophic active biomass concentration in the parent system. As explained in section 5.3.1, the high f_{av} value resulted from inconsistent sampling techniques to determine the VSS concentration in the parent system.

Accepting that the f_{av} value calculated for sewage batch 23 did not yield a valid representation of the "true" active biomass concentration in the parent system, it was decided to compare the active biomass concentrations measured in the batch tests conducted during sewage batch 23, with the average theoretical values obtained for the two preceding sewage batches 21 and 22. In Table 5.6 below, the theoretical heterotrophic active biomass concentration in the parent system for sewage batch 23 was estimated using the *average* parent system Z_{BH} values calculated for sewage batches 21 and 22 in Table 5.5 i.e. (1540 + 1516)/2 = 1528 mgCOD/*l*.

Based on this average mixed liquor concentration in the parent system, the theoretical heterotrophic active biomass concentration in the batch reactor was accordingly calculated for each modified batch test conducted during sewage batch 23. The "assumed" values were calculated using Eq (5.2) and are listed in the right hand side column of Table 5.6 below (Z_{BH} - Theoretical * Batch test):

Table 5.6 : Results for modified batch tests conducted during sewage batch no. 23. Note the *revised* estimations for the theoretical Z_{BH} present in the parent system mixed liquor (ML) and the projected Z_{BH} present the start of each batch test. Revised values based on the average parent system Z_{BH} values calculated for sewage batches 21 and 22 in Table 5.5.

MODIFIED BATCH TESTS : FILTERED WASTEWATER (WW) + MIXED LIQUOR (ML)											
Sew. Batch no.	Batch test no.	Date of test	Volume (l)		COD recov. (%)	Max. specific growth rates (/d)		$Z_{BH(0)}$ (mgCOD/l)			
								Measured		Theoretical *	
			ML	WW		K_{MP}	μ_H	Batch test	ML	ML	Batch test
23	F15	10-04a	0.20	2.80	89.1	0.83	1.10	86	1292	1528	102
	F16	10-04b	0.25	2.75	94.0	0.79	0.73	116	1396	1528	127
	F17	11-04a	0.10	2.90	90.2	0.72	1.03	52	1549	1528	51
	F18	11-04b	0.15	2.85	108.4	0.91	0.86	69	1370	1528	76
	F19	12-04a	0.30	2.70	99.9	1.14	0.98	111	1106	1528	153
	F20	12-04b	0.35	2.65	104.3	0.54	0.79	212	1815	1528	178

The comparison between the "revised" theoretical values obtained in Table 5.6 and the heterotrophic active biomass concentrations measured at the start of batch tests F15 – F20, is shown plotted in Fig 5.8.

- Excluding the rejected batch test results, a reasonably good agreement exists between the measured and theoretical values. The "revised" theoretical values for sewage batch 23 compare consistently better with the measured values, indicating that the high f_{av} value obtained for sewage batch 23 was a misrepresentation of the "true" heterotrophic active biomass fraction in the parent system.

- Although the good correlation between the theoretical and measured values in Fig 5.8 is consistent throughout the range of mixed liquor volumes used in the batch tests, individual data points tend to exhibit some variation from the 100 % correlation line. This variation can be attributed to the sensitivity of the measured heterotrophic active biomass concentration, calculated via Eq (3.9), to the slope of the $ln(OUR_H)$ plots (Fig 5.5). In linear regression fitting lines to the $ln(OUR_H)$-time plot, low values were obtained for the $ln(OUR_H)$ slope. With these low values, even the smallest changes in the slope (magnitude ~ 0.05) can result in marked variations in the heterotrophic active biomass concentration values calculated with Eq (3.9).

To demonstrate the conviction that the modified batch test results provide substantiated support for the heterotrophic active biomass hypothesized in the kinetic simulation model *as well as* the steady state design, a statistical plot of the *projected* heterotrophic active biomass concentration (Z_{BH}) in the parent system was constructed; see Fig 5.9.

The projected values were calculated from the measured values obtained in all the batch tests, using Eq (5.4). The projected values are listed in Table 5.5. The statistical plot (Fig 5.9) represent the results obtained in all the modified batch tests, including the rejected results from batch tests F2 and F4.

- From the statistical plot the projected values appear to be normally distributed with a *mean* projected Z_{BH} of 1587 mgCOD/ℓ and a sample standard deviation of 448 mgCOD/ℓ.

- The mean Z_{BH} value of 1587 mgCOD/ℓ compares remarkably closely with the theoretical steady state design value of 1567 mgCOD/ℓ, calculated for the parent system with $f_{S,us}$ = 0,086 and $f_{S,up}$ = 0,114 (From Chapter 2 - mean wastewater fractions obtained for sewage batches 1 - 23).

- The rather large sample standard deviation value of 448 mgCOD/ℓ underlines the variation associated with the sensitivity of the batch test analysis (described above).

- The remarkable correlation between the projected Z_{BH} mean value, obtained from the modified batch test results, and the theoretical Z_{BH} steady state design value appear to provide substantiated support for the modified batch test results, and the accuracy of the steady state design model to predict the hypothesized value for the heterotrophic active biomass concentration in activated sludge systems.

5.5 CLOSURE

This chapter describes the modifications to the experimental batch test procedure, the interpretation of the recorded data and the evaluation of the batch test results. The following aspects and conclusions are of significance:

- The modifications to the batch test procedure developed by Ubisi *et al.* (1997a,b) involve the physical removal (through pre-filtration) of OHO active biomass from the diluted wastewater used in the batch tests and the adoption of the exponential approach towards nitrification, where it was accepted that the nitrate concentration – time profile follows an exponentially increasing trend, as opposed to a linear increase.

- Separate aerobic batch tests were conducted using only the flocculation filtered wastewater. The fact that no measurable OUR was observed, indicate that the flocculation filtration procedure (using alum flocculation and filtration through a glass fibre filter) effectively removed all active biomass from the wastewater.

- In general, the modified batch tests yielded good %COD recoveries with only 2 out of 20 batch tests rejected on the basis of %COD recovery > 110%. The mean %COD recovery for all the batch tests was 100.5% with sample standard deviation of 7.3%. The good %COD recoveries lend credibility to the measurements and the modified batch test procedure.

- Excluding the rejected batch test results, good agreement existed between the theoretical heterotrophic active biomass concentration (Z_{BH}) and the Z_{BH} values measured in the modified batch tests. The mean Z_{BH} value (1587 mgCOD/ℓ) measured in the batch tests compares remarkably closely with the theoretical steady state design value of 1567 mgCOD/ℓ calculated for the parent system.

- Despite the remarkable agreement between the theoretical Z_{BH} and the mean of the measured Z_{BH} values, the individually measured Z_{BH} values were prone to significant variation. This was attributed mainly to the sensitivity of the measured heterotrophic active biomass values, to the low values measured for the slopes of the $\ln(OUR_H)$-time plots. Even a small change in the slope of the $\ln(OUR_H)$-time plot resulted in a marked variation of the measured Z_{BH} values. The slope of the $\ln(OUR_H)$-time plot was low because the maximum specific growth rates for the heterotrophs were low (mean = 0.84 / d).

The good correlation that exists between the theoretical and measured values in Fig 5.8 is remarkable, considering the sensitivity of the analysis. The results appear to substantiate the modified batch test method as a reliable means of quantifying the heterotrophic active biomass in terms of the mathematical formulations derived from the UCT kinetic simulation model. The results also underline the importance of incorporating the exponential approach to nitrification in the interpretation of the batch test results.

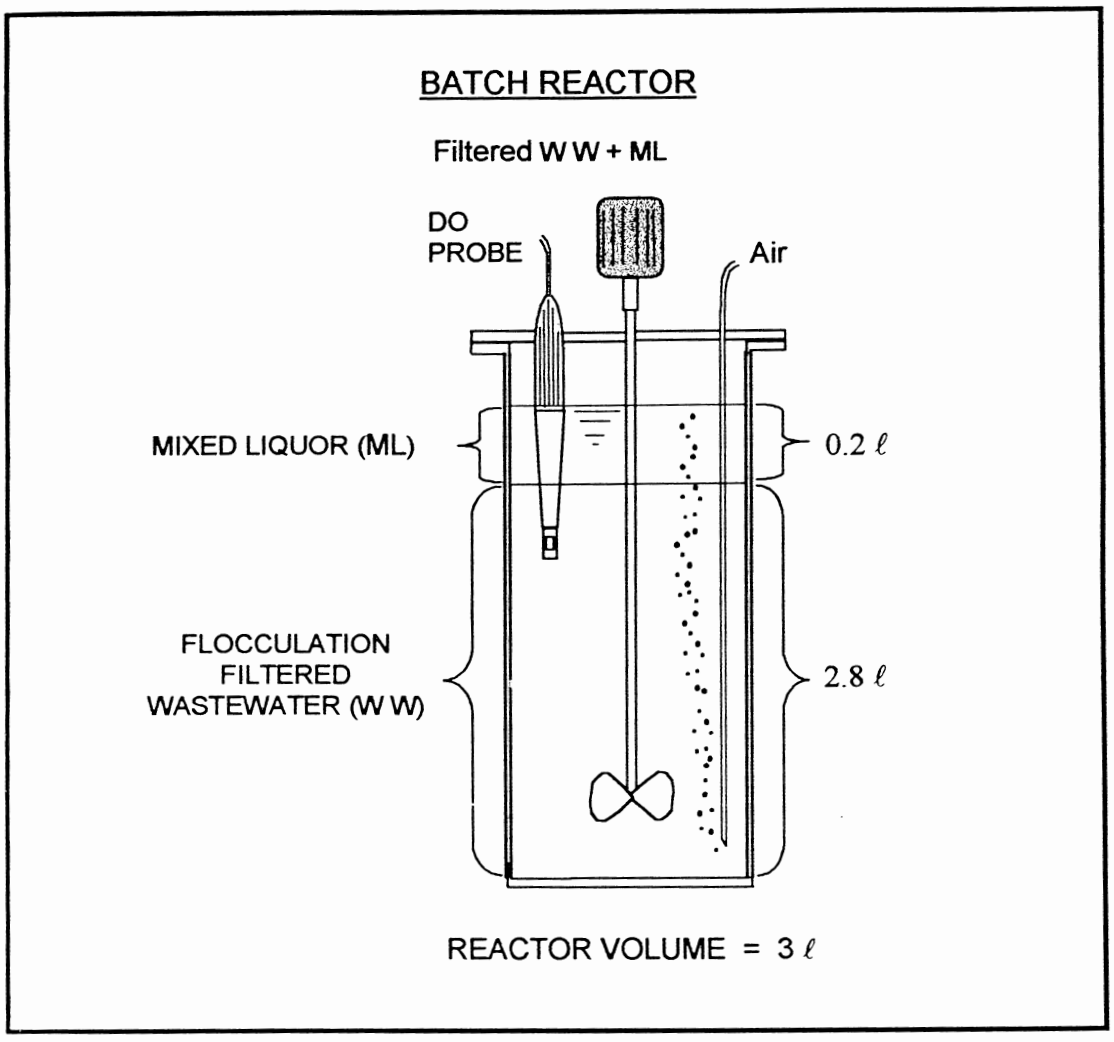


Figure 5.1: Single batch reactor arrangement used to conduct modified batch tests with mixed liquor added to filtered wastewater.

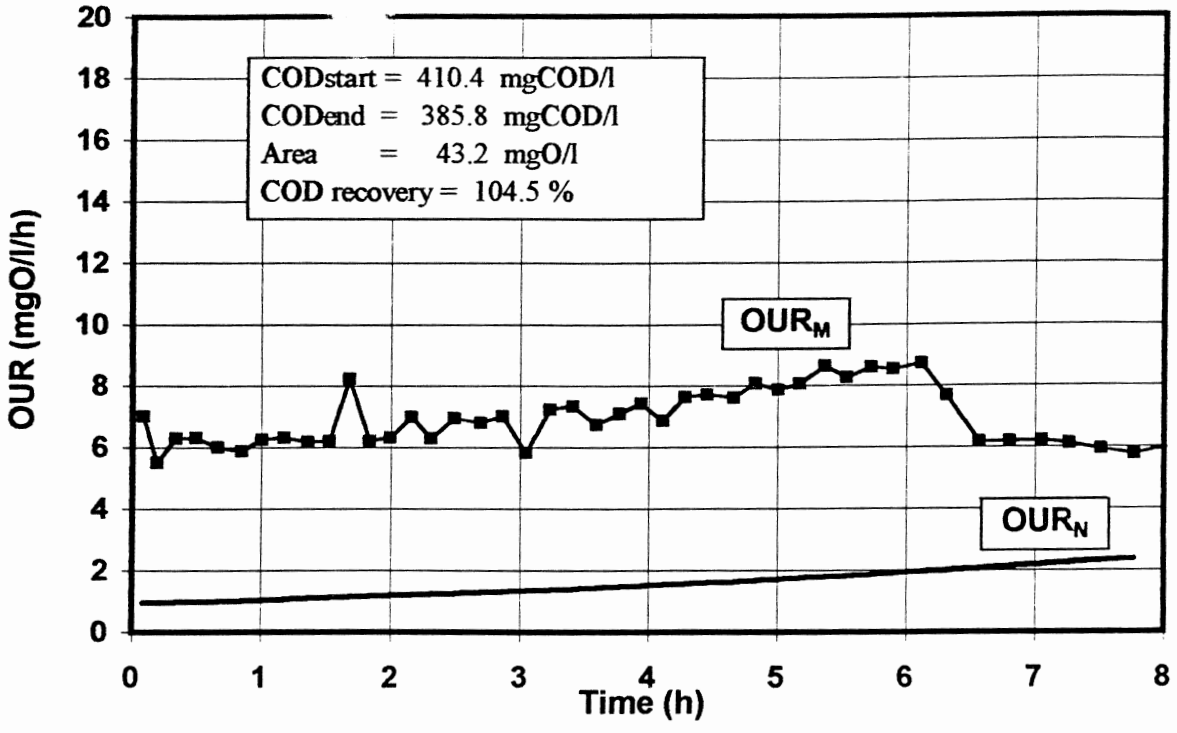


Figure 5.2 : Oxygen utilization rate (OUR) response with time for a modified batch test on a mixture of filtered wastewater (2.8ℓ) and mixed liquor (0.2ℓ) drawn from the aerobic reactor of the parent system. Batch test no. F1, 14-03, sewage batch no.21.

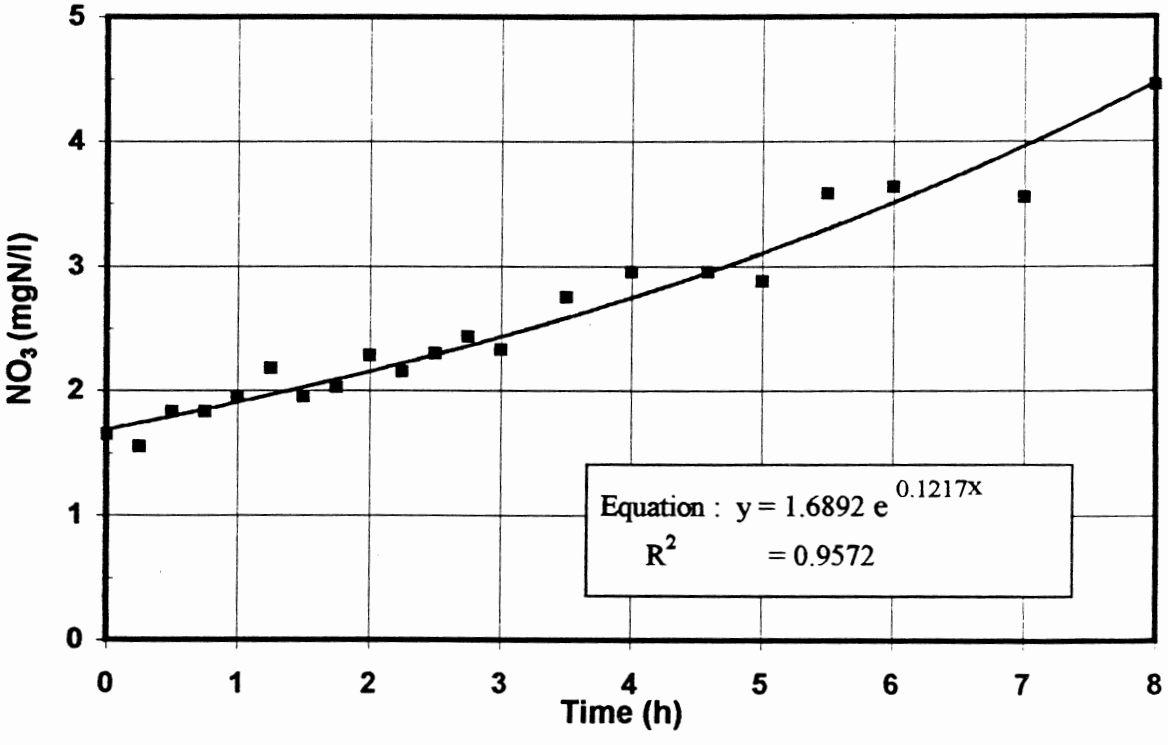


Figure 5.3 : Nitrate variation with time for the modified batch test in Fig 5.2 . Also shown is the exponential expression and correlation coefficient (R^2) , obtained through regression . The nitrification OUR, calculated from $OUR_N = 4.57*(\Delta NO_3/\Delta t)$, is shown in Fig 5.2.

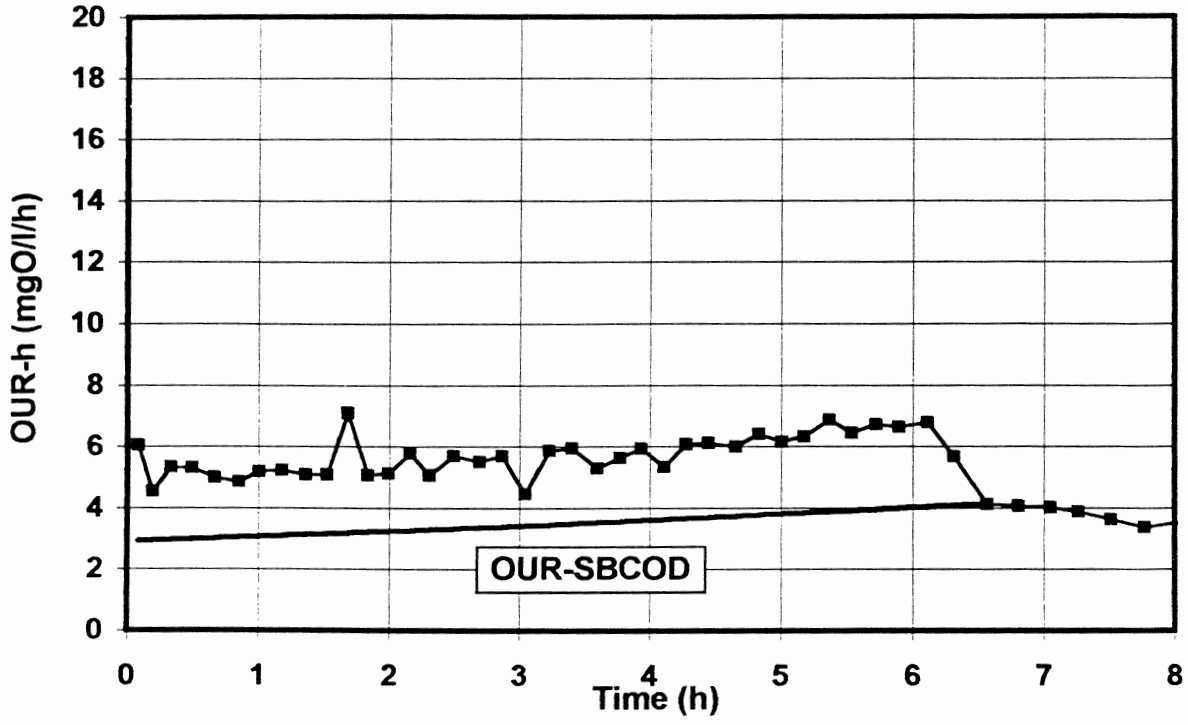


Figure 5.4 : Oxygen utilization rate due to heterotrophic active biomass (OUR-h) versus time for modified batch test no. F1, 14-03 , sewage batch no.21. The OUR due to nitrification (OUR_N) was subtracted from the measured OUR data in Fig. 5.2.

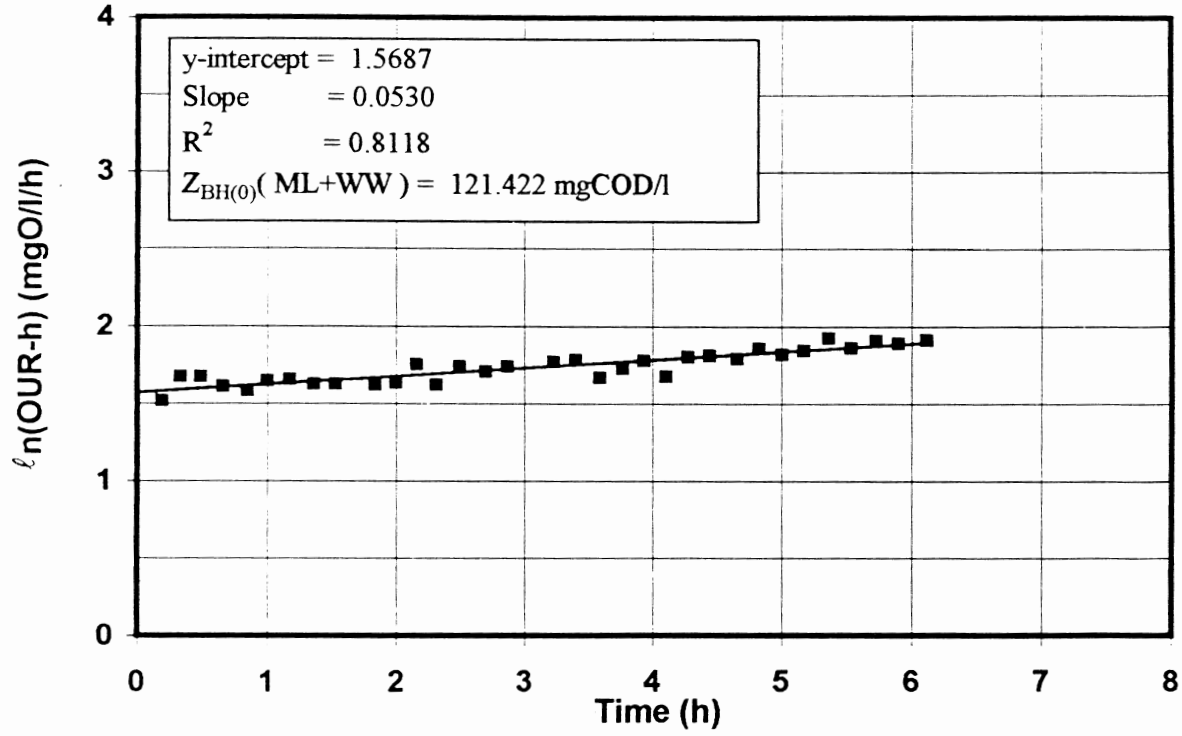


Figure 5.5 : ℓn oxygen utilization rate due to heterotrophic active biomass (ℓn OUR-h) versus time for the OUR-h data in Fig. 5.4 , up to the precipitous drop in OUR. Batch test no. F1 , 14-03 , sewage batch no.21.

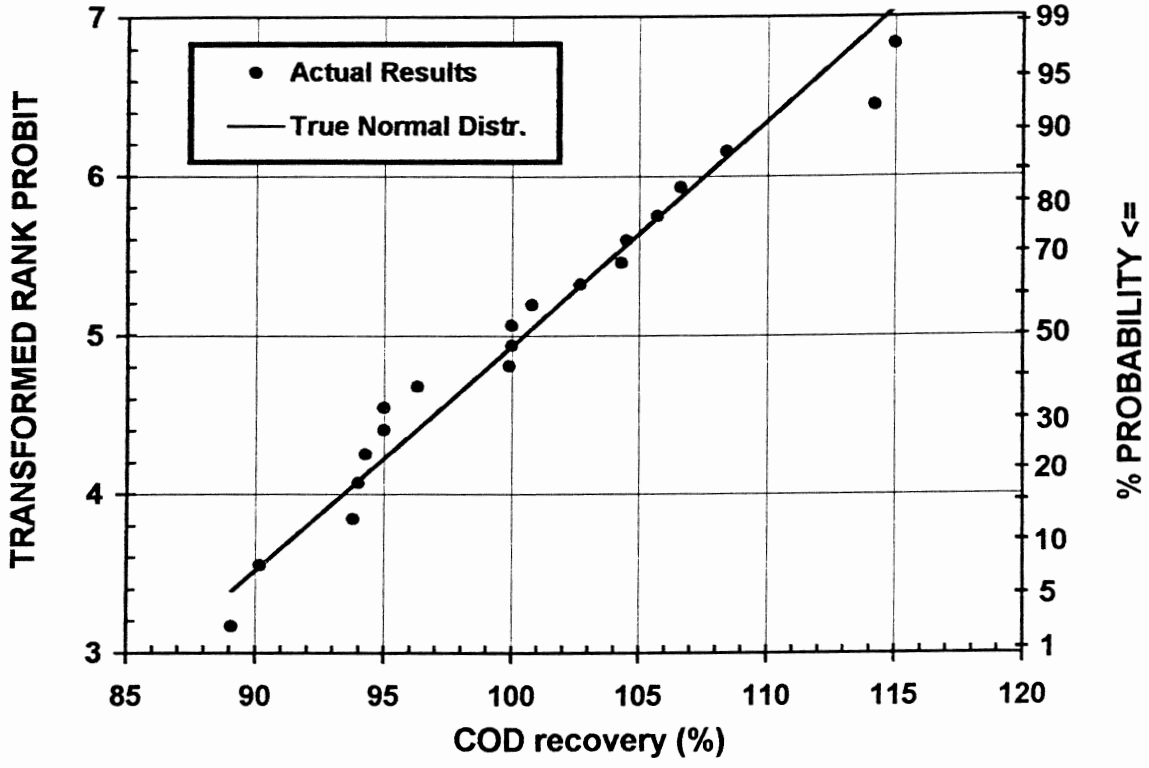


Figure 5.6 : Statistical plot of %COD recovery for all the modified batch tests conducted with filtered wastewater and mixed liquor.

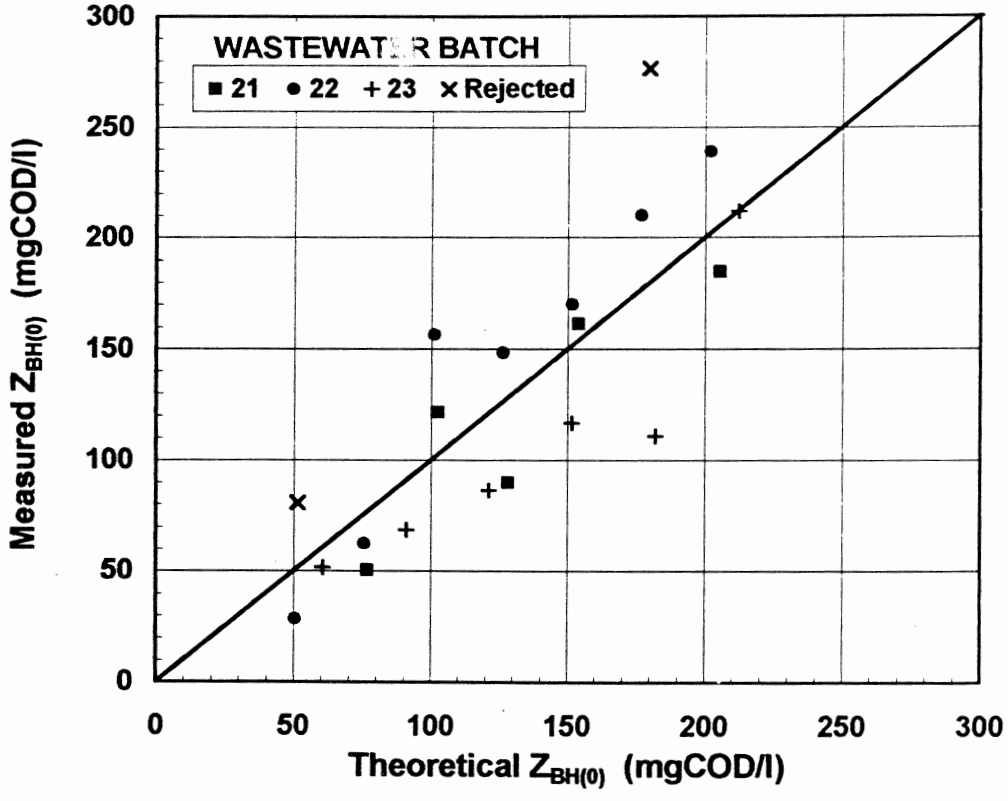


Figure 5.7 : Modified batch test results : graph of measured versus theoretical active biomass $Z_{BH(0)}$(ML) for the various wastewater batches with the parent laboratory-scale system operated at 10 days sludge age.

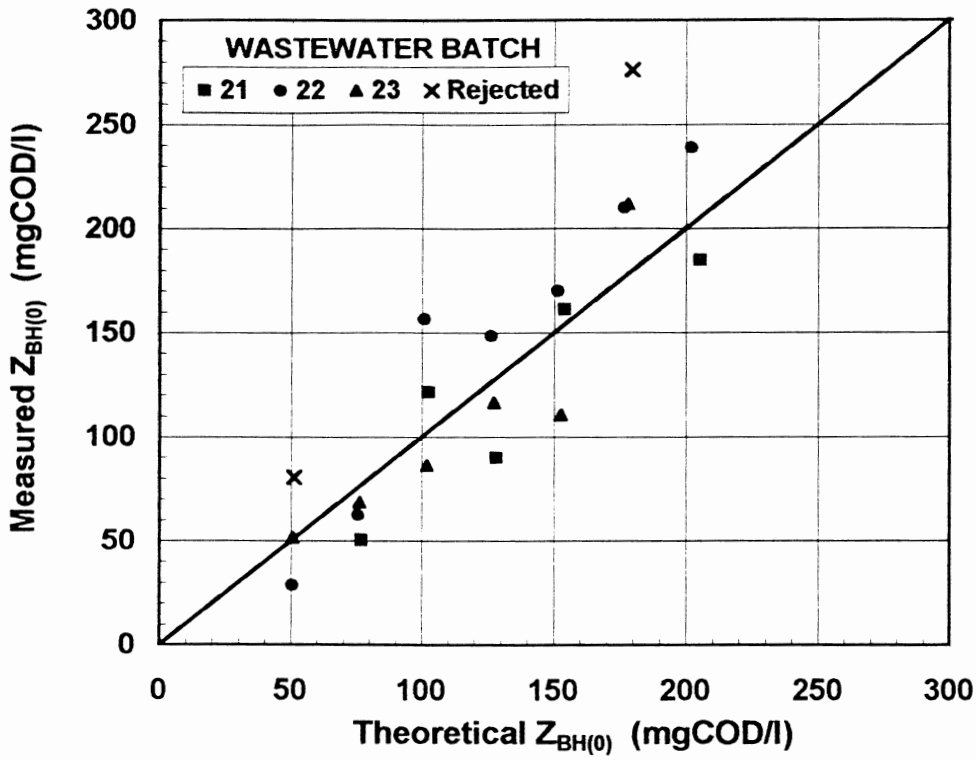


Figure 5.8 : Modified batch test results : graph of measured versus theoretical active biomass $Z_{BH(0)}$(ML) using theoretical ML values for sewage batch no. 23 based on average active fractions calculated for sewage batches 21 and 22.

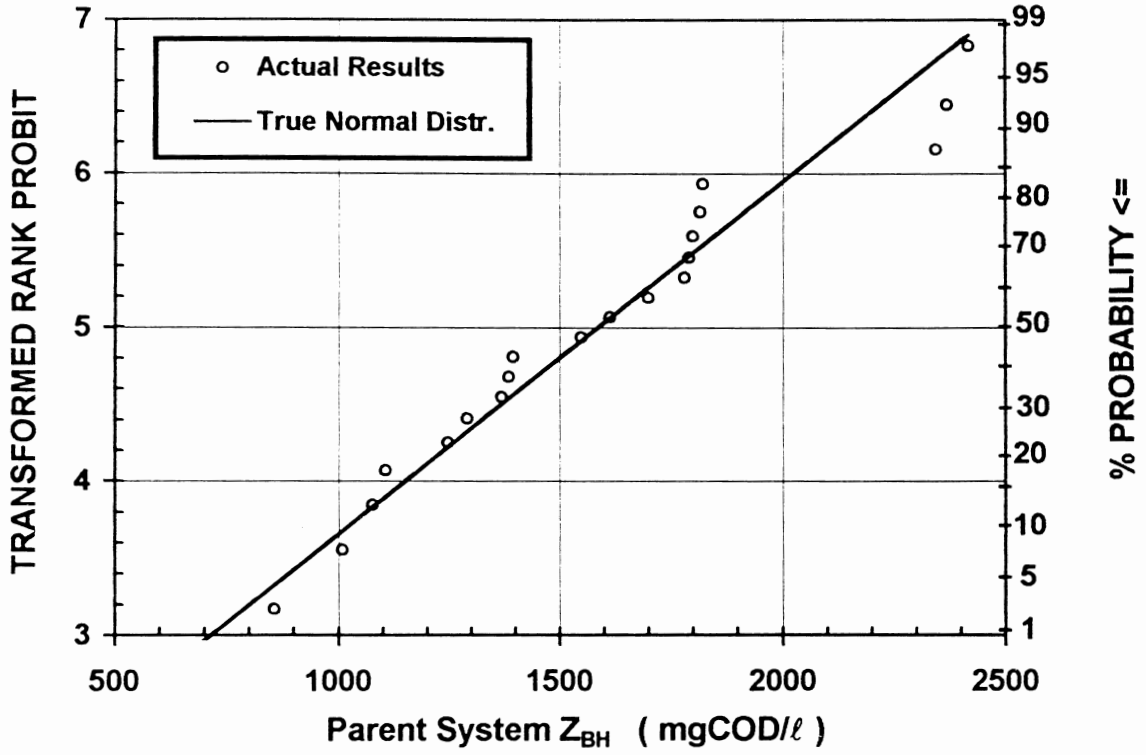


Figure 5.9 : Statistical plot of the projected heterotrophic active biomass concentration (Z_{BH}) present in the parent system operated at a sludge age of 10 days.

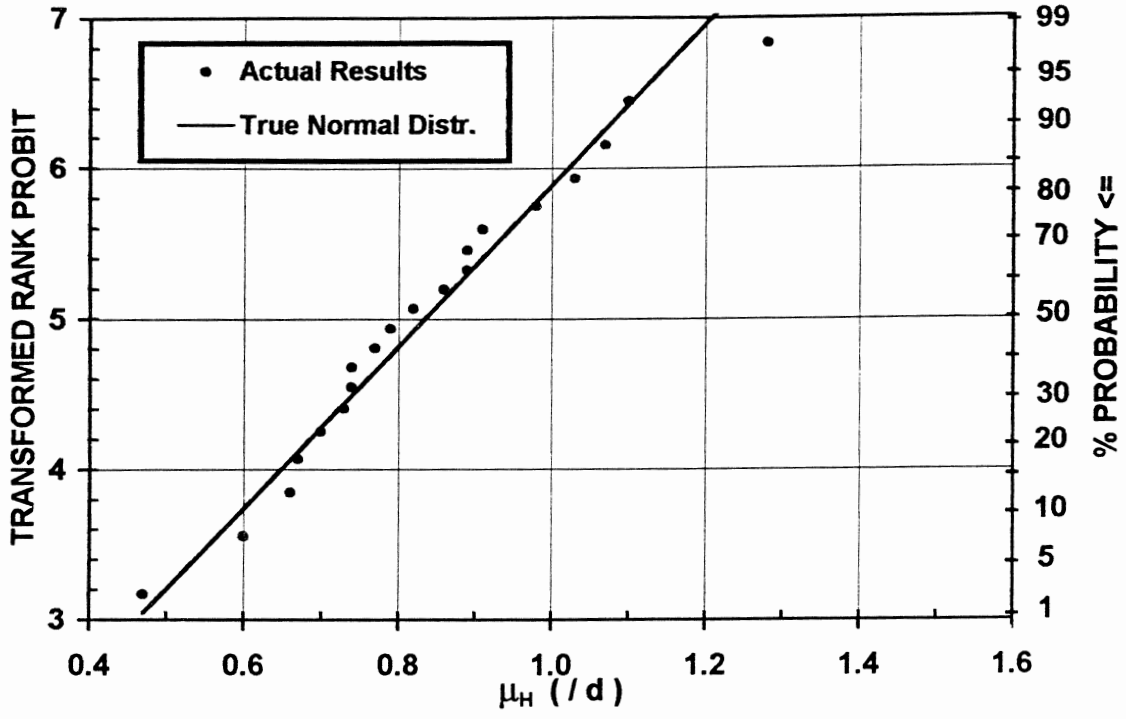


Figure 5.10: Statistical plot of μ_H values measured for all the modified batch tests

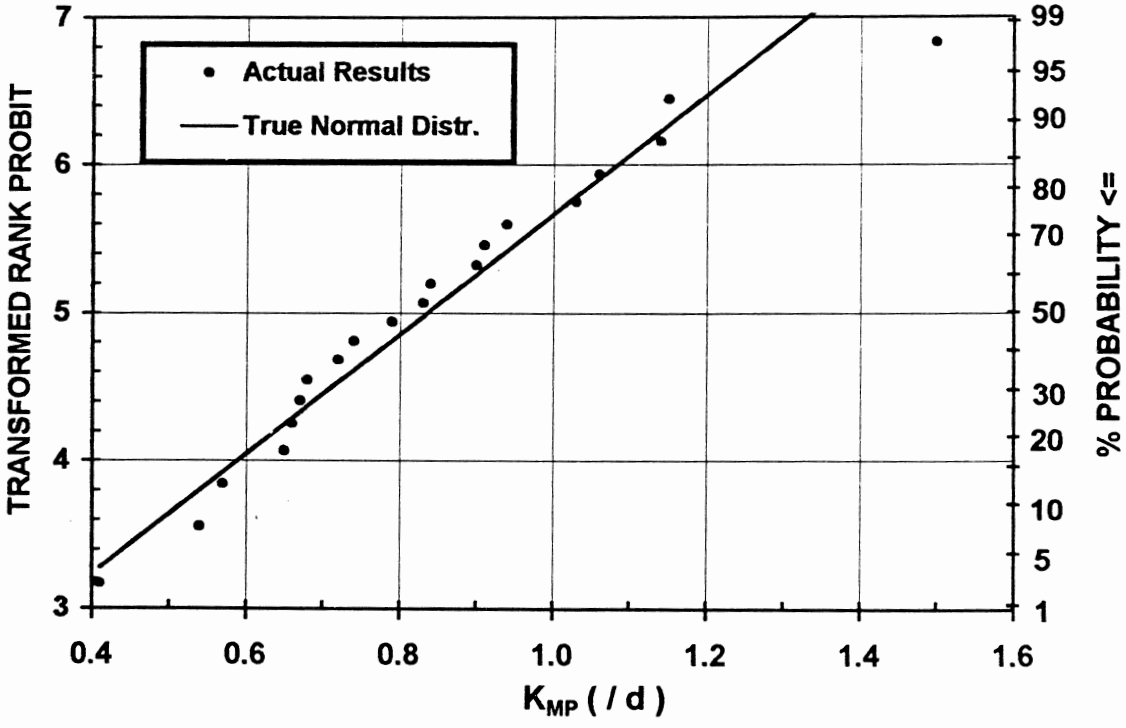


Figure 5.11: Statistical plot of K_{MP} values measured for all the modified batch tests

CHAPTER 6

EXTENSION TO THE MODIFIED BATCH TEST INVESTIGATION :

THE PARENT LABORATORY-SCALE INTERMITTENTLY FED FILL AND DRAW (IFFD) ACTIVATED SLUDGE SYSTEM

6.1 INTRODUCTION

It was concluded in Chapter 5 that the modified batch test method proved to be a reliable means of determining the heterotrophic active biomass concentration present in a parent laboratory-scale nitrification/denitrification activated sludge system. This conclusion was based on the good correlation that existed between the active biomass measured in the batch tests and the theoretical active biomass present in the parent system, calculated on the basis of the steady state design model. However in evaluating the batch test procedure (Chapter 5), it was noted that the maximum specific growth rate for the heterotrophic organisms in the parent system mixed liquor was low (average = 0.84 /d). This caused the slope of the best fit linear line to the ℓn OUR-time data to be low, which resulted in the estimate of heterotrophic active biomass concentration being very sensitive to small changes in the slope of the linear line fit. Accordingly, if the maximum specific growth rate of the heterotrophs can be increased, then the sensitivity of the estimate for heterotrophic active biomass concentration to the slope of the best fit linear line should correspondingly decrease. It was decided to extend the research project to investigate this aspect.

The first task was to identify systems that would stimulate heterotrophic maximum specific growth rates that would be significantly higher than those in the continuously fed parent nitrification/denitrification activated sludge system. Many investigators have shown that sludges subjected to different COD feeding patterns exhibited significantly different characteristics. In particular, Jenkins *et al.* (1983) demonstrated that alternating feed-starve conditions induced in intermittently fed fill and draw (IFFD) systems, promoted higher soluble COD and oxygen uptake rates than do continuously fed activated sludge systems. Ekama *et al.* (1986) found that the higher oxygen uptake rate (OUR) was related to the higher heterotroph maximum specific growth rate on readily biodegradable COD observed in IFFD systems. Thus, the overall growth rate of heterotrophic organisms subjected to IFFD conditions can be expected to be significantly higher than the low growth rate of the heterotrophic organisms observed in the continuously fed MLE activated sludge system from which mixed liquor samples were drawn to evaluate the modified batch tests, described in Chapter 5.

Accordingly, to evaluate the effect of increased heterotroph maximum specific growth rates on the batch test procedure, it was decided to operate a steady state parent laboratory-scale IFFD system at a sludge age of 10 days, receiving the same wastewater as the continuously fed parent system described in Chapter 2. This system would provide the source mixed liquor for addition to the batch tests. In addition to providing the means to evaluate the effect of increased heterotroph maximum specific growth rate on the batch tests procedure, this investigation would also allow evaluation of the batch test method conducted on mixed liquors drawn from systems that are not of a completely mixed reactor configuration. This chapter describes the operation of the parent laboratory-scale IFFD system, and details the response of the system. Details of the batch test procedure and results are set out in Chapter 7.

6.2 PARENT SYSTEM CONDITIONS

The parent laboratory-scale IFFD activated sludge system was operated to supply mixed liquor samples for the modified batch tests (see Chapter 7), to measure the heterotrophic active biomass. In accordance with the steady state design model, the same quantity of heterotrophic active biomass present in a continuously fed system will be established in an IFFD system if the sludge is contained in the same volume and the same mass of biodegradable COD is introduced daily with the same wastewater as influent.

Accordingly, a single aerobic reactor of 10ℓ volume was used (see Fig. 6.1). For the continuously fed parent system (Chapter 2) the influent was introduced at a constant rate of 20 ℓ/d and the COD concentration was controlled at $\pm$ 500 mgCOD/ℓ. To achieve approximately the same daily COD loading (10 000 mgCOD/d), the raw wastewater was diluted to $\pm$ 1000 mgCOD/ℓ and two equal 5ℓ volumes of diluted wastewater was fed twice daily to the system, resulting in a total daily feed volume of 10ℓ/d.

The reactor contents were completely mixed by means of independent stirring, and the reactor was aerated by passing low pressure air through a small bore Perspex tube which terminated at the bottom of the tank (see Fig 6.1). The dissolved oxygen (DO) concentration was measured by means of a DO probe immersed in the solution. The oxygen supply was controlled by an automatic DO meter / OUR logger which also measured the oxygen utilization rate (OUR), using an automated technique (Randall *et al.*, 1991). A typical OUR response from the IFFD system, recorded during a 24 hour period, is shown in Fig 6.2.

The same sewage was used to feed the parent IFFD system and was collected in batches from the Mitchell's Plain Treatment Works. The sewage batches were changed every $\pm$ 2 weeks to prevent degradation under storage. To attain steady state conditions, the system was operated for 3 sewage batches (> 2 sludge ages) before testing was commenced. A total of 5 batches of sewage were fed to the parent system after testing commenced. These sewage batches and the dates they were used as feed are listed in Table 6.1. Also indicated are the sewage batches during which mixed liquor samples were drawn from the system for batch tests. To evaluate the OUR response for different COD loading patterns, the volumes of the wastewater fed twice daily were changed from 5ℓ : 5ℓ to 2.5ℓ : 7.5ℓ during the latter part of the investigation.

In addition, the raw wastewater COD measured for sewage batch 28 was significantly less than 1000 mgCOD/*l*, resulting in an increased daily feed volume of 12.5 *l* to attain the required daily COD mass of 10 000 mgCOD/d. In Table 6.1 reference is given to the periods when the revised feeding patterns were implemented.

From 31 May 2000 to 5 June 2000 (sewage batch 26) no testing was done on the parent system. However, during this period the system was operated and maintained by the technical staff of the Water Research Laboratory.

Table 6.1: Details of the parent laboratory-scale intermittently fed fill and draw (IFFD) activated sludge system; sewage batch number, sewage feed dates, days of operation, batch tests conducted and daily volumes of wastewater fed to system.

Sewage Batch No.	Date of tests (2000)	Days of operation (d)	Batch Tests	Feed volumes	
				am	pm
24	24 Apr – 5 May	day 1 – 10	No	5.0 *l*	5.0 *l*
25	6 May – 25 May	day 11 – 30	Yes	5.0 *l*	5.0 *l*
26	26 May – 5 Jun	day 31 – 41	No	2.5 *l*	7.5 *l*
27	6 Jun – 19 Jun	day 42 – 55	Yes	2.5 *l*	7.5 *l*
28	20 Jun – 23 Jun	day 56 – 59	Yes	5.0 *l*	7.5 *l*

6.3 WASTEWATER COLLECTION AND STORAGE

The influent for the parent laboratory-scale IFFD activated sludge system was raw (unsettled) wastewater from the Mitchell's Plain Treatment Plant in Cape Town (South Africa). This sewage is primarily domestic, with a small (< 25%) industrial component. The sewage was collected in batches from the head of the works, after the course screens, but before the fine screens, the grit removal and primary sedimentation tanks.

The sewage batch was brought to the laboratory and stored in 400 *l* stainless steel tanks in a cold room at 4°C for 10 to 14 days, then discarded and a new batch of sewage collected; experience has shown that storage of sewage for periods longer than 3 weeks leads to hydrogen sulfide build-up in the tanks and a change in the characteristics of the sewage. For each new batch of sewage, immediately after storage in the cold room a COD test was done to determine the COD concentration required for subsequent dilution (see below). The COD of the undiluted sewage usually ranged from 1 000 to 1 500 mgCOD/*l*.

6.4 SYSTEM MAINTENANCE AND OPERATION

In the absence of a continuous rate of feeding and effluent discharge, the operation and maintenance of the intermittently fed fill and draw (IFFD) system was based on a daily routine of operational tasks, performed in a designated sequence to maintain steady state conditions. The different tasks will be described in the sequence that they were performed daily:

6.4.1 Downloading OUR data

The sequence was initiated by downloading to a PC the oxygen utilization rate (OUR) data, recorded when the system was feeding on the previous day's feed. The OUR in the reactor was measured continually by using an automated technique (Randall *et al.*, 1991): A dissolved oxygen (DO) probe (YSI) from an automatic DO meter/OUR logger (Hi Tech Microsystems) was immersed in the mixed liquor. The low and high DO set point of the meter were 2.0 and 4.5 mgO/*l* respectively: When the DO reached ± 4.5mgO/*l* the air switched off automatically and the decrease in DO with time was monitored; when the DO reached ± 2.0 mgO/*l*, the air was switched on again automatically and the cycle repeated. Automatically, for each cycle the slope of the DO-time data during the air off period was determined by linear regression; this gives the OUR, which was stored by the meter (together with regression analysis and time data). OUR results with a regression correlation coefficient less than 0.99 (R^2 = 0.99) were rejected. The number of OUR readings ranged between 120 and 150 per day and a typical OUR response is shown in Fig 6.2. After downloading the OUR data, the OUR meter was reset and data recording for the next 24 hours was commenced automatically.

6.4.2 Sampling from the reactor

To obtain mixed liquor samples that were representative of the steady state conditions, samples were drawn prior to feeding and wasting sludge from the system. As a result of the aeration process, some sludge particles were splashed out of solution and deposited on the reactor wall, above the water surface level. Prior to sampling the internal surfaces of the reactor were thoroughly brushed to dislodge sludge deposits from the tank floor and to re-introduce wall deposits above and below the water surface back into the mixed liquor. After brushing, the following volumes of mixed liquor were drawn for testing:

- A 50 ml sample for TSS, VSS and ISS testing (Standard Methods, 1985).
- A 50 ml sample, diluted 1:10 with distilled water and homogenized in a liquidizer for mixed liquor COD and TKN testing (Standard Methods, 1985).
- A 500 ml mixed liquor sample to perform the Diluted Sludge Volume Index (DSVI) test (Ekama and Marais, 1984).

A total mixed liquor volume of 600 ml was thus drawn from the system for sampling purposes.

6.4.3 Settling and effluent withdrawal

After sampling was completed the aeration was switched off, while the system remained completely mixed. The reason for introducing a mixing period emanated from the fact that the aeration process exhibited a tendency to break up the sludge flocs, compromising the settleability of the sludge particles. A 10 minute mixing period preceding the sludge settling phase, allowed for the re-flocculation of sludge particles which enhanced the sludge settling characteristics. After the 10 minute mixing period had elapsed, the mixer was switched off and the sludge was allowed to settle. After a settling period of ± 30 minutes the clear supernatant (effluent) was siphoned into a clean effluent bucket. An effluent volume of 4.4ℓ was drawn off in this fashion, taking due care not to extract any settled sludge particles.

The total volume of mixed liquor (0.6ℓ) and effluent (4.4ℓ) drawn off consequently amounted to 5ℓ.

6.4.4 Feed preparation

During the 30 minute settling period described above, the wastewater to be used as feed was prepared as follows:

The total COD concentration for the feed to the parent laboratory-scale system was set at 1 000 ± 100 mgCOD/ℓ. Knowing the total COD concentration of the sewage batch collected, volumes of sewage and tap water to dilute the sewage to the required concentration (1 000 mgCOD/ℓ) could be calculated. Daily, the contents of the storage tanks were thoroughly mixed and a volume of sewage was then drawn from the tank: The sewage was drawn from a tap at the bottom of the tank, passed through a 1 mm sieve into a graduated 20 ℓ plastic bucket. Then the appropriate volume of tap water was added to dilute the sewage to the COD concentration required.

The complete nitrification that was achieved under fully aerobic conditions caused a concomitant consumption of alkalinity, resulting in a significant decrease in the parent system pH. The absence of pH recovery (through denitrification) aggravated the situation. A low mixed liquor pH is detrimental to the nitrification process and to prevent the inhibition of nitrification, the influent alkalinity was supplemented by adding a buffer solution of sodium bicarbonate ($NaHCO_3$) to the wastewater, as detailed in section 2.4, Chapter 2. Depending on the intensity of nitrification observed, varying volumes of the buffer solution (50 - 100 mℓ) was added to the 10ℓ diluted wastewater to maintain a minimum pH of ± 7.0 in the parent system.

After thoroughly mixing the 10ℓ of diluted wastewater, the 5ℓ required for the first feed was carefully measured off and stored in a separate 5ℓ container. The remaining 5ℓ of wastewater (for the second feed) was stored in the cold room at 4°C.

6.4.5 Feeding the system

Because the wastewater was brought from storage in the cold room at 4°C, the diluted wastewater in the 5ℓ container was warmed to 20°C in a warm water bath prior to feeding the system. Subsequent to the effluent withdrawal, the 5ℓ of diluted wastewater (at 20°C) was added to the mixed liquor in the reactor. The mixing and aeration was recommenced immediately after the introduction of the wastewater. To ensure that the high initial oxygen demand (refer to Fig 6.2) could be met, the oxygen supply to the system was increased.

The OUR of the system was monitored for substrate depletion. As shown in Fig 6.2 the substrate depletion was evident in the gradual decrease in OUR. In general, the substrate was completely utilized within 6 hours of feeding; this was reflected in the OUR remaining more or less constant and less than 20 mgO/ℓ/h. After ensuring that the substrate from the first feed was completely utilized, the fill and draw sequence described above (sections 6.4.2 to 6.4.5) was repeated for the second feed – using the remaining 5ℓ of wastewater stored earlier.

6.4.6 Sludge age control

The sludge age (R_S) of the system was controlled hydraulically at 10 days, by wasting daily from the aerobic reactor 1ℓ of mixed liquor (including any samples drawn for analysis). On account of drawing 0.6ℓ of mixed liquor from the system for sampling purposes during the first feeding cycle, the remainder of the waste mixed liquor (0.4ℓ) was drawn during the second feeding cycle. When conducting batch tests, the volume of mixed liquor drawn from the reactors usually exceeded the amount to be wasted daily (depending on the volume of mixed liquor used in the batch test): To avoid over wasting, the effluent used in diluting the mixed liquor when conducting the DSVI test was decanted, and the mixed liquor rediluted to the correct volume with effluent and the appropriate volume returned to the system.

6.4.7 Effluent collection

By extracting 0.4ℓ of mixed liquor during the second feeding cycle, 4.6ℓ of effluent was concomitantly drawn off to facilitate the second feed volume of 5. The daily volume of effluent (4.4ℓ + 4.6ℓ = 9.0ℓ) was collected in the effluent bucket and stored for subsequent testing in the cold room at 4°C.

6.5 SAMPLING AND MEASUREMENTS

Daily, the following samples were drawn for analysis:

- ***Influent***: Before the influent was poured into the 10ℓ container, the feed was thoroughly mixed and a 200 mℓ sample was taken in a glass jar and stored at 4°C for analysis the following day. Experience showed that inaccuracies were encountered when the 5 mℓ or 10 mℓ volumes required for the TKN and COD tests were pipetted from the refrigerated 200 mℓ sample the following day. To avoid this, the influent sample was warmed to 20°C just prior to pipetting the samples for COD and TKN testing.

- ***Reactor***: Two 50 mℓ samples as well as a 500 mℓ sample were drawn daily for testing, as described in detail in section 6.4.2.

- ***Effluent***: After retrieving the effluent bucket from the cold room, the contents was thoroughly mixed and a 1ℓ sample was drawn for testing. The effluent sample was warmed to 20°C before testing was commenced.

The following analyses were performed on the samples daily:

1. COD and TKN (Standard Methods, 1985) on the unfiltered influent sample.
2. COD and TKN on the unfiltered effluent sample.
3. COD and TKN on the filtered effluent sample (filtered through 0.45 μm filter paper).
4. COD and TKN on the unfiltered mixed liquor sample.
5. Oxygen utilization rate (OUR) in the reactor (see section 6.4.1 above).
6. pH of the reactor.
7. DSVI (Ekama and Marais, 1984) on the mixed liquor sample.
8. Nitrate and nitrite concentration (Technicon Auto Analyser) on the filtered effluent sample (filtered through 0.45 μm filter paper).
9. Total suspended solids (TSS), volatile suspended solids (VSS) and inorganic suspended solids (ISS) on the mixed liquor sample (Standard Methods, 1985).

Previous experience showed that varying analytical results were obtained during the first two days after the introduction of a new sewage batch. These variations were ascribed to the phenomenon that the active organisms require a certain period to adapt to the changed sewage characteristics of a new sewage batch. As a result, no testing (apart from the influent) was done during the first two days after the introduction of a new sewage batch.

The daily tests conducted on the parent laboratory-scale IFFD system are summarized in Table 6.2.

Table 6.2: Daily tests conducted on parent IFFD steady state system.

Test	Influent	Reactor	Effluent
COD	♦	♦	♦○
TKN	♦	♦	♦○
Nitrate			○
Nitrite			○
OUR		□	
TSS		×	
VSS		×	
ISS		×	
pH		□	
DSVI		□	

♦ Unfiltered sample
○ Sample filtered through 0.45 μm filter paper
□ Direct measurement taken
× Centrifuge pellet

6.6 RESULTS

6.6.1 Steady state periods

Daily results for the parent IFFD activated sludge system are listed in Appendix D, Tables D2 and D3.

Each wastewater batch was accepted as a steady state period. The daily data for each wastewater batch were analysed statistically to determine outliers; data lying outside the 95% confidence interval (i.e. data outside the range mean ± 2* sample standard deviation) were rejected. The rejected data are shown marked in the appropriate tables.

Excluding the rejected data, for each wastewater batch (steady state period) the daily data were averaged and the sample standard deviations calculated. The averages, sample standard deviations and number of data for the different wastewater batches are listed in Table 6.3 (a, b and c).

Table 6.3(a): Parent laboratory-scale IFFD system steady state COD data. For each sewage batch the data have been averaged and the means, sample standard deviations (SSD) and number of tests are listed.

Sew. Batch No.	COD (mgCOD/l)								
	INFLUENT			UNFILT. EFFLUENT			MIXED LIQUOR		
	Mean	SSD	No. of tests	Mean	SSD	No. of tests	Mean	SSD	No. of tests
24	1021	41	9	116	21	9	3839	176	9
25	999	38	13	90	8	13	4094	260	13
27	1026	85	7	90	20	7	4134	147	7
28	785	18	3	67	12	3	3974	38	3

Table 6.3(b): Parent laboratory-scale IFFD system steady state TKN data. For each sewage batch the data have been averaged and the means, sample standard deviations (SSD) and number of tests are listed.

Sew. Batch No.	TKN (mgN/l)								
	INFLUENT			UNFILT. EFFLUENT			MIXED LIQUOR		
	Mean	SSD	No. of tests	Mean	SSD	No. of tests	Mean	SSD	No. of tests
24	89	2	9	8.0	1.2	9	253	12	9
25	85	3	10	3.4	1.4	10	260	11	10
27	121	6	7	1.5	0.5	7	266	4	6
28	126	2	3	2.7	1.2	3	252	9	3

Table 6.3(c): Parent laboratory-scale IFFD system steady state OUR, VSS and effluent nitrate data. For each sewage batch the data have been averaged and the means, sample standard deviations (SSD) and number of tests are listed.

Sew. Batch no.	VSS (mgVSS/ℓ)			OUR Area (mgO/ℓ)			EFFLUENT NITRATE (mgN/ℓ)		
	Mean	SSD	No. of tests	Mean	SSD	No. of tests	Mean	SSD	No. of tests
24	2632	184	9	777.6	14.0	9	58.1	2.5	9
25	2779	73	13	737.5	27.6	12	52.7	2.2	13
27	2713	103	7	1030.0	105.7	5	94.6	6.7	7
28	2709	109	3	1153.6	14.1	3	99.2	7.3	2

6.6.2 COD and N mass balances

The reliability of the experimental measurements was checked by means of COD and N mass balances on the steady state periods (Ekama *et al.*, 1986): The method of mass balance calculation is different to the calculation method described in Chapter 2, section 2.9.2. A brief description of the method for calculating the N and COD mass balances for the parent IFFD system, using the averaged data from sewage batch 24 as an example, is given below. The description is followed by an analysis of the results for the various sewage batches.

Nitrogen (N) mass balance: Calculation method

In the N mass balance, since the IFFD system is fully aerobic and there is no prospect of denitrification, the influent TKN mass must be accounted for by the sum of the mass of TKN and nitrate in the effluent as well as the mass of TKN and nitrate abstracted through the waste sludge:

mass of influent TKN	=	mass of effluent TKN	+	mass of effluent NO_3	+	mass of TKN wasted	+	mass of NO_3 wasted

$$MNti = MNte + MNne + MNw + MNnw \quad (mg\ N/d) \quad (6.1)$$

The influent TKN mass (MNti) was calculated as being equal to the average influent TKN concentration (Nti, mgN/ℓ) * daily volume of wastewater added (Vi, ℓ) :

for sewage batch No. 24: MNti = Nti• Vi = 89•10 = 890 mgN/d

The mass of effluent TKN (MNte) was taken as the average unfiltered TKN concentration of the effluent (Nte, mgN/ℓ) * daily volume of effluent drawn off (Ve, ℓ) :

for sewage batch No. 24: MNte = Nte• Ve = 8.0•9 = 72 mgN/d

The mass of effluent NO_3 (MNne) was calculated as the average nitrate concentration of the effluent (Nne, mgN/ℓ) * daily volume of effluent drawn off (Ve, ℓ) :

for sewage batch No. 24: MNne = Nne• Ve = 58.1•9 = 522.9 mgN/d

The mass of TKN in the waste sludge (MNw) was found by multiplying the average mixed liquor TKN concentration (N_{ML}, mgN/ℓ) by the daily volume of mixed liquor wasted from the system (Vw, ℓ):

for sewage batch No. 24: MNw = N_{ML}• Vw = 253 • 1.0 = 253 mgN/d

The mass of NO_3 in the waste sludge (MNnw) was calculated as the average nitrate concentration of the effluent (Nne, mgN/ℓ) * daily volume of mixed liquor wasted from the system (Vw, ℓ):

for sewage batch No. 24: MNnw = Nnw• Vw = 58.1•1.0 = 58.1 mgN/d

By substituting the masses calculated above into Eq (6.1), the **N mass balance** is given by

$$Nbal\ (\%) = 100 * (MNte + MNne + MNw + MNnw) / MNti \qquad (6.2)$$

for sewage batch No. 24: Nbal (%) = 100•(72+522.9+253+58.1) / 890
Nbal (%) = 101.8 %

The data can be considered acceptable if the N mass balance fall in the range 90 – 110%.

Nitrogen (N) mass balance: Analysis of results

The results of the N mass balances calculated for each sewage batch are listed in Table 6.4 and are shown graphically in Fig. 6.3. The results can be commented on as follows :

- Compared to the variable N mass balances achieved for the continuously fed nitrification/denitrification parent system described in Chapter 2, the N mass balances for all the sewage batches tested during this investigation showed remarkable consistency.

- The N mass balances for the four sewage batches tested 24, 25, 27 and 28 were all in the range 96 % – 102 %. It is evident that the exceptionally good N mass balances lend credibility to the reliability of the measurements and no sewage batches were rejected for further analysis.

COD mass balance: Calculation method

In the COD mass balance, the influent COD mass must be accounted for by the sum of the effluent COD mass, COD mass of the waste sludge and mass of carbonaceous oxygen demand:

mass of influent COD	=	mass of effluent COD	+	mass of COD wasted	+	mass of carbonaceous oxygen demand

$$MSti = MSte + MXsvw + MOc \qquad (mgCOD/d) \qquad (6.3)$$

The mass of influent COD (MSti) was taken as the average influent COD concentration (Sti, mgCOD/ℓ) * daily volume of wastewater added (Vi, ℓ) :

for sewage batch No. 24: MSti = Sti • Vi = 1021 • 10 = 10210 mgCOD/d

The mass of effluent COD (MSte) was found by multiplying the unfiltered effluent COD concentration (Ste, mgCOD/ℓ) by the daily volume of effluent drawn off (Ve, ℓ) :

for sewage batch No. 24: MSte = Ste • Ve = 116 • 9 = 1044 mgCOD/d

The mass of COD in the waste sludge (MX_{SVW}) was found by multiplying the mixed liquor COD concentration (COD_{ML}, mgCOD/ℓ) by the daily volume of mixed liquor wasted from the system (Vw, ℓ):

for sewage batch No. 24: $MX_{SVW} = COD_{ML} \cdot Vw = 3839 \cdot 1.0 = 3839$ *mgCOD/d*

In contrast to a continuously fed system, the OUR response from an IFFD system does not remain constant throughout the day, but shows significant variation over a 24 hour period. From the typical OUR-time response shown in Fig 6.2 it is clear that the OUR response follows the feeding pattern and reflects the sequential feed-starve conditions. The area below the OUR (mgO/ℓ/h) – time (h) plot gives the total daily oxygen demand (MOt) in mgO/ℓ:

$$MOt = \text{Area (integral) under the daily OUR–time plot} = \int_{t=0h}^{t=24h} OUR \cdot dt$$

Graphical representations of the daily OUR responses recorded during this investigation are given in Appendix D5. Accompanying each OUR response is the calculated value for the area below each daily OUR–time plot. The daily OUR areas are listed in Table D3, Appendix D and the mean value for the area below the OUR–time plot, calculated for each sewage batch, is given in Table 6.3 (c).

for sewage batch No. 24: MOt = Mean area under OUR-time plot = 777.6 mgO/ℓ

However, the total oxygen demand (in mgO/d) is the sum of the carbonaceous oxygen demand (MOc) and the oxygen demand for nitrification (MOn):

$$MOt = OURarea * V_{ML} = MOc + MOn \quad \text{(mgO/d)} \quad (6.4)$$

where,

V_{ML} = volume of the reactor (10ℓ)
OURarea = mean area under the daily OUR–time plot (mgO/ℓ)

Therefore, to calculate MOc an estimate for MOn is essential. The oxygen demand for nitrification is related to the mass of nitrate generated (MNc):

$$MOn = 4.57*MNc \quad (mgO/d) \quad (6.5)$$

where,

4.57 = mass of oxygen utilized per mgNH_4-N nitrified.

Given the satisfactory N mass balance, the mass of nitrate generated is equal to the nitrate concentration in the effluent (Nne, mgN/ℓ) * daily volume of wastewater added (Vi, ℓ) :

for sewage batch No. 24: MNc = Nne • Vi = 58.1 • 10 = 581 mgN/d

The carbonaceous oxygen demand (MOc) was calculated by substituting Eq (6.5) into Eq (6.4) and solving for MOc:

$$MOc = (OURarea*V_{ML}) - (4.57*MNc) \quad (mgO/d) \quad (6.6)$$

for sewage batch No. 24: MOc = (777.6 • 10) – (4.57 • 581)
MOc = 5120.8 mgO/d

By substituting the masses calculated above into Eq. (6.3), the **COD mass balance** is given by:

$$CODbal(\%) = 100*(MSte + MX_{SVW} + MOc) / MSti \quad (6.7)$$

for sewage batch No. 24: CODbal(%) = 100 • (1044 + 3839 + 5120.8)/10 210
CODbal(%) = 98.0%

As with the N mass balance, the COD mass balance should also fall within the range 90 – 110% for the experimental data to be acceptable.

COD mass balances: Analysis of results

The results of the COD mass balances calculated for each sewage batch are listed in Table 6.4 and are shown graphically in Fig. 6.4. The results can be commented on as follows:

- The COD mass balances for the four sewage batches tested 24, 25, 27 and 28 were all in the range 98 % – 108 %. Considering the good N mass balance results, the achievement of satisfactory COD mass balances endorsed the reliability of the measurements. No sewage batches were rejected for further analysis.

6.6.3 Determination of mixed liquor COD/VSS and TKN/VSS ratios

Three independent measurements were made on the mixed liquor organic solids, volatile suspended solids (VSS), COD and TKN. To check the reliability of these measurements, the ratios of COD/VSS and TKN/VSS for the parent IFFD system mixed liquor were calculated for each sewage batch, see Table 6.4. Statistical plots for these ratio's were constructed, see Figs. 6.5 and 6.6 respectively. (See Appendix D for interpretation of statistical plots). From the statistical plots it is evident that the COD/VSS data are not truly normally distributed, due to the small sample population. However, the TKN/VSS data appear to be normally distributed, indicating that no single factor has had a dominating influence on the measurements. The means for COD/VSS and TKN/VSS were 1.48 mgCOD/mgVSS and 0.095 mgN/mgVSS respectively, with sample standard deviations 0.03 and 0.002 respectively. These values correspond to the accepted standard values of 1.48 mgCOD/mgVSS and 0.10 mgN/mgVSS respectively (WRC, 1984).

6.6.4 Determination of unbiodegradable soluble and particulate fractions

The unbiodegradable soluble and particulate fractions of the influent COD ($f_{S,us}$ and $f_{S,up}$ respectively) were determined in accordance with the procedures described in Chapter 2, using the methods of Ekama *et al.* (1986).

Unbiodegradable soluble COD fractions ($f_{S,us}$)

According to Ekama *et al.* (1986), the unbiodegradable soluble COD is given by the COD of the filtered effluent of the activated sludge system. Hence from Eq (2.11):

$$f_{S,us} = S_{te} / S_{ti}$$

where

S_{te} = filtered effluent COD concentration (mgCOD/ℓ)
S_{ti} = unfiltered influent COD concentration (mgCOD/ℓ)

From the average data recorded for each sewage batch (Table 6.3), the $f_{S,us}$ was calculated using Eq (2.11), and the values are listed in Table 6.4. The $f_{S,us}$ data from all the sewage batches were analyzed using a statistical plot, see Figure 6.7 (For the interpretation of the statistical plot, see Appendix F). Although the plot comprises only four data points, the data appear to be normally distributed, giving for the raw wastewater a mean $f_{S,us} = 0.073$ and sample standard deviation of 0.004. This value is slightly lower than the value of $f_{S,us} = 0.085$ obtained for the same wastewater via the continuously fed system (see Chapter 2), and that observed by Ubisi *et al.* (1997a,b), $f_{S,us} = 0.09$ for the same Mitchell's Plain wastewater, and probably reflects the small variations from sewage batch to sewage batch.

Unbiodegradable particulate COD fractions ($f_{S,up}$):

Following the method of Ekama *et al.* (1986), the mixed liquor volatile suspended solids concentration (MLVSS) measured in the IFFD system was used to determine $f_{S,up}$. From the average data on each sewage batch (Table 6.3) and the calculated $f_{S,us}$ (Table 6.4), the $f_{S,up}$ was calculated using Eq (2.12):

$$MX_V = \frac{MS_{ti}(1 - f_{S,us} - f_{S,up})\, Y_H^* R_S}{(1 + b_H^* R_S)} (1 + f^* b_H^* R_S) + f_{S,up}\, MS_{ti}\, R_S / f_{cv}$$

MX_V = total volatile solids mass (mgVSS)
= $X_V \cdot V_P$
X_V = MLVSS concentration (mgVSS/ℓ)
= measured value (Table 6.3)
V_P = system volume
= 10ℓ
Y_H^* = heterotrophic active biomass yield (VSS units)
= 0.45 mgVSS/mgCOD
R_S = sludge age (d)
= 10 d
b_H^* = net specific endogenous mass loss rate
= 0.24 / d at 24°C
f^* = endogenous residue fraction
= 0.20
f_{cv} = COD/VSS ratio of mixed liquor (mgCOD/mgVSS)
= 1.48 mgCOD/mgVSS
MS_{ti} = total influent COD mass fed per day (mgCOD/d)
= $S_{ti} \cdot Vi$
Vi = daily volume of wastewater added
= 10ℓ
S_{ti} = average influent COD concentration (mgCOD/ℓ)
= measured value (Table 6.3)

For each batch of wastewater tested, successive values for $f_{S,up}$ were substituted into Eq. (2.12); the $f_{S,up}$ value that gave a theoretical MLVSS mass (MX_V) equal to the measured value was accepted. The $f_{S,up}$ values for the different wastewater batches are listed in Table 6.4. The $f_{S,up}$ data for all the wastewater batches were analyzed using a statistical plot, given in Fig 6.8. The data appear to be normally distributed, giving for the raw wastewater a mean $f_{S,up} = 0.1822$ with sample standard deviation of 0.019. This mean is significantly higher than the value of $f_{S,up} = 0.103$, obtained for the same wastewater via the continuously fed system (see Chapter 2), and that observed by Ubisi *et al.* (1997a,b), $f_{S,up} = 0.12$ for the same Mitchell's Plain wastewater. This difference may be due to the particular sewage batches used, or may arise from the IFFD system operation influencing biodegradability of slowly biodegradable COD.

Table 6.4 : Summary of parent laboratory-scale intermittently fed fill and draw (IFFD) system N and COD mass balances, wastewater fractions and mixed liquor parameters.

Sewage Batch No.	MASS BALANCE (%)		WASTEWATER FRACTIONS		MIXED LIQUOR	
	N	COD	Unbiodegr. Soluble ($f_{s,us}$) (mgCOD / mgCOD)	Unbiodegr. Particulate ($f_{s,up}$) (mgCOD / mgCOD)	COD/VSS (f_{cv}) (mgCOD / mgVSS)	TKN/VSS (f_N) (mgN / mgVSS)
24	102	98	0.0754	0.1598	1.46	0.096
25	96	99	0.0721	0.2009	1.47	0.094
27	101	106	0.0750	0.1735	1.52	0.098
28	97	108	0.0675	0.1945	1.47	0.093
MEAN			0.0725	0.1822	1.48	0.095
Std. Deviation			0.0037	0.0190	0.03	0.002

6.7 EVALUATION OF THE OUR RESPONSE

The shape of the typical IFFD system OUR response shown in Fig. 6.2 deviates from the expected shape of the OUR response observed for similar systems (Still *et al.*, 1986). This prompted a renewed examination of the process kinetics governing the OUR response.

6.7.1 Expected OUR response

The expected OUR response described by Still *et al.* (1986) is also shown in Fig. 6.2. This OUR response can be subdivided into distinct zones, where each zone is characterized by the type of substrate utilization. The derivation of the numerical OUR values, associated with each zone shown in Fig 6.5, is given in Appendix D6. The different zones and their specific relation to substrate utilization will be briefly described here. In this description it should be noted that only the substrate utilization behaviour unique to the particular zone is described. However, substrate utilization behaviour in a particular zone applies also to all preceding zones. For example, prior to the depletion of FSA, nitrification is continually operative.

Initial high OUR response – Area 1

The area below the initial high OUR response curve (Area 1) represents the oxygen consumed in utilizing the wastewater readily biodegradable COD (RBCOD) and the area reflects the magnitude of the RBCOD in relation to the total COD present in the wastewater. The larger the area with respect to the total area under the curve, the greater the fraction of readily biodegradable COD in the influent (Ekama *et al*, 1986). The point

at which the RBCOD is completely utilized is indicated by the precipitous drop in OUR. The shape of the initial high OUR response rectangle reflects the rate of RBCOD utilization (μ_H). A high value of μ_H is indicative of the rapid utilization of RBCOD, hence a high initial OUR response present for a short time is expected.

Second OUR plateau – Area 2

The area between the second plateau and the third plateau in the OUR profile (Area 2) represents the concentration of oxygen required for nitrification. With IFFD conditions favouring uninhibited nitrification it can be expected that the rate of nitrification will remain more or less constant and related to the maximum growth rate of the nitrifiers (μ_H). The constant nitrification rate will induce a constant OUR, which is reflected in the nearly horizontal shape of the second OUR plateau. The second precipitous drop in OUR is the consequence of a cessation of nitrification.

Third OUR plateau – Area 3

The area between the third plateau and the fourth plateau in the OUR curve (Area 3), represents the concentration of oxygen required for the solubilization and utilization of particulate slowly biodegradable COD (SBCOD) which is limited by the rate of SBCOD solubilization. The relatively low OUR response, present for a comparatively long period, reflects the low maximum specific growth rate on SBCOD (K_{MP}).

Fourth OUR plateau

When the influent SBCOD utilization is complete, the OUR reaches a fourth plateau. The fourth OUR plateau represents the oxygen demand associated with death regeneration. The death regeneration concept entails the solubilization of substrate released via organisms death and lysis. Oxygen is consumed in the subsequent utilization of the released substrate for growth, giving rise to the observed death regeneration OUR plateau.

6.7.2 Observed OUR response

From Fig. 6.2 it is clear that during the initial stages of the feeding cycle the shape of the observed OUR response does not follow the expected OUR curve. There is no well defined initial OUR plateau followed by a precipitous drop in OUR as a result of RBCOD depletion, but rather a progressive decrease from the initial high OUR to the second OUR plateau. This difference in response is of significance as it indicates a deviation from the expected process kinetics that govern the OUR response. The deviation can be the result of a multitude of causes and some of the more prominent possibilities will be detailed below.

COD loading rate too low

The COD loading rate (LR) is defined as the ratio of the mass of influent COD, added to the IFFD system during a particular feeding cycle, to the mass of VSS (MX_V) in the parent system i.e.

$$LR = MS_{ti} / MX_V \quad \text{(mgCOD/mgVSS)} \tag{6.8}$$

where

MS_{ti} = mass of wastewater total COD added per feeding cycle (mgCOD)
= $V_{WW} \cdot S_{ti}$

MX_V = mass of parent system volatile suspended solids receiving the wastewater feed (mgVSS).
= $V_{ML} \cdot X_v$

If the LR is too low, the RBCOD would be utilized very rapidly and the shape of the OUR response during the initial stages would be too tall and narrow, resulting in the recording of too few OUR measurements to give reasonable surety of the initial high OUR. Batch tests conducted by Dold *et al.* (1991) on mixed liquor samples drawn from a similar IFFD system, indicated that the initial tall shape of the OUR response is distinctly recognizable with a LR in excess of 0.26 mgCOD/mgVSS. The OUR response shown in Fig. 6.2 was recorded during the period when the parent system was receiving sewage batch 25. The LR was determined by substituting the average values (Table 6.3) for S_{ti} and X_V in Eq. (6.8) for the 5ℓ feeding cycle:

$$LR_{(5\ell)} = \frac{(5\ell \cdot 999 \text{ mgCOD}/\ell)}{(10\ell \cdot 2779 \text{ mgVSS}/\ell)} = 0.18 \text{ mgCOD/mgVSS}$$

It is evident that the implementation of the 5ℓ : 5ℓ feeding cycle represented a cyclic LR that was significantly lower than the rate of 0.26 mgCOD/mgVSS required to establish the initial OUR plateau. As a result of the COD loading rate being too low, there existed a distinct possibility that the expected initial OUR response could not be established due to the insufficient availability of RBCOD. Consequently, during the period when the parent system was receiving sewage batches 26 and 27, the feeding cycle was changed to 2.5ℓ : 7.5ℓ. Using the averaged data for sewage batch 27 (Table 6.3), the LR during the second (7.5ℓ) daily feeding period was calculated, using Eq. (6.8):

$$LR_{(7.5\ell)} = \frac{(7.5\ell \cdot 1026 \text{ mgCOD}/\ell)}{(10\ell \cdot 2713 \text{ mgVSS}/\ell)} = 0.28 \text{ mgCOD/mgVSS}$$

Having implemented a loading rate in excess of the required 0.26 mgCOD/mgVSS, it was anticipated that the increased RBCOD loading would be sufficient to produce the "expected" initial OUR response. However, examination of the OUR profiles recorded

during sewage batches 26 and 27 (given in Appendix D5) reveals that the shape of the OUR profiles did not change significantly. Despite the higher initial OUR, the OUR response still decreased progressively, without the distinct plateau followed by the precipitous drop in OUR due to RBCOD depletion. It was concluded that the LR did not impact significantly on the shape of the OUR response, and that the deviation of the observed OUR response from the "expected" OUR response, could not be attributed to the COD loading rate being too low.

Intracellular RBCOD storage

By mixing different proportions of soluble COD and VSS in a batch test, Van Niekerk (1985) measured the soluble COD concentrations and OUR with time. During the initial stages of the batch tests a high OUR was observed, in association with the utilization of soluble COD. The OUR continued at a constant high rate until approximately 60% of the soluble COD was utilized. Thereafter the OUR declined rapidly, but leveled off at levels higher than the OUR levels at the start of the batch tests, before the soluble COD was added. After the OUR leveled off, only limited amounts of soluble COD were taken up. According to Van Niekerk (1985) the area under the elevated OUR curve directly gave the concentration of soluble COD utilized during the rapid uptake phase. By comparing the concentration of oxygen consumed and soluble COD taken up, it was concluded that only 38% of the soluble COD taken up was utilized and the rest (62%) was stored intracellularly in the form of polyhydroxybutyrate (PHB).

The UCT research group (Still *et al.*, 1996) commented that the 0.38 uptake ratio was not unexpected, but was indicative of conventional heterotrophic metabolic behaviour without significant intracellular soluble COD storage. In terms of the UCT kinetic model (Dold *et al.*, 1980, 1991), heterotrophic metabolism comprises anabolism, in which a fraction of the organic substrate is converted to organism mass (cell synthesis), and catabolism in which the remaining organic substrate fraction is utilized to generate energy for the anabolism process. In terms of the UCT kinetic model, the magnitude of the anabolism (substrate incorporated in organism mass - Y_{ZH}) fraction (COD units) is 0.666 mgCOD/mgCOD and the catabolism (substrate utilized to generate energy) fraction is $(1 - Y_{ZH}) = 0.334$ mgCOD/COD. This fraction correlates well with the 0.38 value observed by Van Niekerk (1985). Although the explanation proposed by Still *et al.* (1996) essentially dismissed the possibility of intracellular RBCOD storage, the concept will still be considered as a possible cause for the deviation in OUR response observed during this investigation. In Chapter 7 a more conclusive deliberation is given.

Decreasing organism growth rates

Another plausible explanation for the OUR response observed in Fig. 6.2 is that the progressively decreasing OUR can be attributed to progressively decreasing organism growth rates. For the heterotrophic organisms, this proposal suggests that the RBCOD uptake and associated OUR do not proceed at a constant rate, defined by the maximum specific growth rate on RBCOD (μ_H), but that IFFD conditions stimulate the organisms to follow a decreasing rate of RBCOD utilization. The result of this would be evident as a progressively decreasing OUR.

The results of earlier research work on simulating the behaviour of the heterotrophic populations present in environments with high COD loading rates (notably contact-stabilization systems subjected to cyclic flow and load) support this concept.

Dold *et al.* (1980, 1991) found that the half saturation constant for RBCOD (K_{SH}) had to be increased from the default value of 5 mgCOD/ℓ (used for continuously fed systems) to 110 mgCOD/ℓ for the UCTOLD kinetic simulations applied to the contact-stabilization system, to obtain better comparisons between the measured and predicted results. The K_{SH} value is incorporated into the UCT kinetic model through the Monod equation for the aerobic growth rate (ρ_1) for heterotrophic organisms on RBCOD:

$$\rho_1 = \frac{\mu_H \cdot S_{bs}}{(K_{SH} + S_{bs})} Z_{BH} \qquad \text{(mgCOD/}\ell\text{/d)} \qquad (6.9)$$

The influence of the higher K_{SH} value on the growth rate is evident from an inspection of Eq. (6.9), where growth rate reduction is expected at much higher RBCOD concentrations (S_{bs}) compared to continuously fed systems. The growth rate is closely related to the maximum growth rate (μ_H) when $S_{bs} >> K_{SH}$. With the low K_{SH} value of 5 mgCOD/ℓ for continuously fed systems, it is expected that heterotrophic growth would proceed at the maximum growth rate (μ_H) for longer periods, until the RBCOD is depleted to a concentration of approximately 10 mgCOD/ℓ, when a reduction in grow rate would occur in accordance with Eq. (6.9). With the higher K_{SH} value simulated for high COD loading systems (such as the IFFD system), it is therefore assumed that growth rate reduction is initiated while the RBCOD concentration is still relatively high (200 mgCOD/ℓ). In accordance with Eq. (6.9) the concomitant decrease in growth rate would also not occur as rapidly as for continuously fed systems, but would be related to the gradual depletion of RBCOD. Because this behaviour correlates with the observed OUR response in Fig. 6.2, the heterotrophic growth rate reduction concept appeared to be the most plausible reason for the progressively decreasing OUR.

In addition, many investigators (Chudoba *et al.*, 1986; Dold *et al.*, 1991; Still *et al.*, 1996) attributed changes in the area below the second OUR plateau, i.e. associated with the utilization of SBCOD and nitrification, principally to changes in the maximum specific growth rate of autotrophs (μ_A). For reasons not yet known, the feed-starve conditions appear to select for autotrophic organisms that nitrify at a higher rate than those under continuously fed conditions. This is reflected in the consistently higher values for μ_A observed in IFFD systems. This observation initiated a specific investigation into the oxygen demand due to nitrification.

6.7.3 Oxygen Demand due to nitrification (OUR_N)

To estimate the oxygen demand for nitrification, the concentration of nitrate generated must be known. Accordingly, the variation in nitrate (NO_3), nitrite (NO_2) and the free and saline Ammonia - NH_4^+ (FSA) concentrations was measured at regular intervals

during the period when the system was feeding on the first feed. A typical pattern for the change in concentrations for these compounds, together with the pH profile, is shown in Fig. 6.10 for testing conducted on the parent system on 23-06-2000. From the data in Fig. 6.10, the NO_2 and NO_3 generation profiles were constructed in Fig. 6.11. From these profiles the OUR_N was calculated and the OUR_N variation with time is shown in Fig. 6.9.

Construction of NO_2 and NO_3 generation profiles

The NO_3 generation profile shown in Fig. 6.11 was derived from the NO_3 profile shown in Fig. 6.10. By subtracting the initial NO_3 concentration (52 mgN/ℓ) from the series of NO_3 data values in Fig. 6.10, a series of data points was obtained reflecting the generation of NO_3. A mathematical expression ($y = 10.5 \times ^{0.77}$) was fitted to the NO_3 generation data, thereby obtaining a theoretical NO_3 generation profile that closely approximated the observed increase in NO_3 with time. A similar NO_2 generation profile with the mathematical expression ($y = -0.247 x^2 + 3.5853 x + 0.4294$) was fitted to the NO_2 data.

In Fig. 6.10 the FSA profile shows a progressive decrease, associated with the oxidation of NH_4^+ to NO_2 and the subsequent oxidation of NO_2 to NO_3. FSA depletion is evident after 4.5 hours. However, the NO_3 concentration continues to increase during the period 4.5 – 6.0 hours. The continued nitrification is the result of the remaining NO_2 being oxidized to NO_3 and is evident in the depletion of NO_2 from 12 mgN/ℓ to zero at time 6.0 hours. Consequently, a NO_2 depletion profile, with the mathematical expression $y = 2 x^2 - 30 x + 109$, was fitted to the decreasing NO_2 data.

Construction of the OUR response due to nitrification

From the NO_3 generation profile (Fig. 6.11) it is clear that the rate of increase in NO_3 is not constant, but gradually levels off after an initial high NO_3 generation rate. The NO_2 generation profile shows a similar trend, although the changing rate is less marked. Because the nitrification rate decreases, it would induce a similar decrease in OUR_N. To derive the OUR_N profile, the OUR_N was sequentially calculated for a number of time intervals, similar to the calculation procedure described in section 5.3.2, Chapter 5. The nitrification OUR profile shown in Fig. 6.9 was constructed from the NO_2 and NO_3 generation profiles as follows:

- During the period 0 – 4.5 hours, the oxygen demand due to nitrification (OUR_N) is associated with the oxidation of NH_4^+ to NO_2 and the subsequent oxidation of NO_2 to NO_3. In terms of nitrification stoichiometry, the OUR_N during any particular time interval is the sum of the OUR_N calculated for each of the separate reactions:

$$OUR_N = 4.57 \cdot \frac{\Delta NO_3}{\Delta t} + 3.43 \cdot \frac{\Delta NO_2}{\Delta t} \quad (mgO/\ell/h) \qquad (6.10)$$

  Due to the decreasing nitrification rate, the OUR_N also follows a decreasing trend up to the time when the substrate (FSA) is depleted (4.5 hours).

- At time = 4.5 hours a precipitous drop in OUR_N occurs due to FSA depletion.

- Subsequent to the precipitous drop in OUR_N, nitrification continues due to the oxidation of the remaining NO_2 concentration. The nitrification rate ($\Delta NO_3 / \Delta t$) is assumed to be equal to the NO_2 depletion rate ($\Delta NO_2 / \Delta t$) and the associated OUR_N was calculated sequentially from the theoretically fitted line for each time interval. During the period 4.5 – 6.0 hours the OUR_N was calculated using the following stoichiometric relationship:

$$OUR_N = 1.14 \cdot \frac{\Delta NO_2}{\Delta t} \qquad (mgO/\ell/h) \qquad (6.11)$$

  The progressive decrease in OUR_N for the second OUR_N plateau (Fig. 6.9) is a reflection of progressive decrease in nitrification, with the NO_2 depletion profile (Fig. 6.11) gradually leveling off with time.

- After the remaining NO_2 is completely utilized, the OUR_N drops rapidly to zero at time = 6.3 h.

6.7.4 Comparison between the OUR_N profile and the observed OUR response

From Fig. 6.9, it is evident that the shape of the OUR_N profile shows remarkable agreement with the shape of the observed OUR response. During the period 0 – 4.5 hours, the progressively decreasing OUR is directly related to the decreasing nitrification rate. It is concluded therefore, that the deviation in observed OUR response from the "expected" OUR response can be attributed principally to the continually decreasing nitrification rate of the autotrophs.

When comparing the OUR_N profile with the observed OUR response, it is interesting to note that the second OUR plateau arises from the oxidation of the remaining NO_2 to NO_3, and not as a result of the solubilization and utilization of SBCOD, as assumed previously. Confirmation of this proposal is found when comparing the OUR response obtained on 24-06-2000 when the system was fed with filtered wastewater (5ℓ), with the OUR response recorded during the previous day (23-06-2000) when the system was normally fed with 5ℓ of unfiltered wastewater. The OUR responses are compared in Fig. 6.12, together with the OUR_N profile obtained in Fig. 6.9 for 23-06-2000.

The measured COD concentration of the filtered wastewater used for the batch test conducted on 21-06-2000 was 247 mgCOD/ℓ. It is reasonable to assume that the COD for the filtered wastewater fed to the system on 24-06-2000 would have been similar. Bearing in mind that filtered effluent COD measured for the parent system on 23-06-2000 was 55 mgCOD/ℓ, the biodegradable COD concentration of the filtered wastewater can be estimated as 247 – 55 mgCOD/ℓ = 192 mgCOD/ℓ at the most Therefore, the maximum mass of biodegradable COD fed to the system would have been:

$$MS_{bs} = 192 \text{ mgCOD}/\ell \cdot 5\ell = \underline{960 \text{ mgCOD}}.$$

It was previously assumed that the area below the second OUR plateau (Area 1 in Fig. 6.12) is associated with the solubilization and utilization of SBCOD. Because the greater part of the SBCOD fraction of the wastewater is in the particulate form (which was removed through filtration), it is unlikely that the small concentration of soluble SBCOD would be able to sustain an OUR of the magnitude observed in Fig. 6.12 for such a long period of time (length of Area 1): Area 1 can be estimated by assuming a constant $OUR_{SBCOD} \sim 10$ mgO/*l*/h and a utilization period of ~ 6 hours (from Fig. 6.12). This gives the area for SBCOD utilization ~ 60 mgO/*l*. Interpreted in terms of the UCT model, the area of SBCOD utilization represents only the fraction of substrate utilized during the catabolism process, i.e. expressed in terms of COD mass:

$$\text{Reactor volume} \cdot \text{Area of } OUR_{SBCOD} = (1 - Y_{ZH}) \cdot \text{SBCOD mass in influent} \qquad (6.12)$$

Solving for the associated SBCOD mass in the influent:

$$\text{SBCOD mass} = \frac{10\,l \cdot 60\ \text{mgO}/l}{(1 - 0.666)} = \underline{1800\ \text{mgCOD}}$$

Therefore, to sustain the OUR represented by Area 1 in Fig. 6.12 requires an influent SBCOD mass of 1800 mg. Since from the above the maximum total biodegradable COD mass that was added with the filtered wastewater amounts to only 960 mgCOD, it is clear that the second OUR plateau in Fig. 6.12 cannot be attributed to the solubilization and utilization of SBCOD, but rather to the oxidation of the NO_2 remaining to NO_3.

6.8 CLOSURE

The parent IFFD system was operated for 60 days and received 5 batches of unsettled municipal wastewater from Mitchell's Plain, Cape Town. From the results obtained for the system:

- Compared to the variable N mass balances achieved for the continuously fed nitrification/denitrification parent system described in Chapter 2, the N mass balances for all the sewage batches tested during this investigation showed remarkable consistency. The N mass balances for the four sewage batches tested were all in the range 96 % – 102 %.

- The COD mass balances calculated for the four sewage batches tested were all in the range 98 % – 108 %. Together with the good N mass balance results, the good COD mass balances lend credibility to the reliability of the measurements. No sewage batches were rejected for further analysis.

- Measurement of the mixed liquor VSS, COD and TKN yielded the following ratios; COD/VSS = 1.48 mgCOD/mgVSS (sample standard deviation = 0.03) and TKN/VSS = 0.095 mgN/mgVSS (sample standard deviation = 0.002). These values correspond to the accepted standard values of 1.48 mgCOD/mgVSS and 0.10 mgN/mgVSS respectively (WRC, 1984), lending further credibility to the reliability of the measurements.

- The wastewater mean unbiodegradable soluble COD fraction ($f_{S,us}$) was determined to be 0.073 (sample standard deviation = 0.004). This value is slightly lower than the value of $f_{S,us}$ = 0.085 obtained for the same wastewater via the continuously fed system (see Chapter 2), and that observed by Ubisi *et al.* (1997a,b), $f_{S,us}$ = 0.09 for the same Mitchell's Plain wastewater, and probably reflects the small variations from sewage batch to sewage batch.

- The wastewater mean unbiodegradable particulate COD fraction ($f_{S,up}$) was determined to be 0.1822 (sample standard deviation = 0.019). This mean is significantly higher than the value of $f_{S,up}$ = 0.103, obtained for the same wastewater via the continuously fed system (see Chapter 2), and that observed by Ubisi *et al.* (1997a,b), $f_{S,up}$ = 0.12 for the same Mitchell's Plain wastewater. This difference may be due to the particular sewage batches used, or may arise from the IFFD system operation influencing biodegradability of slowly biodegradable COD.

- The observed daily OUR response deviated from the expected typical IFFD system OUR response pattern. The expected OUR plateau associated with RBCOD utilization, followed by a well-defined precipitous drop in OUR, were not evident from the observed OUR responses. Instead a high initial OUR with a subsequent progressive decrease in OUR was observed.

- It was concluded that the deviation in OUR response above was mainly the result of continually decreasing growth rates for the organisms when subjected to cyclic feed-starve conditions. The nitrate generation profile, recorded during the period immediately after the system was fed, indicated that the nitrification rate gradually decreased after an initial high rate of nitrification. The decreasing nitrification rate, in particular, had a significant impact on the shape of the OUR profile. Previous research on similar systems subjected to high COD loading rates (Dold *et al.*,1980 and Dold *et al.*, 1991), indicate that the heterotrophic organisms may exhibit a similar decreasing rate of RBCOD utilization. However, during this investigation no direct evidence substantiating this proposal could be identified.

- Although the intracellular storage of RBCOD could have been a contributing factor to the progressive decrease in OUR, this possibility was largely discarded after interpretation of similar results in terms of the heterotrophic metabolism concept contained in the UCT (and IAWQ) kinetic simulation models.

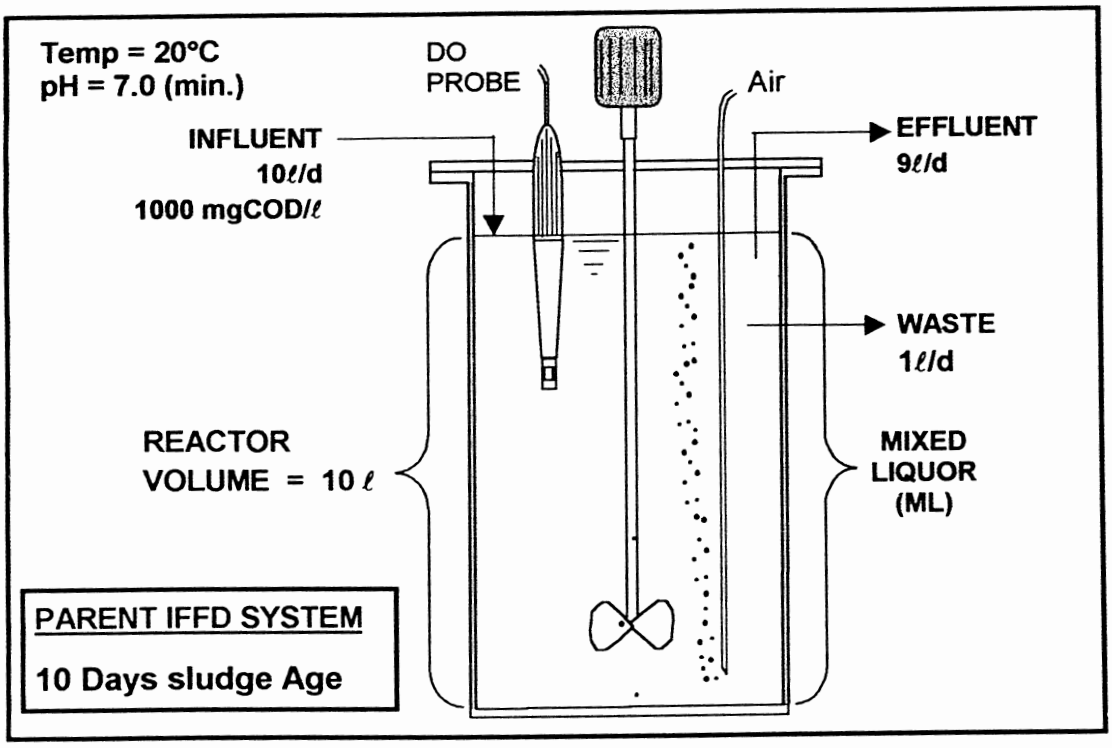


Figure 6.1: Illustration of the parent laboratory-scale intermittently fed fill and draw (IFFD) system, comprising a single aerobic reactor of 10ℓ volume.

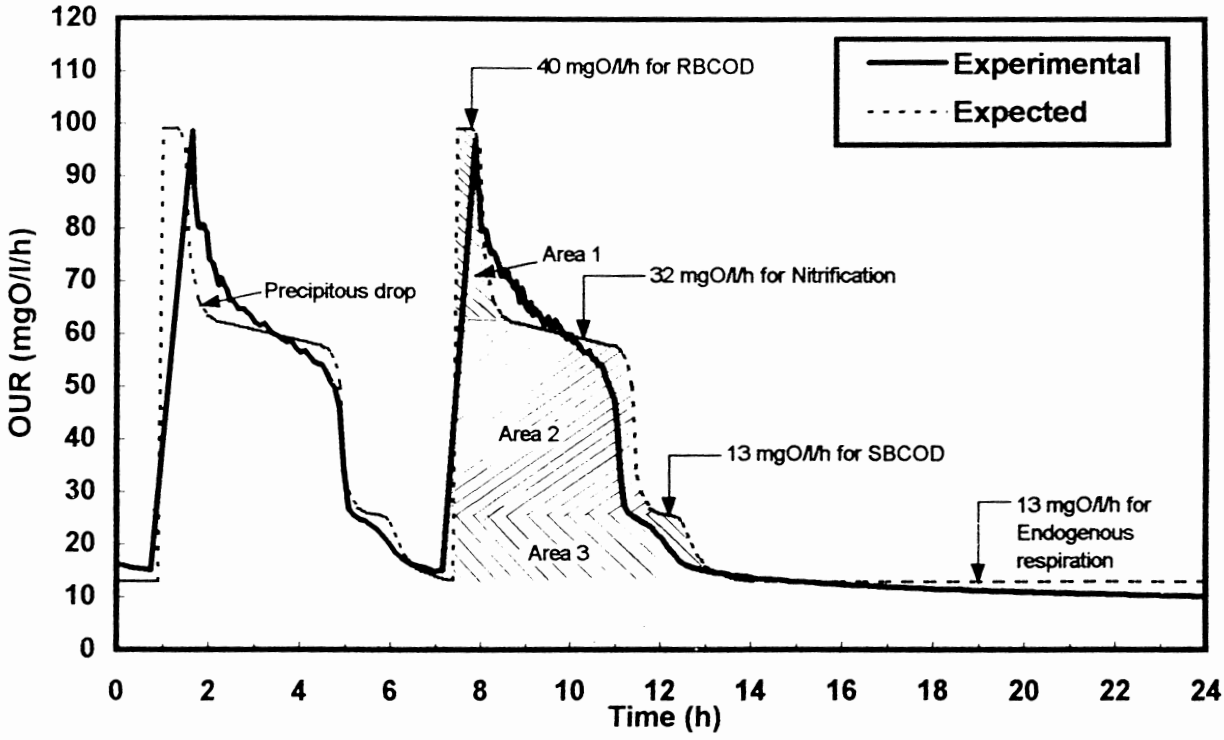


Figure 6.2 : Typical OUR response (16-05-2000) from the intermittently fed fill and draw (IFFD system during a 24 hour period when the system was fed twice daily with equal volumes (5ℓ) of wastewater. Also shown is the theoretically predicted OUR respons

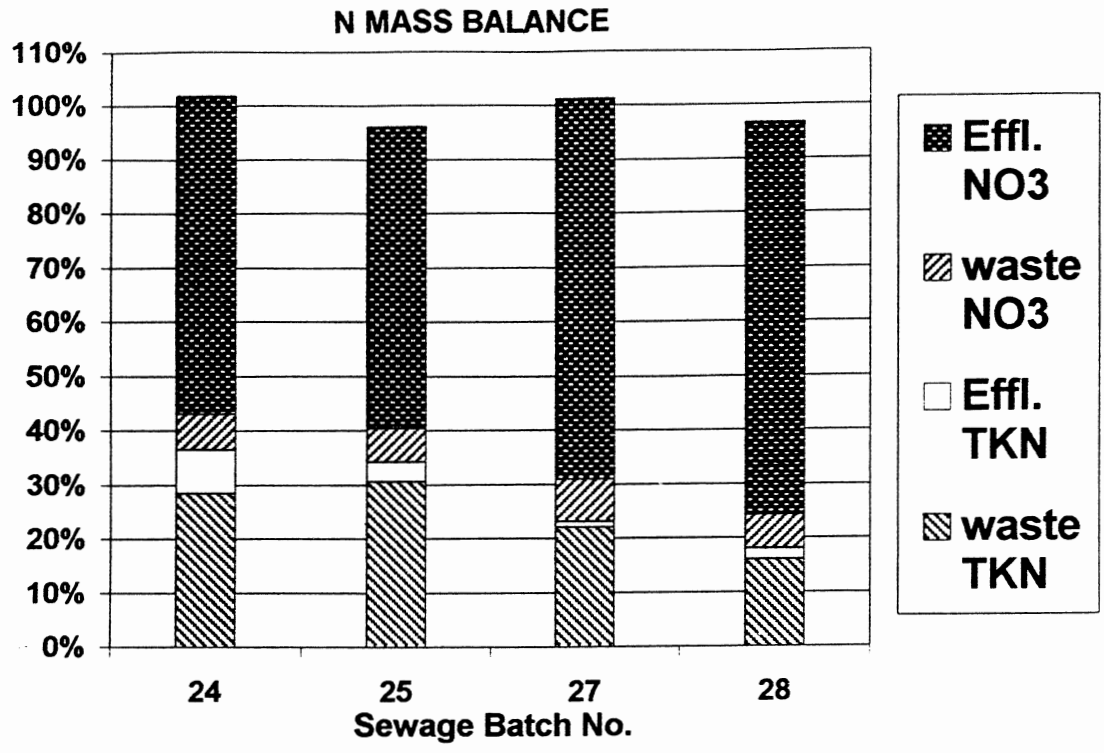


Figure 6.3: Graphical representation of the percentage Nitrogen (N) mass balance for the IFFD parent system. Percentages are shown for : Nitrogen for sludge production (waste) , Effluent TKN , nitrate in the waste mixed liquor and effluent nitrate.

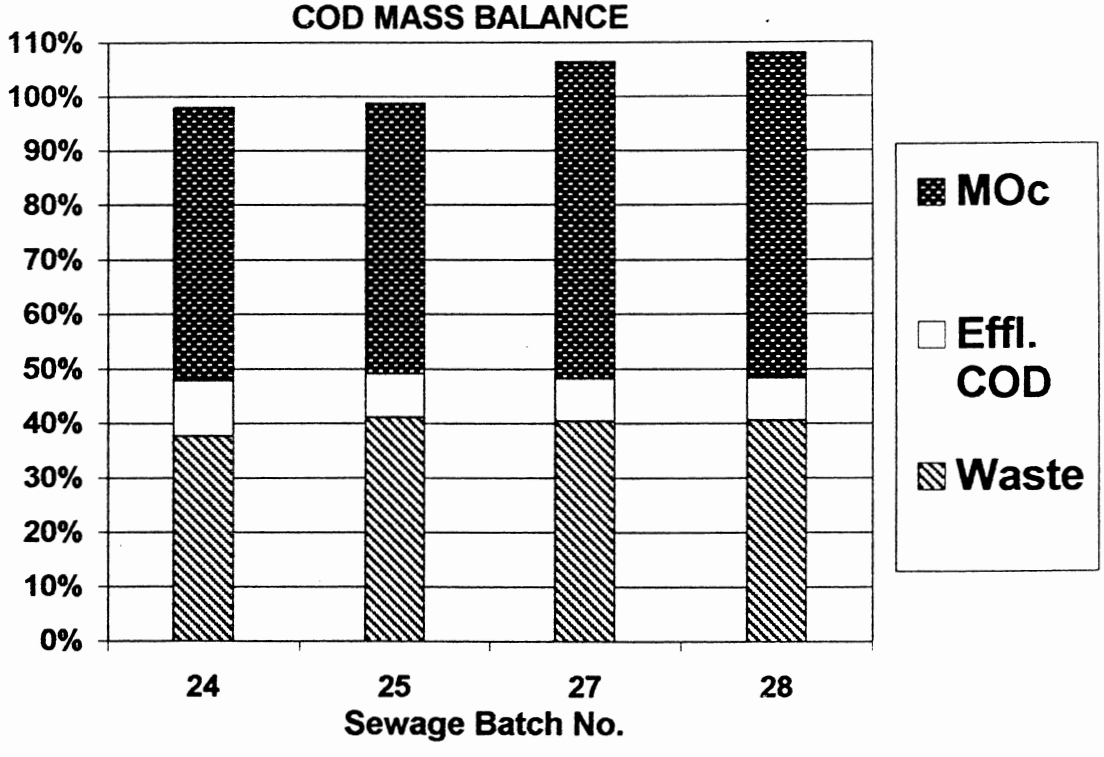


Figure 6.4: Graphical representation of the percentage COD mass balance for the various wastewater batches. Percentages are also shown for : Waste sludge COD , unfiltered effluent COD and carbonaceous oxygen demand (MOc).

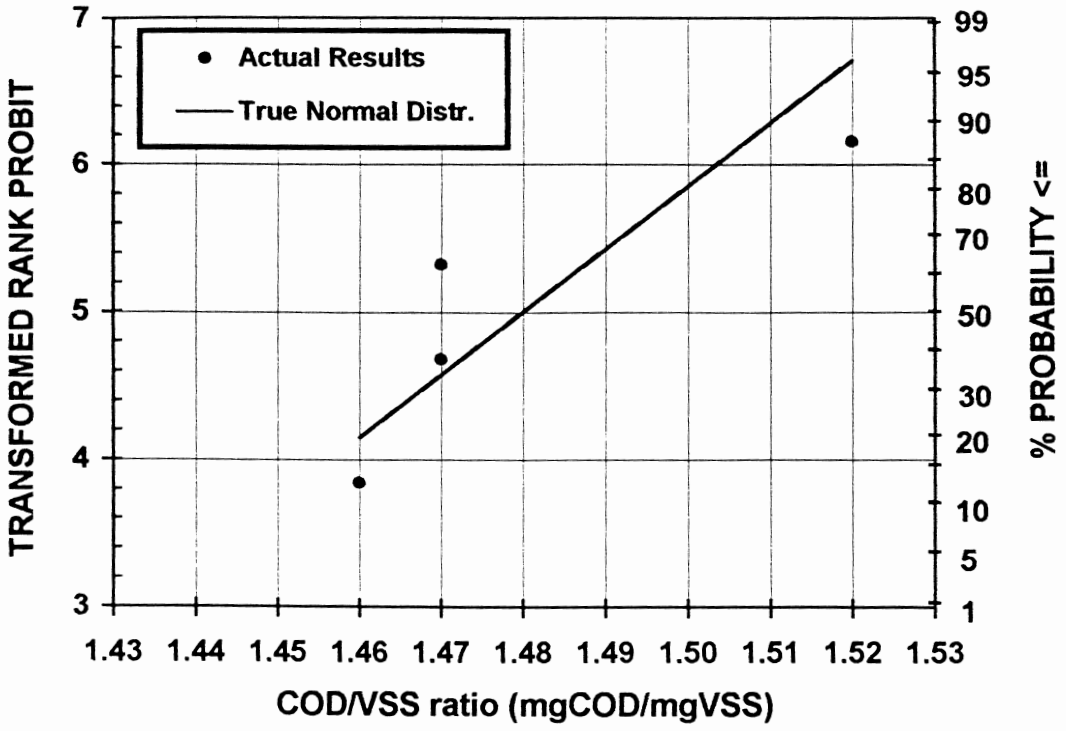


Figure 6.5 : Statistical plot for COD/VSS ratio for the parent laboratory-scale intermittently fed fill and draw (IFFD) system mixed liquor.

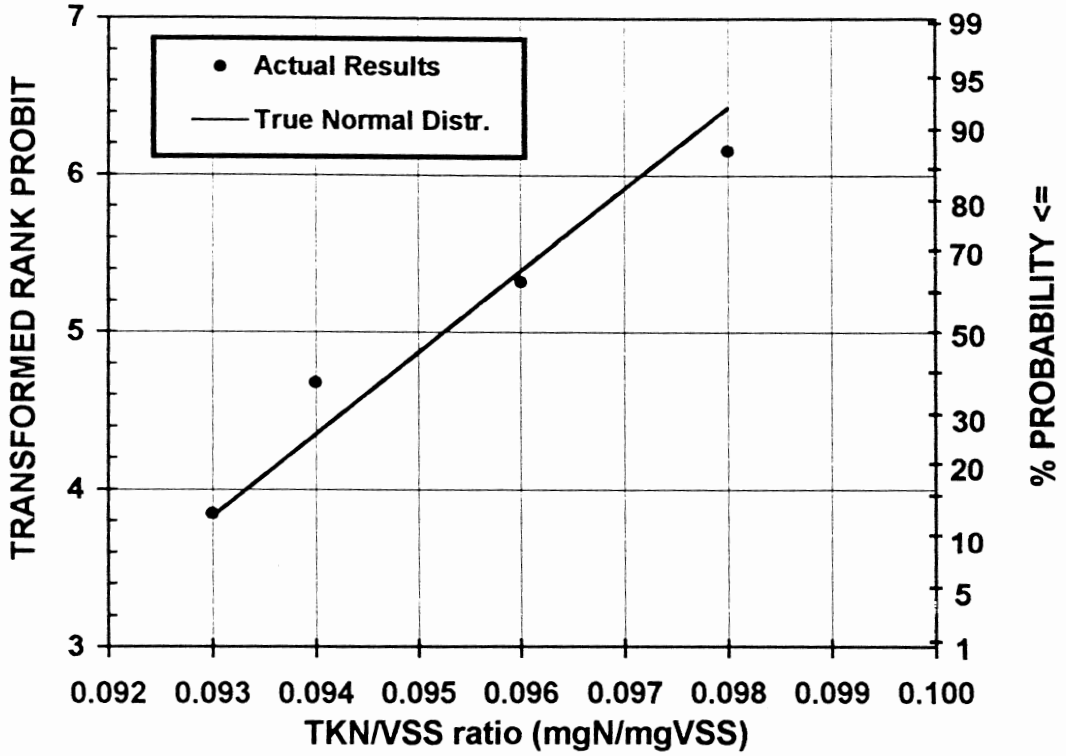


Figure 6.6 : Statistical plot for TKN/VSS ratio for the parent laboratory-scale intermittently fed fill and draw (IFFD) system mixed liquor.

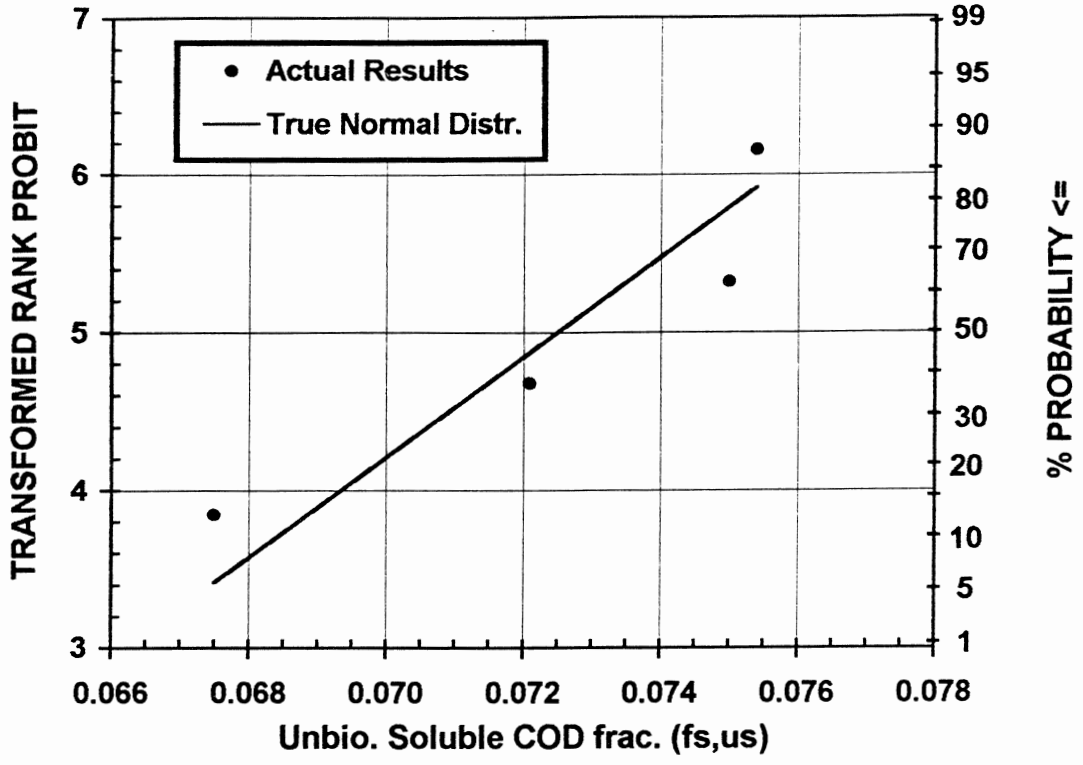


Figure 6.7 : Statistical plot for unbiodegradable soluble COD fractions ($f_{s,us}$) for the parent laboratory-scale IFFD system fed with municipal wastewater from Mitchell's Plain (Cape Town, South Africa).

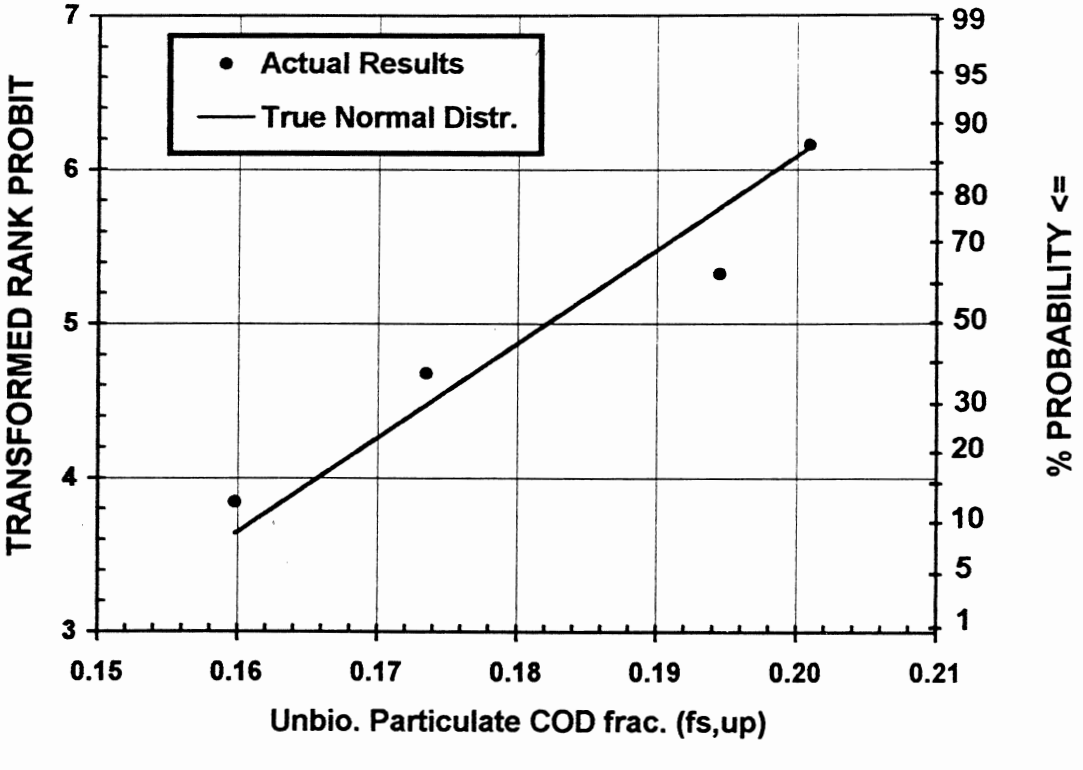


Figure 6.8 : Statistical plot for unbiodegradable particulate COD fractions ($f_{s,up}$) for the parent laboratory-scale IFFD system fed with municipal wastewater from Mitchell's Plain (Cape Town, South Africa).

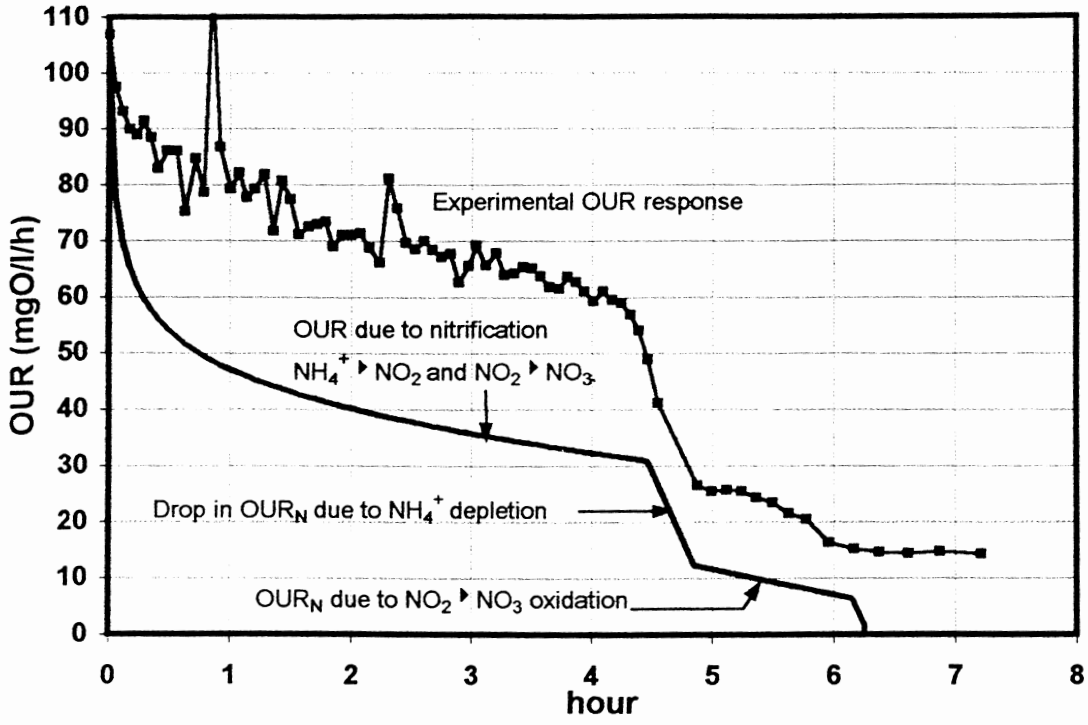


Figure 6.9 : OUR response recorded during the first feeding period (5ℓ) on 23-06-2000. Also shown is the oxygen demand due to nitrification (OUR_N), calculated from the observed NO_2 and NO_3 generation - time profiles given in Fig 6.11.

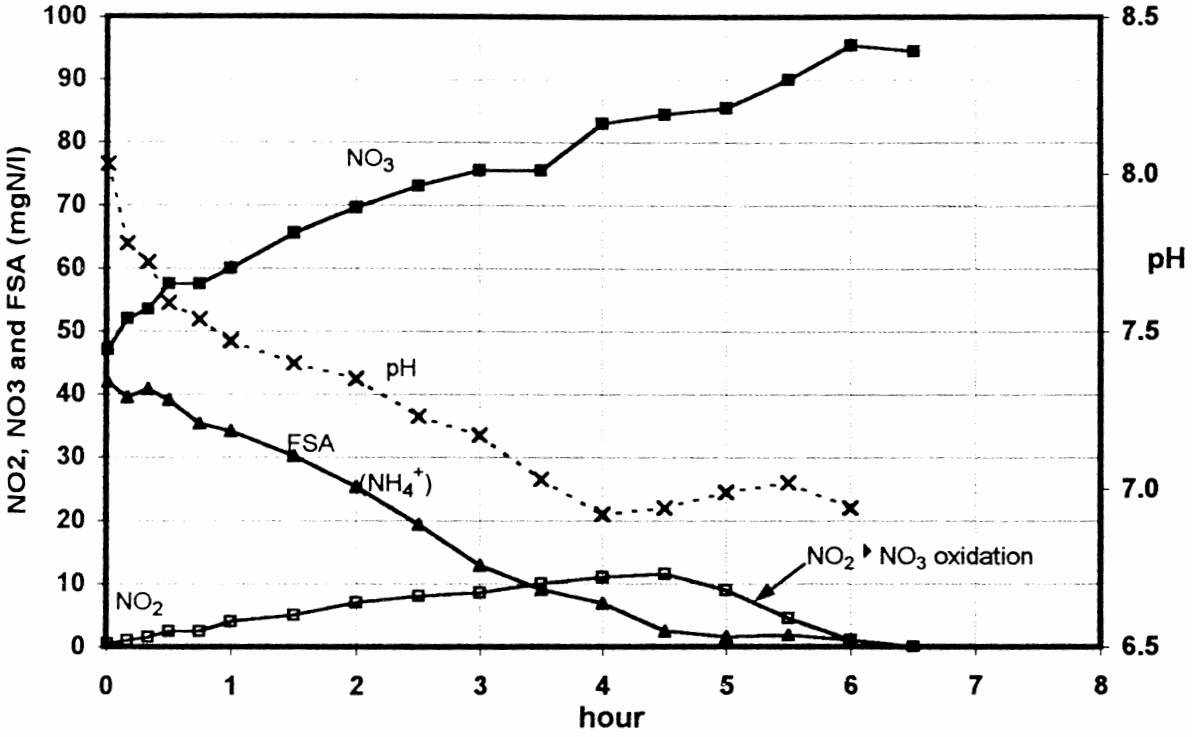


Figure 6.10 : NO_2 and NO_3 versus time profiles recorded during the first feeding cycle (5ℓ) on 23-06-2000. Also shown is the decrease in free and saline Ammonia-NH_4^+ (FSA) concentration with time, together with the pH profile.

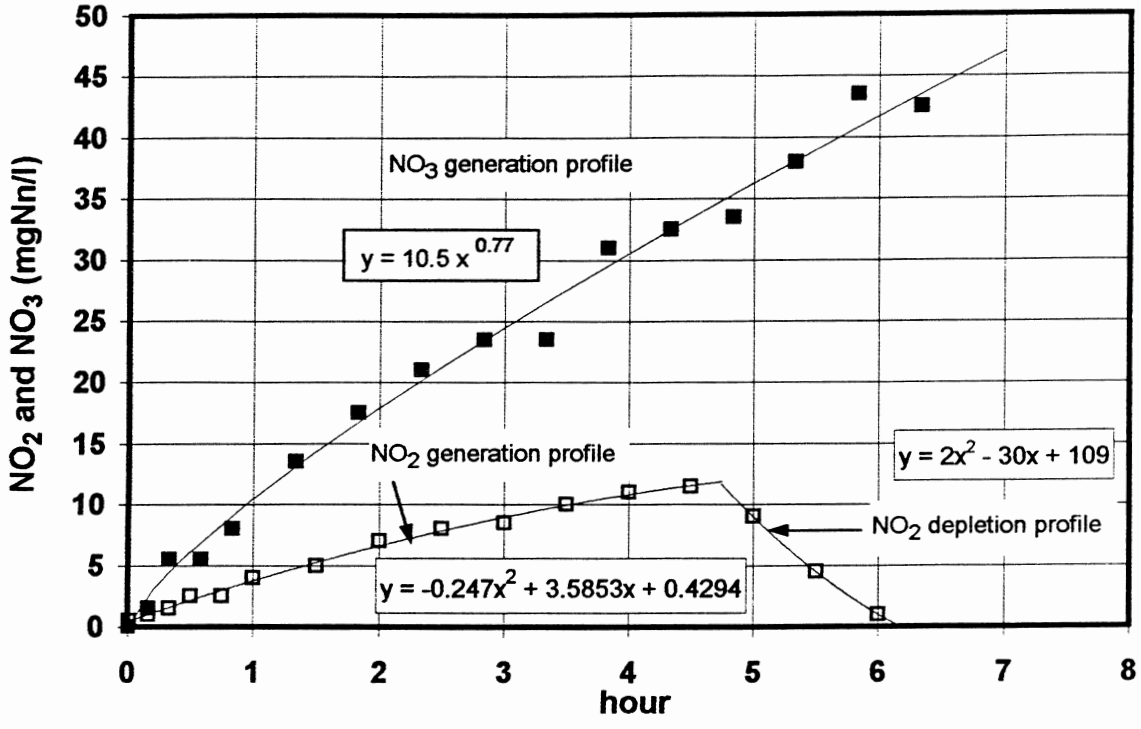


Figure 6.11 : Generation of NO_2 and NO_3 versus time profiles derived from Fig 6.10. Also given are the mathematical formulations used to approximate the generation of NO_2 and NO_3 and to calculate the nitrification oxygen demand (OUR_N) profile in Fig 6.9.

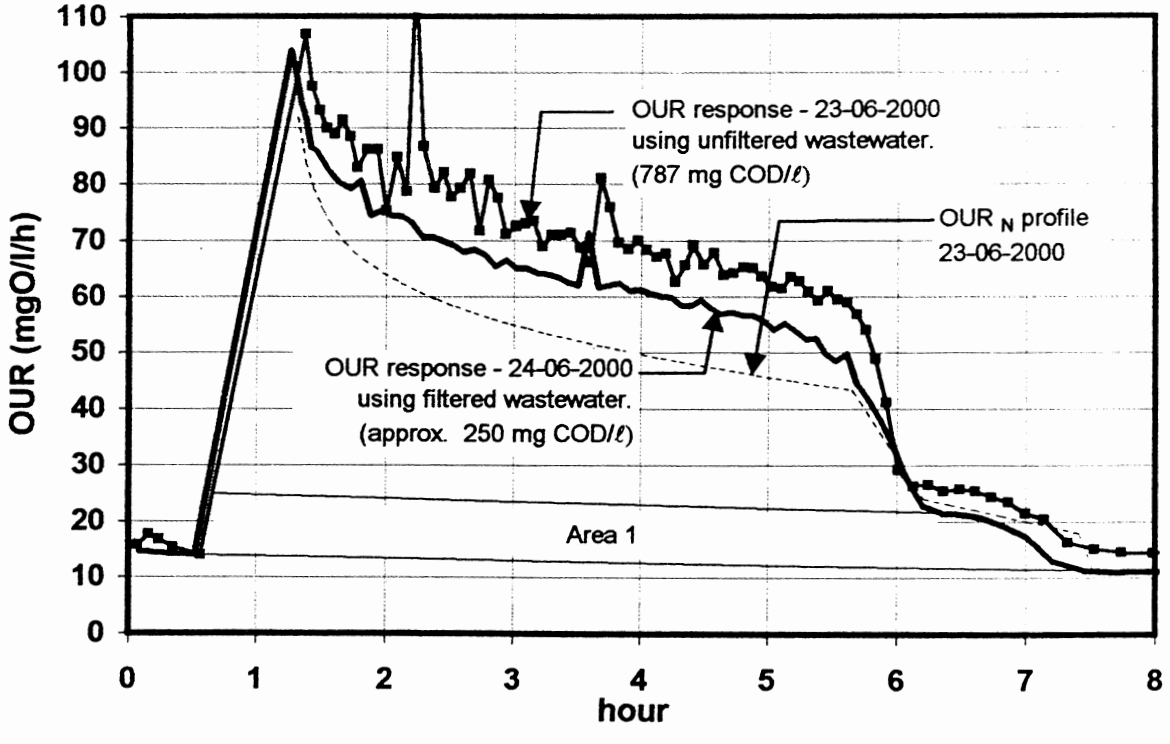


Figure 6.12 : Comparison between the OUR response recorded on 23-06-2000 and the OUR response recorded the following day (24-06-2000), when *filtered* wastewater was used to feed the system. A feed volume of 5ℓ was used on both days.

CHAPTER 7

EXTENSION TO THE MODIFIED BATCH TEST INVESTIGATION:

RESULTS OF BATCH TESTS CONDUCTED ON THE IFFD SYSTEM MIXED LIQUOR

7.1 INTRODUCTION

As described in Chapter 6, the purpose of extending the investigation was to evaluate (i) the effect of increased heterotroph maximum specific growth rates on the batch test procedure, and (ii) the reliability of the modified batch test results when the method is applied to quantify the heterotrophic active biomass present in a variety of parent activated sludge systems. Although the modified batch test method proved to be a reliable means of quantifying the heterotrophic active biomass present in the continuously fed parent system described in Chapter 5, the ability of the method to accommodate a wide range of behavioural responses was unknown. Of particular importance is the capability of the simplified UCT kinetic model incorporated in the analysis of batch test results, to accurately describe the processes and compounds responsible for the OUR-time responses observed in the batch tests.

It is extensively reported that the feeding pattern and load applied in the operation of an activated sludge system, is instrumental in stimulating different responses in terms of active biomass behaviour. On this basis the intermittently fed fill and draw (IFFD) variant of the activated sludge system was chosen to serve as the parent system. The parent system conditions, in terms of COD and TKN loading, are significantly different from the continuously fed system conditions and different active biomass behaviour can be expected, and was indeed observed (see Chapter 6).

This chapter describe the results of modified batch tests conducted on mixed liquor samples harvested from the parent IFFD system. The capacity of the modified batch test method to provide an accurate analysis of the active biomass behaviour observed in Chapter 6, will also be evaluated.

7.2 EXPERIMENTAL PROCEDURE

The experimental approach is identical to the modified batch test approach described in Chapter 5. However, in regard of the harvesting of mixed liquor samples to conduct batch tests, the following variations and limitations were implemented:

- Because it was initially suspected that in the parent system the heterotrophic organisms were storing internally some of the RBCOD for subsequent utilization, mixed liquor samples were drawn from the parent IFFD system during both feed and starve periods to investigate the impact on the batch test OUR response. The majority of the batch test were conducted using mixed liquor samples drawn from the parent system during the endogenous respiration phase, i.e. when the biomass was subjected to "starve" conditions. During the period when the IFFD parent system was receiving sewage batch 25, six batch tests were conducted on mixed liquor samples drawn from the parent system approximately 1 hour after the wastewater was introduced during the first feeding cycle, i.e. significant influent substrate still present. These batch tests (E5 to E10) are indicated in Table 7.4.

- Initially it was intended to conduct the batch tests on mixed liquor samples which were similar in volume to the mixed liquor volumes used for batch tests conducted on the continuously fed parent system, i.e. varying between 100 ml and 400 ml. However, when conducting the first batch test on a 250 ml mixed liquor sample drawn from the parent IFFD system, an excessively high initial oxygen utilization rate (OUR) was observed, resulting in the rapid depletion of RBCOD. The rapid RBCOD depletion resulted in the recording of insufficient OUR data during the initial stages of the batch test, prior to the precipitous drop in OUR. Accordingly, for this investigation the mixed liquor sample volumes were reduced in size and varied between 25 ml and 175 ml.

7.3 DATA ANALYSIS AND RESULTS

7.3.1 Parent system

A well defined and controlled parent laboratory-scale intermittently fed fill and draw (IFFD) system was operated at 10 day's sludge and was continually monitored - see Chapter 6. As described in Chapter 6, the sewage was fed to the parent system in batches which lasted for approximately 2 weeks. To ensure steady state conditions, the parent system received four sewage batches before the modified batch tests were commenced. A total number of 18 modified batch tests were conducted during 3 sewage batch periods. The sewage batches during which the batch tests were conducted are set out in Table 7.1.

Table 7.1: The sewage batch number and the dates it was used as feed together with the number of modified batch tests conducted during each sewage batch.

Sewage batch no.	Dates (2000)	Number of batch tests
25	6 May – 25 May	12
27	6 June – 19 June	4
28	20 June – 23 June	2
TOTAL		18

Although each sewage batch period constituted a steady state period, the wastewater characteristics invariably fluctuated from one sewage batch to the next. As the activated sludge population dynamics are directly related to the wastewater characteristics, the heterotrophic active biomass would exhibit a corresponding fluctuation between different sewage batches. To formulate a theoretical estimate for the heterotrophic active biomass concentration present in the parent system during each sewage batch period, certain key compounds and parameters were monitored on a daily basis. Detailed data on the parent system are given in Appendix D, Tables D3 and D4. For each sewage batch tested, the daily results have been averaged and the average values are listed in Table 7.2 below.

Table 7.2: Steady state results for parent laboratory-scale IFFD activated sludge system receiving sewage batches during which modified batch tests were performed. Averages are listed with sample standard deviations in brackets.

PARENT ANOXIC/AEROBIC STEADY STATE SYSTEM											
WW Batch	No of Tests	COD (mg/ℓ)		TKN (mg/ℓ)		Nitrate (mgN/ℓ)	OUR Area mgO/l/h	Mixed liquor (mg/ℓ)			min pH
		Inf	Eff	Inf	Eff	Effluent		VSS	COD	TKN	
25	12	999 (38)	90 (8)	85 (3)	3.4 (1.4)	52.7 (2.2)	737.5 (27.6)	2779 (73)	4094 (260)	260 (11)	7.19 (0.07)
27	4	1026 (85)	90 (20)	121 (6)	1.5 (0.5)	94.6 (6.7)	1030.0 (105.7)	2713 (103)	4134 (147)	266 (4)	6.82 (0.48)
28	2	785 (18)	67 (12)	126 (2)	2.7 (1.2)	99.2 (7.3)	1153.6 (14.1)	2709 (109)	3974 (38)	252 (9)	6.41 (0.67)

Using the average values in Table 7.2, for each sewage batch the following were determined :

- Influent wastewater unbiodegradable soluble and particulate COD fractions ($f_{S,us}$ and $f_{S,up}$, respectively); system COD and N mass balances (Ekama *et al.* 1986) and the COD and TKN to VSS ratios for the mixed liquor (f_{cv} and f_N, respectively). The calculation procedures are set out in detail in Chapter 2 and the results for each sewage batch are listed in Table 7.3.

- The heterotrophic active biomass fractions of the mixed liquor organic suspended solids (f_{av}) were determined for each sewage batch using the steady state design model (Eq. 3.21), see Chapter 3, section 3.4.1, i.e.:

$$\frac{1}{f_{av}} = 1 + f_E^* b_H^* R_S + \frac{f_{S,up}(1 + b_H^* R_S)}{f_{cv} Y_H^* (1 - f_{S,us} - f_{S,up})}$$

The results are given in Table 7.3.

Table 7.3 : Steady state COD and N mass balances, wastewater fractions and mixed liquor parameters for parent laboratory-scale IFFD activated sludge system. Data calculated from data Table 7.2, either directly or using the steady state (SS) design model (WRC, 1984).

PARENT ANOXIC/AEROBIC STEADY STATE SYSTEM								
WW Batch	No. of tests	Mass Balance (%)		Wastewater fractions		Mixed liquor		
		COD	N	Unbiod Soluble COD ($f_{S,us}$)	Unbiod. Particulate COD ($f_{S,up}$)	COD/VSS ratio (mgN/mgVSS) (f_{cv})	TKN/VSS ratio (mgN/mgVSS) (f_n)	Active Fraction (f_{av})
25	12	96	99	0.0721	0.2009	1.47	0.094	0.3476
27	4	101	106	0.0750	0.1735	1.52	0.098	0.3653
28	2	97	108	0.0675	0.1945	1.47	0.093	0.3571

Examination of the f_{av} values calculated for the three sewage batch periods revealed that, in general a lower mixed liquor active fraction was obtained compared to the average f_{av} values calculated for the continuously fed system (see Chapters 4 and 5). Through Eq (3.21) the low f_{av} values are inversely related to the high wastewater unbiodegradable particulate fractions ($f_{S,up}$) obtained for the particular sewage batch periods. In Chapter 6 the wastewater mean $f_{S,up}$ was determined to be 0.1822. This mean is considerably higher than the value of $f_{S,up} = 0.103$, obtained for the same wastewater via the continuously fed system (see Chapter 2), and that observed by Ubisi *et al.* (1997a,b), $f_{S,up} = 0.12$ for the same Mitchell's Plain wastewater.

In turn, the high $f_{S,up}$ values are the result of the higher average VSS concentrations observed for the parent IFFD system (Table 6.3, Chapter 6). The relationship between $f_{S,up}$ and the VSS concentration (X_v) is given by Eq (2.12):

$$X_V = \frac{S_{ti}\,(1 - f_{S,up} - f_{S,us})Y_H^{*} R_S}{(1 + b_H^{*} R_S)}\,(1 + f_E^{*} b_H^{*} R_S) + f_{S,up} S_{ti} R_S / f_{cv}$$

The higher VSS concentrations could have arisen from two possible sources, (i) change in the nature of the wastewater, or (ii) change in organism response to the wastewater. With regard to the former, it is unlikely that the Mitchell's Plain wastewater would be subject to such a consistent increase in the wastewater unbiodegradable particulate fraction. Therefore, it would appear that the cyclic feed/starve conditions present in the IFFD system induced a change in the behavioural response of the organisms to the wastewater. This change in behaviour is reflected in consistently higher VSS concentrations. In terms of the theory of for activated sludge systems, the higher VSS concentrations could be due to (i) some of the influent slowly biodegradable COD remaining unbiodegraded in the system, or (ii) a change in the kinetics and stoichiometry of the heterotrophic organisms. With regard to the former, in Eq (2.12), any

unbiodegraded slowly biodegradable COD would be added to the influent unbiodegradable particulate fraction (this parameter is a measure of the unbiodegradability of the particulates ***in the system being assessed***), and hence Eq (2.12) would still provide a reasonable estimate for heterotrophic active biomass. With regard to the latter, the relationship between the measured VSS concentration and $f_{S,up}$ is governed by the kinetic and stoichiometric constants in Eq (2.12) and it would imply that under IFFD conditions different values for the heterotrophic active biomass yield (Y_H^* = 0.45 mgVSS/mgCOD), net specific mass loss rate (b_H^* = 0.24/d at 20°C) and/or the endogenous residue fraction (f_E^* = 0.2) need to be incorporated. Different values for these constants would similarly affect the value of f_{av} through Eq (3.21).

Alternatively, during the feed period the heterotrophic active biomass stores part of the influent RBCOD as some internally stored polymer which is not completely utilized in the IFFD system. This stored polymer will add to the measured VSS, but is not taken into account in the activated sludge modelling theory kinetics and stoichiometry. If the values for the constants in Eq (2.12) have changed, or RBCOD is internally stored which is not taken into account in the theory, the estimate for f_{av} from Eq (3.21) very likely will be in error. From the data available on the parent IFFD system, it is not possible to make a definitive judgement as to which of the possibilities above is most likely. However, these will be considered further when the batch test results are analyzed.

Despite the uncertainty surrounding the accuracy of the $f_{S,up}$ and f_{av} values, the *theoretical* heterotrophic active biomass concentrations in the parent system [X_{BH}(theor), COD units] were calculated for each sewage batch using Eq. (5.1):

$$X_{BH}(\text{theor}) = f_{av}\, X_v\, f_{cv} \qquad (\text{mgCOD}/\ell)$$

The calculated values are given in Table 7.4 below (X_{BH} - Theoretical ML). Accordingly, the *theoretical* heterotrophic active biomass concentration in each batch test, due to the addition of the mixed liquor samples drawn from the parent system, was calculated using Eq. (5.2):

$$Z_{BH}\,(\text{theor})_{BT} = X_{BH}\,(\text{theor}) \cdot V_{ML} / (V_{ML} + V_{WW}) \qquad (\text{mgCOD}/\ell)$$

where,

$Z_{BH}\,(\text{theor})_{BT}$ = theoretical heterotrophic active biomass concentration in batch reactor due to added mixed liquor, COD units (mgCOD/ℓ batch reactor)

$X_{BH}\,(\text{theor})$ = theoretical heterotrophic active biomass concentration in the parent system from Eq (5.1) (mgCOD/ℓ)

V_{ML} = volume of parent system mixed liquor added to the batch test (ℓ)

V_{WW} = volume of filtered wastewater added to the batch test (ℓ)

The theoretical heterotrophic active biomass concentration in the batch reactor was correspondingly calculated for each modified batch test and the values are listed in the right hand side column of Table 7.4 (Z_{BH} - Theoretical Batch test).

7.3.2 Modified batch tests

Eighteen modified batch tests with flocculated filtered wastewater and mixed liquor drawn from the IFFD parent system, were conducted during 3 sewage batch periods 25 to 28. The OUR versus time plots are set out in detail in Appendix E. As an example, the OUR (mg/O/ℓ/h) and nitrate concentration (mgN/ℓ) versus time responses for the modified batch test E1 conducted on 9 May 2000 (09-05a, sewage ba 25) with a mixture of flocculated filtered wastewater (2.9ℓ) and mixed liquor (0.1ℓ) are shown in Fig 7.1 and Fig 7.2 respectively.

The observed OUR – time responses did not conform to the expected OUR profile described by Wentzel et al. (1995) and that observed in the batch tests conducted earlier during this investigation on mixed liquor samples harvested from the continuously fed parent system (Chapter 5). In contrast to the expected exponential increase in OUR during the first period of the batch test, the OUR initially decreased, before gradually increasing. This phenomenon is illustrated in Fig 7.1: A relatively high initial OUR was observed, followed by a progressive decrease in OUR during the first period (< 2.0h). During the following period (2.0h – 7.5h) the OUR exhibited the expected pattern associated with the exponential heterotrophic active biomass growth, until the precipitous drop in OUR occurred at time ~ 7.5h.

To a large extent the shape of OUR response recorded during the first period of each batch test, correlated well with the shape of the daily OUR profiles observed in the parent system and described in Chapter 6. The consistency of the observed OUR response indicated that the deviation in observed OUR from the expected OUR behaviour noted in the parent system was replicated in the batch tests. In Chapter 6 it was concluded that the deviation could be ascribed principally to a continually decreasing nitrification rate. However, the high initial rate of NO_3 generation, followed by a gradual decrease in the rate of NO_3 generation observed in the parent system, was not explicitly evident in the majority of the nitrate concentration (mgN/ℓ) versus time profiles recorded during each batch test. Further, although nitrite (NO_2) generation was detected in most of the batch tests, the small magnitude and the fact that the increase in NO_2 was predominantly linear (i.e. constant nitrification rate) excluded unaccounted nitrite generation as a major contributing factor towards the observed deviation in OUR response.

An alternative possible explanation was that the heterotrophic organisms were storing RBCOD substrate while feeding in the parent system and then utilizing the stored RBCOD substrate when introduced into the batch reactor. This could possibly account for the initial high OUR which decreases with time as stored RBCOD is depleted. The storage of RBCOD was also identified as a possible explanation for the high VSS

concentrations observed in the parent IFFD system. To investigate the possibility of RBCOD storage and subsequent utilization in the batch test, two batch tests were conducted on 23-05-2000 with a mixture of unfiltered *effluent* from the parent system and mixed liquor samples drawn from the parent system during the period when the system was feeding on the first feed.

By using unfiltered effluent accepted to contain negligible amounts of biodegradable COD, the OUR response was expected to represent only the OUR associated with endogenous respiration, unless the organisms were capable of utilizing stored substrate derived from the parent system. The OUR versus time plots obtained during these batch tests are given in Appendix E2. As an example, the OUR (mg/O/ℓ/h) versus time response for batch test 23-05a (sewage batch 25), with a mixture of unfiltered effluent (2.825ℓ) and mixed liquor (0.175ℓ) is shown in Fig 7.7. Although the initial OUR (~2.5 mg/O/ℓ/h) is marginally higher than the ultimate OUR associated with endogenous respiration (~0.8 mg/O/ℓ/h), the magnitude of the difference in OUR is not significant compared to the initial OUR observed in batch tests conducted with flocculated filtered wastewater and the same volume of mixed liquor, i.e. batch tests E6 and E12 (OUR ~ 14 mg/O/ℓ/h). Based on these observations it was concluded that the high initial OUR's observed in the batch tests were not related to intracellular substrate storage in the parent system and utilization of this stored substrate in the batch test. (This also excluded RBCOD storage as a possible reason for the high VSS concentrations in the parent IFFD system).

Through a process of elimination, it was thus concluded that the shape of OUR response in the batch tests could only result from progressively decreasing substrate utilization / growth rates exhibited by the heterotrophic active biomass. For reasons not yet known, the heterotrophic organisms originating from feed/starve conditions, are stimulated to yield a high initial OUR which gradually decreases with time, until the point is reached where this oxygen demand is superceded by the OUR associated with nitrification and heterotrophic growth. The fact that the magnitude of the high initial OUR is directly related to the size of the mixed liquor sample (compare OUR responses in Appendix E), demonstrated that the OUR is directly related to the active biomass. This conclusion complicates the interpretation of results in terms of the simplified UCT model.

Considering only the utilization of RBCOD, in the UCT kinetic model the aerobic growth rate (ρ_1) of heterotrophic organisms is given by the Monod equation incorporating the half saturation constant for RBCOD (K_{SH}):

$$\frac{dZ_{BH}}{dt} = \rho_1 = \frac{\mu_H \cdot S_{bs}}{(K_{SH} + S_{bs})} Z_{BH} \qquad \text{(mgCOD/ℓ/d)}$$

During the initial stages of the batch test it was accepted that $S_{bs} >> K_{SH}$ and the expression was simplified to:

$$\frac{dZ_{BH}}{dt} = \mu_H \cdot Z_{BH}$$

The observations from this investigation suggest that this simplification is not justified when analyzing the OUR response from batch tests conducted on mixed liquor samples originating from an IFFD system. In particular, in the discussion on the OUR response observed in the parent system (Chapter 6, section 6.7.2) it was noted that the value for K_{SH} appeared to increase in the IFFD parent system. If this increase in K_{SH} is true, in the interpretation of the batch test data the simplification $S_{bs} >> K_{SH}$ may not be reasonable. Accepting that K_{SH} does increase for the IFFD mixed liquor, in the batch tests this will result in a continual decrease in substrate utilization rate and associated OUR as RBCOD decreases due to utilization. This corresponds to the observed initial behaviour. However, at some point in the batch test, the observed OUR does exhibit an exponential increase. This cannot be explained in terms of a change in K_{SH}. Thus, the observed response in the batch tests cannot be simply explained in terms of the currently accepted kinetic models. It can be concluded, however, that the IFFD system produces a mixed liquor whose response deviates from that expected from activated sludge theory.

Despite the fact that the simplified UCT approach proved to be inadequate to accurately analyze the behaviour during the initial stages of the batch test, it was decided to investigate its applicability to the time intervals where the OUR response did conform to the characteristic exponential increase, i.e. the period (2.0h – 7.5h) in Fig 7.1.

For this investigation the interpretation of batch test data and subsequent calculation procedures are identical to that described in section 5.3.2, Chapter 5. For elucidation purposes the procedures are briefly summarized below:

Oxygen utilization rate due to nitrification (OUR_N)

For the modified batch tests it was accepted that the nitrate concentration - time profile follows an exponentially increasing trend. A detailed description of the exponential approach to nitrification and the determination of the oxygen demand due to nitrification (OUR_N) is given in Chapter 5, section 5.3.2, but a brief summary of the application is given here.

- From the measured nitrate concentration – time profile for example, (Fig 7.2) the most appropriate general exponential expression was obtained through non-linear regression:

  $$S_{NO} = 1.8345\ e^{0,0890\,t} \qquad (mgN/\ell)$$

  From Fig 7.2 it is evident that the general exponential expression provides a good representation of the observed nitrate concentration data with time, which is reflected in the good correlation coefficient (R^2) of 0.9345.

However, some modified batch tests conducted with the IFFD system mixed liquor, showed variable nitrate concentration – time results, with 6 out of 18 batch tests yielding $R^2 < 0.9$.

- Using the general exponential expression, approximated nitrate concentrations were sequentially calculated for a series of time intervals to estimate the increasing OUR_N response. The sequential calculation procedure is described in Chapter 5, section 5.3.2, and the calculated OUR_N profile is shown in Fig 7.1.

Subtracting the calculated OUR_N values from the OUR data measured in the batch test (OUR_M) gives the estimated OUR response due to the heterotrophic active biomass (OUR_H), shown plotted in Fig 7.3.

Determining the heterotrophic active biomass concentration

For each batch test the $OUR_{H(t)}$ data recorded during the initial stages, when the OUR values exhibited the decreasing trend, were disregarded and the remaining $OUR_{H(t)}$ data up to the precipitous drop in OUR were plotted ℓn (OUR) versus time. For example, the $OUR_{H(t)}$ data in Fig 7.3 are shown plotted ℓn ($OUR_{H(t)}$) in Fig 7.4. Linear regression was applied to the linear section of the ℓn ($OUR_{H(t)}$) time data to determine the y-intercept, slope and correlation coefficient (R^2).

It was observed that the slopes determined from the $\ell n(OUR_{H(t)})$ plots were variable, but in general the measured slopes did not differ significantly in magnitude from the $\ell n(OUR_{H(t)})$ slopes measured in the batch tests conducted with mixed liquor samples drawn from the continuously fed parent system. This observation was unexpected, as it is generally accepted that the heterotrophic growth rate on RBCOD (μ_H) is much higher for IFFD systems than the μ_H for continuously fed systems (Jenkins *et al.*, 1984; Still *et al.*, 1986, 1996; Gabb *et al.*, 1989; Dold *et al.*, 1991). It was expected that a higher μ_H would result in a higher overall growth rate, which would be reflected in higher values for the slopes measured from the $\ell n(OUR_{H(t)})$ plots.

From the y-intercepts and slopes, the heterotrophic active biomass concentration at the start of each batch test was calculated using Eq (3.9):

$$Z_{BH(0)} = \frac{(e^{\text{y-intercept}}) \cdot 24}{\frac{1-Y_{ZH}}{Y_{ZH}} \cdot (\text{slope} \cdot 24 + b_H)} \quad (\text{mgCOD}/\ell)$$

The heterotrophic active biomass values measured at the start of each modified batch test are listed in Table E1, Appendix E. The results are summarized in Table 7.4. From the measured $Z_{BH(0)}$ values, the projected heterotrophic active biomass concentrations in the parent IFFD system were calculated for each modified batch test using Eq (5.4):

$$Z_{BH(ML)} = Z_{BH(0)BT} \cdot (V_{ML} + V_{WW}) / V_{ML} \quad (mgCOD/\ell)$$

The projected mixed liquor heterotrophic active biomass concentrations in the parent system values are listed in Table 7.4 (Z_{BH} - Measured ML).

Determining the maximum specific growth rates

From the analysis of an $OUR_{H(t)}$ versus time plot the maximum specific growth rates on slowly biodegradable COD (K_{MP}) and readily biodegradable COD (μ_H) can be calculated. As described in Chapter 5, it has to be assumed that the filtered wastewater will contain some soluble SBCOD. Thus, up to the precipitous drop in OUR_H (Fig 7.3), the OUR is the sum of both the SBCOD and RBCOD utilization. When the precipitous drop in OUR_H occurs, the RBCOD is depleted and the OUR is that for SBCOD only.

If the precipitous drop in OUR_H occurs at t = s hours, at which time the OUR is $OUR_{SBCOD(t=s)}$, K_{MP} can be calculated via Eq (3.18):

$$K_{MP} = \frac{OUR_{SBCOD(t=s)} \cdot 24}{\frac{1-Y_{ZH}}{Y_{ZH}} \cdot Z_{BH(0)} \cdot e^{(\mu_H + K_{MP} - b_H) \cdot (t=s)/24}}$$

Disregarding the $\ell n(OUR_H)$ data recorded during the initial period, the overall growth rate ($\mu_H + K_{MP} - b_H$) is given by the slope of the $\ell n(OUR_H)$ plot in Fig 7.4. For the simplified UCT model the value for the maximum specific growth rate on RBCOD (μ_H) can be calculated from the value for K_{MP} derived above and the slope of the $\ell n(OUR_H)$ versus time plot, as given by Eq (3.19):

$$\mu_H = \text{slope} \cdot 24 - K_{MP} + b_H$$

For each batch test the values for K_{MP} and μ_H were calculated using Eq (3.18) and Eq (3.19). The results are given in Table E1, Appendix E, and are summarized in Table 7.4 :

- From Table 7.4 statistical plots for μ_H and K_{MP} were constructed, see figs 7.8 and 7.9 respectively. From the statistical plots it is evident that the K_{MP} values (*mean* K_{MP} = 0.91/d) are of the same magnitude as the K_{MP} and μ_H values measured for the continuously fed system (*mean* K_{MP} = μ_H = 0.84 /d). However, the μ_H values calculated for all the batch tests were slightly higher, with a *mean* μ_H of 1.35 /d. As described earlier, the μ_H values were significantly lower than expected. UCTOLD batch simulations conducted by Dold *et al.* (1991) on mixed liquor samples harvested from a similar IFFD system, gave a μ_H value of 4.3 /d for satisfactory correlation between the simulated and experimental results.

Table 7.4 : Results from modified batch tests with a mixture of filtered wastewater (WW) and mixed liquor (ML) drawn from parent IFFD system : Batch test numbers, dates of batch tests, volumes added, COD recoveries, max. specific growth rates, *measured* heterotrophic active biomass at the start of the batch test ($Z_{BH(0)}$) and that projected in the parent system mixed liquor (ML). Also shown are the *theoretical* $Z_{BH(0)}$ in the parent system mixed liquor (ML) and that present at the start of the batch test.

MODIFIED BATCH TESTS : FILTERED WASTEWATER (WW) + IFFD system MIXED LIQUOR (ML)											
Sew. Batch no.	Batch test no.	Date of test	Volume (ℓ)		COD recov. (%)	Max. specific growth rates (/d)		$Z_{BH(0)}$ (mgCOD/ℓ)			
								Measured		Theoretical	
			ML	WW		K_{MP}	μ_H	Batch test	ML	ML	Batch test
25	E1	09-05a	0.100	2.900	86.2	1.01	1.39	82	2452	1423	47
	E2	09-05b	0.150	2.850	96.9	0.81	0.95	136	2725	1423	71
	E3	10-05a	0.075	2.925	91.0	1.13	1.48	59	2363	1423	36
	E4	10-05b	0.125	2.875	98.0	0.86	1.00	128	3066	1423	59
	E5 *	12-05a	0.150	2.850	100.5	0.81	0.84	264	5275	1423	71
	E6 *	12-05b	0.175	2.825	99.4	0.31	0.34	690	11825	1423	83
	E7 *	16-05a	0.075	2.925	83.9	1.42	1.16	57	2267	1423	36
	E8 *	16-05b	0.125	2.875	101.5	0.73	0.74	129	3098	1423	59
	E9 *	17-05a	0.100	2.900	77.7	1.11	1.15	112	3358	1423	47
	E10 *	17-05b	0.150	2.850	88.9	0.99	1.04	124	2489	1423	71
	E11	19-05a	0.125	2.875	89.7	0.82	0.91	191	4585	1423	59
	E12	19-05b	0.175	2.825	95.0	0.76	0.76	217	3724	1423	83
27	E13	16-06a	0.025	2.975	71.4	0.58	1.68	28	3394	1510	13
	E14	16-06b	0.050	2.950	82.2	0.54	1.45	45	2724	1510	25
	E15	19-06a	0.075	2.925	86.8	1.16	2.68	48	1924	1510	38
	E16	19-06b	0.100	2.900	90.5	1.61	2.77	41	1245	1510	50
28	E17	21-06a	0.025	2.975	88.7	0.66	2.00	59	7046	1419	12
	E18	21-06b	0.050	2.950	98.2	1.06	2.02	35	2075	1419	24

* Batch tests on ML samples drawn from IFFD system after the system was fed.

COD recovery

The acceptability of the data from the batch test can be evaluated by doing a COD mass balance, as defined by Eq (3.1):

$$\% \text{ COD recovery} = \frac{COD_{t=T} + \int_{t=0}^{t=T} OUR_{H(t)} \cdot dt}{COD_{t=0}} \cdot 100$$

%COD recoveries ranging from 90 – 100 % provide support for the reliability of the measurements. The %COD recoveries for all the batch tests were calculated using Eq (3.1) and the results are listed in Table E1, Appendix E. The %COD recovery for each modified batch test is summarized in Table 7.4.

- In general, the %COD recoveries were variable with 9 batch tests yielding %COD recoveries < 90%. A statistical plot of the %COD recovery for all the modified batch tests was constructed; see Fig 7.5. From Fig 7.5 the mean %COD recovery was 90.4% with sample standard deviation of 8.2%. The variable %COD recoveries appear to reflect the uncertainty surrounding the OUR responses observed in the modified batch tests conducted on the IFFD system mixed liquor.

7.4 COMPARISON BETWEEN MEASURED AND THEORETICAL ACTIVE BIOMASS

In Table 7.4 the *measured* heterotrophic active biomass concentration at the start of each modified batch test is compared with the *theoretical* heterotrophic active biomass concentration contained in the mixed liquor sample drawn from the parent system, predicted via the steady state design model. To illustrate the comparison, the measured versus theoretical mixed liquor heterotrophic active biomass data for all the modified batch tests are shown plotted in Fig 7.6.

The comparison shows poor agreement between the measured and theoretical results. The measured values were consistently higher than the theoretically predicted values and the poor correlation seems to be accentuated with increasing volumes of mixed liquor used in the batch tests. The fact that the measured values were consistently higher indicate that the low theoretical active fraction (f_{av}) calculated in section 7.3.1, may indeed constitute an under-prediction of the "true" heterotrophic active biomass concentration in the parent system. As explained in section 7.3.1, the low f_{av} value resulted from the steady state design model being incapable of providing an accurate interpretation of the VSS concentrations observed for the parent IFFD system. The comparisons therefore support the assumption that the steady state design model, through Eq (2.12), is inadequate in characterizing the behavioural response in the IFFD system.

However, the under-prediction of the theoretical heterotroph active biomass values is unlikely to be the single reason for the poor correlation between the measured and theoretical values. From Fig 7.5 it is evident that the measured values are more than twice the theoretical values for batch tests conducted on mixed liquor samples in excess of 100 mℓ. This can only be ascribed to the uncertainty regarding the OUR profile during the initial stages of the batch tests. It is obvious from the comparison that the process(es) present during the initial stages of the batch tests do indeed have an impact on the subsequent exponential increase in the OUR response. It is therefore incorrect to assume that an accurate reflection of the heterotrophic a e biomass at the start of the batch test can be obtained by disregarding the initial OUR data and applying the simplified UCT kinetic model in the analysis of the remaining data.

7.5 CONCLUSIONS

In this Chapter it was hoped to evaluate the effect of increased heterotroph maximum specific growth rates on the batch test procedure. To generate mixed liquor with higher maximum specific growth rates, an IFFD system was operated as parent system. Observations in the literature indicated that such systems stimulate growth of organisms with high maximum specific growth rates (e.g. Ekama *et al.*, 1986). Further, this would provide the opportunity to assess the reliability of the modified batch test method to quantify the heterotrophic active biomass present in an (IFFD) activated sludge system dissimilar in operating conditions to the continuously fed system. From the modified batch tests conducted on mixed liquor samples drawn from the intermittently fed fill an draw (IFFD) system, the following can be concluded:

- The parent IFFD system produced VSS concentrations that were consistently higher than the continuously fed system despite receiving the same COD load daily and having the same sludge age and system volume. In terms of the steady state design model, the high VSS concentration reflects in a consistently high wastewater unbiodegradable particulate COD fraction ($f_{S,up}$), which in turn gives a low mixed liquor heterotrophic active biomass fraction (f_{av}). Previous research indicates that it is unlikely that the Mitchell's Plain wastewater had a consistently high $f_{S,up}$ fraction. One possibility is that slowly biodegradable COD remains unbiodegraded in the IFFD system, and therefore adds to the unbiodegradable particulates. If this possibility is true, then the theoretical estimate for heterotrophic active biomass concentration in the parent system mixed liquor will be acceptable. However, the comparison between the theoretical and measured heterotrophic active biomass concentration in the modified batch tests suggests an under-prediction of the "true" theoretical heterotrophic active biomass in the parent system.

- It was thus concluded that the steady state design model does not accurately reflect the behavioural response emanating from the cyclic feed / starve conditions present in the IFFD system.

- In the batch test on IFFD system mixed liquor, the OUR response differed from that observed on continuously fed system mixed liquor : The initial OUR was high and gradually decreased with time before increasing exponentially. To a large extent the shape of OUR response recorded during the first period of each batch test, correlated well with the shape of the daily OUR profiles observed in the parent IFFD system and described in Chapter 6. The consistency of this observation indicated that the observed OUR deviation from the expected OUR behaviour was replicated in the batch tests.

- In contrast to the parent system observations described in Chapter 6, where the deviation in OUR response could be attributed principally to a continually decreasing nitrification rate, the nitrate generation profiles recorded during the batch tests showed only a moderate influence of the nitrification oxygen demand on the observed OUR response.

- Batch tests conducted with filtered effluent demonstrated that the high initial OUR levels observed in the batch tests were not related to intracellular RBCOD storage in the parent system and its subsequent utilization in the batch tests. This was one possible explanation advanced to explain the high VSS concentrations observed in the parent IFFD system, but from the batch test data this explanation should be rejected..

- Through a process of elimination it was thus concluded that the shape of OUR response during the initial stages of the batch tests resulted from progressively decreasing substrate utilization / growth rates of the heterotrophic active biomass.

- When disregarding the initial batch test OUR data, the analysis of the subsequent exponentially increasing OUR data revealed that the IFFD mixed liquor μ_H values (mean 1.35/d) were only slightly higher than the values μ_H calculated for the continuously fed system (mean 0.84/d). However, the mean μ_H value of 1.35/d was significantly lower than the 4.3 /d obtained via an earlier investigation by Dold *et al.* (1991) for mixed liquor samples harvested from a similar IFFD system.

- In general, the variable %COD recoveries achieved in the batch tests appear to reflect the uncertainty surrounding the OUR responses observed in the modified batch tests conducted on the IFFD system mixed liquor.

It is therefore apparent that the modified batch test in its present format is unable to provide reliable estimations of the heterotrophic active biomass present in a parent system subjected to cyclic feed/starve conditions, such as the IFFD system. It appears that the IFFD system induced a behaviour in the mixed liquor that cannot be accommodated within the current activated sludge system modelling theory.

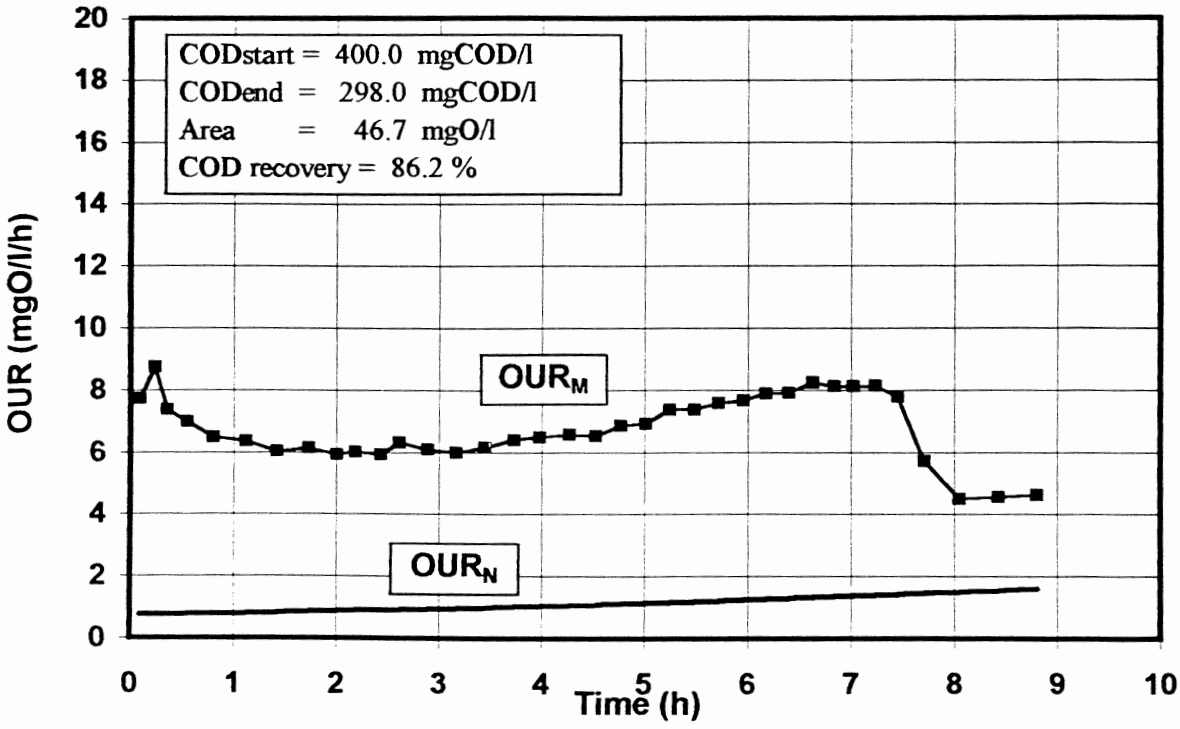


Figure 7.1 : Oxygen utilization rate (OUR) response with time for a modified batch test on a mixture of filtered wastewater (2.9ℓ) and mixed liquor (0.1ℓ) drawn from the parent IFFD system. Batch test no. E1, 09-05a, sewage batch no.25.

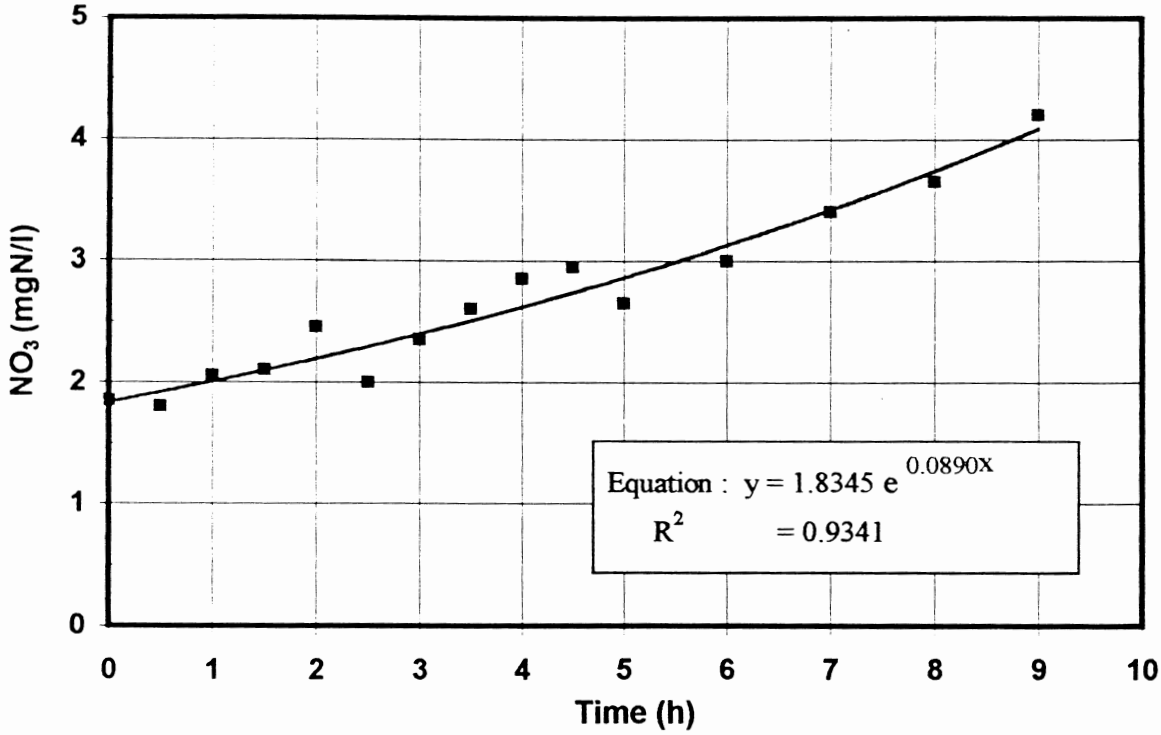


Figure 7.2 : Nitrate variation with time for the modified batch test in Fig 7.1 . Also shown is the exponential expression and correlation coefficient (R^2) , obtained through regression . The nitrification OUR, calculated from $OUR_N = 4.57*(\Delta NO_3/\Delta t)$, is shown in Fig 7.1.

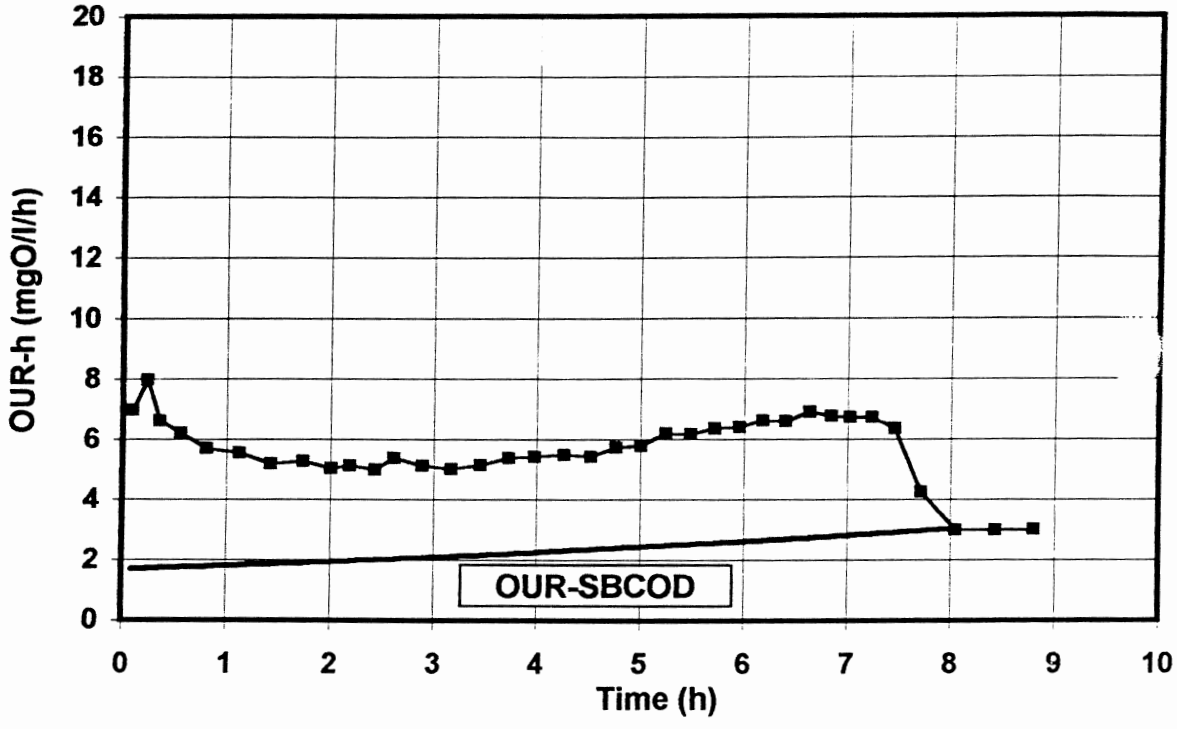


Figure 7.3 : Oxygen utilization rate due to heterotrophic active biomass (OUR-h) versus time for modified batch test no. E1, 09-05a , sewage batch no.25. The OUR due to nitrification (OUR_N) was subtracted from the measured OUR data in Fig. 7.1.

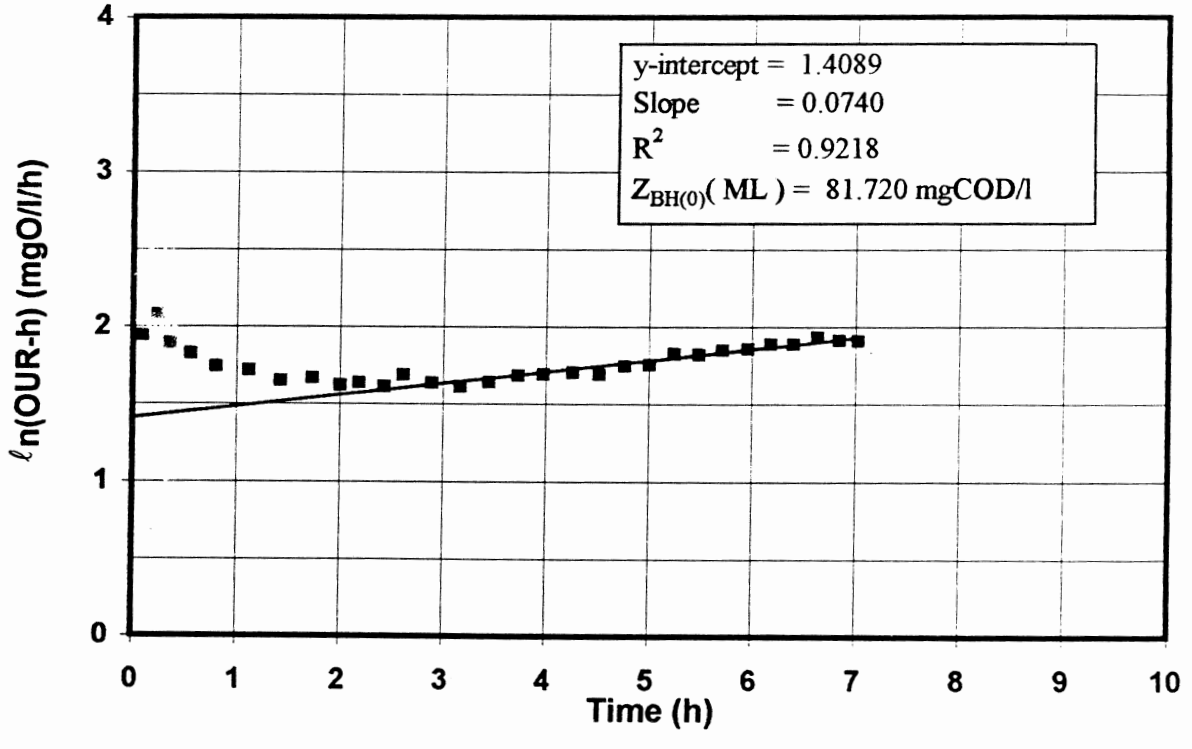


Figure 7.4 : ℓn oxygen utilization rate due to heterotrophic active biomass (ℓn OUR-h) versus time for the OUR-h data in Fig. 7.3 , up to the precipitous drop in OUR. Batch test no. E1 , 09-05a , sewage batch no.25.

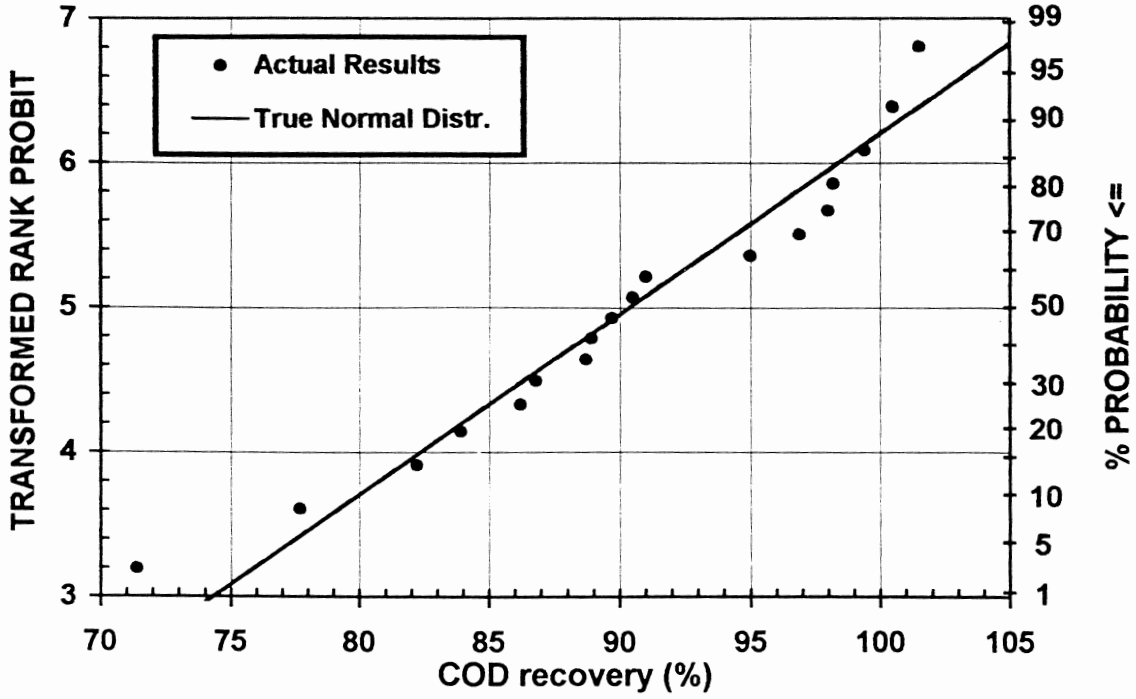


Figure 7.5 : Statistical plot of the %COD recovery for the modified batch tests conducted with filtered wastewater and mixed liquor drawn from the parent IFFD system.

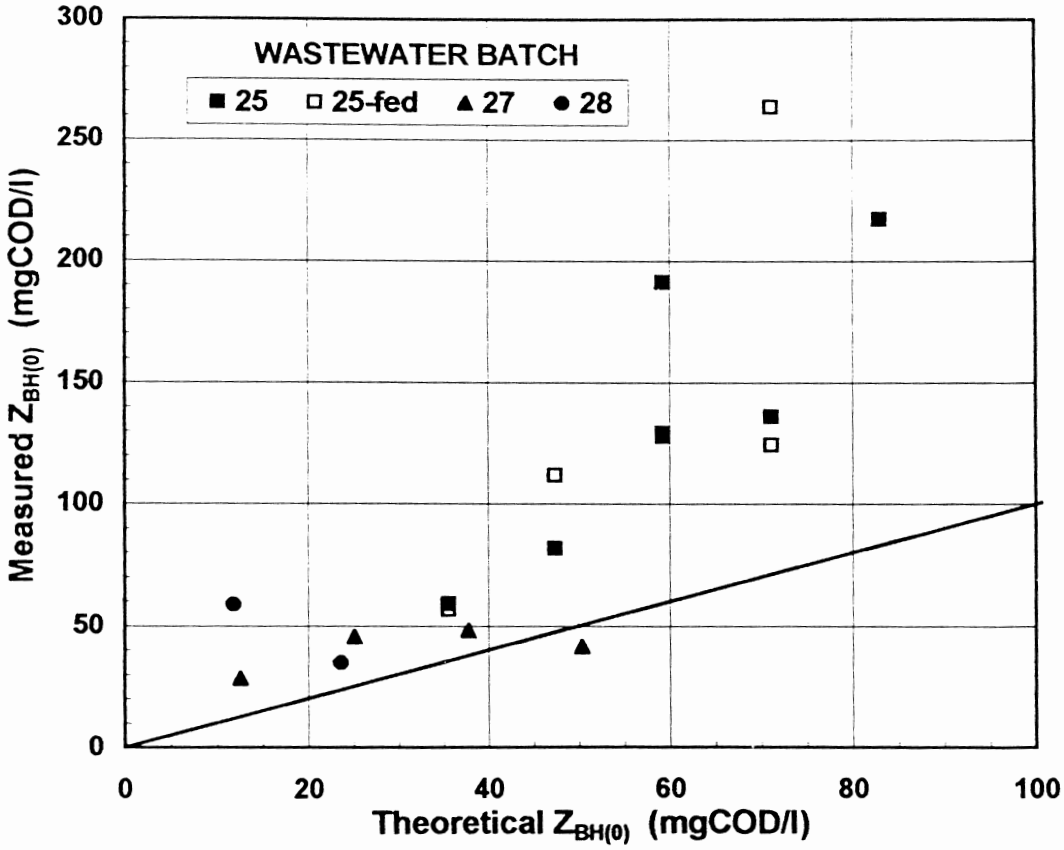


Figure 7.6 : Modified batch test results : graph of measured versus theoretical active biomass $Z_{BH(0)}$(ML) for the various wastewater batches with the parent laboratory-scale IFFD system operated at 10 days sludge age.

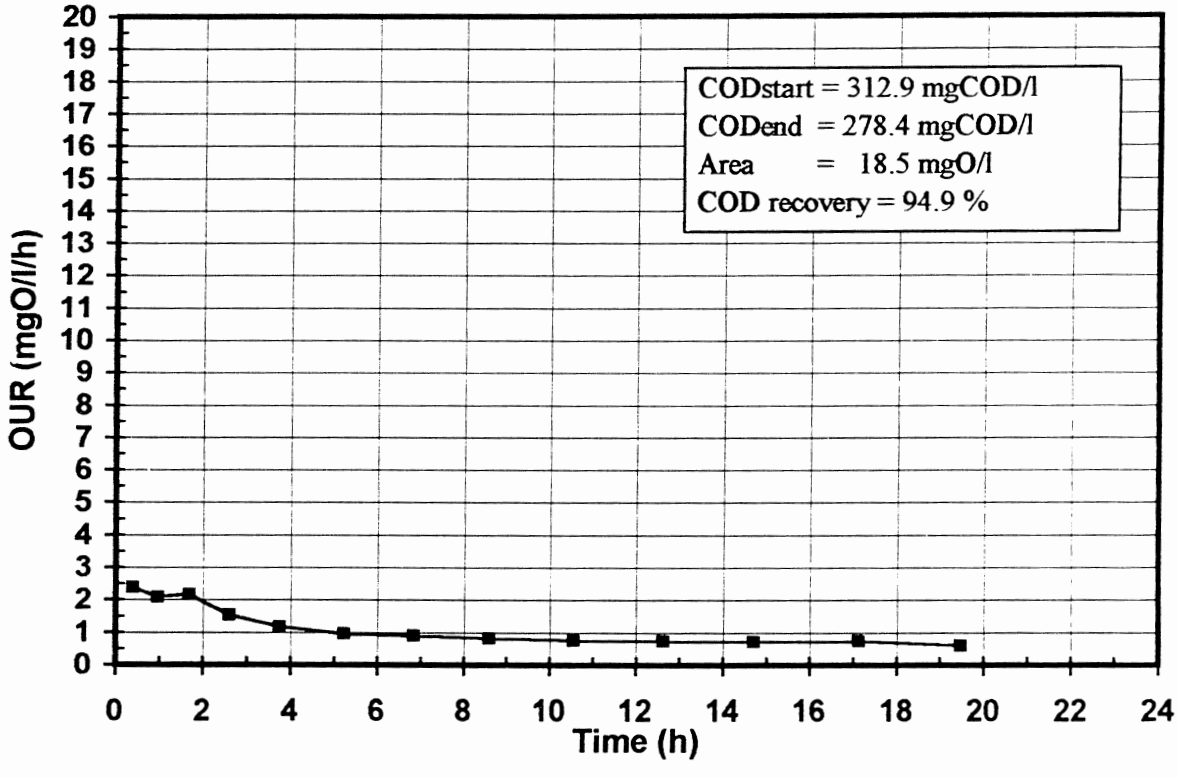


Figure 7.7 : Oxygen utilization rate (OUR) versus time response for a batch test conducted with a mixture of unfiltered *effluent* (2.825ℓ) and mixed liquor (0.175ℓ) drawn from the parent IFFD system. Batch test 23-05b, wastewater batch 25.

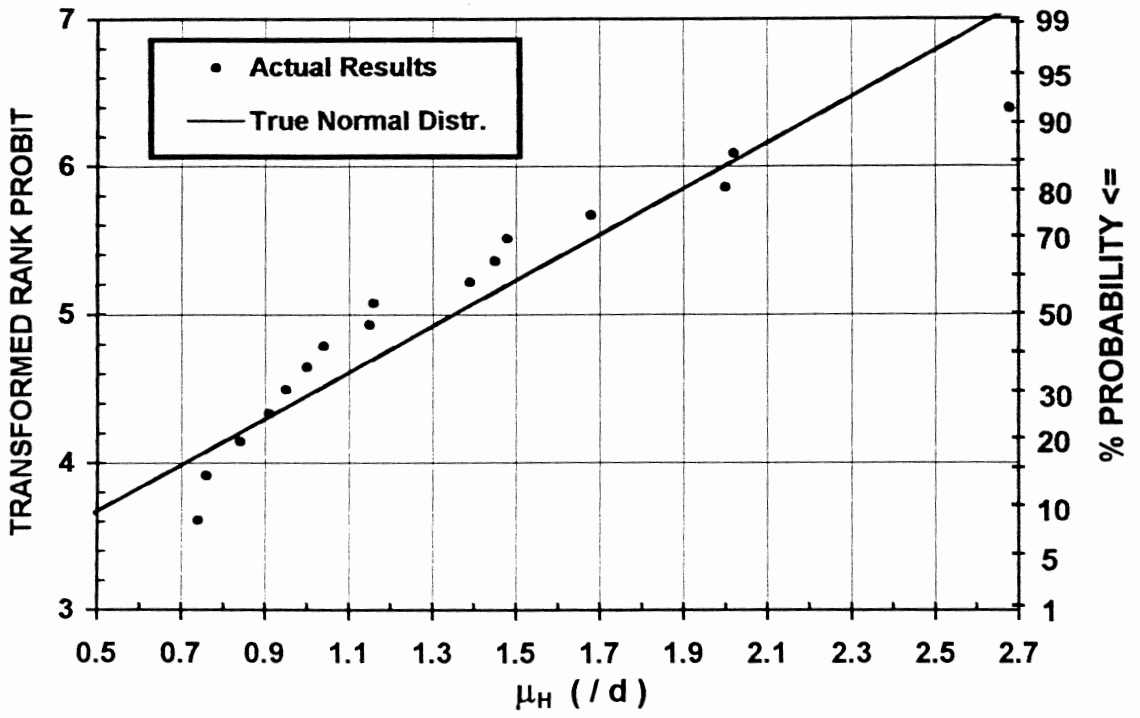


Figure 7.8 : Statistical plot of μ_H values measured for all the modified batch tests conducted with IFFD system mixed liquor.

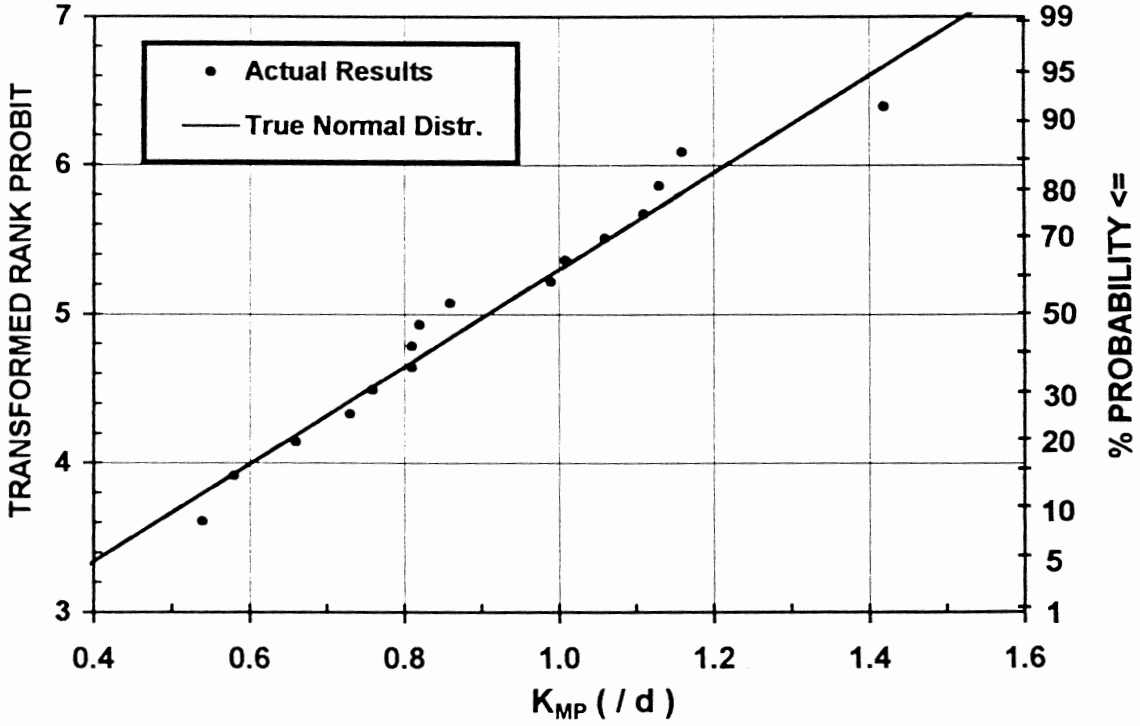


Figure 7.9 : Statistical plot of K_{MP} values measured for all the modified batch tests conducted with IFFD system mixed liquor.

CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS

8.1 OBJECTIVES

Fundamental to the steady state design and kinetic simulation models for activated sludge systems is the parameter heterotrophic active biomass. This mixed liquor organic suspended solids component mediates the biodegradation processes of COD removal and denitrification (and associated processes). In addition, in the models all the relevant specific process rates are expressed in terms of it. Due to the lack of suitable experimental measurement techniques, the heterotrophic active biomass exists only hypothetically within the structure of the design procedures and kinetic models; this casts a measure of uncertainty on the entire framework within which the models have been developed and is a major weakness in the models. Although recently the heterotrophic active biomass concept has gained more acceptance than in the past, it would seem that this is due to the convenience of the computer programmes based on these models, rather than substantive proof of its validity. Thus, to promote confidence in the application of the models for design, operation and control of activated sludge systems, and indeed the models themselves, it is essential that the heterotrophic active biomass parameter is validated by experimental measurement.

Recently, the batch test method developed by Ubisi *et al.* (1997a,b) has shown considerable promise as an *independent* means to quantify the hypothesized concentration of heterotrophic active biomass present in an activated sludge system. However, the batch test has produced inconsistent results for heterotrophic active biomass concentrations in mixed liquor drawn from a well defined parent nitrification / denitrification activated sludge system: The measured heterotrophic active biomass concentrations were in close agreement with those calculated theoretically for the parent system at 12d sludge age, but were about ½ the theoretical values for the parent system at 20d sludge age. Thus, due to this inconsistency, the uncertainty around the active biomass concept largely remained. ***The principle aim of this investigation was to evaluate the batch test method as a reliable means of quantifying the concentration of heterotrophic active biomass.*** To achieve this aim, three primary objectives for this research project have been identified.

8.1.1 Verification of inconsistent batch test results

Ubisi *et al.* (1997a,b) developed a batch test method to quantify the concentration of heterotrophic active biomass present in an activated sludge system. Wentzel *et al.* (1998) evaluated this method by applying the batch test to mixed liquor samples drawn from a well-defined laboratory-scale anoxic/aerobic activated sludge system operated at sludge ages of 12 and 20 d and comparing the measured heterotrophic active biomass values to those calculated theoretically with the models.

From the comparison they concluded that the results obtained were both encouraging and perplexing. With the parent system at 12d sludge age, the agreement between measured and theoretical values was remarkably good. However, with the parent system at 20d sludge age, the agreement was poor, with the theoretical values being about 2 times those measured. Wentzel *et al.* could provide no explanation for this inconsistency.

Thus, ***the first objective of this research project was to repeat the batch test evaluation***, using an identical parent laboratory-scale anoxic/aerobic activated sludge system. The intention was to establish whether the inconsistent batch test results were due to deficiencies in the experimental technique, the misinterpretation of the batch test data, or due to a deficiency in the models themselves. Another possibility was that the parent system from which the mixed liquor was drawn, simply exhibited aberrant behaviour.

8.1.2 Development and evaluation of a modified batch test method

The fact that the theoretical models have been successfully applied to predict the behaviour of a wide variety of system configurations operated under a range of conditions (e.g. Dold *et al.*, 1991), indicated that most likely the deficiency lay in the batch test method, either in the method itself or in the techniques applied in analyzing the batch test data. On the premise that the deficiencies in the batch test method could be identified (first objective above), ***the second objective of this investigation was to develop a modified batch test method*** that would effectively eliminate sources of error in the batch test method developed by Ubisi *et al.* (1997a,b). Integral to this development would be an evaluation of the reliability of the modified batch test results.

8.1.3 Extension of the modified batch test investigation

In addressing the tasks above, it was found that maximum specific growth rate measured for the heterotrophs was low. In analysis of these batch test data, this caused the estimate for heterotroph active biomass concentration to be overly sensitive to small variations in fitting best fit lines to the measured data. It was concluded that this sensitivity could be reduced if the heterotroph maximum specific growth rate could be increased. Accordingly, the effect of increased heterotroph maximum specific growth rate was investigated. From the literature, operating systems as intermittently fed fill and draw (IFFD) induces high heterotroph maximum specific growth rates (Ekama *et al.*, 1986). Thus, it was decided to change operation of the continuously fed system above to IFFD. This would also have provided the opportunity to evaluate the applicability of the modified batch test procedure to provide a reliable quantification of the heterotrophic active biomass present in other activated sludge systems, subjected to dissimilar operating conditions.

In this regard the modified batch tests were to be applied to an intermittently fed fill and draw (IFFD) parent system, operated at the same sludge age of 10 d as the continuously fed parent system with the same bioreactor volume of 10ℓ and receiving the same wastewater and COD load.

Thus, ***the third objective of this investigation was twofold: To evaluate the effect of increased heterotroph maximum specific growth rates on the batch test procedure, and the capacity of the modified batch test method to quantity heterotrophic active biomass in activated sludge systems operated under significantly different conditions.***

8.2 RESEARCH APPROACH

During the first part of this project, the research approach adopted was to operate and monitor a well-defined and controlled anoxic/aerobic activated sludge system. This parent system provided the mixed liquor samples for measuring the heterotrophic active biomass to address first objective. In addition, to address the second objective, this parent system also provided the mixed liquor samples required for evaluating the modified batch test method.

During the latter part of this research project the research approach adopted was to operate and monitor a well-defined and controlled intermittently fed fill and draw (IFFD) activated sludge system. This parent system provided the mixed liquor samples for measuring the heterotrophic active biomass to address the third objective.

8.3 VERIFICATION OF THE INCONSISTENT BATCH TEST RESULTS

To determine if the inconsistent batch test results were related to the sludge age of the system, a well defined and controlled parent laboratory-scale nitrification / denitrification activated sludge system identical to that of Ubisi *et al.* (1997a,b) was operated, but at a different sludge age of 10 days. The system was monitored closely and provided the mixed liquor samples required for measuring heterotrophic active biomass. From the batch tests conducted in accordance with the procedures employed by Ubisi *et al.* (1997a,b), the following was concluded:

- The correlation coefficients (R^2) in linear regression of the $ln OUR_{H(t)}$-time plots for the wastewater (WW) only batch tests were generally very good. For the vast majority of the batch tests $R^2 > 0.95$, which indicates that the observed responses conform to the hypothesized behaviour.

- For the WW only batch tests the mean %COD recovery of 93% is relatively good. However, 9 out of 23 batch tests yielded %COD recoveries which were marginally less than 90%. The cause for these low % COD recoveries was traced to inadequate sampling for measurement of the final total COD concentration in the batch test. When this deficiency was rectified, acceptable COD mass balances > 90% were obtained. Since the source of the error lay in the measurement of COD and not OUR, the low % COD recoveries do not impact the batch test analysis as this relies on OUR measurements.

- No nitrification was observed in the batch tests with wastewater only, indicating the inactivity of autotrophic organisms in the wastewater.

- For the WW only batch tests, the wastewater heterotrophic active biomass ranged from 1.3 to 7.4 % of the total wastewater COD, with a mean of 3.6 % and a sample standard deviation of 2.0 %. This mean compares favourably with the values measured by Ubisi *et al.* (1997a,b) and Mbewe *et al.* (1995) of 4.5 % and 6.1 % respectively, for the same wastewater type.

- For the batch tests conducted with a mixture of wastewater and mixed liquor (WW+ML), the correlation coefficients (R^2) were generally lower than the R^2 values obtained in the wastewater only batch tests. However the R^2 values were generally still acceptable, with the vast majority of the R^2 values > 0.90.

- All the WW+ML batch tests yielded %COD recoveries in the range 90 – 110 % with a mean of 98.0 % and a sample standard deviation of 4.6 %. The good %COD recoveries lend credibility to the reliability of the results.

- For the WW+ML batch tests, nitrification was observed since the mixed liquor added to the batch tests was drawn from a nitrifying activated sludge system.

- With the parent system operated at 10 days sludge age, the heterotrophic active biomass measured in the batch tests showed poor correlation with the theoretically predicted values. The measured values were consistently lower than the theoretical values and the comparison showed remarkable similarity to the comparison obtained by Ubisi *et al.* for batch tests conducted on the parent system operated at a sludge age of 20 days.

- The similarity in the results obtained from batch tests conducted on the two independent parent systems, operated at sludge ages of 10 and 20 days, coupled with the successful application of the models to a wide variety of systems, indicated that in all likelihood the steady state design and kinetic simulation models gave reasonable theoretical predictions, and that limitations within the batch test method compromised the accuracy of the measured values.

From an evaluation of the batch test procedure and data interpretation, it was concluded that the batch test method developed by Ubisi *et al.* (1997a,b) proved to be inadequate as a reliable means to quantify the heterotrophic active biomass present in activated sludge systems. The poor correlation between the active biomass measured in the batch tests and the theoretical active biomass predicted via the steady state design model was attributed to mainly two factors:

(i) It was observed that the ordinary heterotrophic organisms (OHO) present in the wastewater exhibited a growth rate that was much faster than the growth rate exhibited by the heterotrophic organisms present in the mixed liquor of the parent laboratory-scale activated sludge system. In examining the OUR responses of the batch tests conducted with a mixture of wastewater and mixed liquor, it was observed that the wastewater OHO active mass partially masked the OUR response of the OHO active mass from the mixed liquor. Accordingly, a potential

source of error in the batch test arose when subtracting the wastewater OHO active biomass concentration (determined from the wastewater only batch test OUR) from that for the mixed liquor and wastewater OHO active biomass concentration, to derive the mixed liquor OHO active biomass concentration.

(ii) The premise that nitrification in the batch test with wastewater and mixed liquor could be associated with a linear increase in the nitrate concentration with time, proved to be unduly simplified. In agreement with the observations of Antoniou *et al.* (1990) and Sözen *et al.* (1996), it was observed that the generation of nitrate in the batch reactor was better represented by an exponential increase than the linear increase with time followed by Ubisi et al. (1997a,b). Thus, in the present batch test concept when the oxygen demand due to nitrification (OUR_N), with a constant value in terms of the linear approach, is subtracted from the measured OUR response to obtain the OUR response due to the heterotrophic active biomass (OUR_H), another potential source of error is introduced.

8.4 MODIFIED BATCH TEST METHOD AND EVALUATION

To eliminate the above two possible sources of error, the present batch test procedure and data analysis were modified: (i) the OHO active biomass of the diluted wastewater used in the batch tests was physically removed (through pre-filtration), and (ii) nitrification was described by an exponential increase rather than a linear increase. With these modifications, from analysis of the modified batch test results, the following were concluded:

- The flocculation filtration procedure (using alum flocculation and filtration through a glass fibre filter) effectively removed all active biomass from the wastewater. This was demonstrated by no measurable OUR being observed in separate aerobic batch tests, conducted on the flocculated filtered wastewater only.

- In general, the modified batch tests yielded good %COD recoveries with only 2 out of 20 batch tests rejected on the basis of %COD recovery > 110%. The mean %COD recovery for all the batch tests was 100.5% with sample standard deviation of 7.3%. The good %COD recoveries lend credibility to the measurements and the modified batch test procedure.

- Excluding the rejected batch test results, good agreement existed between the theoretical heterotrophic active biomass concentration (Z_{BH}) and the Z_{BH} values measured in the modified batch tests. The <u>mean</u> Z_{BH} value (1587 mgCOD/ℓ) measured in the batch tests compared remarkably closely with the theoretical steady state design value of 1567 mgCOD/ℓ calculated for the parent system.

- Despite the remarkable agreement between the theoretical Z_{BH} and the mean of the measured Z_{BH} values, the individually measured Z_{BH} values were prone to significant variation. This was attributed mainly to the sensitivity of the measured heterotrophic active biomass values to the low values measured for the slopes of the $\ell n(OUR_H)$-time plots. Even a small change in the slope of the $\ell n(OUR_H)$-time plot resulted in a marked variation of the measured Z_{BH} values. The slope of the $\ell n(OUR_H)$-time plot was low because the maximum specific growth rates for the heterotrophs were low (mean K_{MP} and $\mu_H = 0.84/d$ for both parameters).

The good correlation that existed between the theoretical and measured values was remarkable, considering the sensitivity of the analysis. The results appear to substantiate the modified batch test method as a reliable means of quantifying the heterotrophic active biomass in terms of the mathematical formulations derived from the UCT kinetic simulation model. The results also underline the importance of incorporating the exponential approach to nitrification in the interpretation of the batch test results.

8.5 EXTENSION OF THE MODIFIED BATCH TEST INVESTIGATION

In evaluating the modified batch test procedure above, it was found that the low values for the maximum specific growth rate of the heterotrophs drawn from the continuously fed parent system caused undue sensitivity in the analytical procedure. If the maximum specific growth rate of the heterotrophs can be increased, this sensitivity can be correspondingly decreased. Accordingly, the effect of increased heterotroph maximum specific growth rate was investigated. From the literature, operating activated sludge systems in an intermittently fed fill and draw (IFFD) mode induces high heterotroph maximum specific growth rates (Ekama *et al.*, 1986). Thus, it was decided to change operation of the continuously fed system above to IFFD. This would also have provided the opportunity to evaluate the capacity of the modified batch test method to provide a reliable estimate for heterotrophic active biomass concentrations in activated sludge systems operated under significantly different conditions. Accordingly, a well-defined and controlled laboratory-scale intermittently fed fill and draw (IFFD) parent system was operated at the same sludge age of 10 days and bioreactor volume of 10ℓ as the continuously fed system and received the same COD mass load per day. This parent system provided the mixed liquor samples required for measuring heterotrophic active biomass. However, the parent IFFD system response exhibited the following deviations from the expected steady state behaviour:

- Because the parent IFFD system received the same daily mass of COD and was operated at the same sludge age and bioreactor volume as the continuously fed system, it was expected that the two systems would produce equal concentrations of volatile suspended solids (VSS). However, the IFFD system yielded a consistently higher VSS concentration. In the steady state design model the high VSS concentration is ascribed to a concomitantly high wastewater unbiodegradable particulate fraction ($f_{S,up}$), which causes the theoretical heterotrophic active biomass fraction (f_{av}) to be low. Previous research indicates that it is uncharacteristic of the

Mitchell's Plain wastewater to consistently sustain such an unusually high $f_{S,up}$ fraction. In addition, the comparison between the theoretical and measured heterotrophic active biomass concentration in the modified batch tests suggested an under-prediction of the "true" theoretical heterotrophic active biomass fraction (f_{av}) in the parent system.

- The observed daily OUR response in the parent IFFD system deviated from the typical IFFD system OUR response pattern. The expected OUR plateau associated with RBCOD utilization, followed by a well-defined precipitous drop in OUR, was not evident from the observed OUR responses. Instead a high initial OUR with a subsequent progressive decrease in OUR was observed. It was concluded that the deviation in OUR response was mainly the result of continually decreasing growth rates of the organisms when subjected to cyclic feed-starve conditions. The nitrate generation profile, recorded during the period when the system was feeding, indicated that the nitrification rate gradually leveled off after an initial high rate of nitrification. This decreasing nitrification rate, in particular, had a significant impact on the shape of the OUR profile. Previous research projects on similar systems subjected to high COD loading rates (Dold *et al.*,1980 and Dold *et al.*, 1991), indicate that the heterotrophic organisms may exhibit a similar decreasing rate of RBCOD utilization.

- It was thus concluded that the IFFD system induced a behavioural response that could not be accurately interpreted in terms of the current activated sludge modelling theory.

From the modified batch tests conducted on the mixed liquor samples drawn from the parent IFFD system, the following was concluded:

- The shape of OUR response curve recorded during each of the modified batch tests, did not conform to the expected OUR profile described by Wentzel *et al.* (1995) and that observed for the batch tests conducted on the continuously fed parent system mixed liquor. In contrast to the expected exponential increase in OUR, a relatively high initial OUR was established, followed by a progressive decrease in OUR during the first period of the batch test. To a large extent the shape of OUR response recorded during the first period of each batch test correlated well with the unexpected daily OUR profiles observed for the parent IFFD system after feeding. The consistency of occurrence indicated that the deviation of the observed response from the expected behaviour was replicated in the batch tests.

- In contrast to the parent system observations, where the deviation in OUR response could be attributed principally to a continually decreasing nitrification rate, the nitrate generation profiles recorded during the batch tests revealed only a moderate impact of the nitrification oxygen demand on the observed OUR response.

- Batch tests conducted with filtered effluent demonstrated that the high initial OUR levels observed in the batch tests were not related to intracellular COD storage in the parent IFFD system, and subsequent utilization of this stored COD in the batch tests.

- Through a process of elimination it was thus concluded that the shape of OUR response during the initial stages of the batch tests could only be result of progressively decreasing substrate utilization / growth rates of the heterotrophic active biomass.

- When disregarding the initial batch test OUR data, the analysis of the subsequent exponentially increasing OUR data revealed that the IFFD mixed liquor heterotroph maximum specific growth rate (μ_H) values (mean = 1.35/d) were only slightly higher than the values μ_H calculated for the continuously fed system (mean = 0.84/d). However, the mean μ_H value of 1.35/d was significantly lower than the 4.3 /d obtained via an earlier investigation by Dold *et al.* (1991) for mixed liquor samples harvested from a similar IFFD system. That the μ_H for the heterotrophs was not significantly increased by the IFFD operation negated one of the principle objectives for this part of the investigation, namely to investigate the effect of increased μ_H on the batch test procedure.

- In general, the variable %COD recoveries achieved in the batch tests appear to reflect the uncertainty surrounding the OUR responses observed in the modified batch tests conducted on the IFFD system mixed liquor.

It is therefore apparent that the modified batch test in its present format is unable to provide reliable estimations of the heterotrophic active biomass present in a parent system subjected to cyclic feed/starve conditions, such as the IFFD system. It appears that the IFFD system induced a behaviour in the mixed liquor that cannot be accommodated within the current activated sludge system modelling theory.

Furthermore, in systems that include biological excess phosphorus removal the batch test method will not be effective. In these systems the heterotrophic organisms mediating the phosphorus removal (phosphorus accumulating organisms, PAO) are present also and the batch test will not be able to distinguish between the two groups of heterotrophic organisms. In addition, experimental evidence from such systems indicates that there are behaviours in these systems that are not recognised in the models (Ekama and Wentzel, 1997). For example, for the same wastewater there is an inconsistency in the sludge production between aerobic and anoxic/aerobic systems on the one hand and anaerobic/anoxic/aerobic systems on the other; also, the sludge production in anaerobic/anoxic/aerobic systems has been found to be linked to filamentous bulking. In the current models both these observations have to be taken into account by using different influent unbiodegradable particulate fractions ($f_{S,up}$) which markedly affects the heterotrophic active biomass as a fraction of the mixed liquor organic solids (f_{av}), clearly an unacceptable solution.

Despite the deficiencies of the batch test method to accommodate the behavioural responses observed in the systems mentioned above, the results do indicate that the batch test method will (and already has) prove to be a valuable tool that can be used to provide greater insight into the behaviour of the various activated sludge systems. The good correlation that exists between the theoretical and measured values, indicate that the

method can be successfully applied as a reliable means to quantify the heterotrophic active biomass present in the continuously fed anoxic/aerobic activated sludge systems. The quantification of heterotrophic active biomass within the engineering and technology (modelling) paradigm, will establish a possible common link with the information arising from the new analytical techniques developed within the microbiology and biochemistry paradigm, thereby facilitating future comparisons and integration of the two paradigm sets.

8.6 RECOMMENDATIONS

From this investigation the following recommendations can be made:

- Although the modifications to the batch test method constitute a significant advance, the modified batch test was only evaluated with mixed liquor drawn from a parent system at 10d sludge age. The modified batch test method needs to be tested on mixed liquor drawn from parent systems at different sludge ages, say at 20d where the heterotrophic active biomass fraction of the mixed liquor is significantly different.

- The results of this thesis underlined the behavioural differences between sludges originating from different parent systems and the influence of the behavioural differences on the batch test results. For the IFFD system the batch test procedure proved to be inadequate in providing an accurate quantification of the heterotrophic active biomass due to the variability of the results and behavioural responses that did not conform to the current modeling theories. No detailed microbiological investigations were conducted during the course of this project to identify the types of organisms present in the different systems, which could have shed some light on the observed behavioural differences. For instance, the contributions of organisms that also mediate carbon transformations, such as glycogen accumulating organisms (GAO's), are not considered in the present models and the detection of such organisms could greatly assist in the evaluation of results. It is therefore recommended that any future research projects into the quantification of the heterotrophic active biomass should be conducted in collaboration with research groups with the ability to carry out detailed microbiological investigations such as fluorescence *in situ* hybridization (FISH) to assist with the identification of the organism populations present in the different systems.

- The results contained in this thesis revealed that the OUR response curves emanating from batch tests conducted on IFFD system sludge samples did not conform to the expected OUR profiles, rendering ineffectual the results and the batch test procedure itself. Several possible causes for the deviations were sought, but no single factor could be conclusively assigned to the unexpected behaviour. Insofar as the possibility of substrate storage is concerned, it is recommended that future research into this subject should include chemical analytical tests for PHB or glycogen to definitively determine whether substrate storage was occurring or not.

- Quantifying heterotrophic active biomass within the engineering and technology (modelling) paradigm provides the ideal platform for cross-linking and overlap with the microbiological and biochemistry paradigm. In particular, the latest developments in the *in situ* analytical techniques within the microbiological and biochemistry paradigm has the potential to provide quantitative information (a prerequisite for modelling) that can be compared to the measured heterotrophic active biomass in the models. This will facilitate integration of the microbiological and biochemistry paradigm into the models. Some initial integration between modelling and the new microbiological and biochemistry techniques has been started (e.g. Urbain *et al.*, 1998; Wagner *et al.*, 1998), but this is still in its infancy. Integrating the microbiological and biochemistry information into the current design and simulation models would inevitably lead to improved system design and optimization which can definitely contribute to a better understanding of the activated sludge processes and may even facilitate the integration of these techniques into the models. Exploration of ways to integrate the engineering and technology paradigm with the microbiological and biochemistry paradigm should receive serious attention.

8.7 CLOSURE

Due to their convenience as a tool to aid the research, design and operation of activated sludge systems, the design and kinetic simulation models have achieved widespread acceptance and have had a significant impact on the approach to design, operation and control of the activated sludge system, and on research into its' behaviour. However this acceptance should not inhibit critical evaluation of the principles on which these models are based; the models will always need to be used with great circumspection. The results obtained should be interpreted in terms of experience of real systems; the models should not be regarded as a substitute for knowledge and experience. The limitations of the models need to be comprehensively understood and taken into account in their application.

Parallel to the developments in the field of engineering and technology, significant advances have been made in the microbiological and biochemical areas of activated sludge. These advances have been driven by the development of new analytical techniques to allow microbial communities to be studied *in situ* in the activated sludge environment. However, there has been little cross-linking between the engineering and technology and the microbiological and biochemical paradigms. In particular the microbiological and biochemical information has not been integrated into the engineering and technology paradigm, to enable improved design and optimization. One area that can form a starting point to build bridges between the two paradigm sets is active biomass. It is hoped that this project will initiate the development of links and overlap between the two paradigm sets.

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APPENDIX A

OUR vs TIME PLOTS FOR WASTEWATER ONLY BATCH TESTS AND BATCH TESTS CONDUCTED WITH WASTEWATER PLUS MIXED LIQUOR

TABLE OF CONTENTS

Table A1 Summary of the daily wastewater (WW) only batch test data with calculated COD recovery, maximum specific growth rates and measured heterotrophic active biomass.

- OUR vs time and ln(OUR) vs time profiles for wastewater (WW) only batch tests W1 – W23.

Table A2 Summary of the daily wastewater plus mixed liquor (WW+ML) batch test data with mixed liquor sample volumes, COD recovery, maximum specific growth rates and measured heterotrophic active biomass.

- OUR vs time and ln(OUR) vs time profiles for wastewater plus mixed liquor (WW+ML) batch tests M1 – M23. The nitrate concentration vs time profiles recorded during each batch test is also given.

Table A3 Summary of results for the heterotrophic active biomass due to the mixed liquor addition only ($Z_{BH(0)}$ ML*), calculated through analytical separation of the WW only and WW+ML batch test results.

- Combined OUR vs time profiles for WW+ML batch tests M1 – M23 and WW only batch tests W1 – W23. The ln(OUR) vs time profiles obtain from analytically separating the WW+ML and WW only OUR responses are also given.

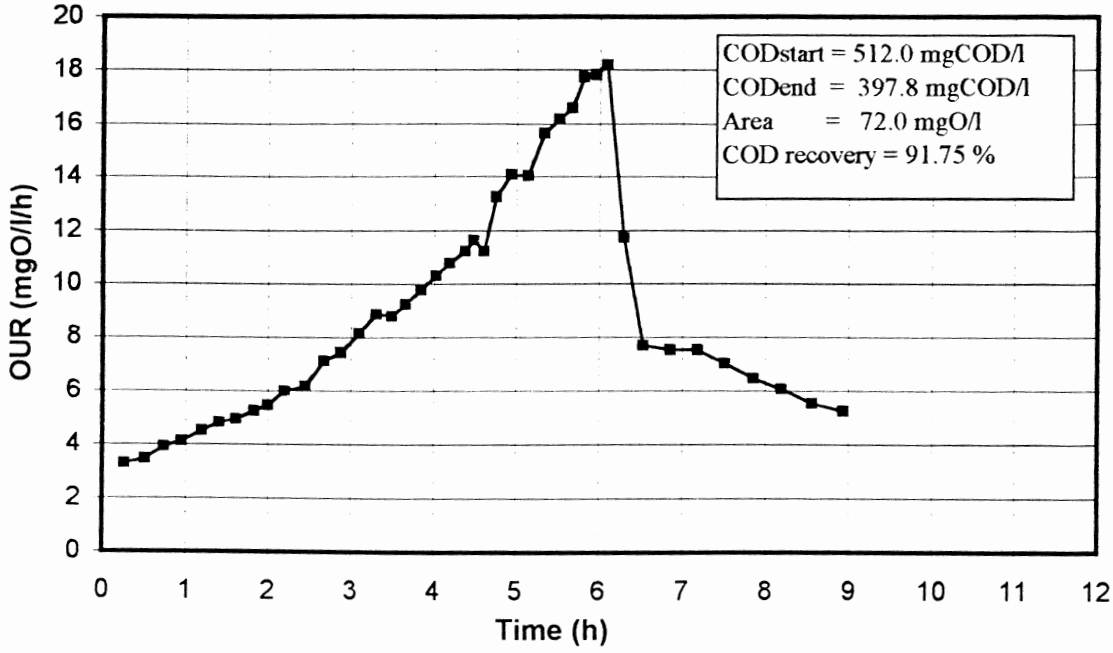
Table A4 Summary of results for the het The nitrate concentration vs time profiles erotrophic active biomass due to the mixed liquor addition only ($Z_{BH(0)}$ ML*), calculated through analytical separation of the WW only and WW+ML batch test results *and* assuming an exponential increase in the nitrate concentration – time data recorded during the WW+ML batch tests.

- Combined OUR vs time profiles for WW+ML batch tests M1 – M23 and WW only batch tests W1 – W23. The exponential expressions obtained from the nitrate concentration vs time profiles and the analytically separated ln(OUR) vs time profiles for the ML only OUR responses are also given.

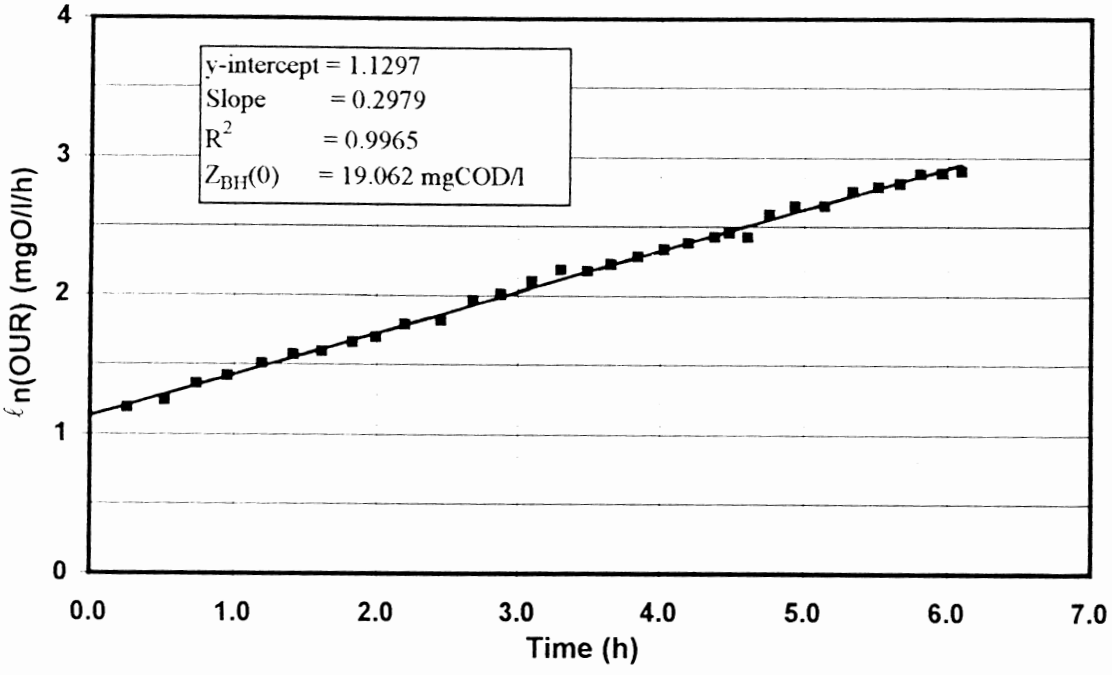
Table A1 : Daily wastewater only (WW) batch test data with calculated COD recovery, maximum specific growth rates and heterotrophic active biomass.

Sew. batch no.	Batch Test No.	Date of test	COD (mgCOD/l)		Area (mgO/l)	%COD Recov.	Linear regression data			Max. specific growth rates (/d)		$Z_{BH(0)}$ (WW) (mgCOD/l)
			Start	End			y-int.	slope	R^2	K_{MP}	μ_H	
11	W1	29-10	512	398	72	92	1.1297	0.2979	0.9965	3.0	4.8	19.062 *
	W2	03-11	494	384	65	91	0.9421	0.3414	0.9963	3.5	5.3	13.929 *
	W3	05-11	467	356	53	88	1.5292	0.3038	0.9866	3.6	4.3	27.914 *
12	W4	15-11	428	323	57	89	0.5243	0.3507	0.9928	3.2	5.8	8.946 *
13	W5	30-11	401	389	47	109	1.6209	0.3144	0.9478	3.9	4.3	29.641
	W6	01-12	450	405	51	101	1.6241	0.3524	0.9860	4.5	4.6	26.749
	W7	03-12	417	376	35	99	1.7731	0.3537	0.9473	4.4	4.7	30.940
14	W8	08-12	450	403	50	101	0.9877	0.3420	0.9624	4.2	4.7	14.556
18	W9	07-02	459	339	46	84	0.8935	0.2863	0.9478	3.4	4.1	15.611
	W10	08-02	467	345	55	86	0.1869	0.3557	0.9889	2.1	7.0	6.300
	W11	09-02	461	335	49	83	1.0017	0.2702	0.9636	3.1	4.0	18.341
	W12	10-02	429	278	42	75	1.4181	0.2448	0.9372	3.3	3.2	30.424
19	W13	15-02	421	327	35	86	0.2125	0.3725	0.9885	4.3	5.3	6.191
	W14	16-02	429	339	32	87	0.3189	0.3830	0.9946	4.4	5.4	6.709
	W15	17-02	435	337	45	88	0.7594	0.2892	0.9797	3.2	4.3	13.526
	W16	22-02	449	410	49	102	0.6996	0.3504	0.9740	3.5	5.6	10.668
	W17	23-02	430	383	40	98	1.2587	0.3931	0.9652	3.2	6.9	16.758
20	W18	29-02	447	363	51	93	0.1225	0.3788	0.9590	3.9	5.8	5.570
	W19	01-03	459	381	58	96	0.2168	0.3836	0.9739	3.5	6.3	6.049
	W20	02-03	455	395	59	100	0.2235	0.3786	0.9673	3.6	6.1	6.165
	W21	06-03	444	389	39	96	0.7039	0.2907	0.9194	2.7	4.9	12.735
	W22	07-03	423	372	39	97	1.1310	0.2961	0.7531	2.2	5.5	19.193
	W23	08-03	414	397	25	102	1.1163	0.3343	0.8384	2.0	6.6	16.907

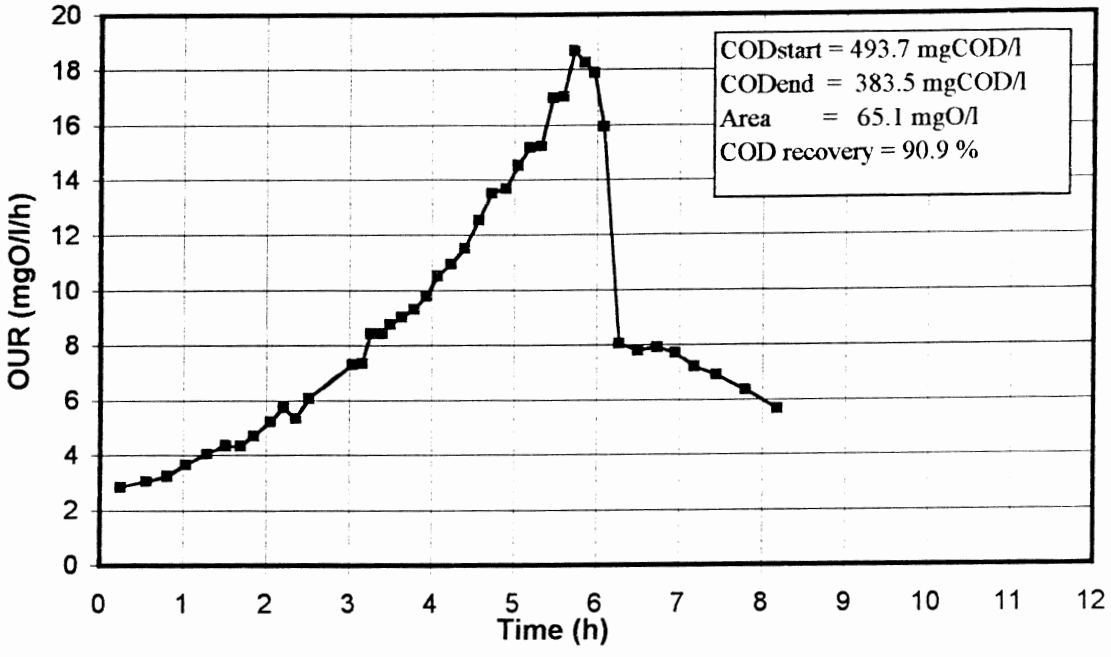
* Batch tests using 3ℓ wastewater, i.e. when compared to WW+ML batch tests, dilution must be taken into account : $Z_{BH(0)}(WW)_D = Z_{BH(0)}(WW) * V_{WW} / (V_{ML} + V_{WW})$



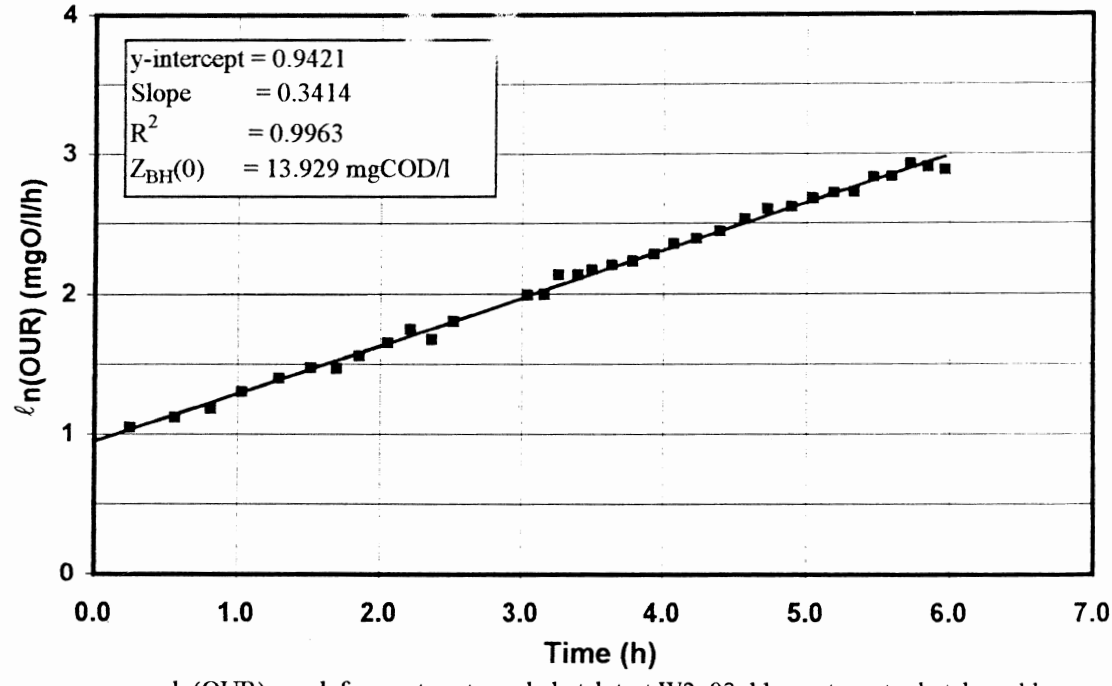
OUR Graph for wastewater only (3ℓ) batch testW1, 29-10, waste water batch no. 11



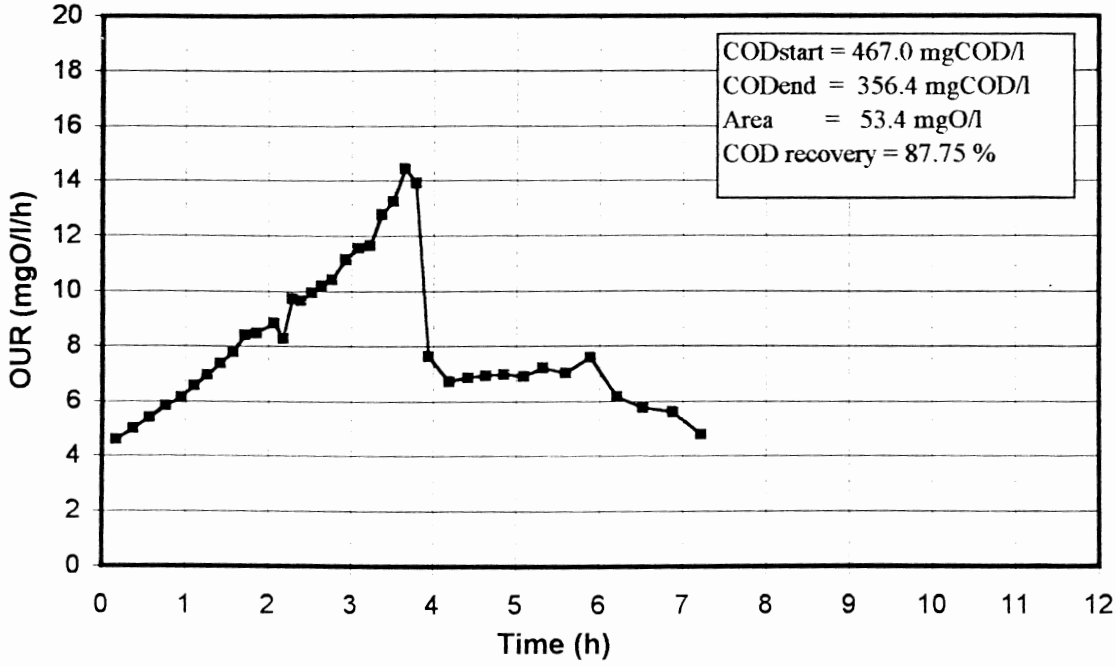
ln(OUR) graph for wastewater only batch test W1, 29-10, waste water batch no.11



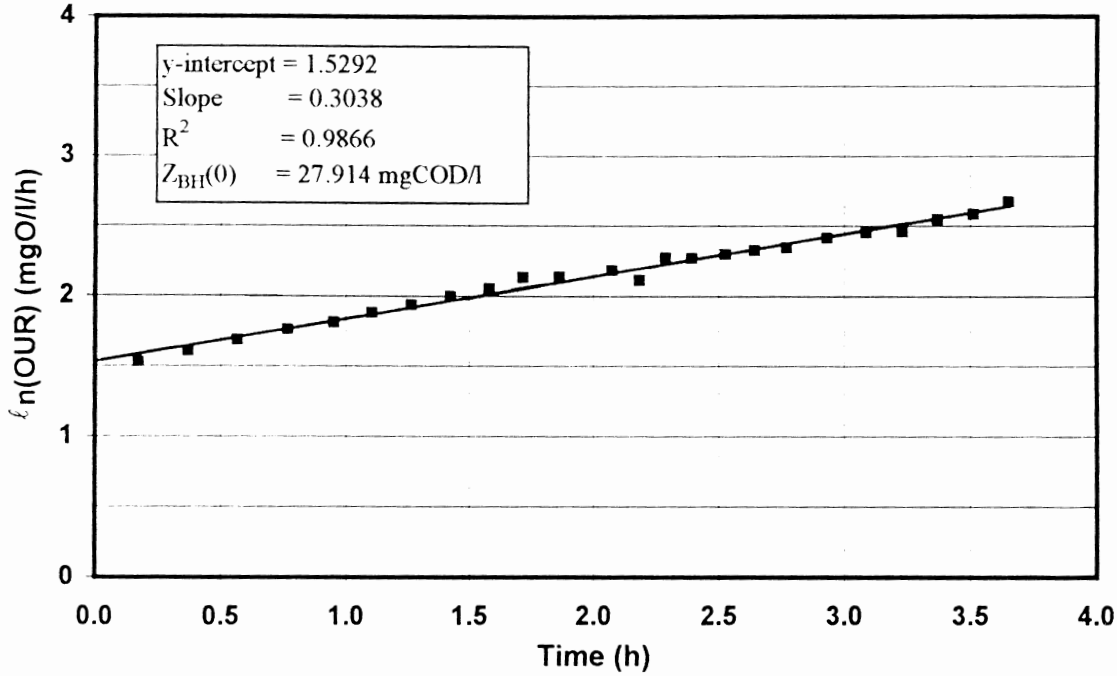
OUR Graph for wastewater only (3ℓ) batch test W2, 03-11, wastewater batch no. 11



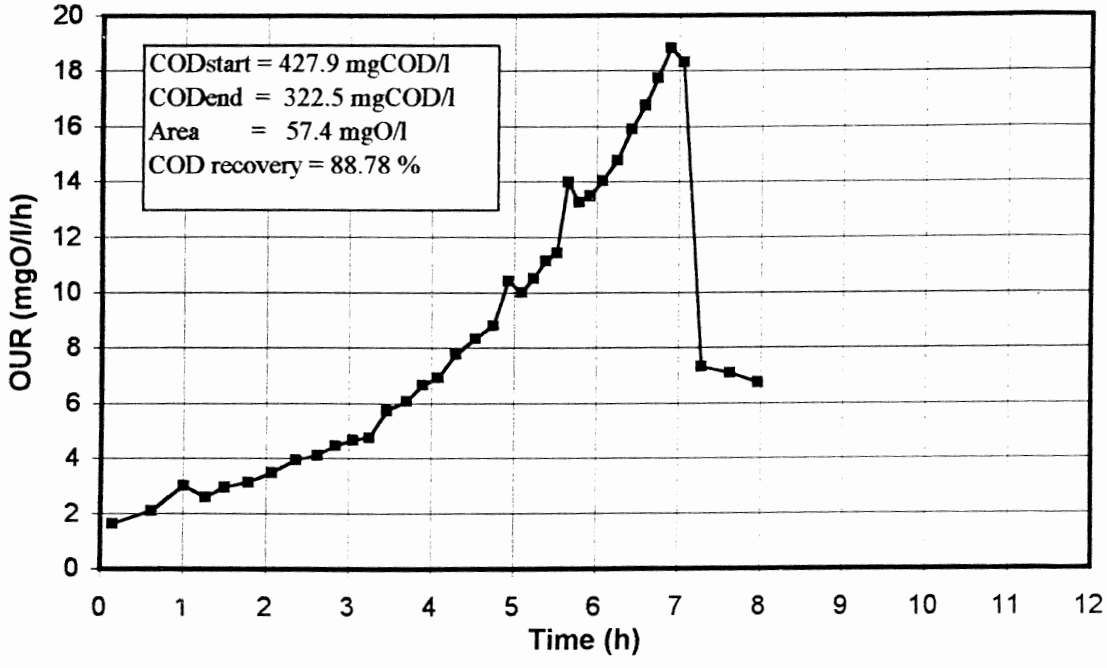
ln(OUR) graph for wastewater only batch test W2, 03-11, waste water batch no.11



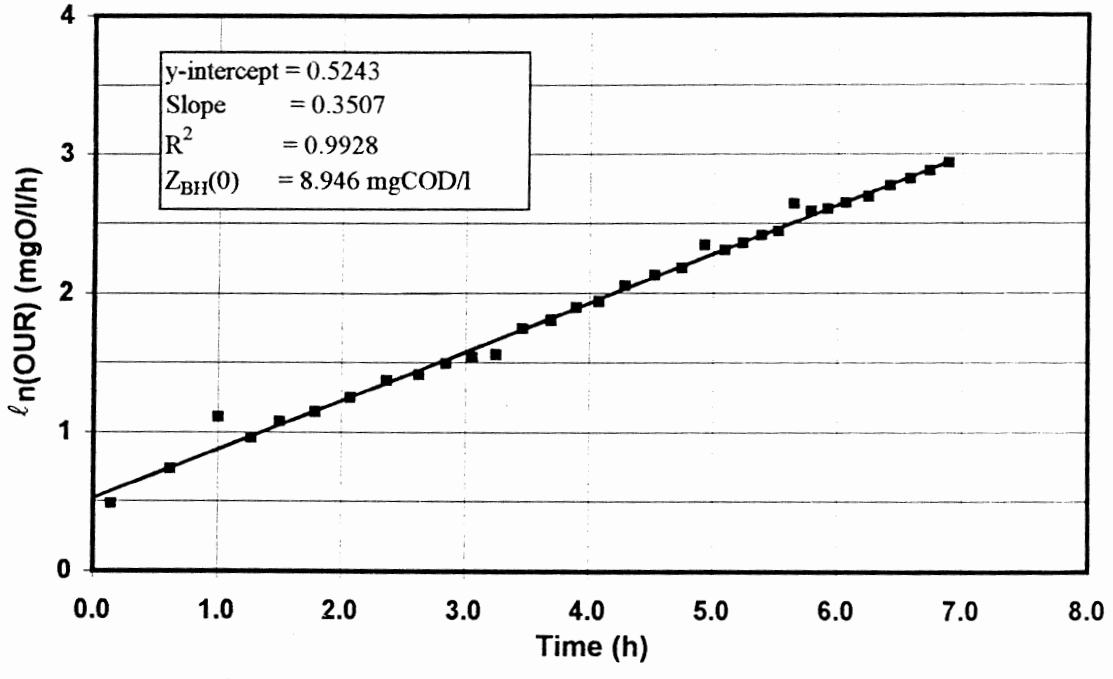
OUR Graph for wastewater only (3ℓ) batch test W3, 05-11, wastewater batch no. 11



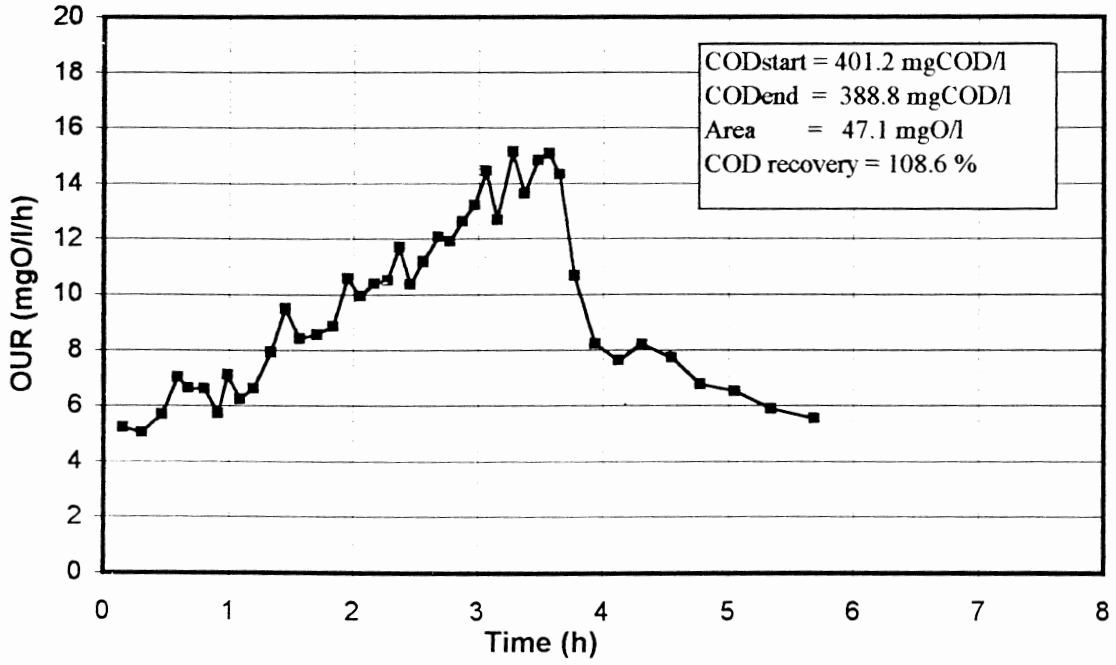
ln(OUR) graph for wastewater only batch test W3, 05-11, wastewater batch no.11



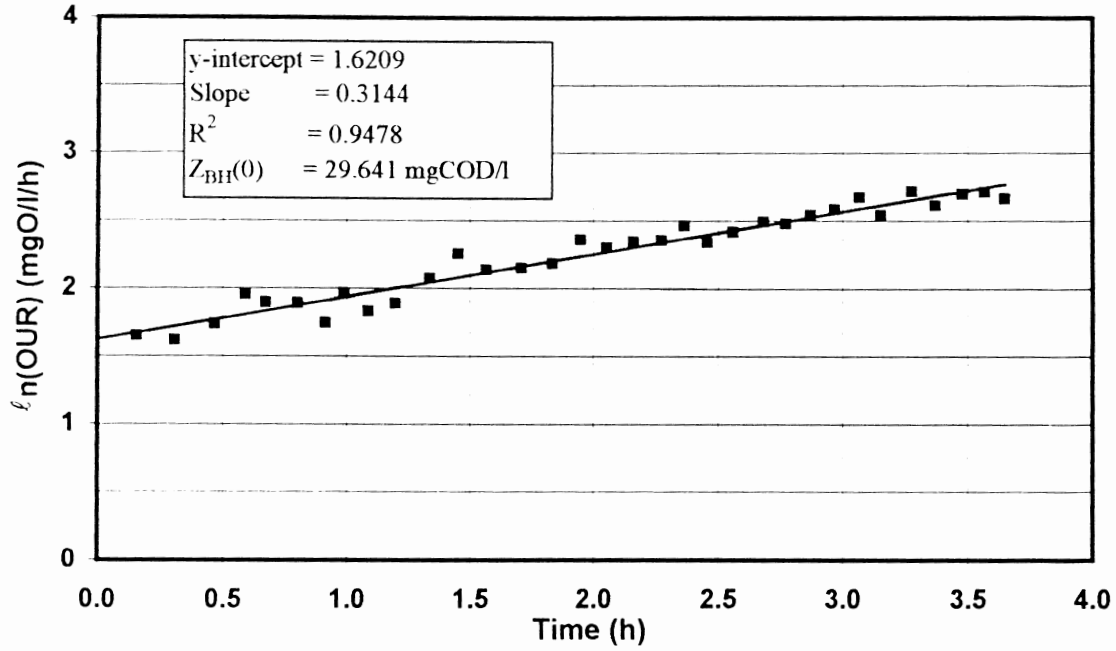
OUR Graph for wastewater only (3ℓ) batch testW4, 15-11, wastewater batch no. 12



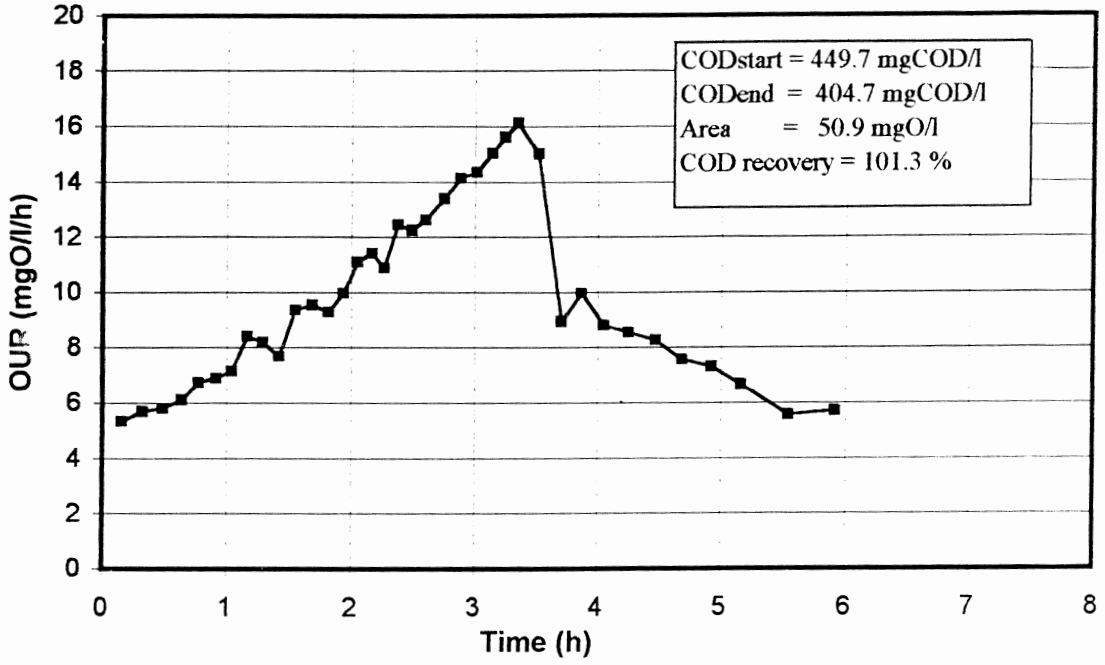
ln(OUR) graph for wastewater only batch test W4, 15-11, wastewater batch no.12



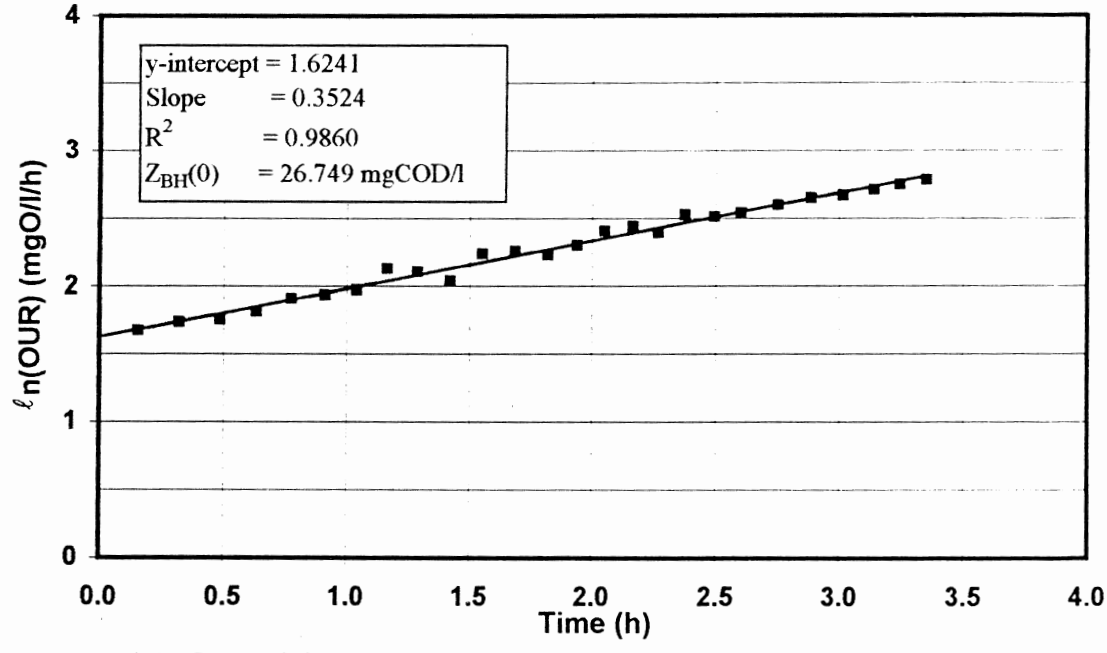
OUR Graph for wastewater (2.8ℓ) plus filtered effluent (0.2ℓ) batch test W5, 30-11, wastewater batch no. 13



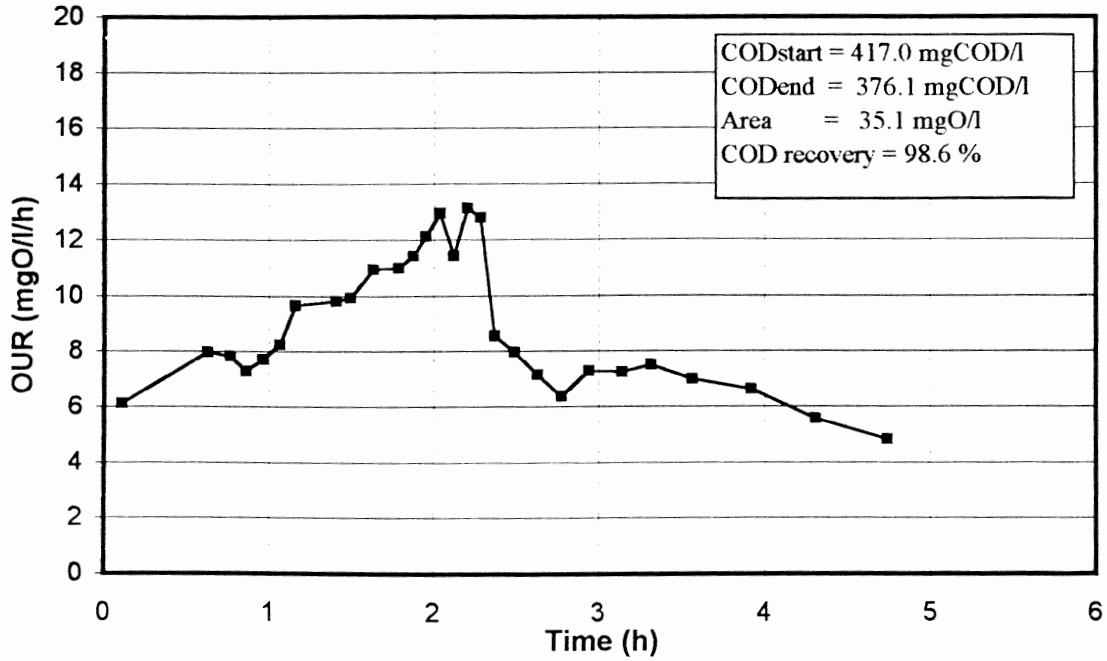
ln(OUR) graph for wastewater (2.8ℓ) plus filtered effluent (0.2ℓ) batch test W5, 30-11, wastewater batch no.13



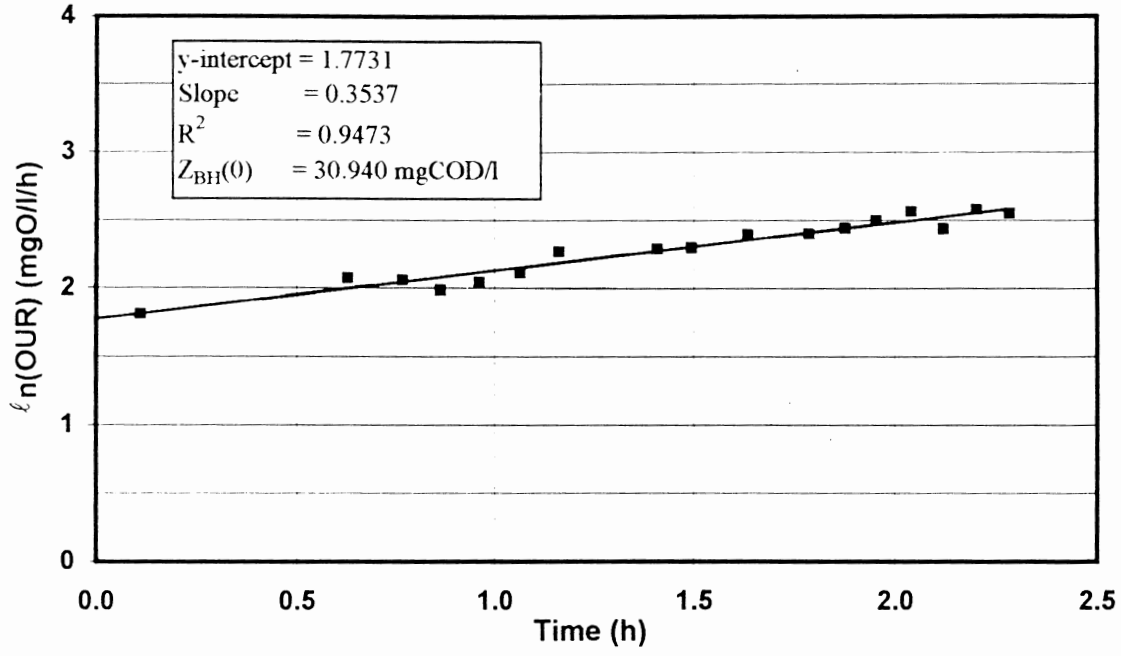
OUR Graph for wastewater (2.9ℓ) plus filtered effluent (0.1ℓ) batch test W6, 01-12, wastewater batch no. 13



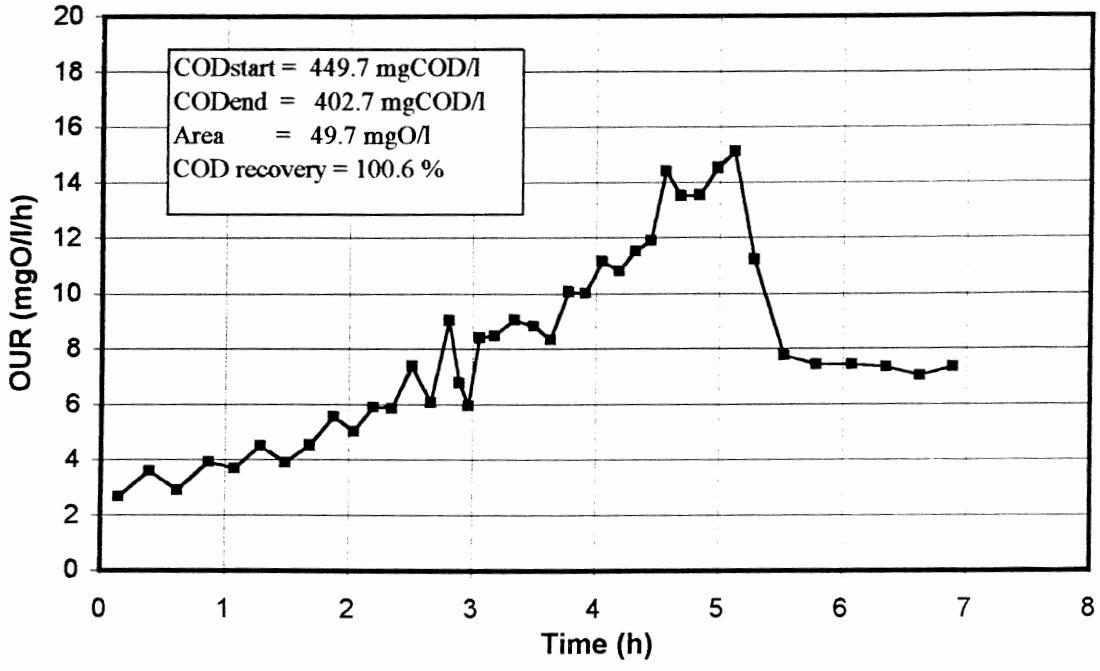
ln(OUR) graph for wastewater (2.9ℓ) plus filtered effluent (0.1ℓ) batch test W6, 01-12, wastewater batch no.13



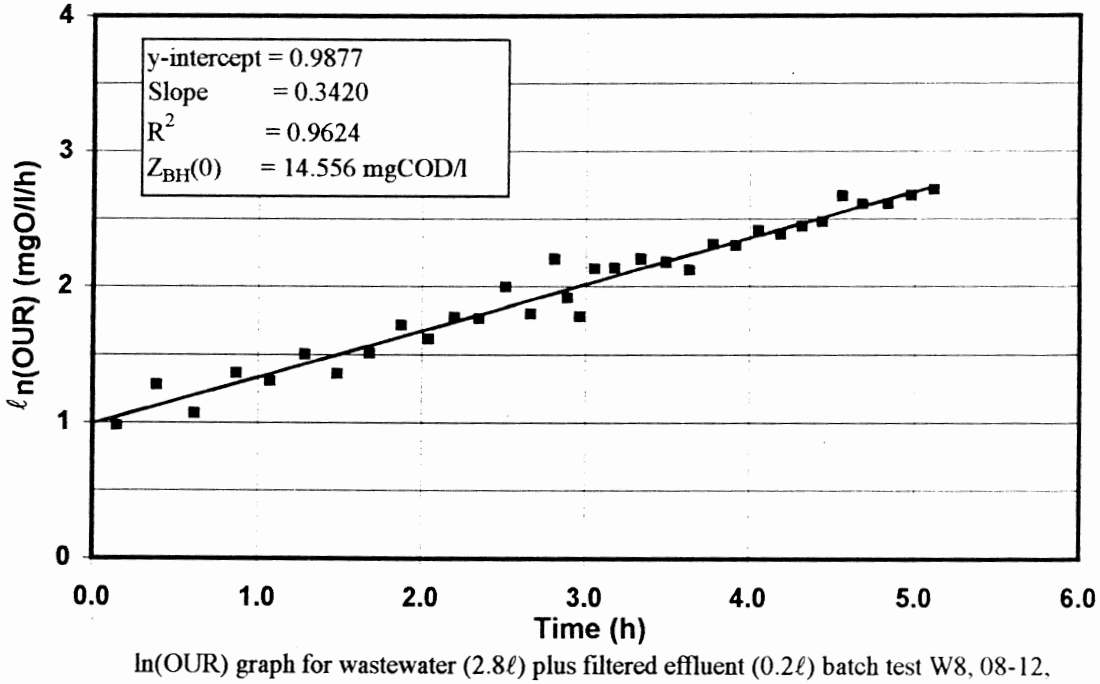
OUR Graph for wastewater (2.75ℓ) plus filtered effluent (0.25ℓ) batch test W7, 03-12, wastewater batch no. 13



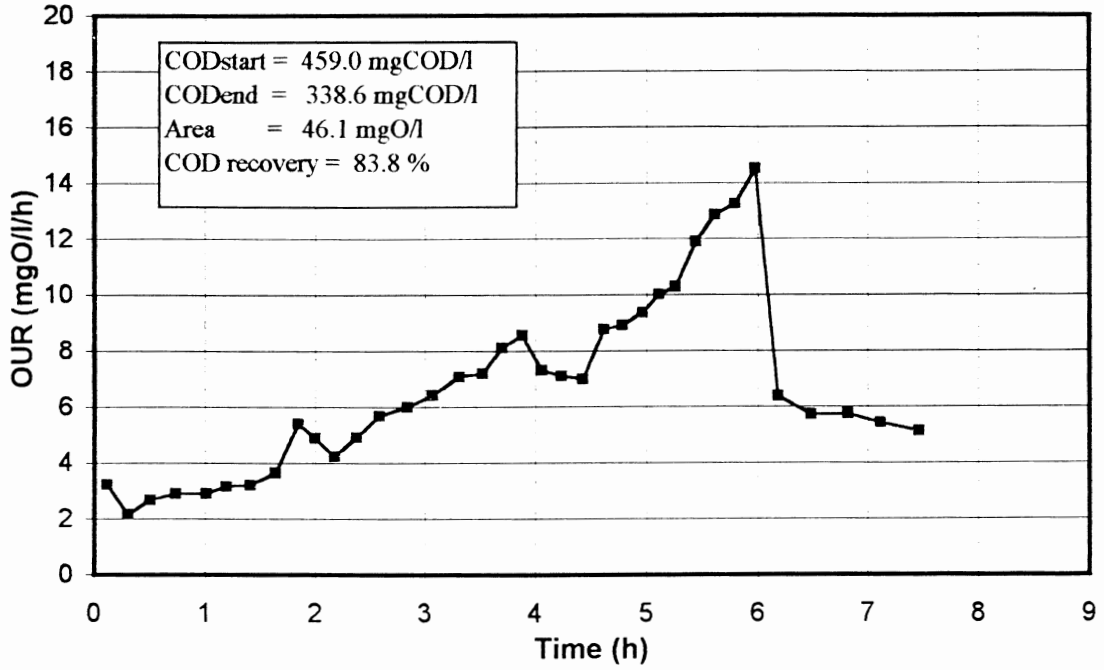
ln(OUR) graph for wastewater (2.75ℓ) plus filtered effluent (0.25ℓ) batch test W7, 03-12, wastewater batch no.13



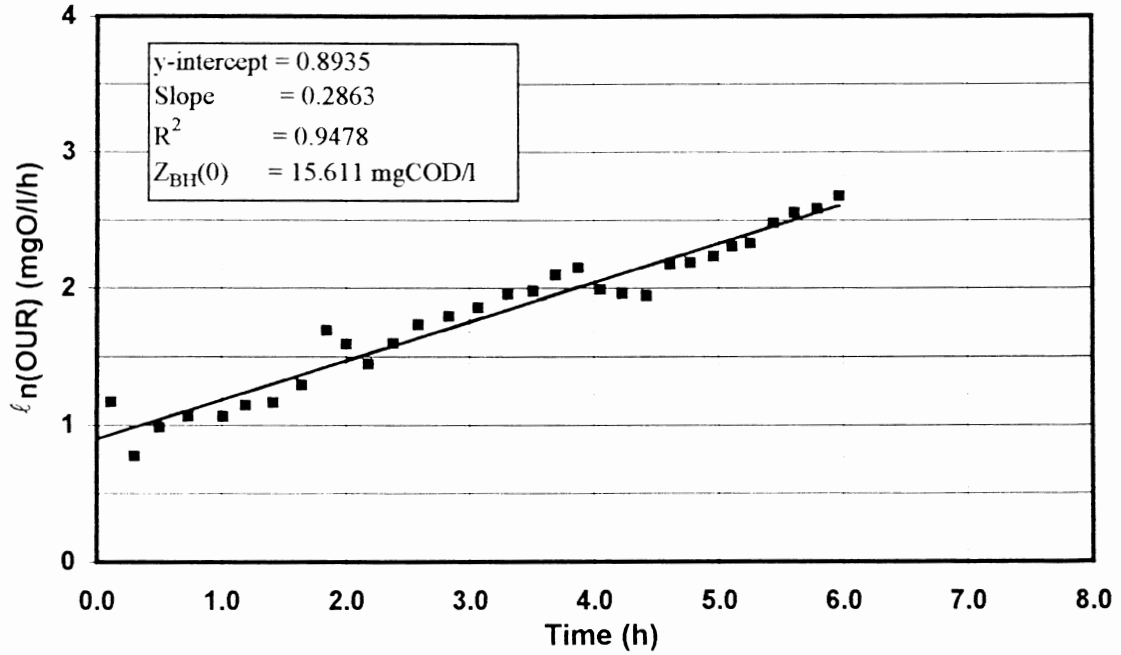
OUR Graph for wastewater (2.8ℓ) plus filtered effluent (0.2ℓ) batch test W8, 08-12, wastewater batch no. 14



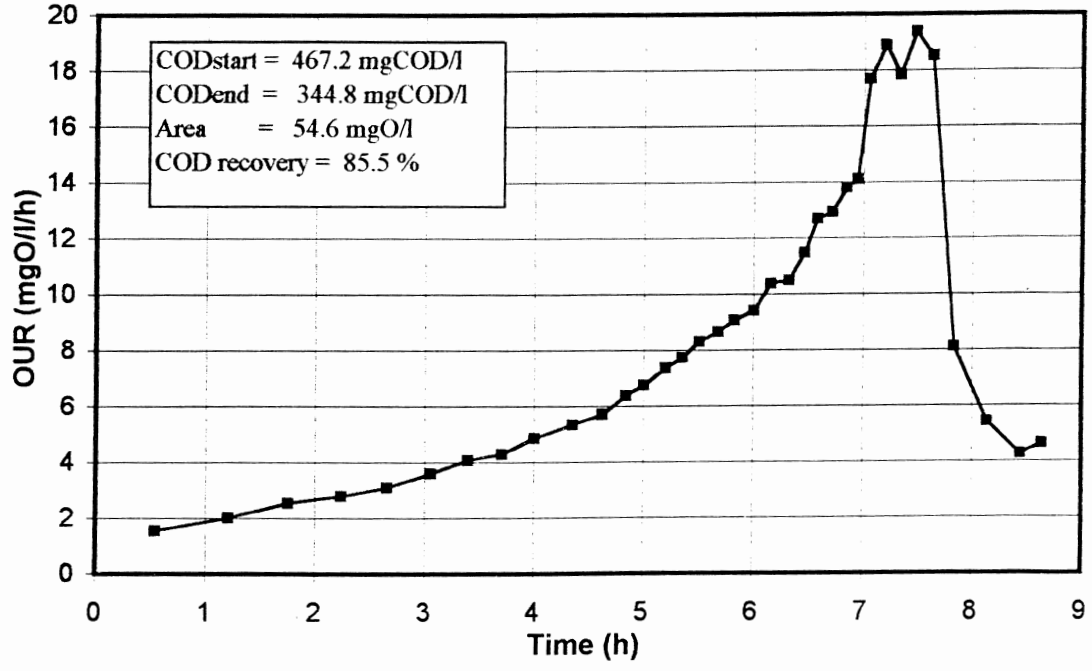
ln(OUR) graph for wastewater (2.8ℓ) plus filtered effluent (0.2ℓ) batch test W8, 08-12, wastewater batch no.14



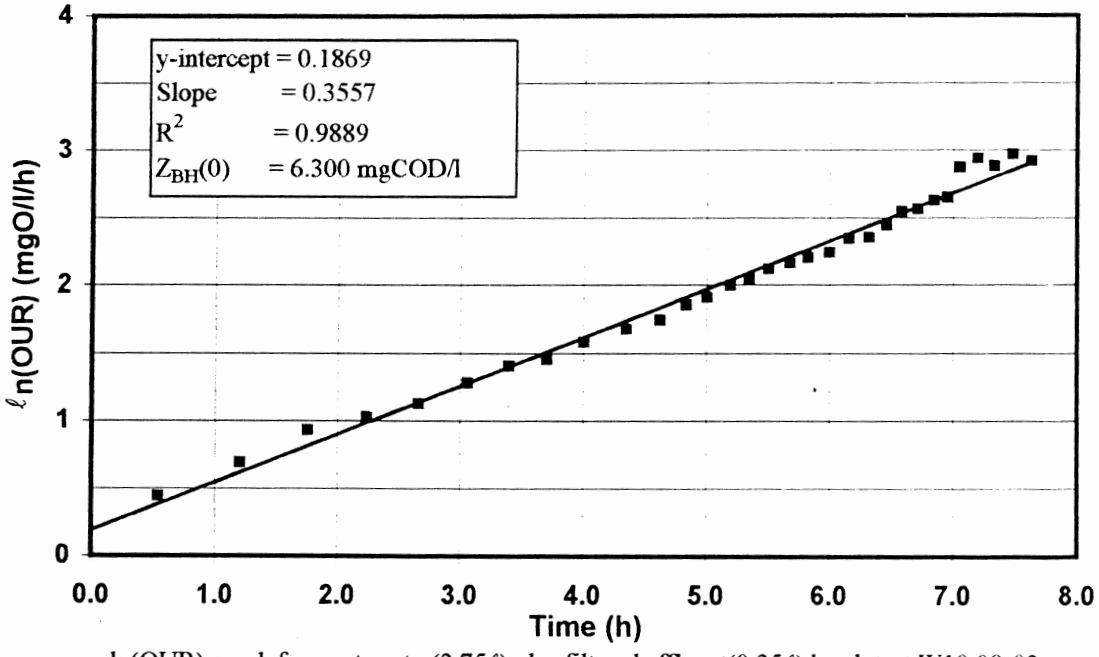
OUR Graph for wastewater (2.8ℓ) plus filtered effluent (0.2ℓ) batch test W9, 07-02, wastewater batch no. 18



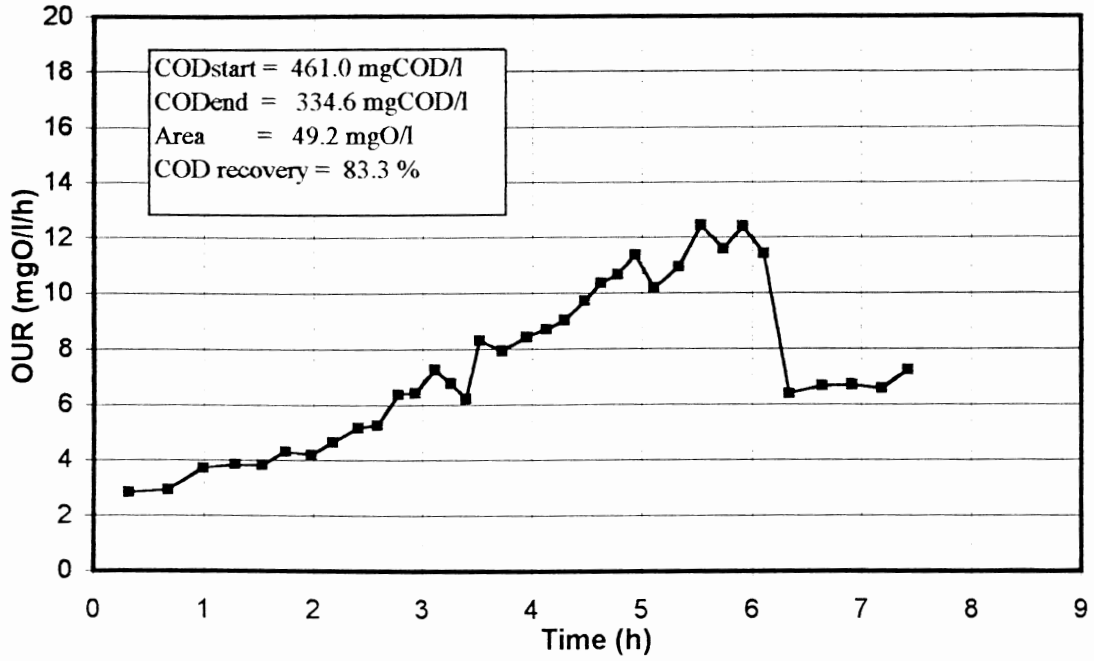
ln(OUR) graph for wastewater (2.8ℓ) plus filtered effluent (0.2ℓ) batch test W9, 07-02, waste water batch no.18



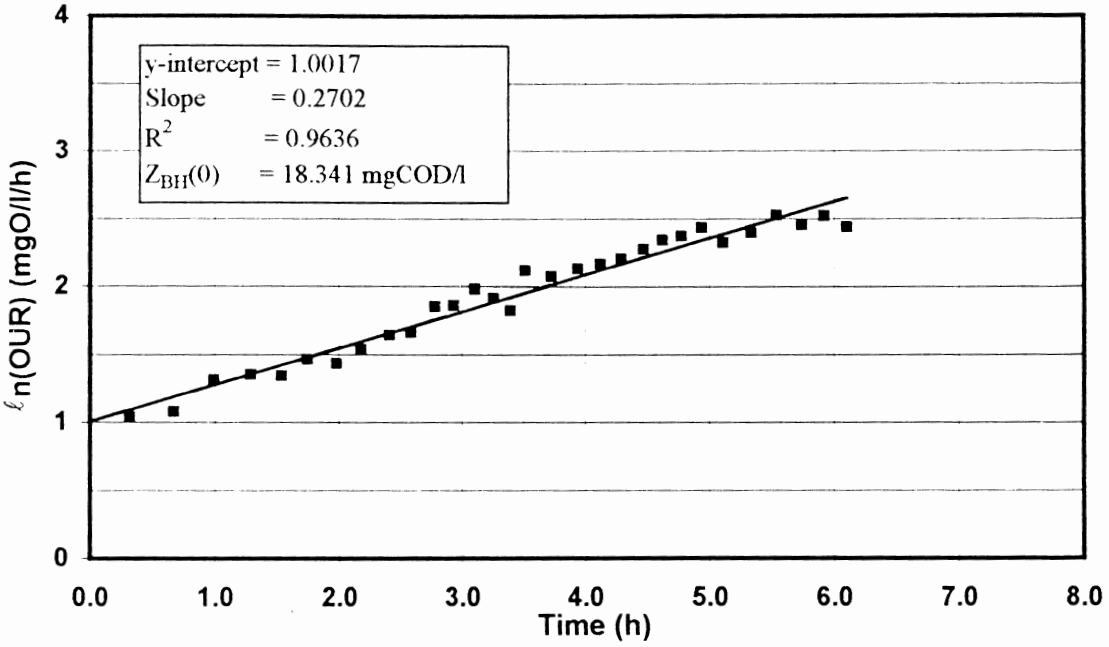
OUR Graph for wastewater (2.75ℓ) plus filtered effluent (0.25ℓ) batch test W10, 08-02, wastewater batch no. 18



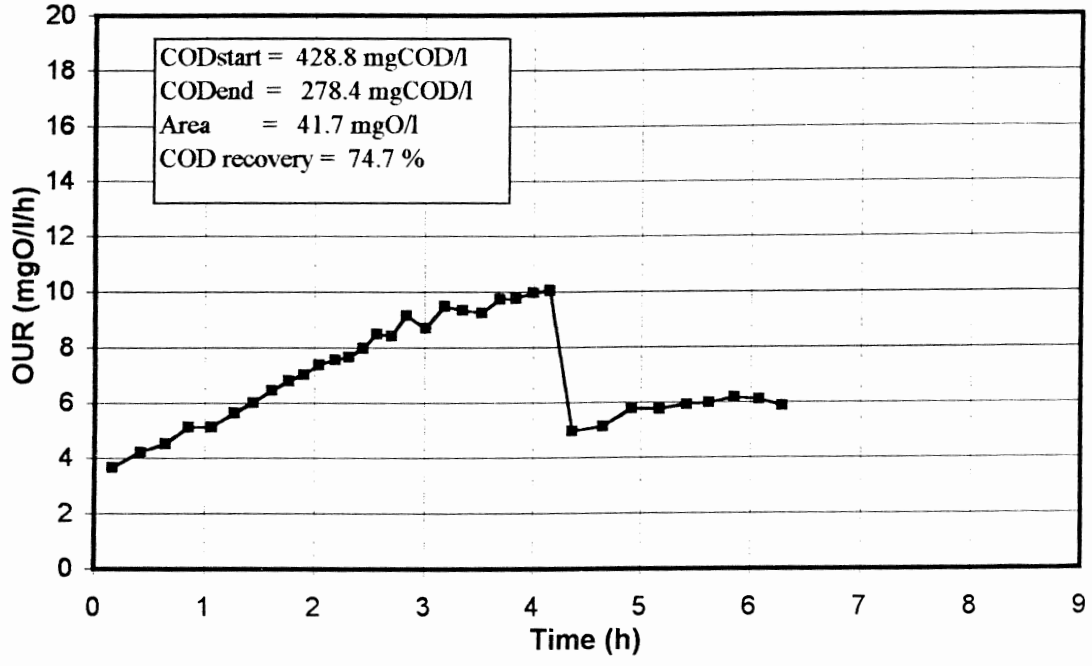
ln(OUR) graph for wastewater(2.75ℓ) plus filtered effluent(0.25ℓ) batch test W10,08-02, wastewater batch no.18



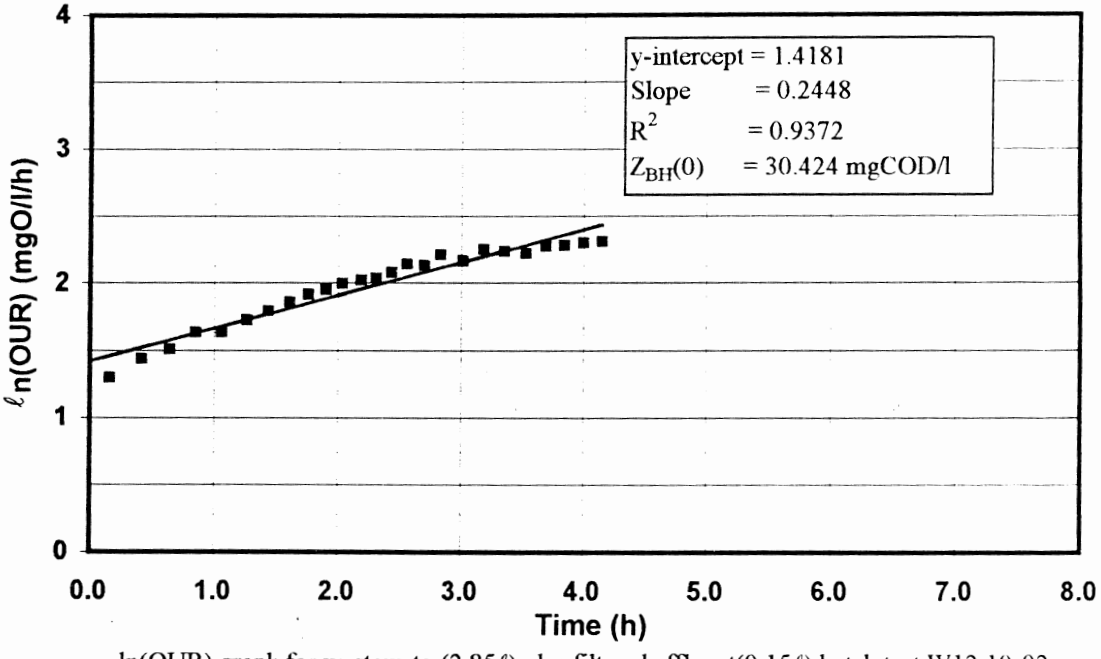
OUR Graph for wastewater (2.7ℓ) plus filtered effluent (0.3ℓ) batch test W11, 09-02, wastewater batch no. 18



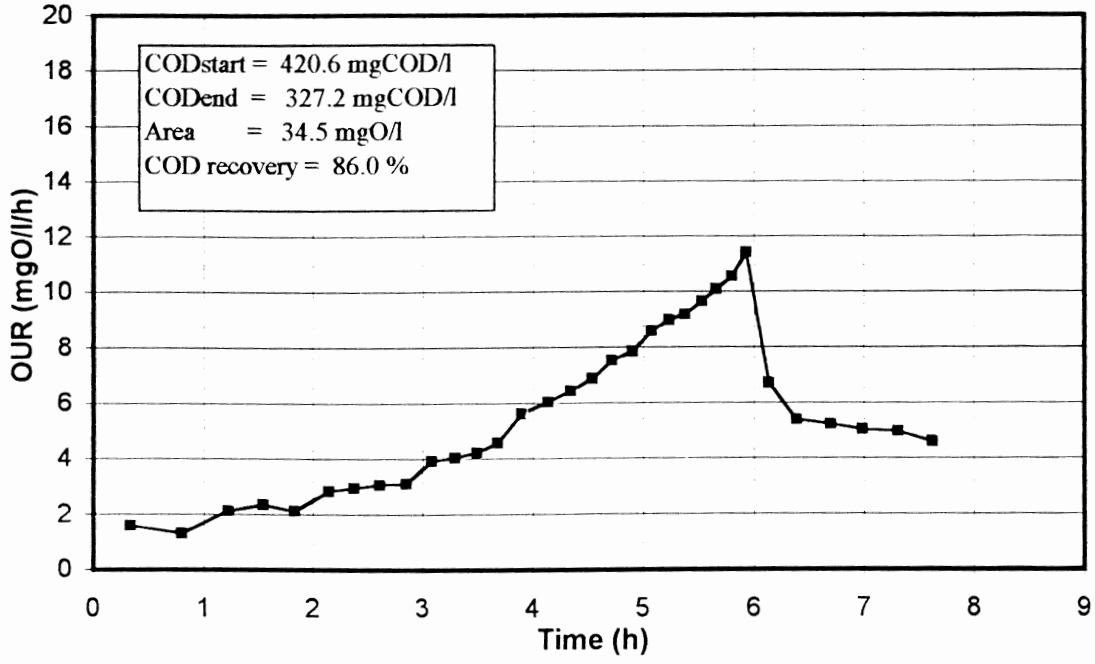
ln(OUR) graph for wastewater (2.7ℓ) plus filtered effluent (0.3ℓ) batch test W11, 09-02, wastewater batch no.18



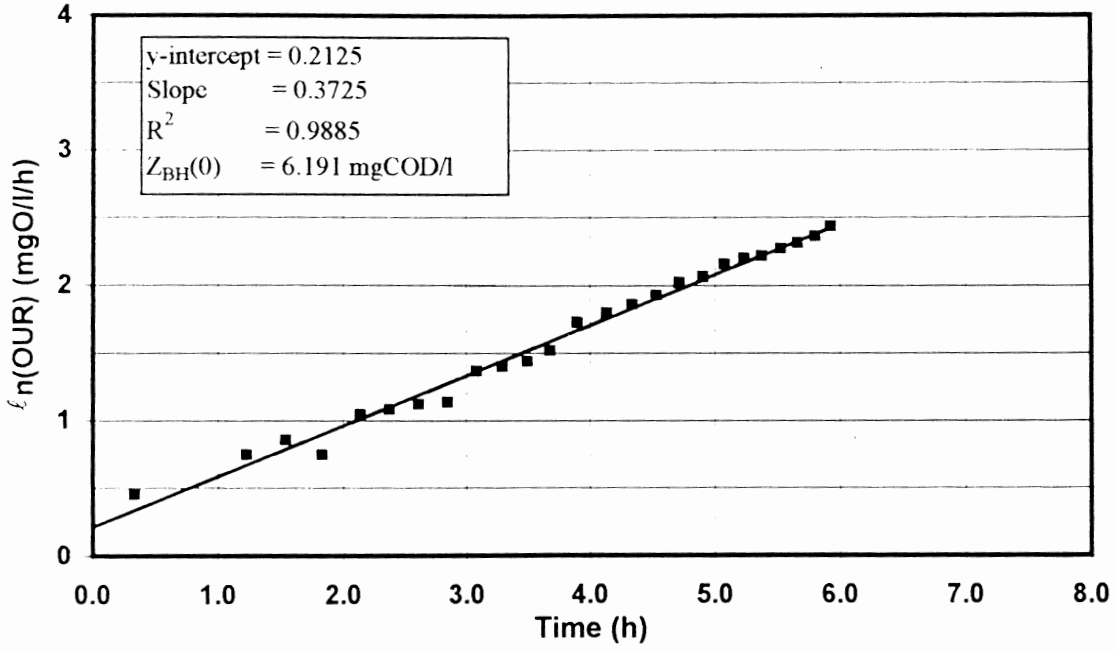
OUR Graph for wastewater (2.85ℓ) plus filtered effluent (0.15ℓ) batch test W12, 10-02, wastewater batch no. 18



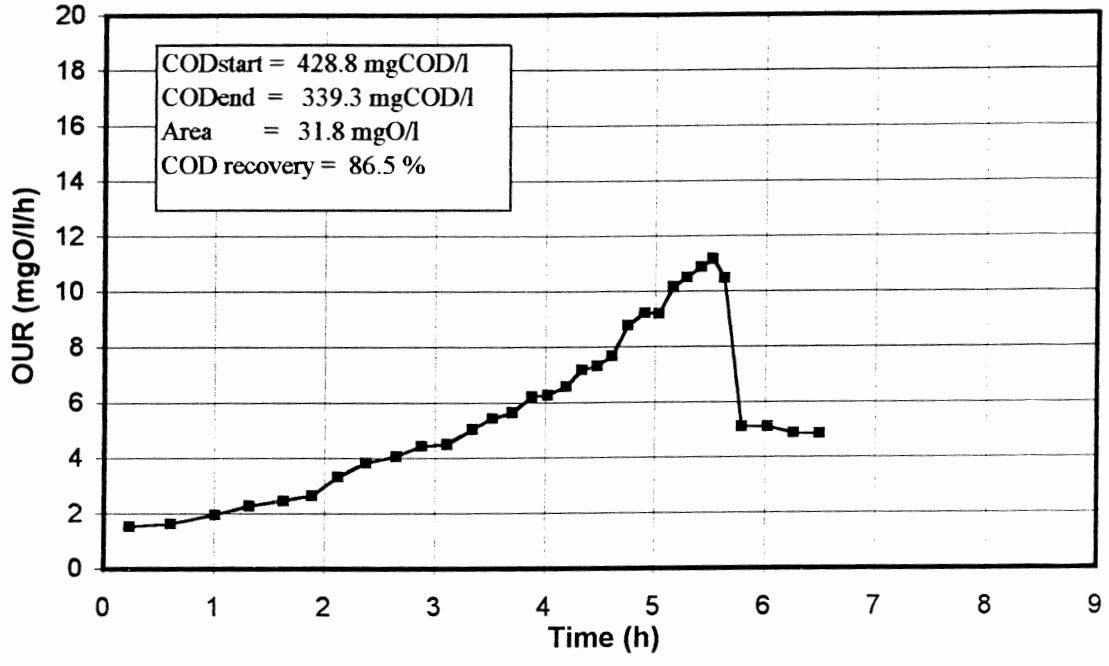
ln(OUR) graph for wastewater(2.85ℓ) plus filtered effluent(0.15ℓ) batch test W12,10-02, wastewater batch no.18



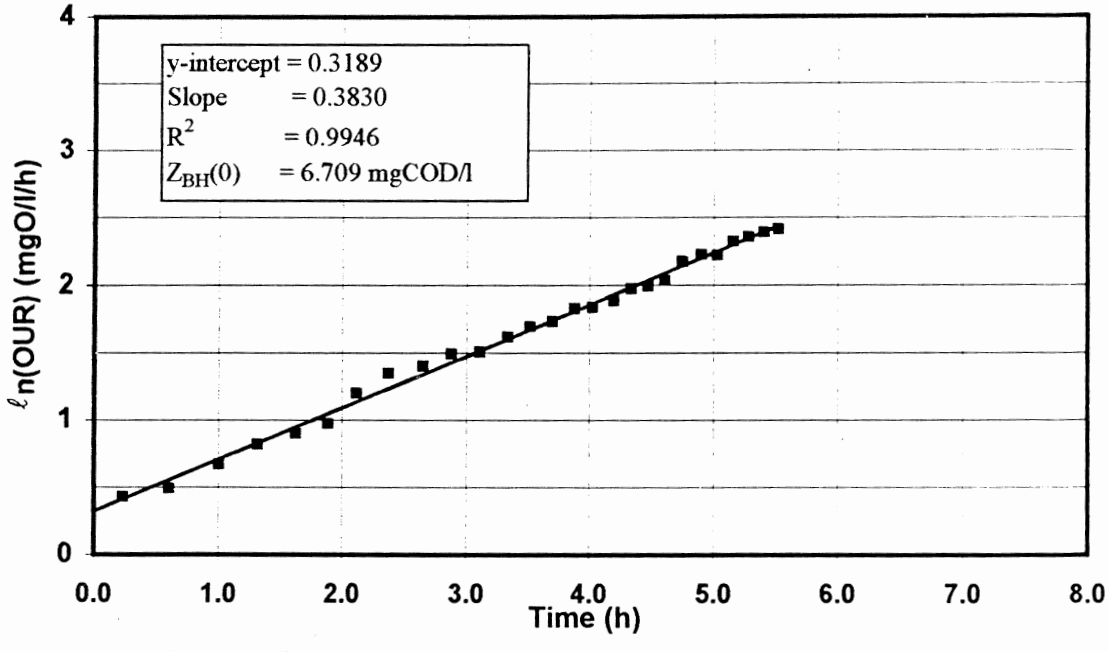
OUR Graph for wastewater (2.7ℓ) plus filtered effluent (0.3ℓ) batch test W13, 15-02, wastewater batch no. 19



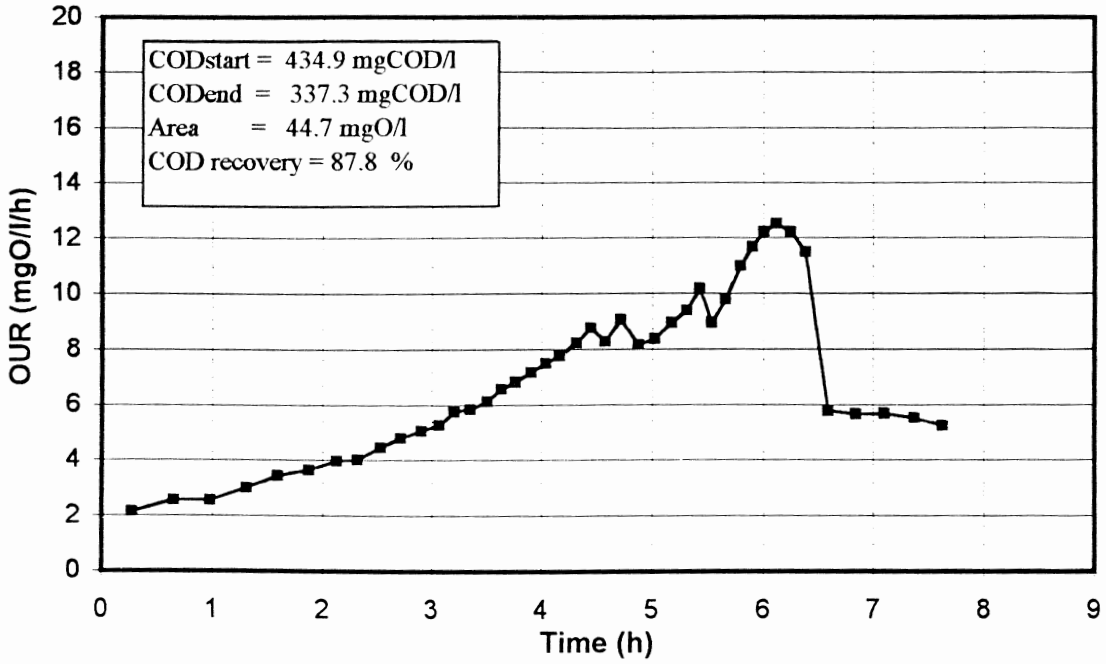
ln(OUR) graph for wastewater (2.7ℓ) plus filtered effluent (0.3ℓ) batch test W13, 15-02, wastewater batch no.19



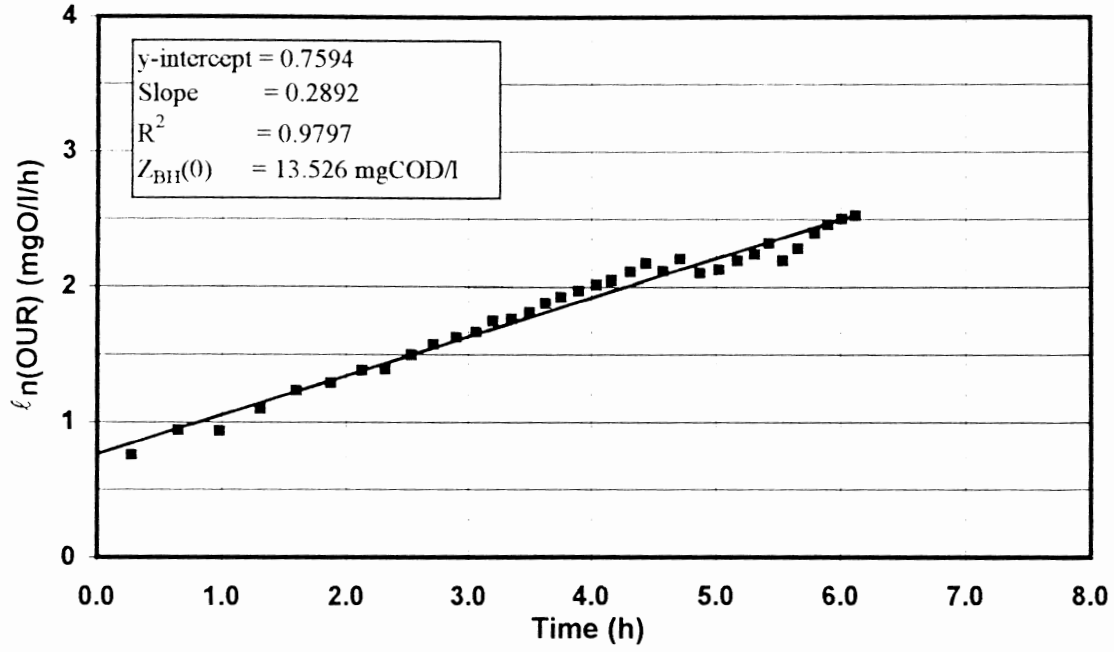
OUR Graph for wastewater (2.65ℓ) plus filtered effluent (0.35ℓ) batch test W14, 16-02, wastewater batch no. 19



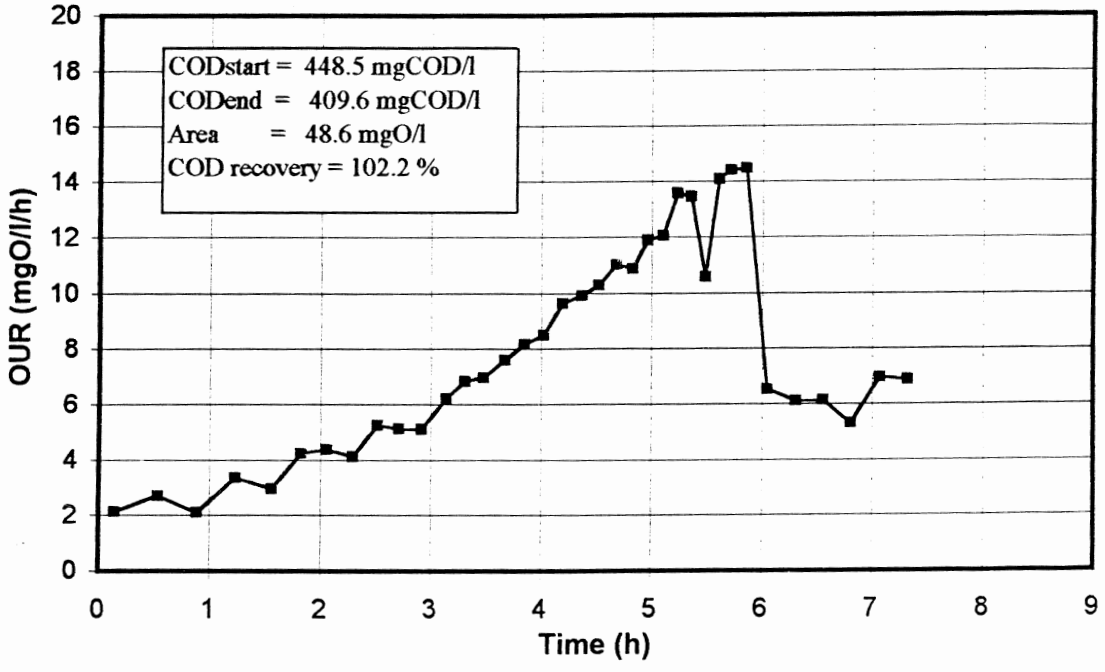
ln(OUR) graph for wastewater(2.65ℓ) plus filtered effluent(0.35ℓ) batch test W14,16-02, wastewater batch no.19



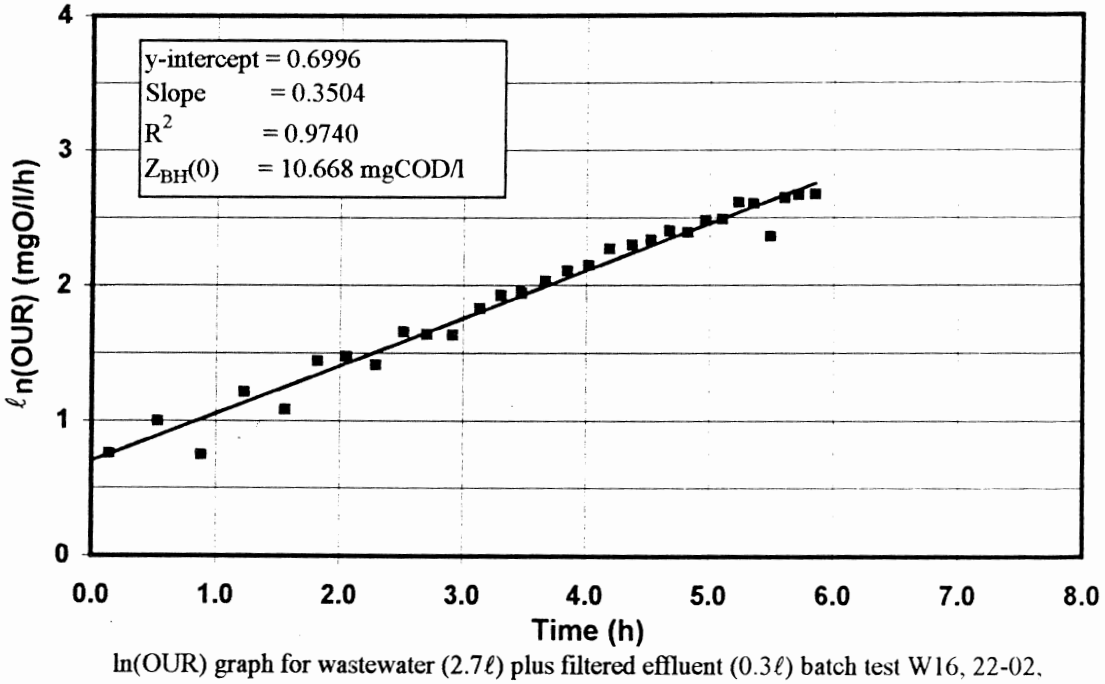
OUR Graph for wastewater (2.6ℓ) plus filtered effluent (0.4ℓ) batch test W15, 17-02, wastewater batch no. 19



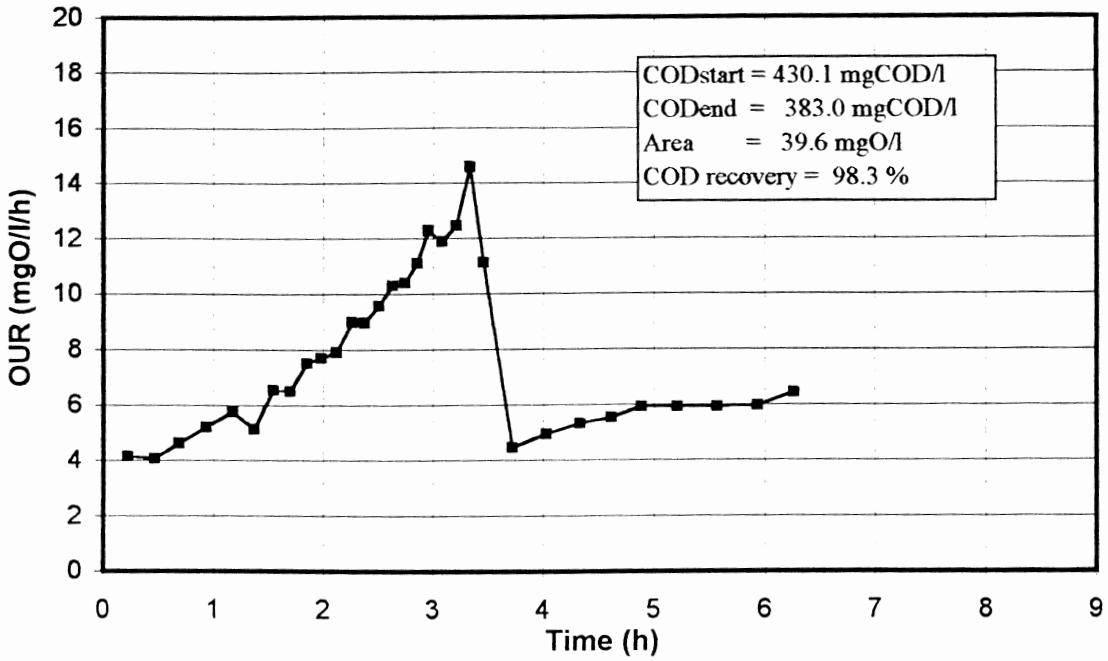
ln(OUR) graph for wastewater (2.6ℓ) plus filtered effluent (0.4ℓ) batch test W15, 17-02, waste water batch no.19



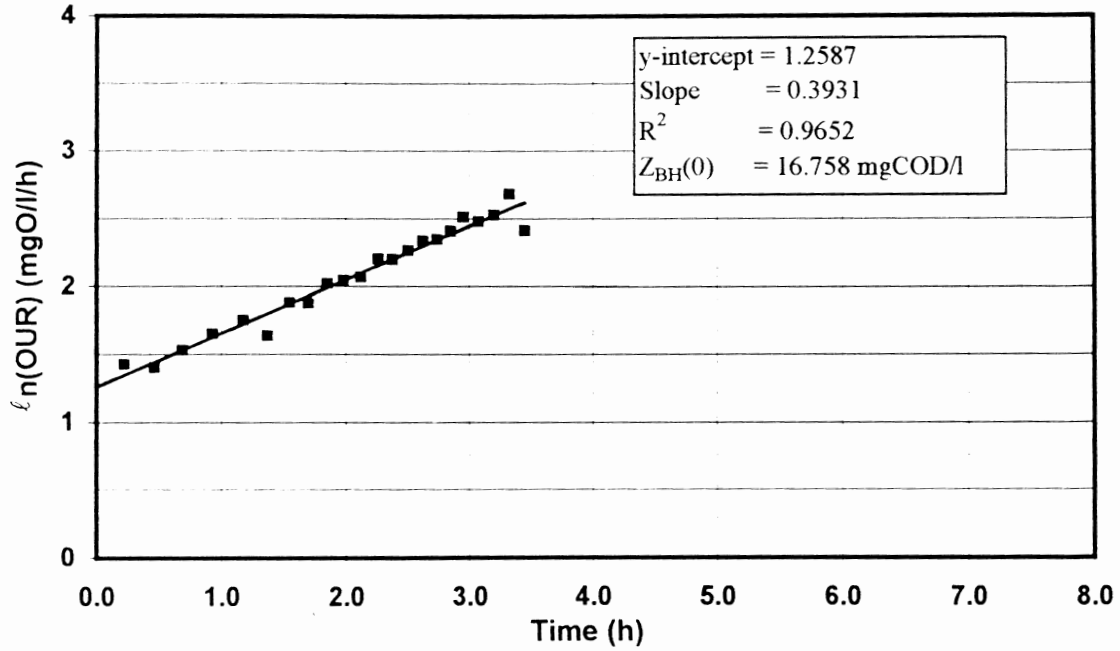
OUR Graph for wastewater (2.7ℓ) plus filtered effluent (0.3ℓ) batch test W16, 22-02, waste water batch no. 19



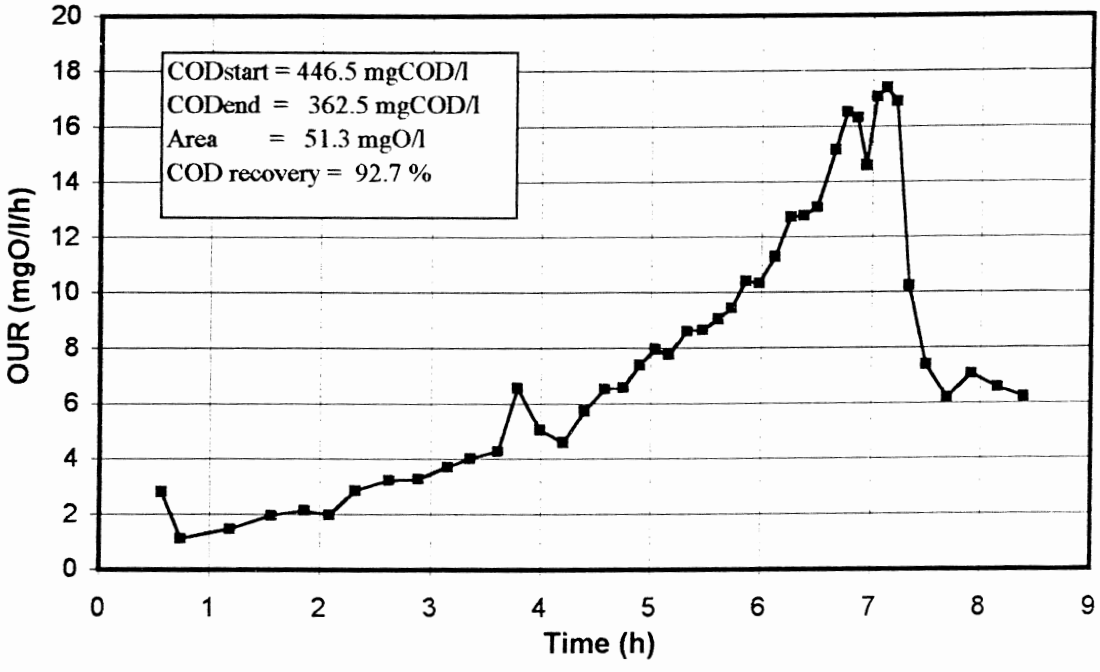
ln(OUR) graph for wastewater (2.7ℓ) plus filtered effluent (0.3ℓ) batch test W16, 22-02, waste water batch no.19



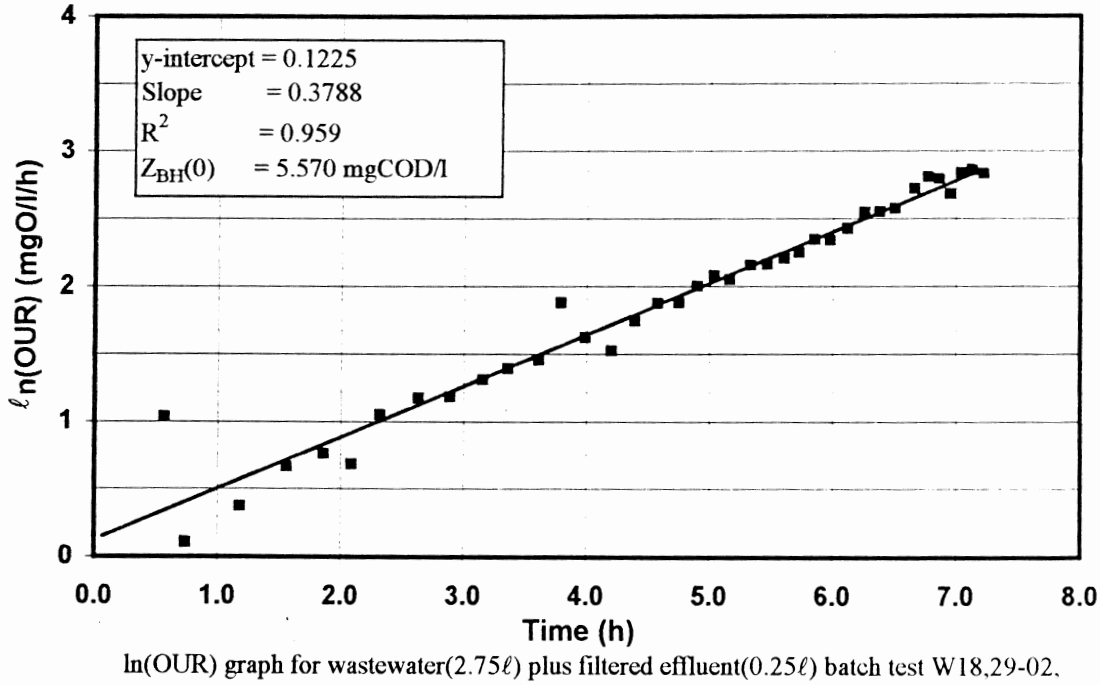
OUR Graph for wastewater (2.65ℓ) plus filtered effluent (0.35ℓ) batch test W17, 23-02, waste water batch no. 19



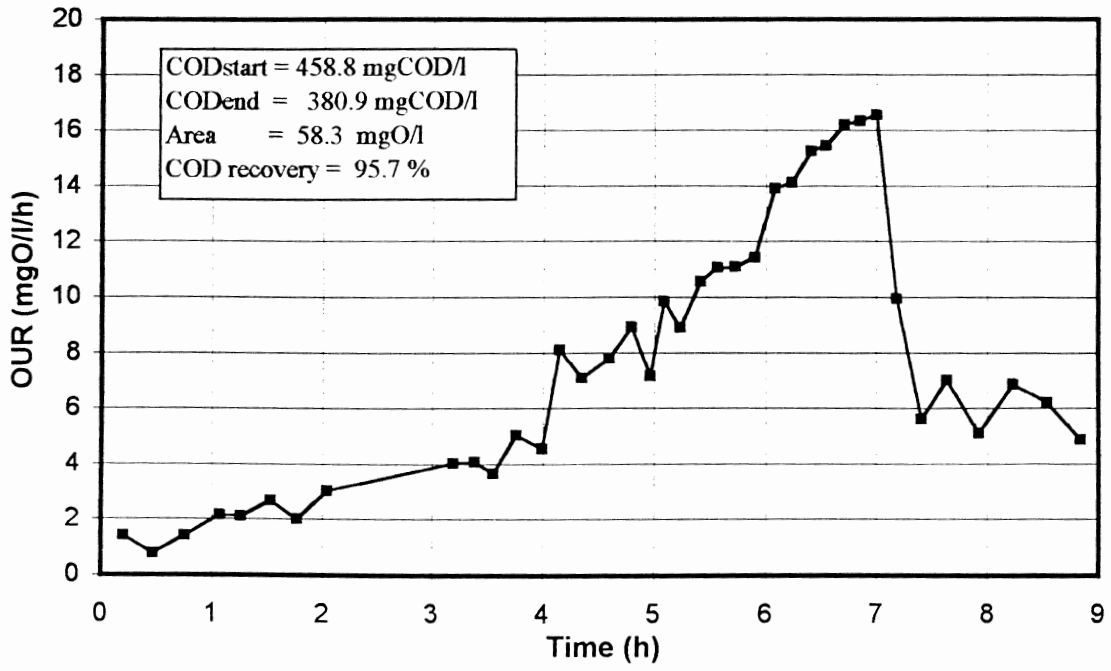
ln(OUR) graph for wastewater(2.65ℓ) plus filtered effluent(0.35ℓ) batch test W17,23-02, wastewater batch no.19



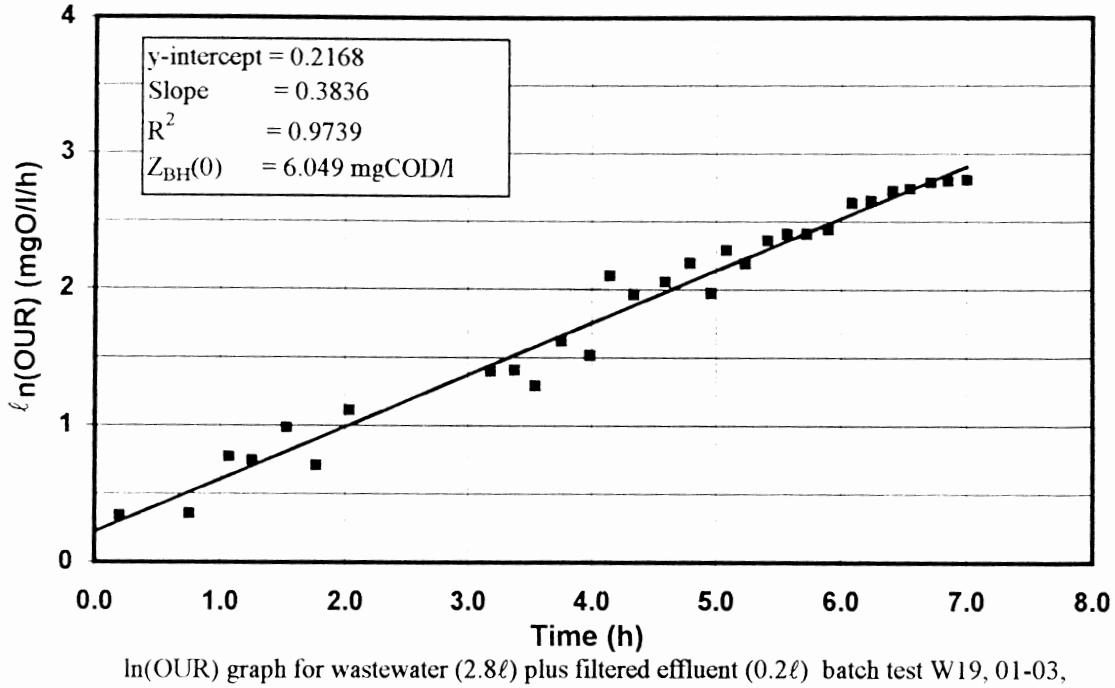
OUR Graph for wastewater (2.75ℓ) plus filtered effluent (0.25ℓ) batch test W18, 29-02, wastewater batch no. 20



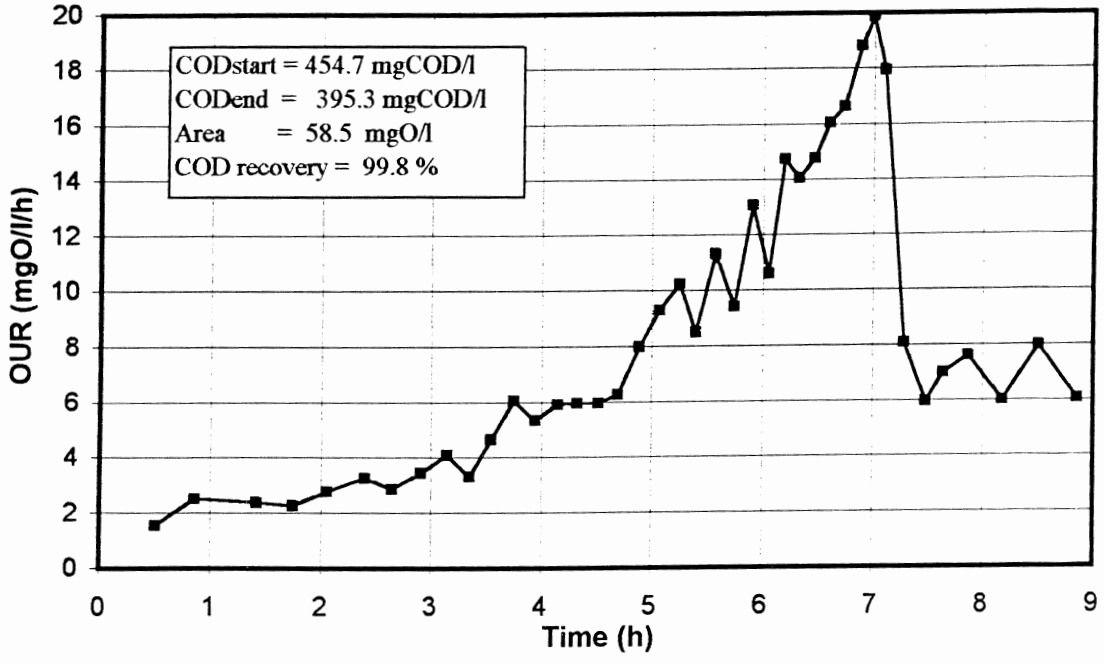
ln(OUR) graph for wastewater(2.75ℓ) plus filtered effluent(0.25ℓ) batch test W18,29-02, waste water batch no.20



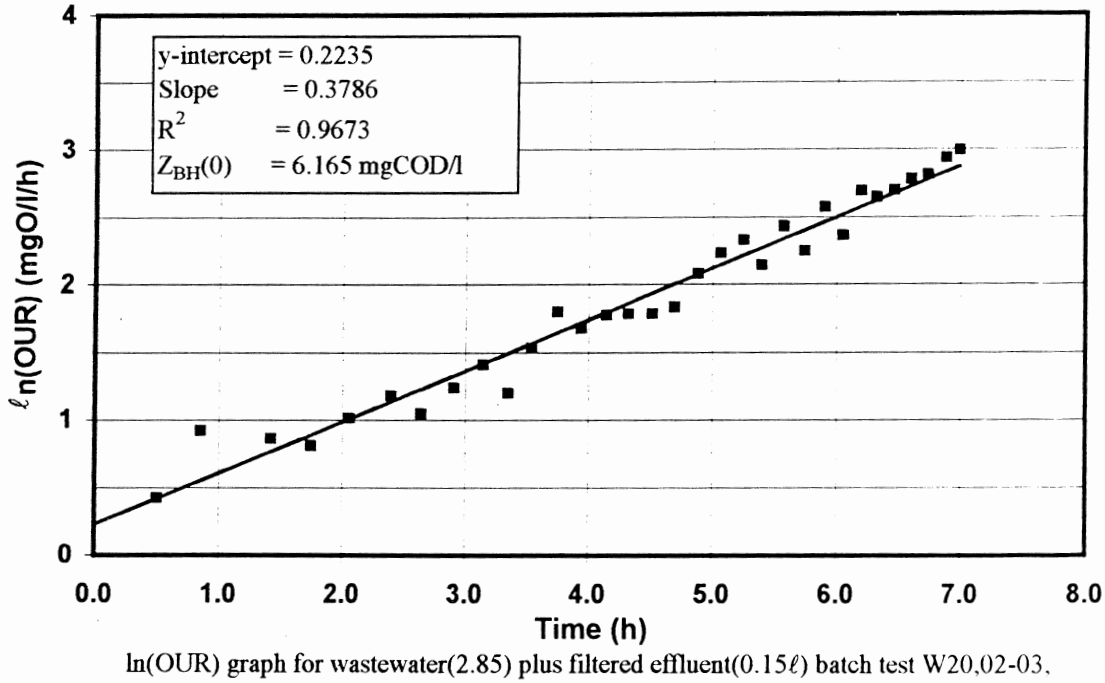
OUR Graph for wastewater (2.8ℓ) plus filtered effluent (0.2ℓ) batch test W19, 01-03, wastewater batch no. 20



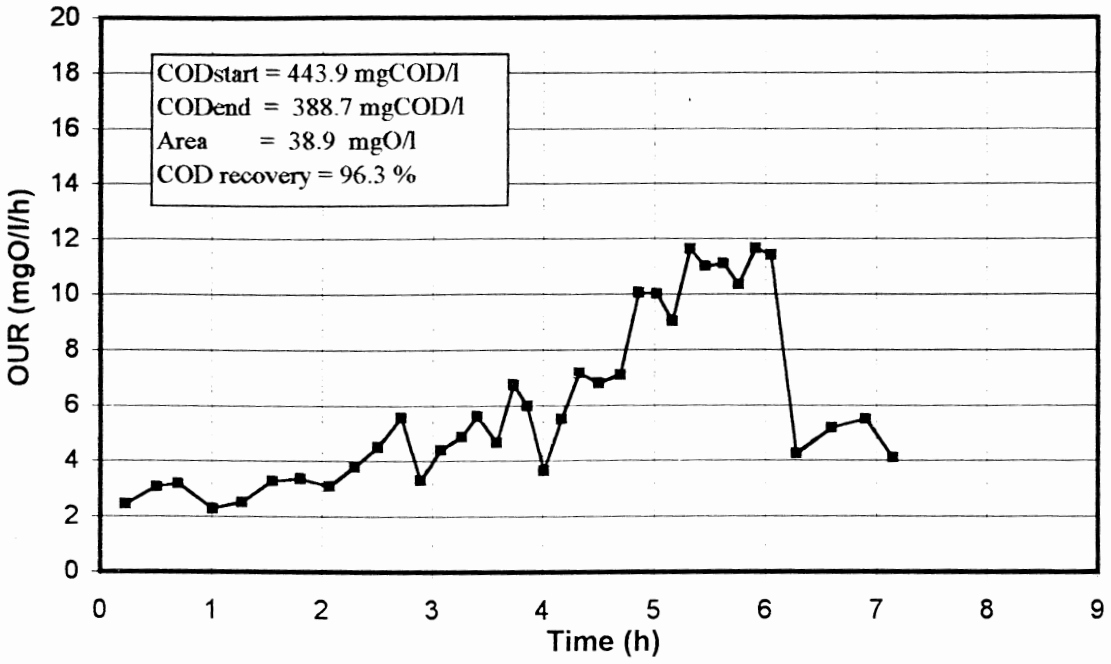
ln(OUR) graph for wastewater (2.8ℓ) plus filtered effluent (0.2ℓ) batch test W19, 01-03, wastewater batch no.20



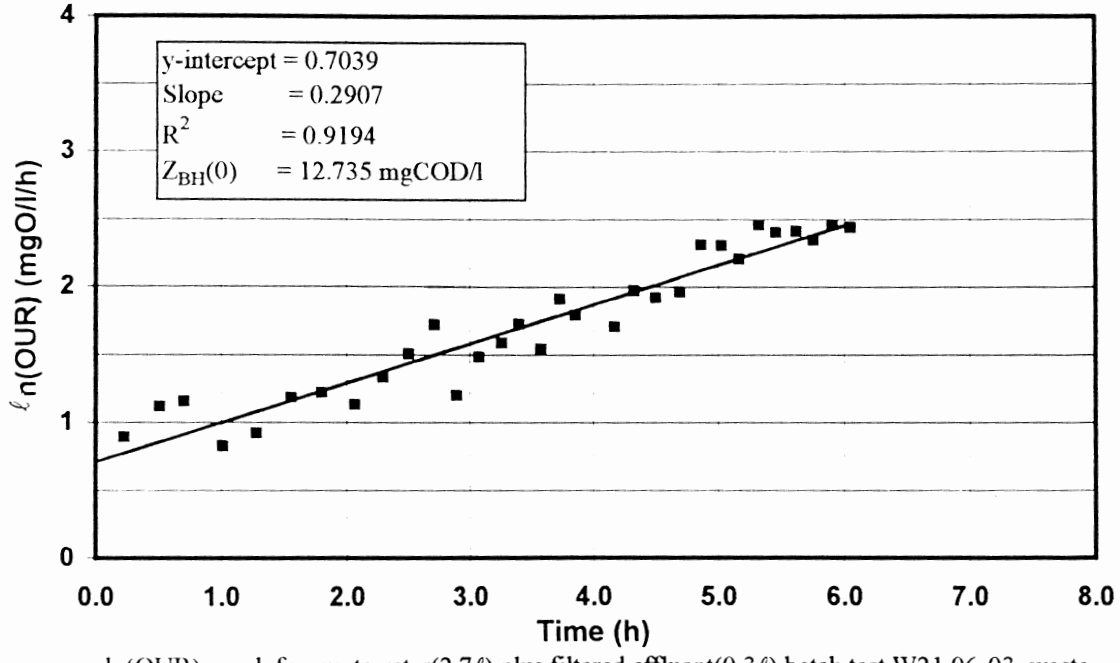
OUR Graph for wastewater (2.85ℓ) plus filtered effluent (0.15ℓ) batch test W20, 02-03, waste water batch no. 20



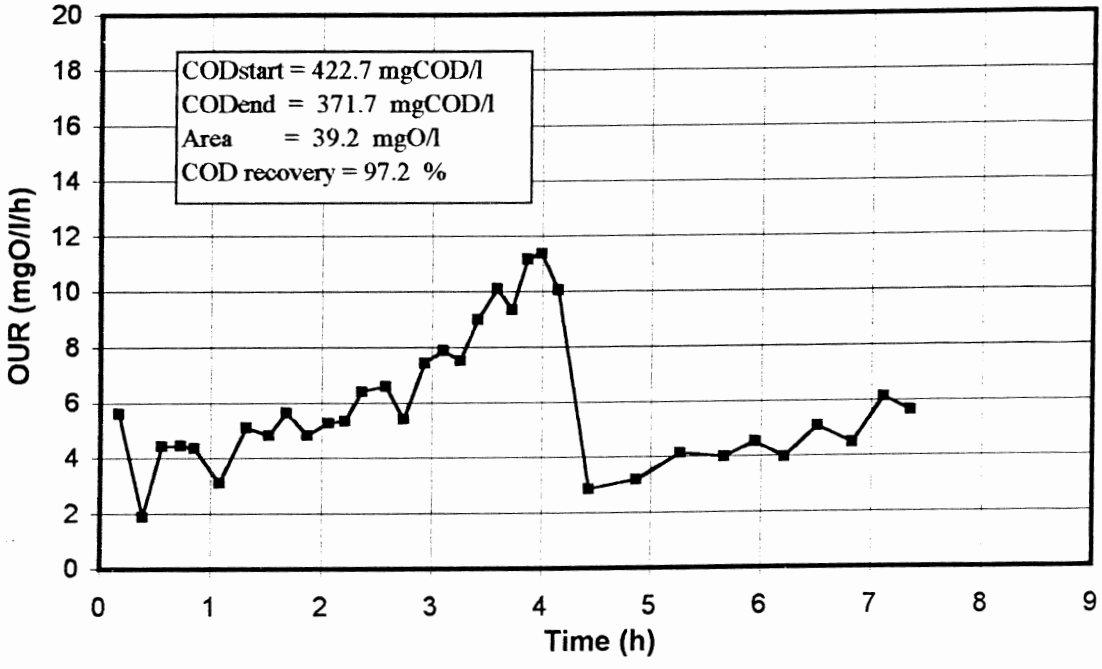
ln(OUR) graph for wastewater(2.85) plus filtered effluent(0.15ℓ) batch test W20,02-03, waste water batch no.20



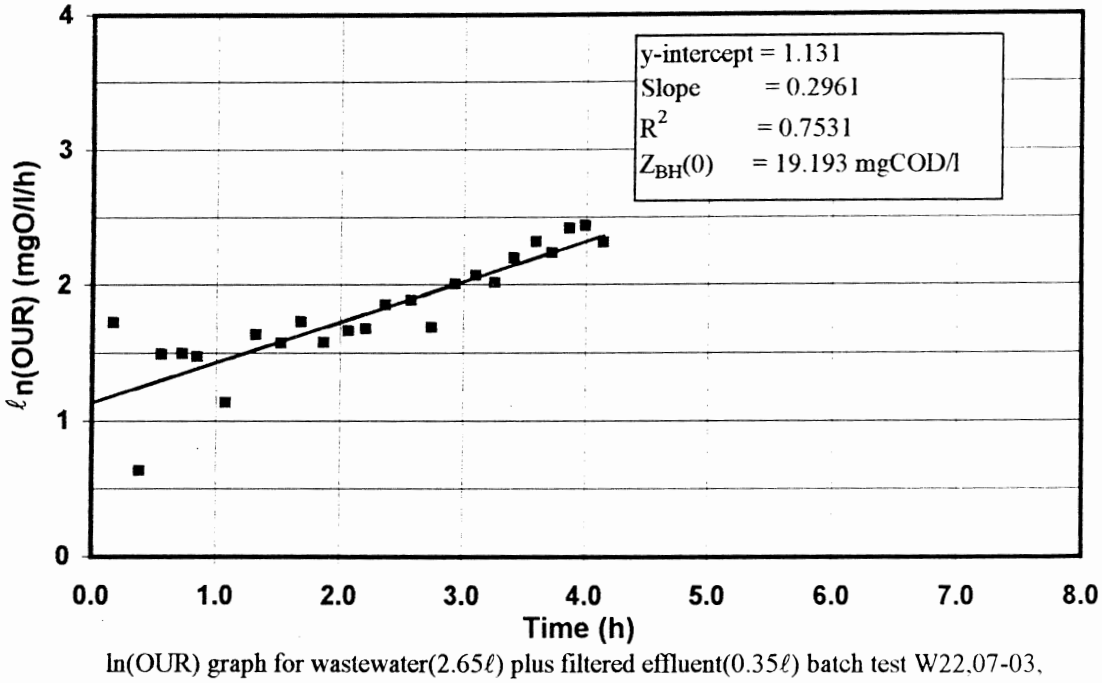
OUR Graph for wastewater (2.7ℓ) plus filtered effluent (0.3ℓ) batch test W21, 06-03, waste water batch no. 20



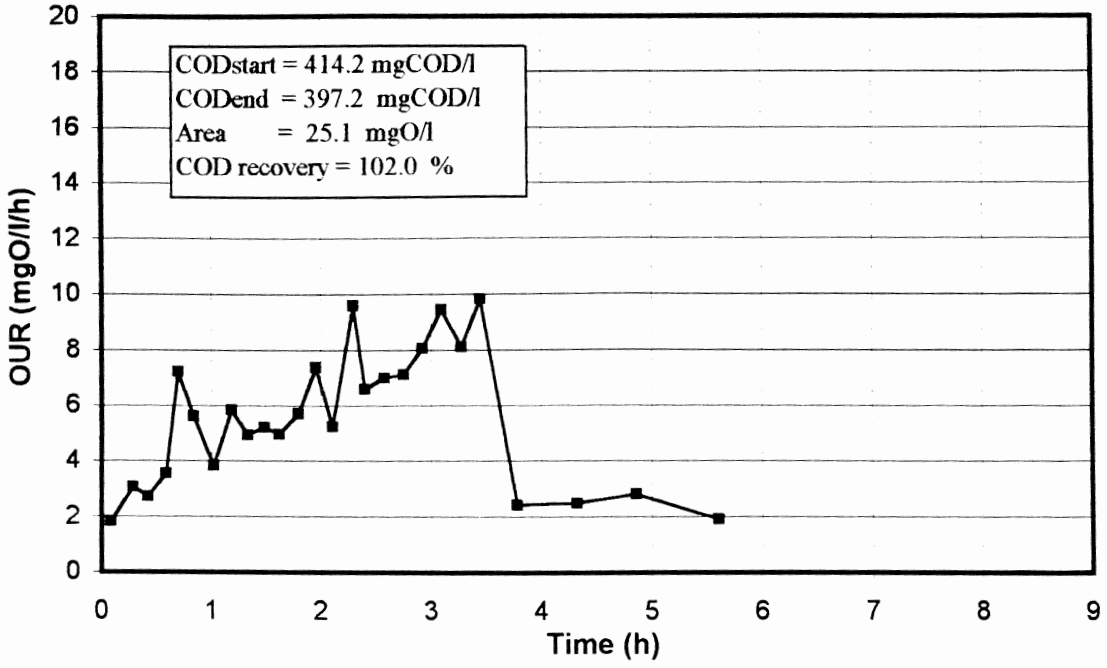
ln(OUR) graph for wastewater(2.7ℓ) plus filtered effluent(0.3ℓ) batch test W21,06-03, waste water batch no.20



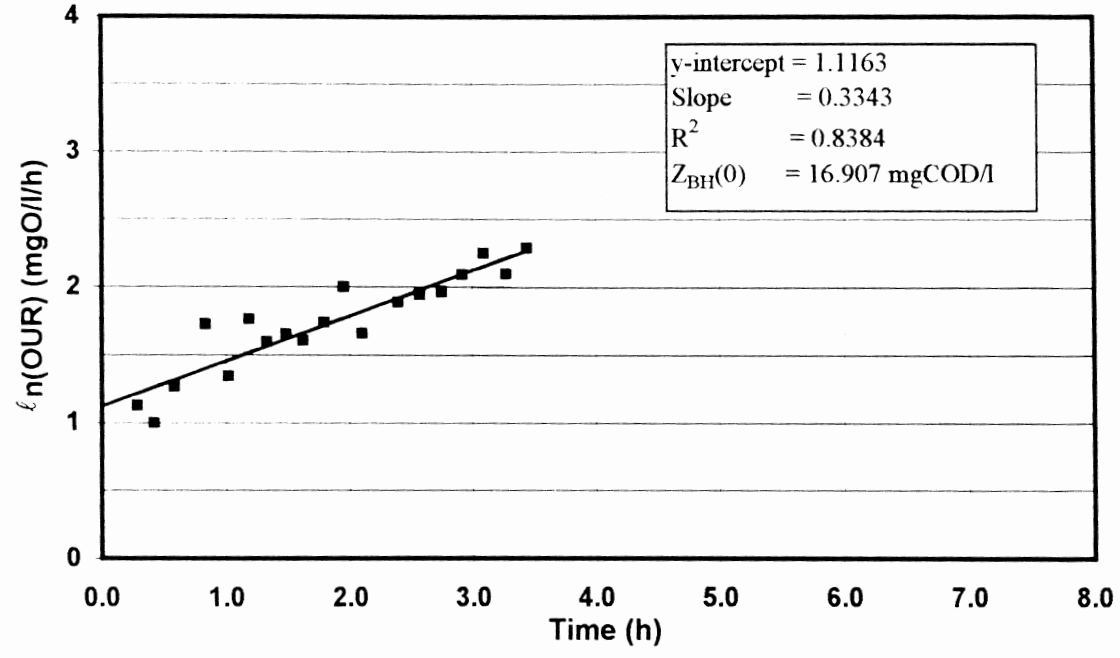
OUR Graph for wastewater (2.65ℓ) plus filtered effluent (0.35ℓ) batch test W22, 07-03, waste water batch no. 20



ln(OUR) graph for wastewater(2.65ℓ) plus filtered effluent(0.35ℓ) batch test W22,07-03, waste water batch no.20



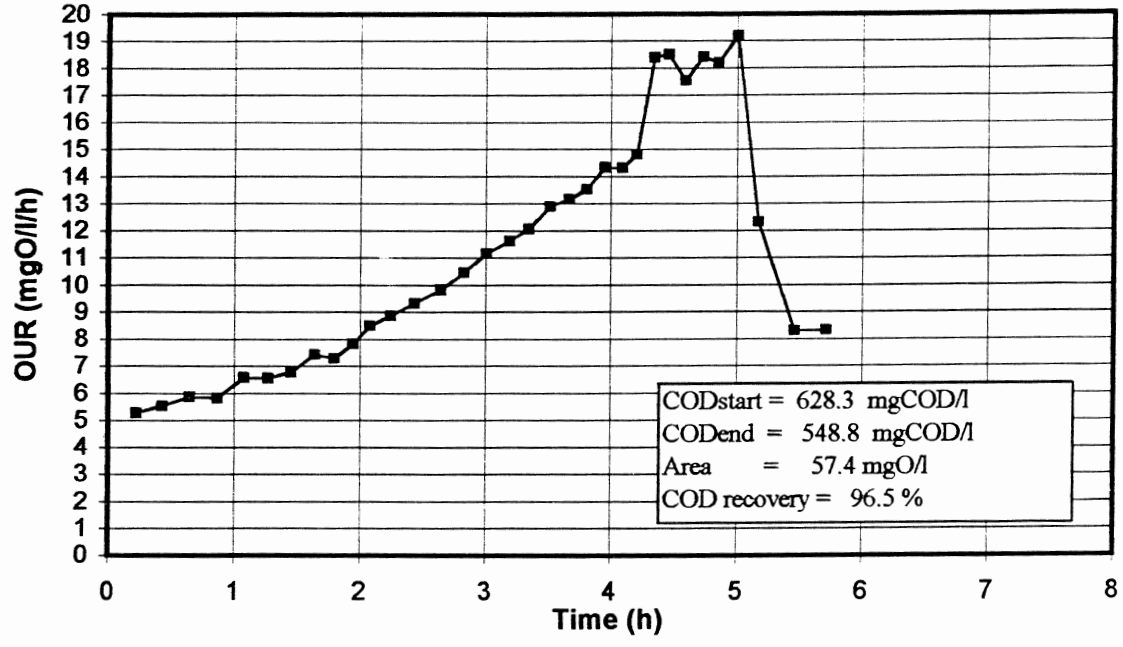
OUR Graph for wastewater (2.6ℓ) plus filtered effluent (0.4ℓ) batch test W23, 08-03, waste water batch no. 20



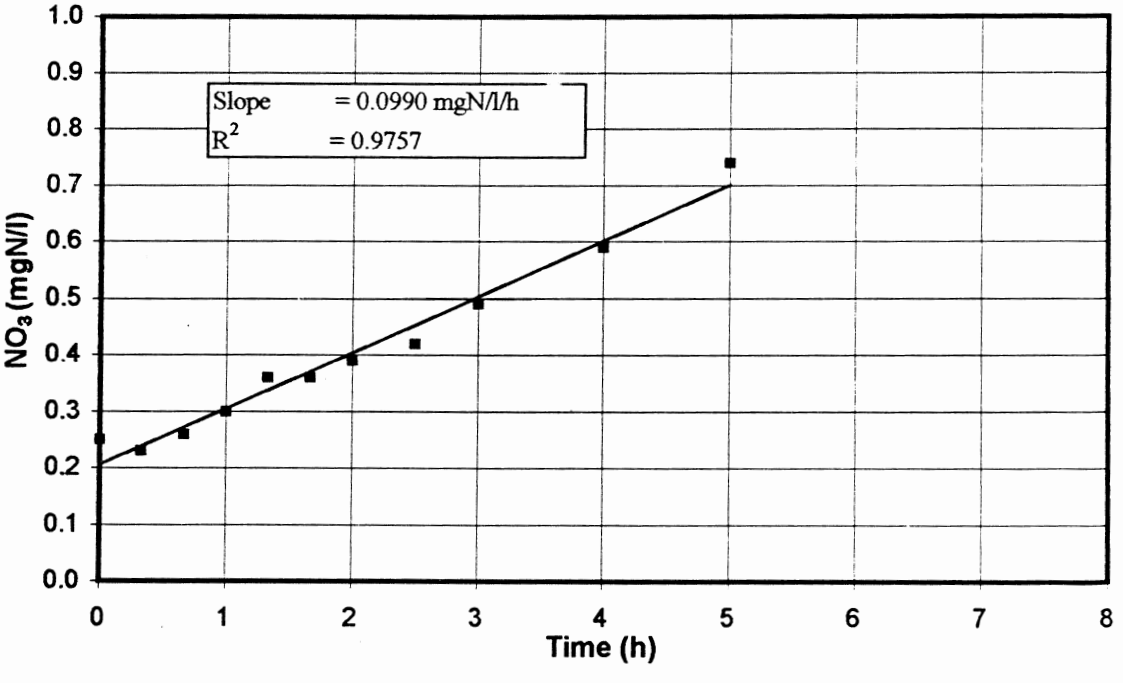
ln(OUR) graph for wastewater (2.6ℓ) plus filtered effluent (0.4ℓ) batch test W23, 08-03, waste water batch no.20

Table A2 : Daily wastewater and mixed liquor (WW+ML) batch test data with mixed liquor sample volume, COD recovery, max. specific growth rates and heterotrophic active biomass.

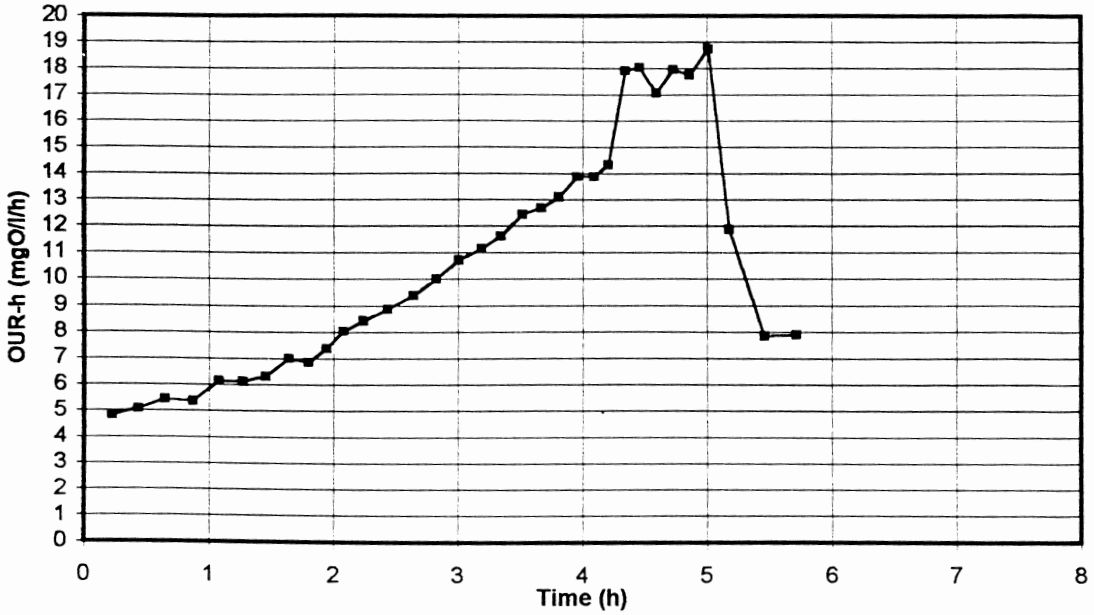
Sew. batch no.	Batch Test No.	Date of test	M L vol.	COD (mgCOD/l)		Area (mgO/l)	%COD Recov.	Linear regression data			Max. specific growth rates (/d)		$Z_{BH(0)}$ (WW+ML) (mgCOD/l)
				Start	End			y-int.	slope	R^2	K_{MP}	μ_H	
11	M1	29-10	0.10ℓ	628	549	57	97	1.4550	0.2999	0.9902	3.2	4.6	26.228
	M2	03-11	0.15ℓ	653	583	62	99	1.5450	0.2995	0.9734	3.7	4.1	28.733
	M3	05-11	0.30ℓ	873	778	53	95	2.3422	0.1786	0.9637	3.3	1.6	101.480
12	M4	15-11	0.30ℓ	771	706	65	100	2.0838	0.1669	0.9517	2.8	1.8	83.129
13	M5	30-11	0.20ℓ	720	678	54	102	2.3129	0.1977	0.7954	3.4	2.0	90.129
	M6	01-12	0.10ℓ	626	519	47	90	1.8699	0.3608	0.9583	5.1	4.2	33.459
	M7	03-12	0.25ℓ	724	626	31	91	2.3147	0.2544	0.8907	3.7	3.0	72.023
14	M8	08-12	0.20ℓ	703	605	50	93	1.6540	0.2568	0.9787	3.9	2.9	36.883
18	M9	07-02	0.20ℓ	673	641	53	103	1.5715	0.2953	0.8414	4.1	3.6	29.891
	M10	08-02	0.25ℓ	714	690	64	106	1.7904	0.2009	0.9001	3.2	2.2	52.695
	M11	09-02	0.30ℓ	763	718	53	101	1.9937	0.2319	0.9393	3.6	2.6	56.808
	M12	10-02	0.15ℓ	597	557	42	100	1.8010	0.2563	0.9077	3.7	3.1	42.799
19	M13	15-02	0.30ℓ	711	683	47	103	1.5950	0.2395	0.9083	3.4	3.0	37.037
	M14	16-02	0.35ℓ	833	744	44	95	2.1418	0.1732	0.8261	2.9	1.8	85.305
	M15	17-02	0.40ℓ	914	845	60	99	2.2600	1.6090	0.6509	3.0	1.5	102.332
	M16	22-02	0.30ℓ	827	721	56	94	1.9608	0.1730	0.9596	2.7	2.0	71.253
	M17	23-02	0.35ℓ	803	758	40	99	2.1179	0.1918	0.9316	2.7	2.5	76.172
20	M18	29-02	0.25ℓ	754	651	59	94	1.5325	0.2147	0.9480	3.1	2.7	38.380
	M19	01-03	0.20ℓ	672	590	64	97	1.3431	0.2500	0.9673	3.2	3.5	27.694
	M20	02-03	0.15ℓ	619	504	56	91	1.2635	0.2162	0.9423	2.4	3.4	29.146
	M21	06-03	0.30ℓ	743	675	47	97	2.1164	0.0994	0.8302	1.4	1.6	132.175
	M22	07-03	0.35ℓ	794	752	43	100	2.2090	0.2033	0.9909	2.4	3.1	79.249
	M23	08-03	0.40ℓ	790	807	46	108	2.4791	0.1584	0.9196	2.2	2.2	129.128



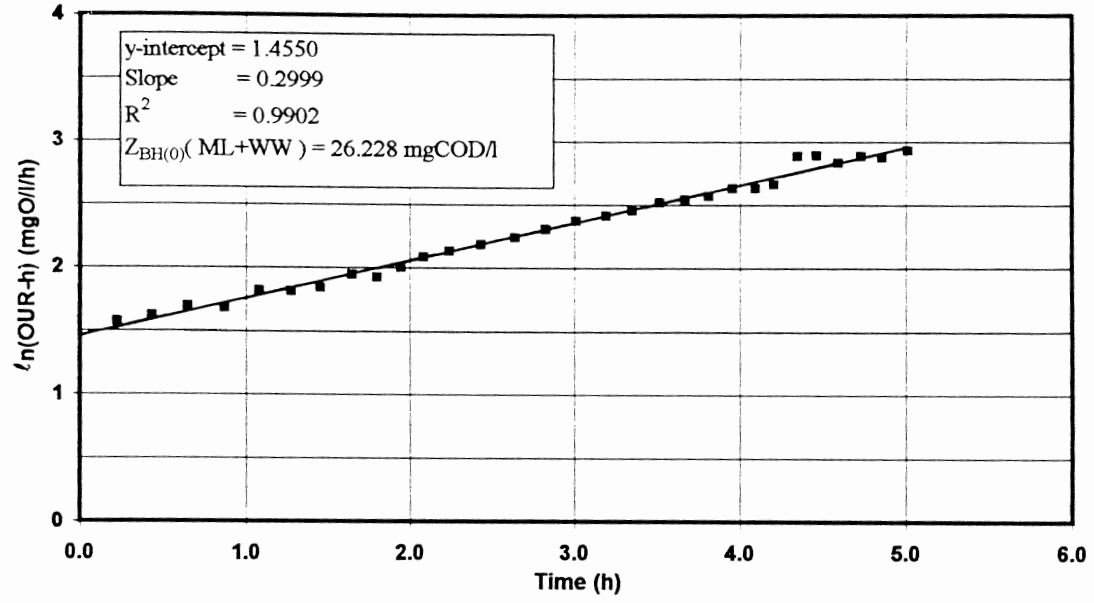
OUR Graph for wastewater (2.9ℓ) plus mixed liquor (0.1ℓ) batch test M1, 29-10, wastewater batch no. 11



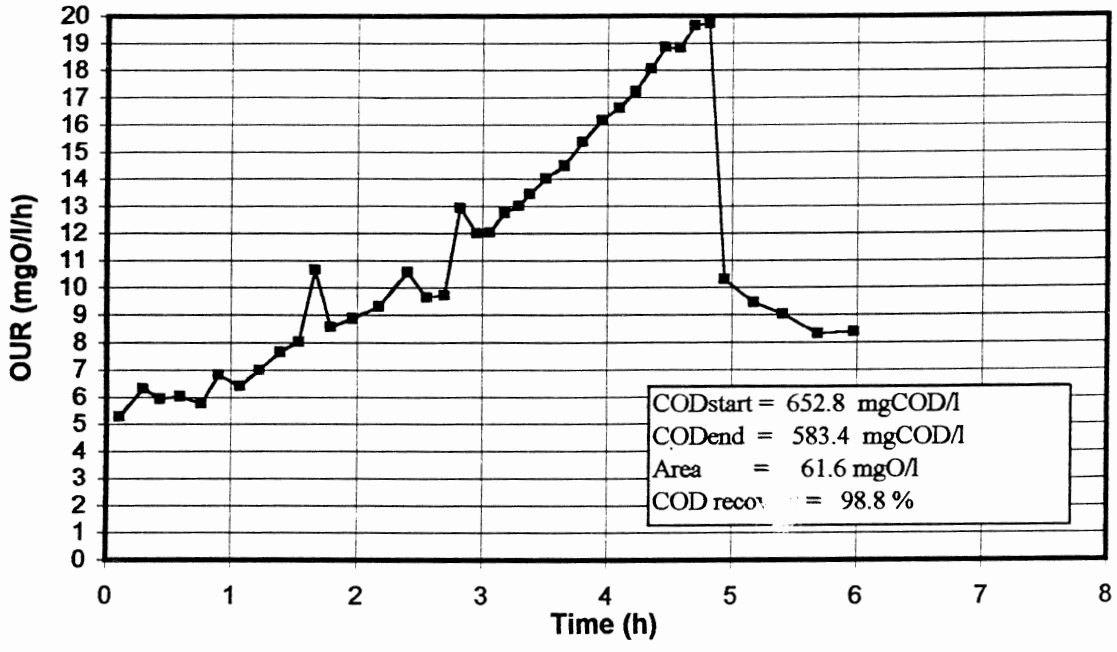
NO_3 Graph for wastewater (2.9ℓ) plus mixed liquor (0.1ℓ) batch test M1, 29-10, wastewater batch no. 11



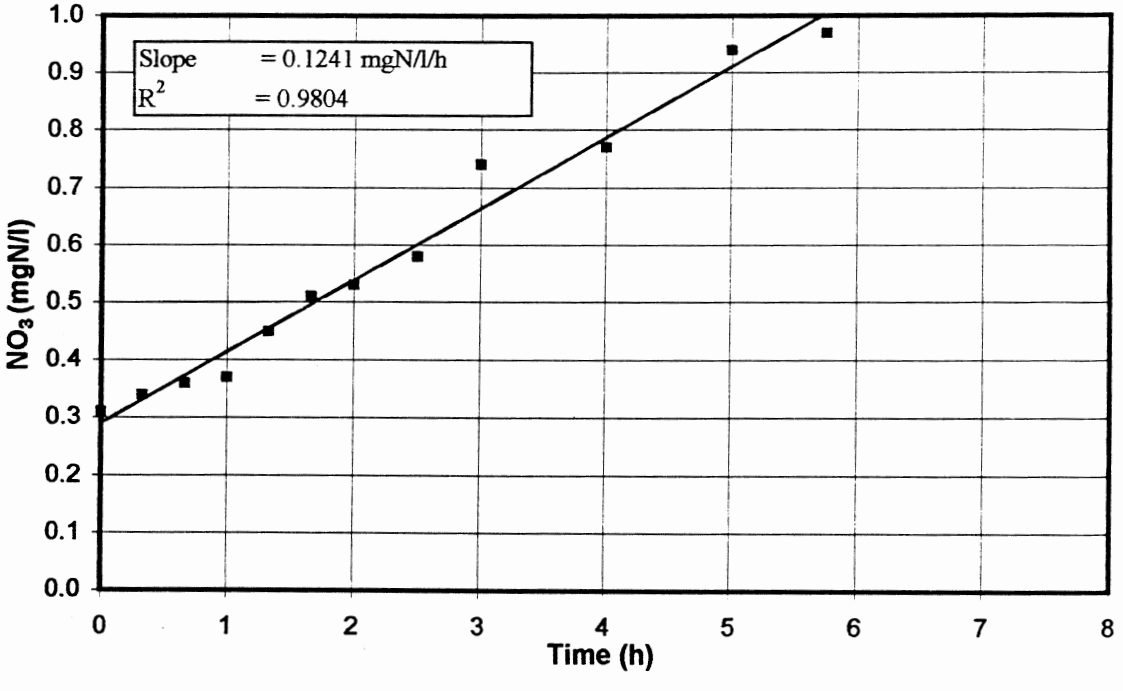
OUR-h Graph for wastewater (2.9ℓ) plus mixed liquor (0.1ℓ) batch test M1, 29-10, wastewater batch no. 11



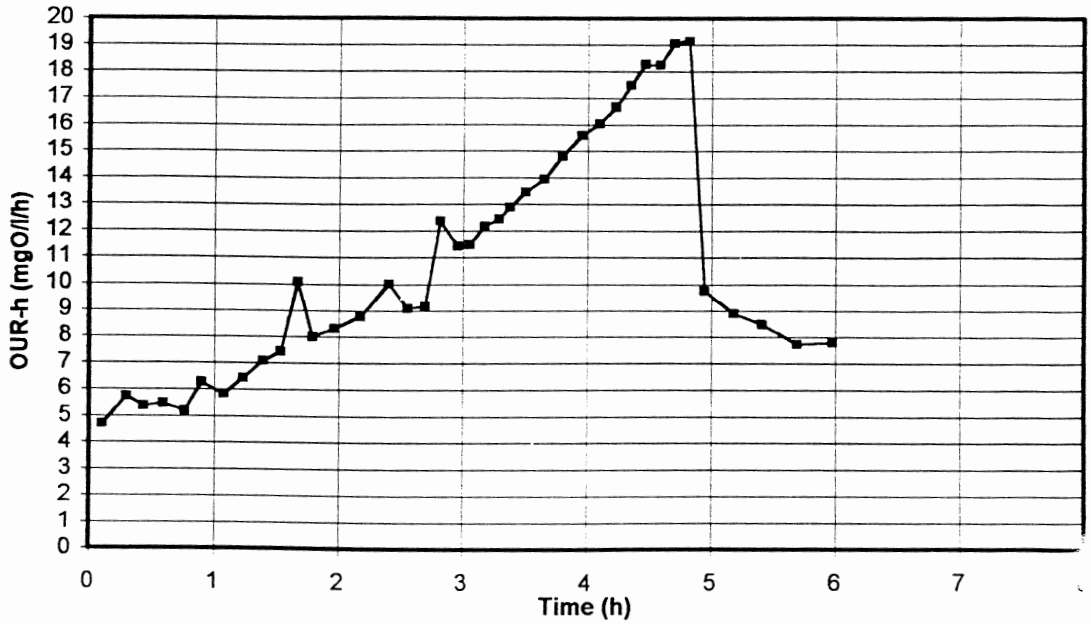
ℓn(OUR-h) graph for wastewater (2.9ℓ) plus mixed liquor (0.1ℓ) batch test M1, 29-10, wastewater batch no.11



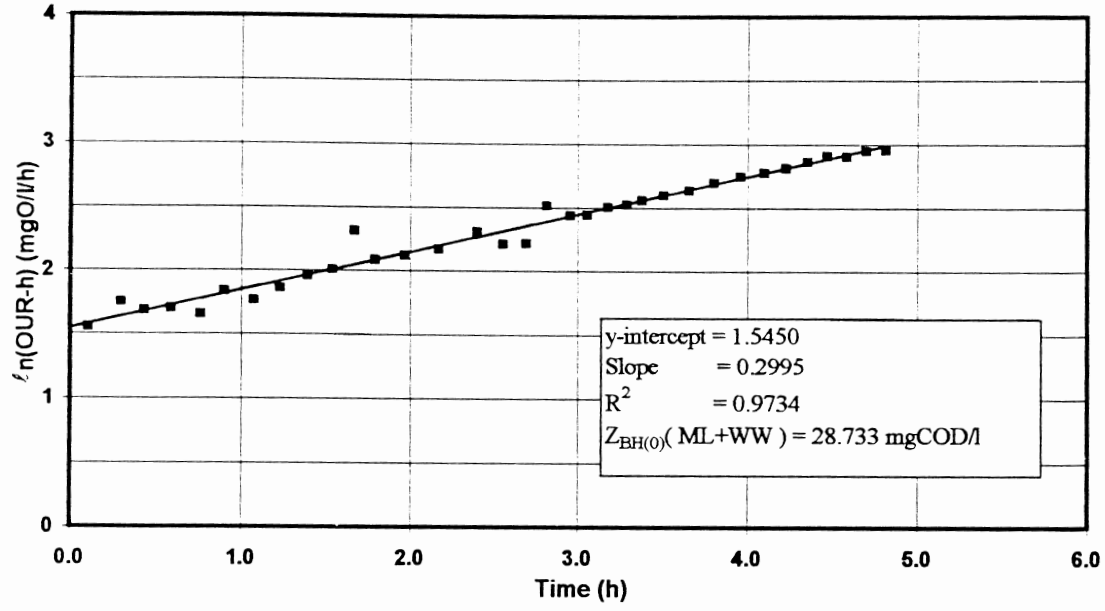
OUR Graph for wastewater (2.85ℓ) plus mixed liquor (0.15ℓ) batch test M2, 03-11, wastewater batch no. 11



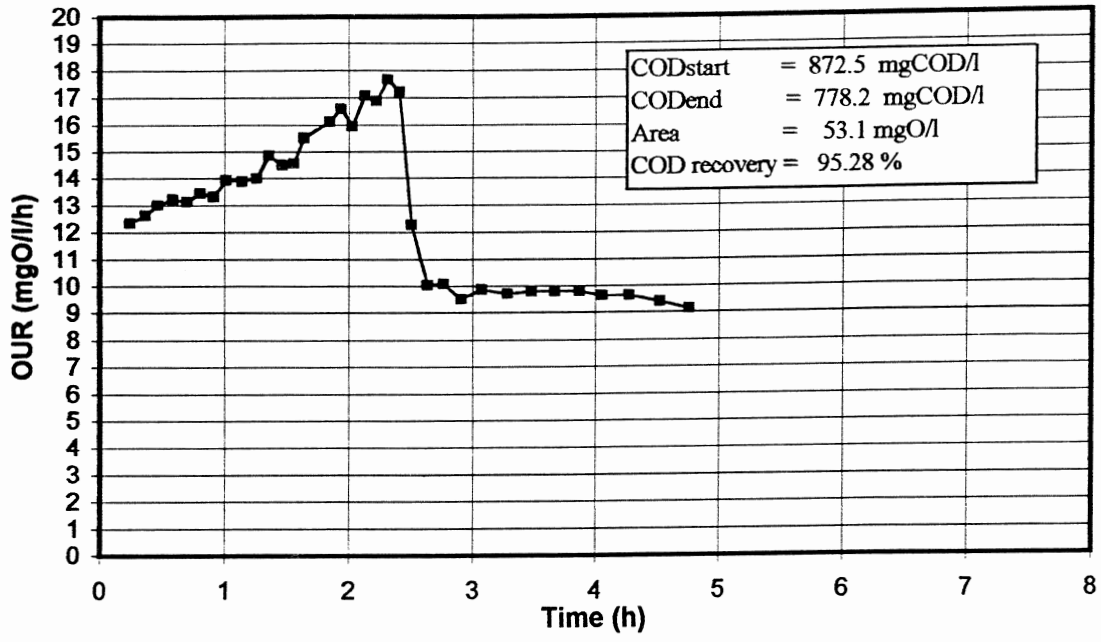
NO_3 Graph for wastewater (2.85ℓ) plus mixed liquor (0.15ℓ) batch test M2, 03-11, wastewater batch no. 11



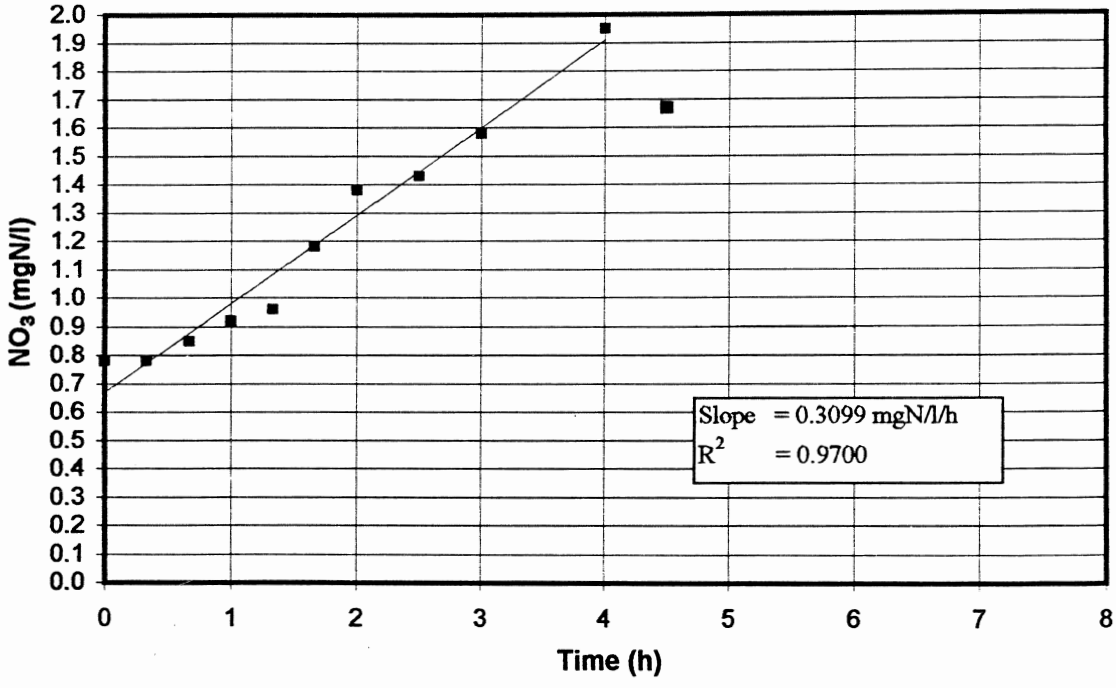
OUR-h Graph for wastewater (2.85ℓ) plus mixed liquor (0.15ℓ) batch test M2, 03-11, wastewater batch no. 11



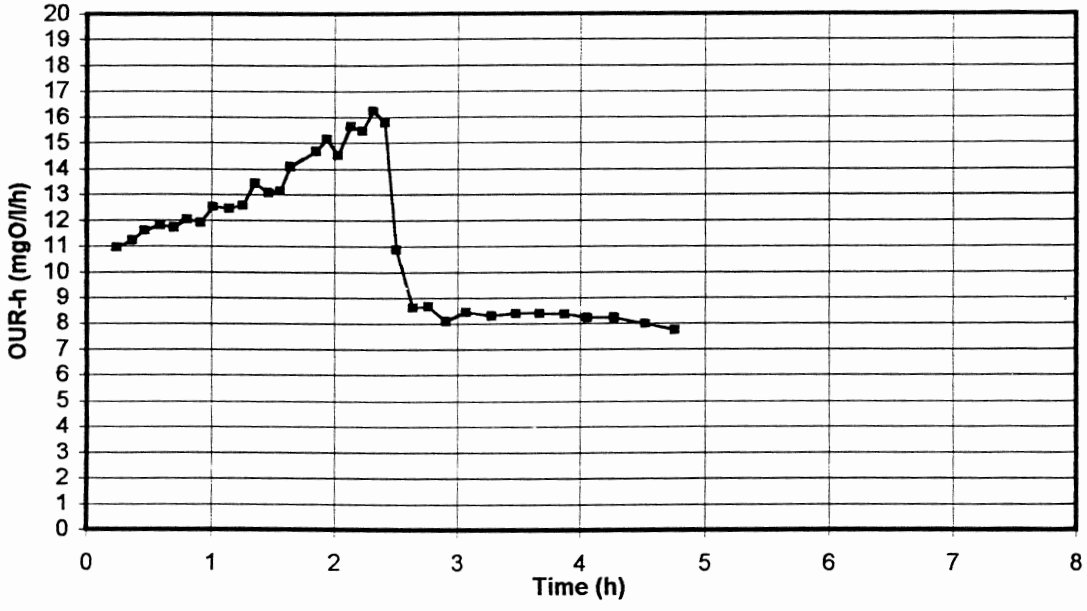
ℓn(OUR-h) graph for wastewater (2.85ℓ) plus mixed liquor(0.15ℓ) batch test M2, 03-11, wastewater batch no.11



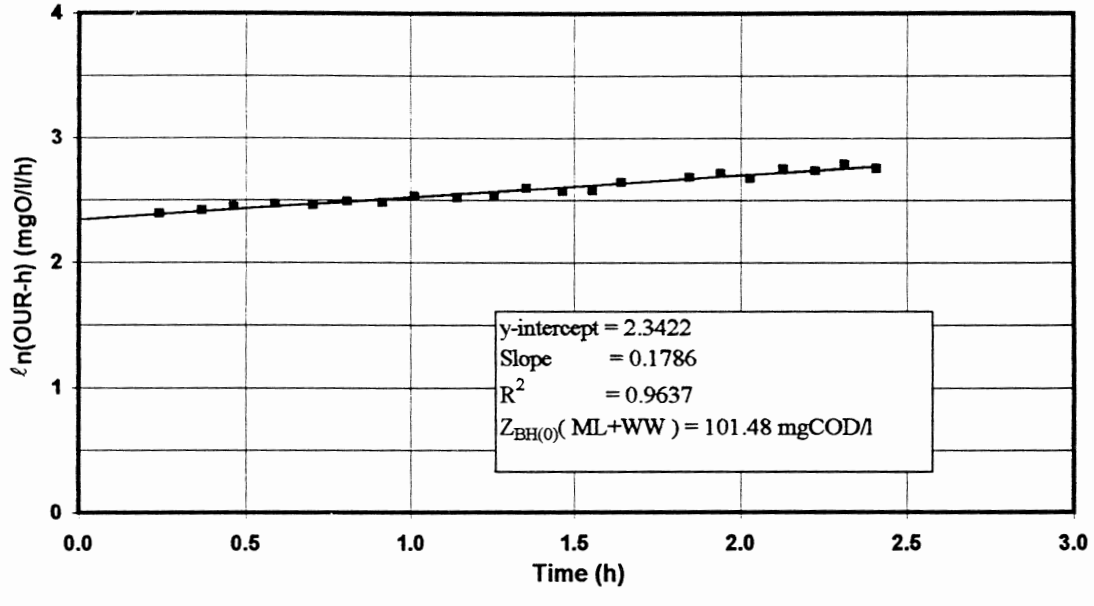
OUR Graph for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M3, 05-11, wastewater batch no. 11



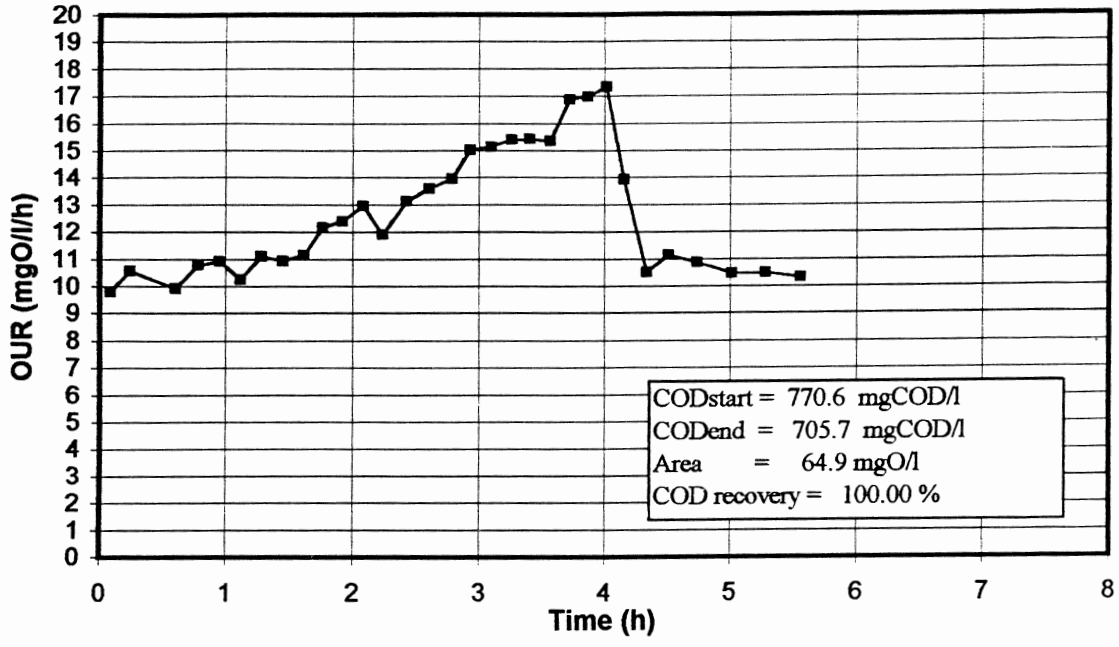
NO_3 Graph for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M3, 05-11, wastewater batch no. 11



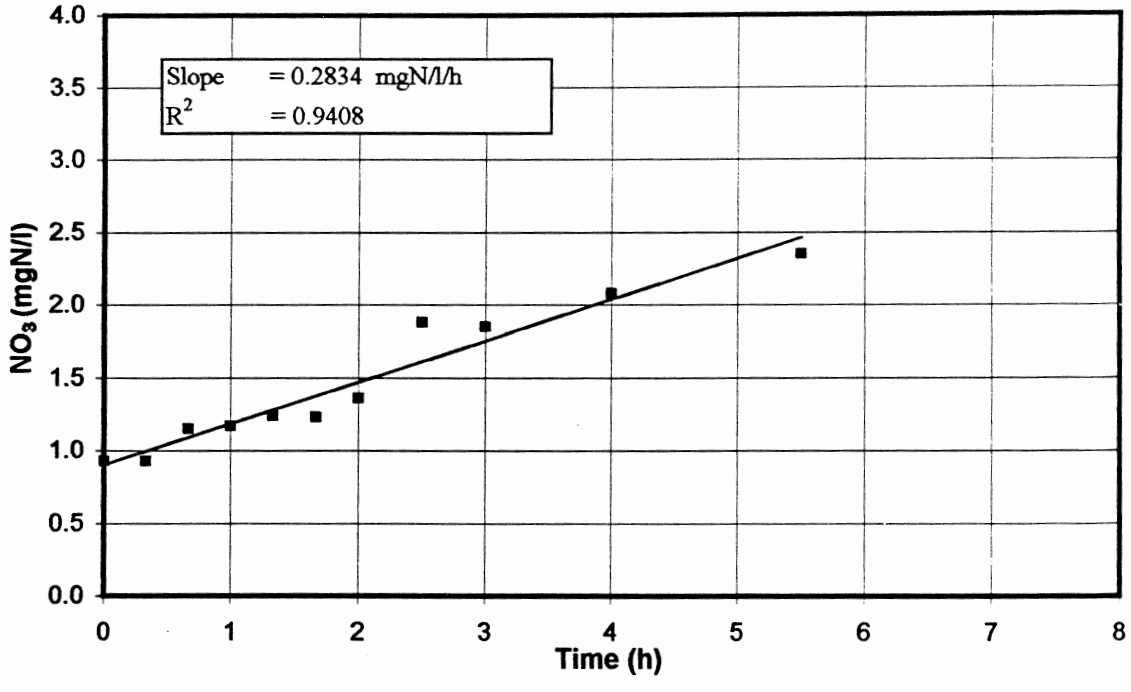
OUR-h Graph for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M3, 05-11, wastewater batch no. 11



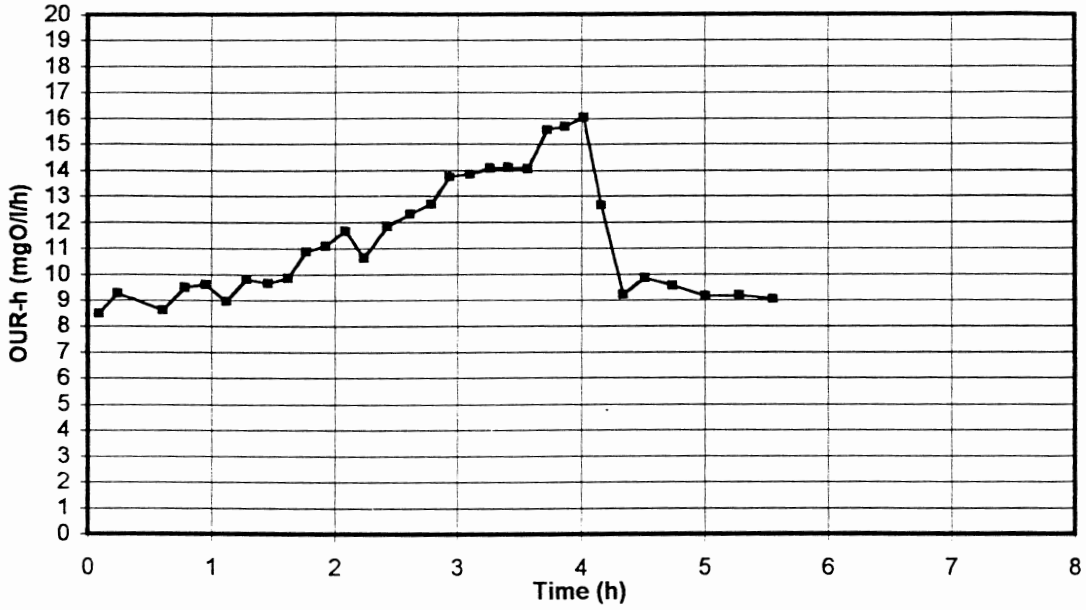
ℓn(OUR-h) graph for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M3, 05-11, waste water batch no.11



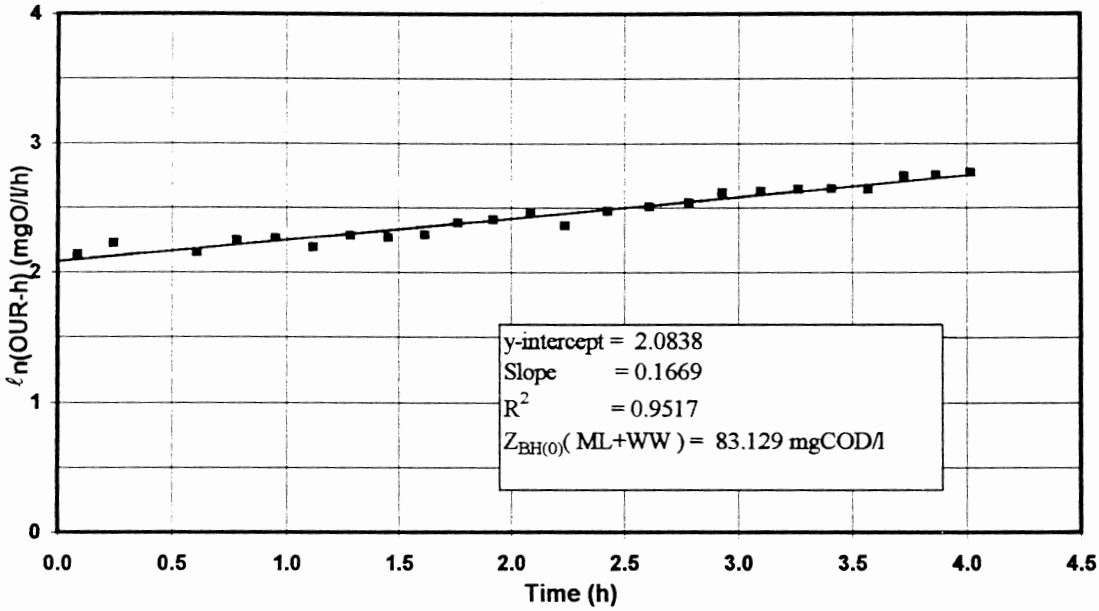
OUR Graph for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M4, 15-11, wastewater batch no. 12



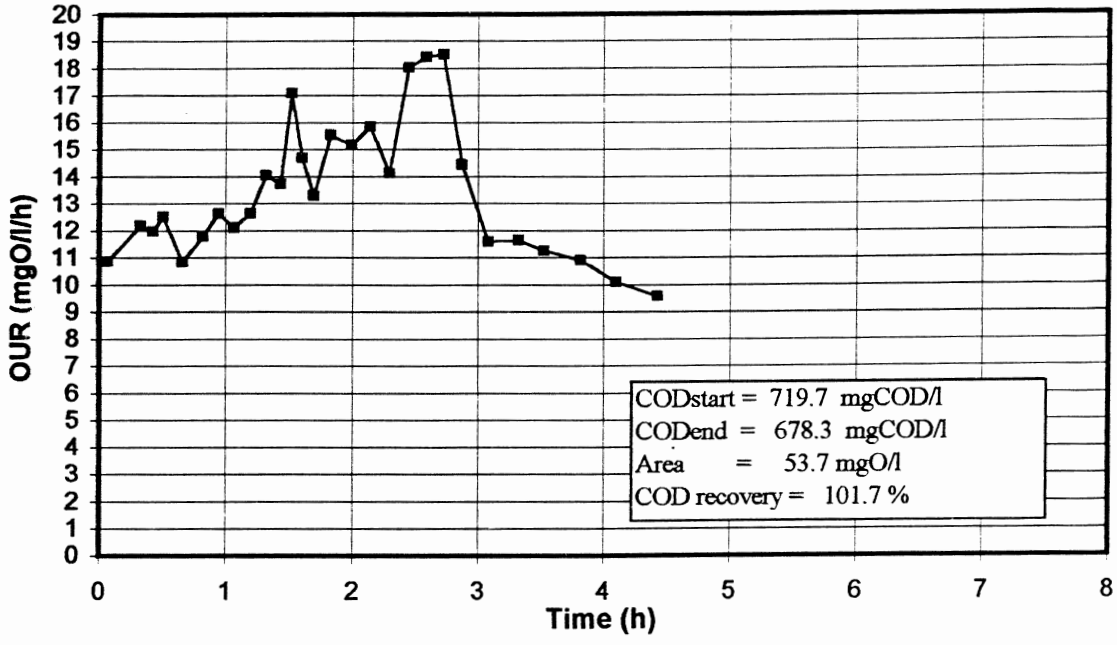
NO_3 Graph for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M4, 15-11, wastewater batch no. 12



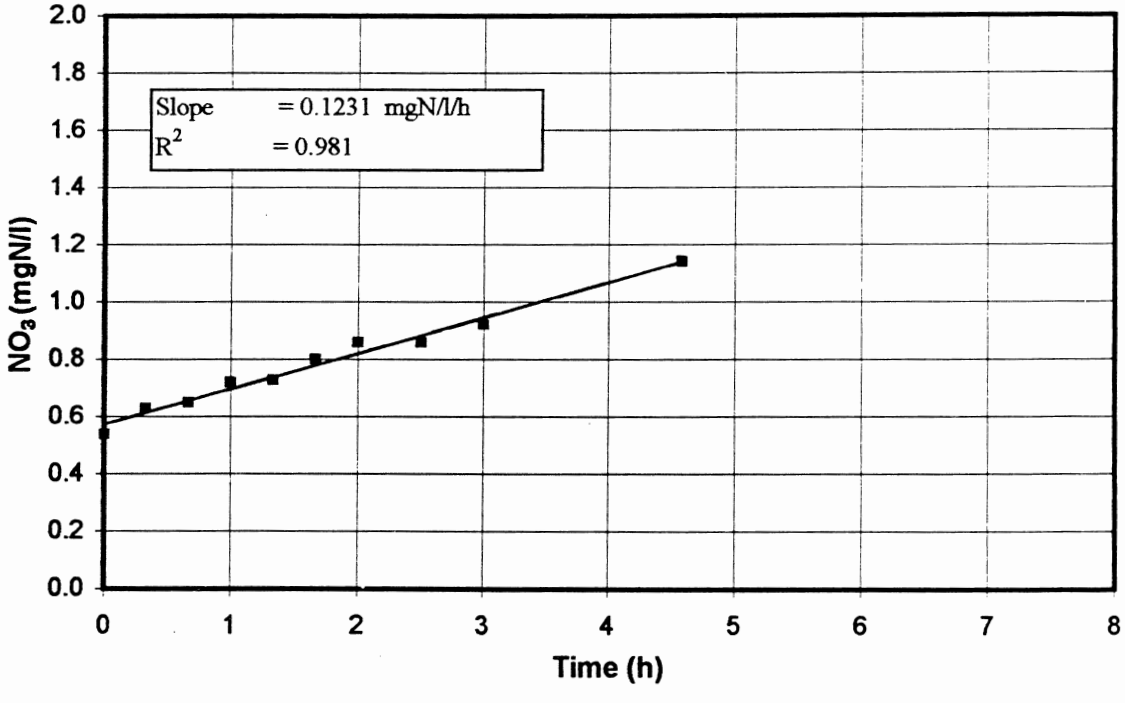
OUR-h Graph for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M4, 15-11, wastewater batch no. 12



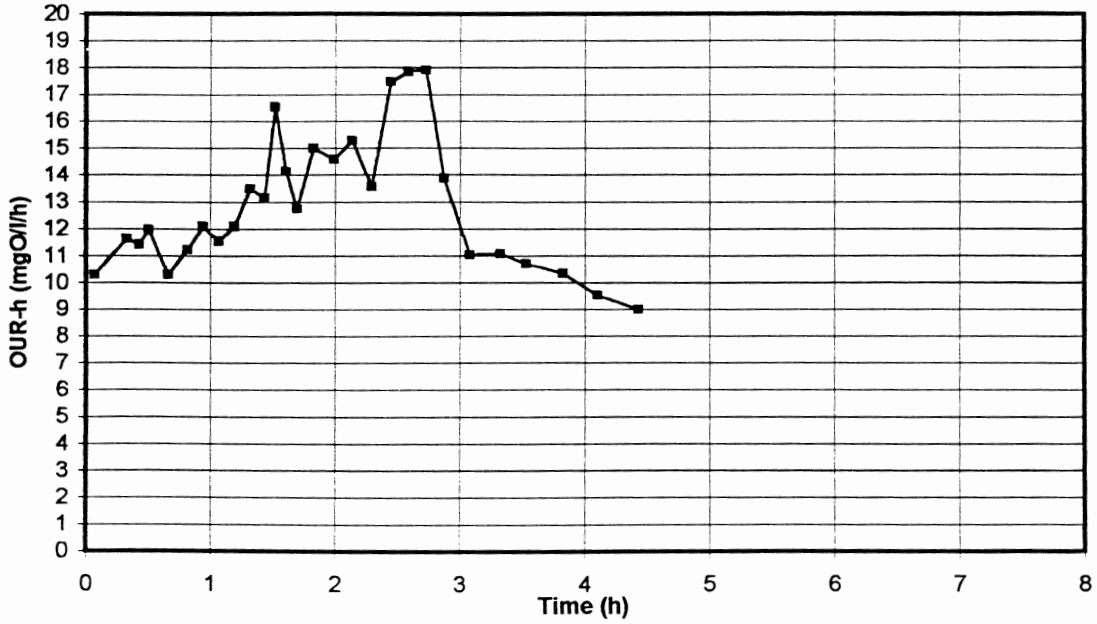
ℓn(OUR-h) graph for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M4, 15-11, waste water batch no.12



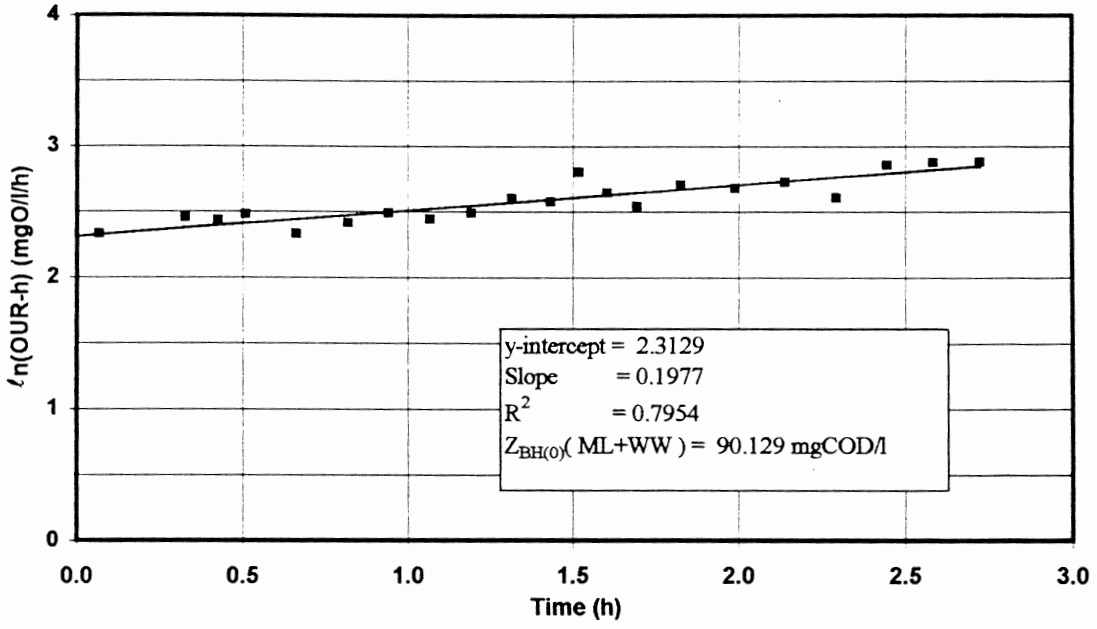
OUR Graph for wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test M5, 30-11, wastewater batch no. 13



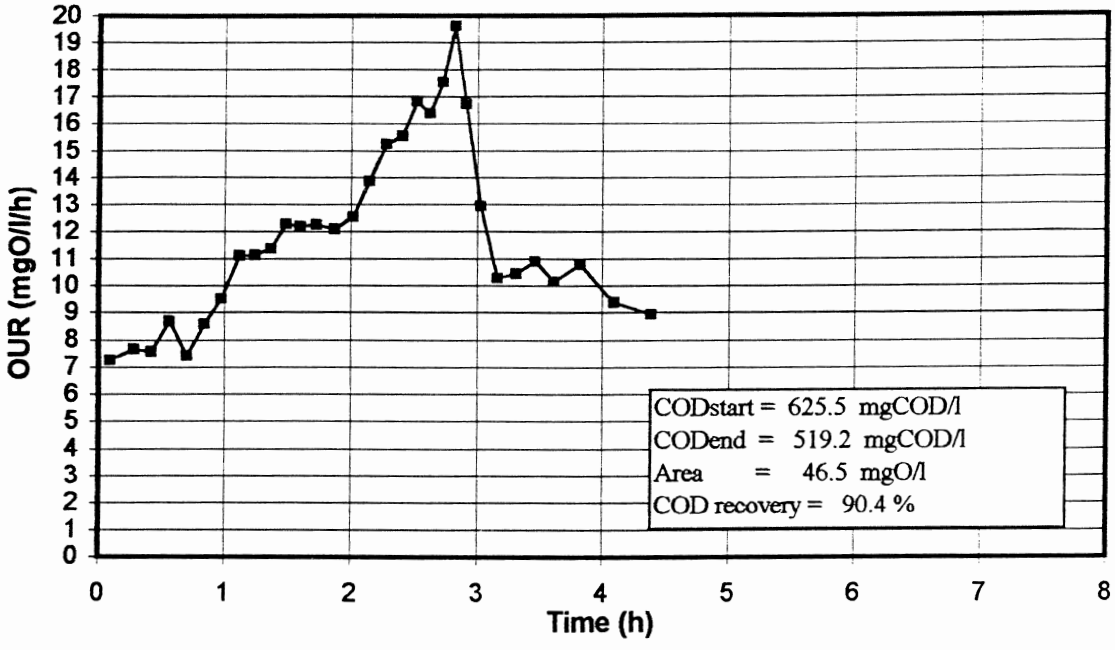
NO_3 Graph for wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test M5, 30-11, wastewater batch no. 13



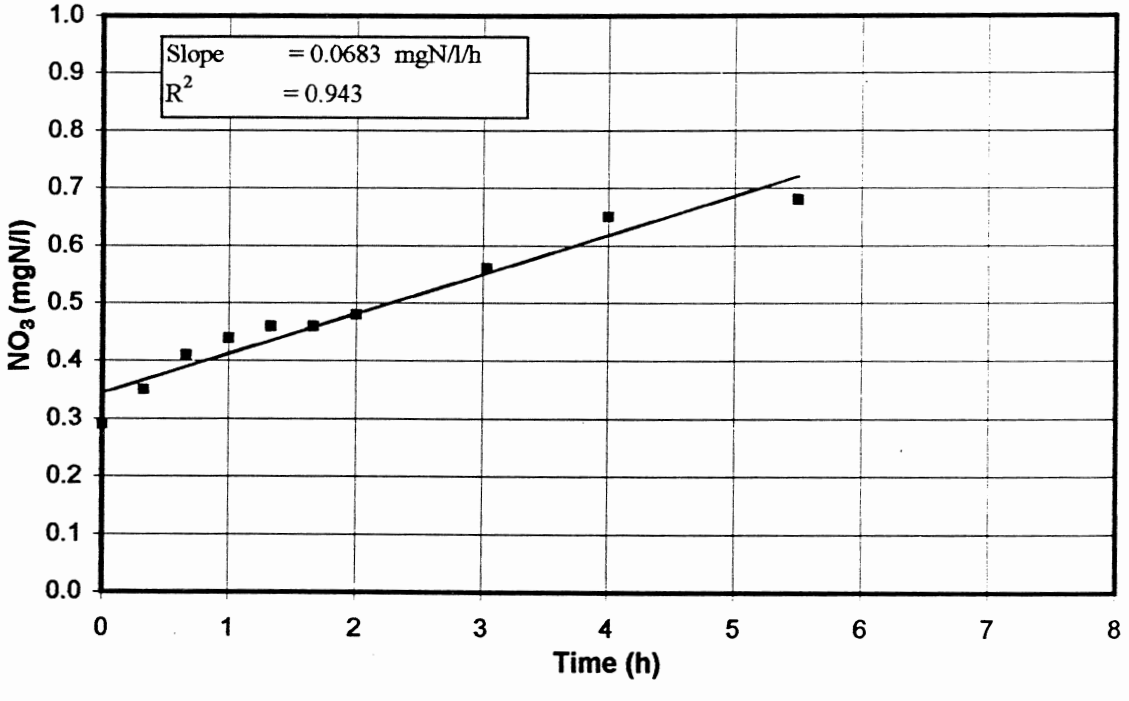
OUR-h Graph for wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test M5, 30-11, wastewater batch no. 13



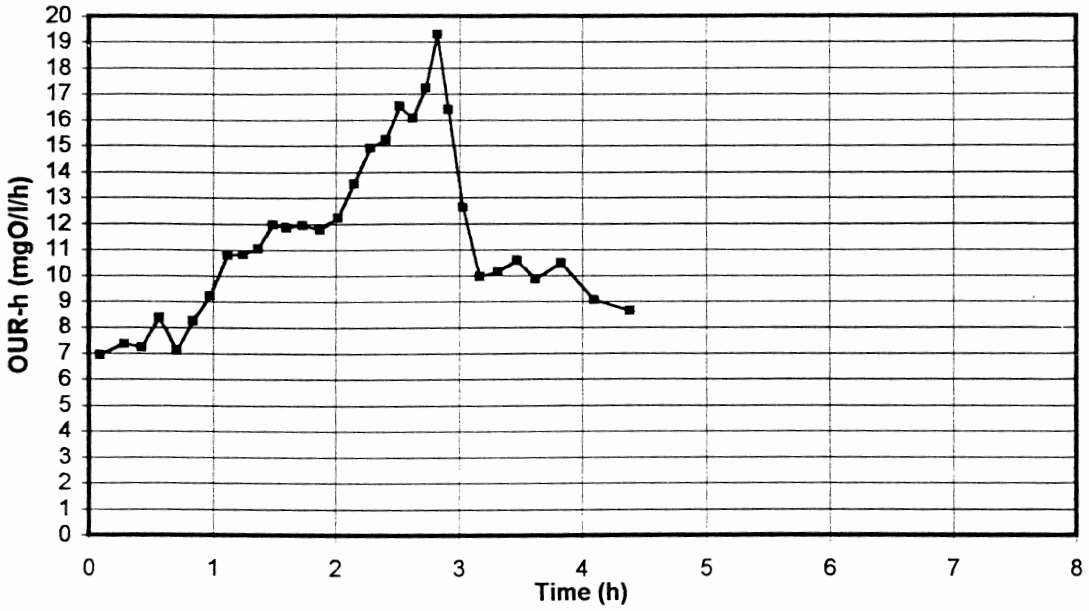
ℓn(OUR-h) graph for wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test M5, 30-11, waste water batch no.13



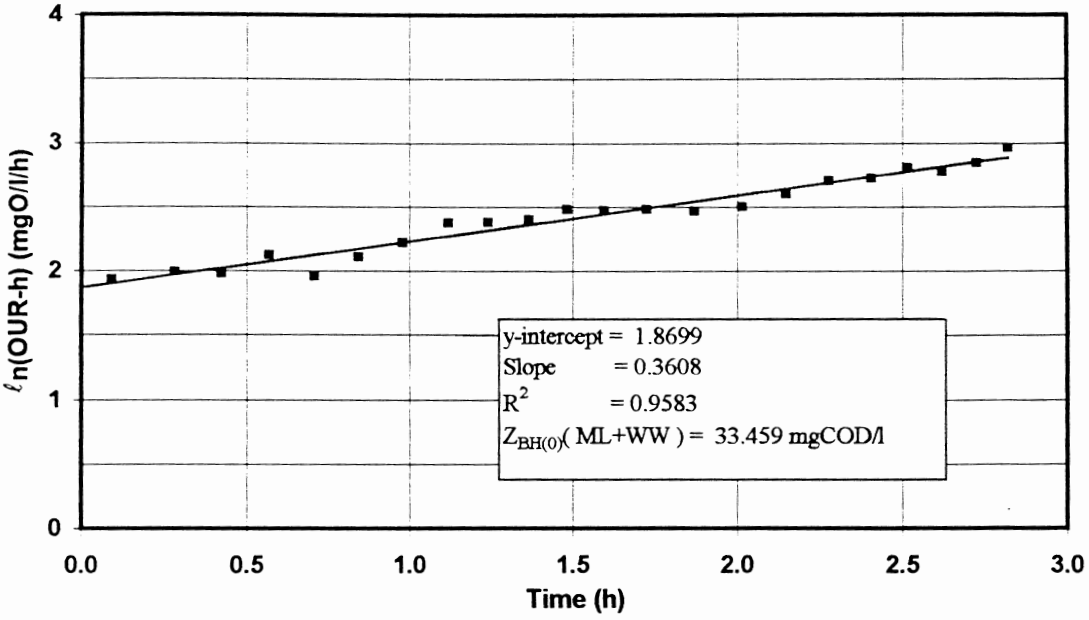
OUR Graph for wastewater (2.9ℓ) plus mixed liquor (0.1ℓ) batch test M6, 01-12, wastewater batch no. 13



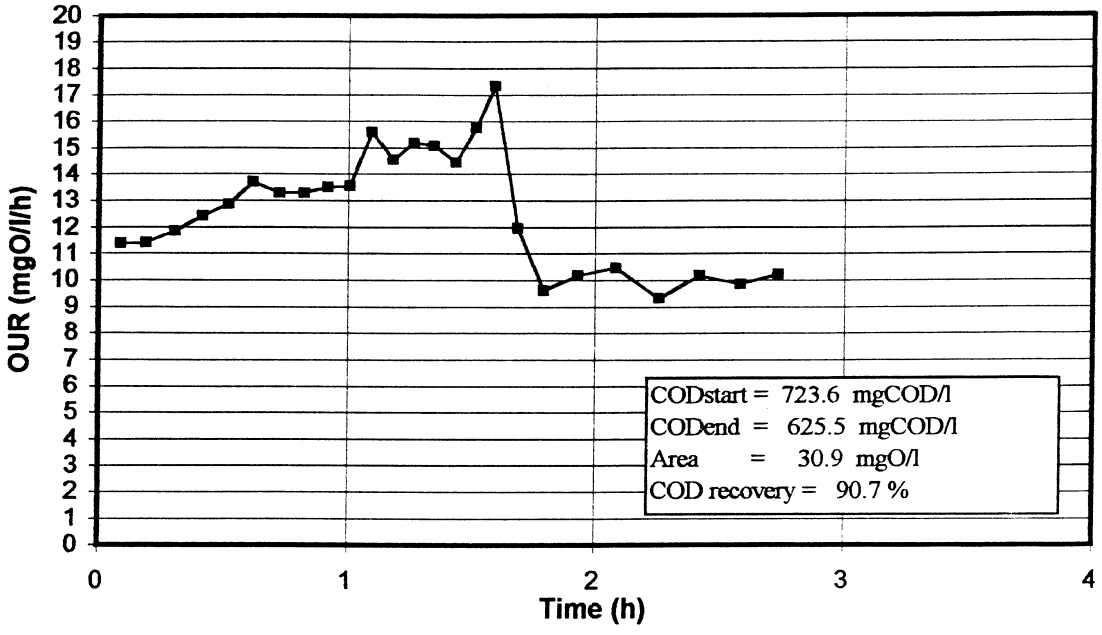
NO_3 Graph for wastewater (2.9ℓ) plus mixed liquor (0.1ℓ) batch test M6, 01-12, wastewater batch no. 13



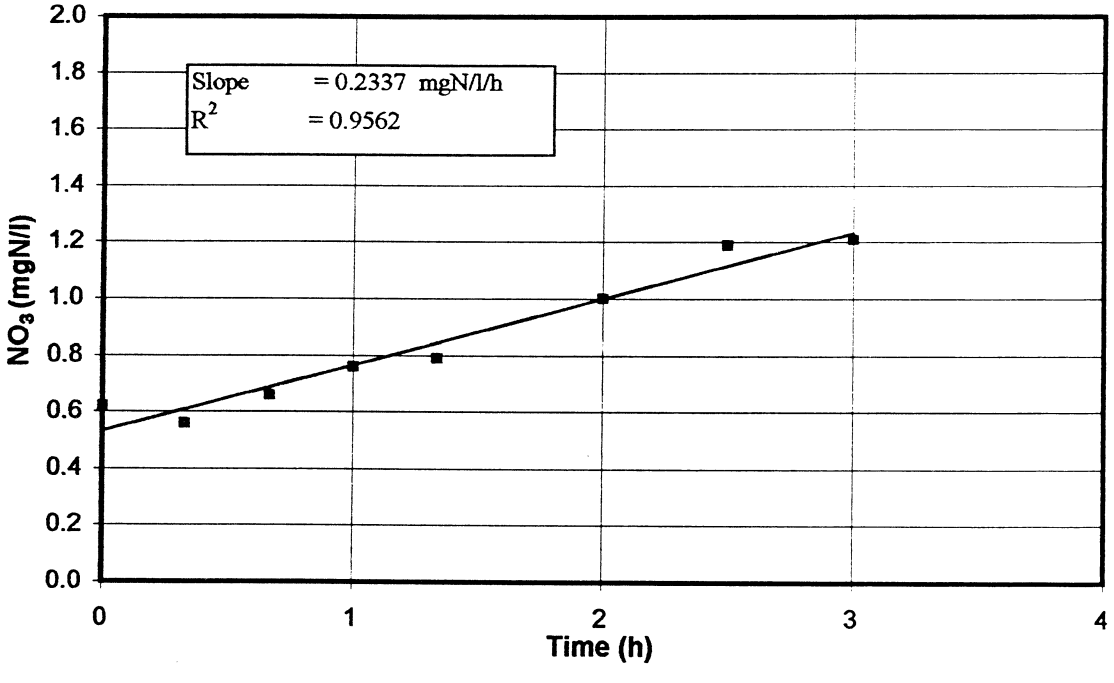
OUR-h Graph for wastewater (2.9ℓ) plus mixed liquor (0.1ℓ) batch test M6, 01-12, wastewater batch no. 13



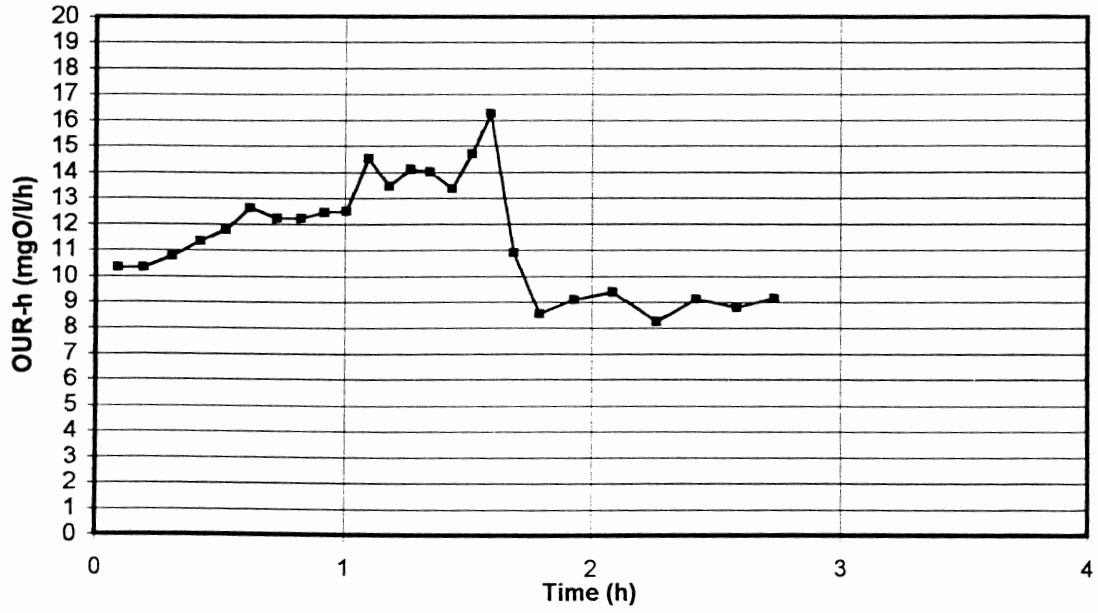
ℓn(OUR-h) graph for wastewater (2.9ℓ) plus mixed liquor (0.1ℓ) batch test M6, 01-12, wastewater batch no.13



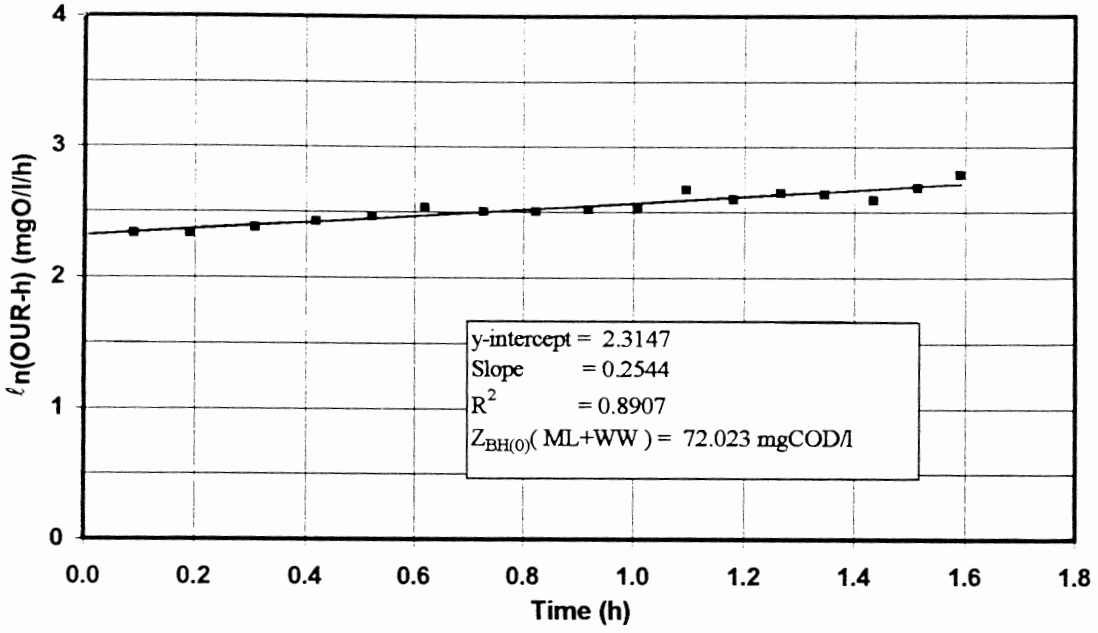
OUR Graph for wastewater (2.75ℓ) plus mixed liquor (0.25ℓ) batch test M7 , 03-12 , wastewater batch no. 13



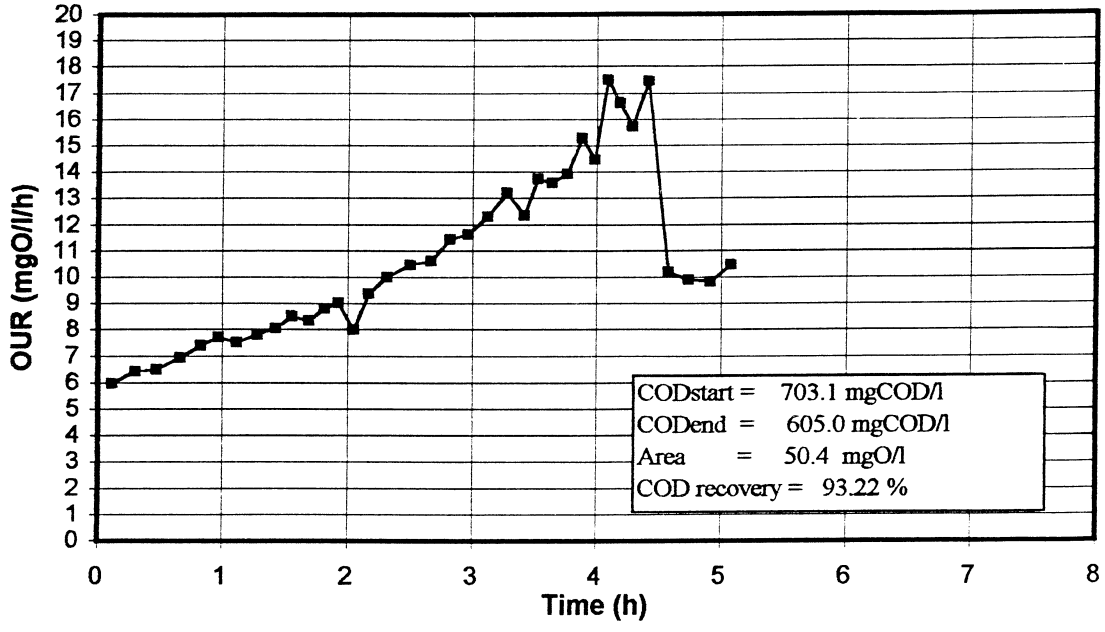
NO_3 Graph for wastewater (2.75ℓ) plus mixed liquor (0.25ℓ) batch test M7 , 03-12 , wastewater batch no. 13



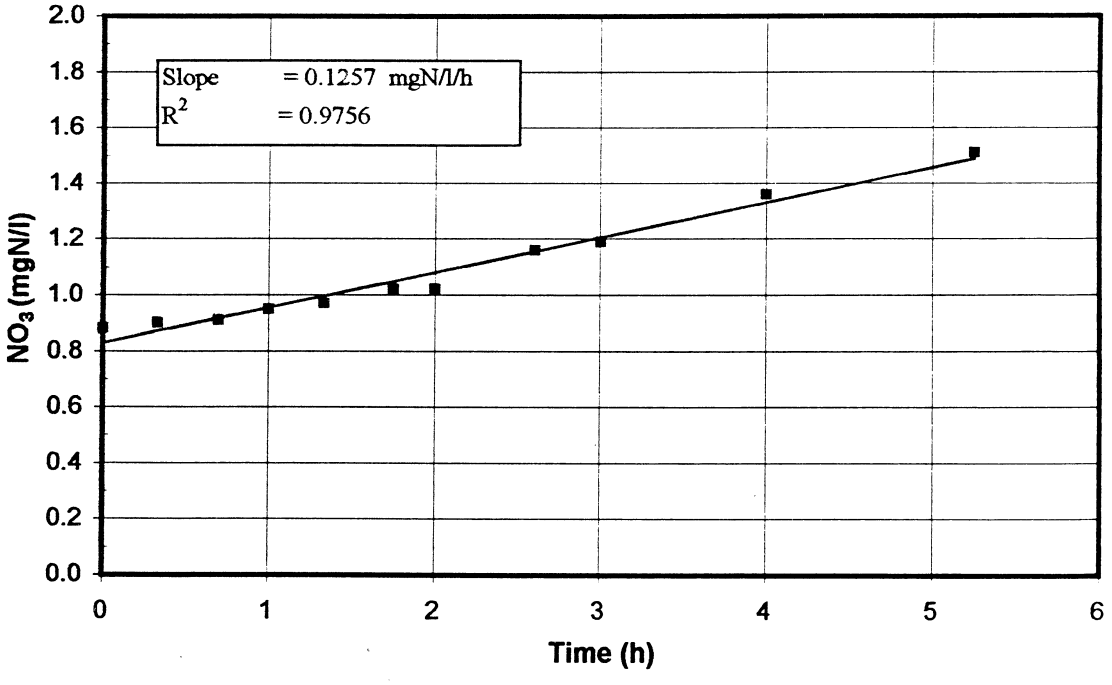
OUR-h Graph for wastewater (2.75ℓ) plus mixed liquor (0.25ℓ) batch test M7 , 03-12 , wastewater batch no. 13



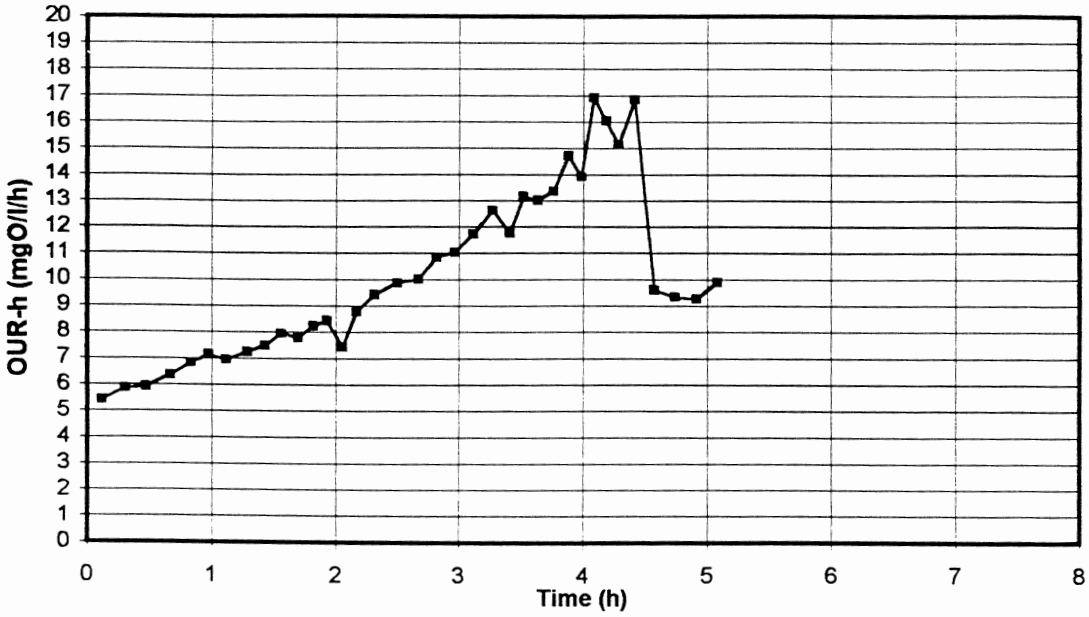
ℓn(OUR-h) graph for wastewater(2.75ℓ) plus mixed liquor (0.25ℓ) batch test M7, 03-12, wastewater batch no.13



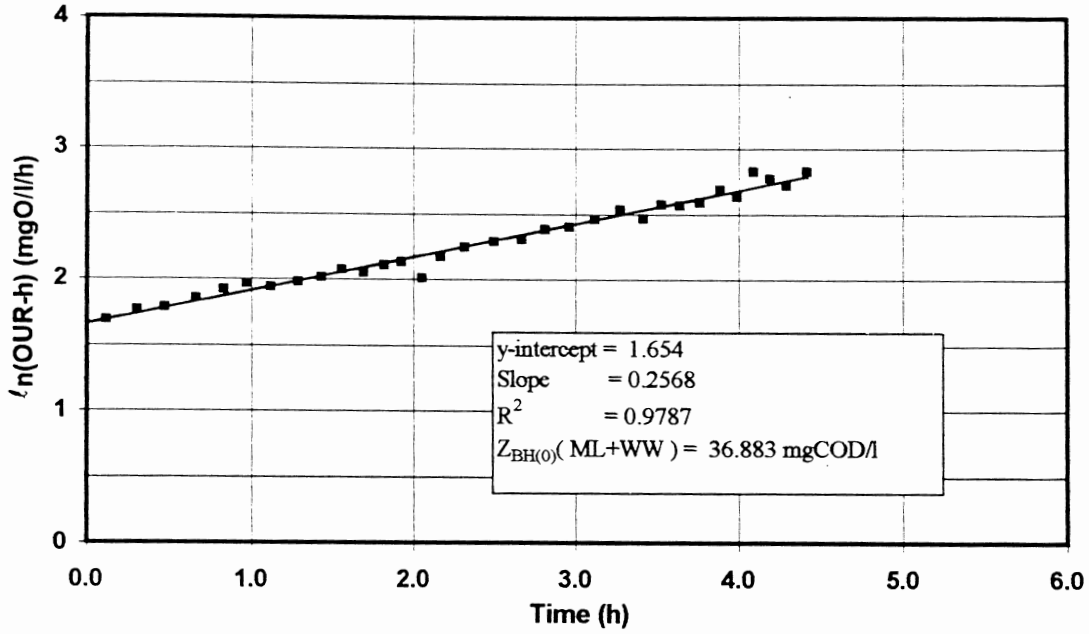
OUR Graph for wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test M8 , 08-12 , wastewater batch no. 14



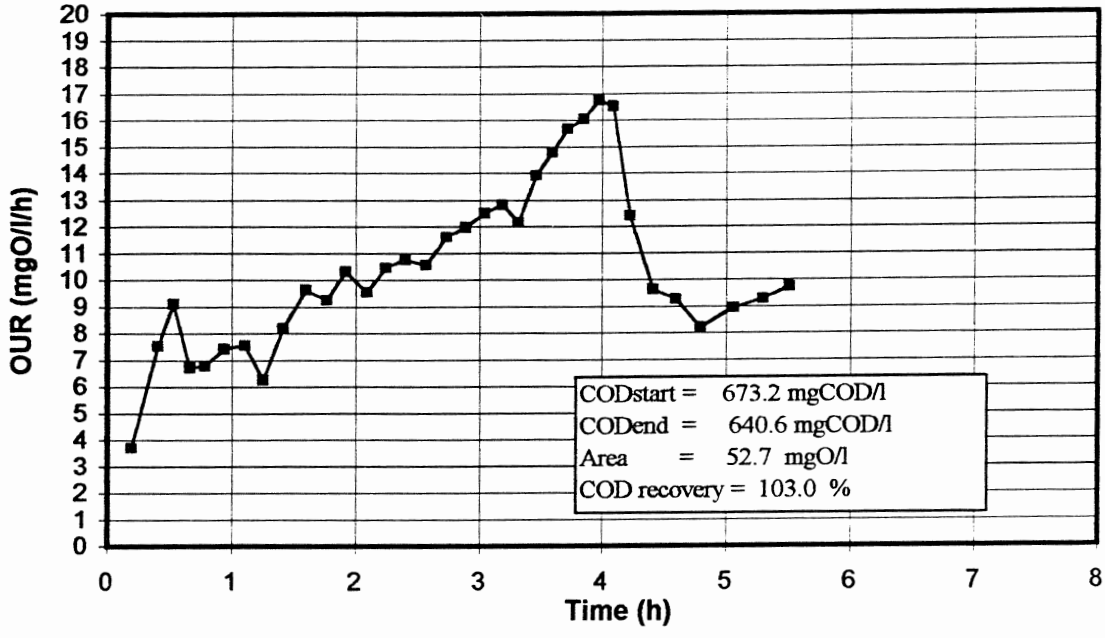
NO_3 Graph for wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test M8 , 08-12 , wastewater batch no. 14



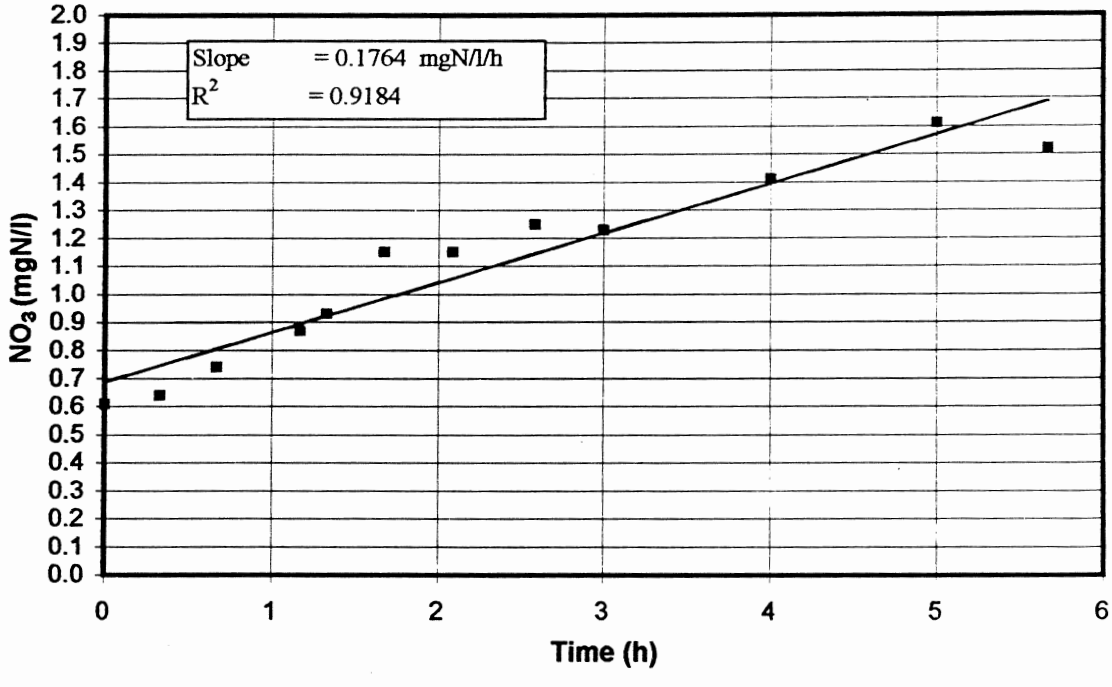
OUR-h Graph for wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test M8 , 08-12 , wastewater batch no. 14



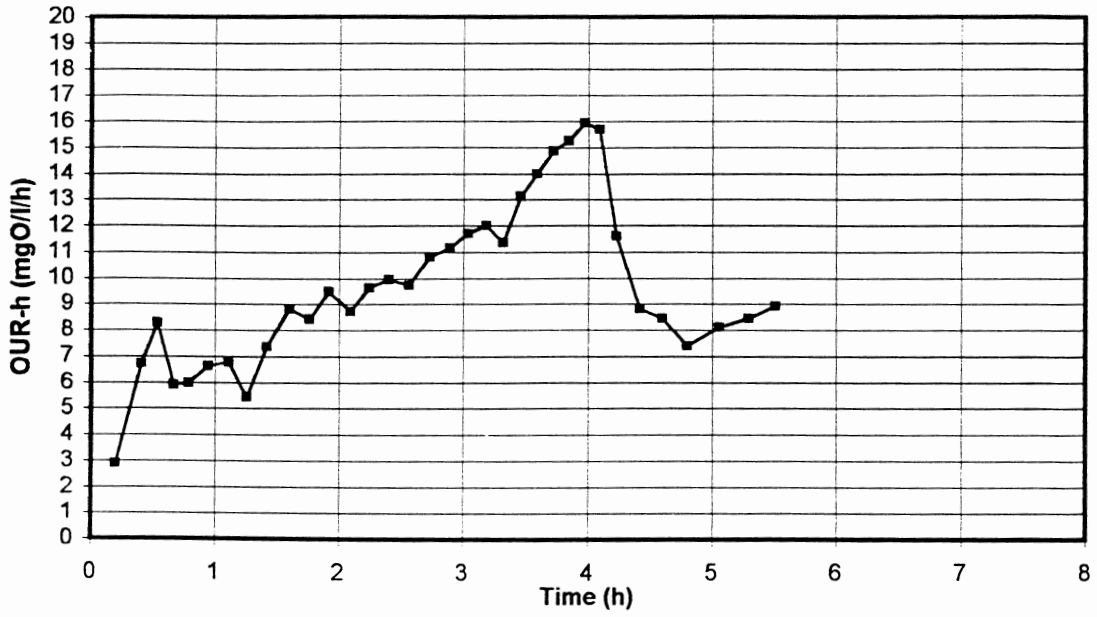
ℓn(OUR-h) graph for wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test M8 , 08-12 , waste water batch no.14



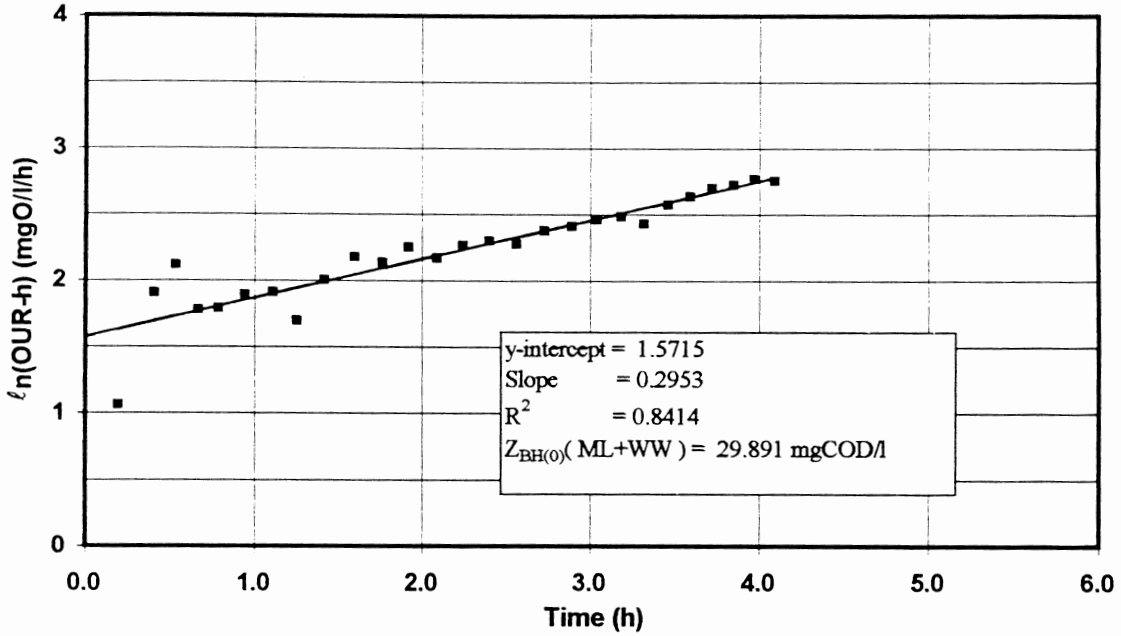
OUR Graph for wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test M9 , 07-02 , wastewater batch no. 18



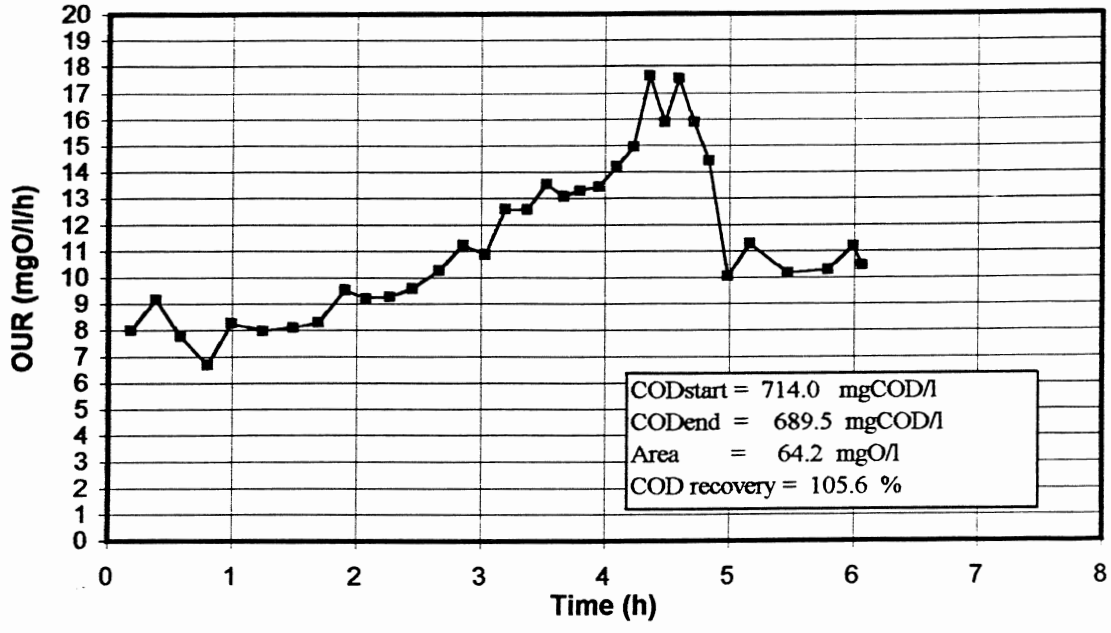
NO_3 Graph for wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test M9 , 07-02 , wastewater batch no. 18



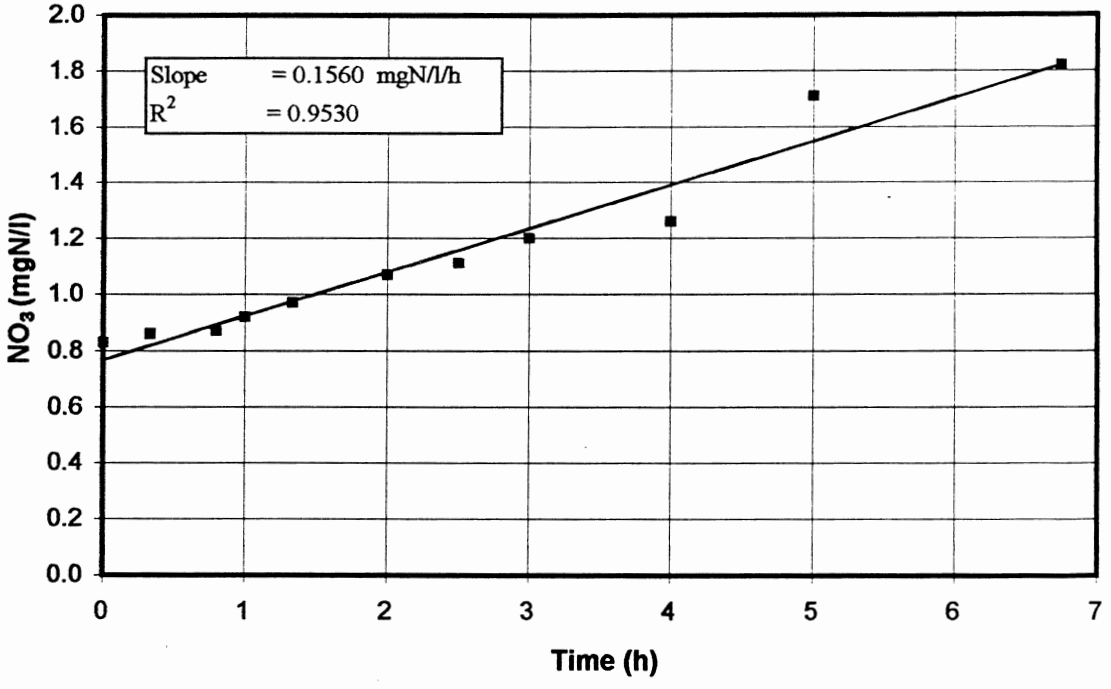
OUR-h Graph for wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test M9 , 07-02 , wastewater batch no. 18



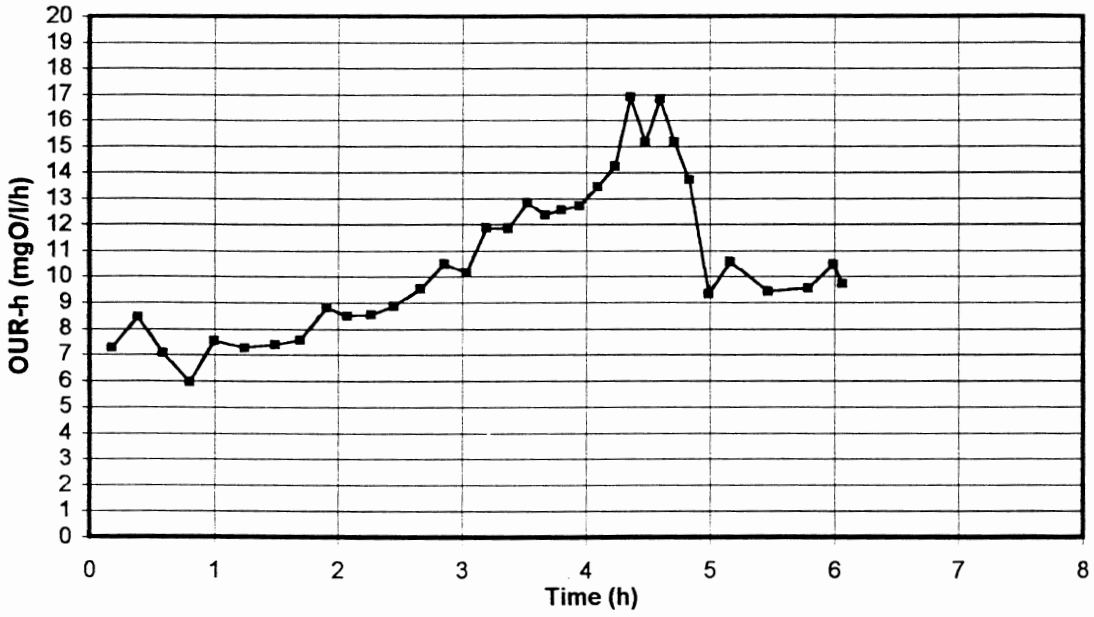
ℓn(OUR-h) graph for wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test M9 , 07-02 , wastewater batch no.18



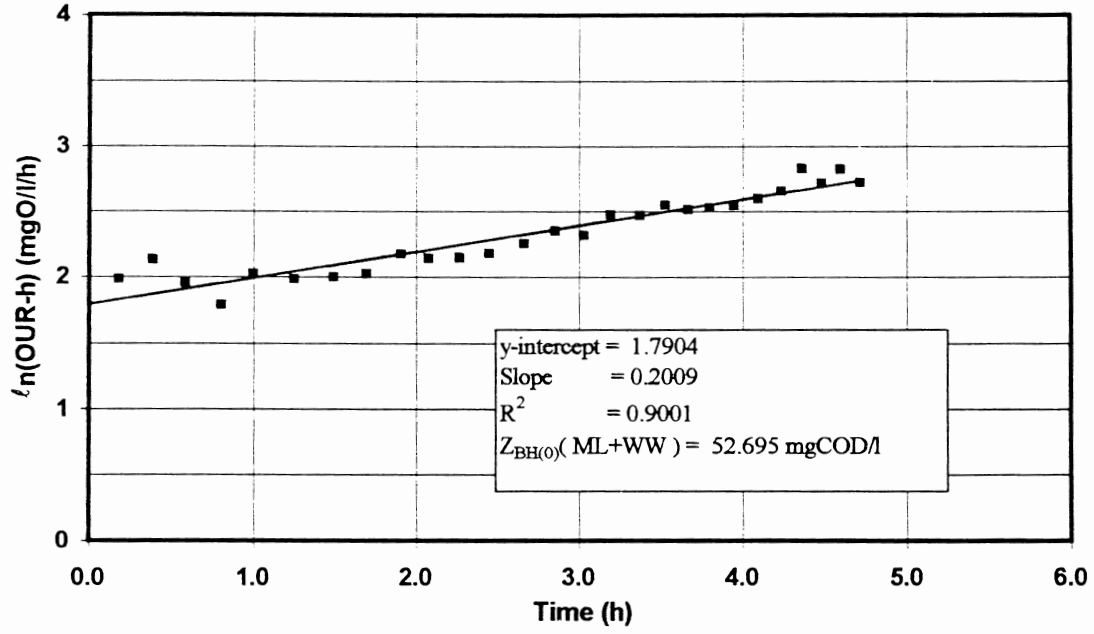
OUR Graph for wastewater (2.75ℓ) plus mixed liquor (0.25ℓ) batch test M10 , 08-02 , wastewater batch no. 18



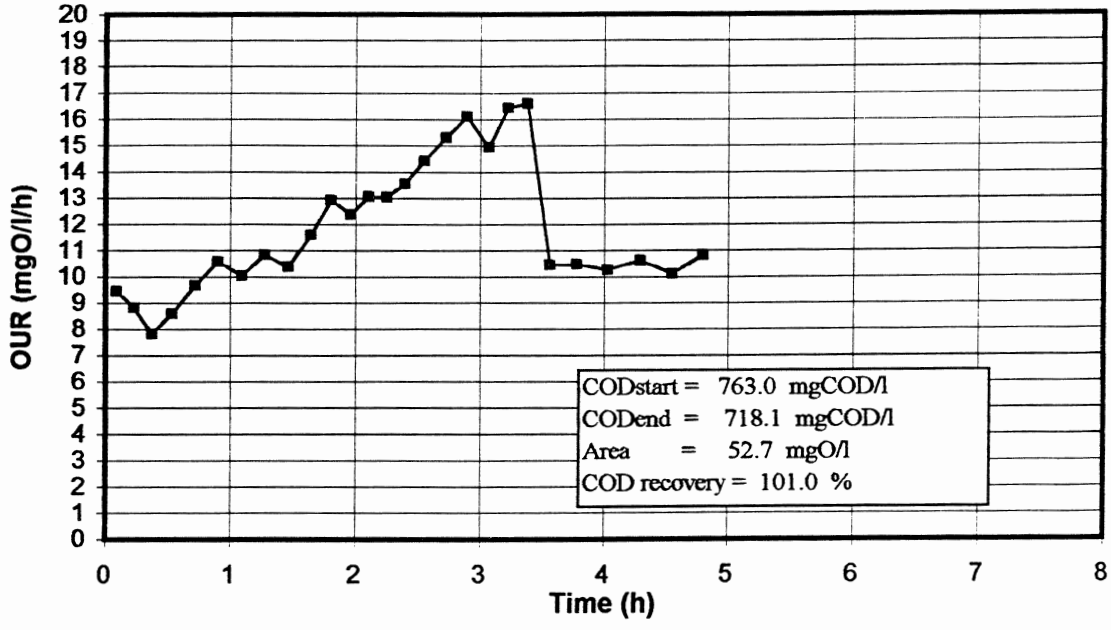
NO_3 Graph for wastewater (2.75ℓ) plus mixed liquor (0.25ℓ) batch test M10 , 08-02 , wastewater batch no. 18



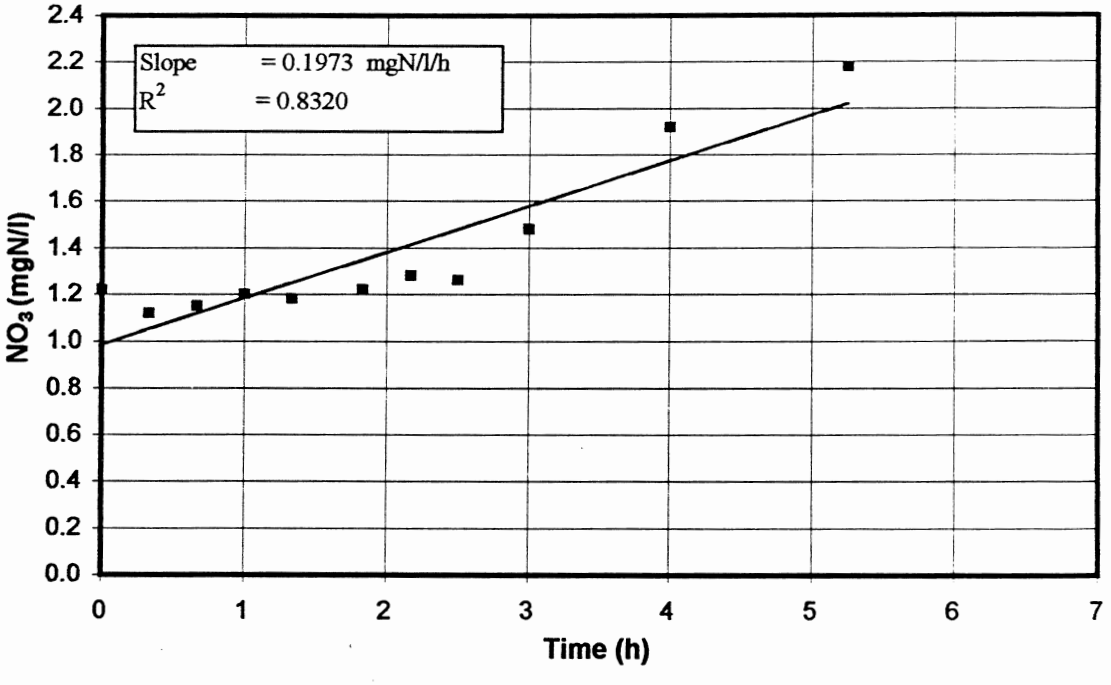
OUR-h Graph for wastewater (2.75ℓ) plus mixed liquor (0.25ℓ) batch test M10 , 08-02 , wastewater batch no. 18



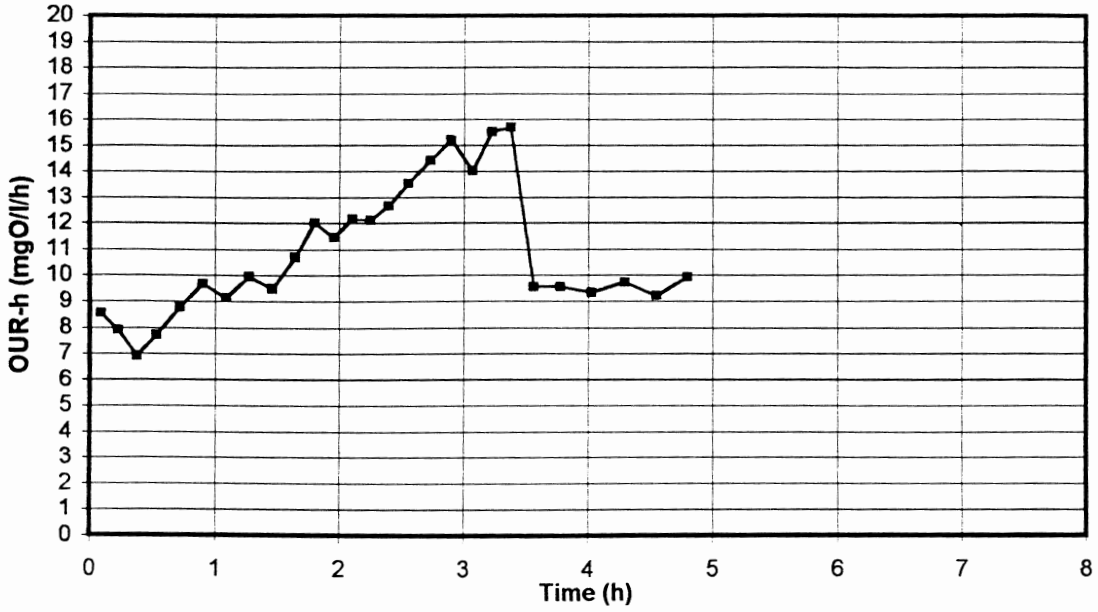
ℓn(OUR-h) graph for wastewater(2.75ℓ) plus mixed liquor(0.25ℓ) batch test M10,08-02, wastewater batch no.18



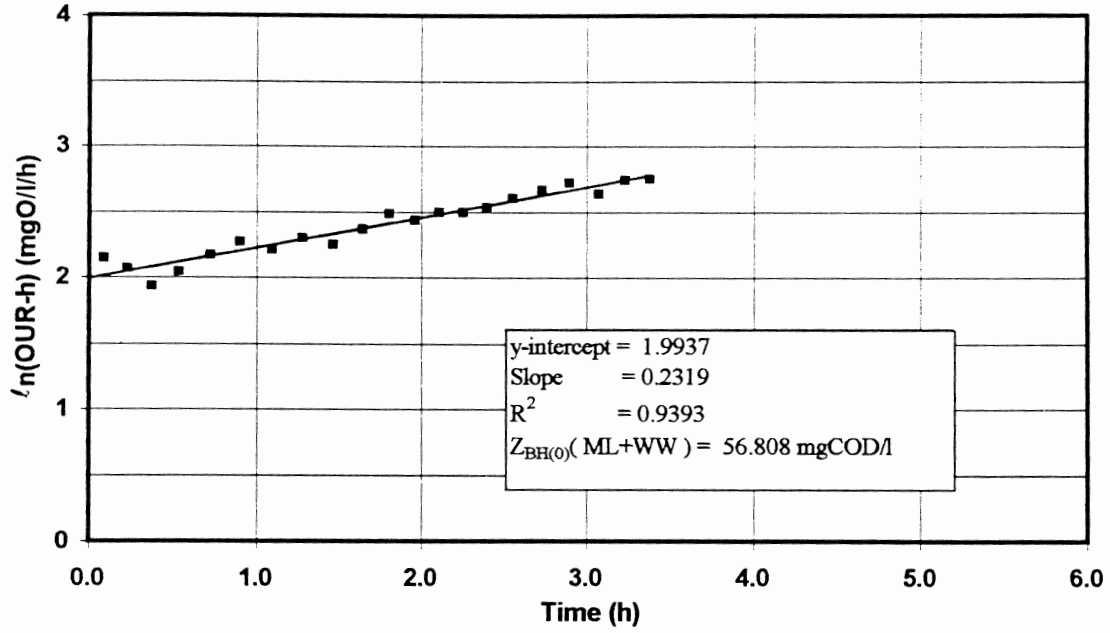
OUR Graph for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M11 , 09-02 , wastewater batch no. 18



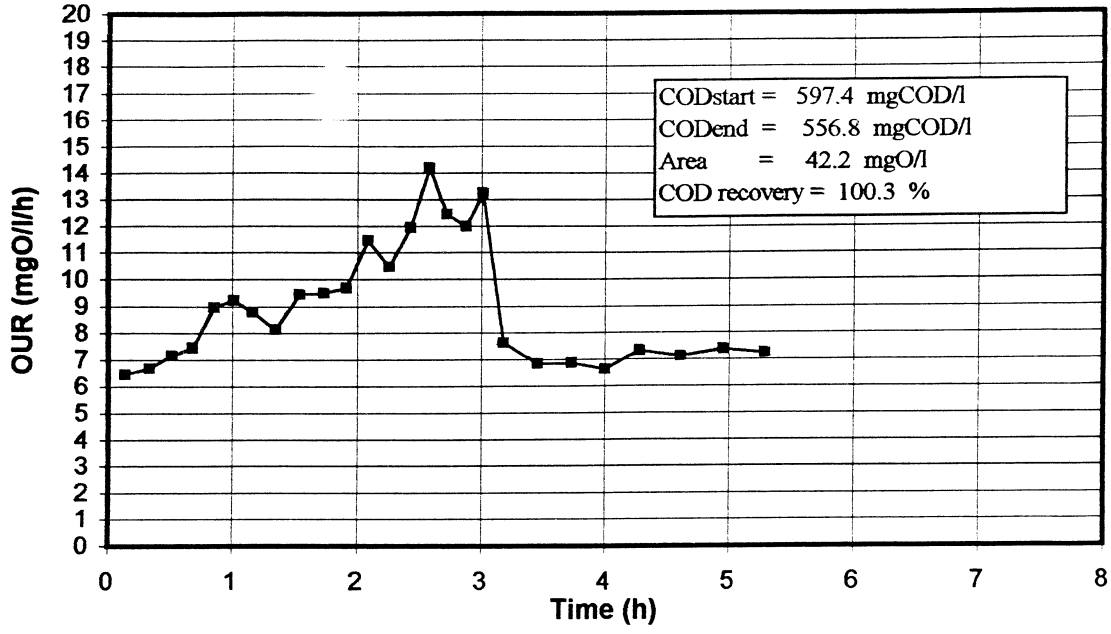
NO_3 Graph for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M11 , 09-02 , wastewater batch no. 18



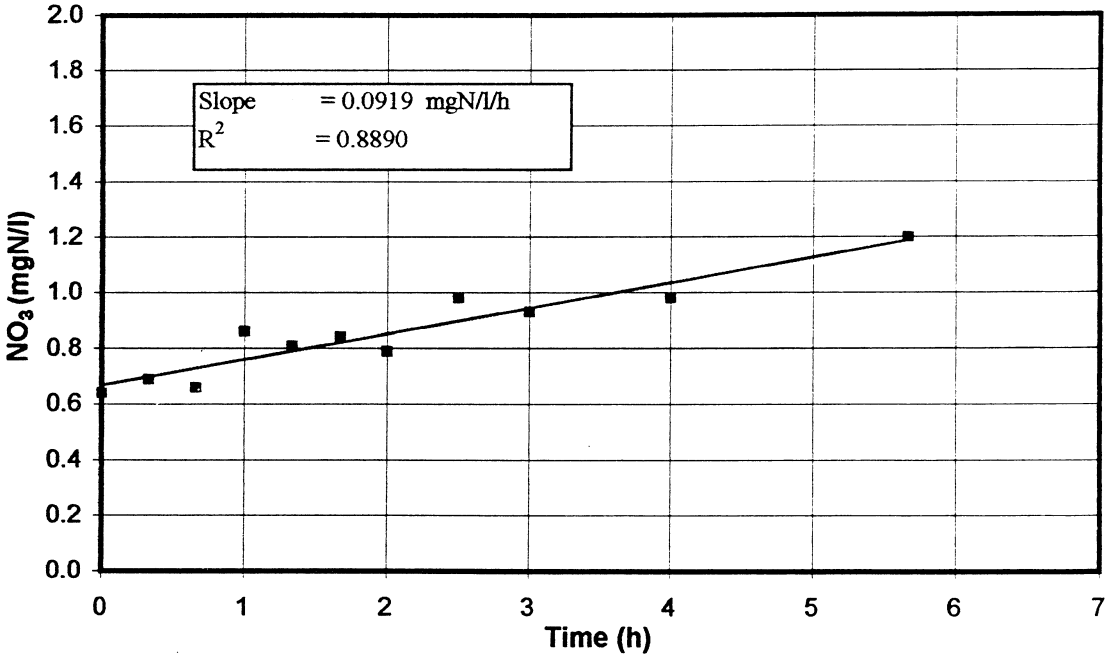
OUR-h Graph for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M11 , 09-02 , wastewater batch no. 18



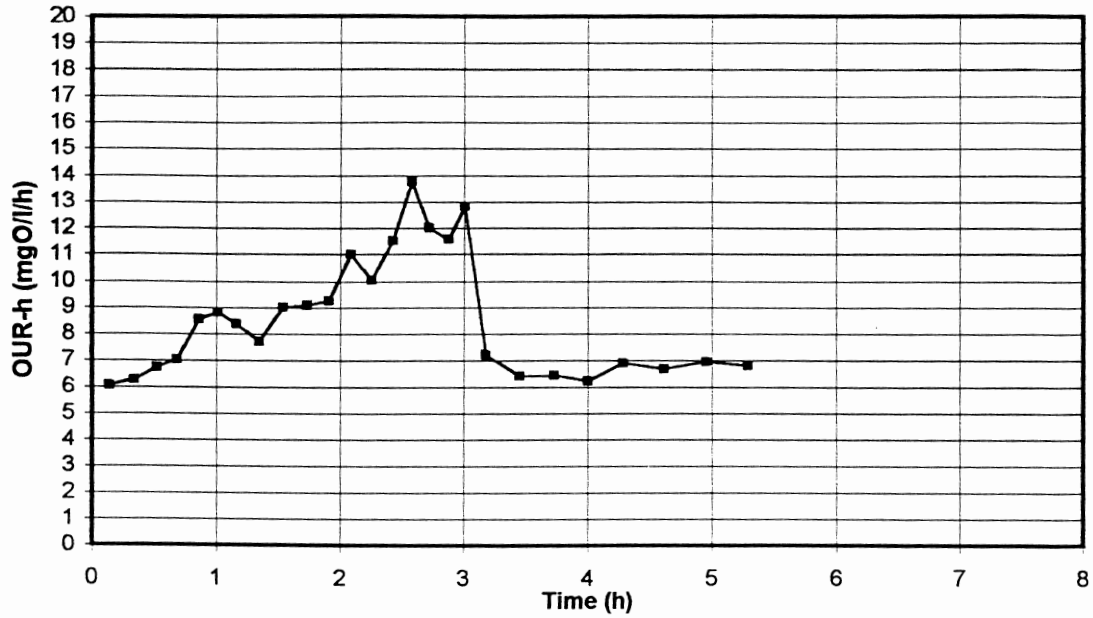
ℓn(OUR-h) graph for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M11, 09-02, waste water batch no.18



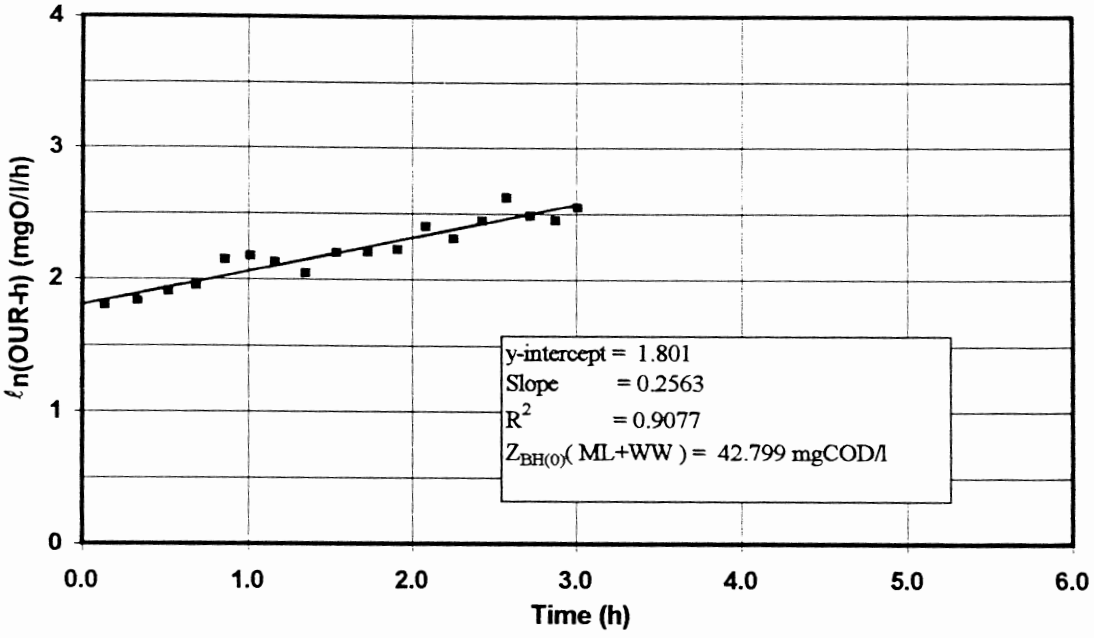
OUR Graph for wastewater (2.85ℓ) plus mixed liquor (0.15ℓ) batch test M12 , 10-02 , wastewater batch no. 18



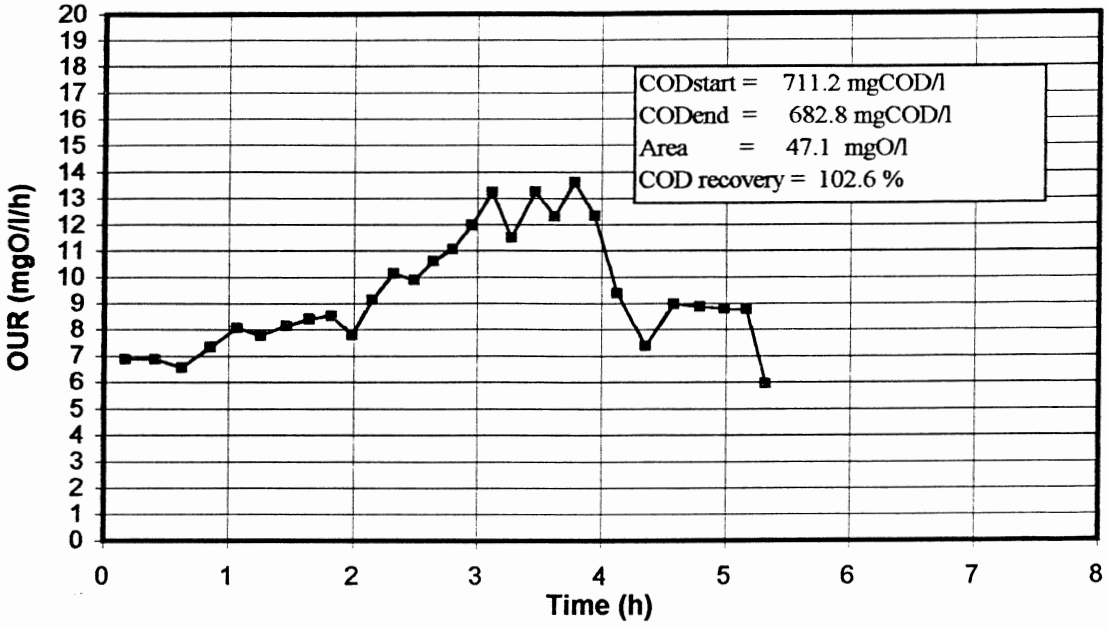
NO_3 Graph for wastewater (2.85ℓ) plus mixed liquor (0.15ℓ) batch test M12 , 10-02 , wastewater batch no. 18



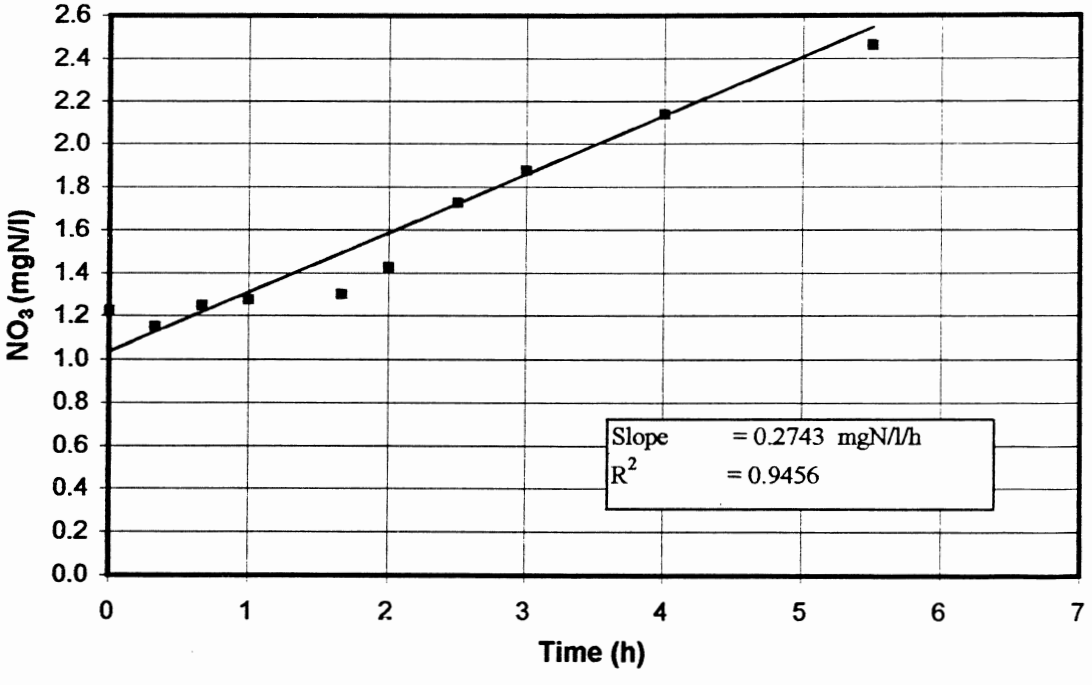
OUR-h Graph for wastewater (2.85ℓ) plus mixed liquor (0.15ℓ) batch test M12, 10-02, wastewater batch no. 18



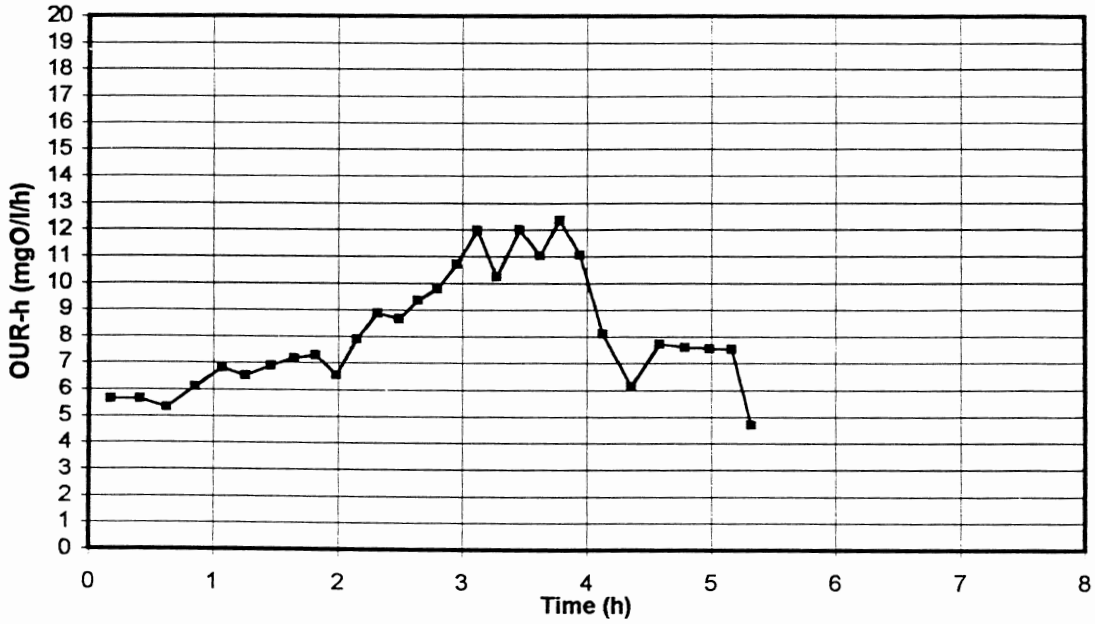
ℓn(OUR-h) graph for wastewater(2.85ℓ) plus mixed liquor(0.15ℓ) batch test M12,10-02, waste water batch no.18



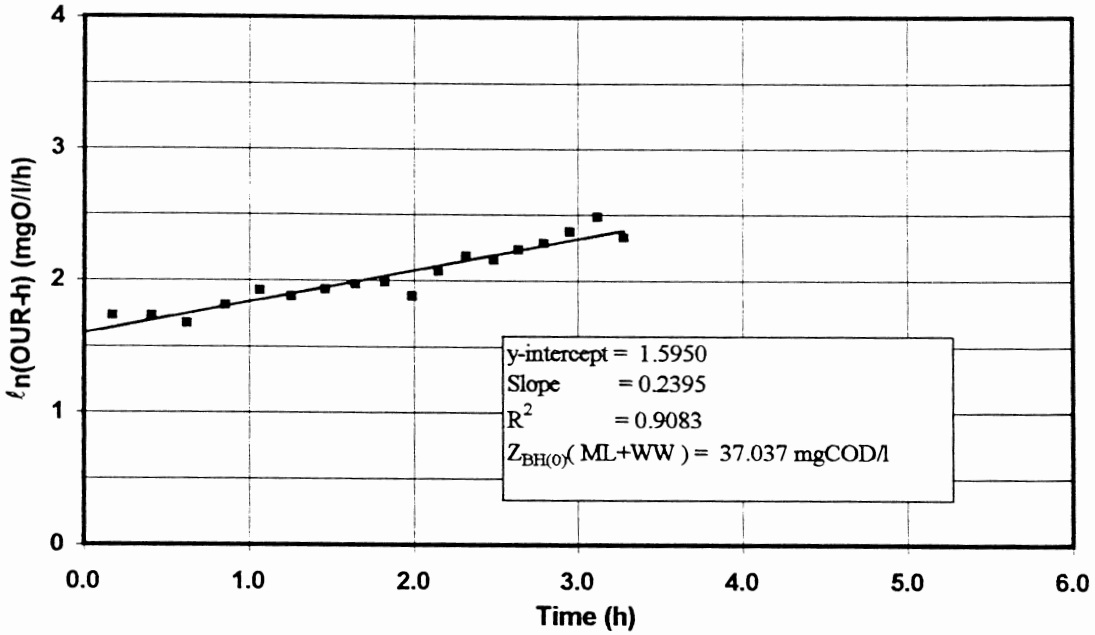
OUR Graph for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M13 , 15-02 , wastewater batch no. 19



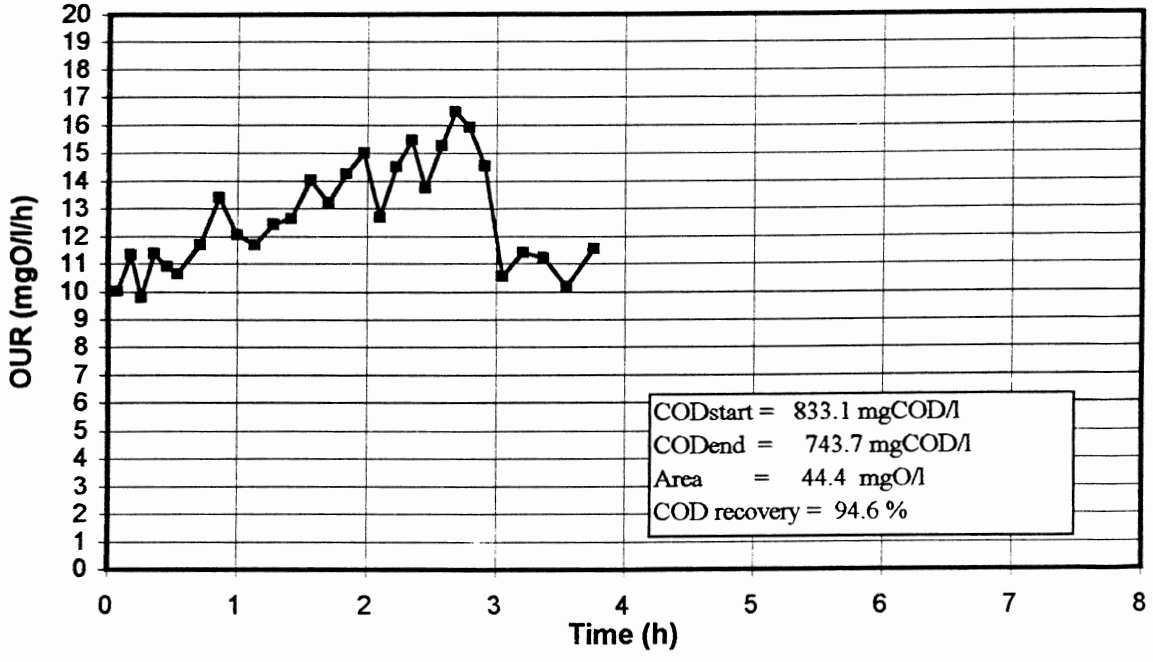
NO_3 Graph for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M13 , 15-02 , wastewater batch no. 19



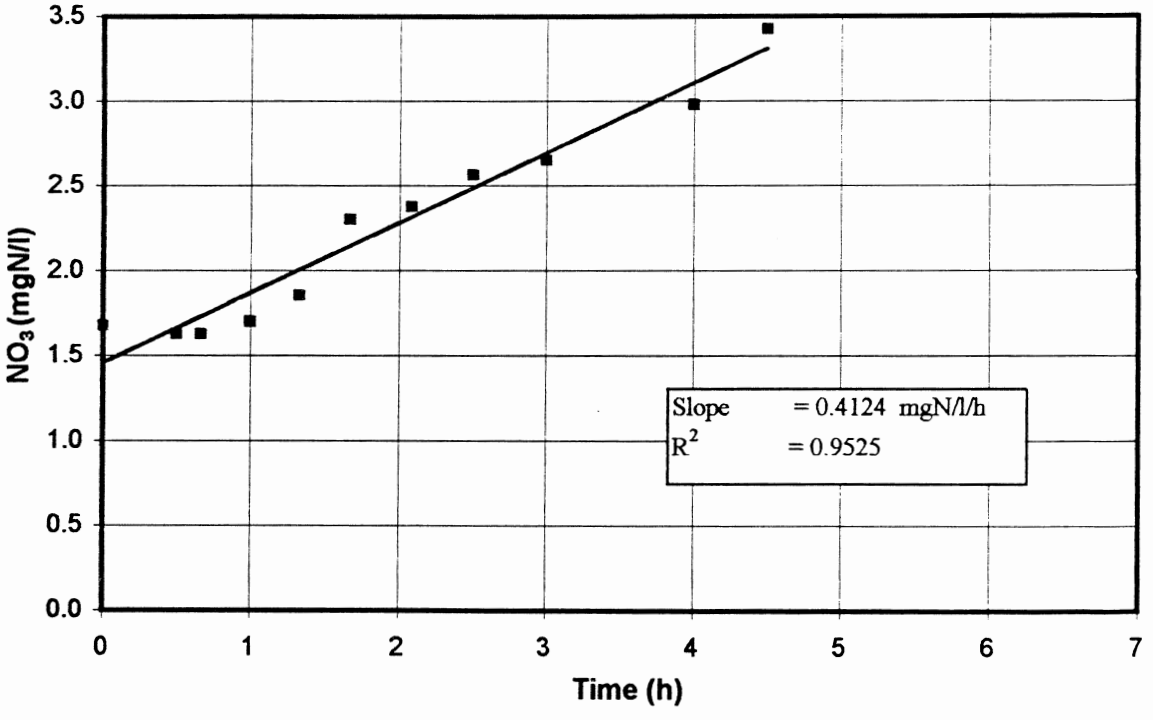
OUR-h Graph for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M13 , 15-02 , wastewater batch no. 19



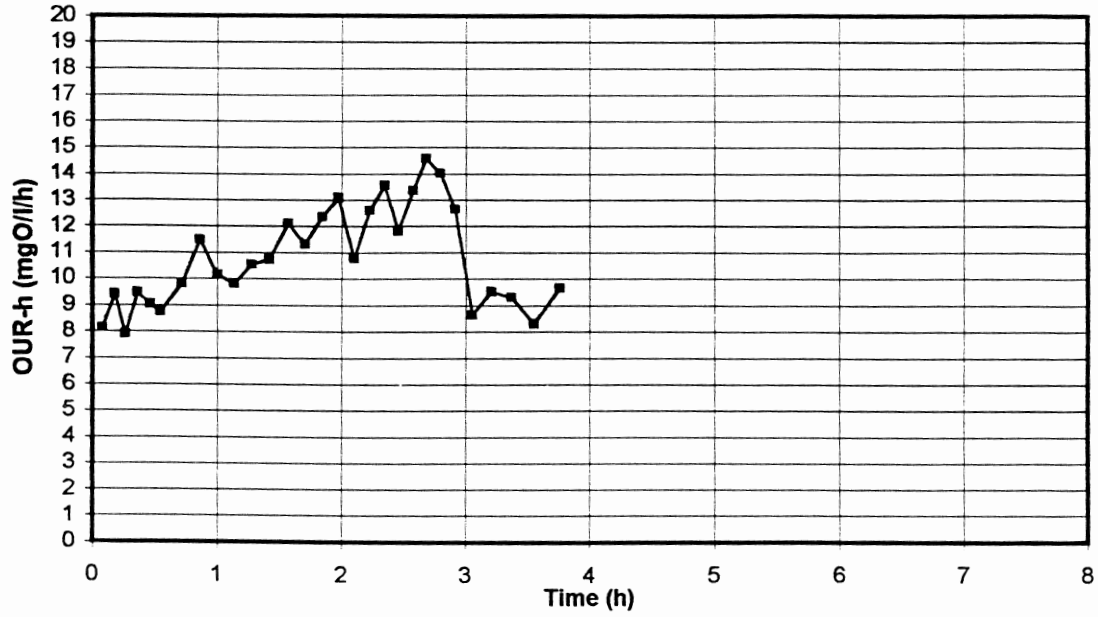
ℓn(OUR-h) graph for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M13 , 15-02, waste water batch no.19



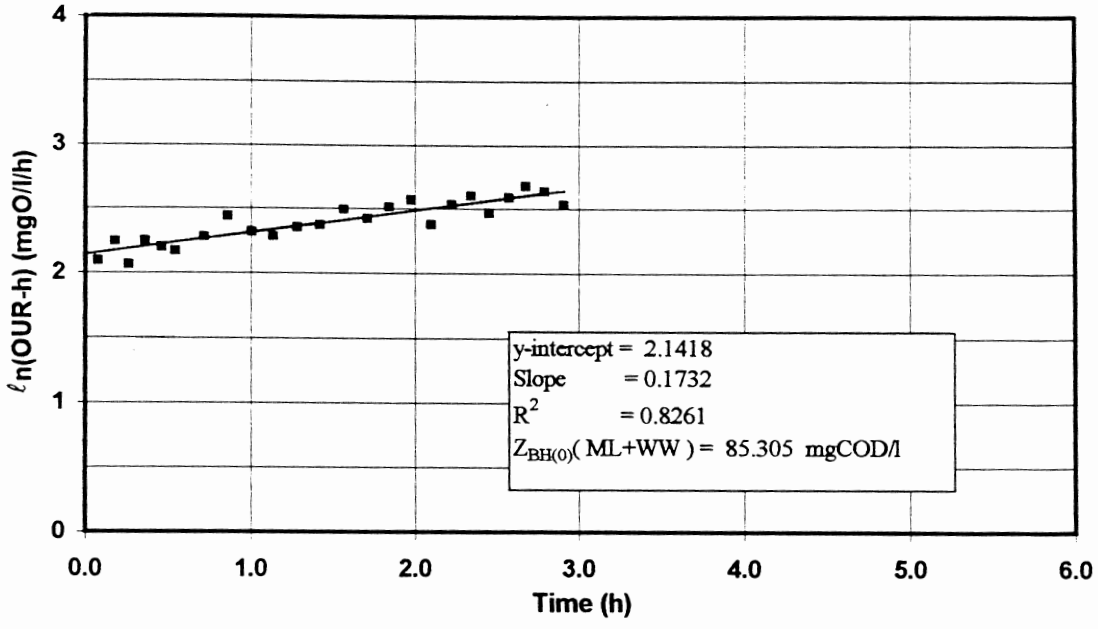
OUR Graph for wastewater (2.65ℓ) plus mixed liquor (0.35ℓ) batch test M14 , 16-02 , wastewater batch no. 19



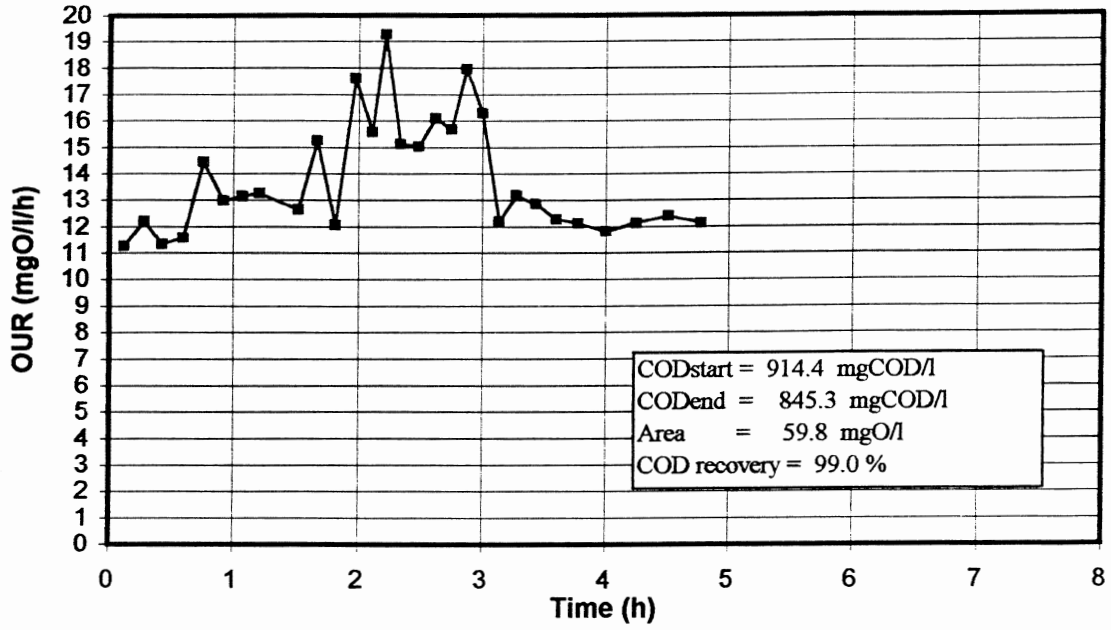
NO_3 Graph for wastewater (2.65ℓ) plus mixed liquor (0.35ℓ) batch test M14, 16-02 , wastewater batch no. 19



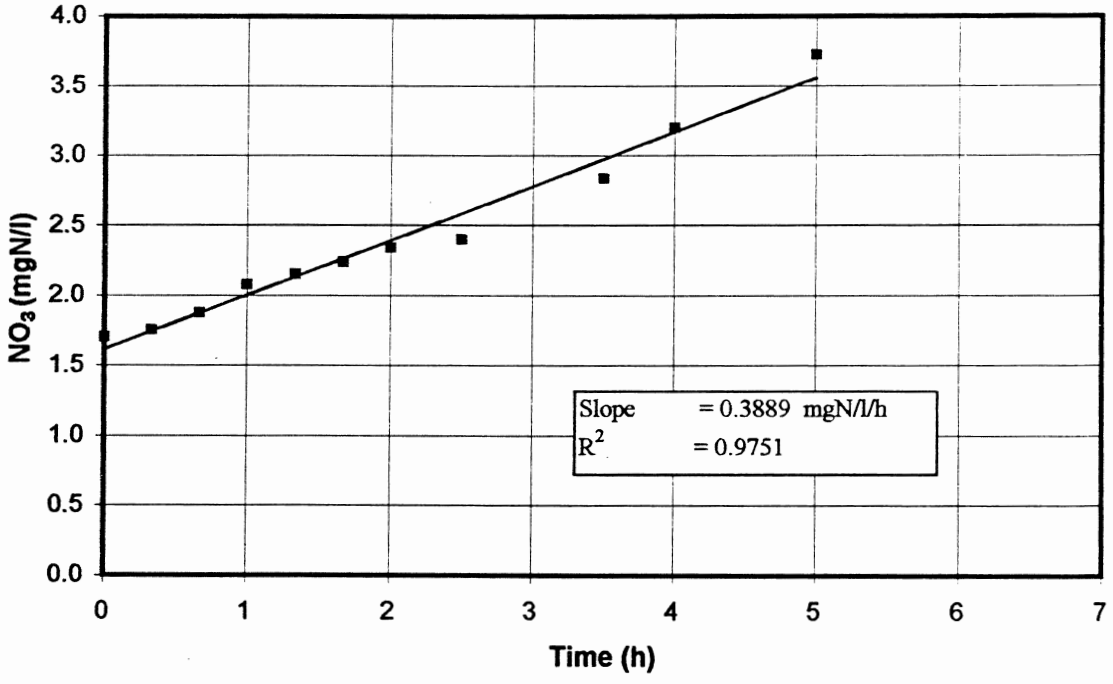
OUR-h Graph for wastewater (2.65ℓ) plus mixed liquor (0.35ℓ) batch test M14 , 16-02 , wastewater batch no. 19



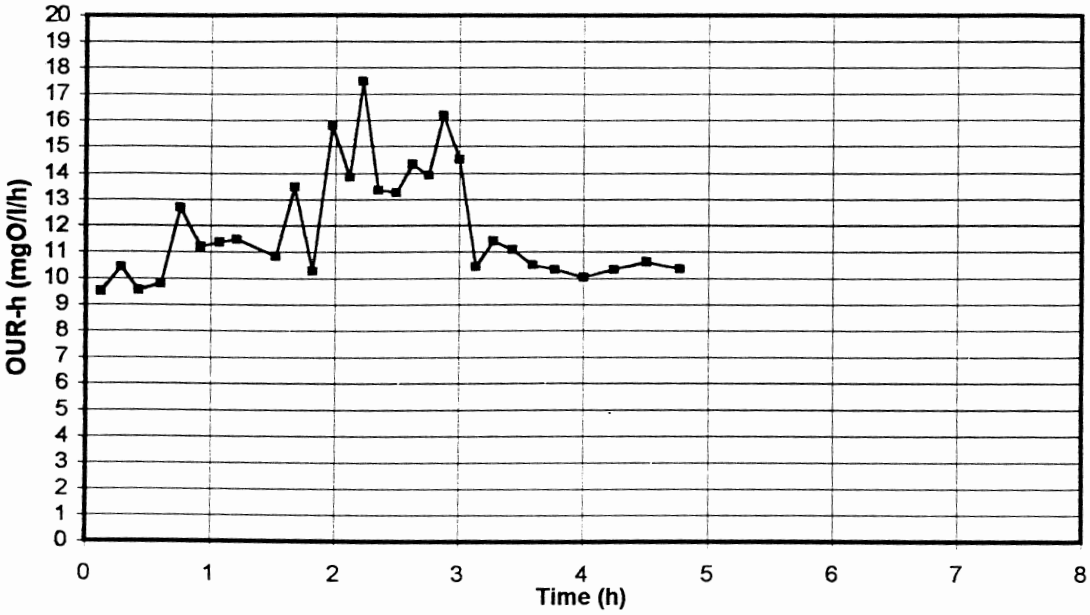
ℓn(OUR-h) graph for wastewater(2.65ℓ) plus mixed liquor(0.35ℓ) batch test M14,16-02, waste water batch no.19



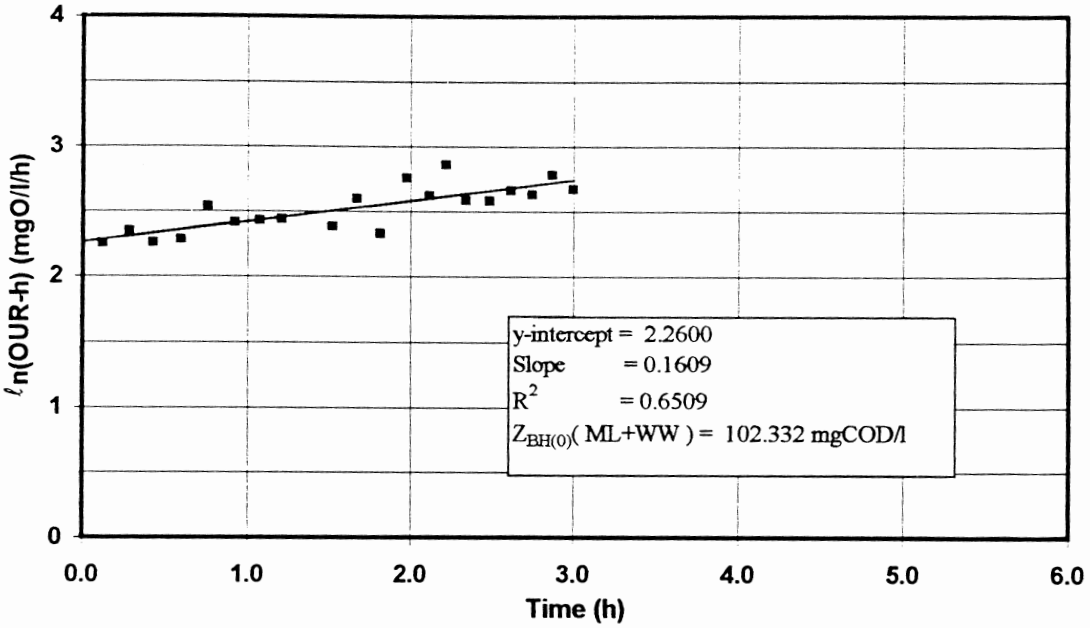
OUR Graph for wastewater (2.6ℓ) plus mixed liquor (0.4ℓ) batch test M15 , 17-02 , wastewater batch no. 19



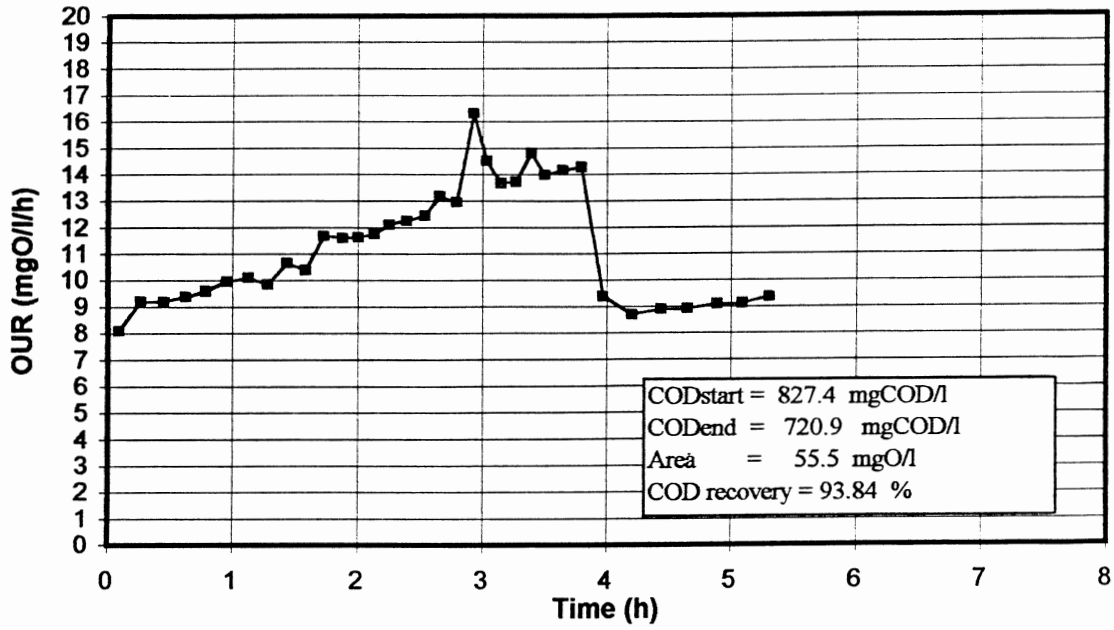
NO_3 Graph for wastewater (2.6ℓ) plus mixed liquor (0.4ℓ) batch test M15 , 17-02 , wastewater batch no. 19



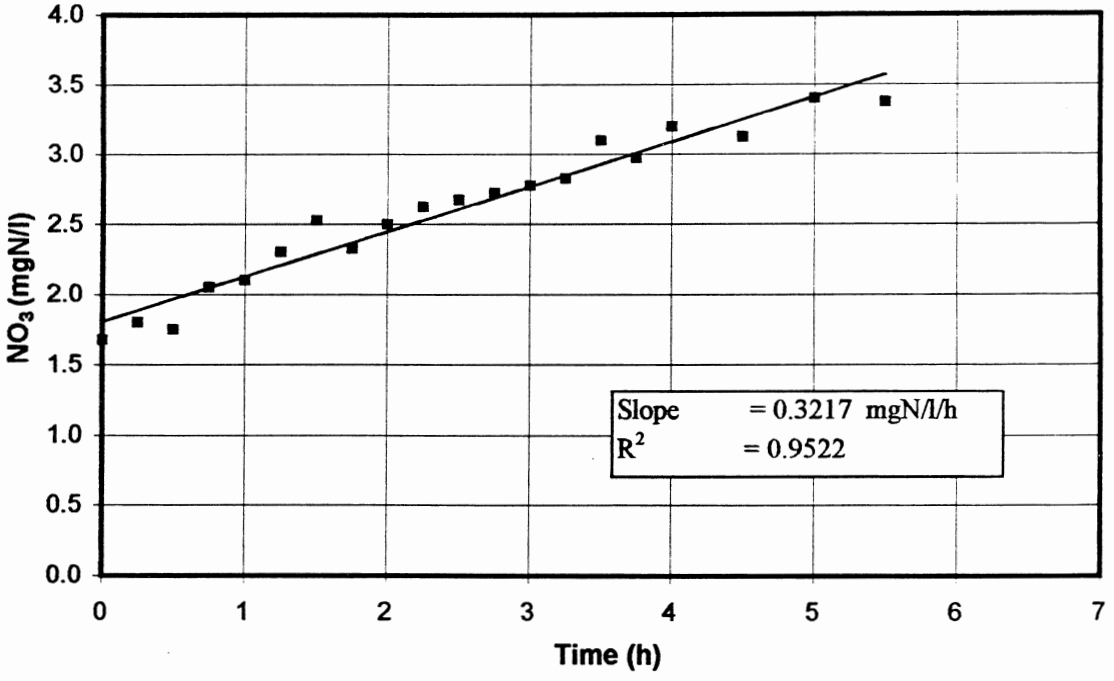
OUR-h Graph for wastewater (2.6ℓ) plus mixed liquor (0.4ℓ) batch test M15 , 17-02 ,
wastewater batch no. 19



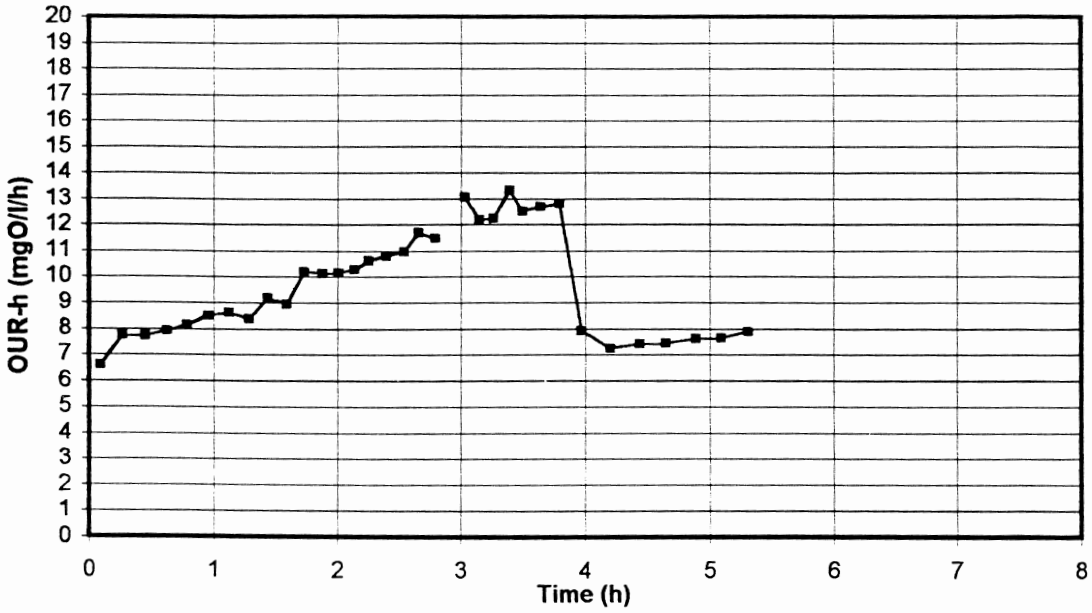
ℓn(OUR-h) graph for wastewater (2.6ℓ) plus mixed liquor (0.4ℓ) batch test M15 , 17-02,
waste water batch no.19



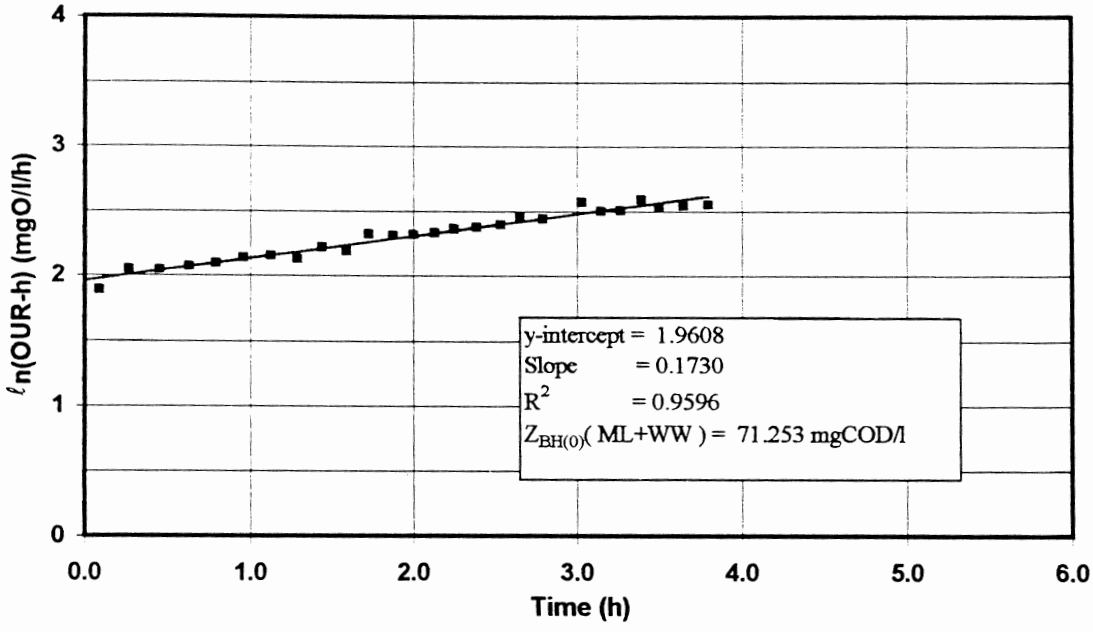
OUR Graph for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M16 , 22-02 , wastewater batch no. 19



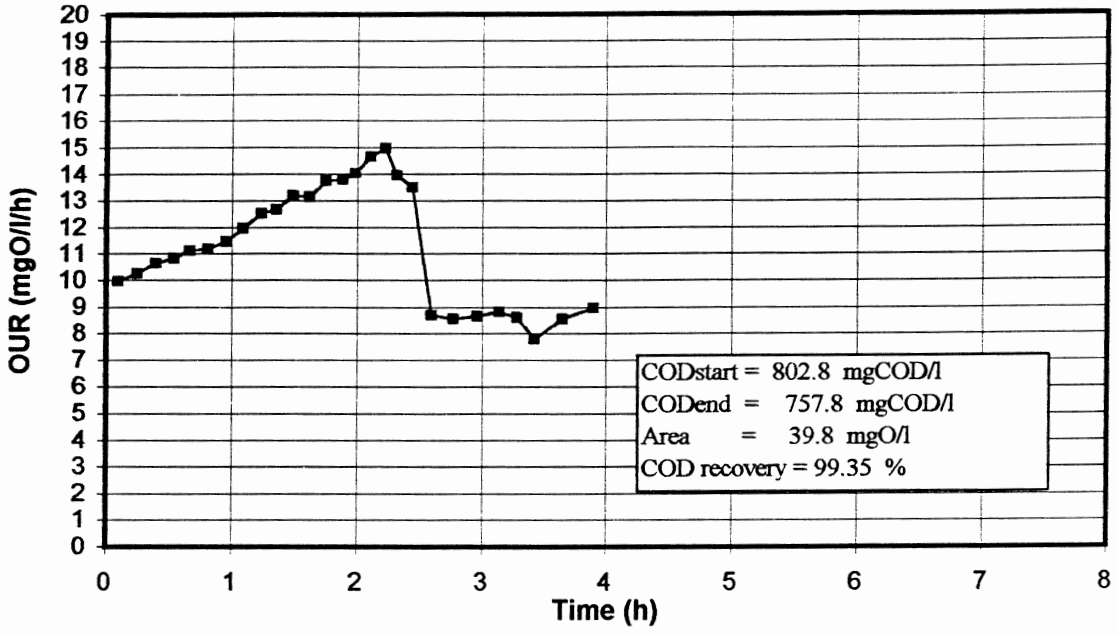
NO_3 Graph for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M16 , 22-02 , wastewater batch no. 19



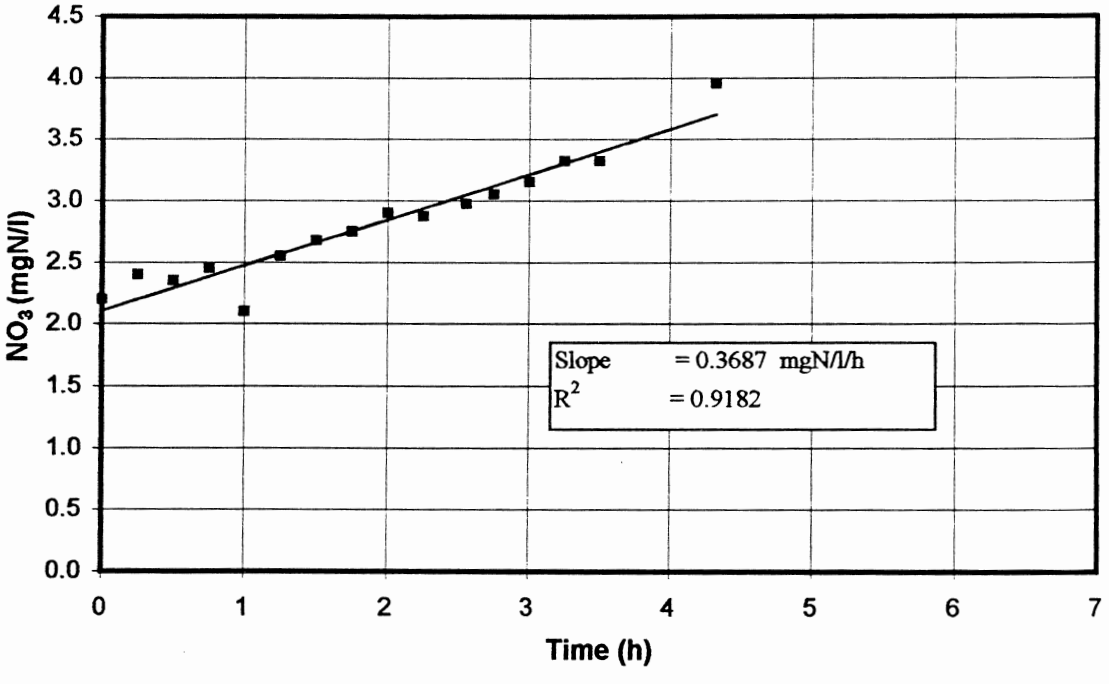
OUR-h Graph for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M16 , 22-02 , wastewater batch no. 19



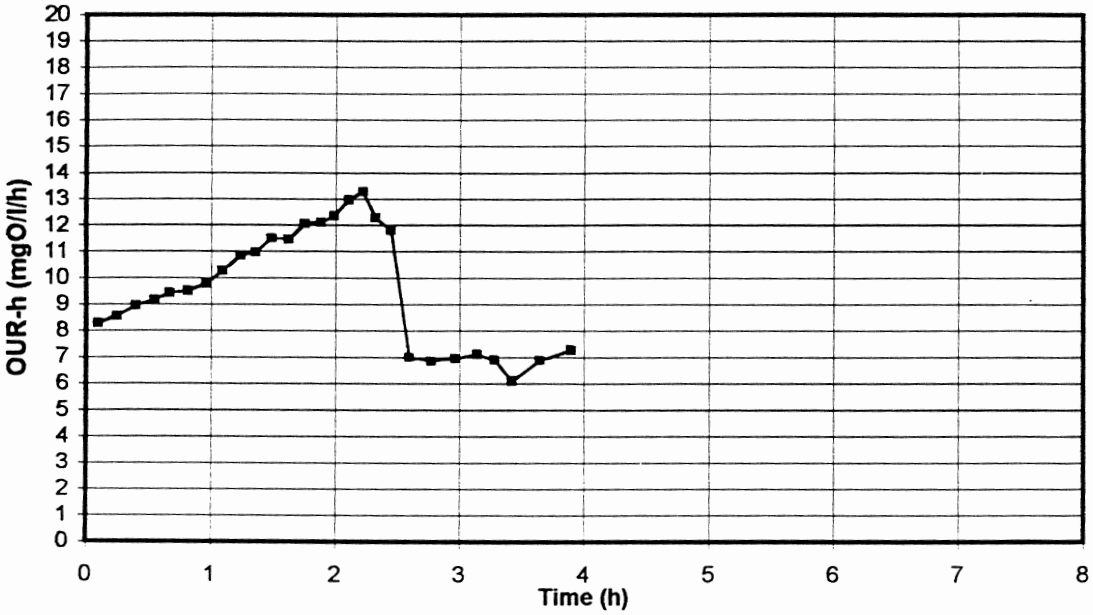
ℓn(OUR-h) graph for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M16 , 22-02, waste water batch no.19



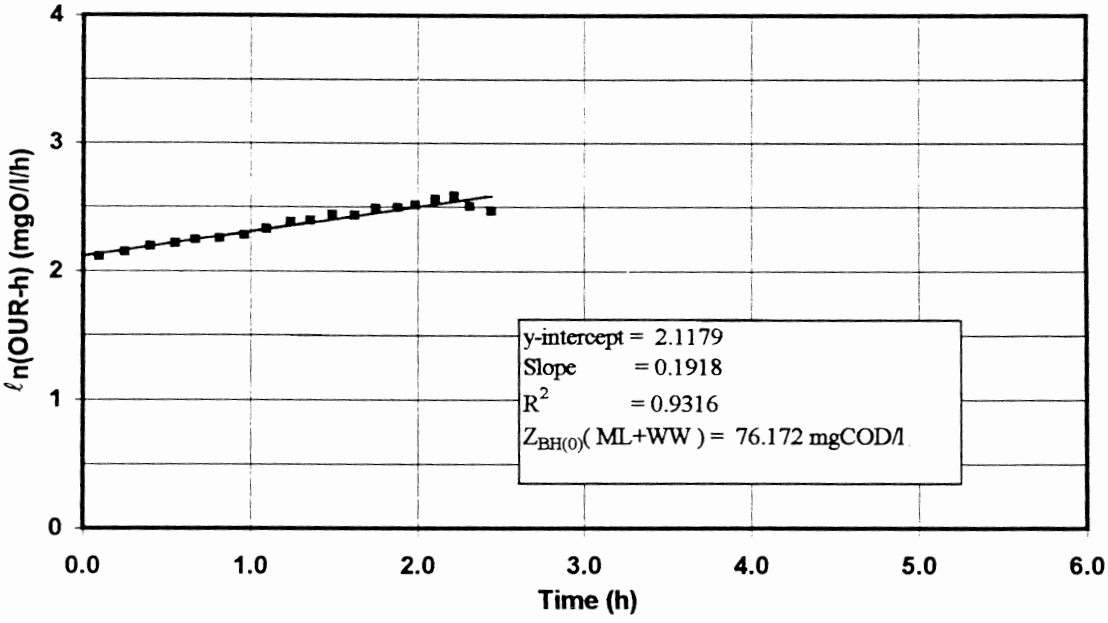
OUR Graph for wastewater (2.65ℓ) plus mixed liquor (0.35ℓ) batch test M17 , 23-02 , wastewater batch no. 19



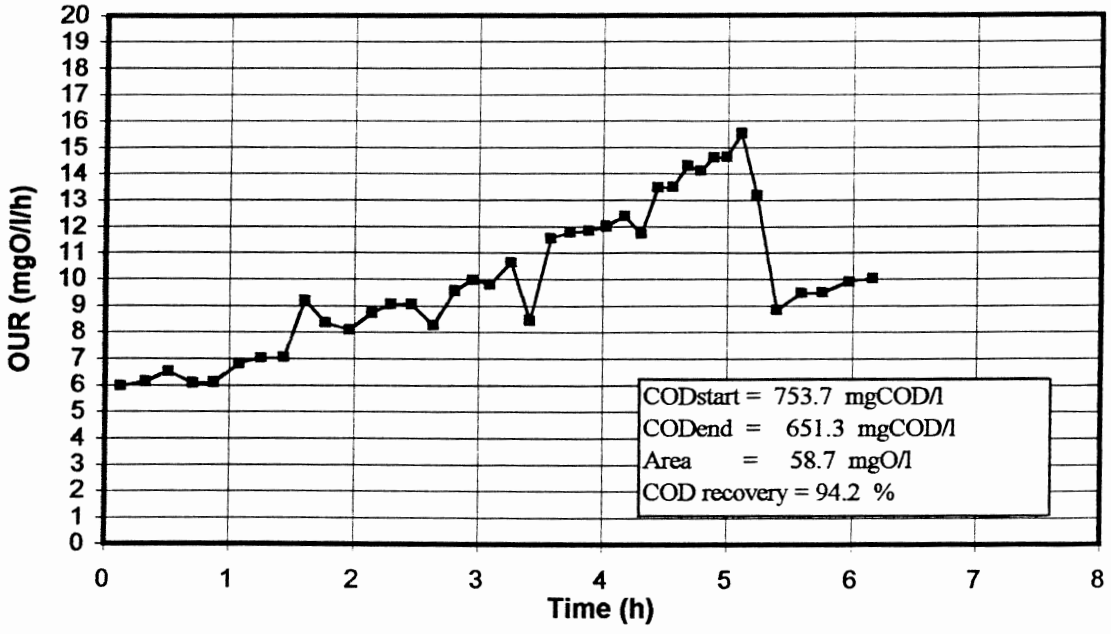
NO_3 Graph for wastewater (2.65ℓ) plus mixed liquor (0.35ℓ) batch test M17, 23-02 , wastewater batch no. 19



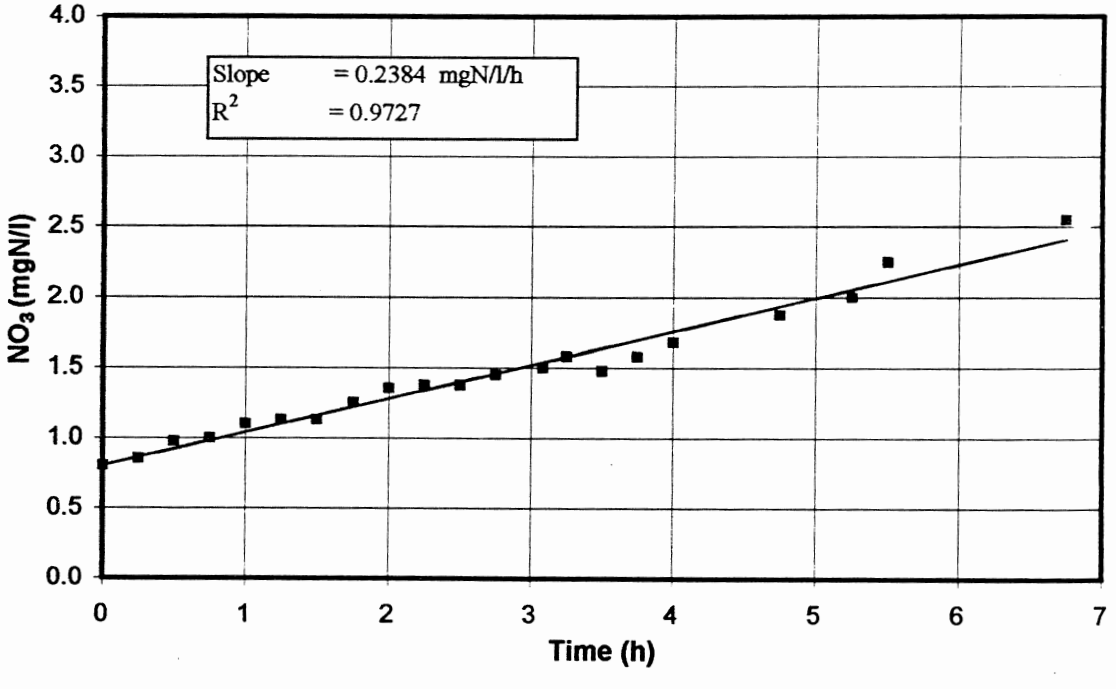
OUR-h Graph for wastewater (2.65ℓ) plus mixed liquor (0.35ℓ) batch test M17 , 23-02 , wastewater batch no. 19



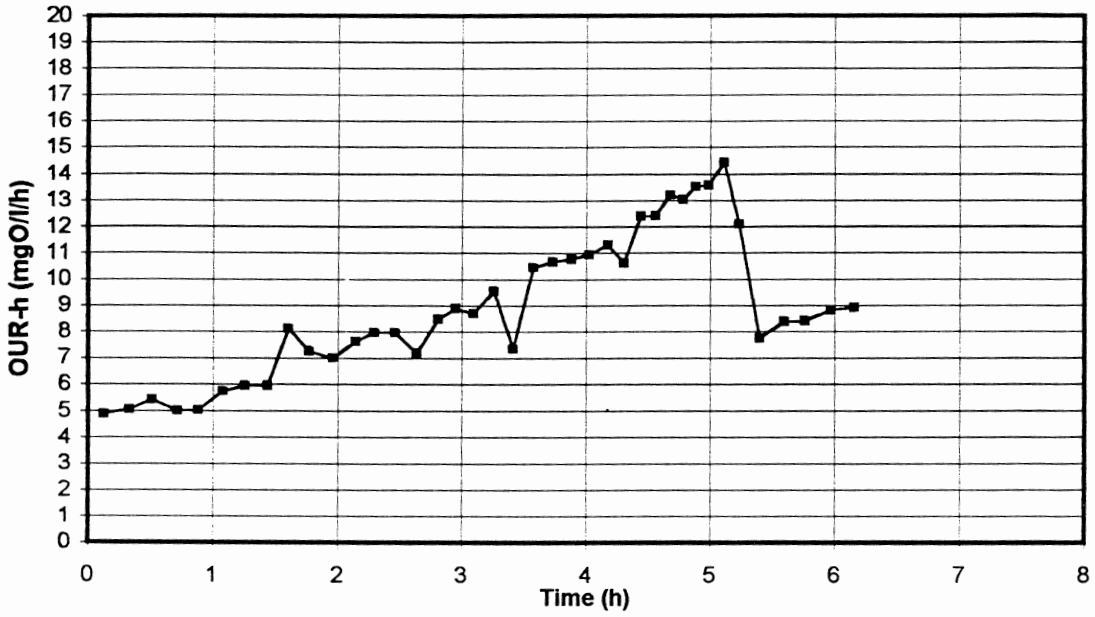
ℓn(OUR-h) graph for wastewater(2.65ℓ) plus mixed liquor(0.35ℓ) batch test M17,23-02, waste water batch no.19



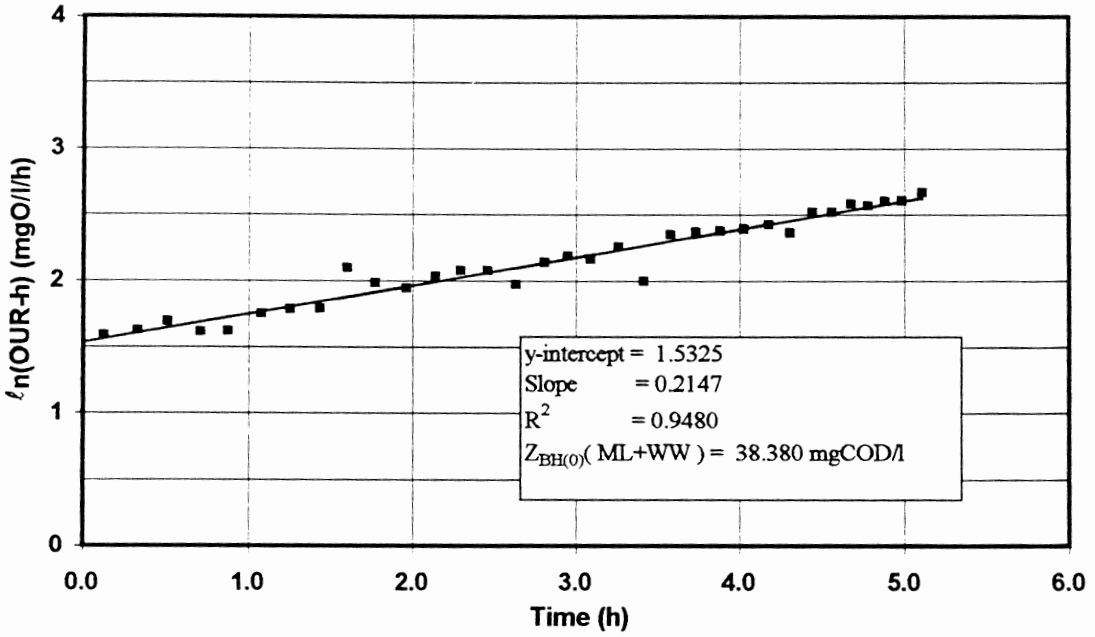
OUR Graph for wastewater (2.75ℓ) plus mixed liquor (0.25ℓ) batch test M18 , 29-02 , wastewater batch no. 20



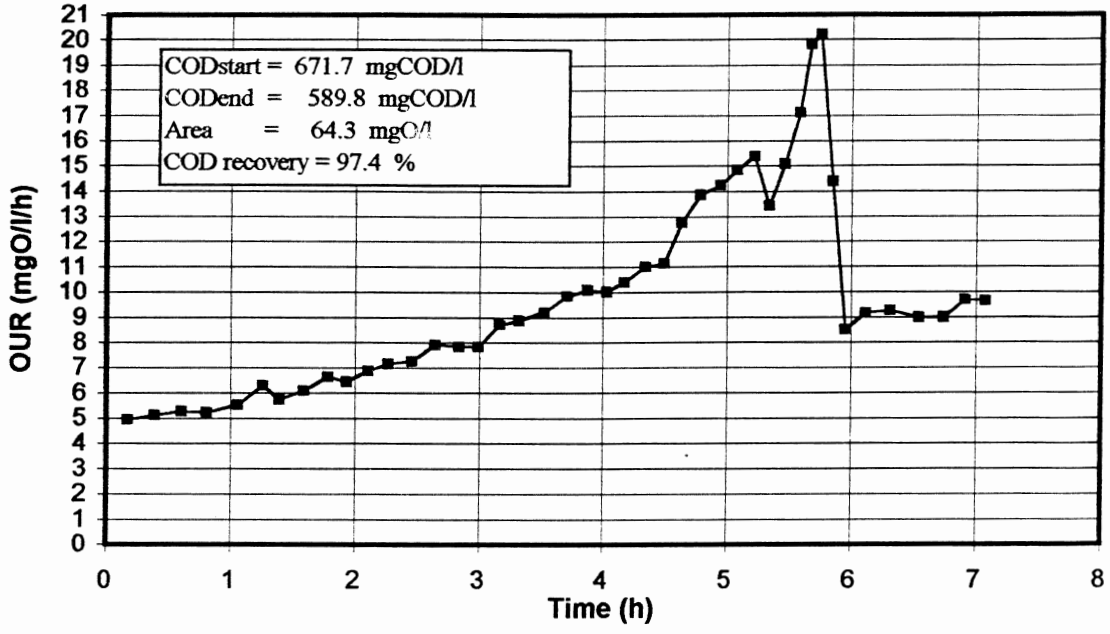
NO_3 Graph for wastewater (2.75ℓ) plus mixed liquor (0.25ℓ) batch test M18 , 29-02 , wastewater batch no. 20



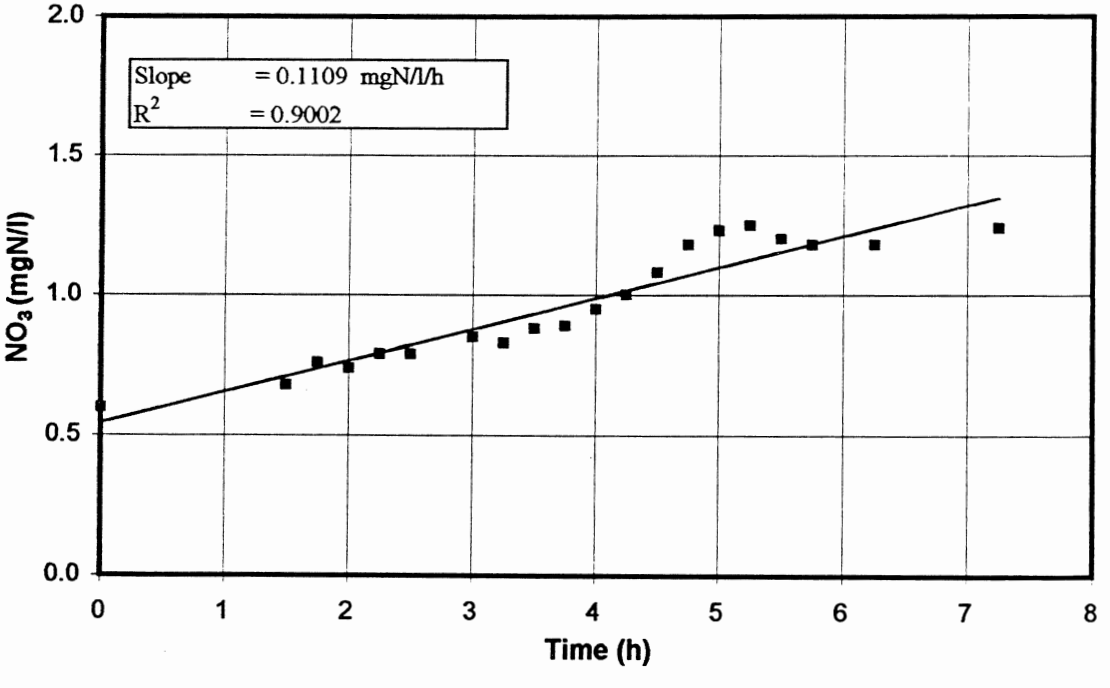
OUR-h Graph for wastewater (2.75ℓ) plus mixed liquor (0.25ℓ) batch test M18, 29-02, wastewater batch no. 20



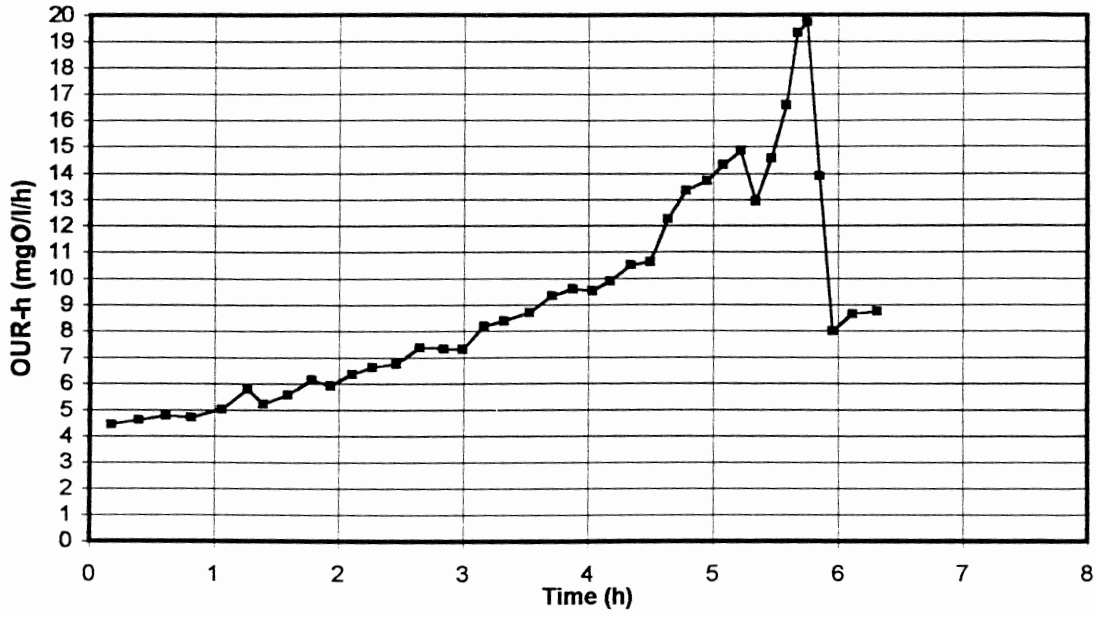
ℓn(OUR-h) graph for wastewater(2.75ℓ) plus mixed liquor(0.25ℓ) batch test M18,29-02, waste water batch no.20



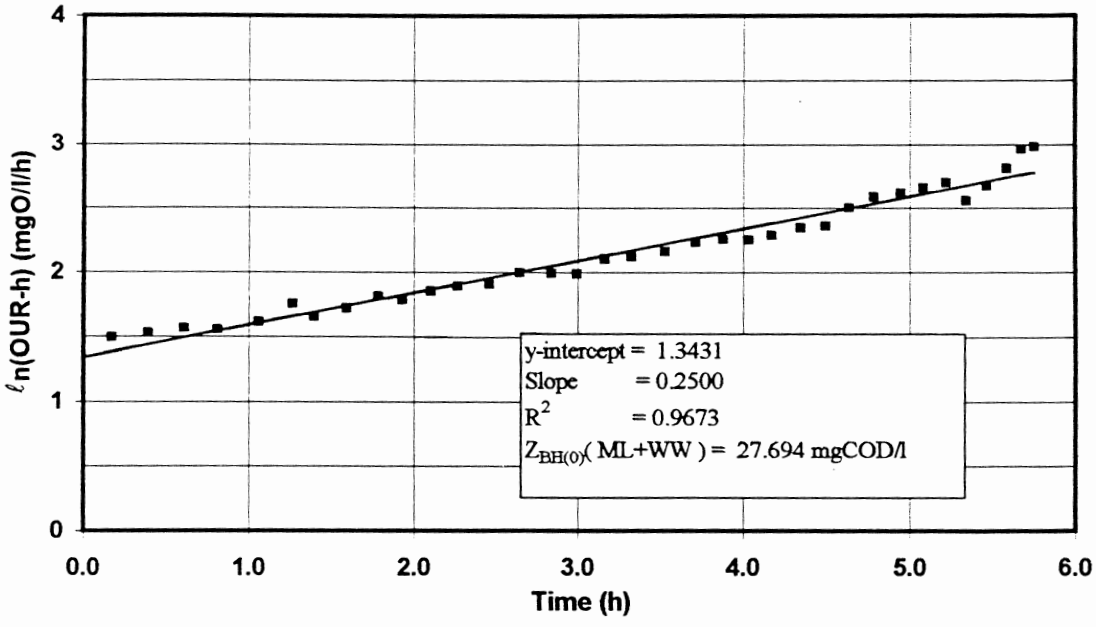
OUR Graph for wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test M19 , 01-03 , wastewater batch no. 20



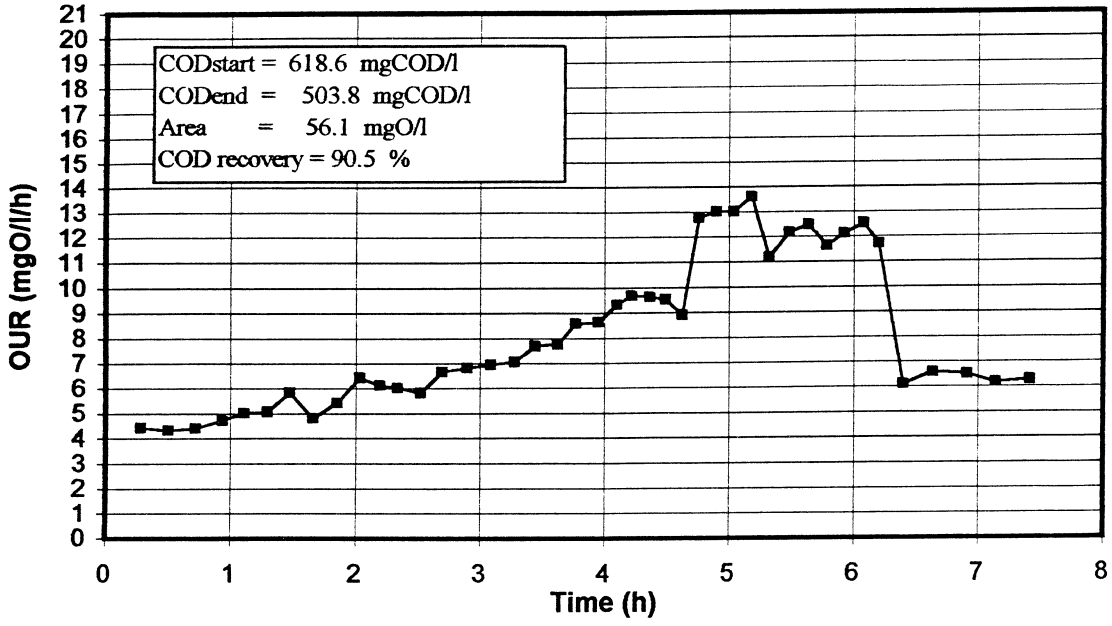
NO_3 Graph for wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test M19 , 01-03 , wastewater batch no. 20



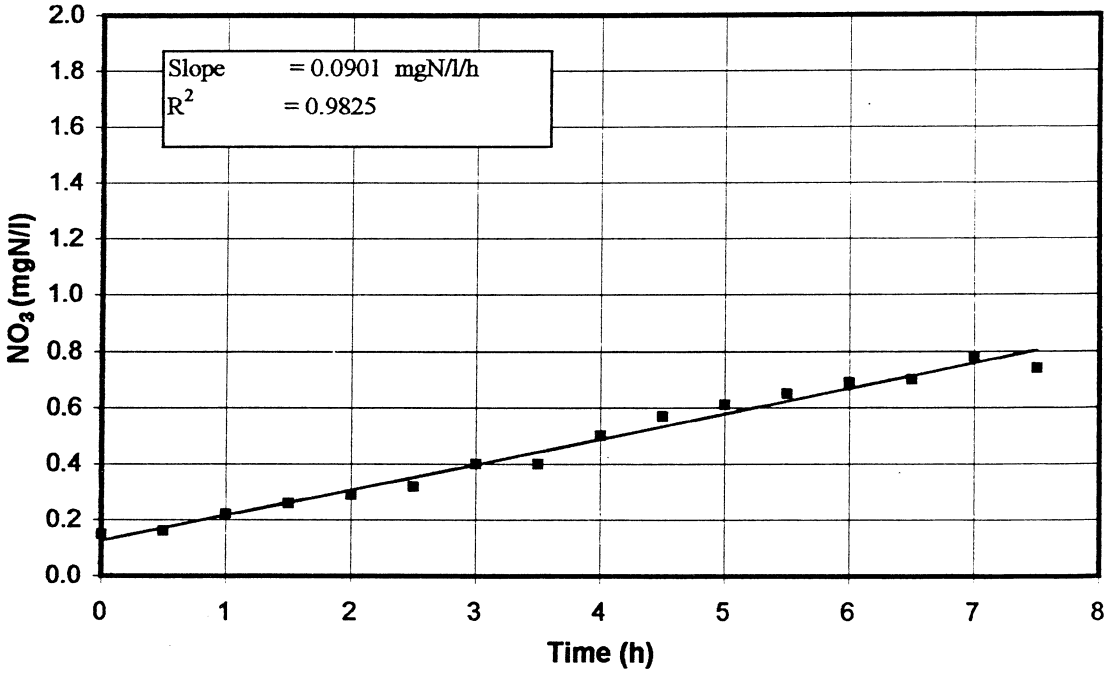
OUR-h Graph for wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test M19 , 01-03 , wastewater batch no. 20



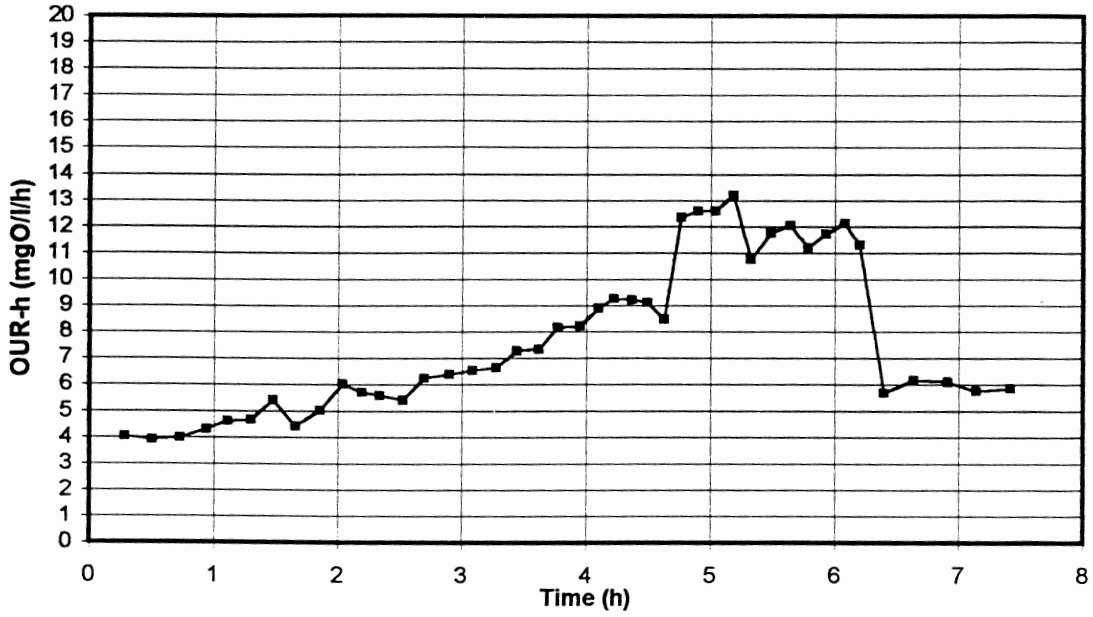
ℓn(OUR-h) graph for wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test M19 , 01-03, waste water batch no.20



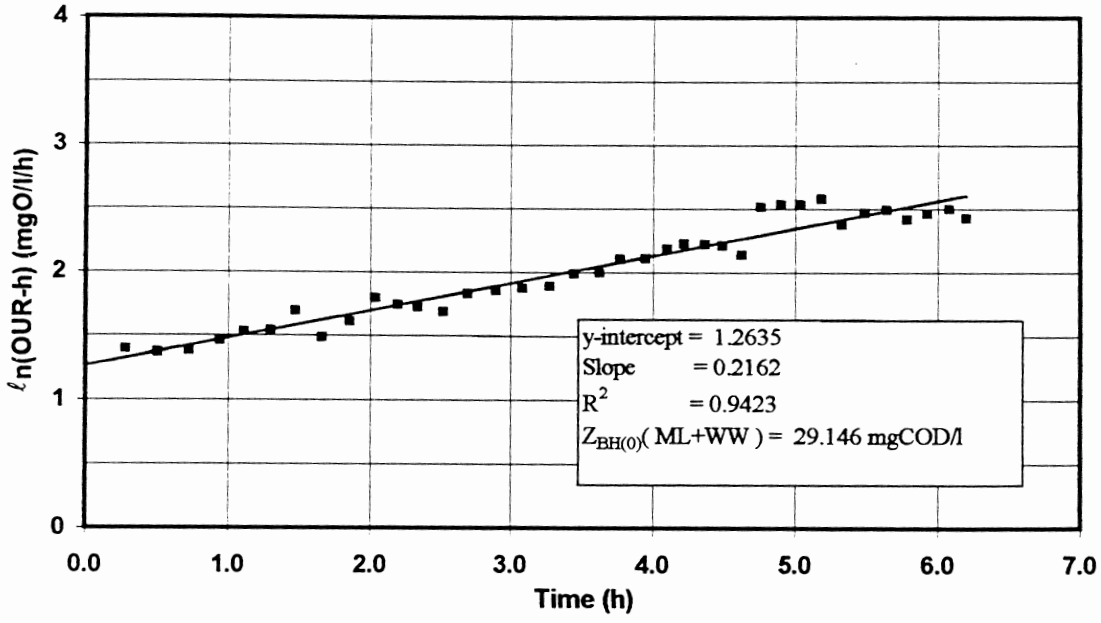
OUR Graph for wastewater (2.85ℓ) plus mixed liquor (0.15ℓ) batch test M20 , 02-03 , wastewater batch no. 20



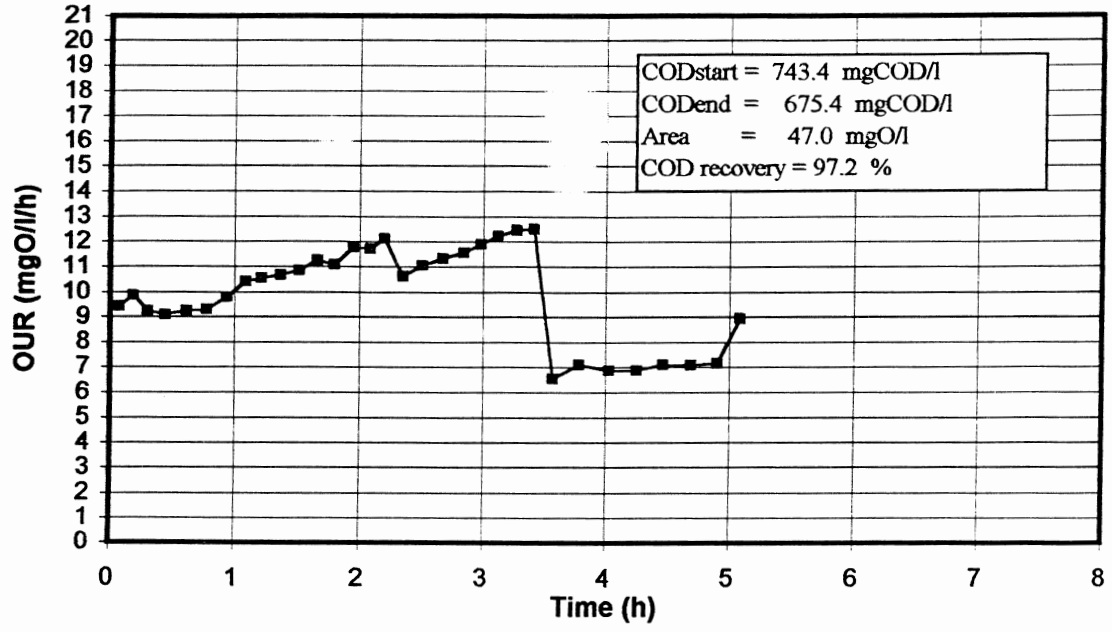
NO_3 Graph for wastewater (2.85ℓ) plus mixed liquor (0.15ℓ) batch test M20 , 02-03 , wastewater batch no. 20



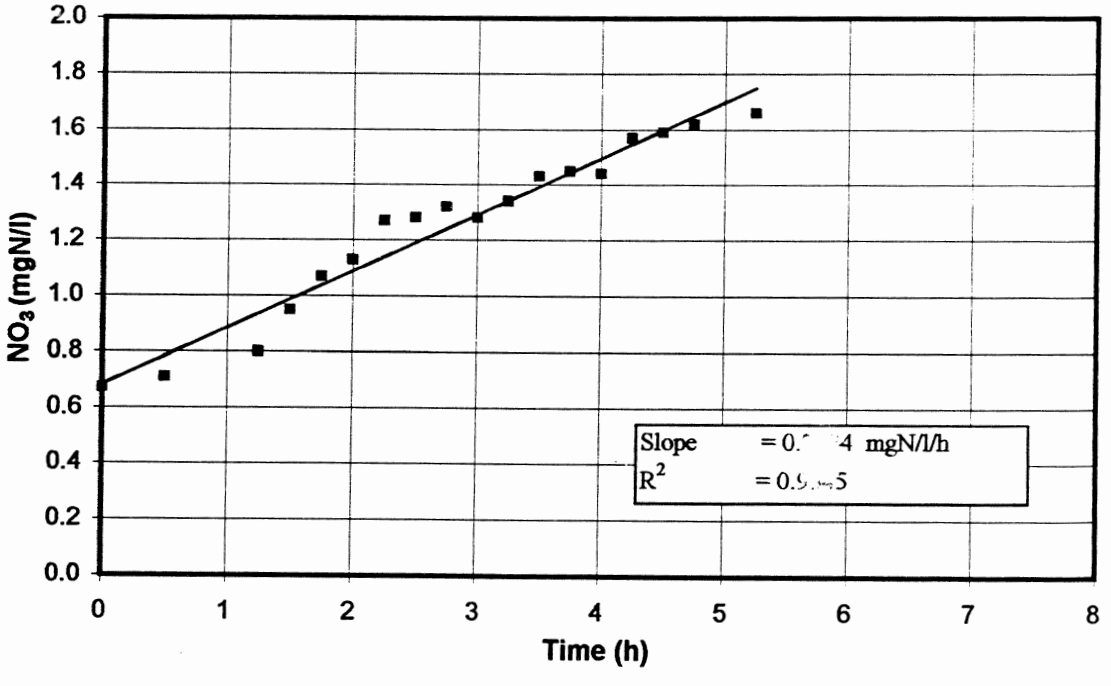
OUR-h Graph for wastewater (2.85ℓ) plus mixed liquor (0.15ℓ) batch test M20 , 02-03 , wastewater batch no. 20



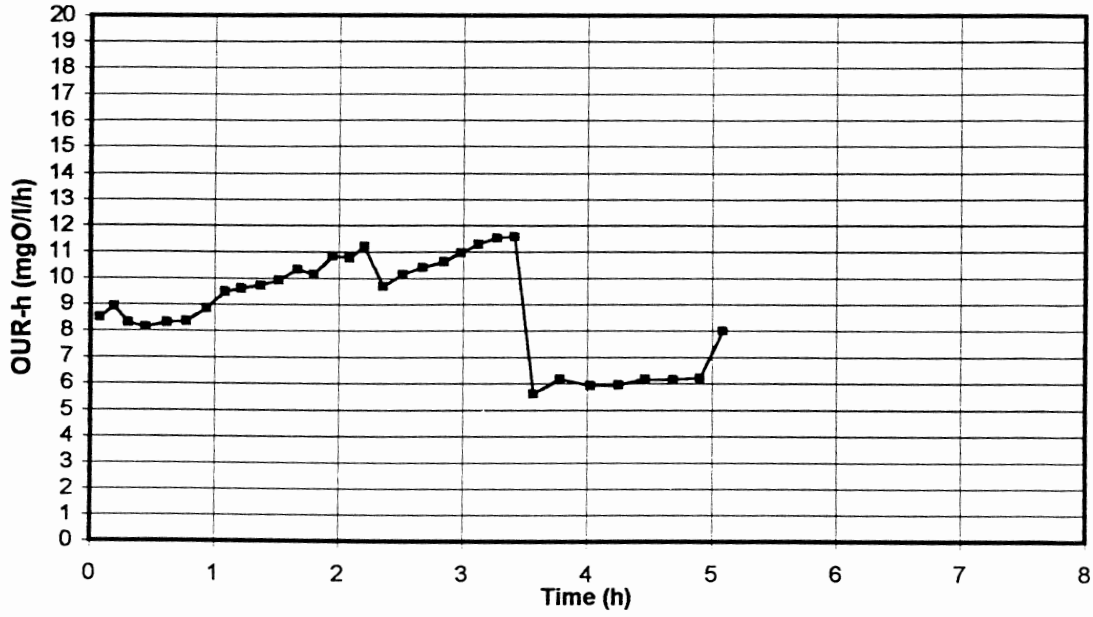
ℓn(OUR-h) graph for wastewater(2.85ℓ) plus mixed liquor(0.15ℓ) batch test M20,02-03, wastewater batch no.20



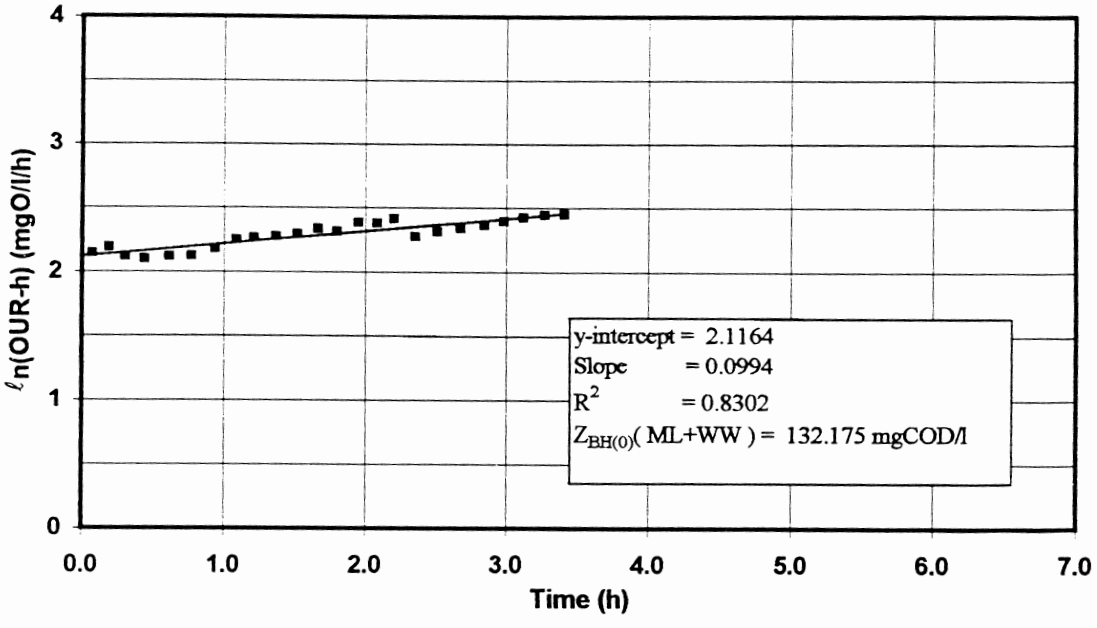
OUR Graph for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M21 , 06-03 , wastewater batch no. 20



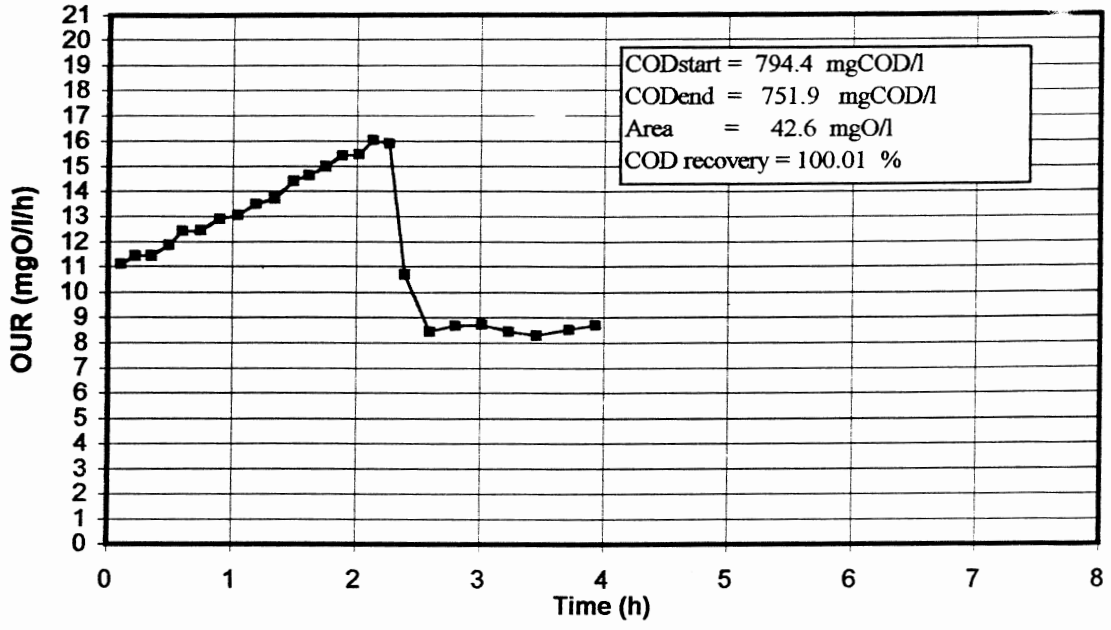
NO_3 Graph for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M21 , 06-03 , wastewater batch no. 20



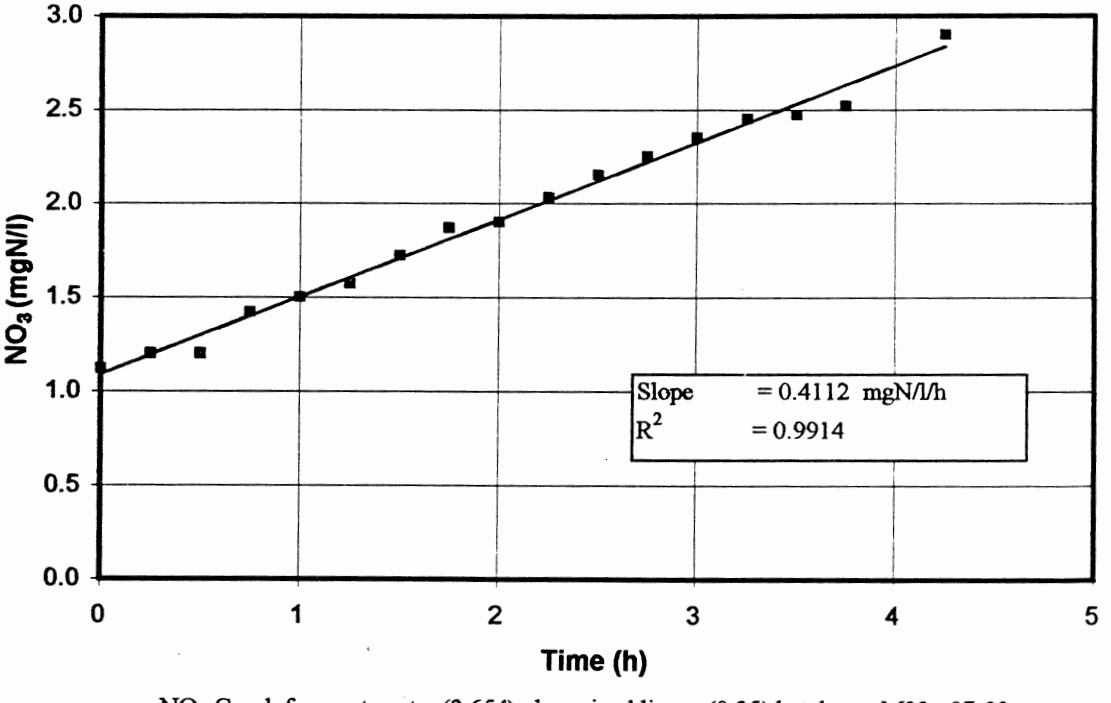
OUR-h Graph for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M21, 06-03 , wastewater batch no. 20



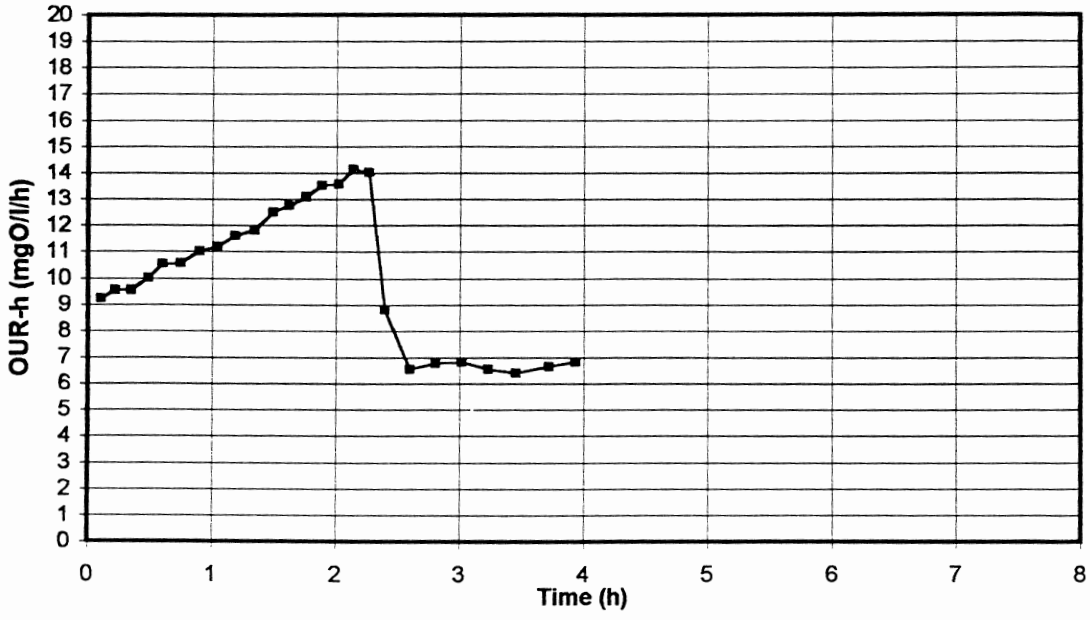
ℓn(OUR-h) graph for wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test M21 , 06-03, waste water batch no.20



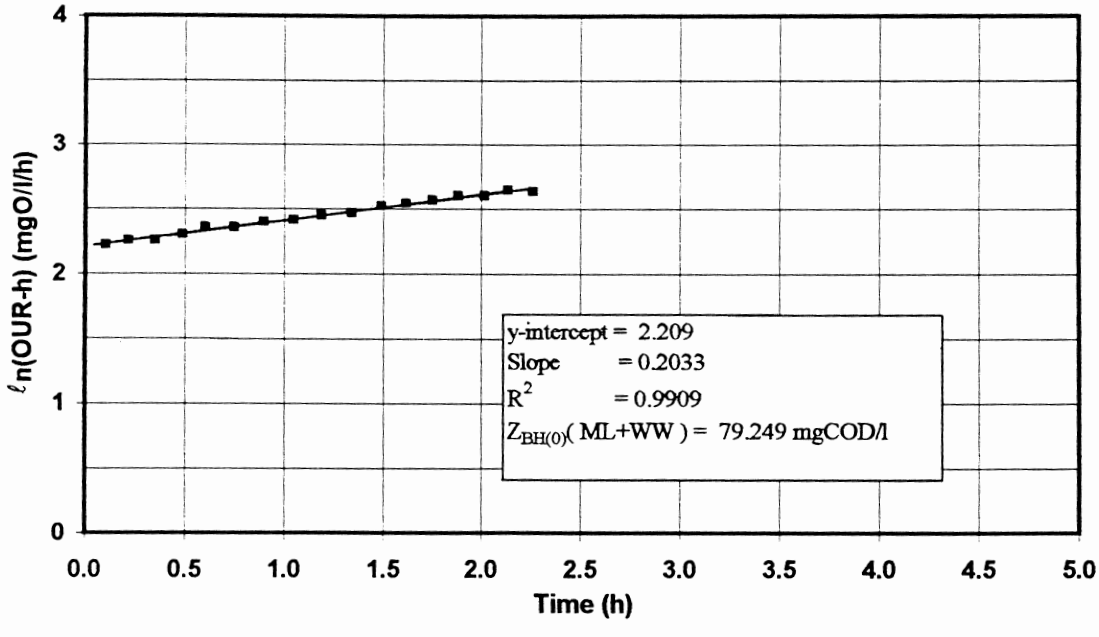
OUR Graph for wastewater (2.65ℓ) plus mixed liquor (0.35ℓ) batch test M22 , 07-03 , wastewater batch no. 20



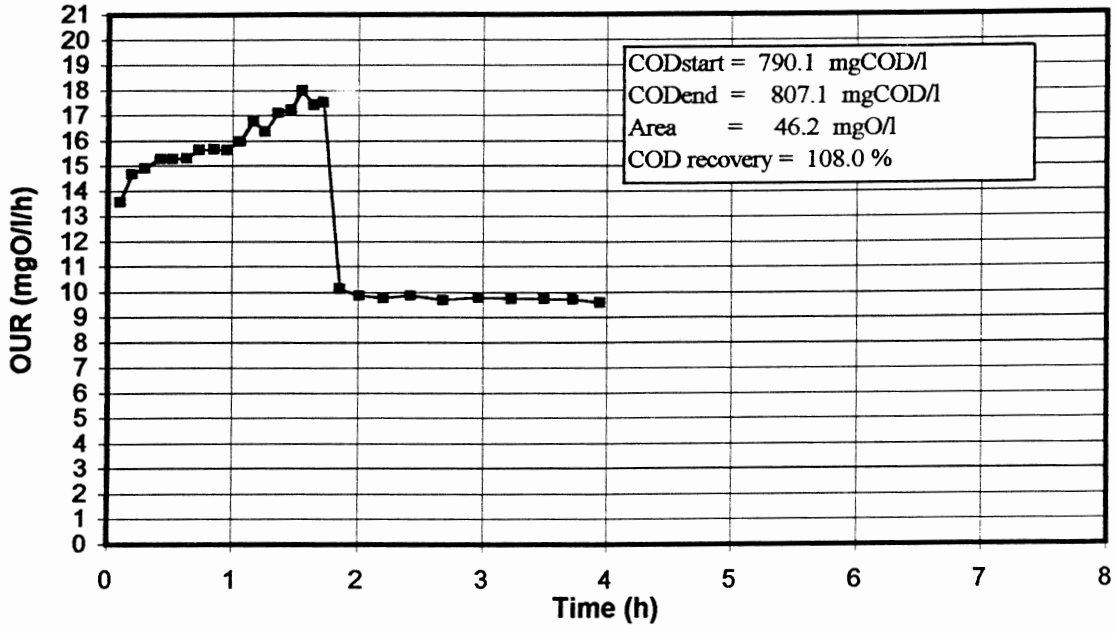
NO_3 Graph for wastewater (2.65ℓ) plus mixed liquor (0.35) batch test M22 , 07-03 , wastewater batch no. 20



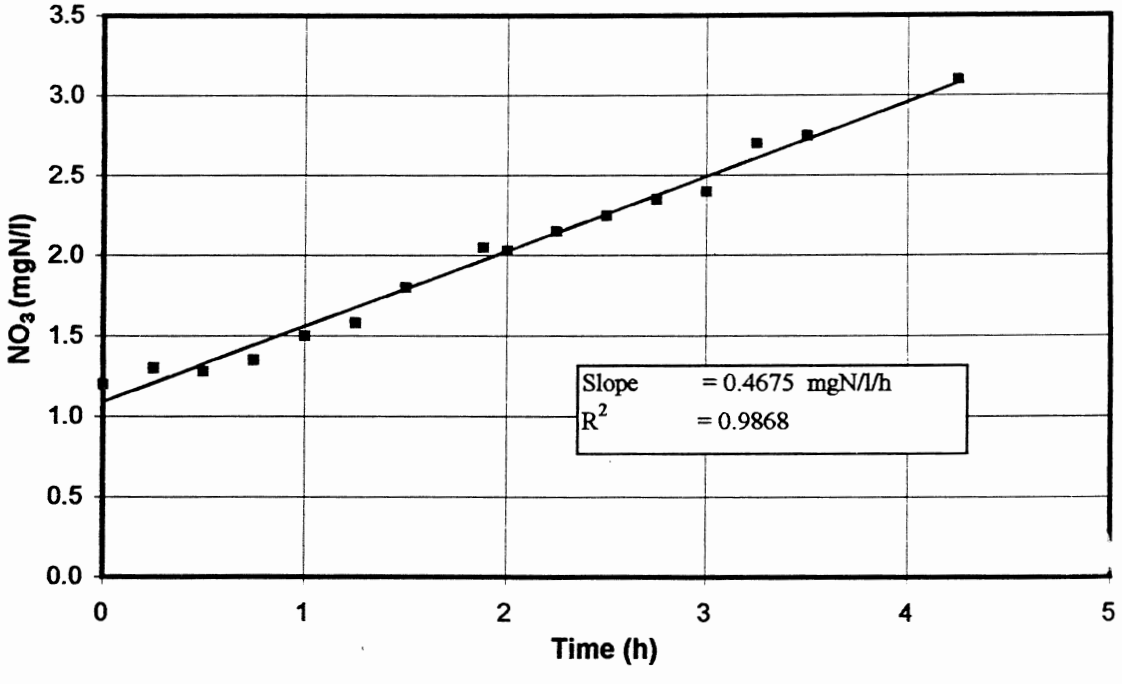
OUR-h Graph for wastewater (2.65ℓ) plus mixed liquor (0.35ℓ) batch test M22 , 07-03 , wastewater batch no. 20



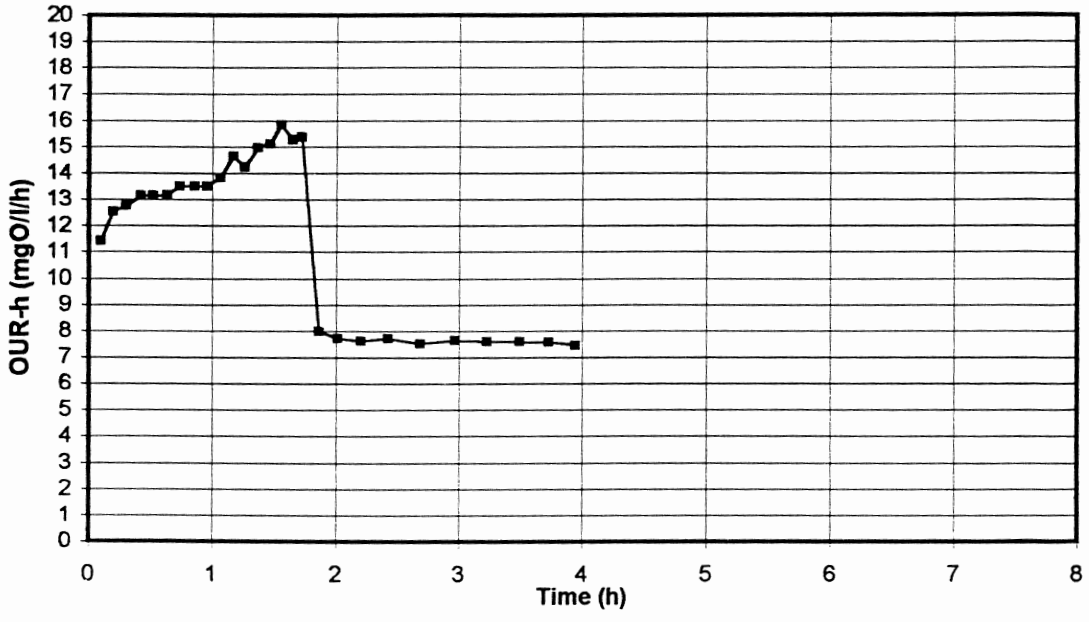
ℓn(OUR-h) graph for wastewater(2.65ℓ) plus mixed liquor(0.35ℓ) batch test M22,07-03, wastewater batch no.20



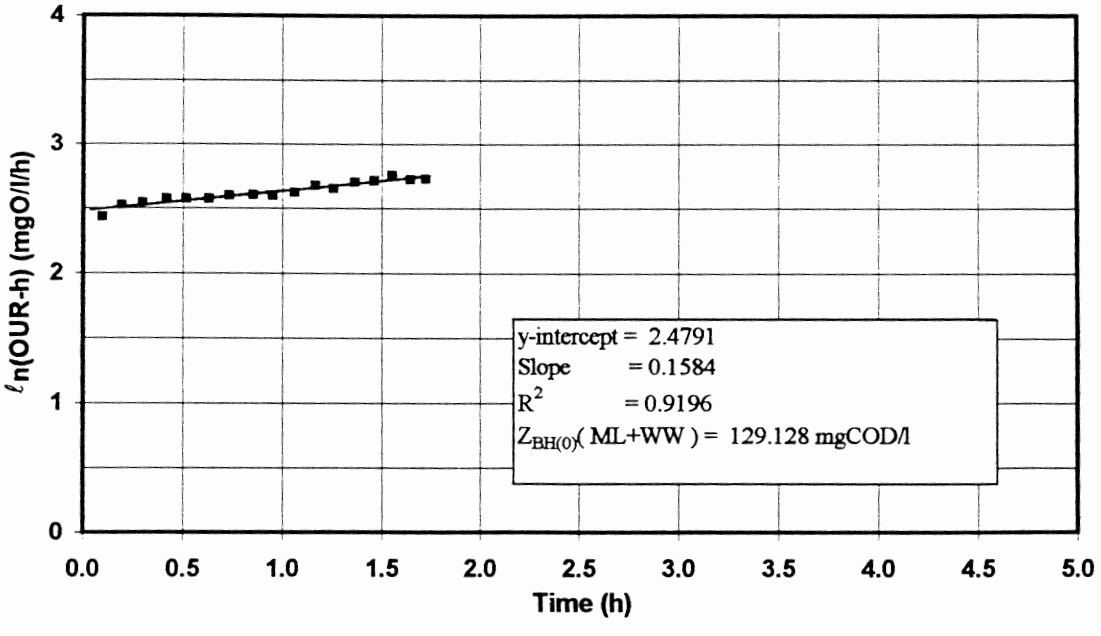
OUR Graph for wastewater (2.6ℓ) plus mixed liquor (0.4ℓ) batch test M23 , 08-03 , wastewater batch no. 20



NO_3 Graph for wastewater (2.6ℓ) plus mixed liquor (0.4) batch test M23 , 08-03 , wastewater batch no. 20



OUR-h Graph for wastewater (2.6ℓ) plus mixed liquor (0.4ℓ) batch test M23 , 08-03 , wastewater batch no. 20



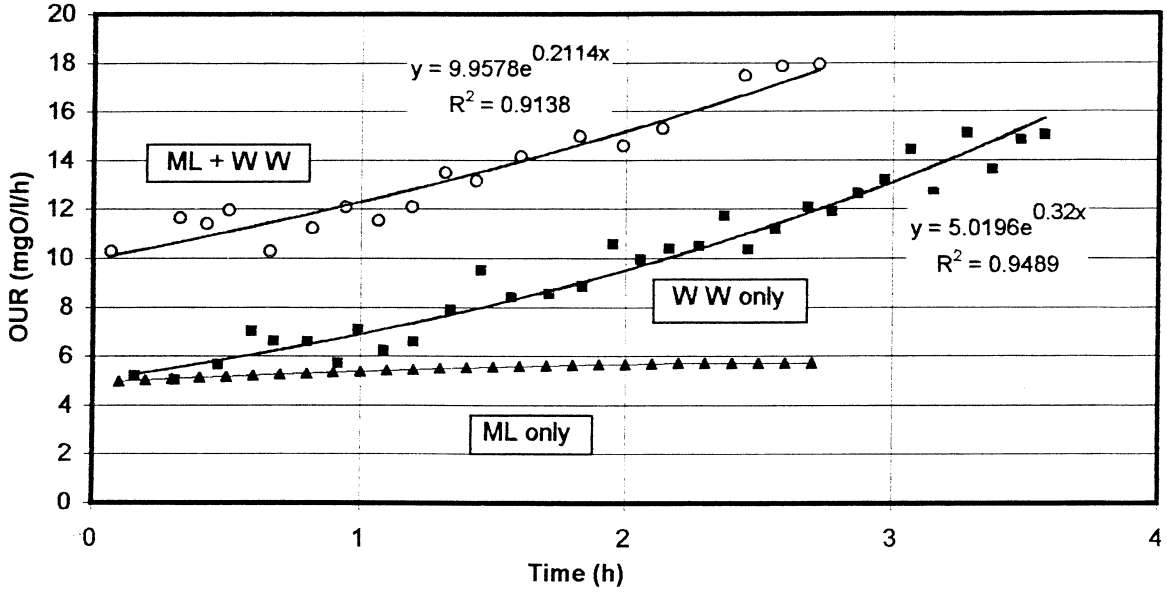
ℓn(OUR-h) graph for wastewater (2.6ℓ) plus mixed liquor (0.4ℓ) batch test M23 , 08-03, wastewater batch no.20

Table A3 : Summary of results for theoretical heterotrophic active biomass due to the mixed liquor addition only ($Z_{BH(0)}ML$), calculated through analytical separation of WW only and WW+ML batch test results. The linear regression data for nitrification in the WW+ML batch tests is also shown.

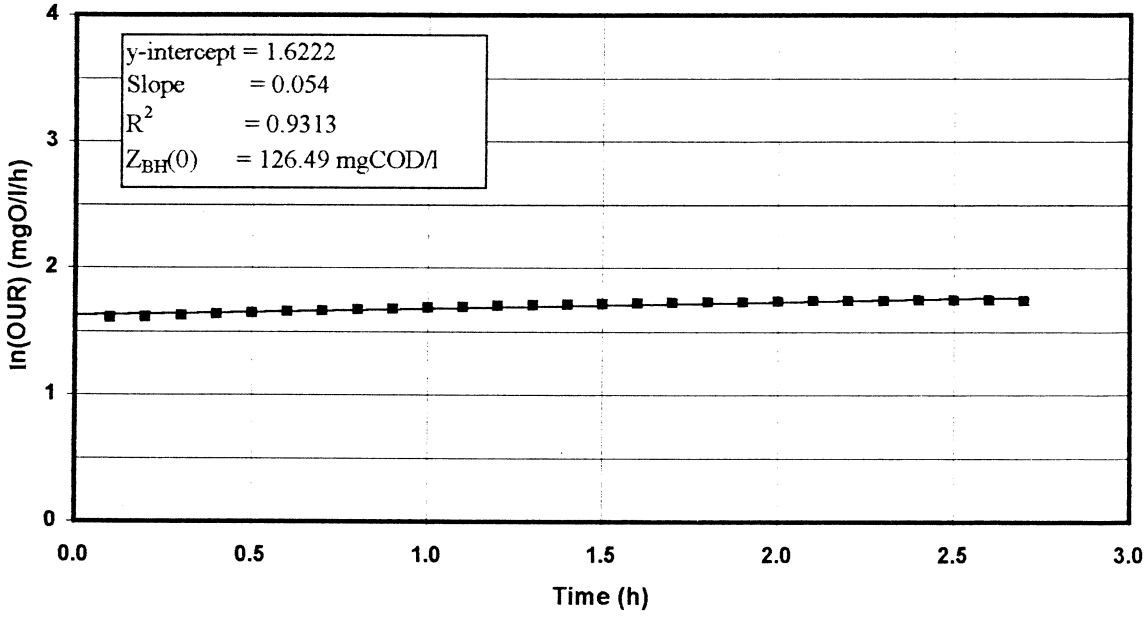
Sew. batch no.	*Batch Test No.*	*Date of test*	*M L vol.*	*%COD Recov.*	*Linear regression data for nitrification (NO3 vs time)*		*Linear regression data for M L only (ln(OUR) vs time)*			$Z_{BH(0)}$ *(M L only) (mgCOD/l)*
					slope	R^2	*y-int.*	*slope*	R^2 *	
11	M1	29-10	0.10ℓ	–	–	–	–	–	–	–
	M2	03-11	0.15ℓ	–	–	–	–	–	–	–
	M3	05-11	0.30ℓ	–	–	–	–	–	–	–
12	M4	15-11	0.30ℓ	–	–	–	–	–	–	–
13	M5	30-11	0.20ℓ	102	0.1231	0.9810	1.6222	0.0540	0.9313	126.49
	M6	01-12	0.10ℓ	90	0.0683	0.9430	0.4506	0.3451	0.9778	8.436
	M7	03-12	0.25ℓ	91	0.2337	0.9562	1.4408	0.1172	0.9330	58.887
14	M8	08-12	0.20ℓ	93	0.1257	0.9756	1.0046	0.1078	0.9672	40.748
18	M9	07-02	0.20ℓ	103	0.1764	0.9184	1.1380	0.1904	0.9554	28.776
	M10	08-02	0.25ℓ	106	0.1560	0.9530	1.6218	0.1150	0.9427	71.674
	M11	09-02	0.30ℓ	101	0.1973	0.8320	1.5449	0.2013	0.9553	41.152
	M12	10-02	0.15ℓ	100	0.0919	0.8890	0.8364	0.1510	0.9429	26.026
19	M13	15-02	0.30ℓ	103	0.2743	0.9456	1.3539	0.1615	0.9562	41.220
	M14	16-02	0.35ℓ	95	0.4124	0.9525	1.9815	0.1088	0.9104	107.431
	M15	17-02	0.40ℓ	99	0.3889	0.9751	2.0451	0.0850	0.9052	139.070
	M16	22-02	0.30ℓ	94	0.3217	0.9522	1.6720	0.0549	0.9502	131.467
	M17	23-02	0.35ℓ	99	0.3687	0.9182	1.5679	0.0138	0.9862	241.322
20	M18	29-02	0.25ℓ	94	0.2384	0.9727	1.3545	0.1105	0.9765	56.673
	M19	01-03	0.20ℓ	97	0.1109	0.9002	1.0758	0.1206	0.9666	39.930
	M20	02-03	0.15ℓ	91	0.0901	0.9825	0.8310	0.0744	0.9544	45.668
	M21	06-03	0.30ℓ	97	0.2034	0.9545	1.7973	0.0815	0.8567	112.086
	M22	07-03	0.35ℓ	100	0.4112	0.9914	1.7618	0.1533	0.9374	64.817
	M23	08-03	0.40ℓ	108	0.4675	0.9868	2.2575	0.0683	0.9231	202.490

* R^2 is ***average*** between R^2 for "best fit" ML+WW exponential curve and R^2 for "best fit" WW only exponential curve - see graphs below.

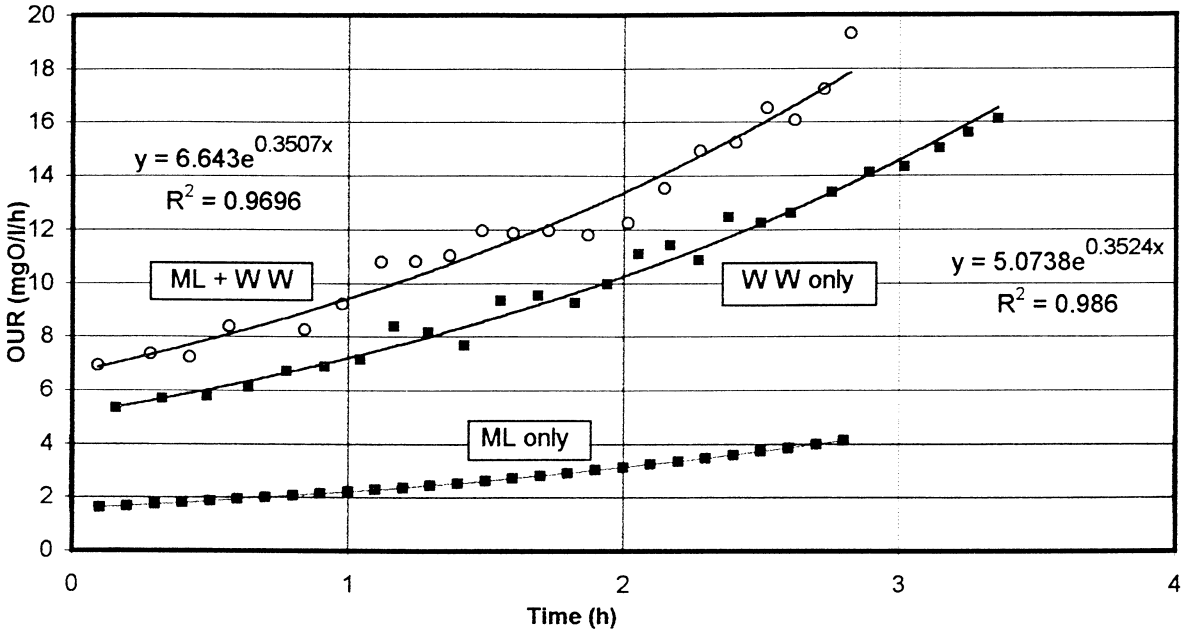
– 3ℓ of wastewater used in WW only batch tests - direct comparison of OUR data for WW only and WW+ML batch tests not possible.



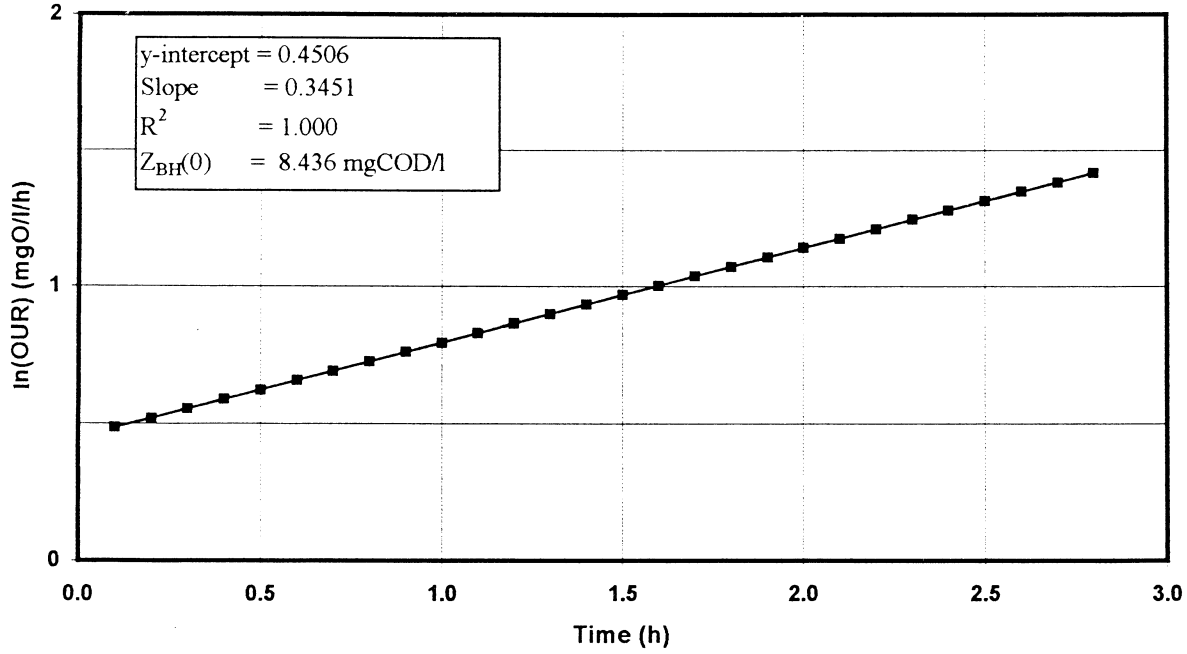
Combined OUR Graph for batch test M5, 30-11, (0.2ℓ ML), wastewater batch no. 13



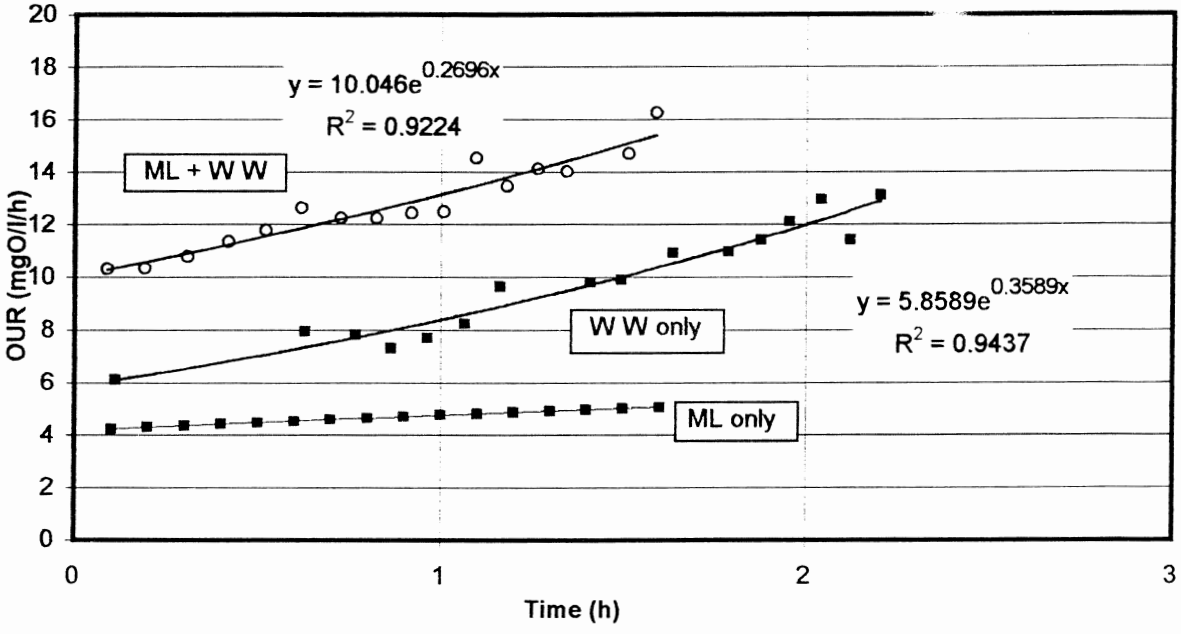
ln(OUR) graph for ML only, batch test M5, 30-11, (0.2ℓ ML), wastewater batch no.13



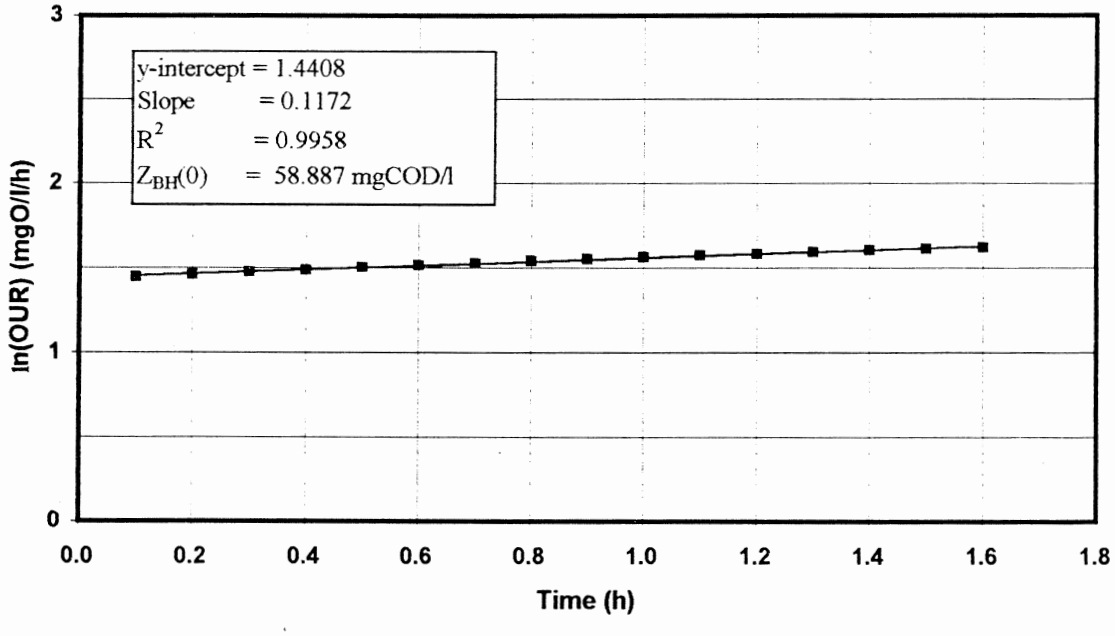
Combined OUR Graph for batch test M6, 01-12, (0.1ℓ ML), waste water batch no. 13



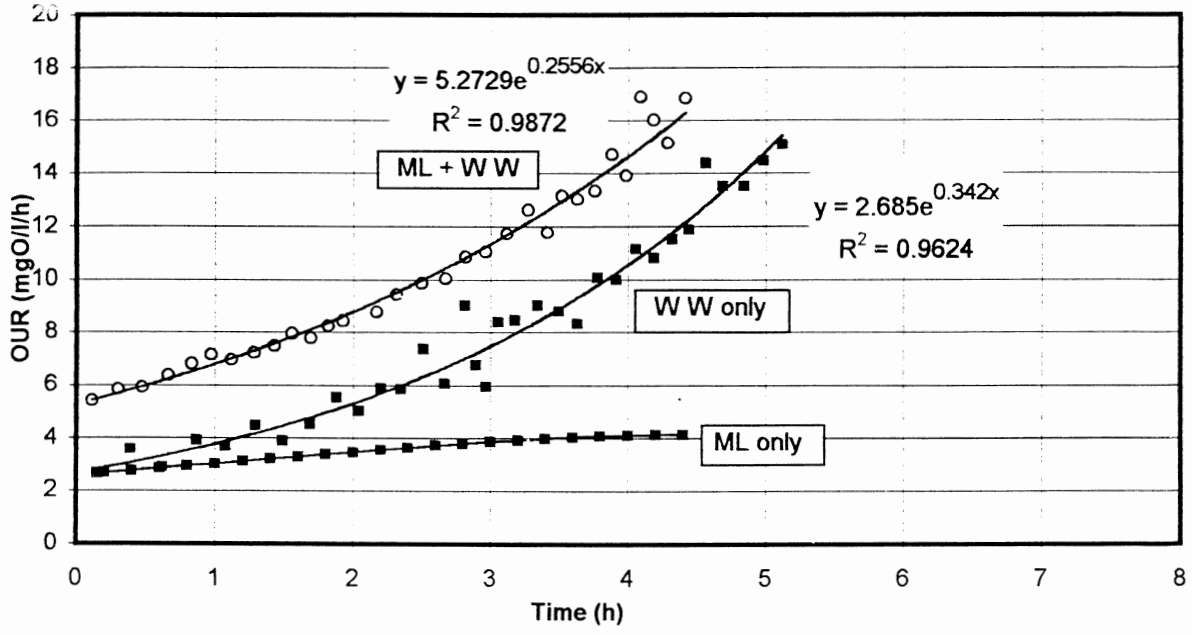
ln(OUR) graph for ML only, batch test M6, 01-12, (0.1ℓ ML), waste water batch no.13



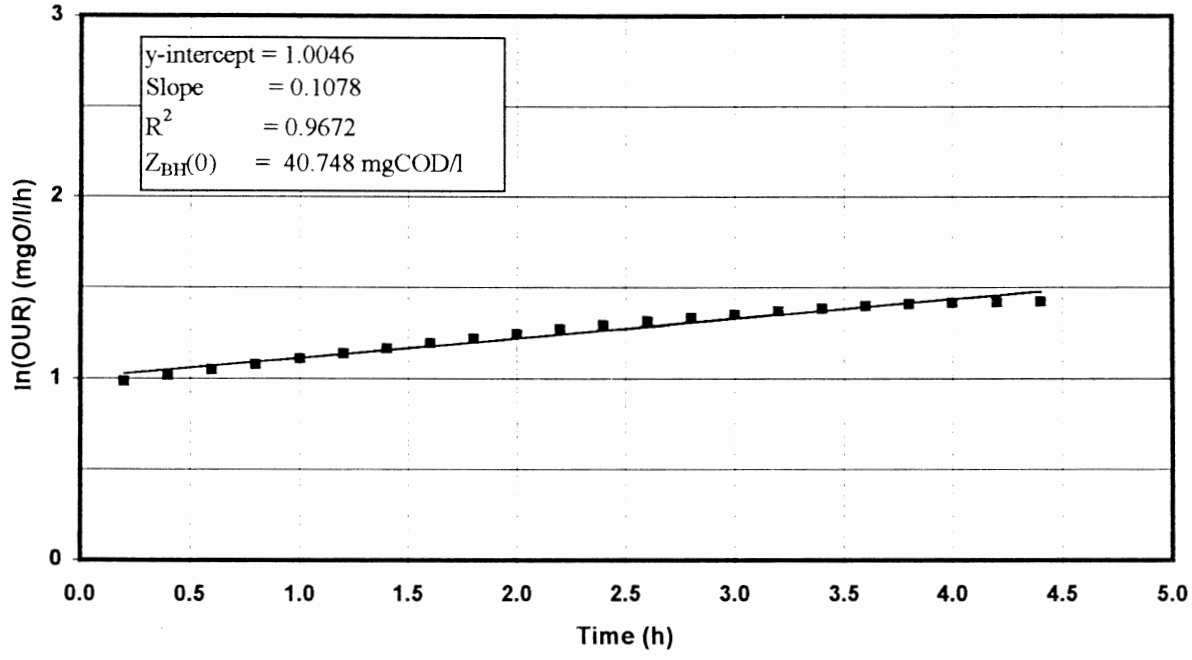
Combined OUR Graph for batch test M7, 03-12, (0.25ℓ ML), wastewater batch no. 13



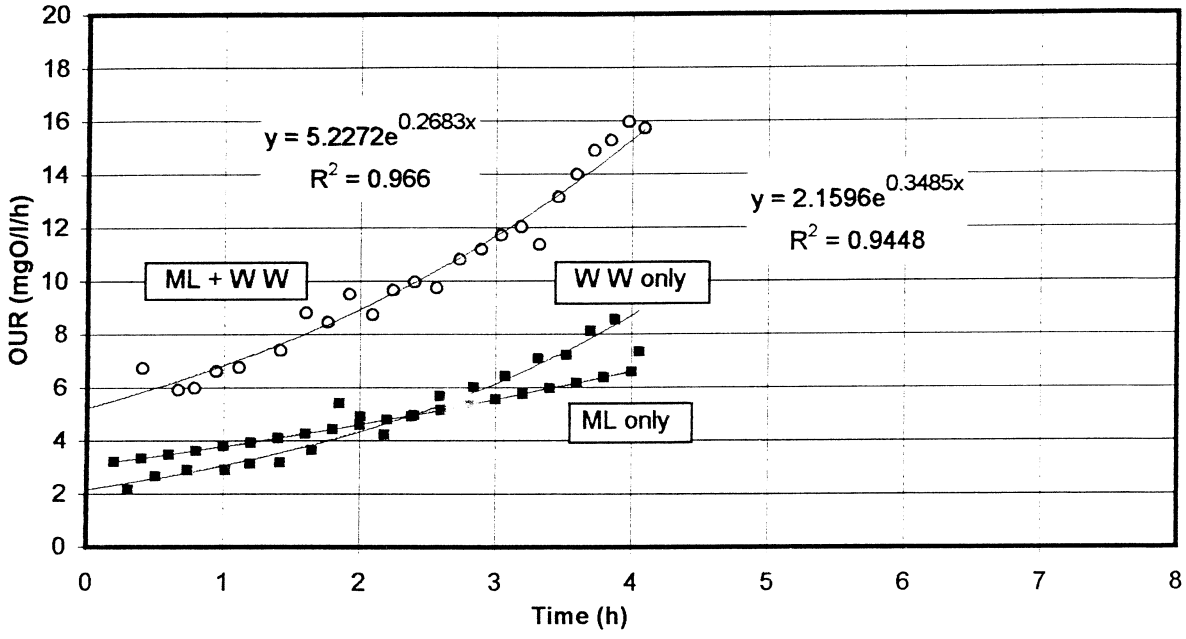
ln(OUR) graph for ML only batch test M7, 03-12, (0.25ℓ ML), wastewater batch no.13



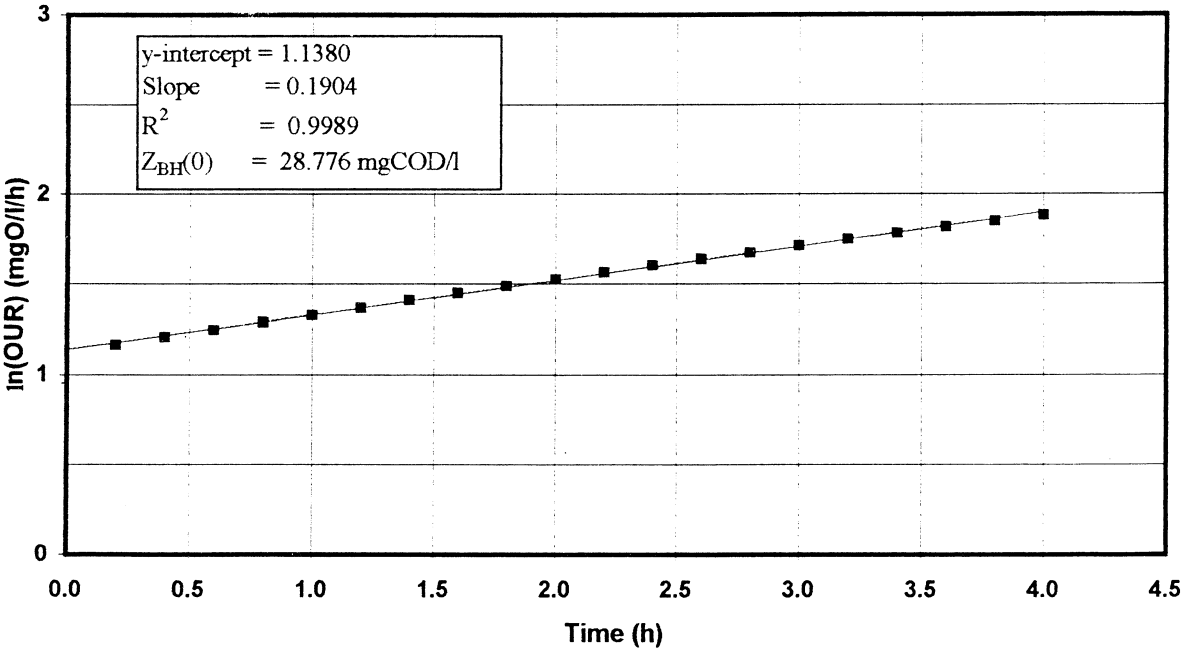
Combined OUR Graph for batch test M8, 08-12, (0.2ℓ ML), wastewater batch no. 14



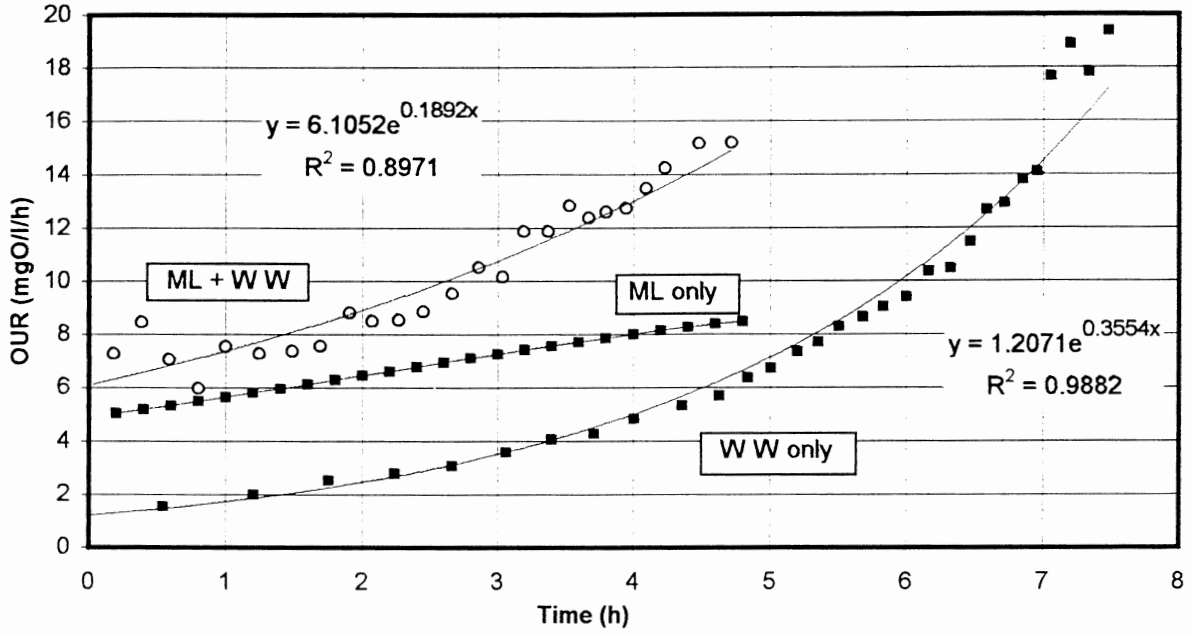
ln(OUR) graph for ML only batch test M8, 08-12, (0.2ℓ ML), wastewater batch no.14



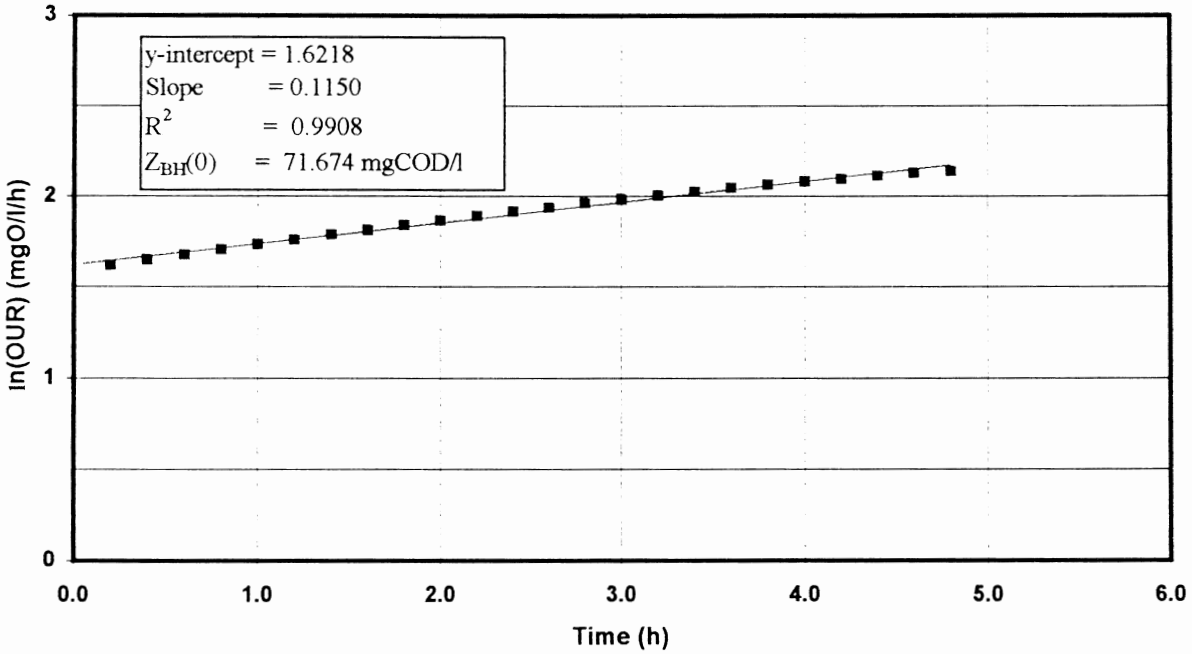
Combined OUR Graph for batch test M9, 07-02, (0.2ℓ ML), wastewater batch no. 18



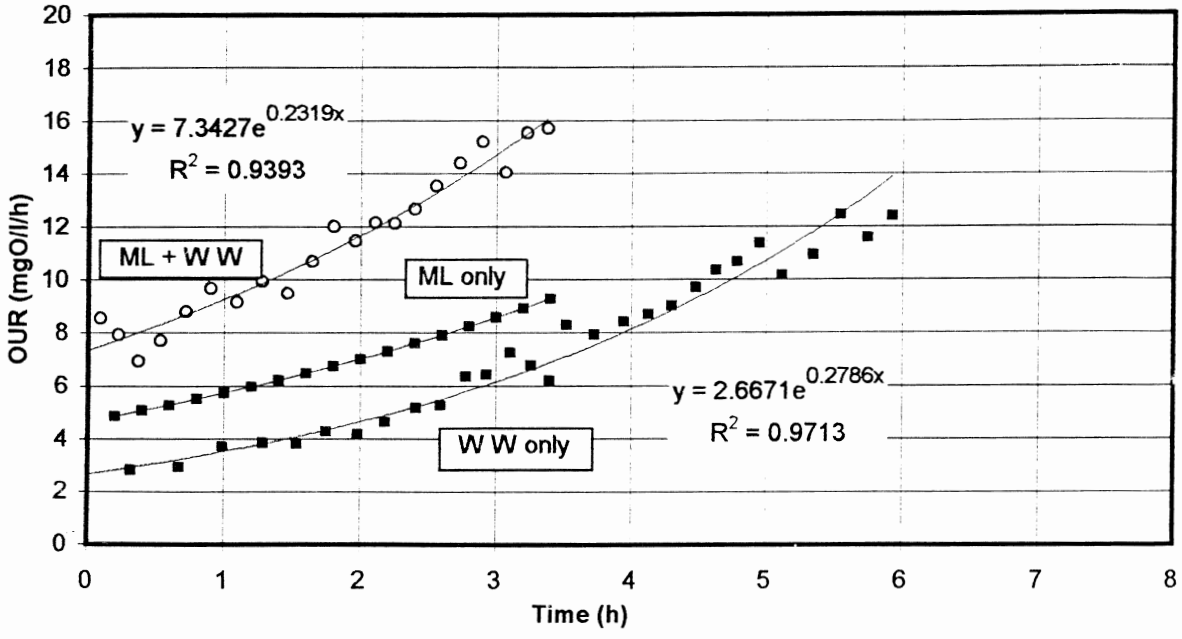
ln(OUR) graph for ML only batch test M9, 07-02, (0.2ℓ ML), wastewater batch no.18



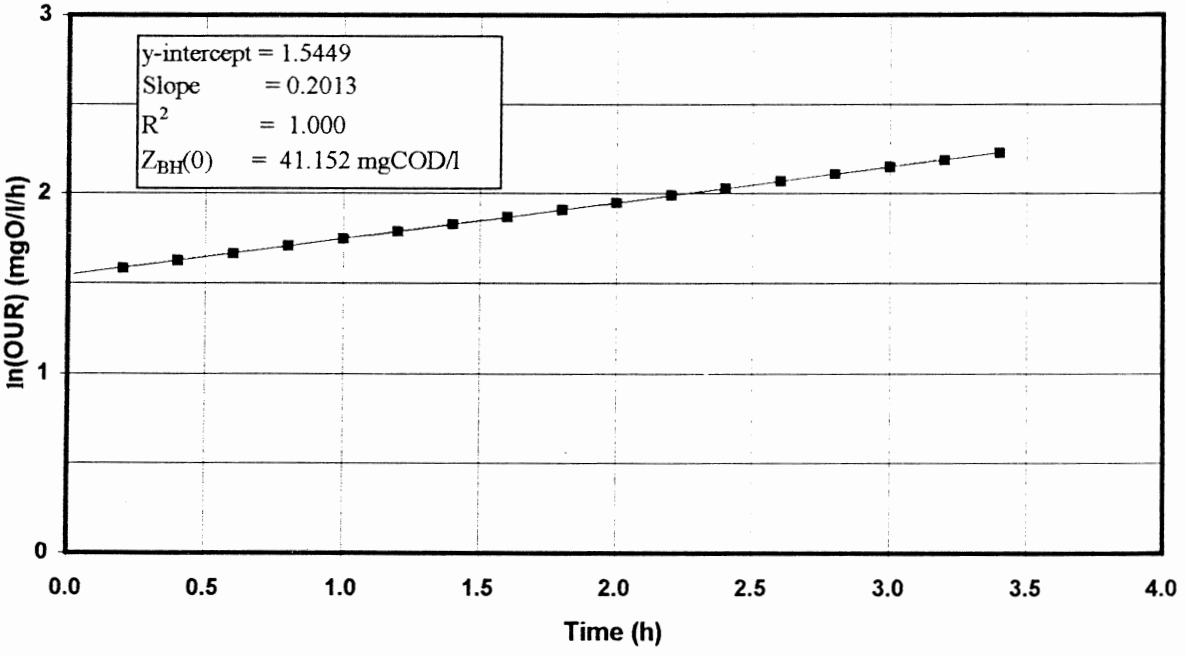
Combined OUR Graph for batch test M10, 08-02, (0.25ℓ ML), wastewater batch no. 18



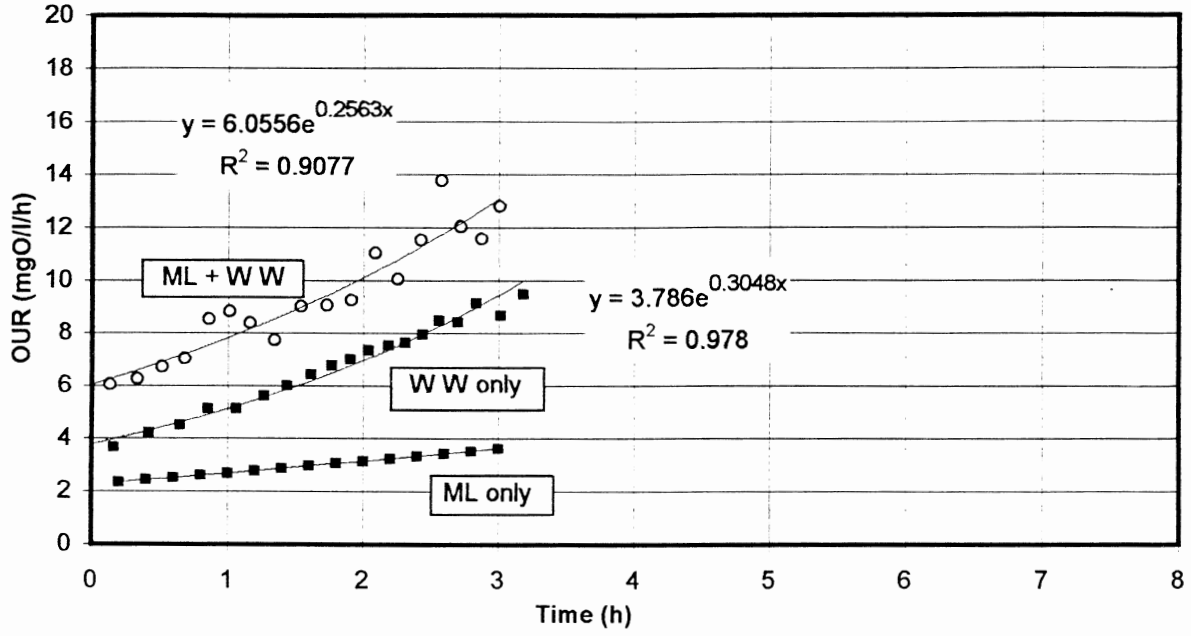
ln(OUR) graph for ML only batch test M10, 08-02, (0.25ℓ ML), wastewater batch no.18



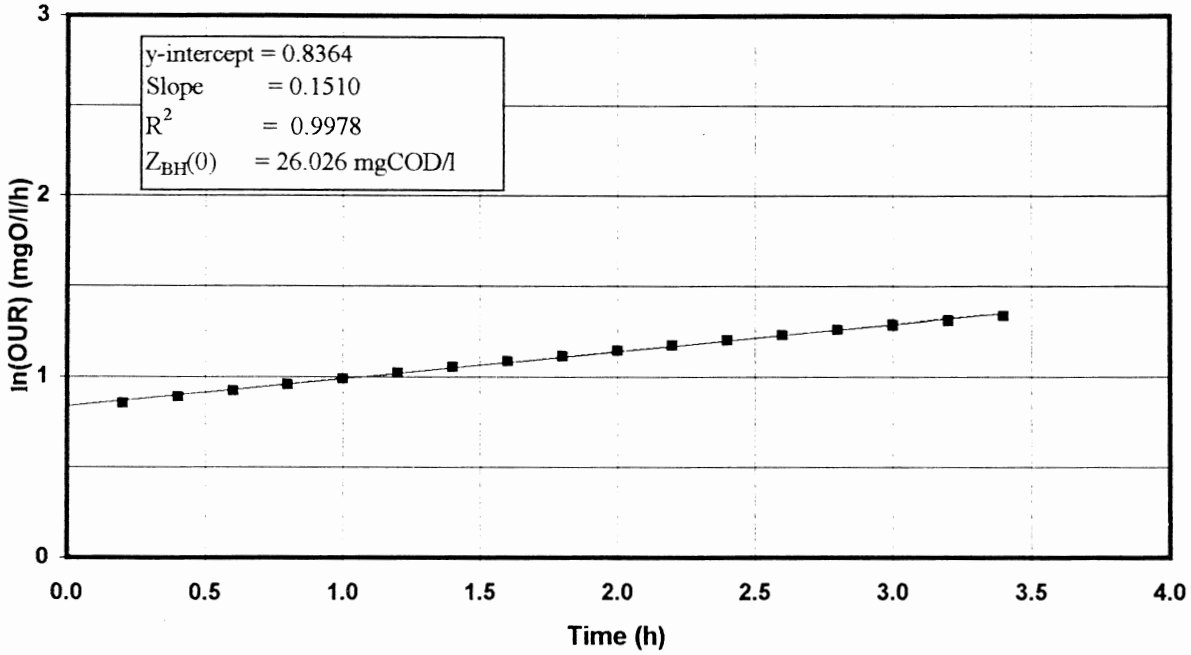
Combined OUR Graph for batch test M11, 09-02, (0.3ℓ ML), wastewater batch no. 18



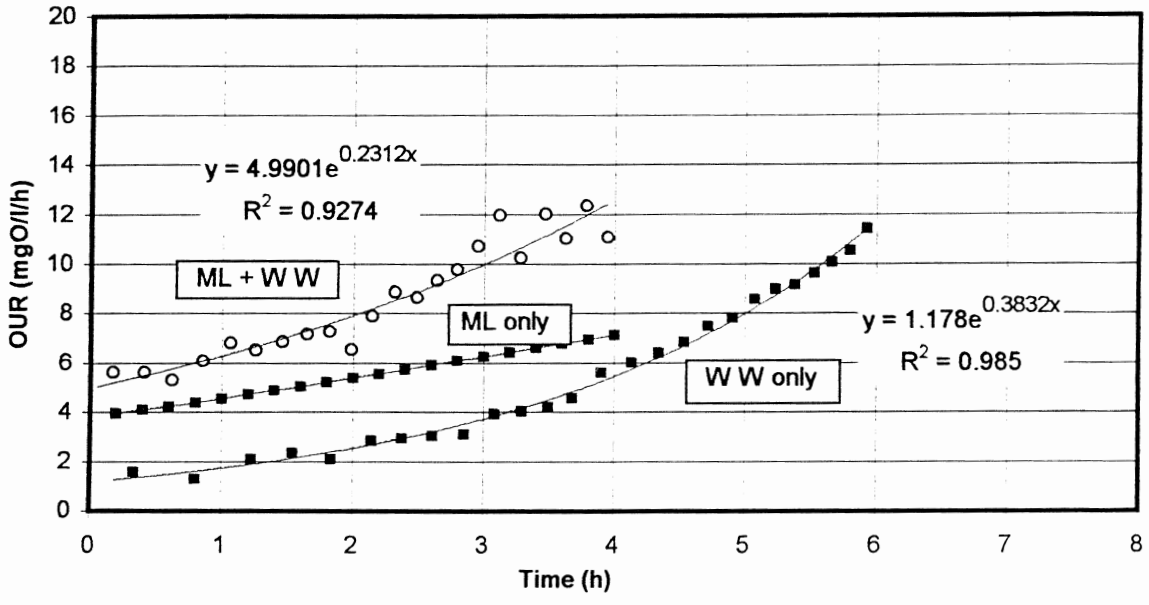
ln(OUR) graph for ML only batch test M11, 09-02, (0.3ℓ ML), wastewater batch no.18



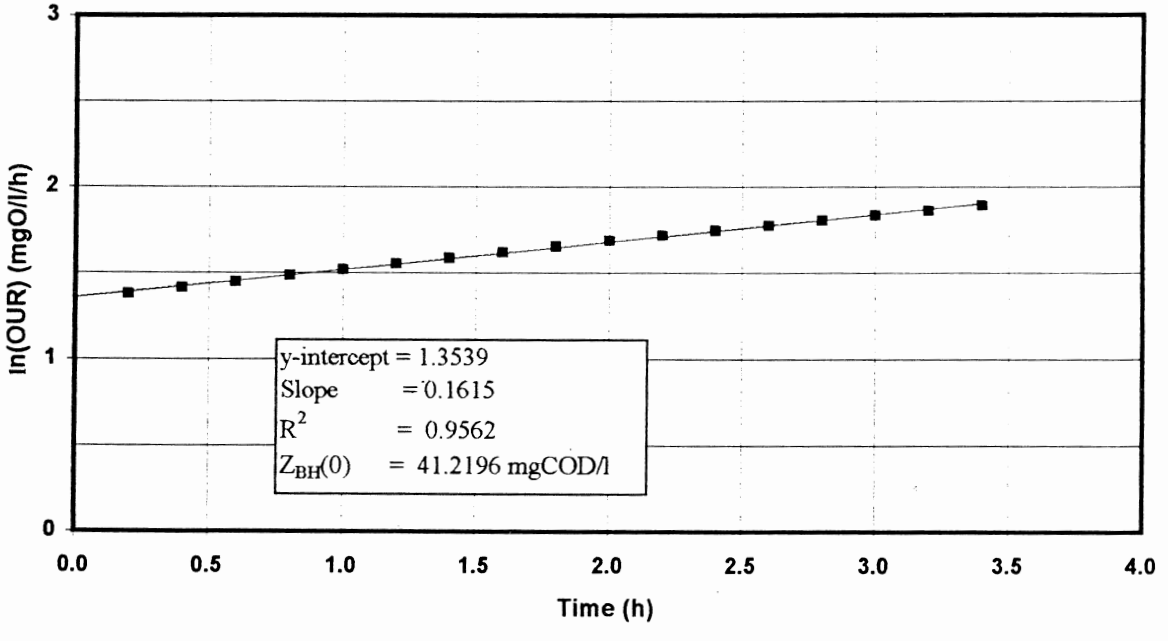
Combined OUR Graph for batch test M12, 10-02, (0.15ℓ ML), wastewater batch no. 18



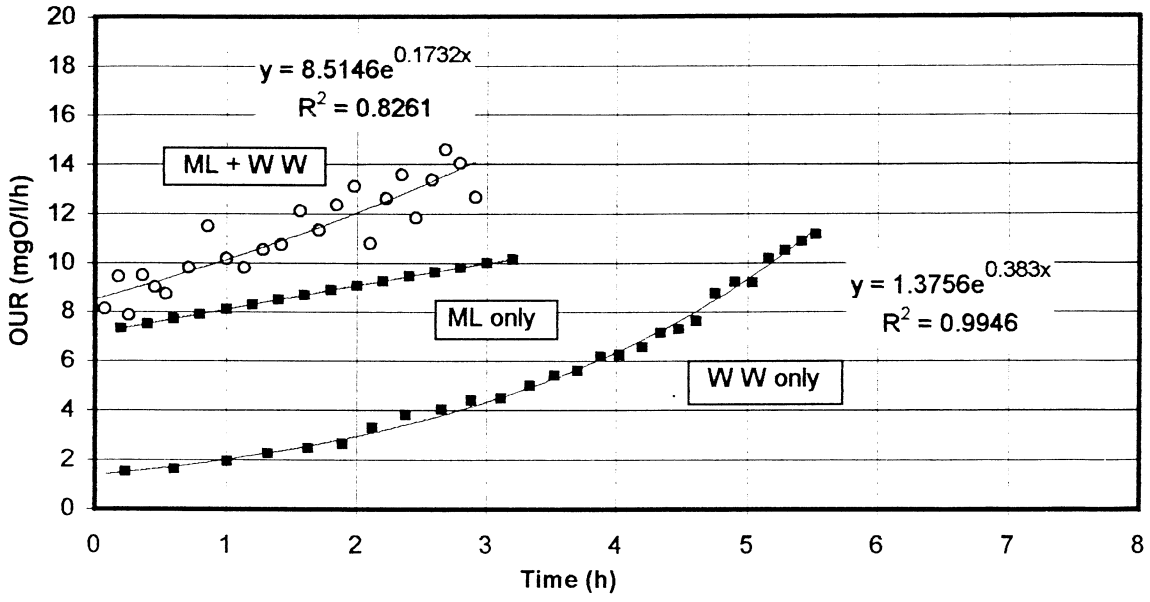
ln(OUR) graph for ML only batch test M12, 10-02, (0.15ℓ ML), wastewater batch no.18



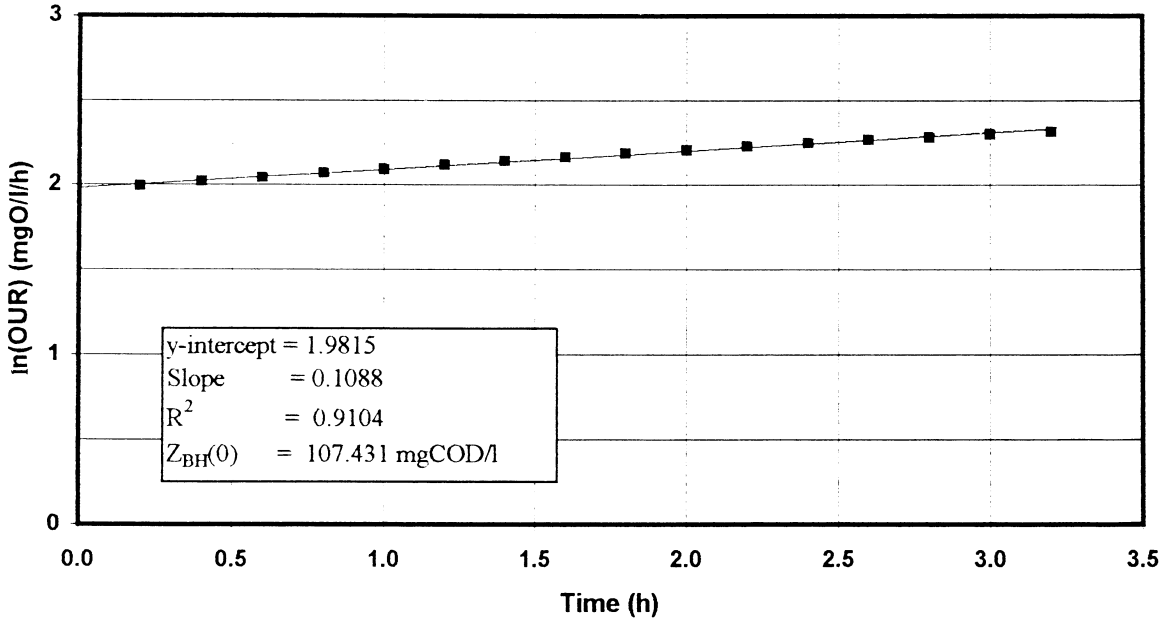
Combined OUR Graph for batch test M13, 15-02, (0.3ℓ ML), wastewater batch no. 19



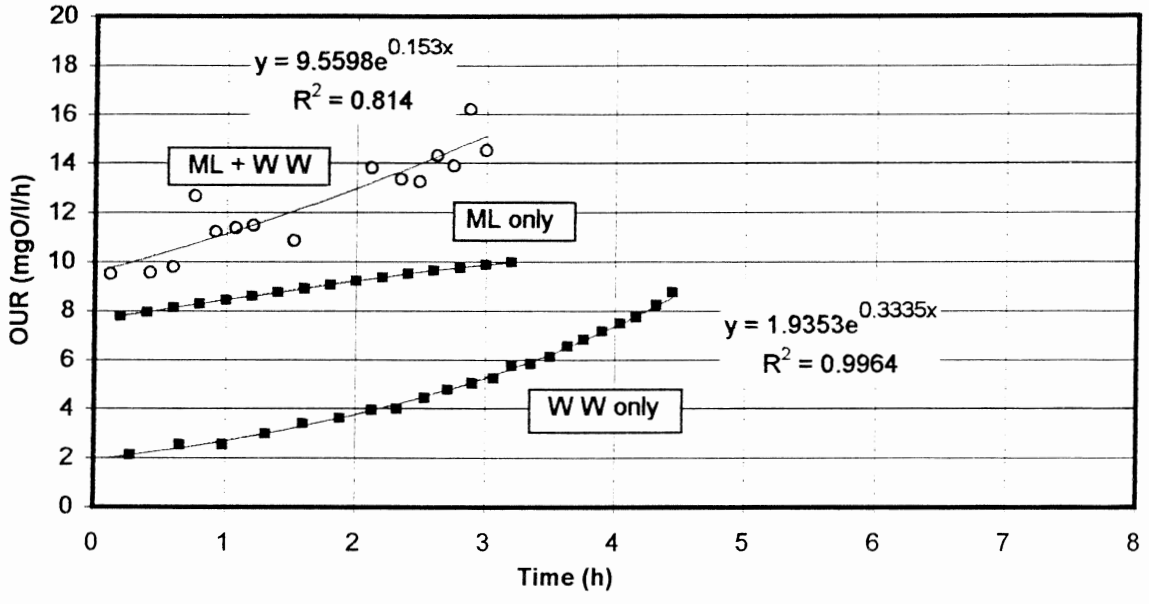
ln(OUR) graph for ML only batch test M13, 15-02, (0.3ℓ ML), wastewater batch no.19



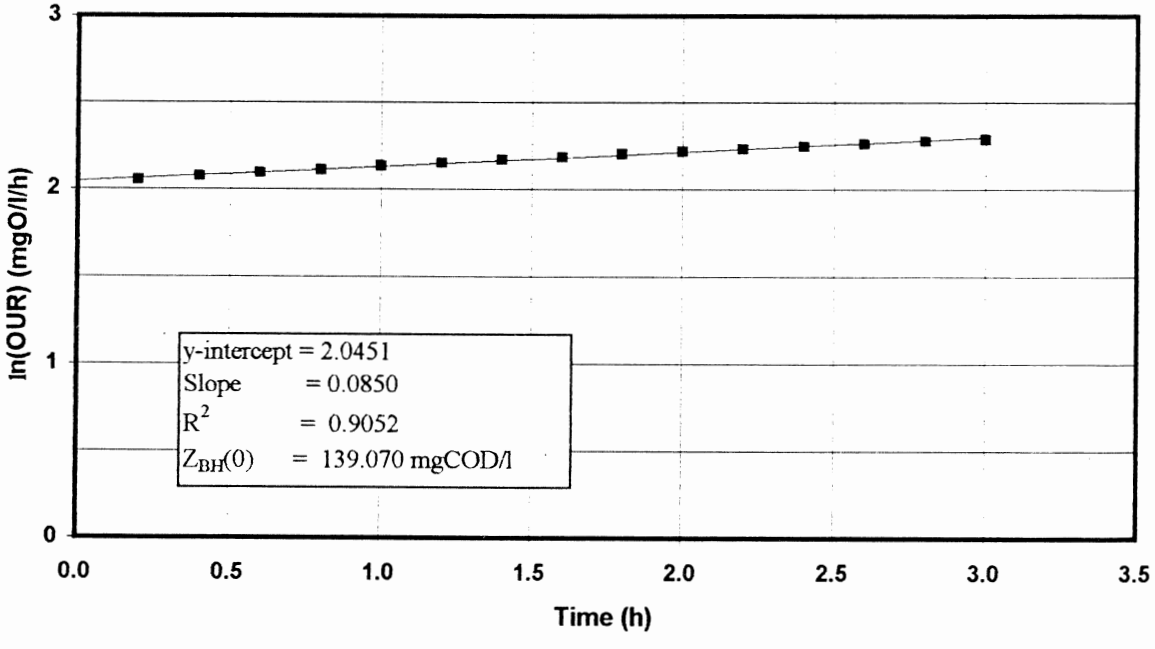
Combined OUR Graph for batch test M14, 16-02, (0.35ℓ ML), wastewater batch no. 19



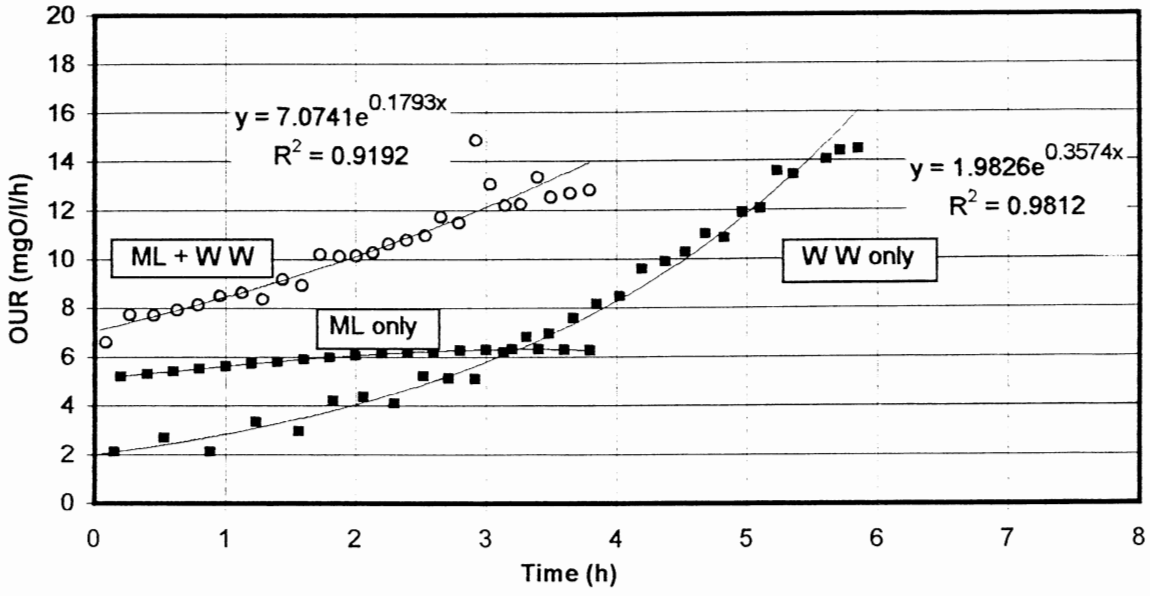
ln(OUR) graph for ML only batch test M14, 16-02, (0.35ℓ ML), wastewater batch no.19



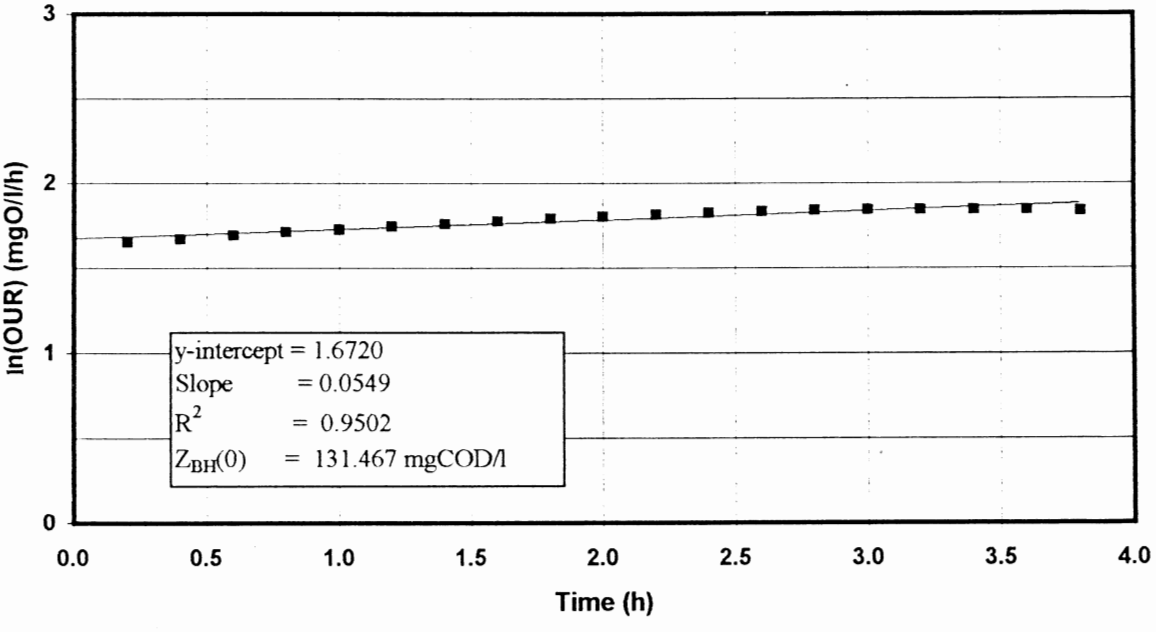
Combined OUR Graph for batch test M15, 17-02, (0.4ℓ ML), wastewater batch no. 19



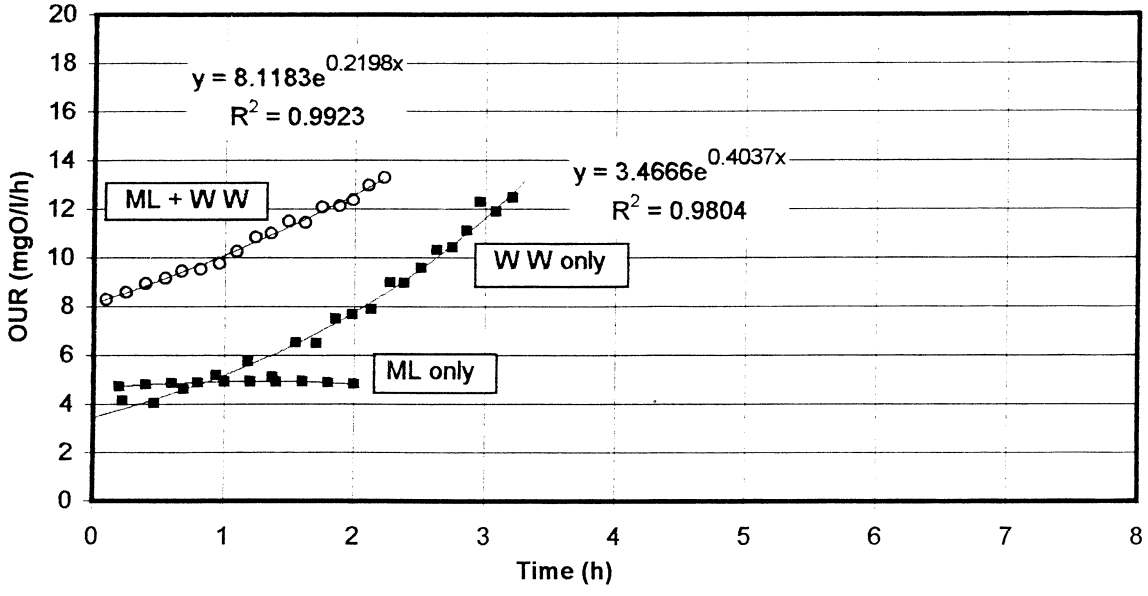
ln(OUR) graph for ML only batch test M15, 17-02, (0.4ℓ ML), wastewater batch no.19



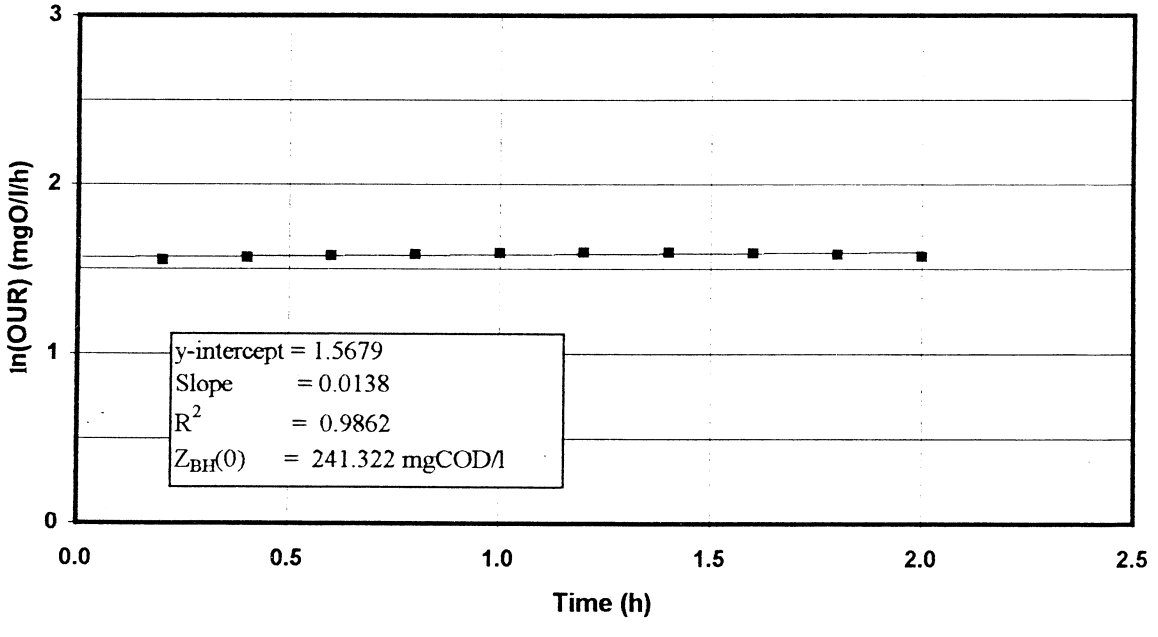
Combined OUR Graph for batch test M16, 22-02, (0.3ℓ ML), wastewater batch no. 19



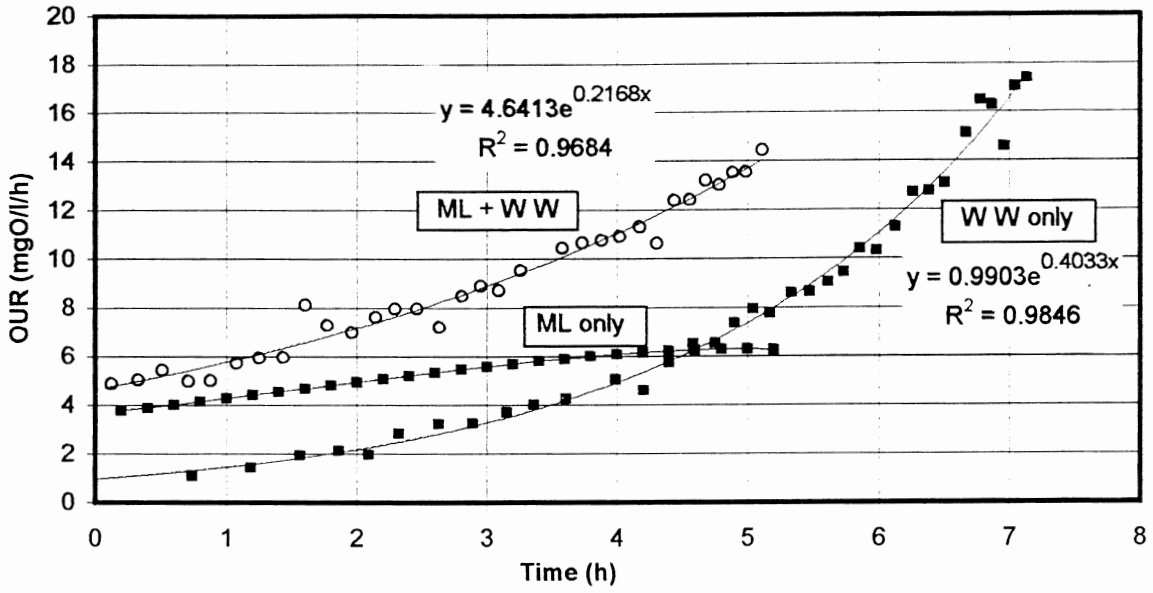
ln(OUR) graph for ML only batch test M16, 22-02, (0.3ℓ ML), wastewater batch no.19



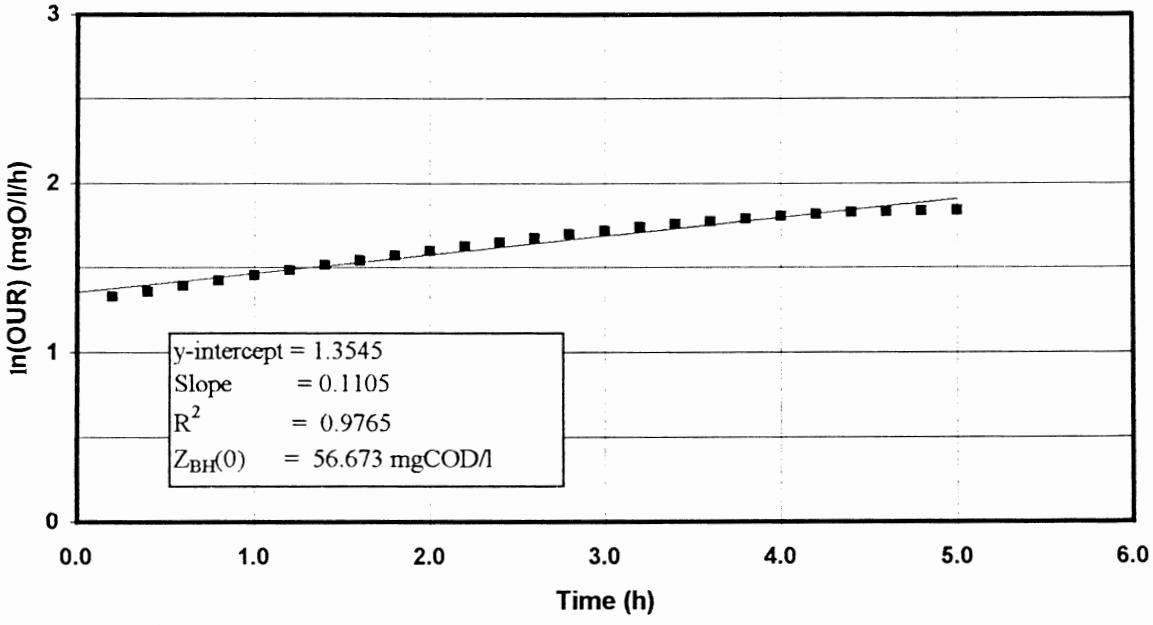
Combined OUR Graph for batch test M17, 23-02, (0.35ℓ ML), wastewater batch no. 19



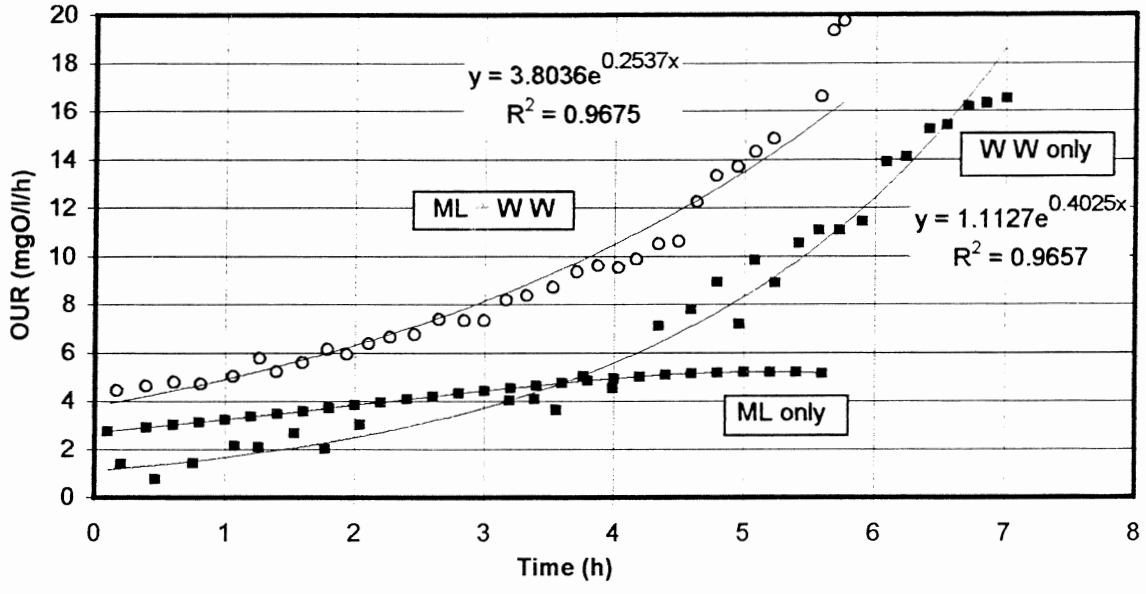
ln(OUR) graph for ML only batch test M17, 23-02, (0.35ℓ ML), wastewater batch no.19



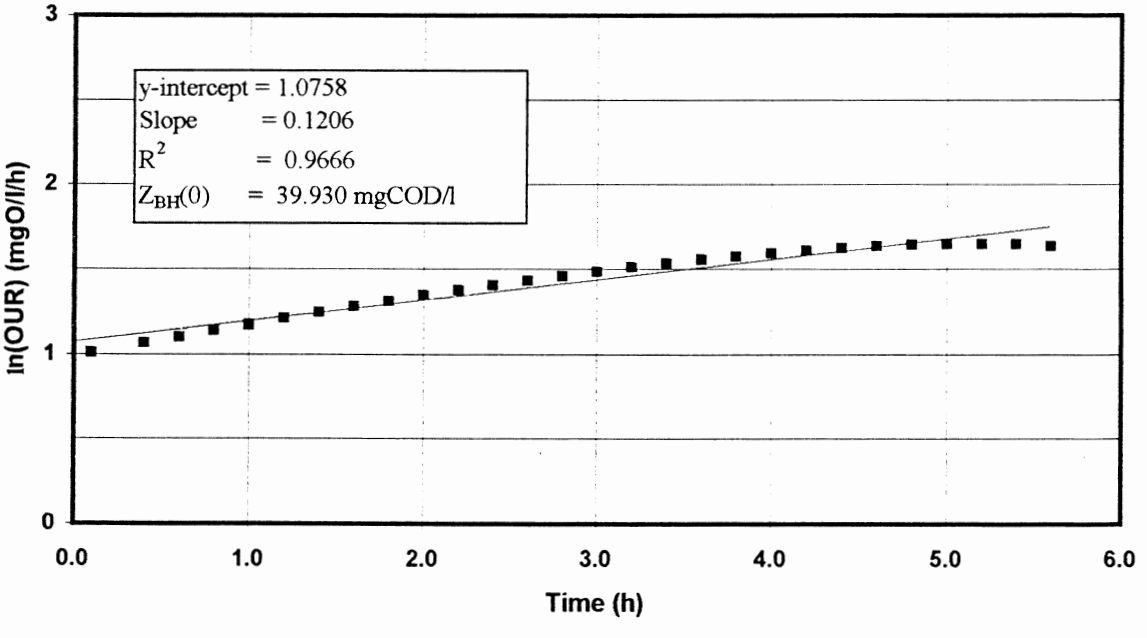
Combined OUR Graph for batch test M18, 29-02, (0.25ℓ ML), wastewater batch no. 20



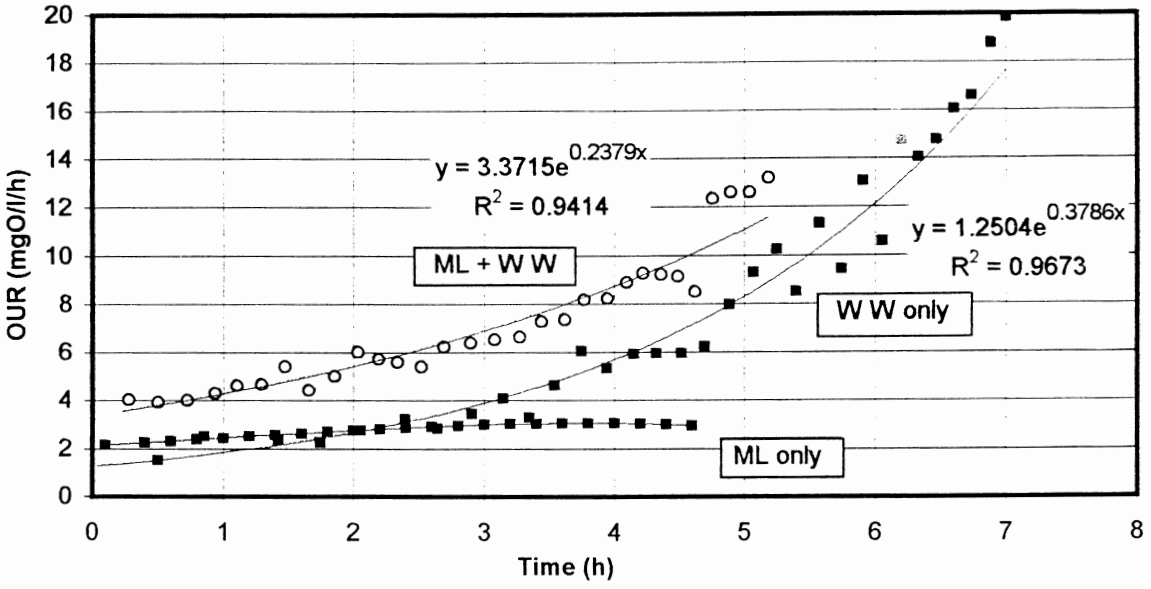
ln(OUR) graph for ML only batch test M18, 29-02, (0.25ℓ ML), wastewater batch no.20



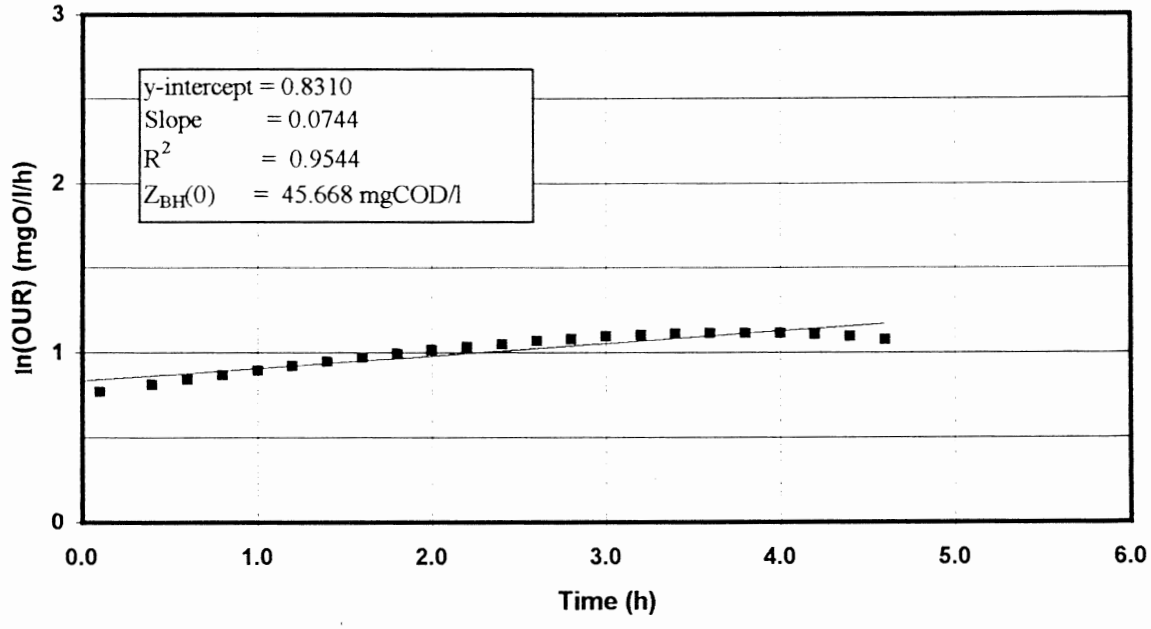
Combined OUR Graph for batch test M19, 01-03, (0.2ℓ ML), wastewater batch no. 20



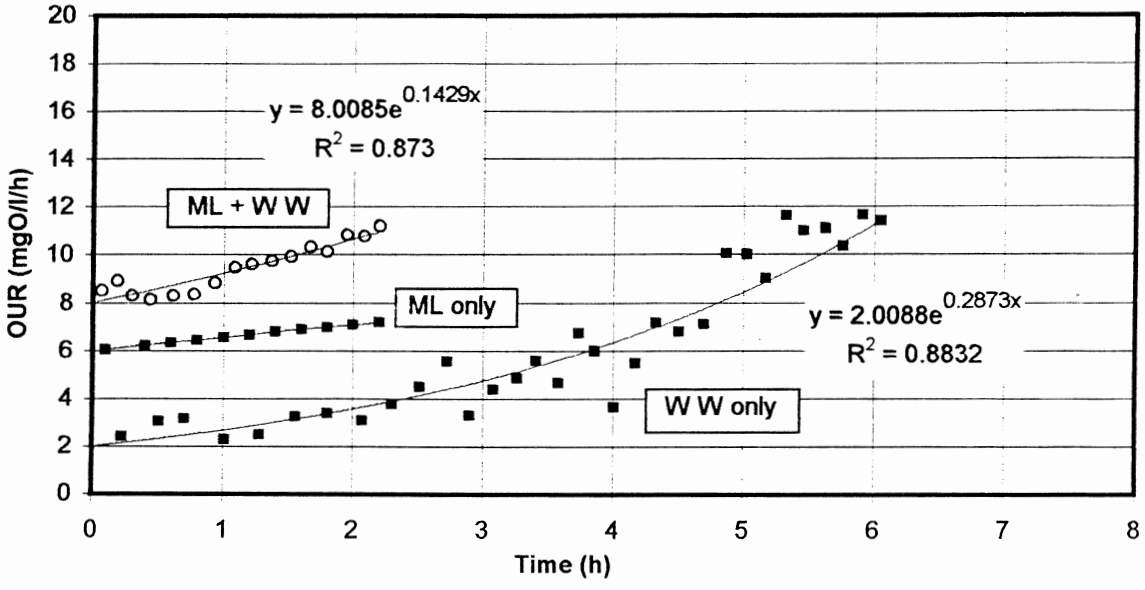
ln(OUR) graph for ML only batch test M19, 01-03, (0.2ℓ ML), wastewater batch no.20



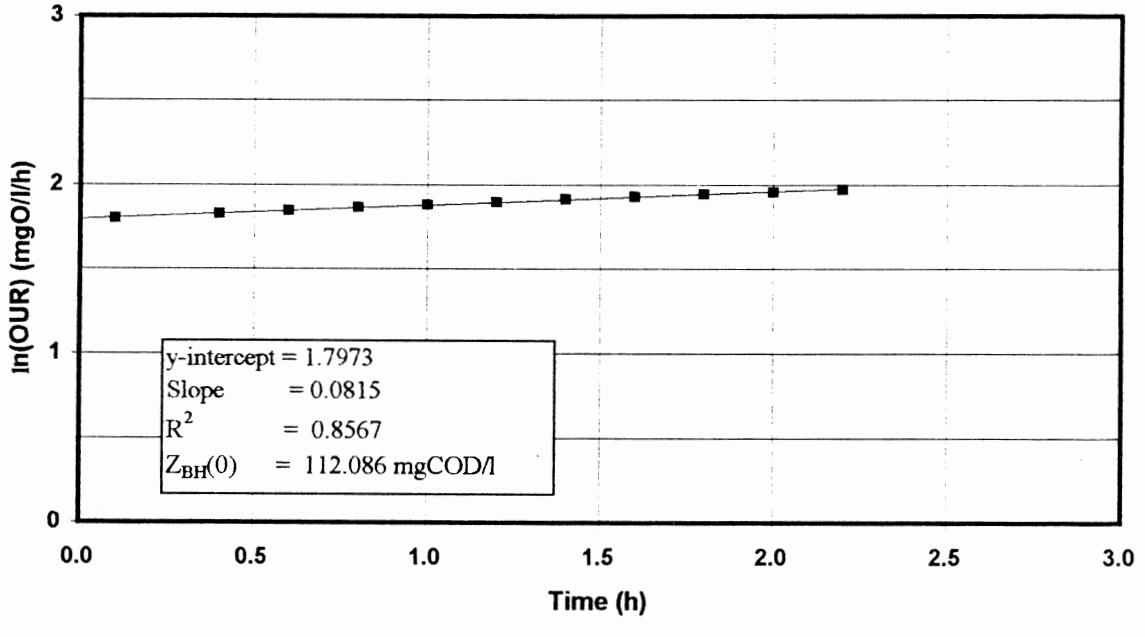
Combined OUR Graph for batch test M20, 02-03, (0.15ℓ ML), wastewater batch no. 20



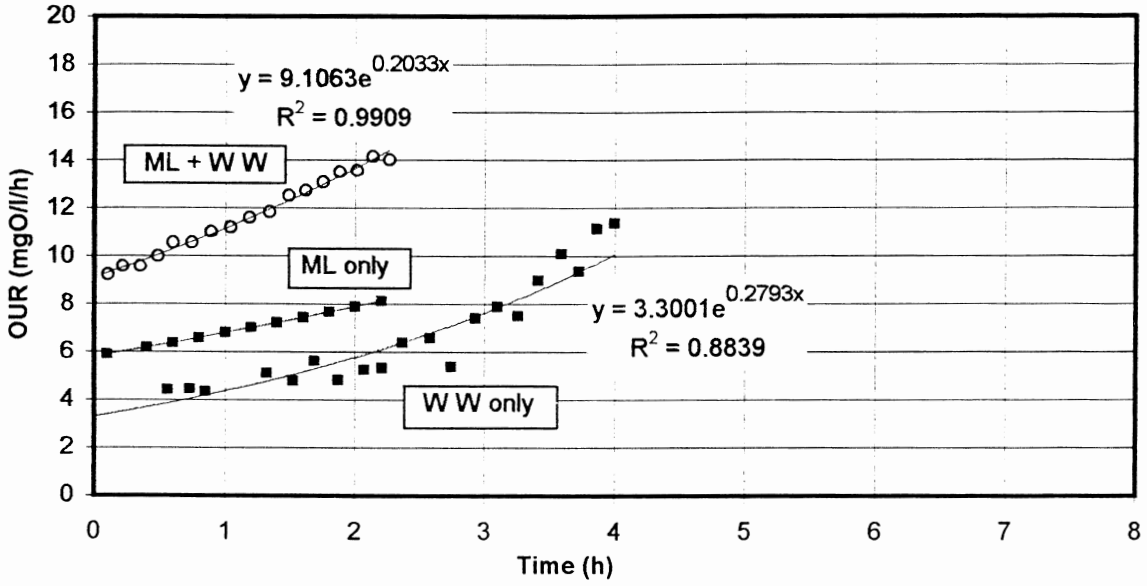
ln(OUR) graph for ML only batch test M20, 02-03, (0.15ℓ ML), wastewater batch no.20



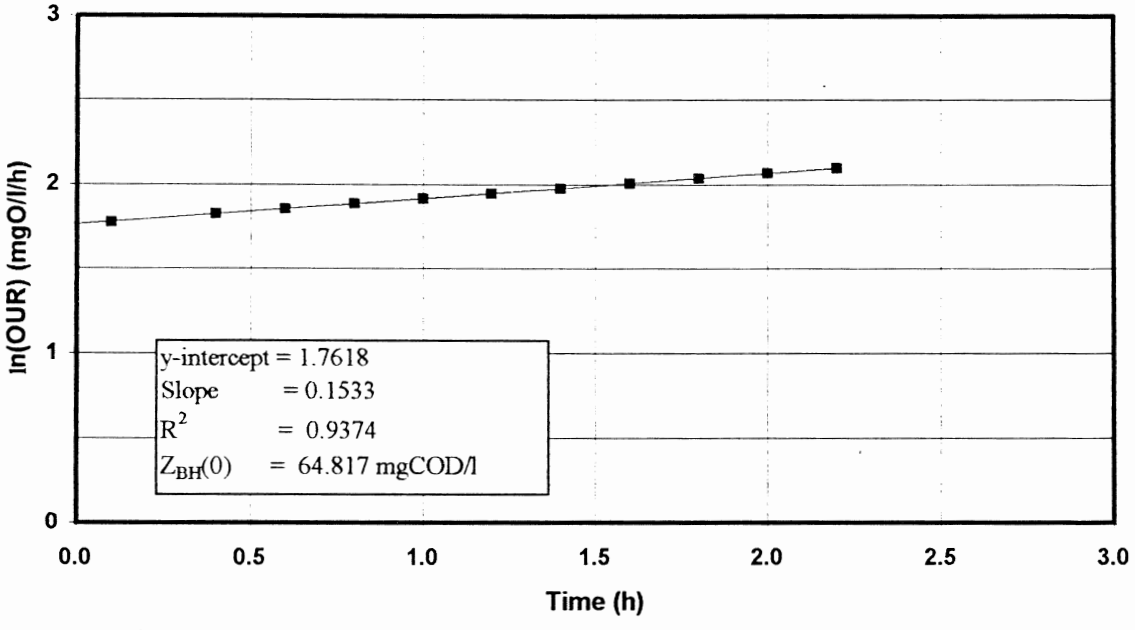
Combined OUR Graph for batch test M21, 06-03, (0.3ℓ ML), wastewater batch no. 20



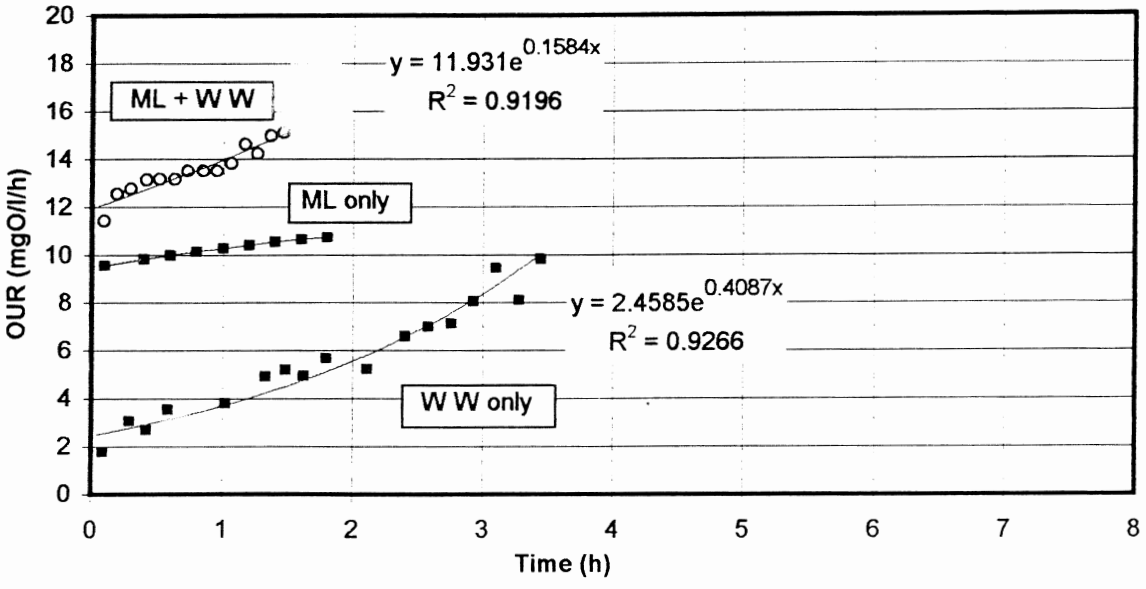
ln(OUR) graph for ML only batch test M21, 06-03, (0.3ℓ ML), wastewater batch no.20



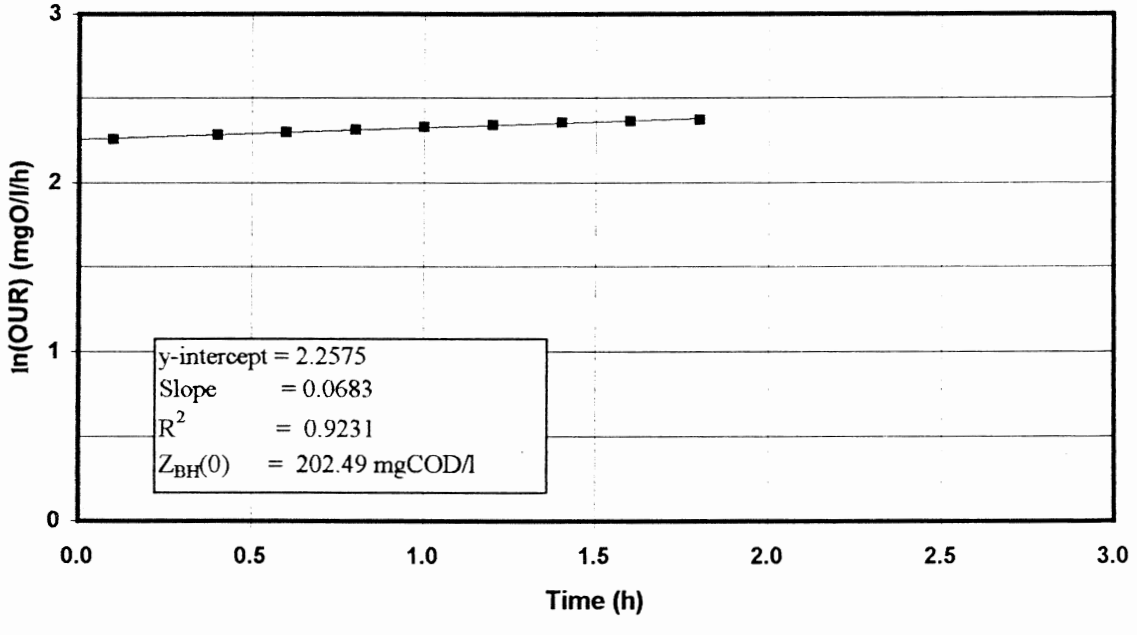
Combined OUR Graph for batch test M22, 07-03, (0.35ℓ ML), wastewater batch no. 20



ln(OUR) graph for ML only batch test M22, 07-03, (0.35ℓ ML), wastewater batch no.20



Combined OUR Graph for batch test M23, 08-03, (0.4ℓ ML), wastewater batch no. 20



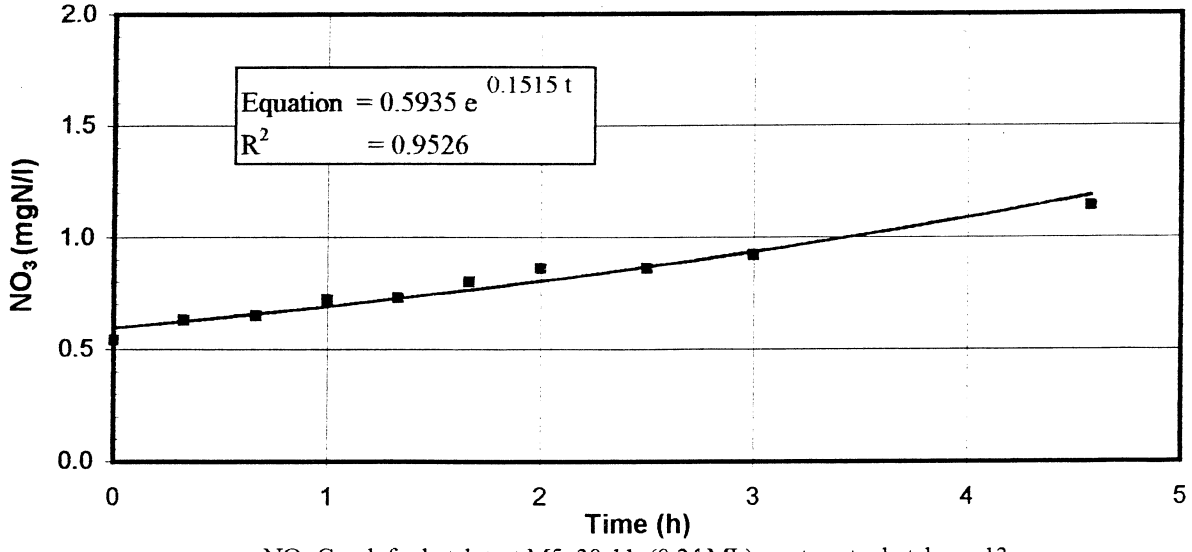
ln(OUR) graph for ML only batch test M23, 08-03, (0.4ℓ ML), wastewater batch no.20

Table A4 : Summary of results for theoretical heterotrophic active biomass due to the mixed liquor addition only ($Z_{BH(0)}ML$), calculated through analytical separation of WW only and (WW+ML) batch test results ***and*** assuming an exponential increase in NO3 concentration in the (WW+ML) batch test.

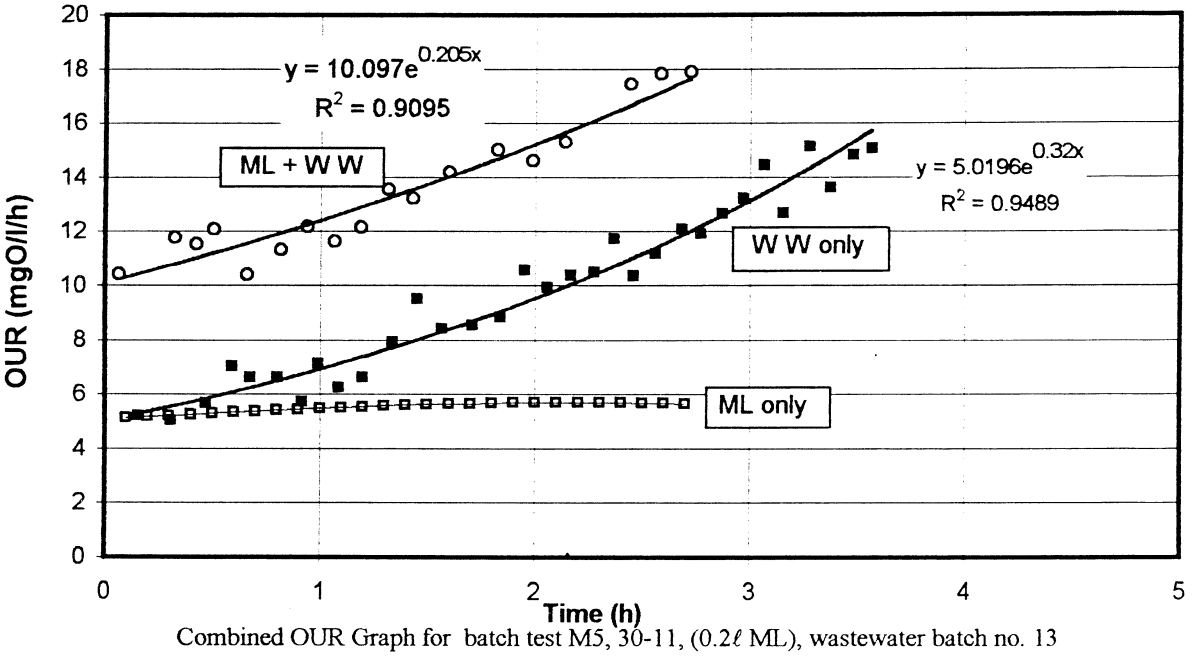
Sew. batch no.	*Batch Test No.*	*Date of test*	*M L vol.*	*%COD Recov.*	*Exponential regression data for nitrification (NO3 vs time)*			*Linear regression data for M L only (ℓn(OUR) vs time)*			$Z_{BH(0)}$ *(M L only) (mgCOD/l)*
					y-int.	*(μ_A - b_A)*	*R^2*	*y-int.*	*slope*	*R^2 **	
11	M1	29-10	0.10ℓ	–	–	–	–	–	–	–	–
	M2	03-11	0.15ℓ	–	–	–	–	–	–	–	–
	M3	05-11	0.30ℓ	–	–	–	–	–	–	–	–
12	M4	15-11	0.30ℓ	–	–	–	–	–	–	–	–
13	M5	30-11	0.20ℓ	102	0.5935	0.1515	0.9526	1.6525	0.0399	0.9292	158.348
	M6	01-12	0.10ℓ	90	0.3785	0.1313	0.9802	0.4969	0.3257	0.9778	9.323
	M7	03-12	0.25ℓ	91	0.5803	0.2619	0.9548	1.5443	0.0277	0.9092	174.496
14	M8	08-12	0.20ℓ	93	0.8540	0.1097	0.9847	1.0159	0.0964	0.9696	45.054
18	M9	07-02	0.20ℓ	103	0.9838	0.0859	0.9168	1.2726	0.1549	0.9606	39.389
	M10	08-02	0.25ℓ	106	0.8179	0.1251	0.9700	1.6561	0.1070	0.9388	78.642
	M11	09-02	0.30ℓ	101	1.1255	0.0627	0.6280	1.7218	0.1572	0.9617	60.949
	M12	10-02	0.15ℓ	100	0.6804	0.1038	0.8582	0.9031	0.0965	0.9498	40.216
19	M13	15-02	0.30ℓ	103	1.1171	0.1560	0.9544	1.4604	0.1239	0.9485	57.366
	M14	16-02	0.35ℓ	95	1.5464	0.1775	0.9386	2.0591	0.0787	0.8845	149.530
	M15	17-02	0.40ℓ	99	1.7460	0.1470	0.9839	2.1173	0.0613	0.8865	190.141
	M16	22-02	0.30ℓ	94	2.0069	0.1059	0.9312	1.7527	0.0369	0.9442	183.408
	M17	23-02	0.35ℓ	99	2.1718	0.1283	0.9150	1.6117	0.0244	0.9712	198.924
20	M18	29-02	0.25ℓ	94	0.9340	0.1502	0.9798	1.4566	0.0813	0.9742	79.872
	M19	01-03	0.20ℓ	97	0.5978	0.1198	0.9176	1.1124	0.1124	0.9644	43.876
	M20	02-03	0.15ℓ	91	0.1770	0.2241	0.9466	0.9070	0.0584	0.9500	58.634
	M21	06-03	0.30ℓ	97	0.7295	0.1803	0.9114	1.8505	0.0603	0.8676	147.305
	M22	07-03	0.35ℓ	100	1.1431	0.2487	0.9825	1.8584	0.0905	0.9354	109.930
	M23	08-03	0.40ℓ	108	1.2029	0.2401	0.9789	2.3058	0.0493	0.9130	266.251

* R^2 is ***average*** between R^2 for "best fit" ML+WW exponential curve and R^2 for "best fit" WW only exponential curve - see graphs below.

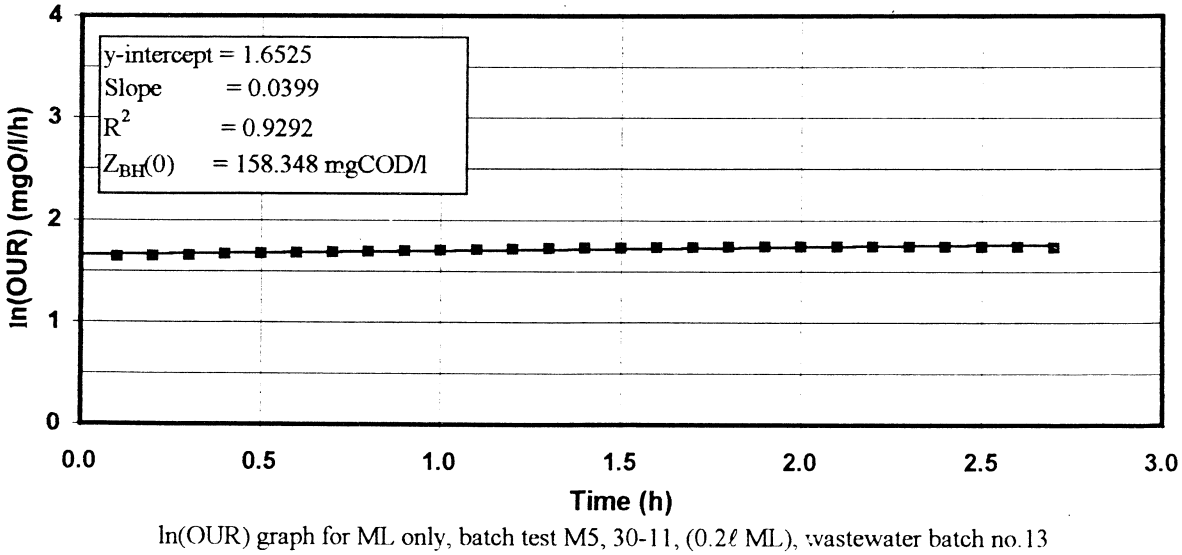
– 3ℓ of wastewater used in WW only batch tests - direct comparison of OUR data for WW only and WW+ML batch tests not possible.



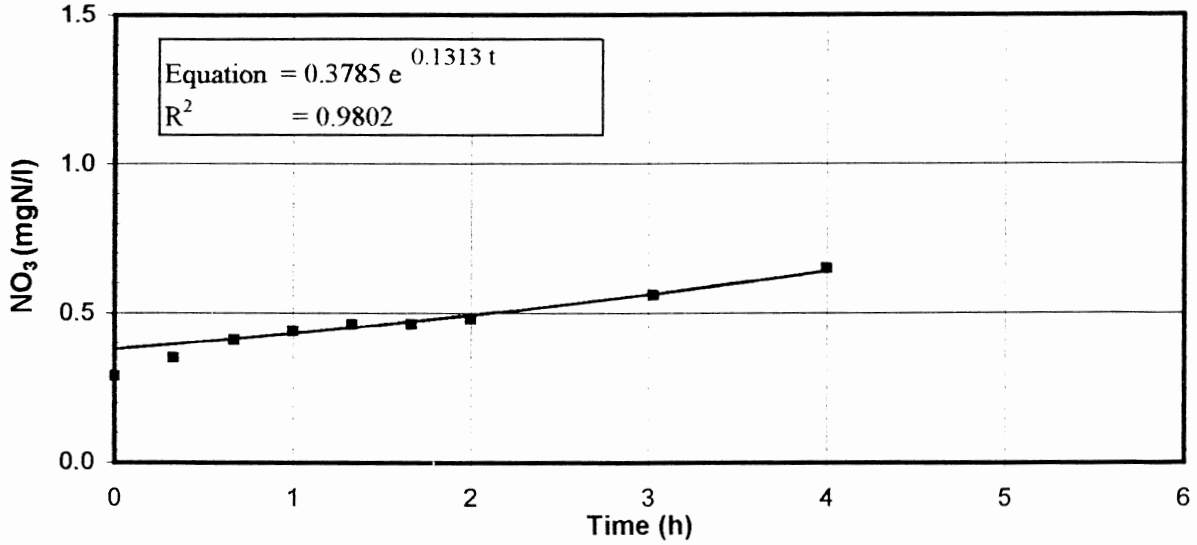
NO_3 Graph for batch test M5, 30-11, (0.2ℓ ML) wastewater batch no. 13



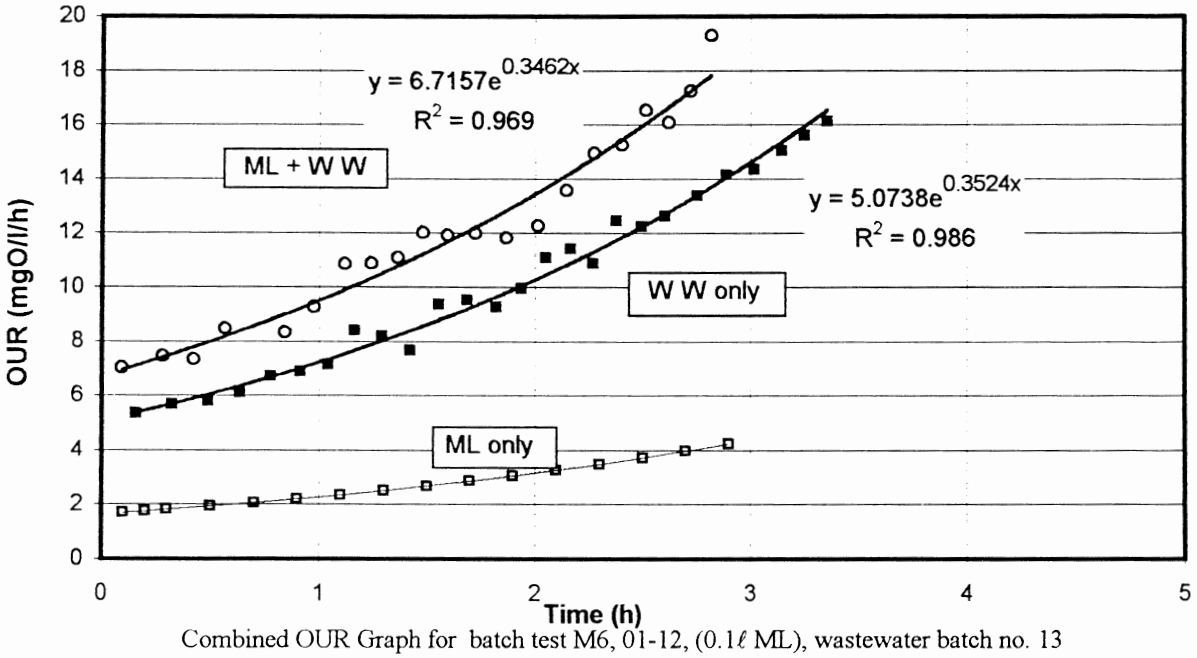
Combined OUR Graph for batch test M5, 30-11, (0.2ℓ ML), wastewater batch no. 13



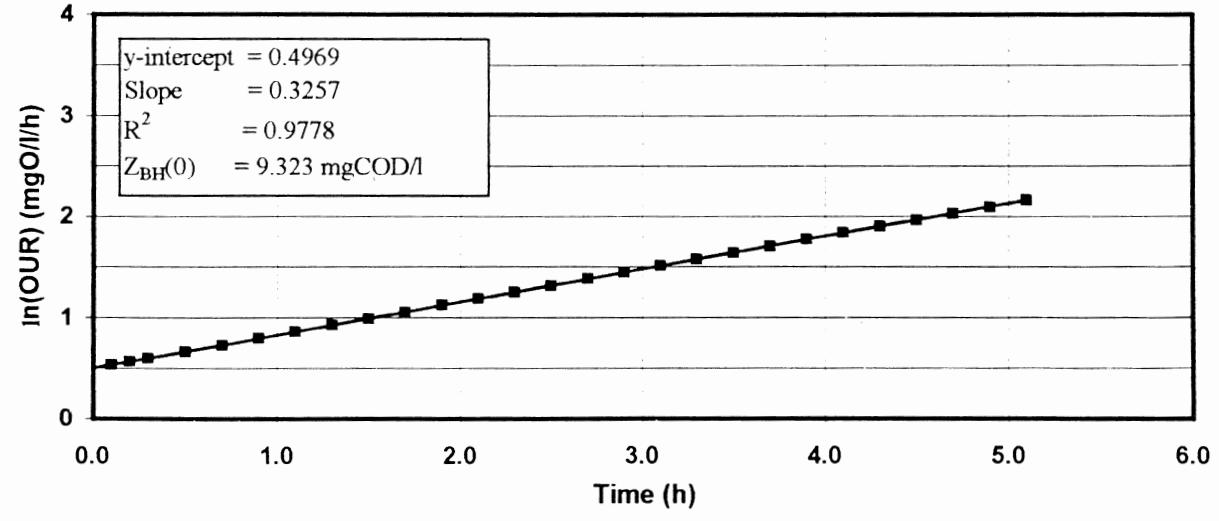
ln(OUR) graph for ML only, batch test M5, 30-11, (0.2ℓ ML), wastewater batch no.13



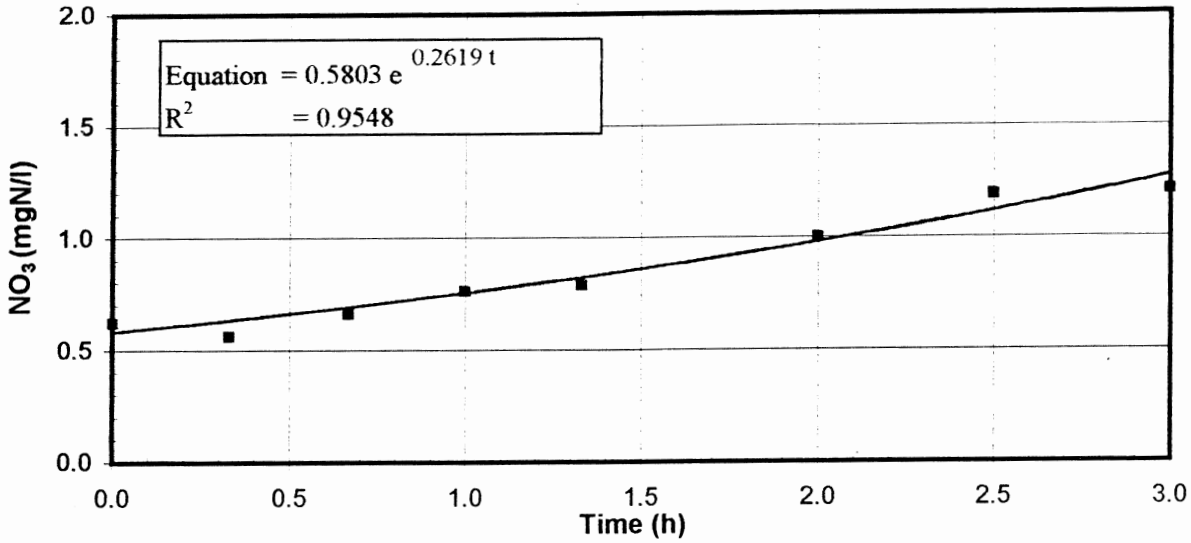
NO_3 Graph for batch test M6, 01-12, (0.1ℓ ML) wastewater batch no. 13



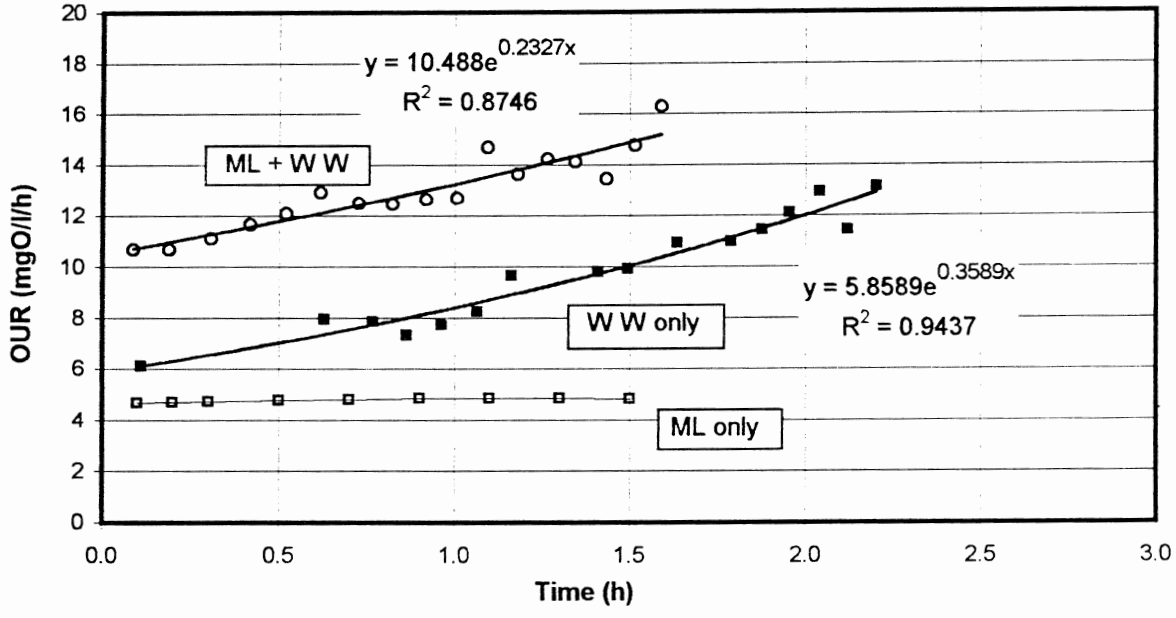
Combined OUR Graph for batch test M6, 01-12, (0.1ℓ ML), wastewater batch no. 13



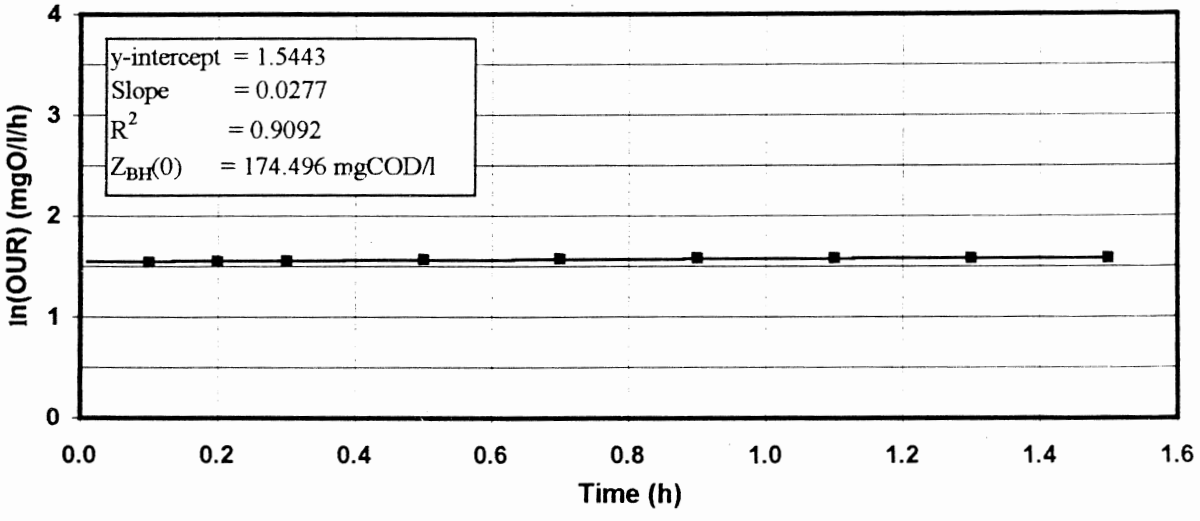
ln(OUR) graph for ML only, batch test M6, 01-12, (0.1ℓ ML), wastewater batch no.13



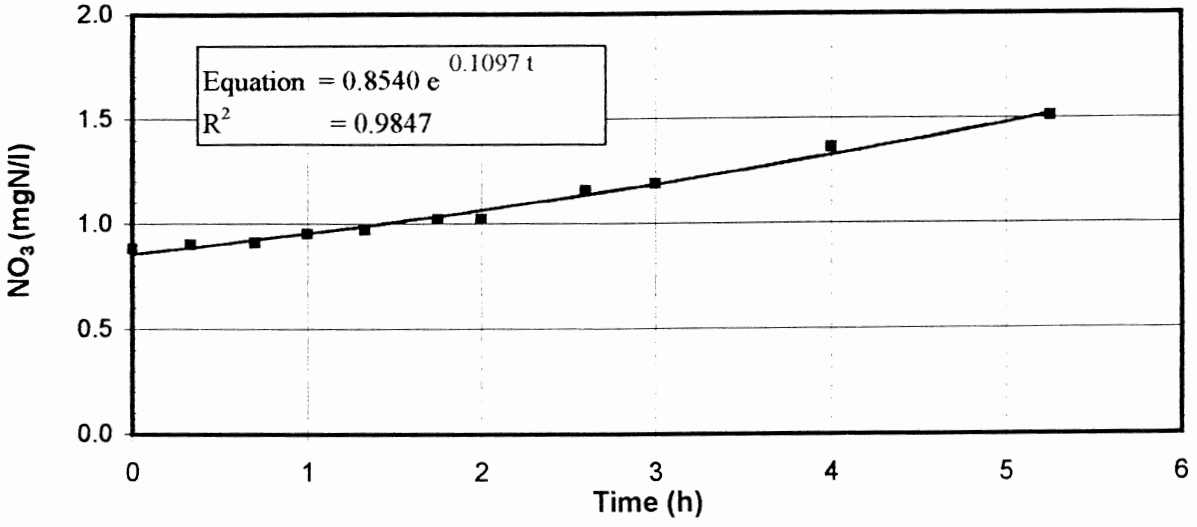
NO_3 Graph for batch test M7, 03-12, (0.25ℓ ML) wastewater batch no. 13



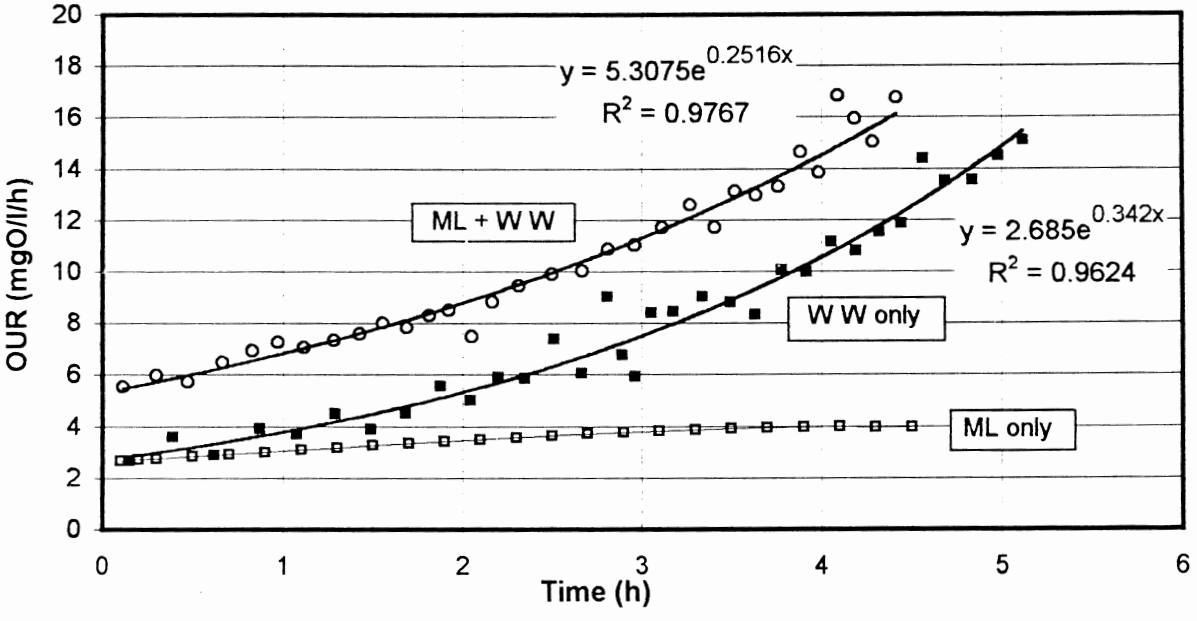
Combined OUR Graph for batch test M7, 03-12, (0.25ℓ ML), wastewater batch no. 13



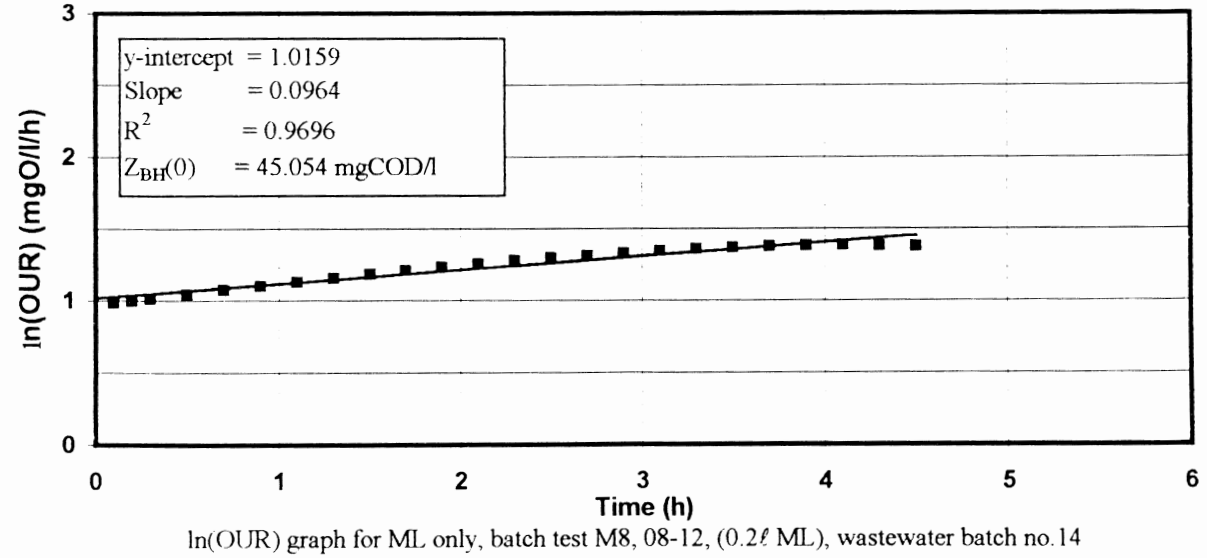
ln(OUR) graph for ML only, batch test M7, 03-12, (0.25ℓ ML), wastewater batch no.13



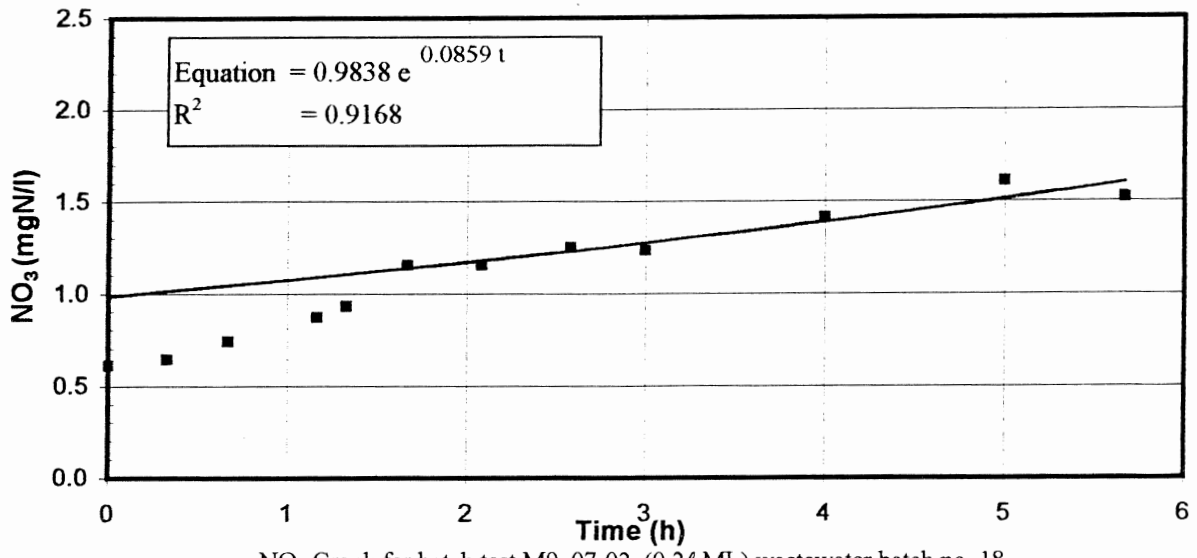
NO_3 Graph for batch test M8, 08-12, (0.2ℓ ML) wastewater batch no. 14



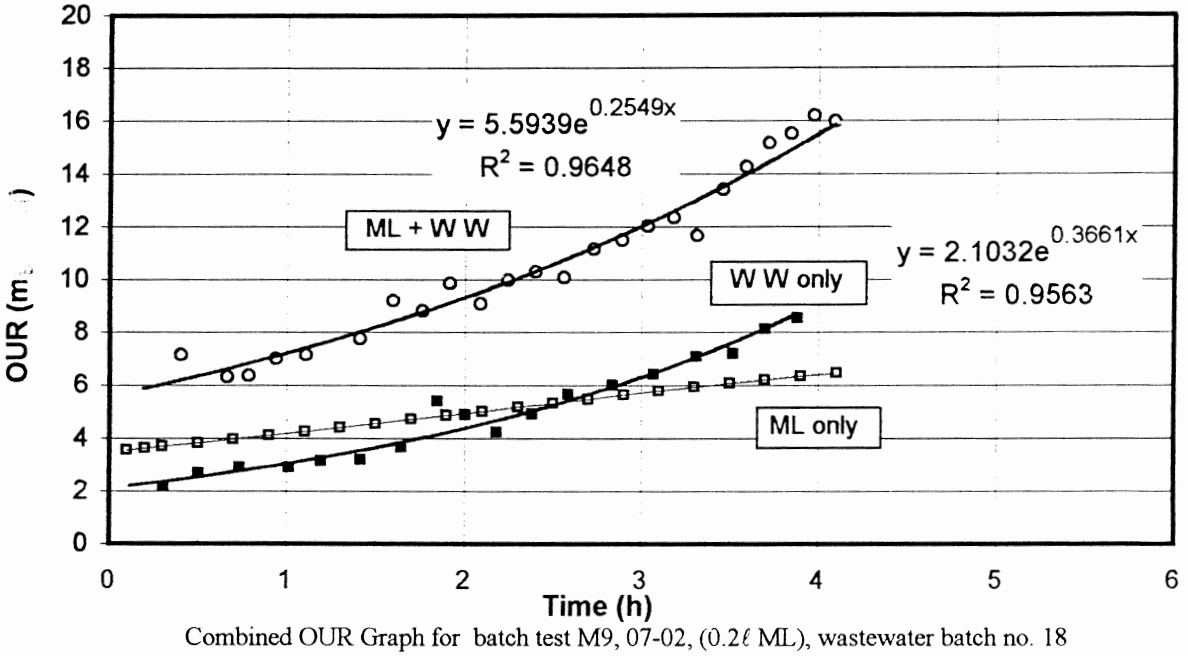
Combined OUR Graph for batch test M8, 08-12, (0.2ℓ ML), wastewater batch no. 14



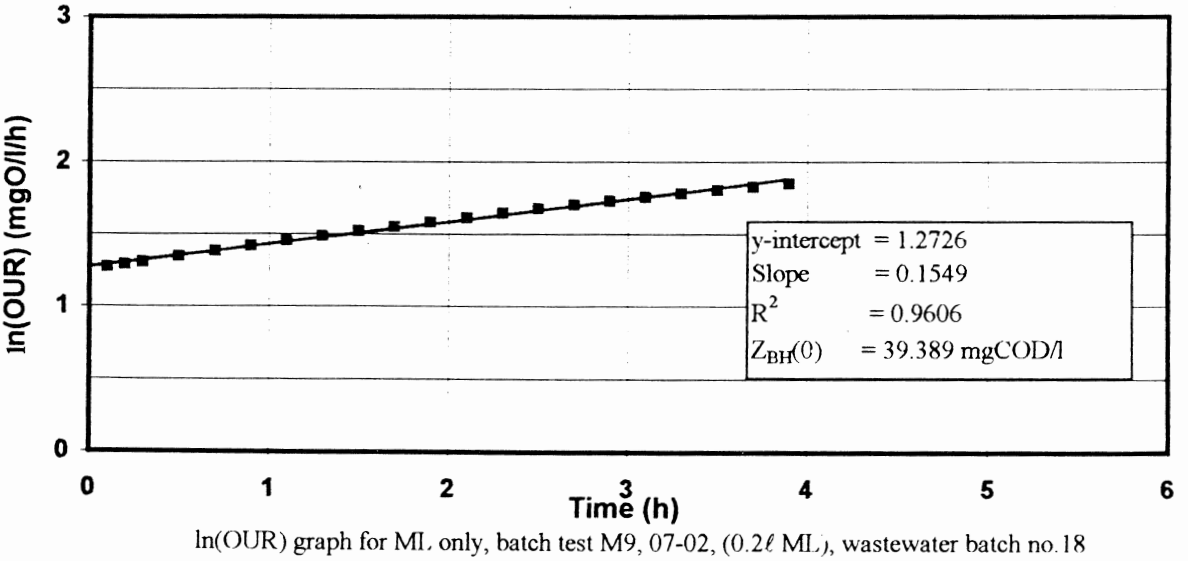
ln(OUR) graph for ML only, batch test M8, 08-12, (0.2ℓ ML), wastewater batch no.14



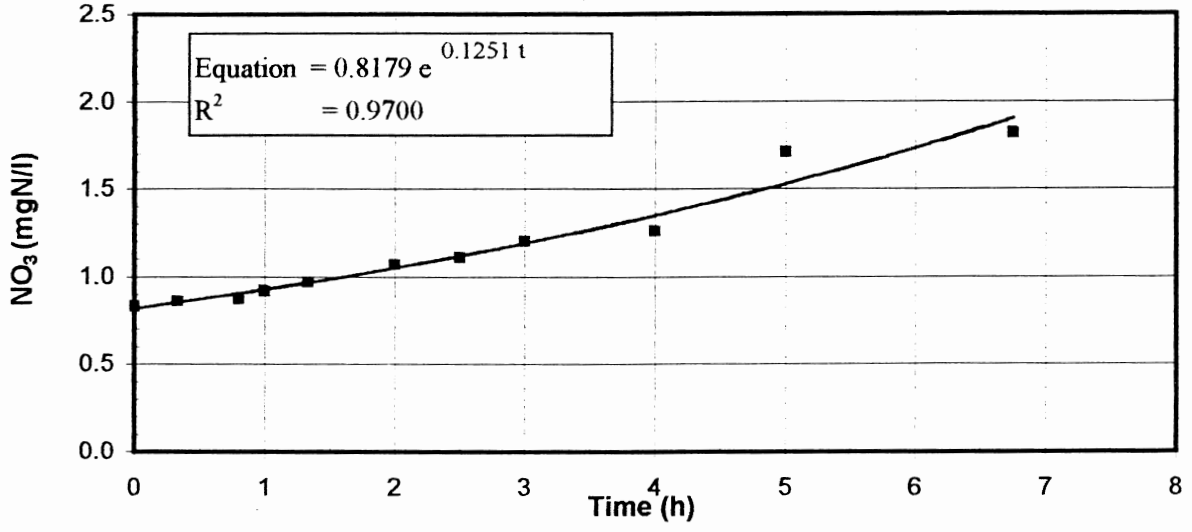
NO_3 Graph for batch test M9, 07-02, (0.2ℓ ML) wastewater batch no. 18



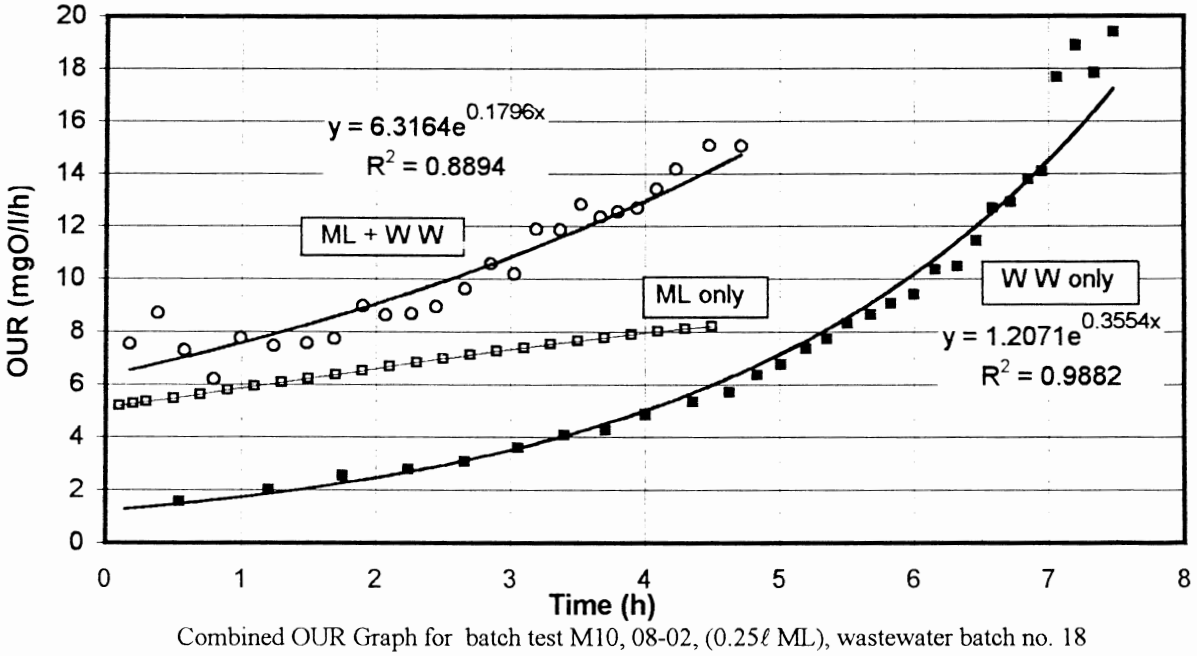
Combined OUR Graph for batch test M9, 07-02, (0.2ℓ ML), wastewater batch no. 18



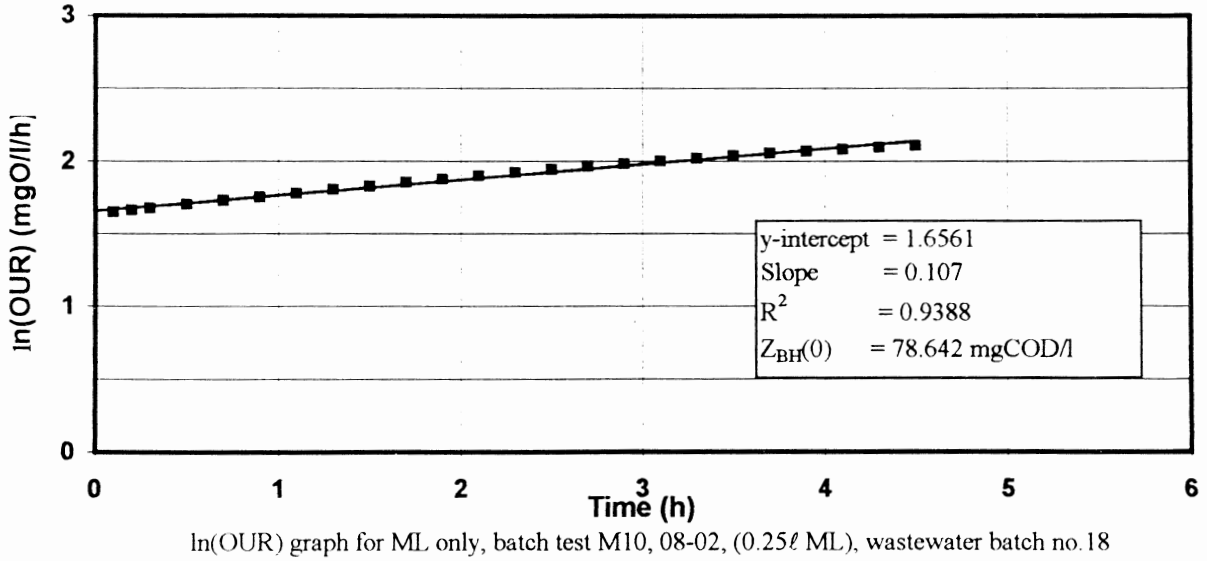
ln(OUR) graph for ML only, batch test M9, 07-02, (0.2ℓ ML), wastewater batch no.18



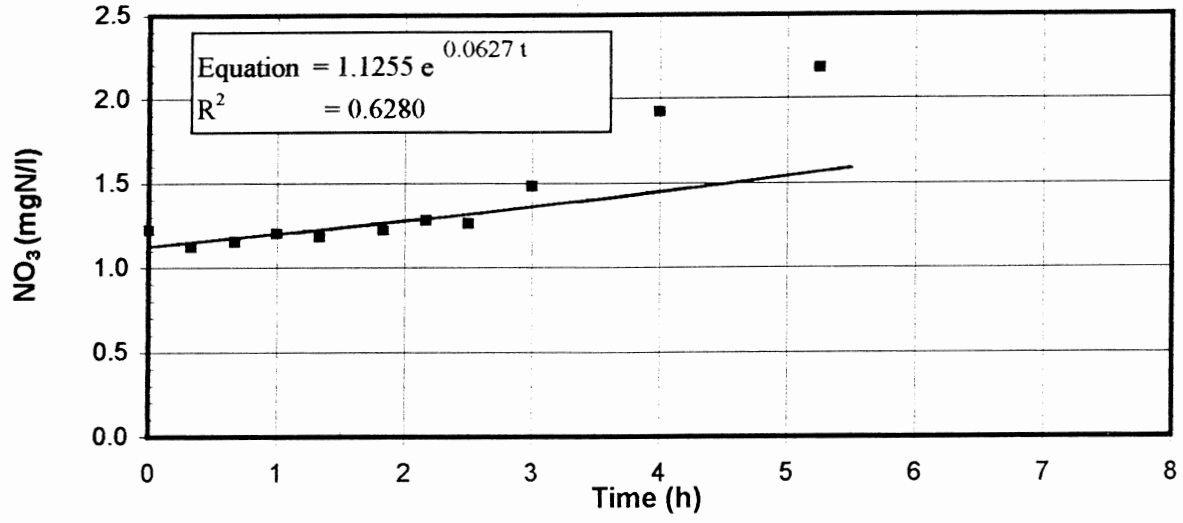
NO_3 Graph for batch test M10, 08-02, (0.25ℓ ML) wastewater batch no. 18



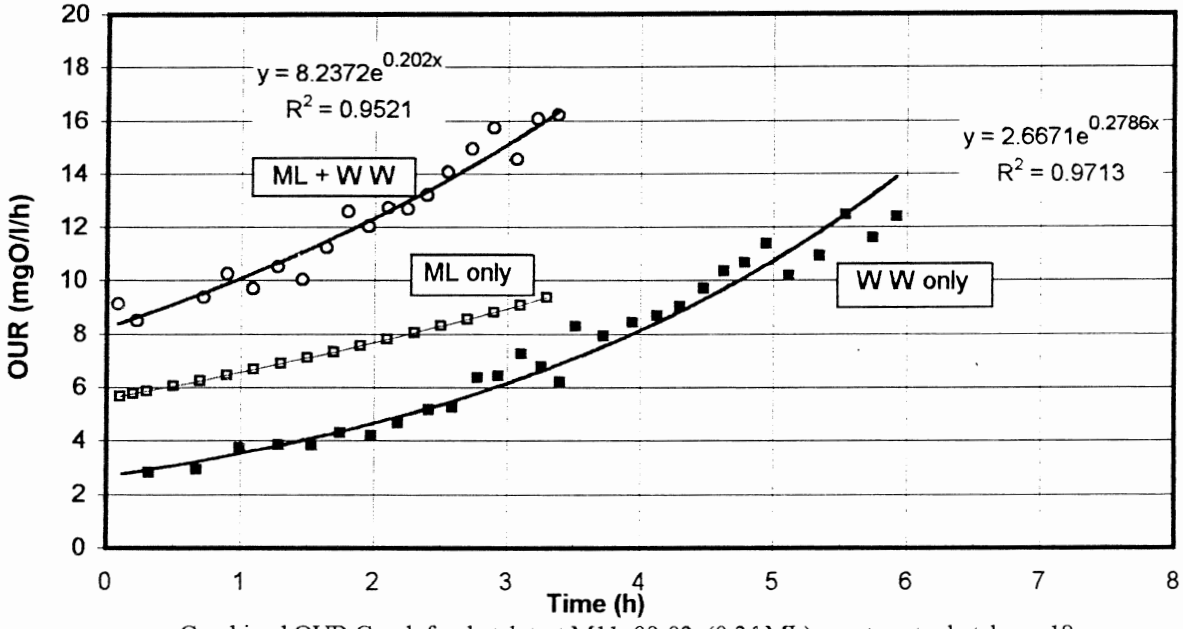
Combined OUR Graph for batch test M10, 08-02, (0.25ℓ ML), wastewater batch no. 18



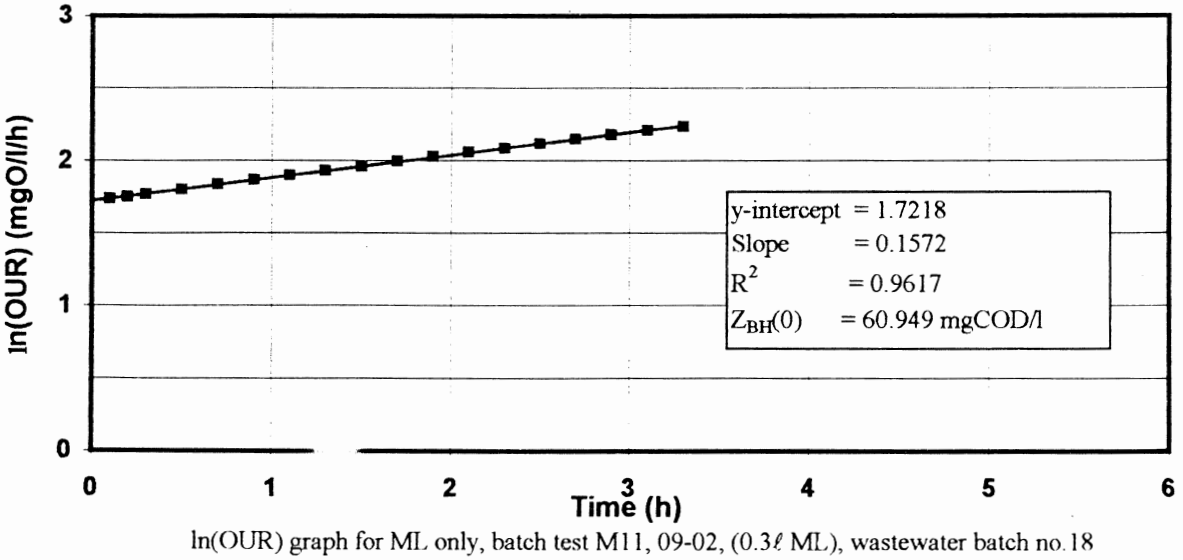
ln(OUR) graph for ML only, batch test M10, 08-02, (0.25ℓ ML), wastewater batch no.18



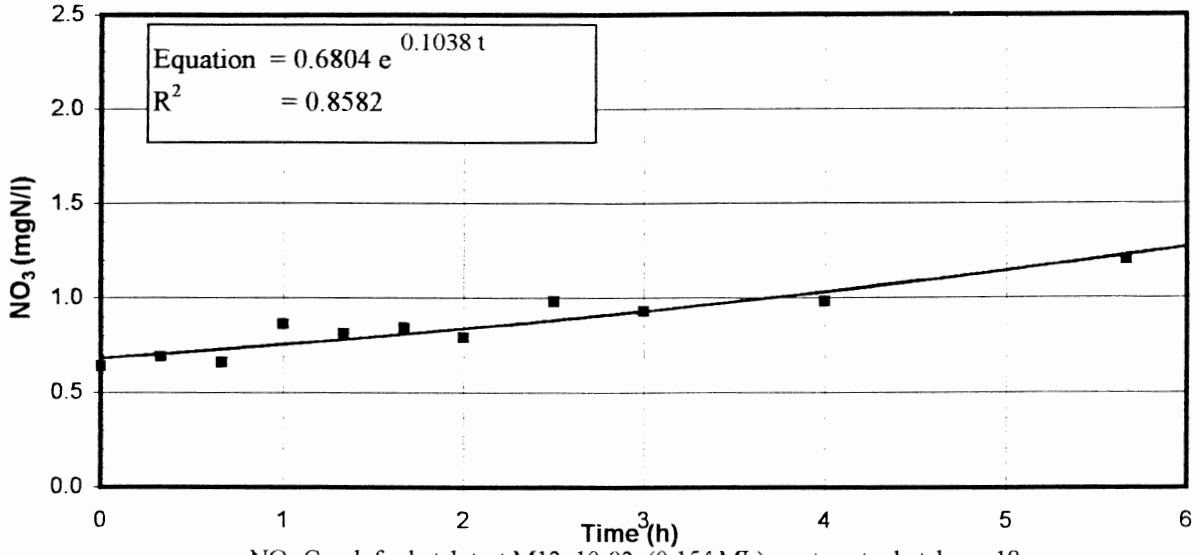
NO_3 Graph for batch test M11, 09-02, (0.3ℓ ML) wastewater batch no. 18



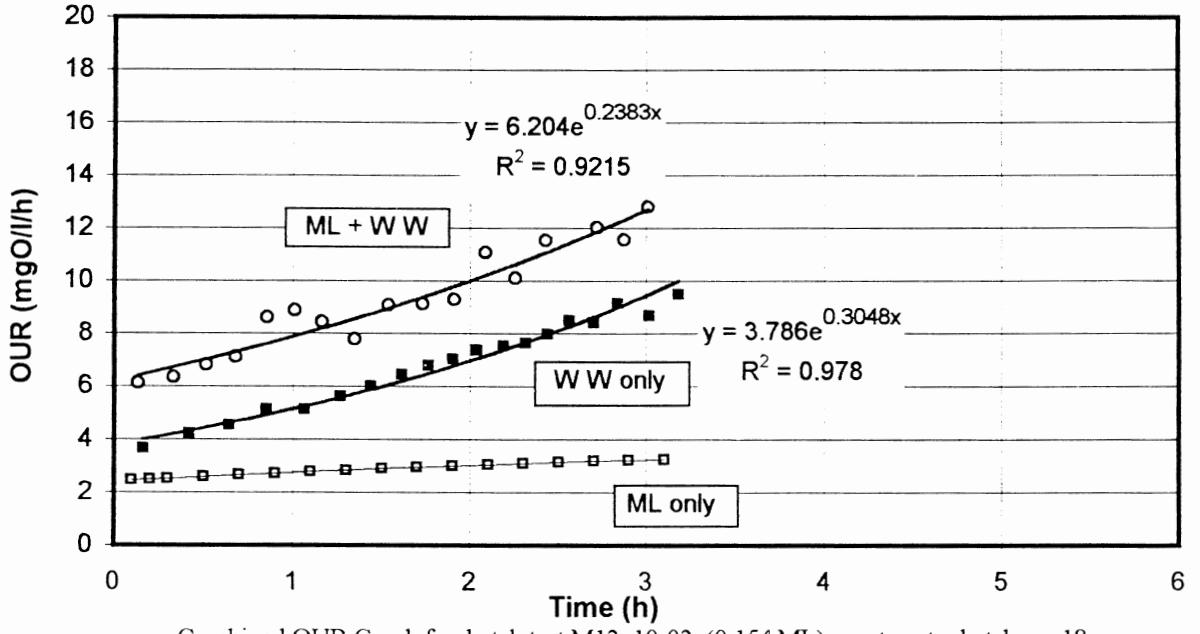
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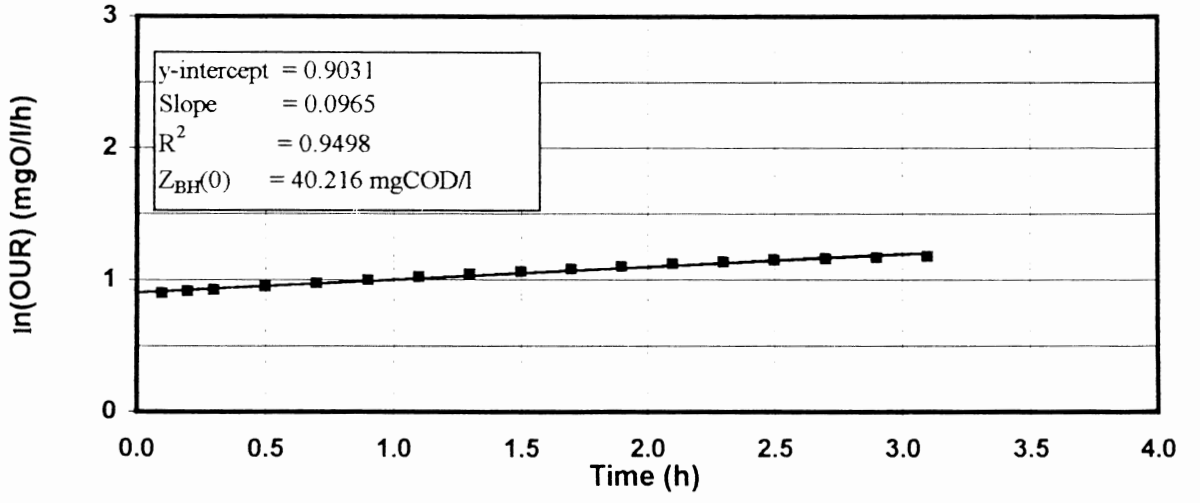
ln(OUR) graph for ML only, batch test M11, 09-02, (0.3ℓ ML), wastewater batch no.18



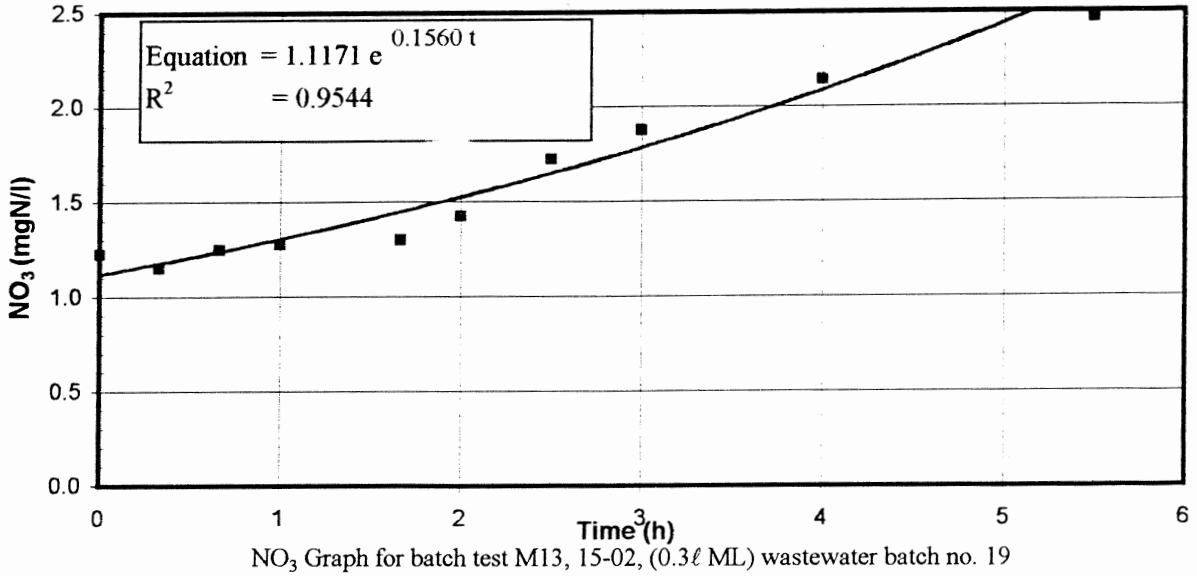
NO_3 Graph for batch test M12, 10-02, (0.15ℓ ML) wastewater batch no. 18



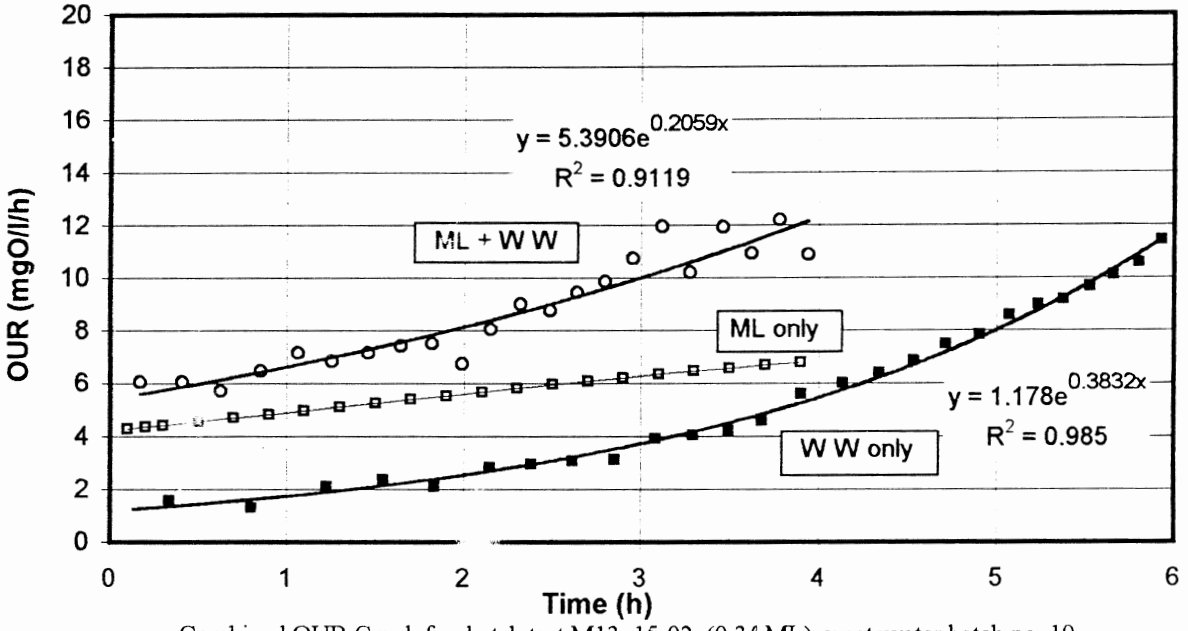
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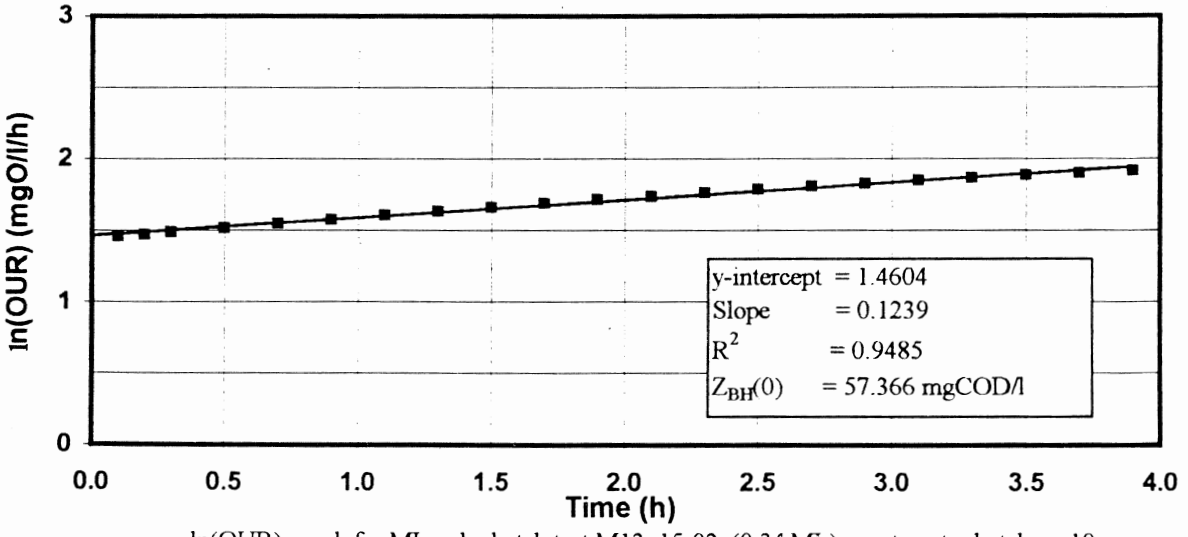
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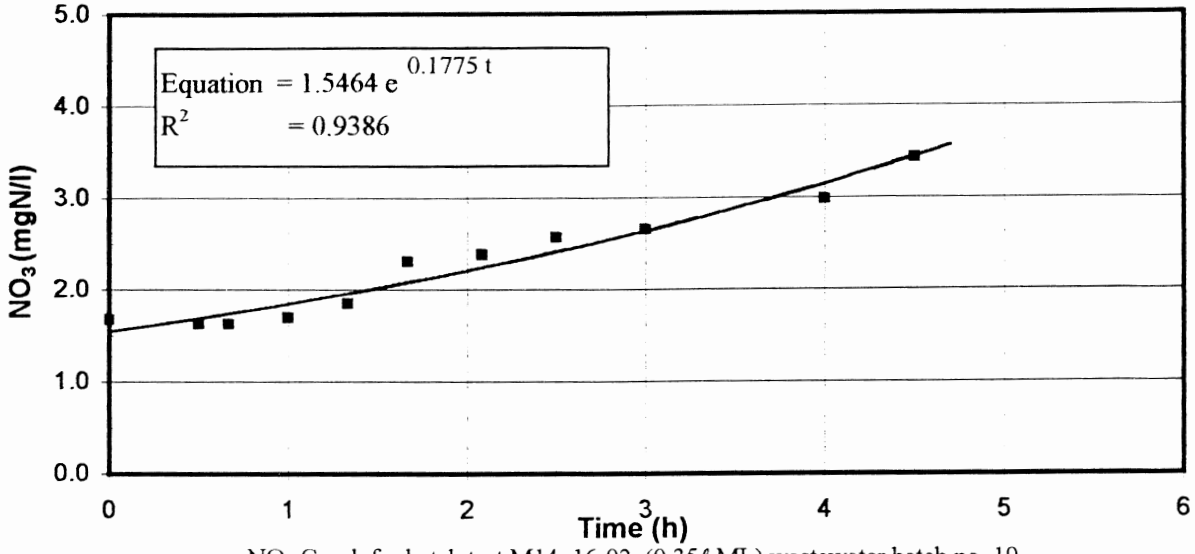
NO_3 Graph for batch test M13, 15-02, (0.3ℓ ML) wastewater batch no. 19



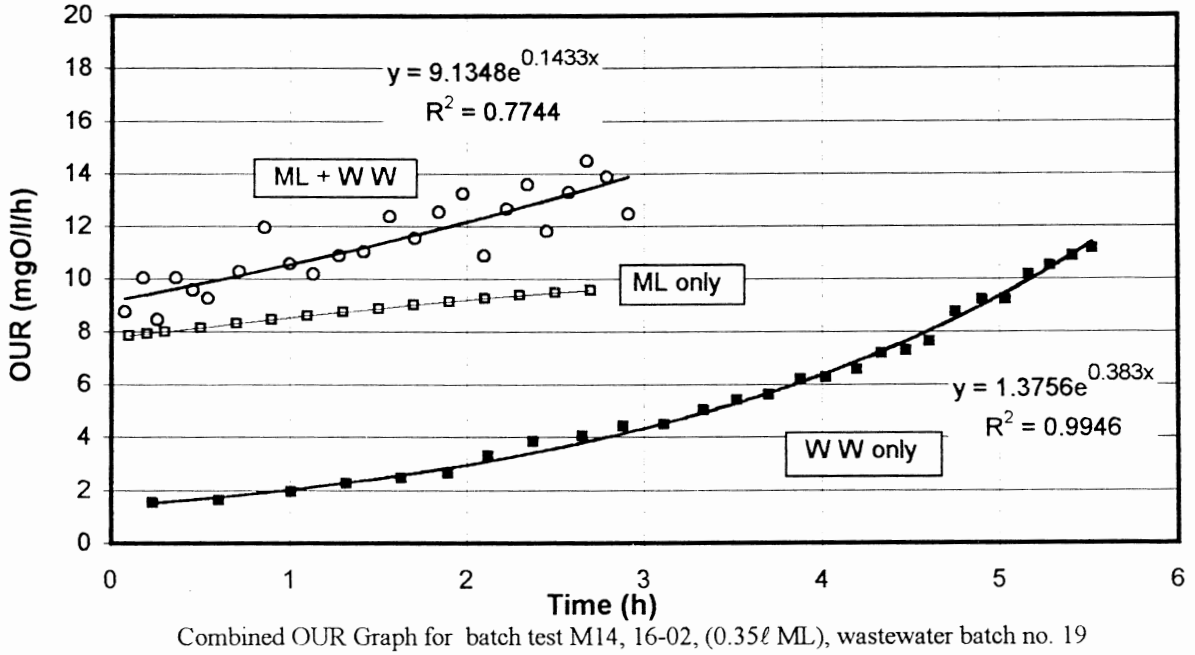
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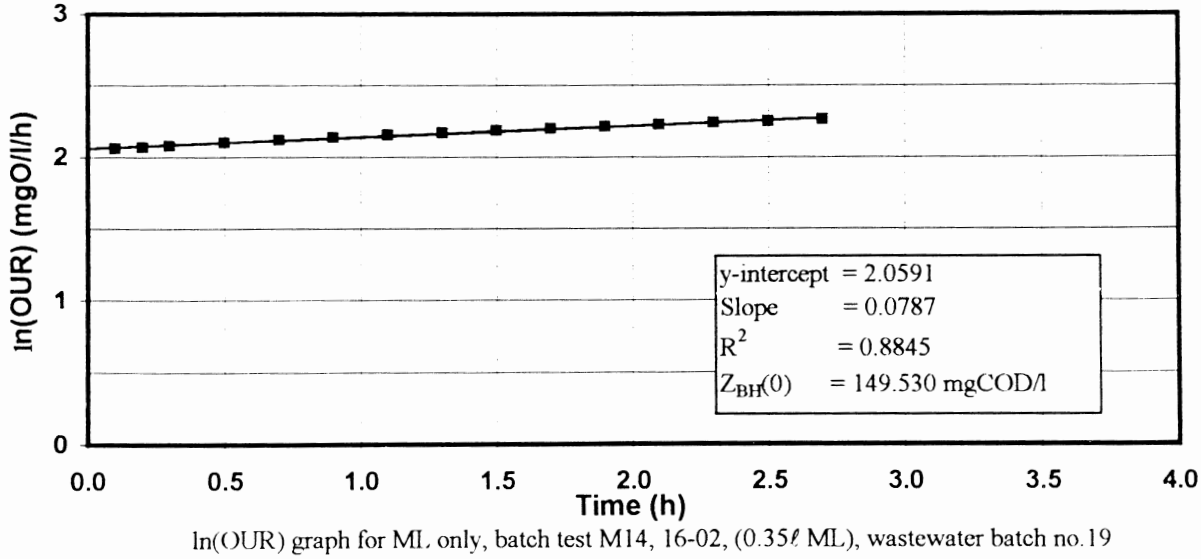
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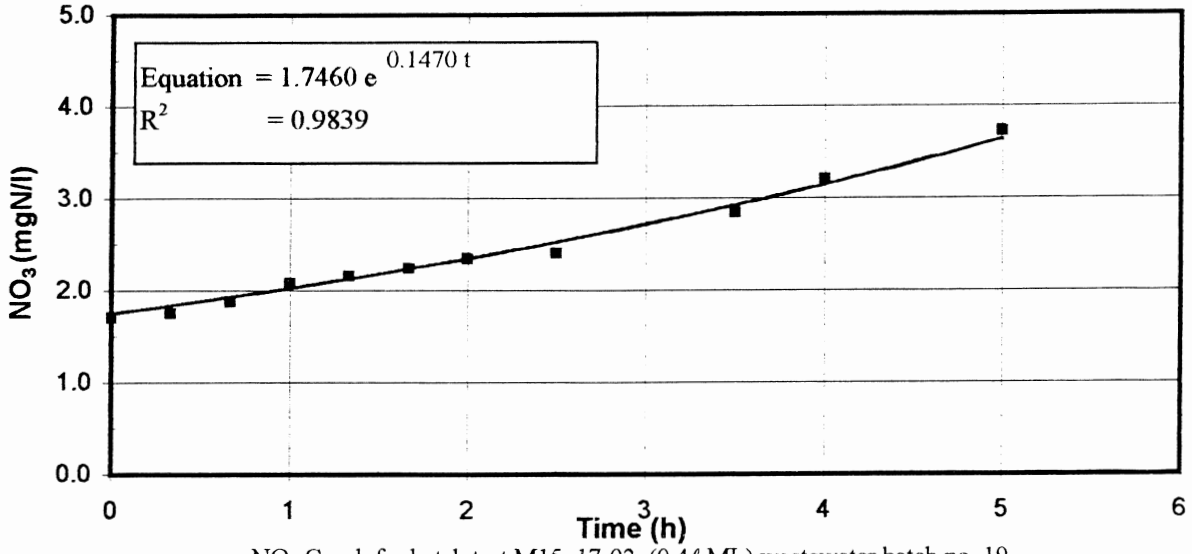
NO_3 Graph for batch test M14, 16-02, (0.35ℓ ML) wastewater batch no. 19



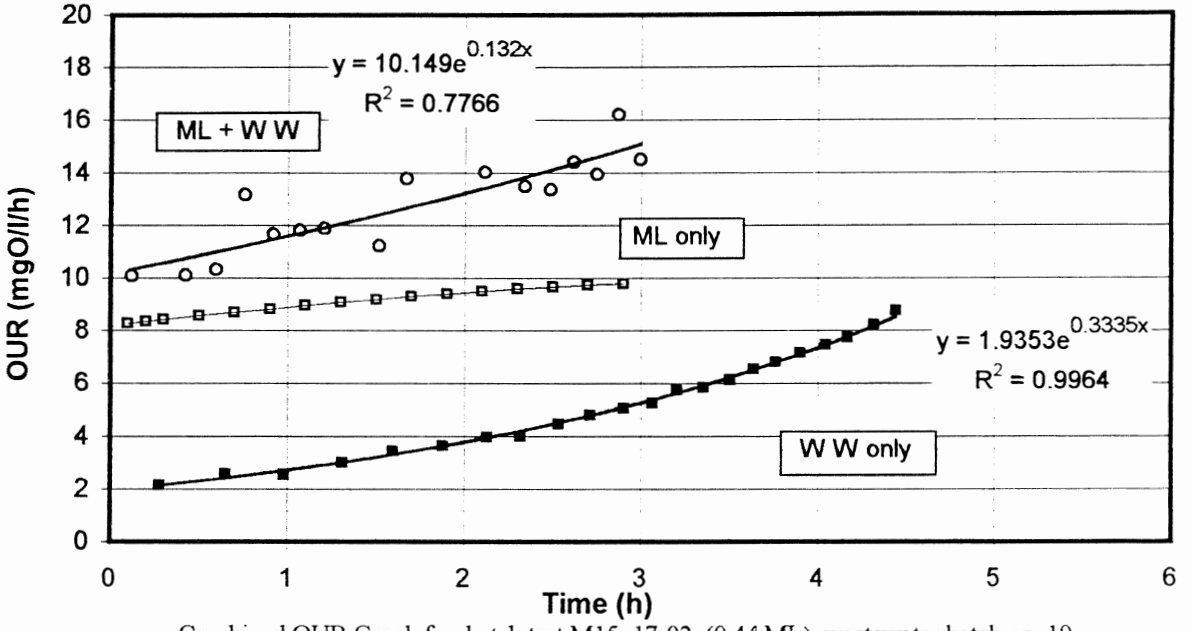
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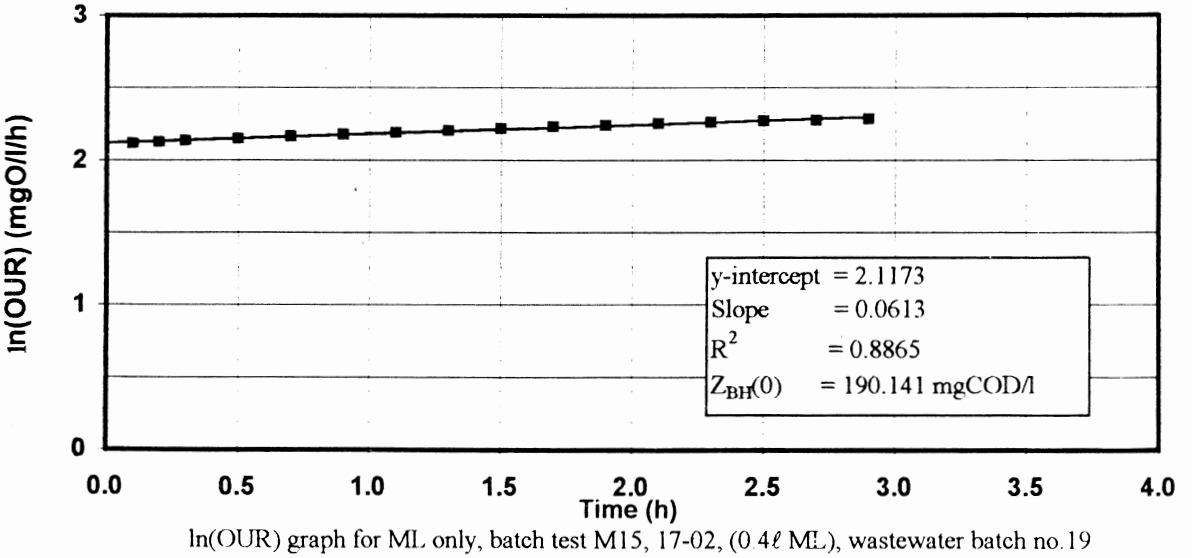
ln(OUR) graph for ML only, batch test M14, 16-02, (0.35ℓ ML), wastewater batch no.19



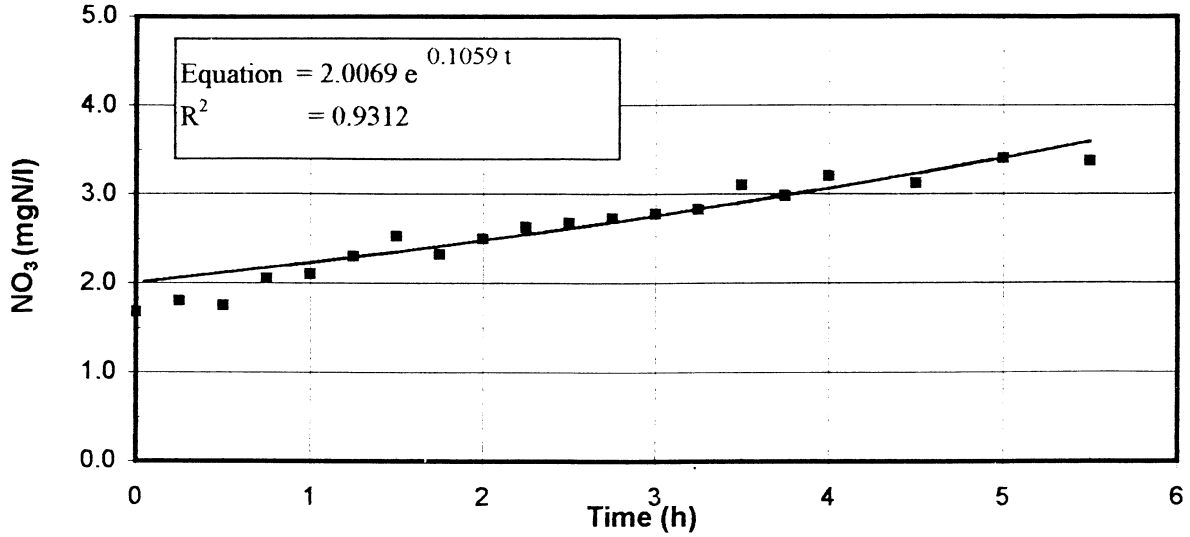
NO_3 Graph for batch test M15, 17-02, (0.4ℓ ML) wastewater batch no. 19



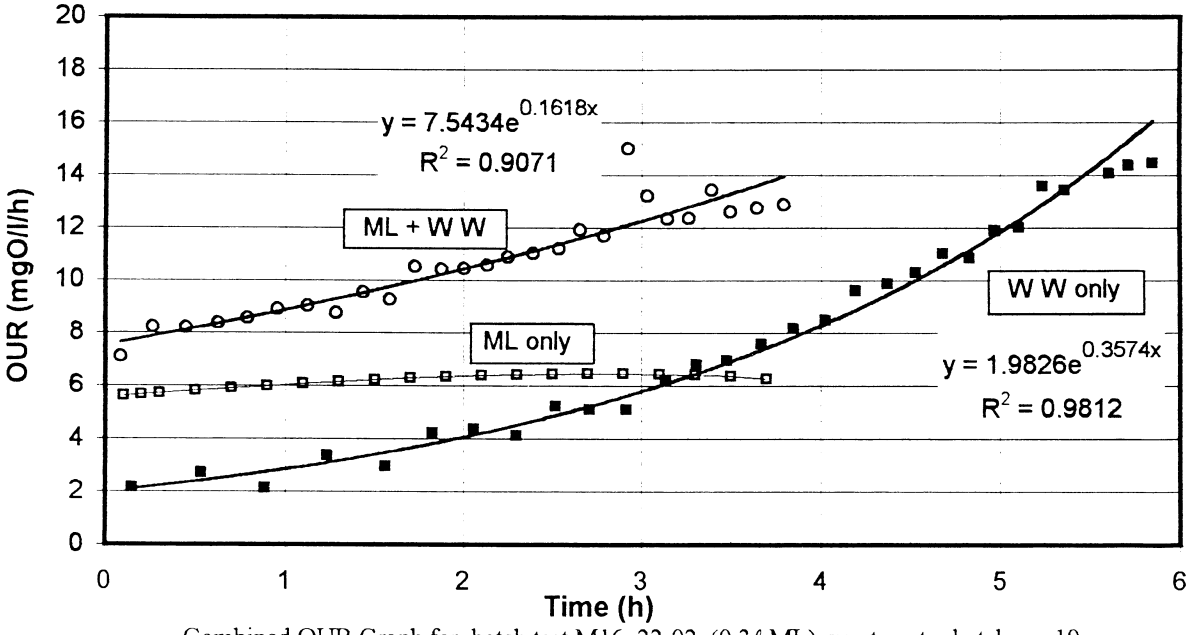
Combined OUR Graph for batch test M15, 17-02, (0.4ℓ ML), wastewater batch no. 19



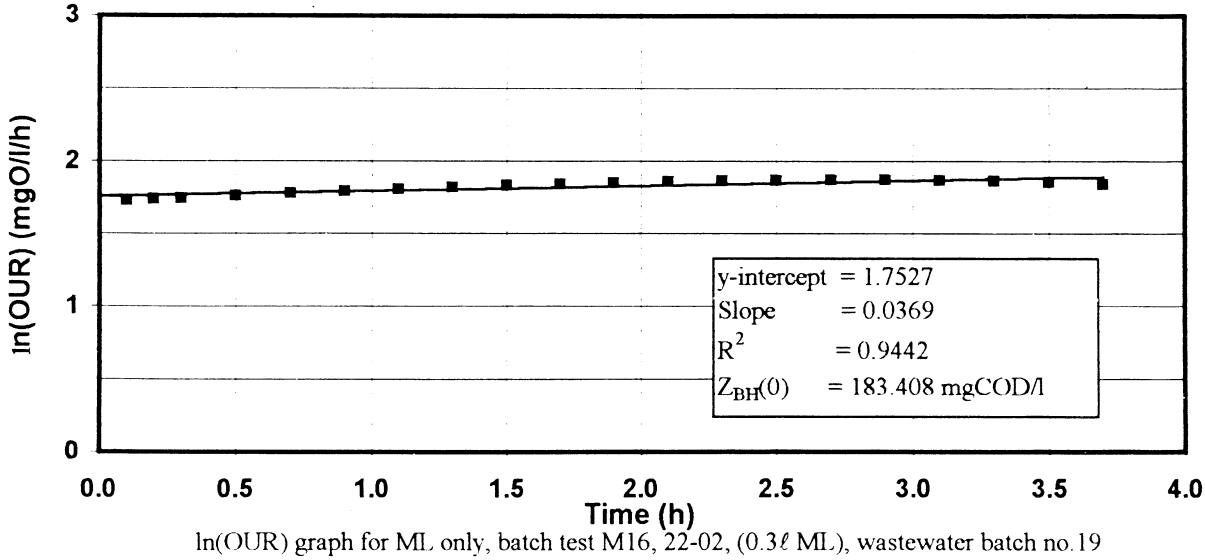
ln(OUR) graph for ML only, batch test M15, 17-02, (0.4ℓ ML), wastewater batch no.19



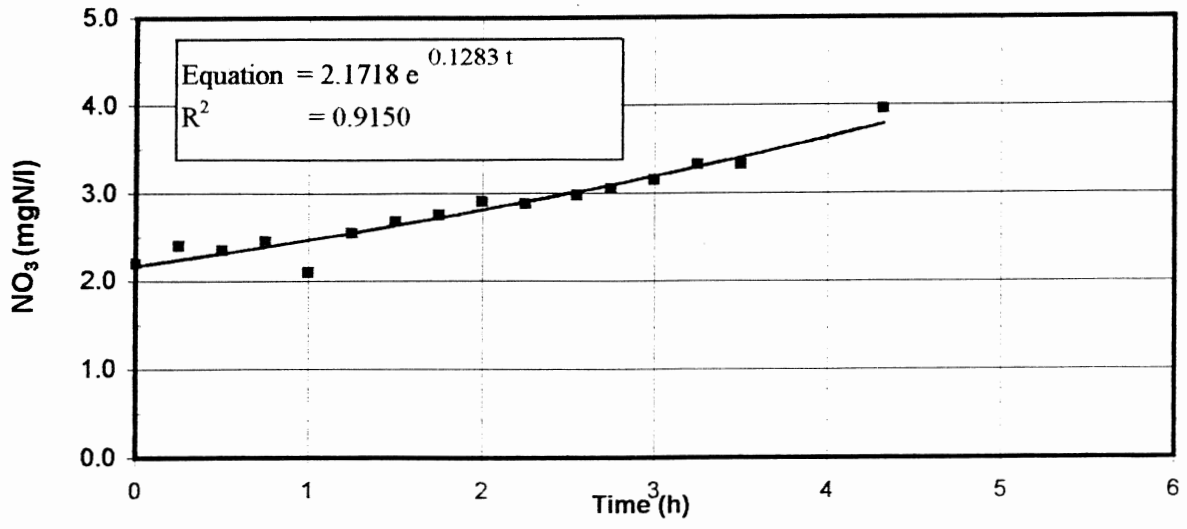
NO_3 Graph for batch test M16, 22-02, (0.3ℓ ML) wastewater batch no. 19



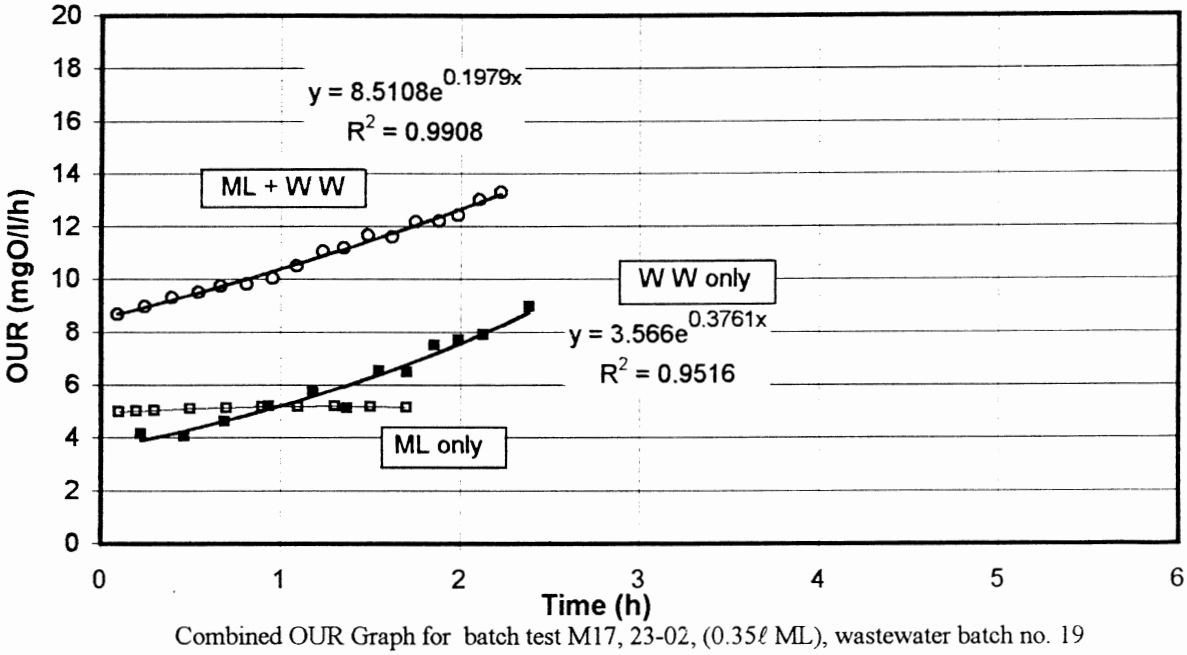
Combined OUR Graph for batch test M16, 22-02, (0.3ℓ ML), wastewater batch no. 19



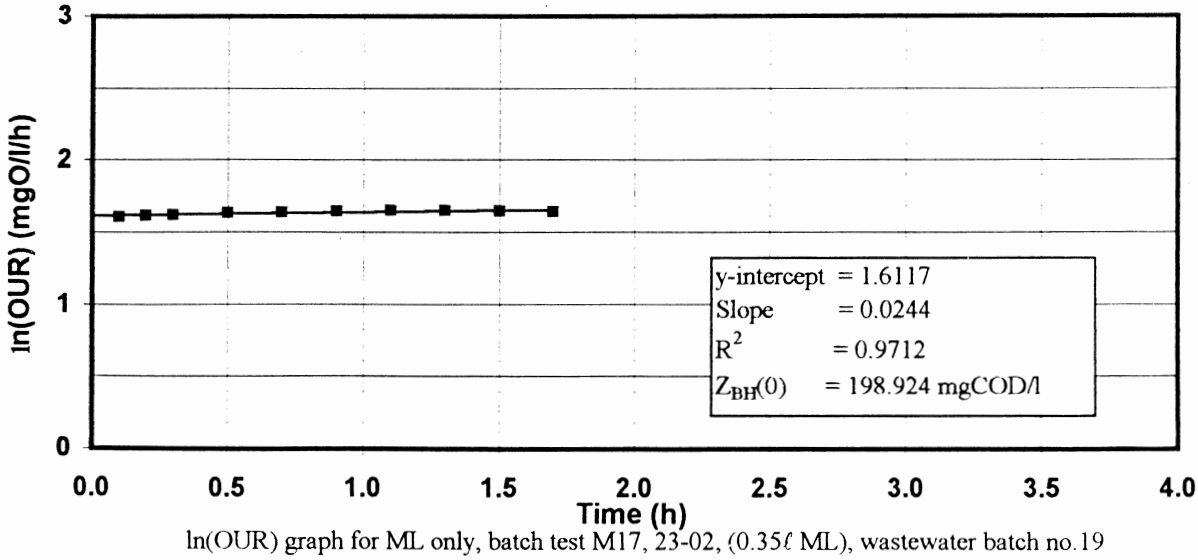
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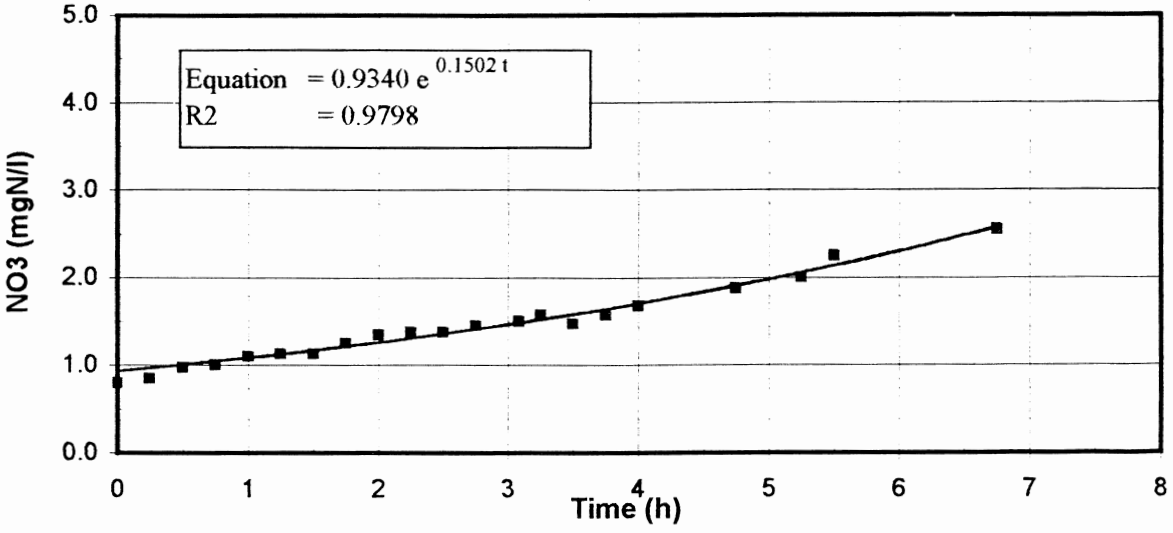
NO_3 Graph for batch test M17, 23-02, (0.35ℓ ML) wastewater batch no. 19



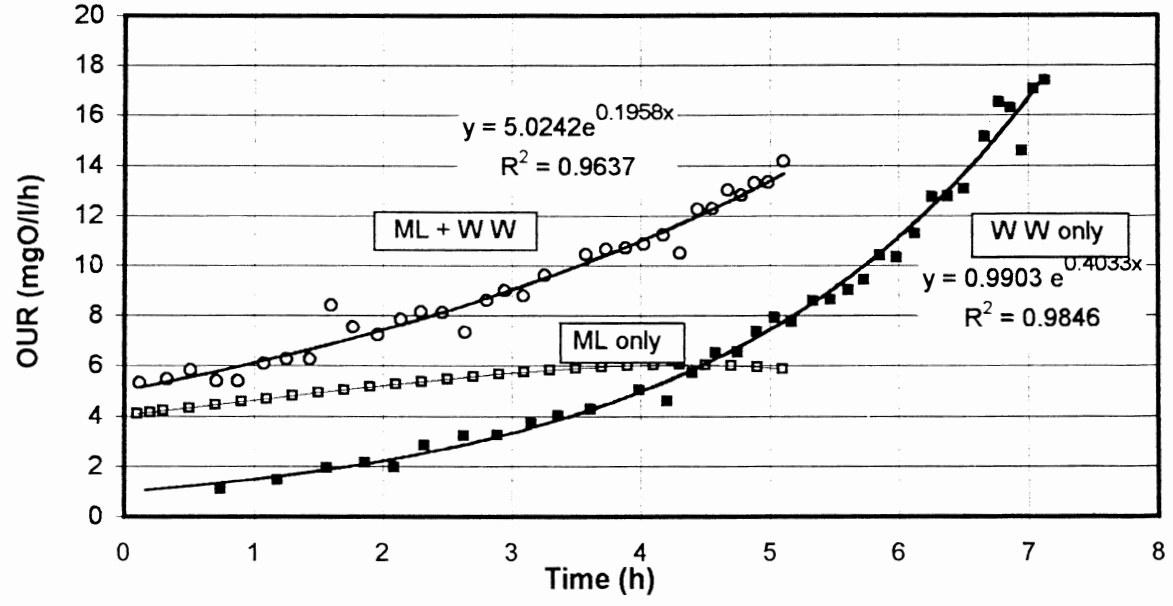
Combined OUR Graph for batch test M17, 23-02, (0.35ℓ ML), wastewater batch no. 19



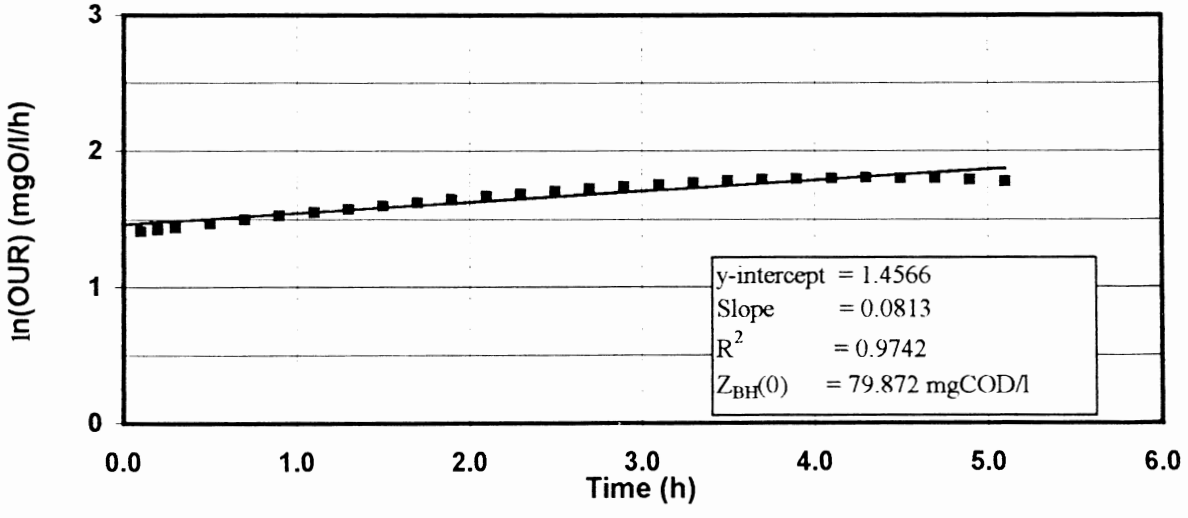
ln(OUR) graph for ML only, batch test M17, 23-02, (0.35ℓ ML), wastewater batch no.19



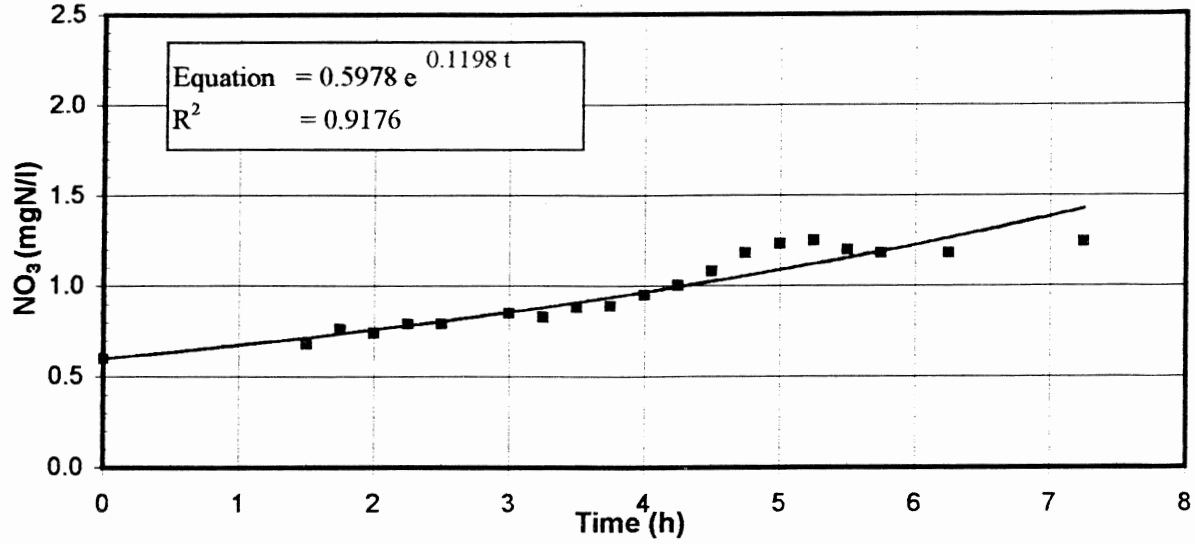
NO3 Graph for batch test M18, 29-02, (0.25ℓ ML) wastewater batch no. 20



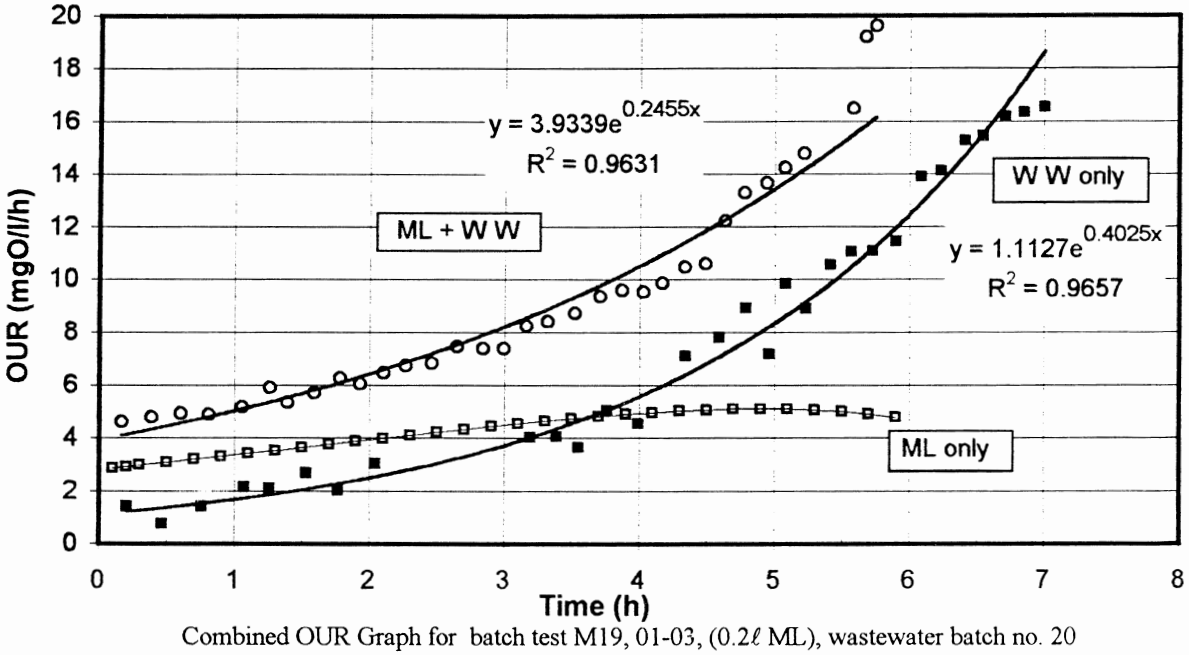
Combined OUR Graph for batch test M18, 29-02, (0.25ℓ ML), wastewater batch no. 20



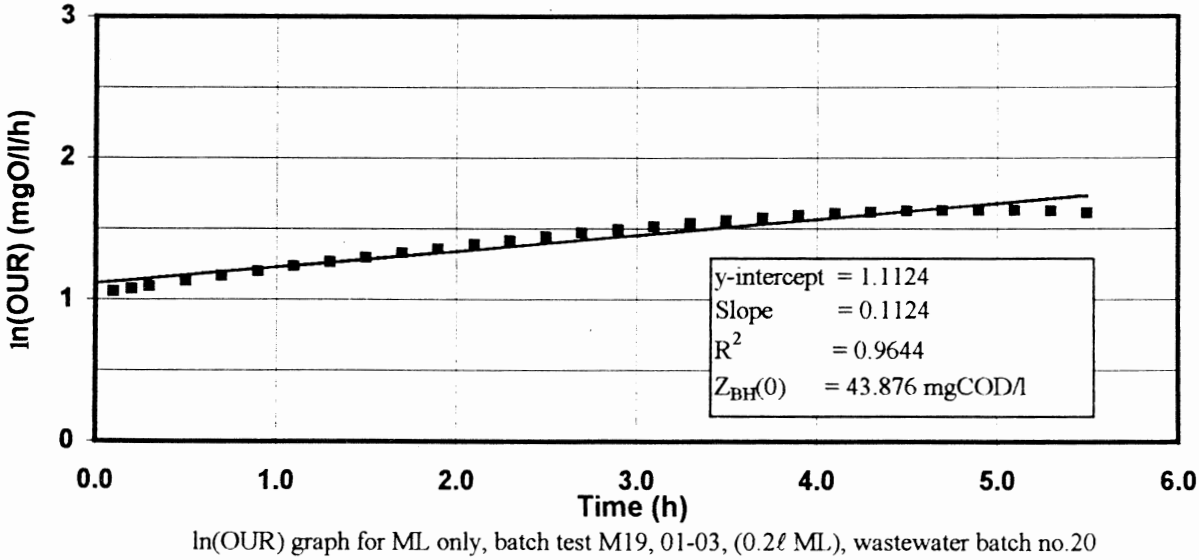
ln(OUR) graph for ML only, batch test M18, 29-02, (0.25ℓ ML), wastewater batch no.20



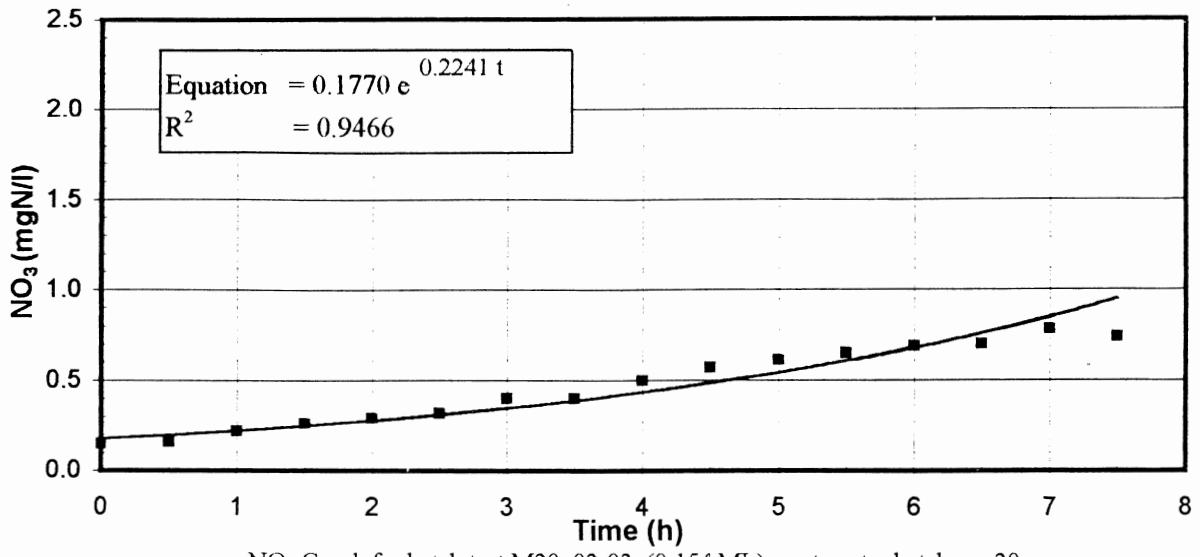
NO_3 Graph for batch test M19, 01-03, (0.2ℓ ML) wastewater batch no. 20



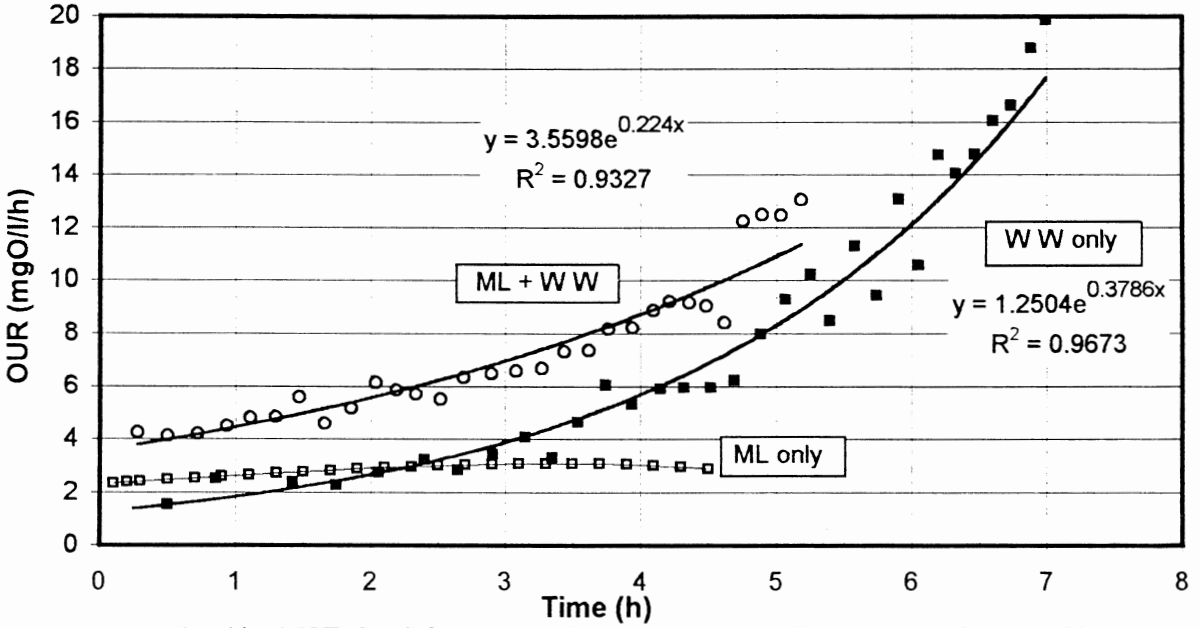
Combined OUR Graph for batch test M19, 01-03, (0.2ℓ ML), wastewater batch no. 20



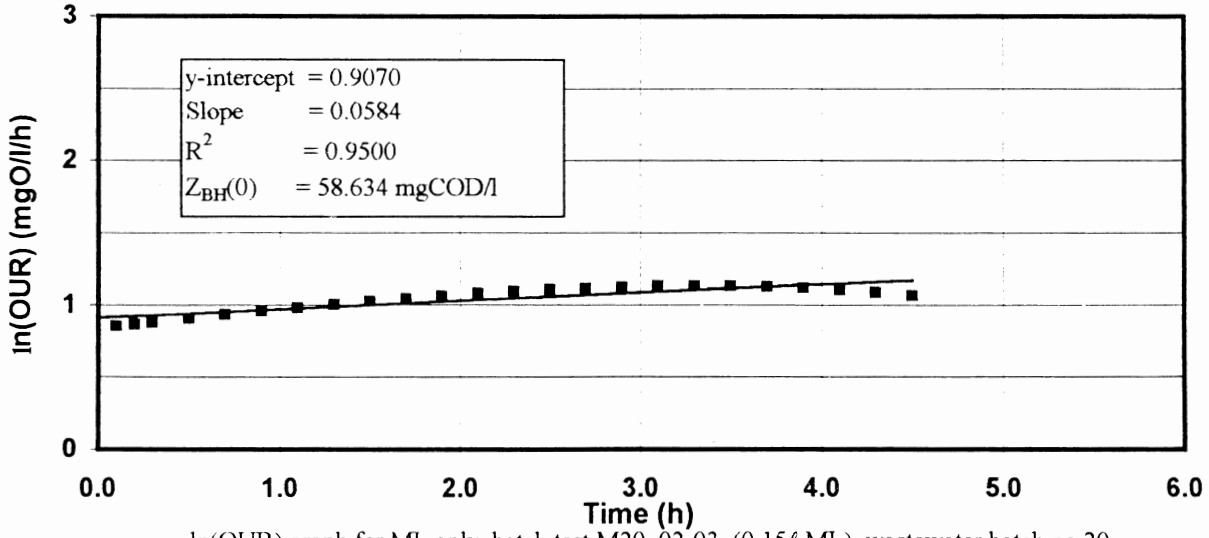
ln(OUR) graph for ML only, batch test M19, 01-03, (0.2ℓ ML), wastewater batch no.20



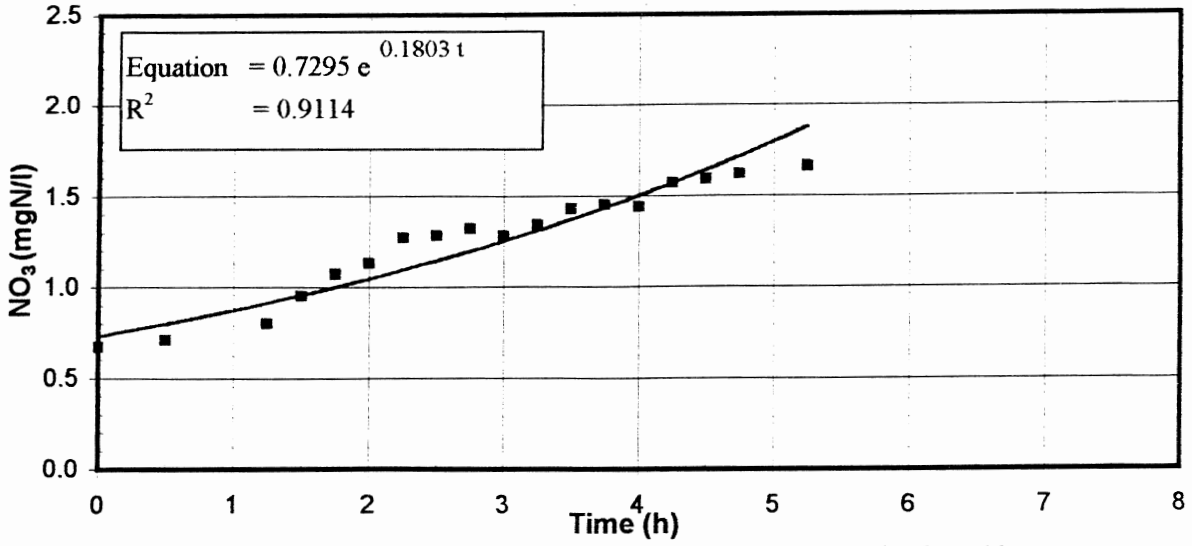
NO_3 Graph for batch test M20, 02-03, (0.15ℓ ML) wastewater batch no. 20



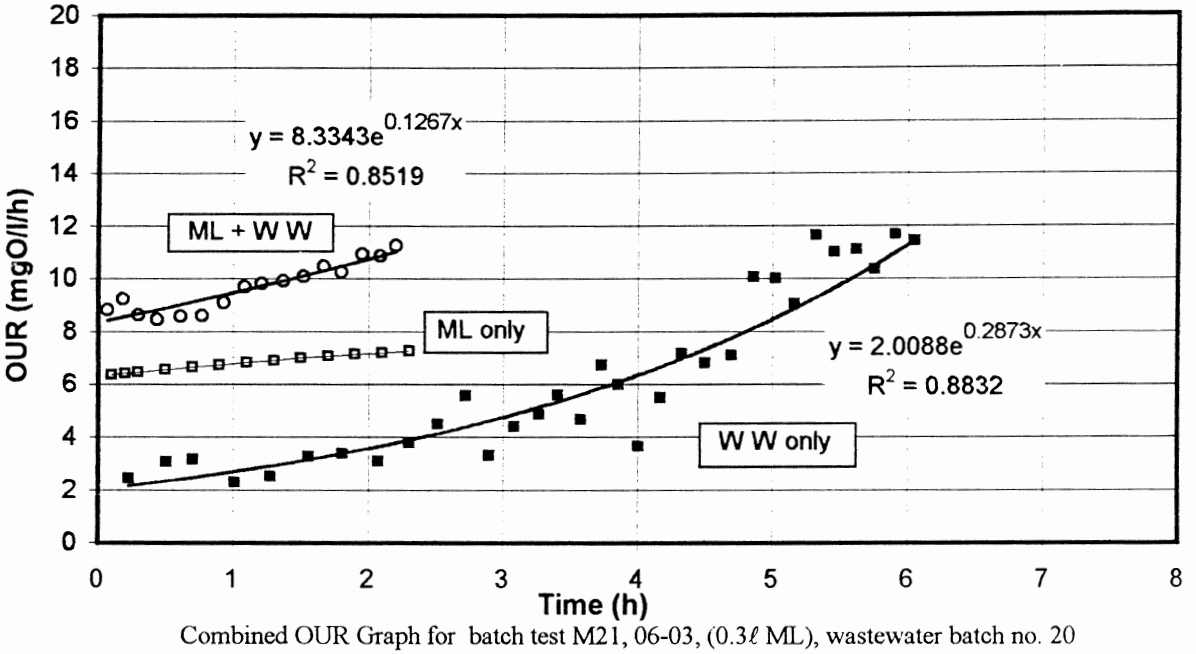
Combined OUR Graph for batch test M20, 02-03, (0.15ℓ ML), wastewater batch no. 20



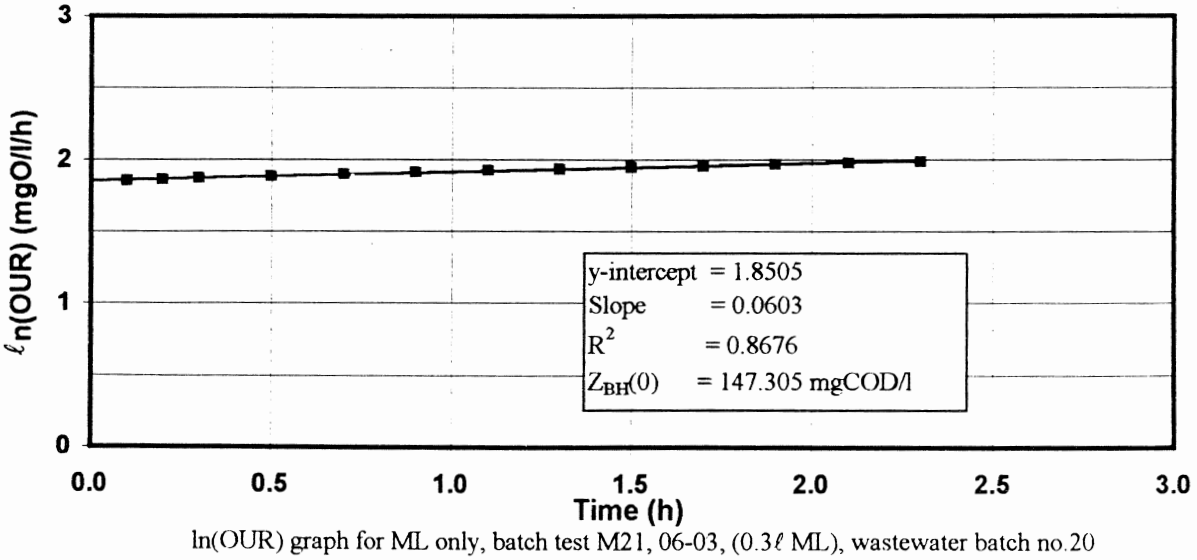
ln(OUR) graph for ML only, batch test M20, 02-03, (0.15ℓ ML), wastewater batch no.20



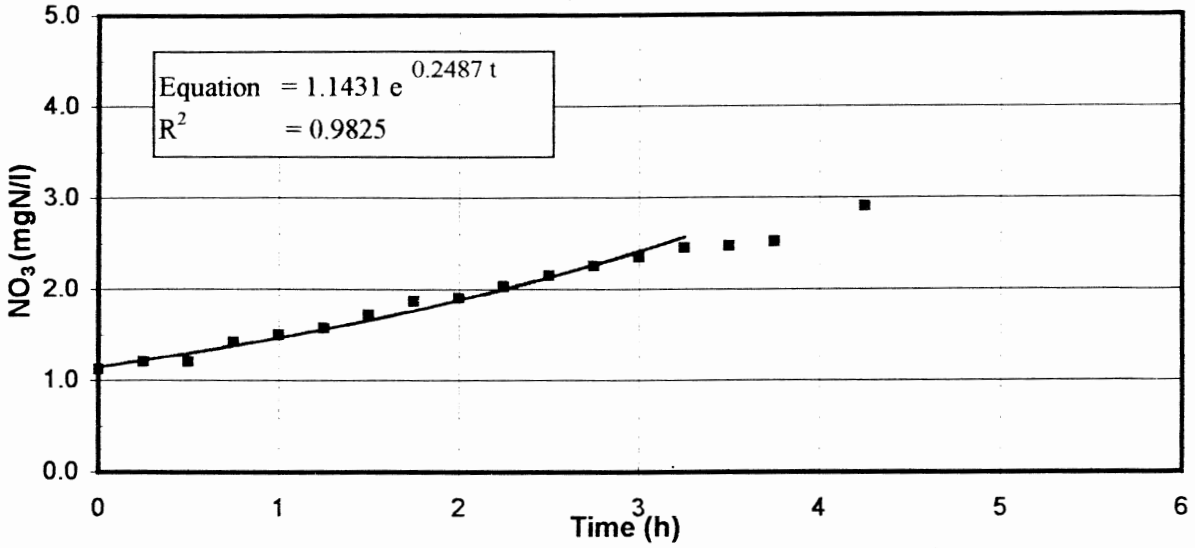
NO_3 Graph for batch test M21, 06-03, (0.3ℓ ML) wastewater batch no. 20



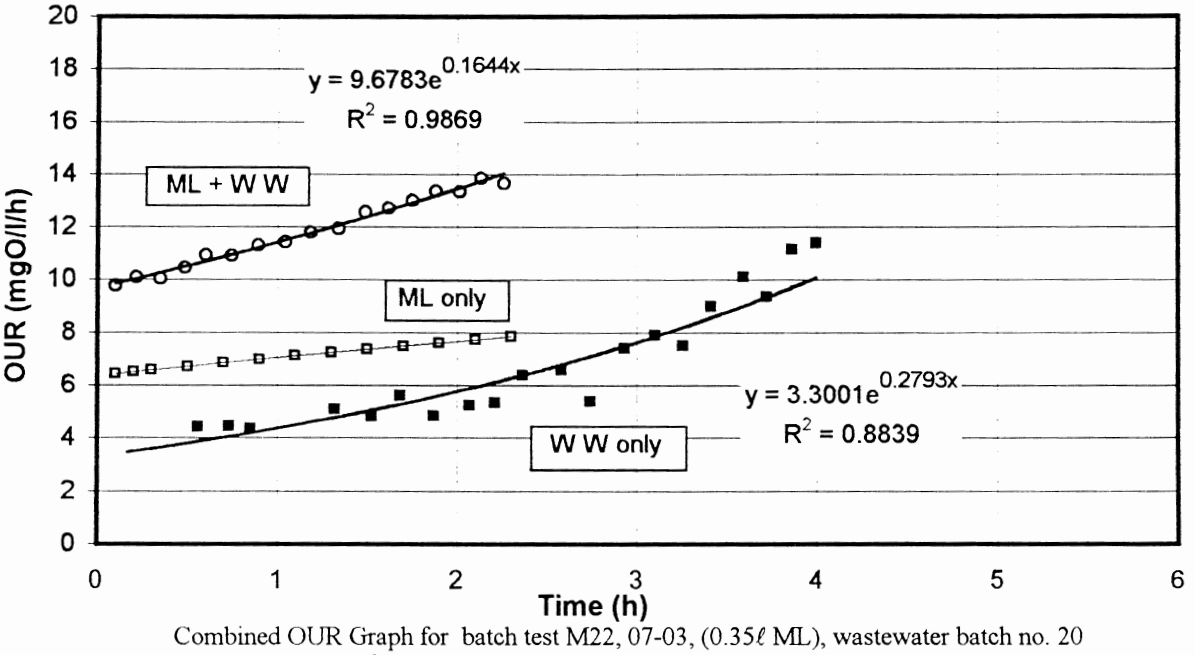
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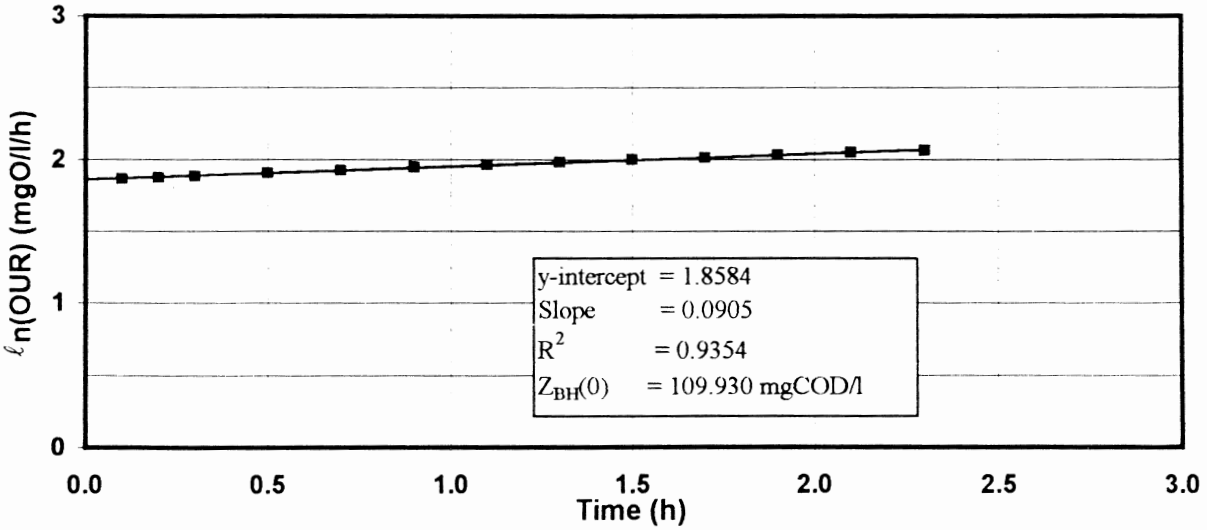
ln(OUR) graph for ML only, batch test M21, 06-03, (0.3ℓ ML), wastewater batch no.20



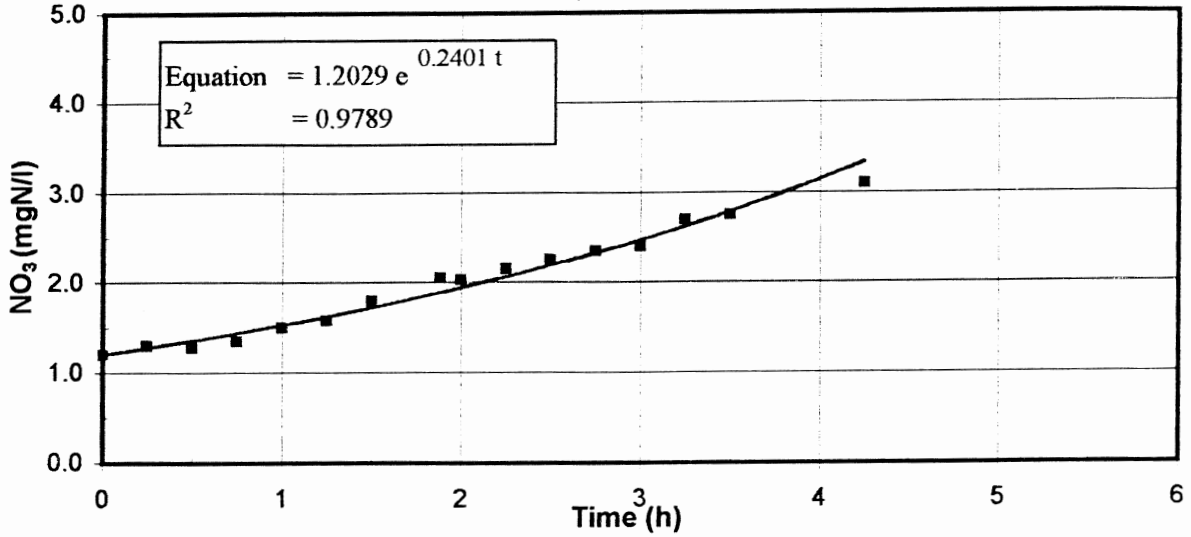
NO_3 Graph for batch test M22, 07-03, (0.35ℓ ML) wastewater batch no. 20



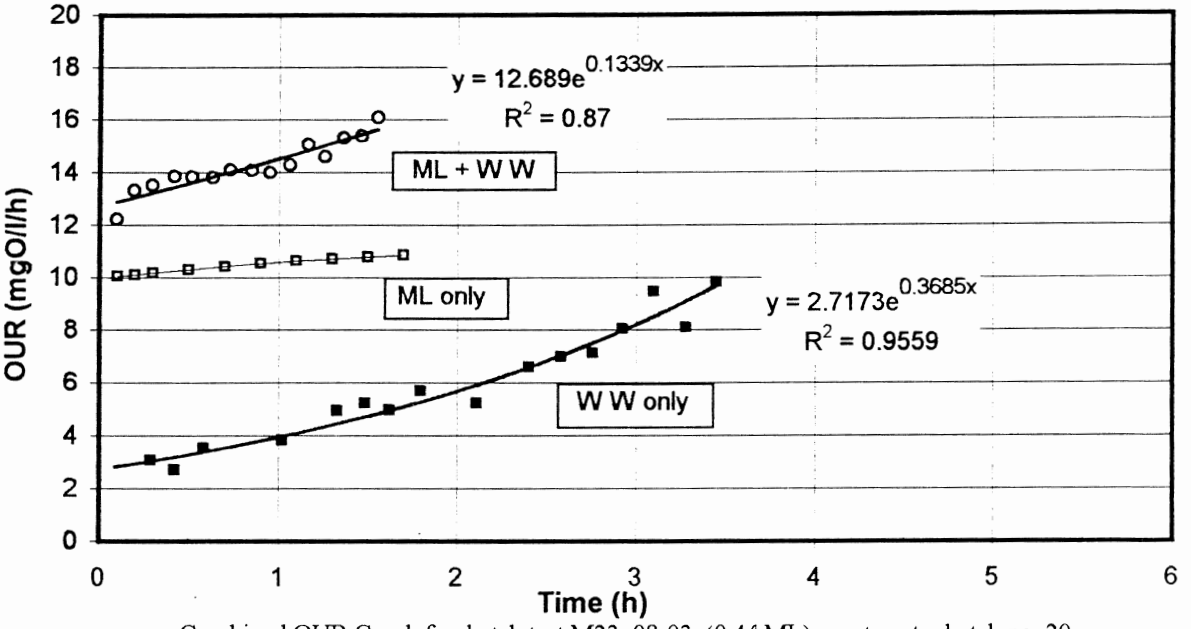
Combined OUR Graph for batch test M22, 07-03, (0.35ℓ ML), wastewater batch no. 20



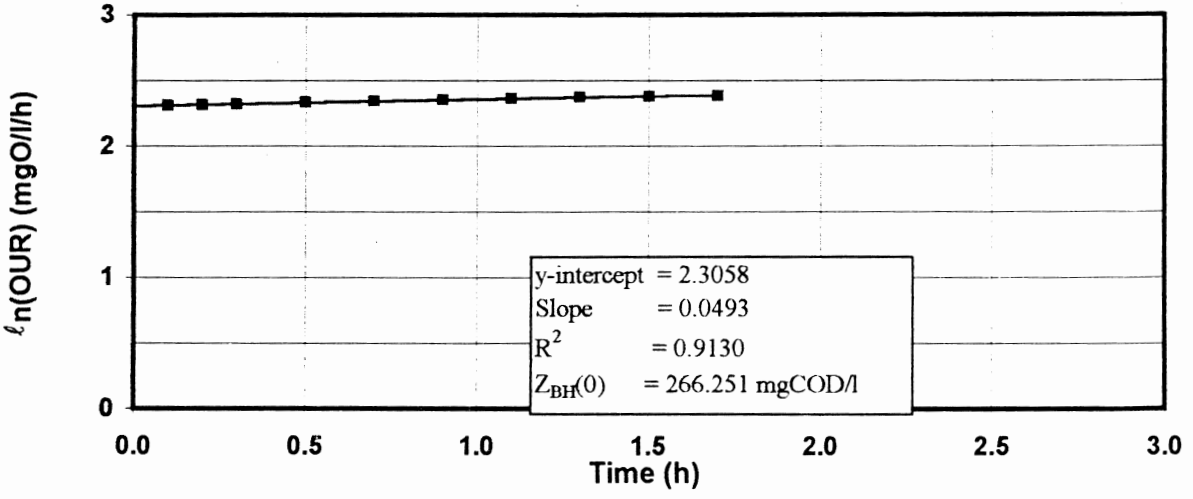
ln(OUR) graph for ML only, batch test M22, 07-03, (0.35ℓ ML), wastewater batch no.20



NO_3 Graph for batch test M23, 08-03, (0.4ℓ ML) wastewater batch no. 20



Combined OUR Graph for batch test M23, 08-03, (0.4ℓ ML), wastewater batch no. 20



ln(OUR) graph for ML only, batch test M23, 08-03, (0.4ℓ ML), wastewater batch no.20

APPENDIX B

OUR vs TIME PLOTS FOR MODIFIED BATCH TESTS CONDUCTED WITH FLOCCULATED FILTERED WASTEWATER PLUS MIXED LIQUOR

TABLE OF CONTENTS

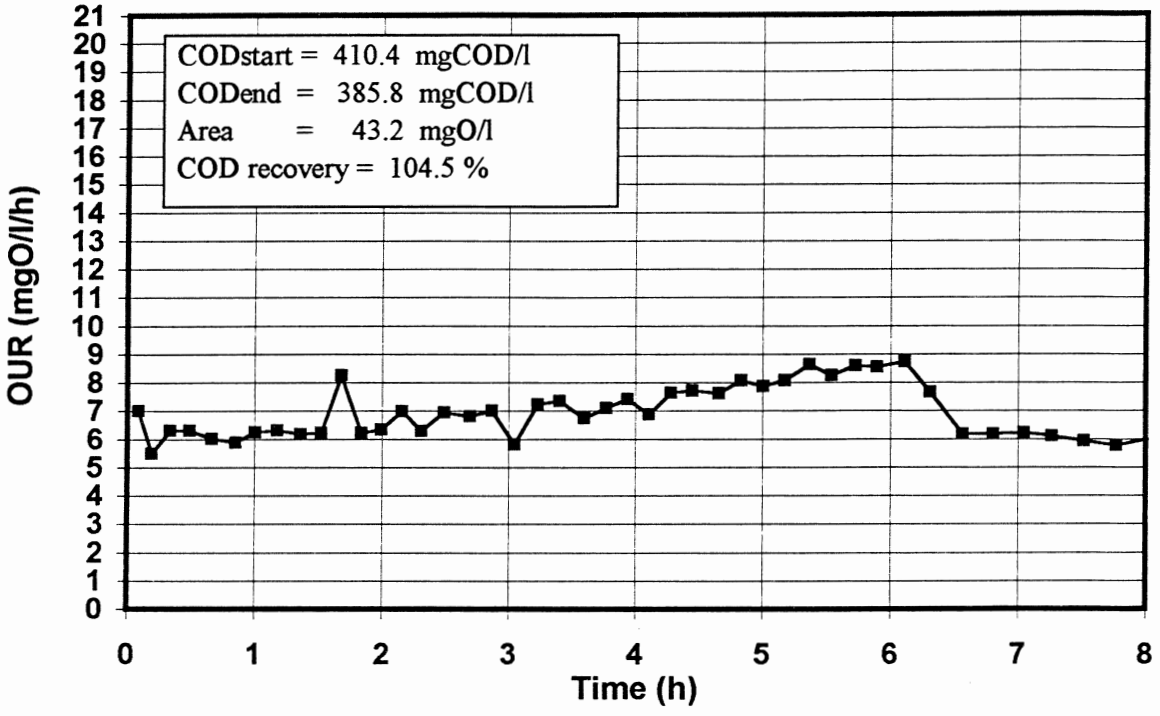
Table B1 Summary of the data recorded for modified batch tests, conducted with a mixture of flocculated filtered wastewater and mixed liquor. Mixed liquor sample volumes, calculated COD recovery, maximum specific growth rates and measured heterotrophic active biomass.

- OUR vs time and ln(OUR) vs time profiles for modified batch tests F1 – F20. The nitrate concentration vs time profiles recorded during each batch test are also given.

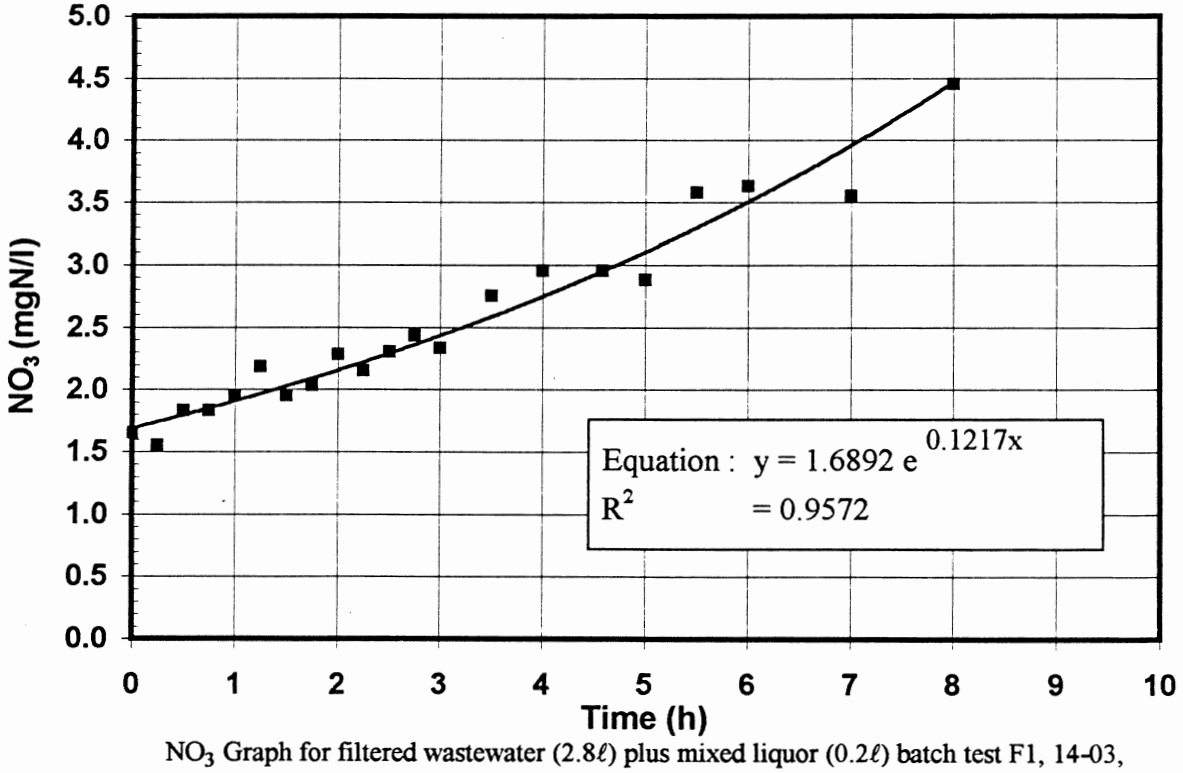
Table B1 : Summarized data for modified batch tests with filtered wastewater and mixed liquor ; mixed liquor volume, COD recovery, maximum specific growth rates and heterotrophic active biomass.

Sew. batch no.	Batch Test No.	Date of test	M L vol.	COD (mgCOD/l)		Area mgO/l	% COD Recov	Linear regression data			Max. specific growth rates (/d)		$Z_{BH(0)}$ (ML) mgCOD/l
				Start	End			y-int.	slope	R^2	K_{MP}	μ_H	
21	F1	14-03	0.20ℓ	410.4	385.8	43.2	104.5	1.5687	0.0530	0.8118	1.15	0.74	121.421
	F2	15-03a	0.10ℓ	207.3	199.0	37.7	114.2	1.1057	0.0489	0.6820	1.06	0.74	80.614 *
	F3	15-03b	0.15ℓ	262.7	242.1	27.6	102.7	0.8031	0.0624	0.8972	0.84	1.28	50.452
	F4	20-03a	0.35ℓ	440.1	468.5	37.6	115.0	1.8873	0.0218	0.2211	0.67	0.47	276.351 *
	F5	20-03b	0.40ℓ	515.1	454.3	35.0	95.0	1.9486	0.0499	0.7524	0.90	0.91	184.802
	F6	21-03a	0.25ℓ	438.0	369.1	41.9	93.8	1.5037	0.0741	0.9299	1.50	0.89	89.757
	F7	21-03b	0.30ℓ	464.4	423.9	42.3	100.0	1.7051	0.0422	0.6721	0.94	0.70	161.258
22	F8	28-03	0.35ℓ	534.6	466.7	37.5	94.3	1.7788	0.0304	0.4031	0.68	0.67	210.018
	F9	29-03a	0.30ℓ	469.2	410.0	35.7	95.0	1.6491	0.0352	0.5618	0.65	0.82	169.964
	F10	29-03b	0.40ℓ	489.6	485.5	36.2	106.6	1.8370	0.0266	0.4080	0.66	0.60	238.747
	F11	30-03a	0.20ℓ	330.5	287.6	30.8	96.3	1.2548	0.0189	0.3749	0.41	0.66	156.332
	F12	30-03b	0.25ℓ	359.0	326.4	33.1	100.0	1.4273	0.0302	0.5783	0.57	0.77	148.303
	F13	04-04a	0.10ℓ	221.2	180.2	42.8	100.8	0.2251	0.0616	0.9694	1.03	1.07	28.563
	F14	04-04b	0.15ℓ	264.2	231.4	47.8	105.7	0.7544	0.0421	0.5013	0.74	0.89	62.413
23	F15	10-04a	0.20ℓ	335.9	264.2	35.1	89.1	1.2444	0.0545	0.9369	0.83	1.10	86.153
	F16	10-04b	0.25ℓ	344.1	290.8	32.7	94.0	1.3041	0.0373	0.6380	0.79	0.73	116.368
	F17	11-04a	0.10ℓ	258.0	198.7	33.9	90.2	0.6357	0.0471	0.9553	0.72	1.03	51.628
	F18	11-04b	0.15ℓ	266.2	251.9	36.6	108.4	0.9310	0.0480	0.8678	0.91	0.86	68.518
	F19	12-04a	0.30ℓ	419.8	378.9	40.3	99.9	1.5893	0.0625	0.9833	1.14	0.98	110.618
	F20	12-04b	0.35ℓ	436.2	415.7	39.2	104.3	1.7711	0.0295	0.8028	0.54	0.79	211.797
			MEAN				100.5			0.6974	0.84	0.84	
			Std Deviation				7.3			0.2302	0.25	0.19	

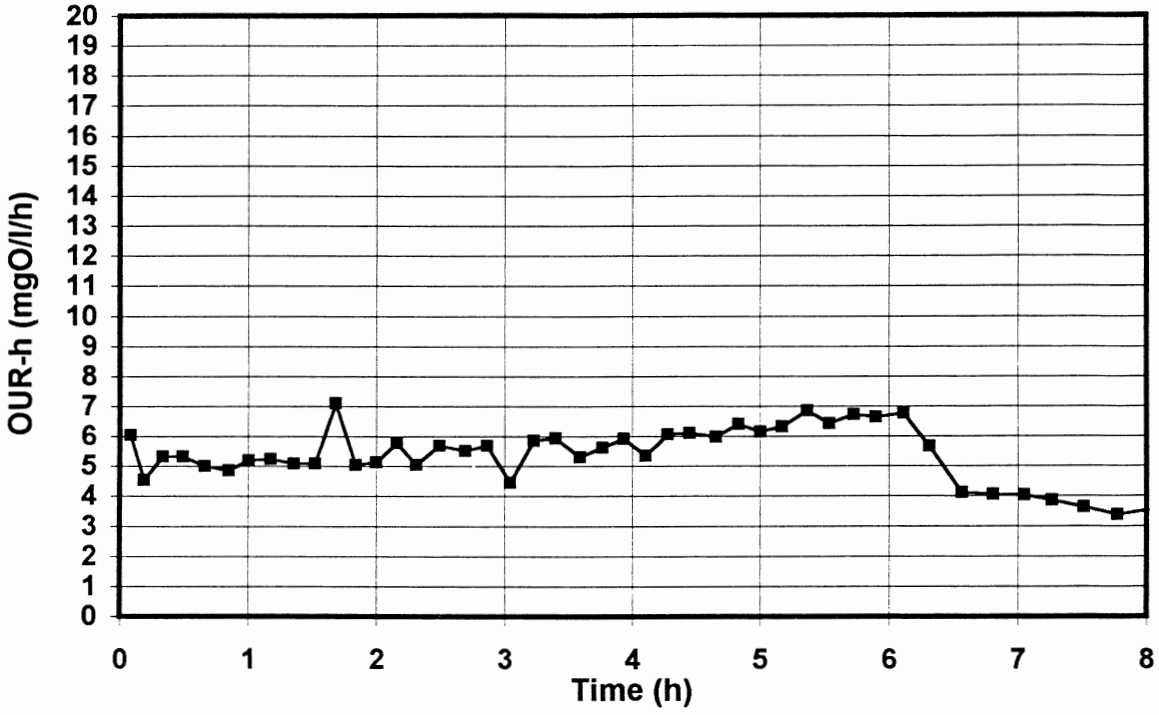
* Batch test rejected on account of poor %COD recovery



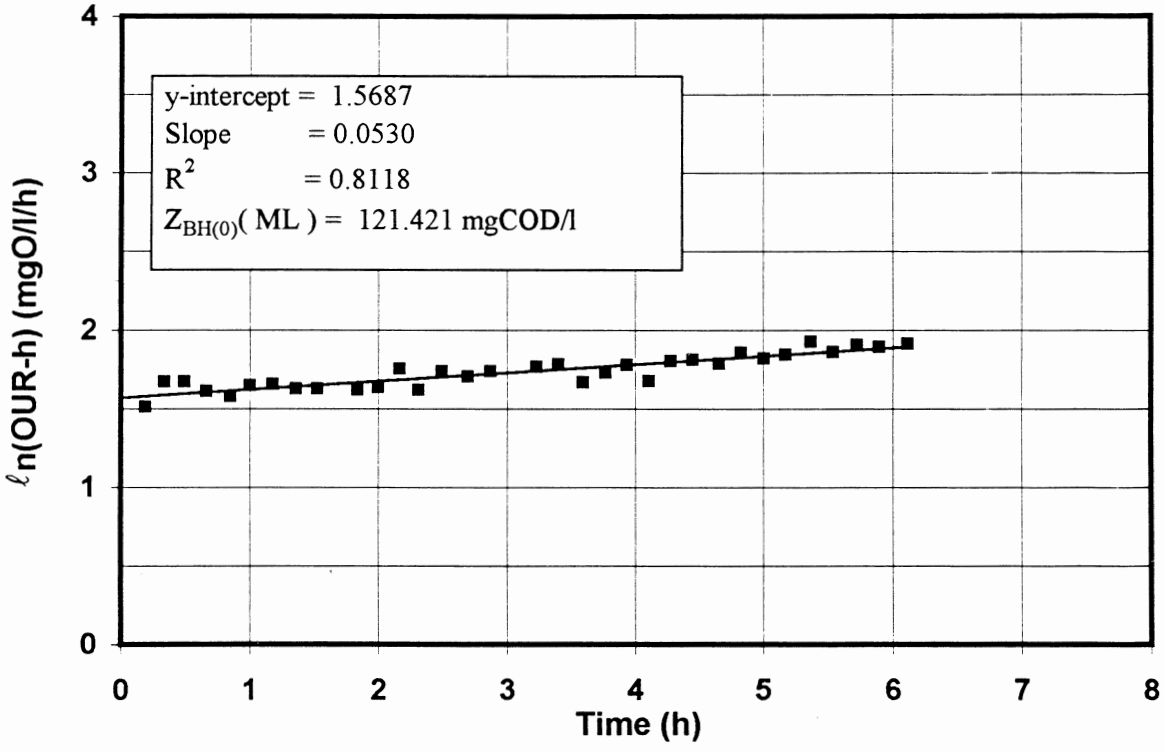
OUR Graph for filtered wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test F1, 14-03, wastewater batch no. 21



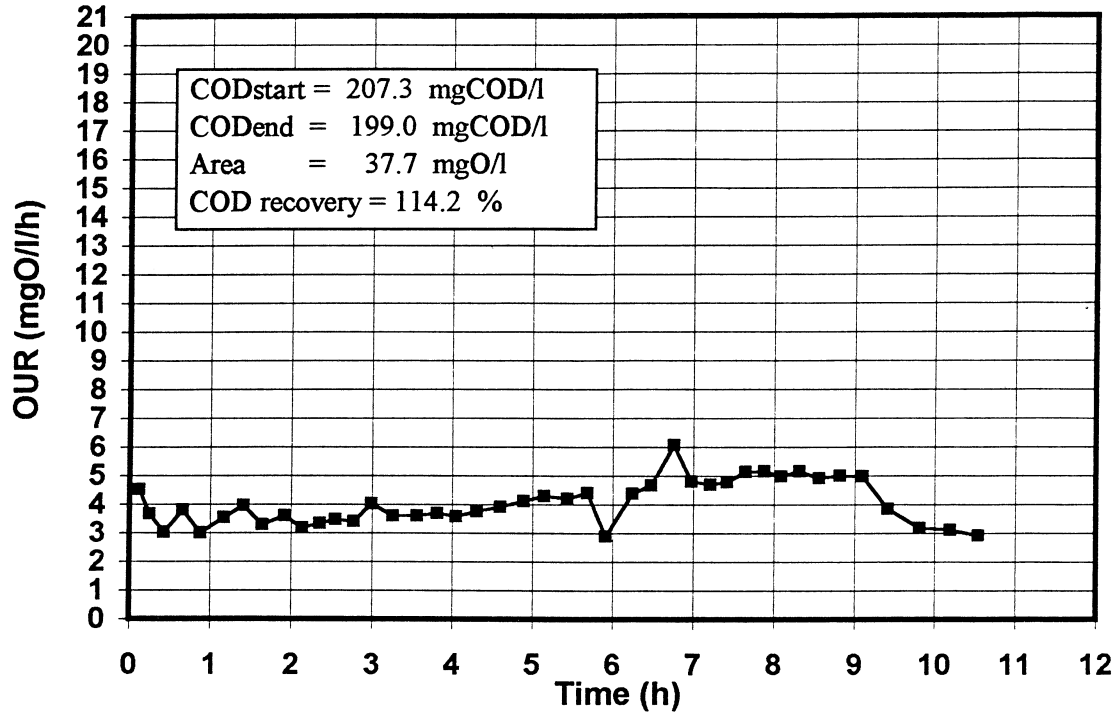
NO_3 Graph for filtered wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test F1, 14-03, wastewater batch no. 21



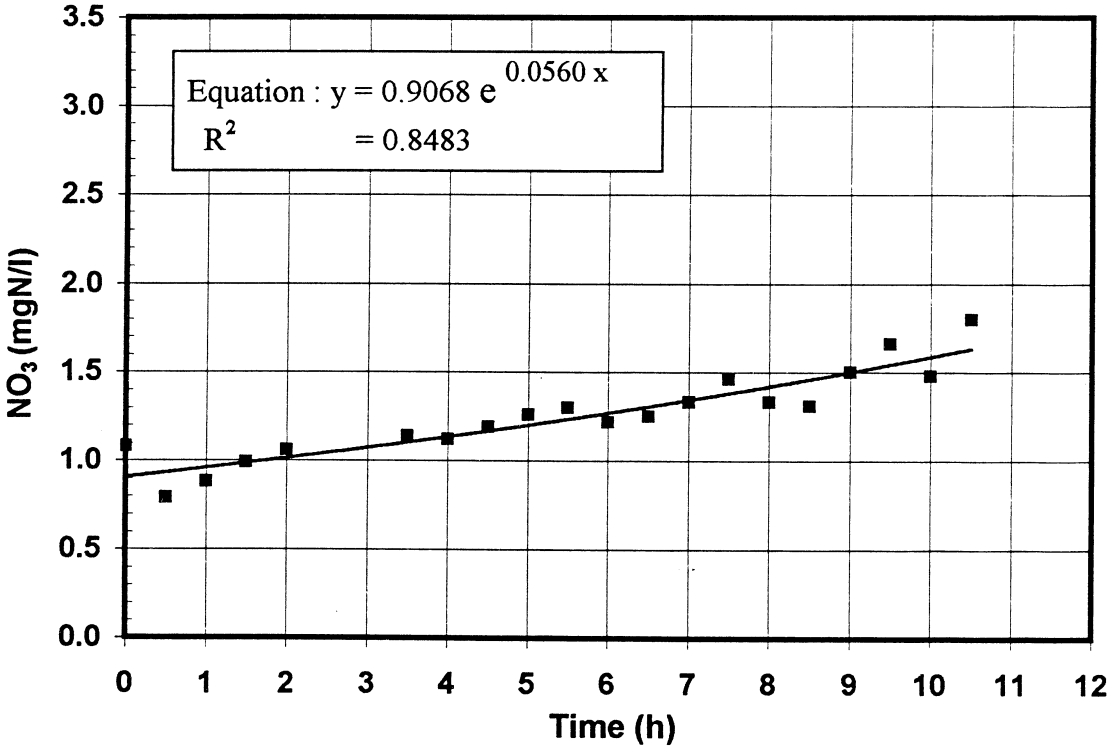
OUR-h Graph for filtered wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test F1, 14-03, wastewater batch no. 21



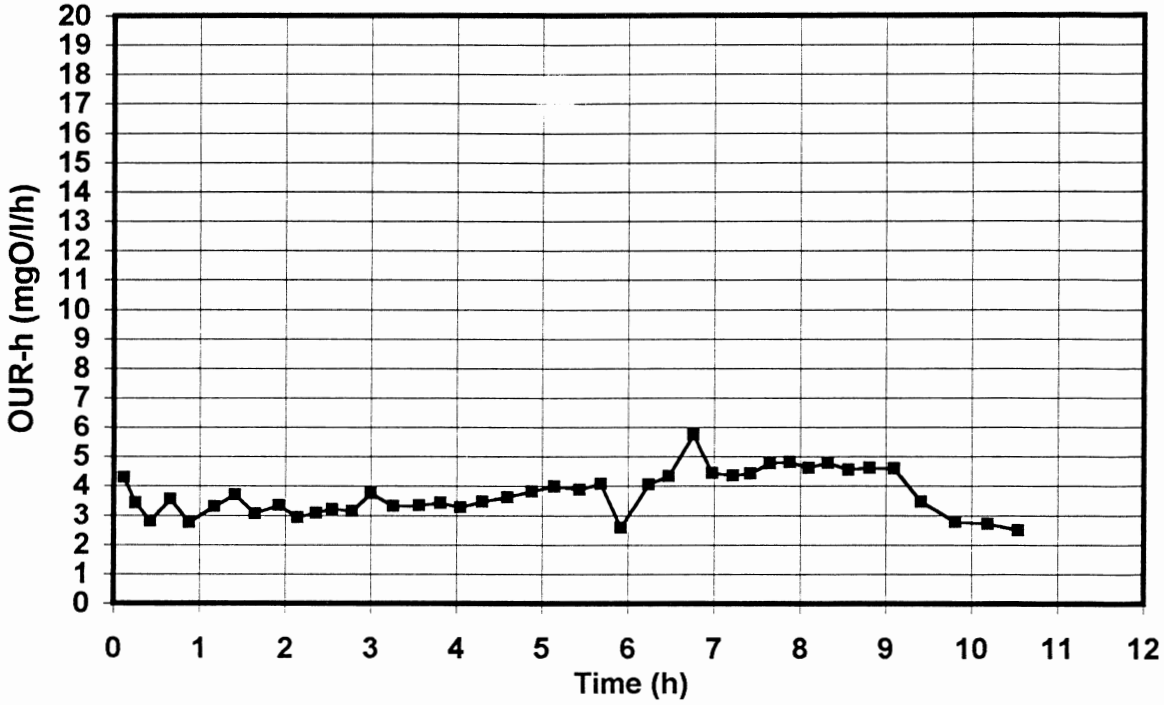
ℓn(OUR-h) graph for filtered wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test F1, 14-03, waste water batch no.21



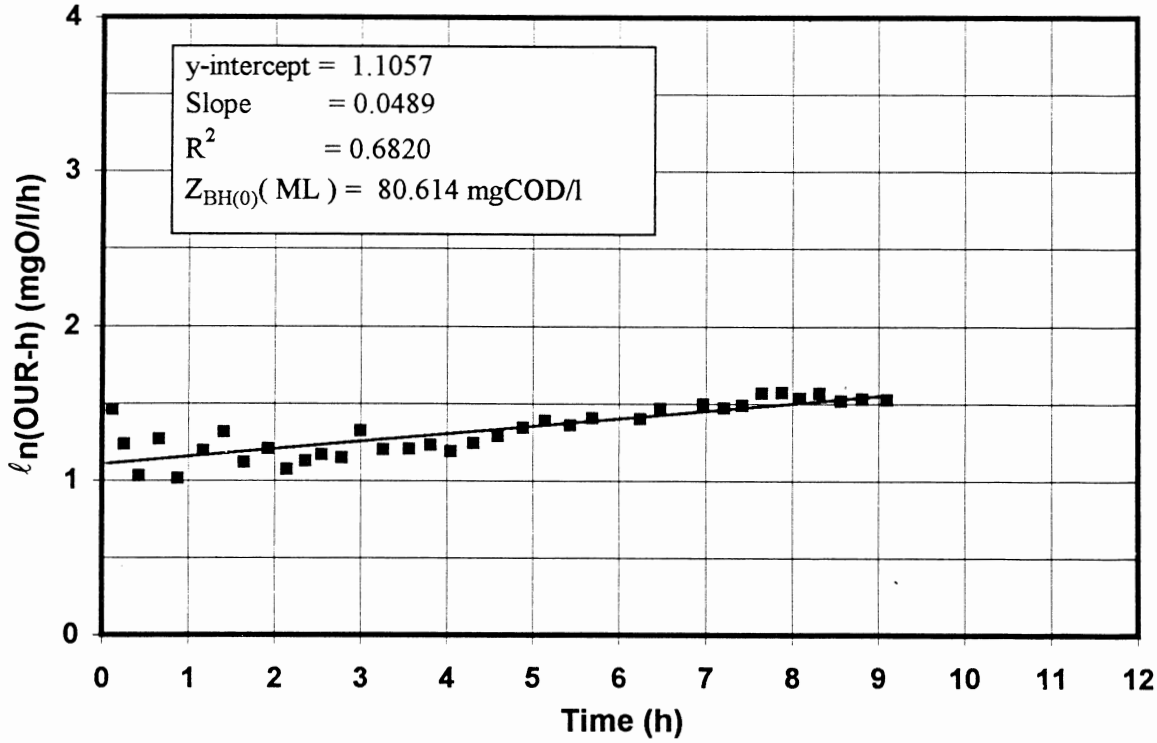
OUR Graph for filtered wastewater (2.9ℓ) plus mixed liquor (0.1ℓ) batch test F2, 15-03a, wastewater batch no. 21



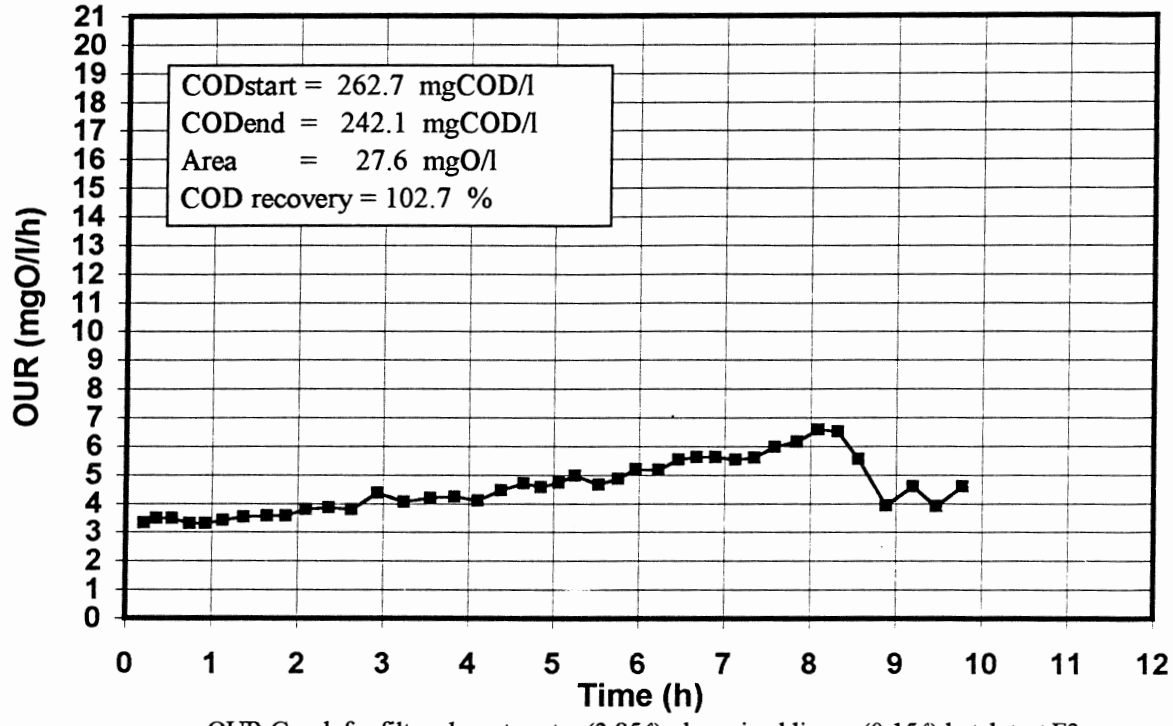
NO_3 Graph for filtered wastewater (2.9ℓ) plus mixed liquor (0.1ℓ) batch test F2, 15-03a, wastewater batch no. 21



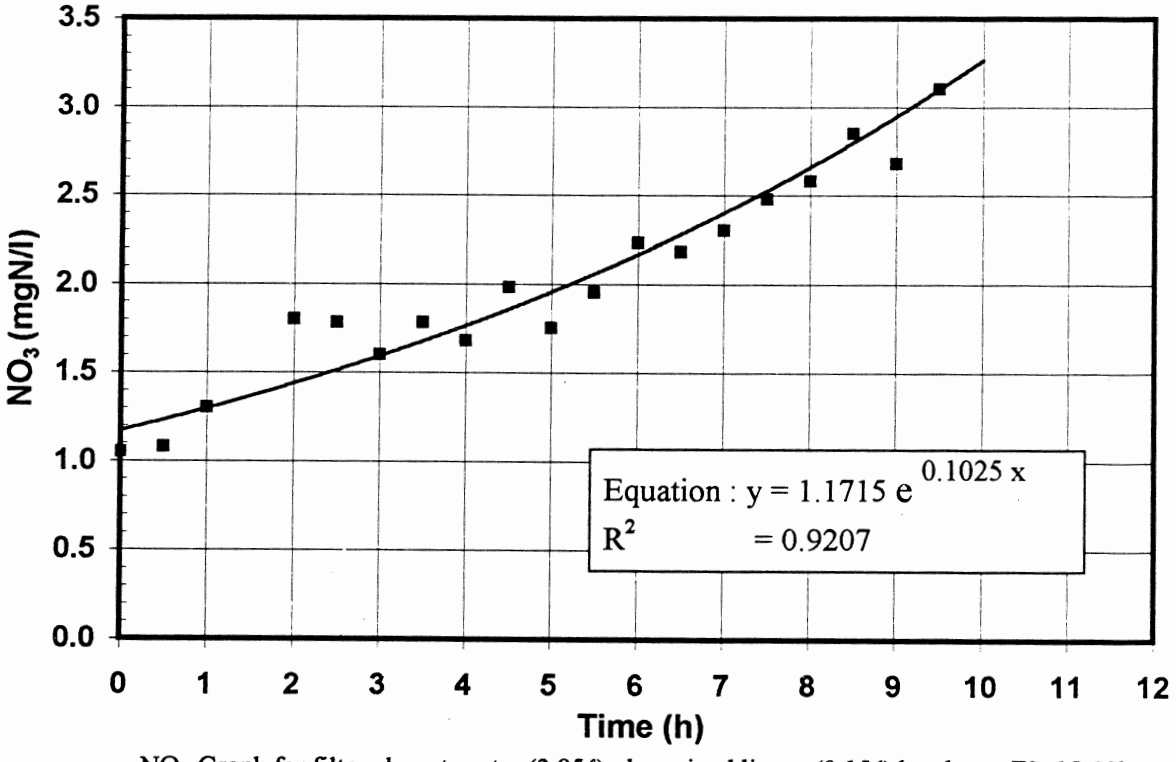
OUR-h Graph for filtered wastewater (2.9ℓ) plus mixed liquor (0.1ℓ) batch test F2, 15-03a, wastewater batch no. 21



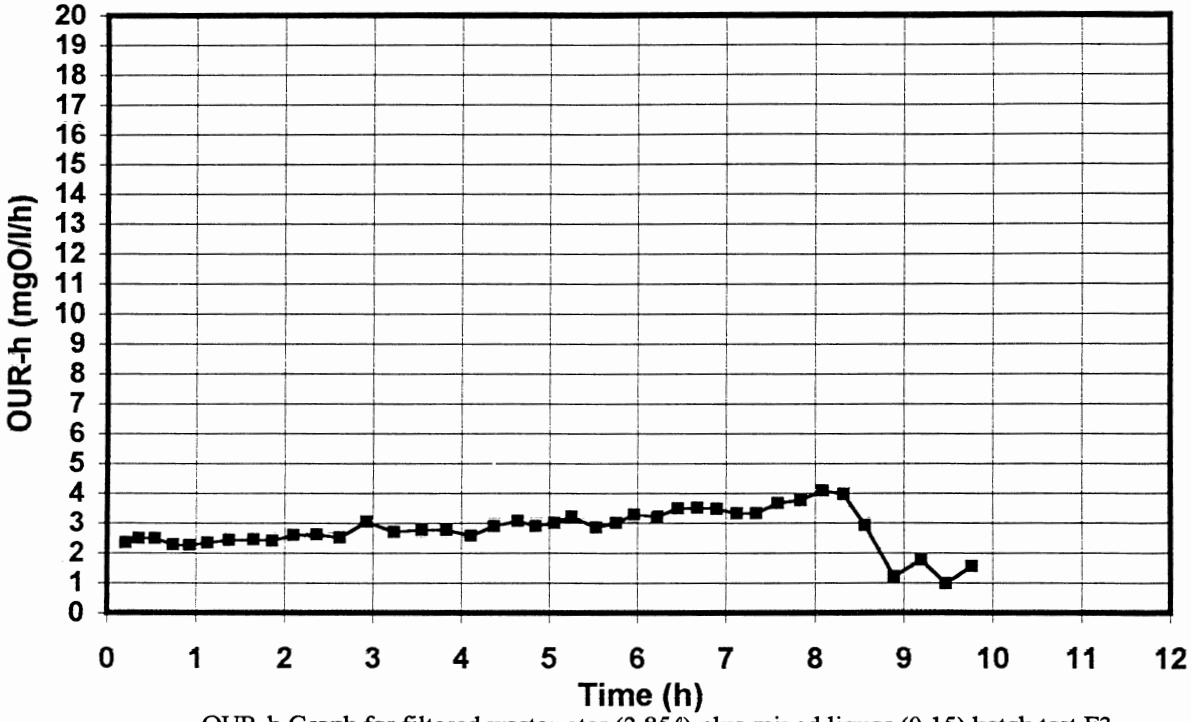
ℓn(OUR-h) graph for filtered wastewater (2.9ℓ) plus mixed liquor (0.1ℓ) batch test F2, 15-03a, waste water batch no.21



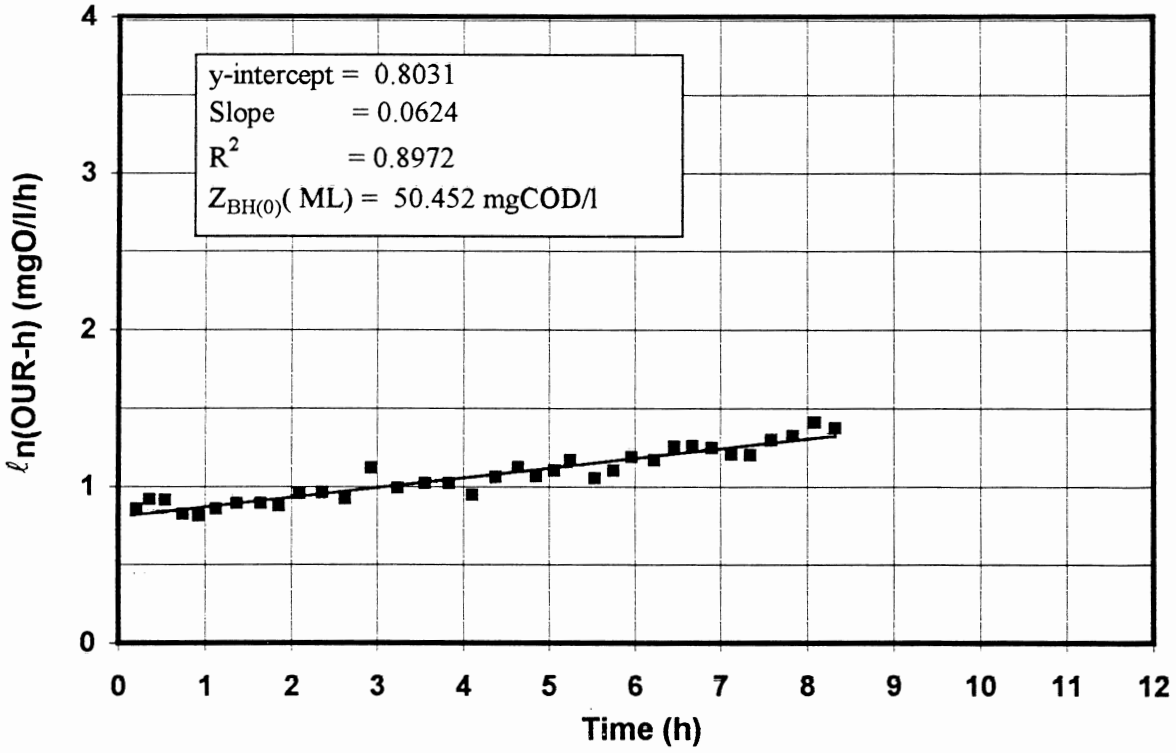
OUR Graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ) batch test F3, 15-03b, wastewater batch no. 21



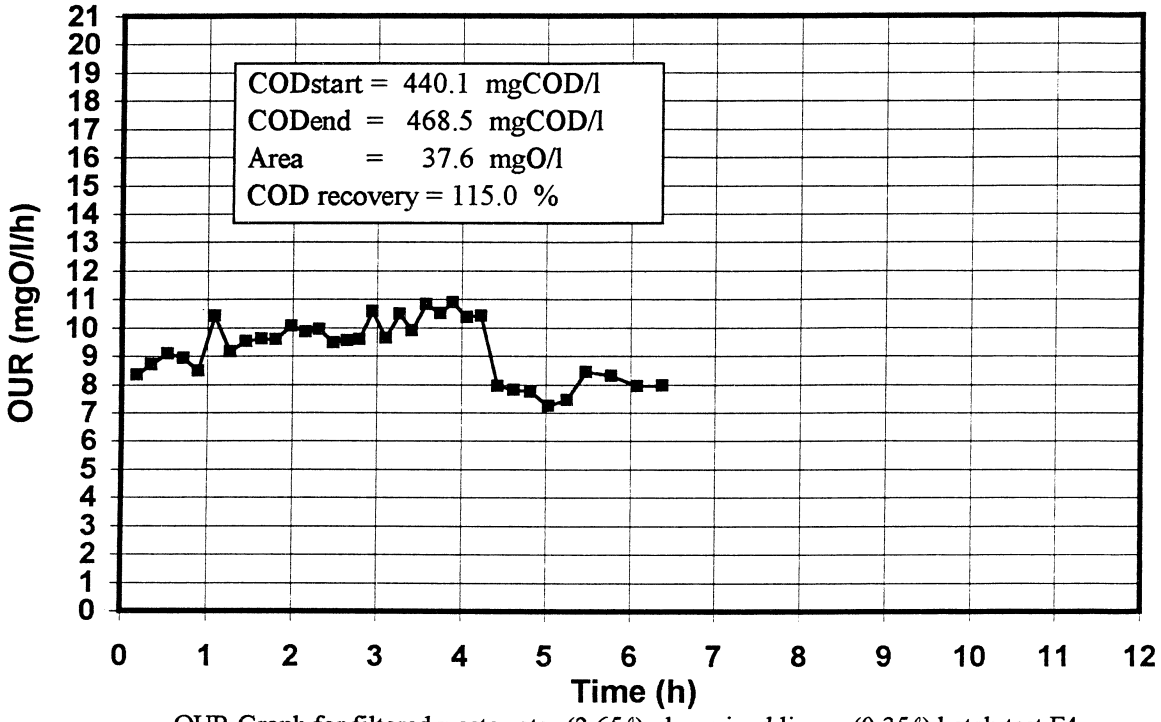
NO_3 Graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ) batch test F3, 15-03b, wastewater batch no. 21



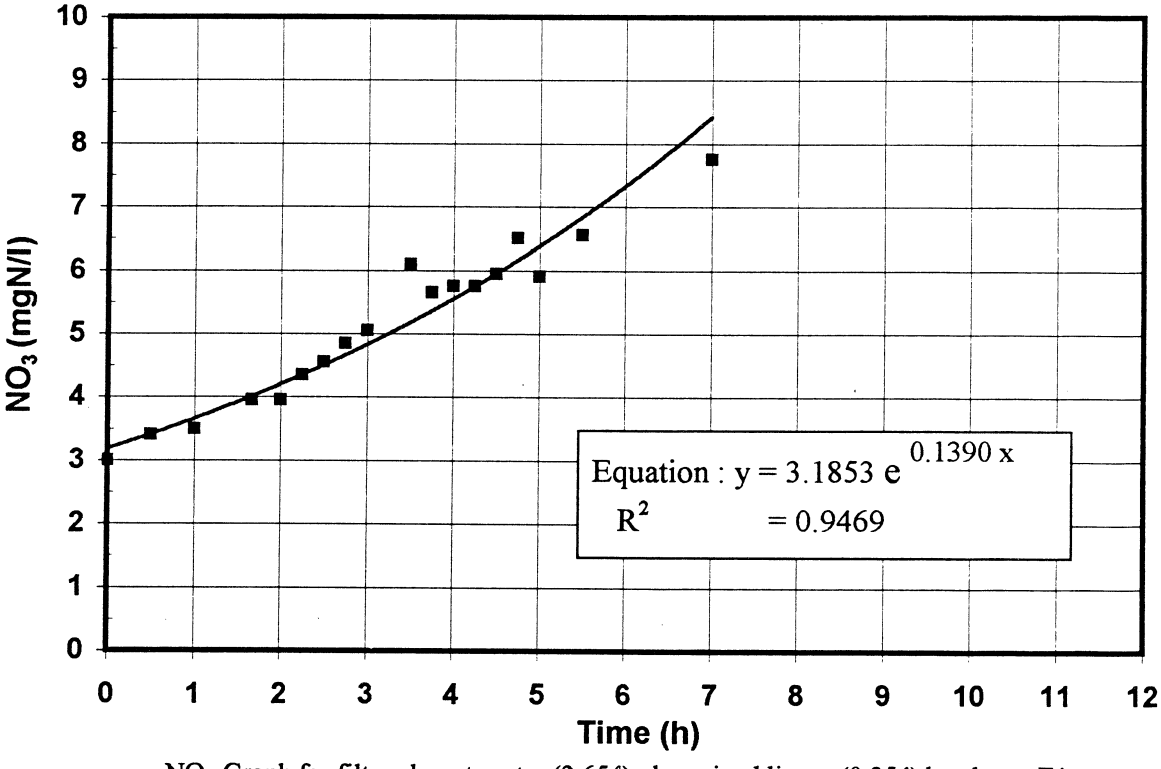
OUR-h Graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15) batch test F3, 15-03b, wastewater batch no. 21



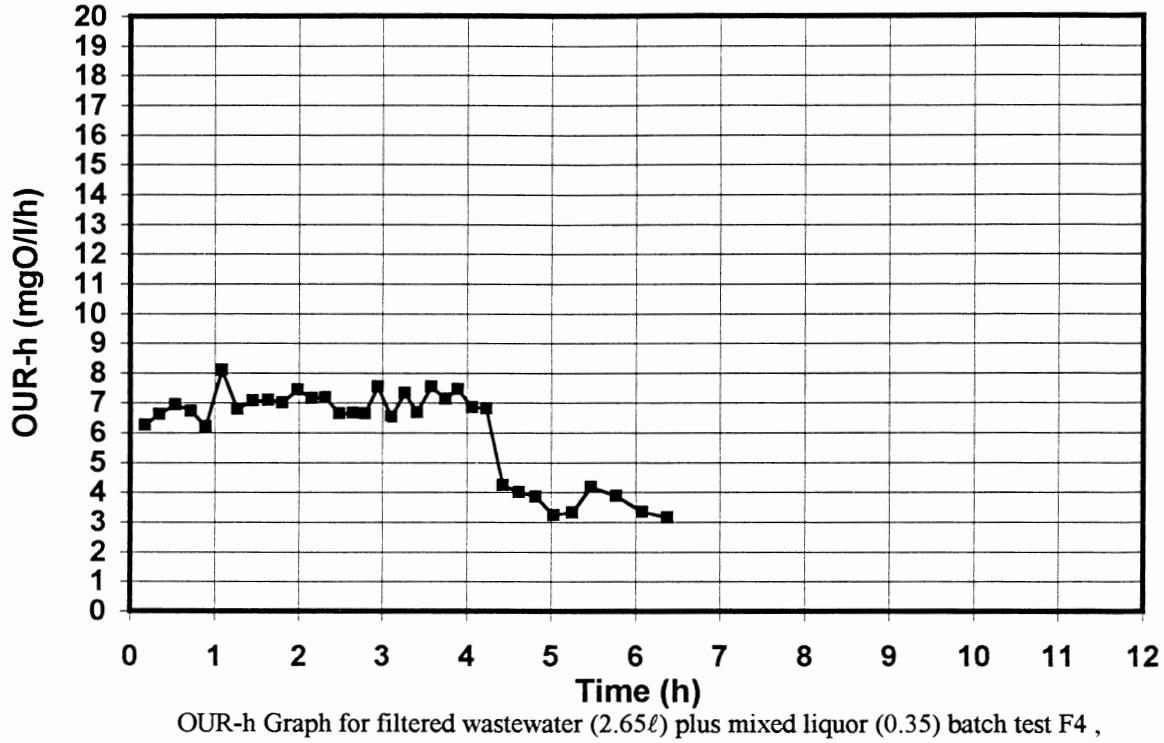
ℓn(OUR-h) graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ) batch test F3, 15-03b, waste water batch no.21



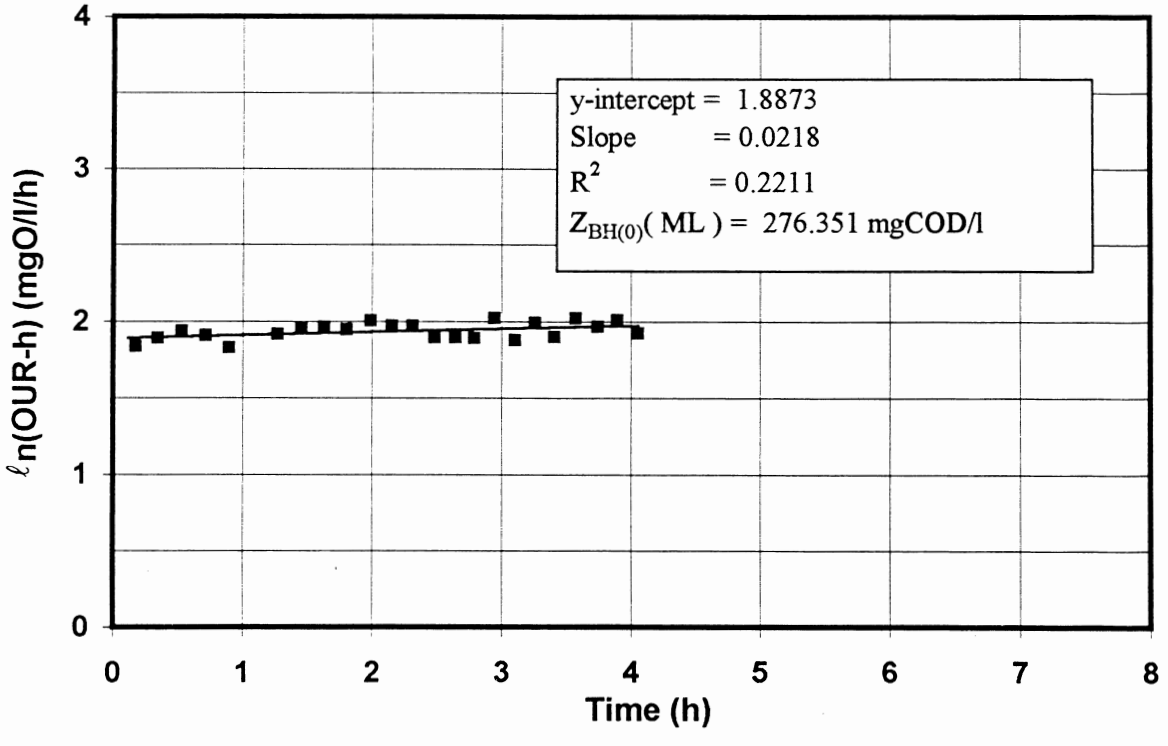
OUR Graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ) batch test F4 , 20-03a , wastewater batch no. 21



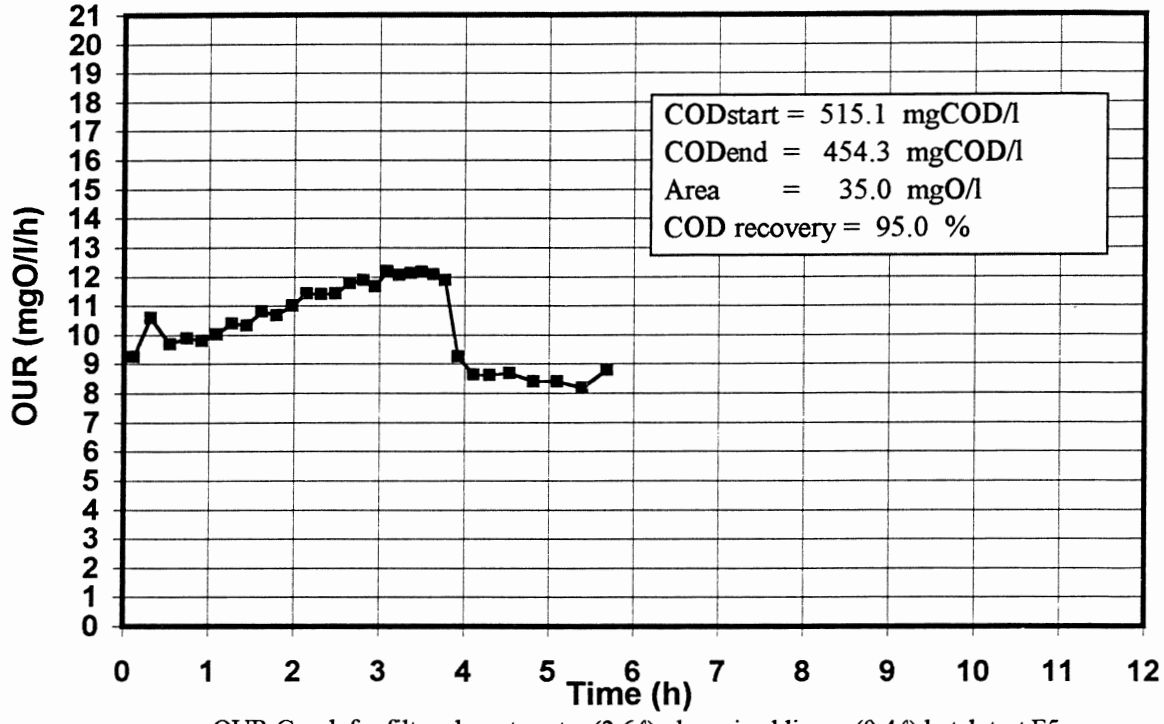
NO_3 Graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ) batch test F4 , 20-03a , wastewater batch no. 21



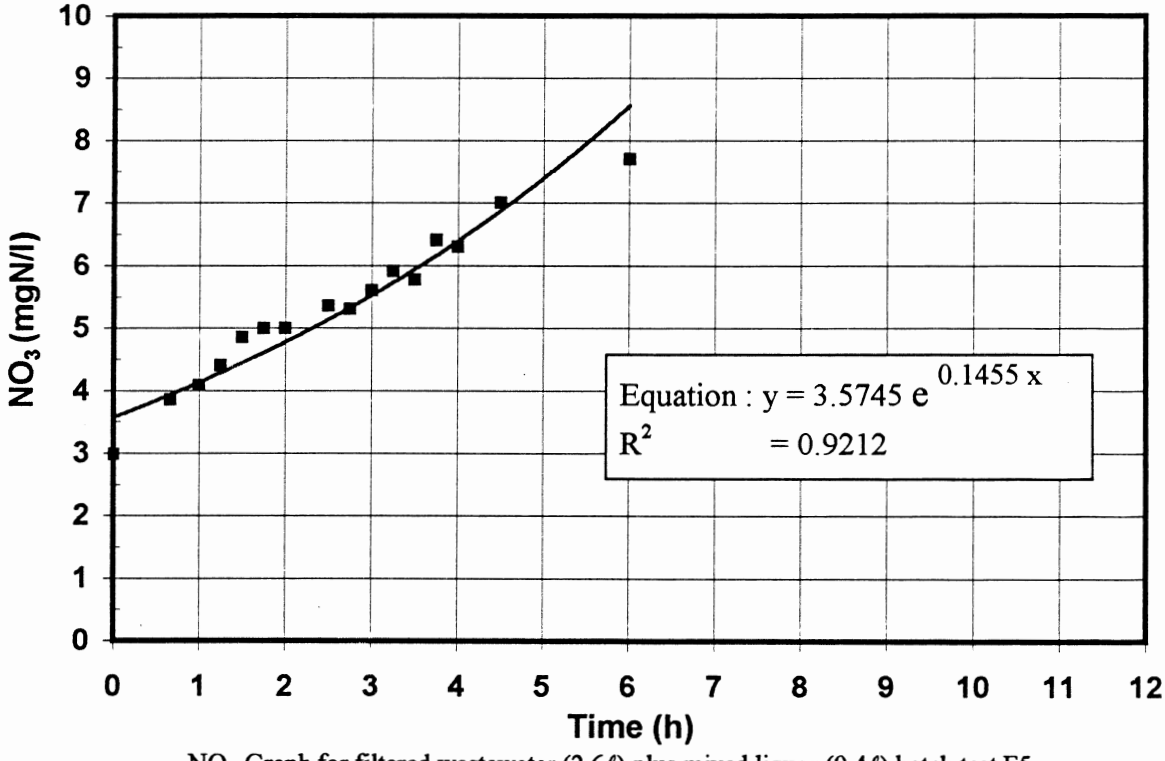
OUR-h Graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35) batch test F4 , 20-03a, wastewater batch no. 21



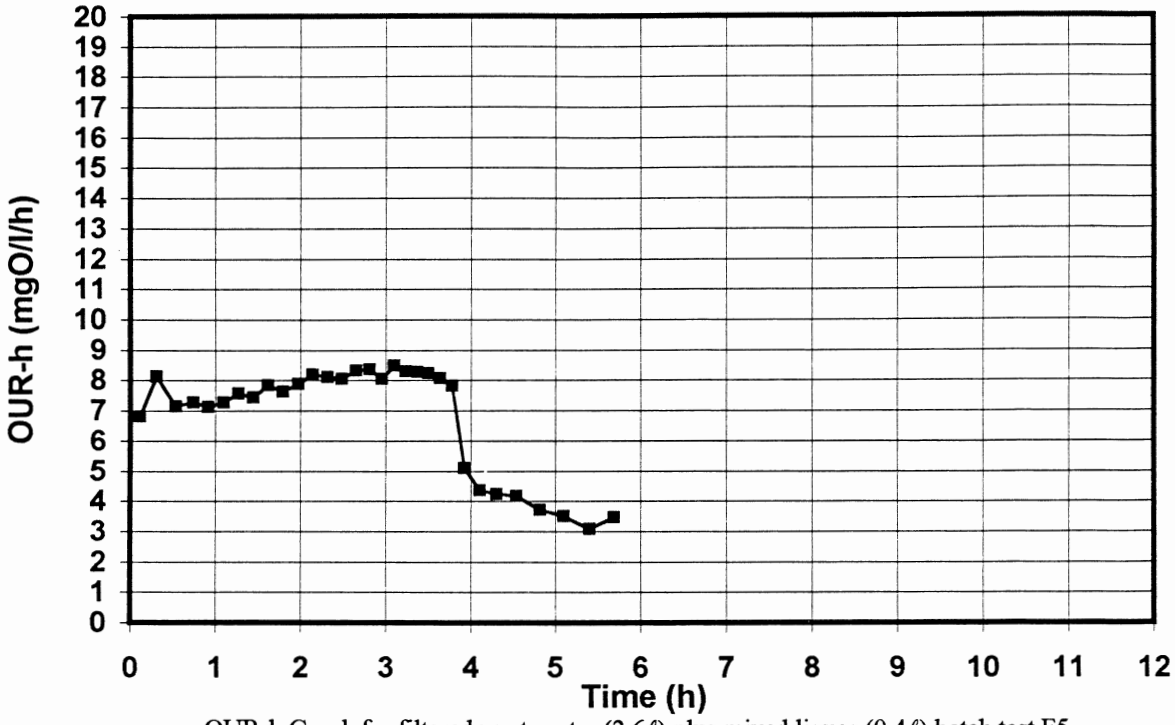
ℓn(OUR-h) graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ) batch test F4 , 20-03a, waste water batch no.21



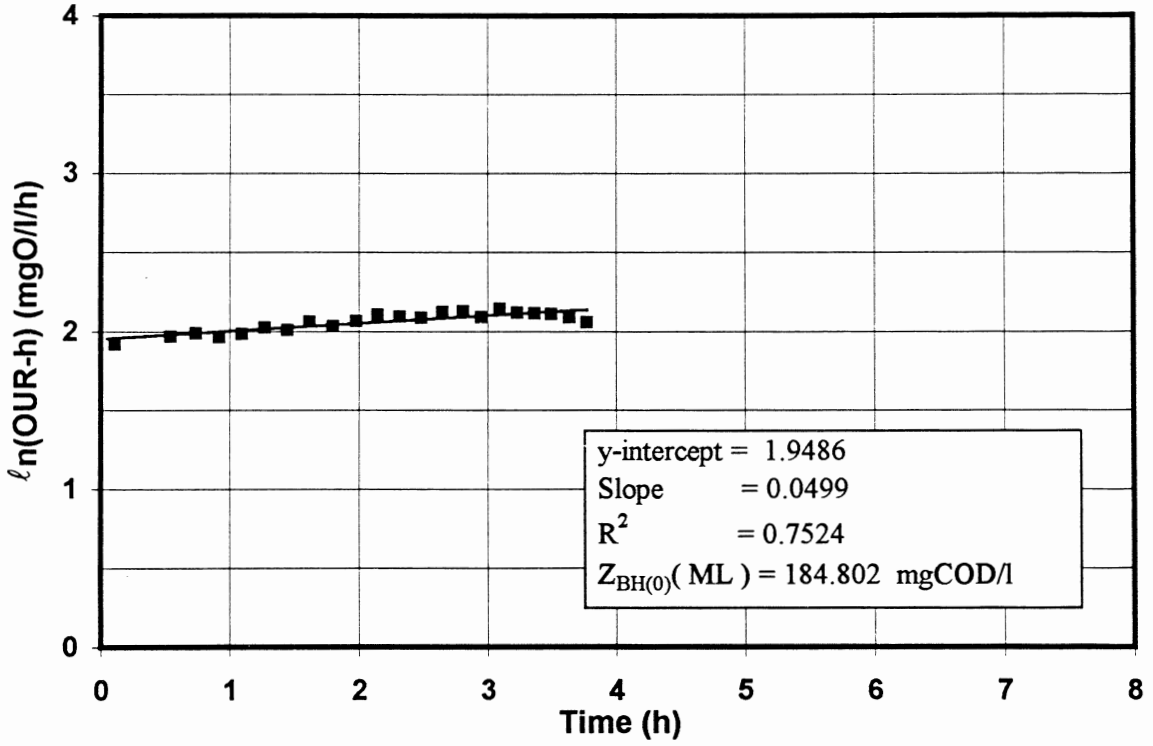
OUR Graph for filtered wastewater (2.6ℓ) plus mixed liquor (0.4ℓ) batch test F5 , 20-03b , wastewater batch no. 21



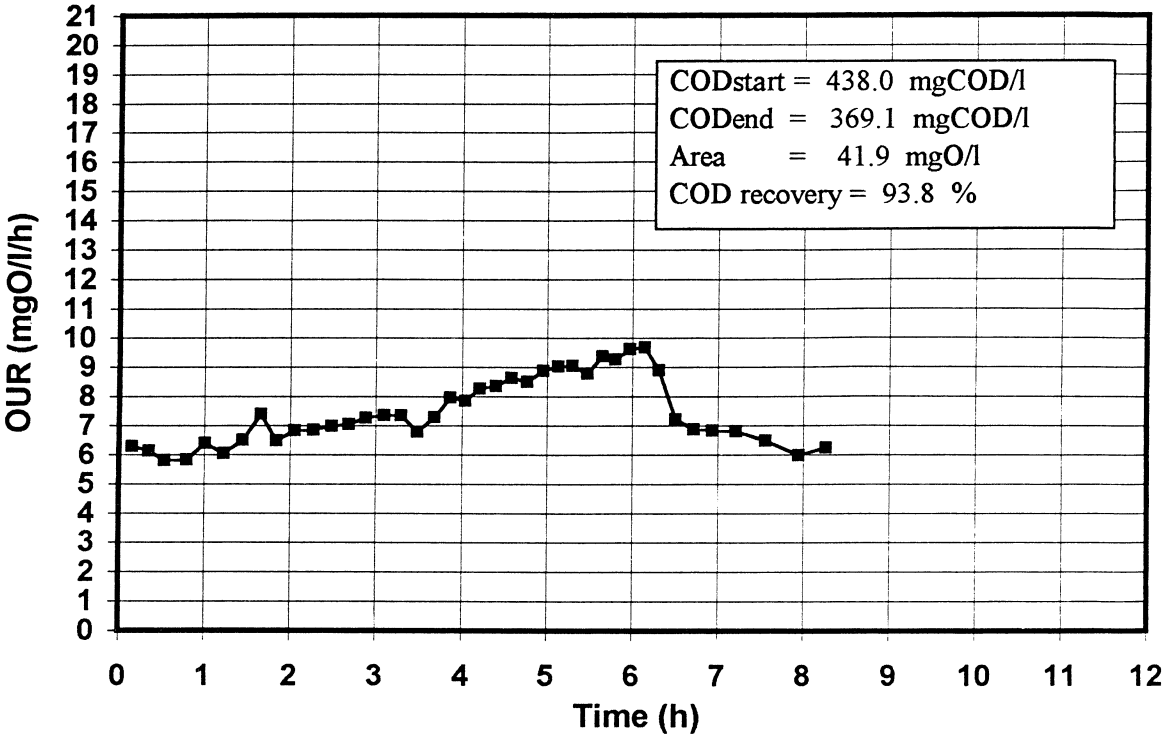
NO_3 Graph for filtered wastewater (2.6ℓ) plus mixed liquor (0.4ℓ) batch test F5 , 20-03b , wastewater batch no. 21



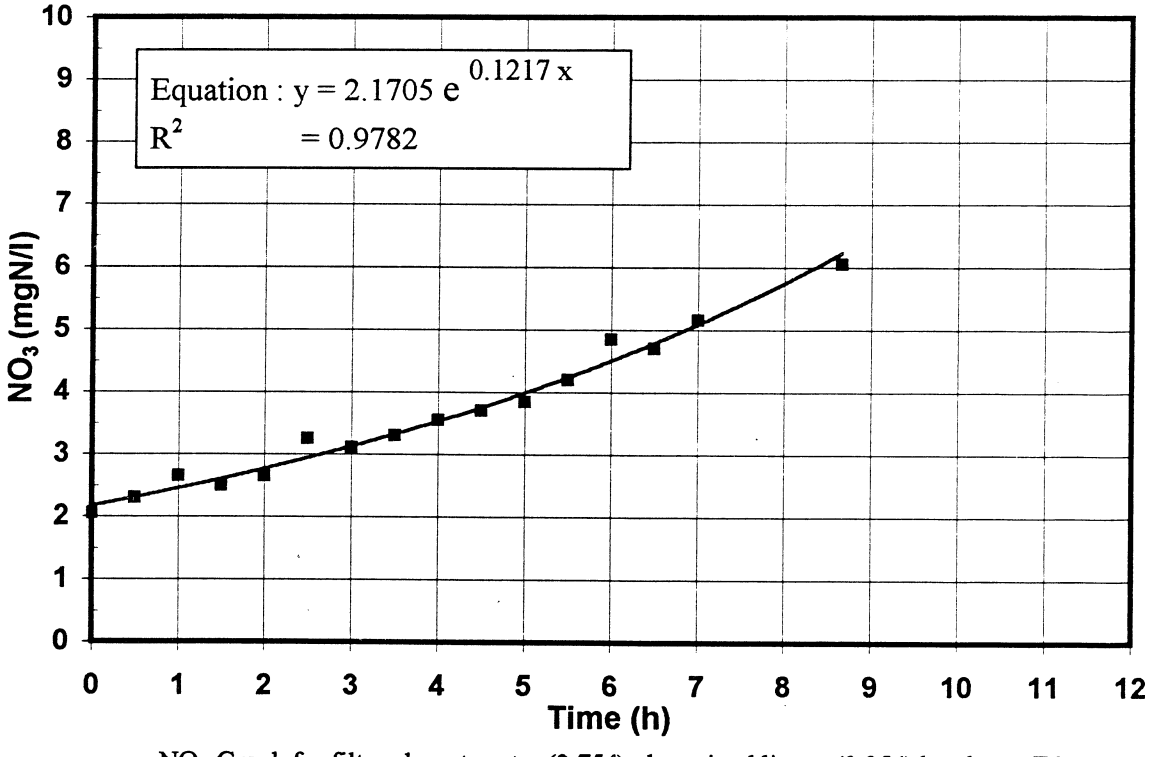
OUR-h Graph for filtered wastewater (2.6ℓ) plus mixed liquor (0.4ℓ) batch test F5 , 20-03b , wastewater batch no. 21



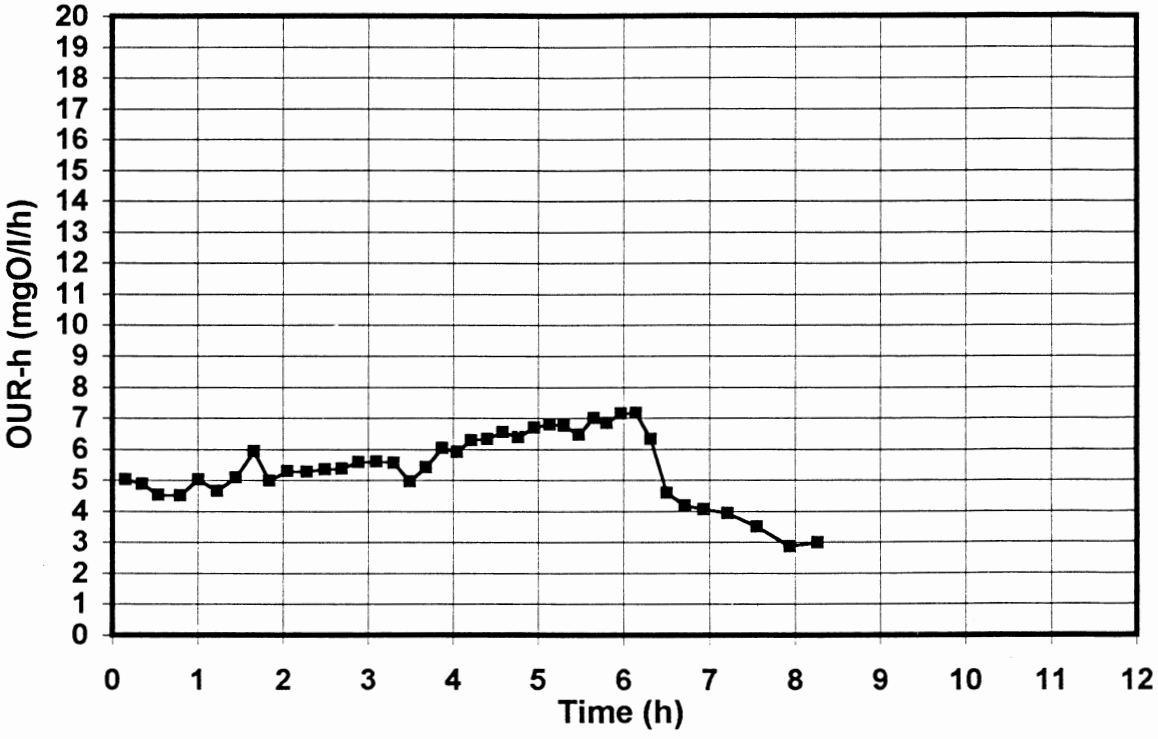
ℓn(OUR-h) graph for filtered wastewater (2.6ℓ) plus mixed liquor (0.4ℓ) batch test F5 , 20-03b , waste water batch no.21



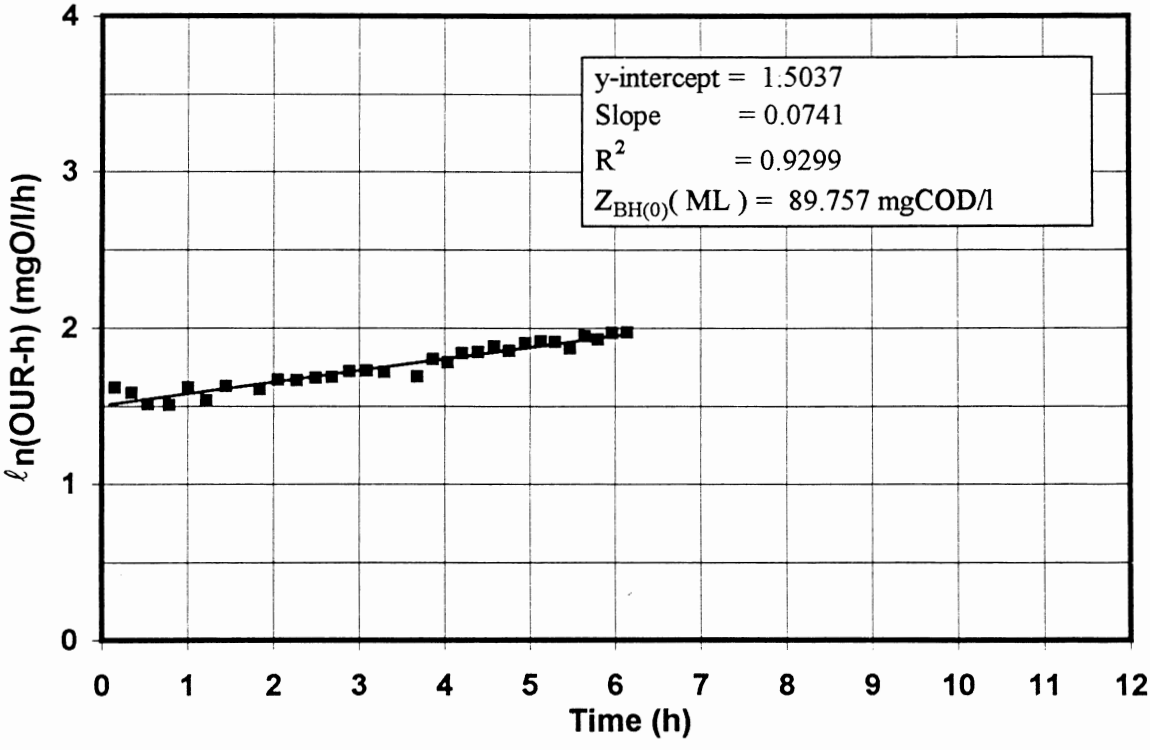
OUR Graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ) batch test F6 , 21-03a , wastewater batch no. 21



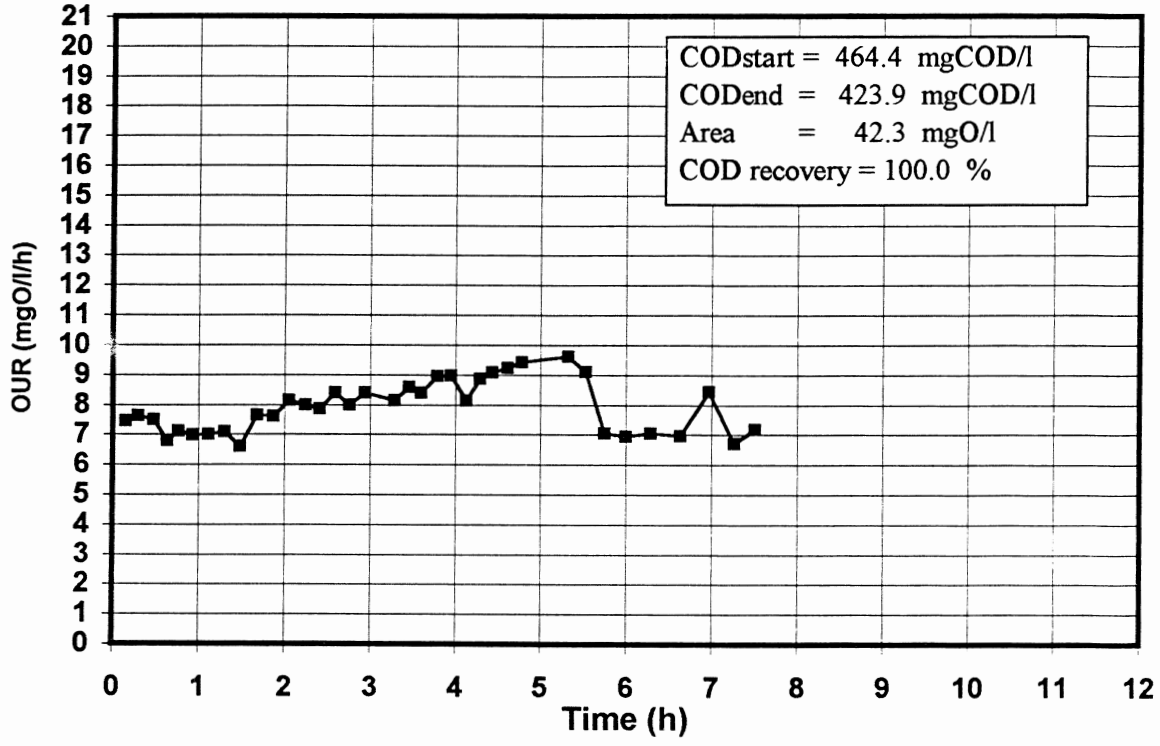
NO_3 Graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ) batch test F6 , 21-03a , wastewater batch no. 21



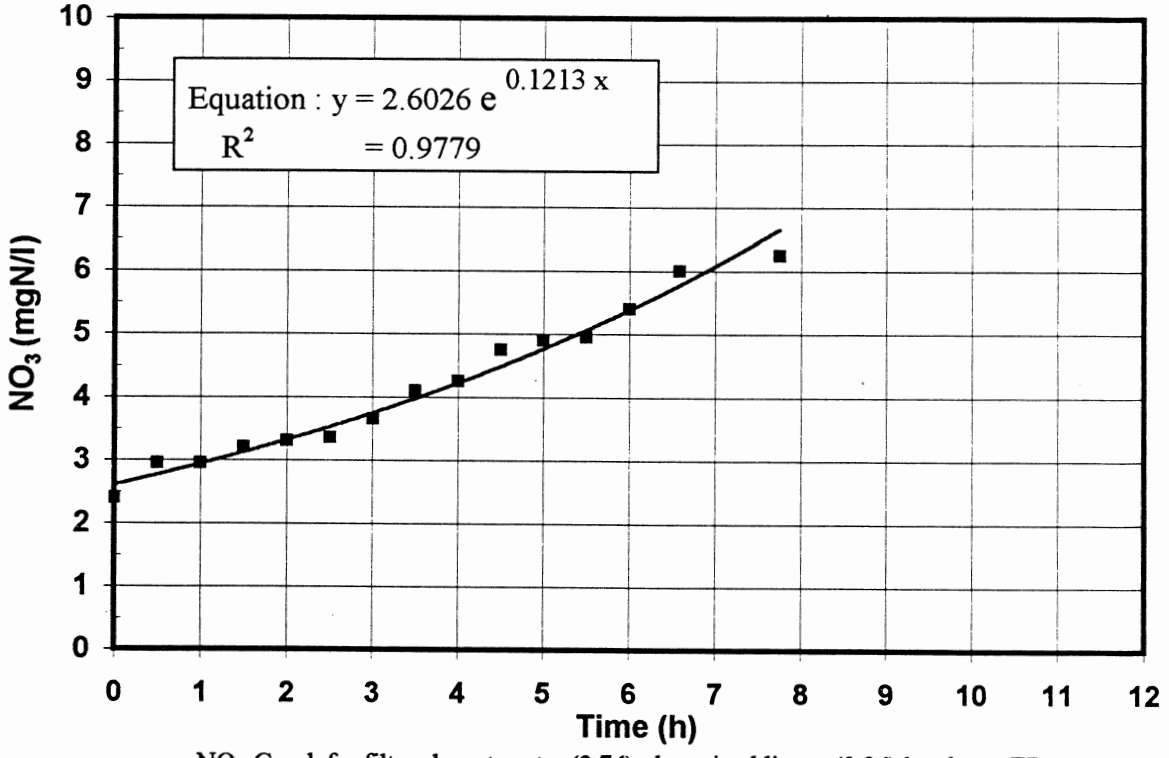
OUR-h Graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25) batch test F6 , 21-03a , wastewater batch no. 21



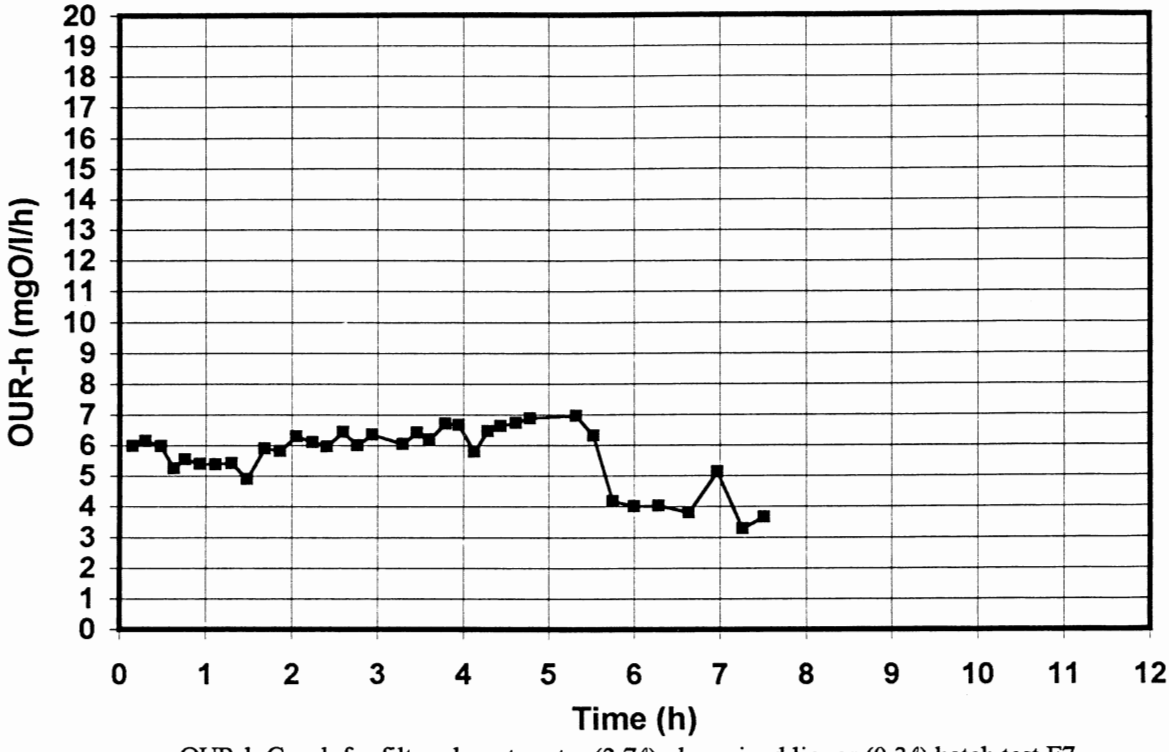
ℓn(OUR-h) graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ) batch test F6 , 21-03a , waste water batch no.21



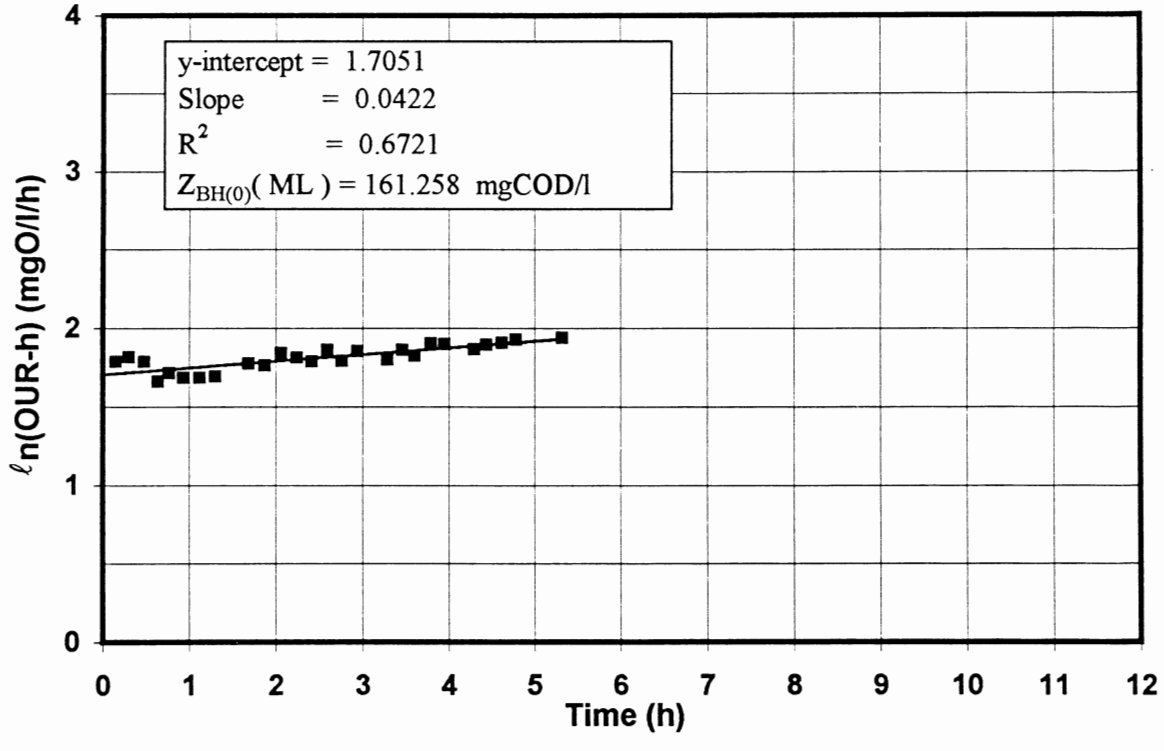
OUR Graph for filtered wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test F7 , 21-03b , wastewater batch no. 21



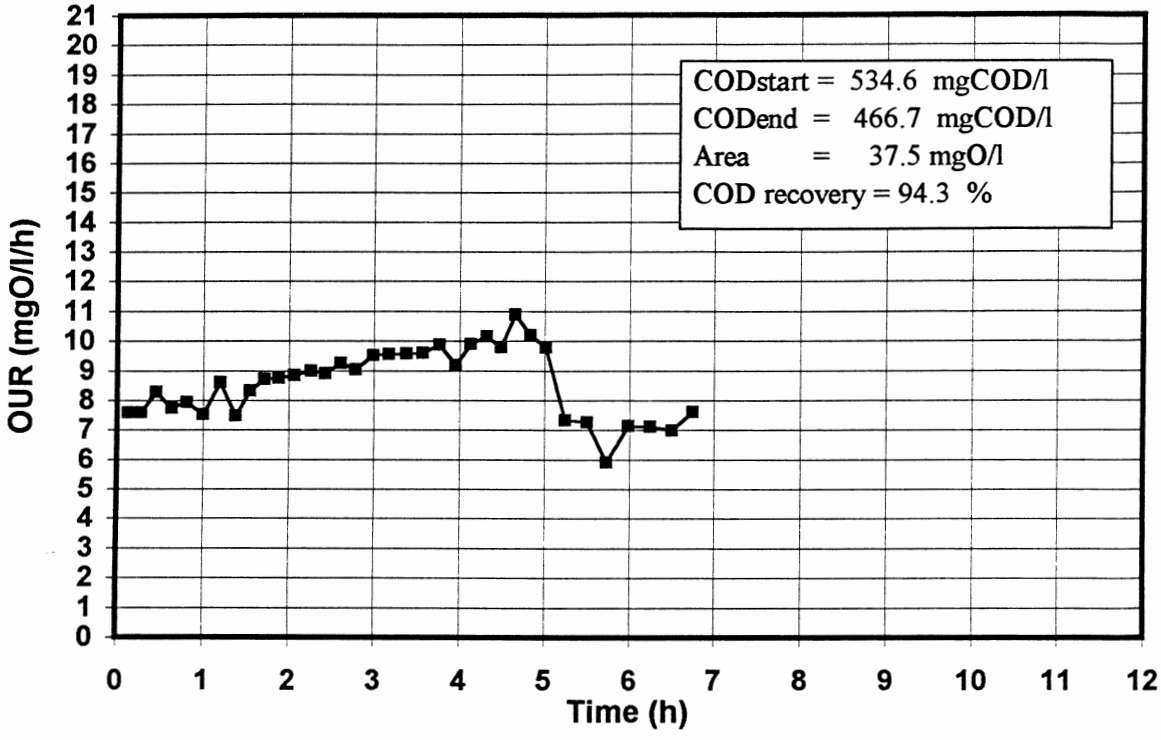
NO_3 Graph for filtered wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test F7 , 21-03b , wastewater batch no. 21



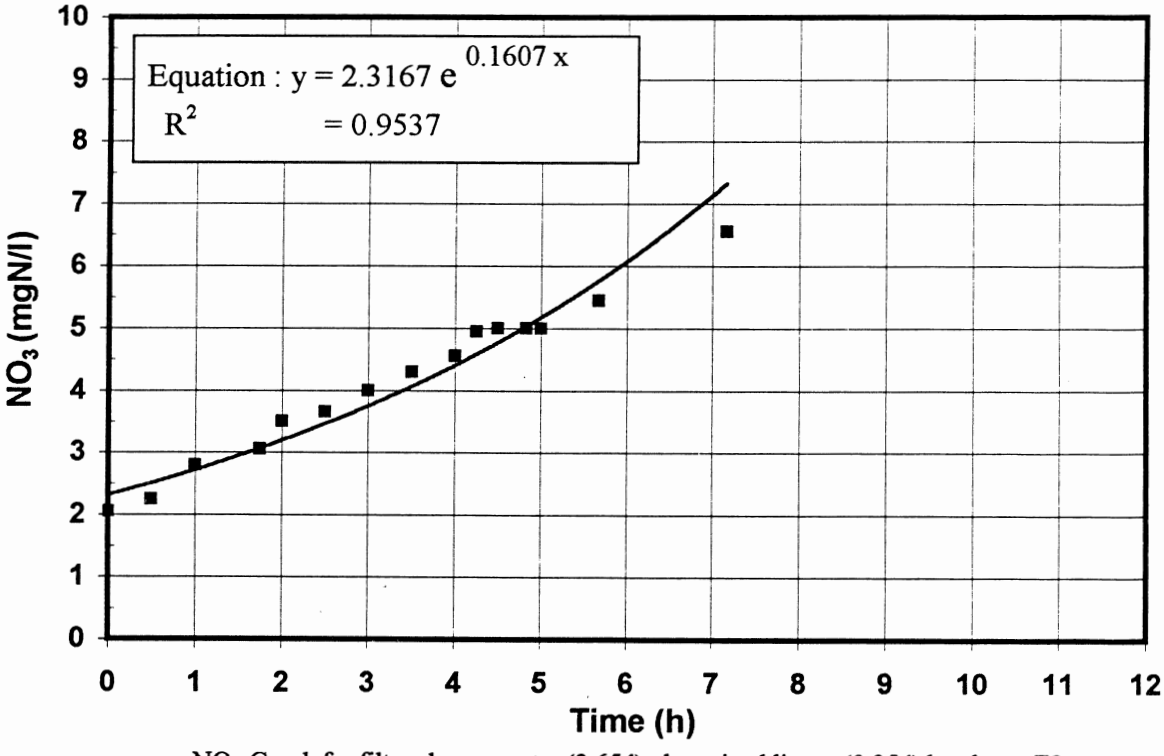
OUR-h Graph for filtered wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test F7 , 21-03b , wastewater batch no. 21



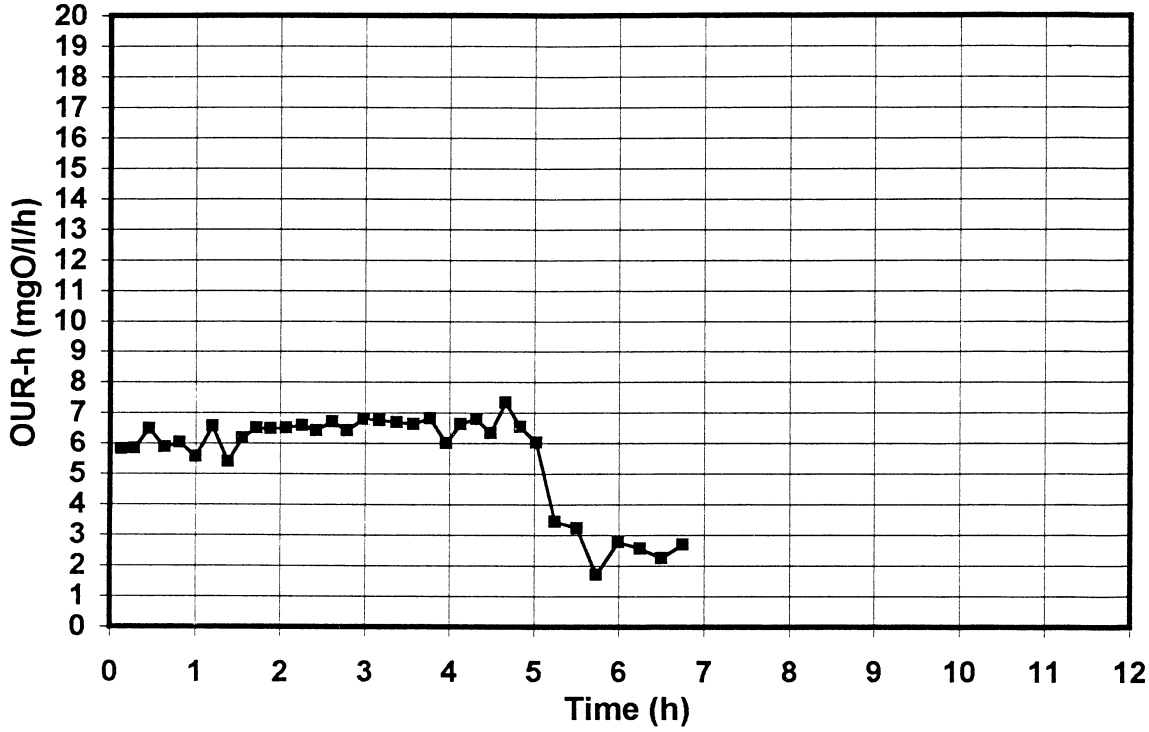
ℓn(OUR-h) graph for filtered wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test F7 , 21-03b , waste water batch no.21



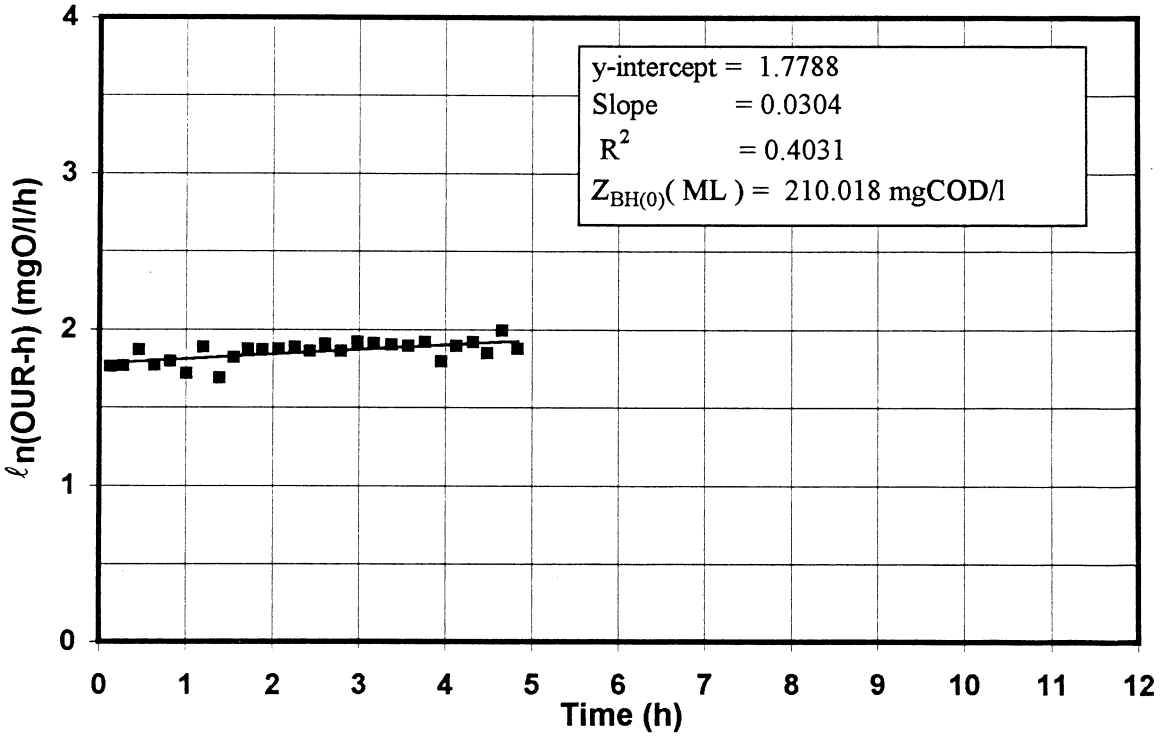
OUR Graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ) batch test F8 , 28-03 , wastewater batch no. 22



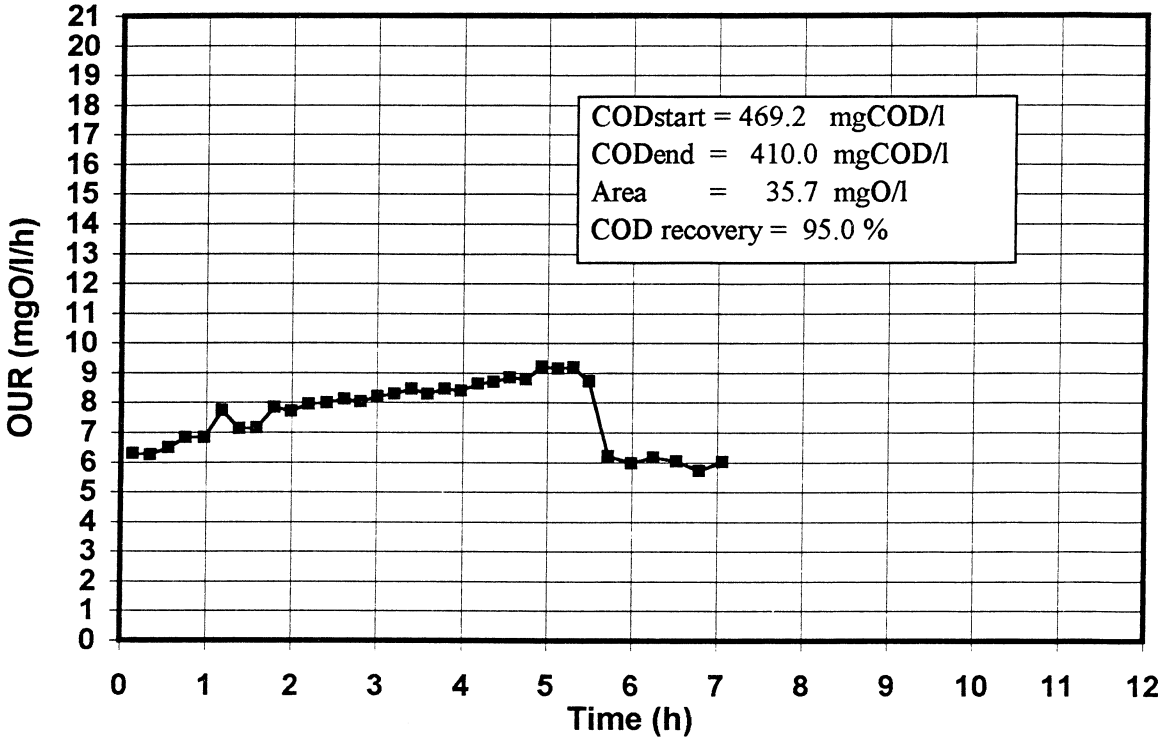
NO_3 Graph for filtered w water (2.65ℓ) plus mixed liquor (0.35ℓ) batch test F8 , 03 , wastewater batch no. 22



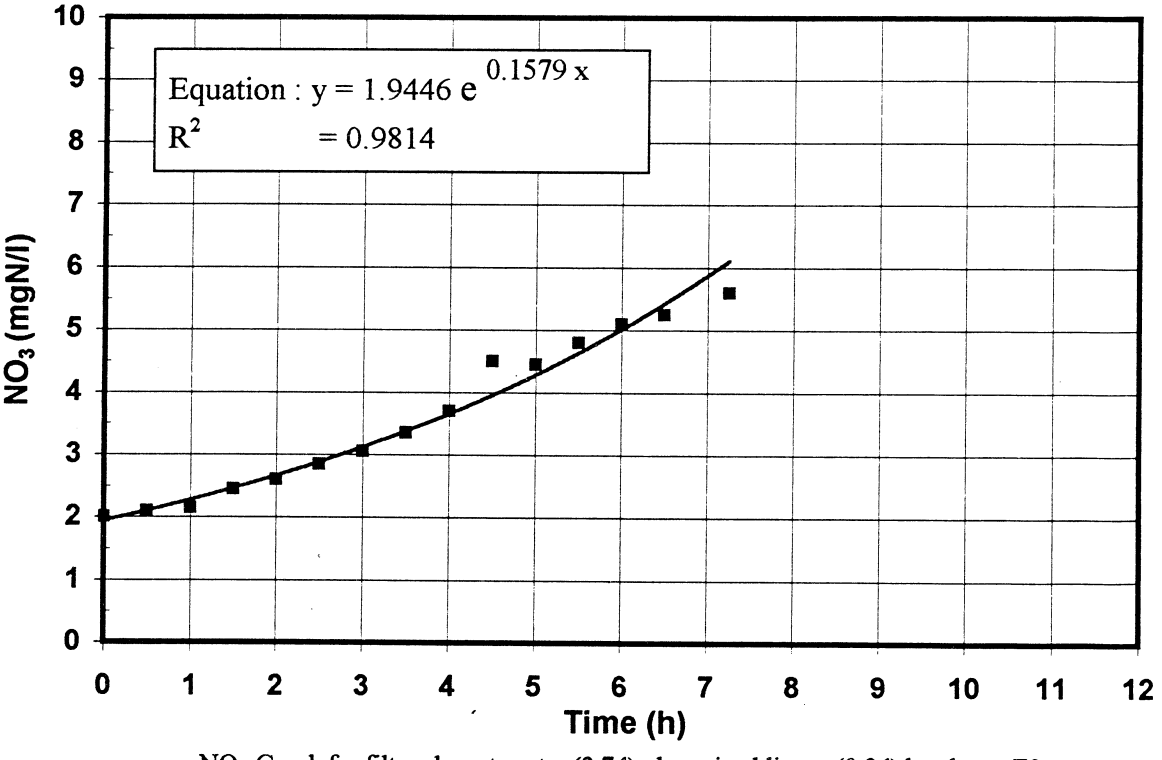
OUR-h Graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35) batch test F8 , 28-03 , wastewater batch no. 22



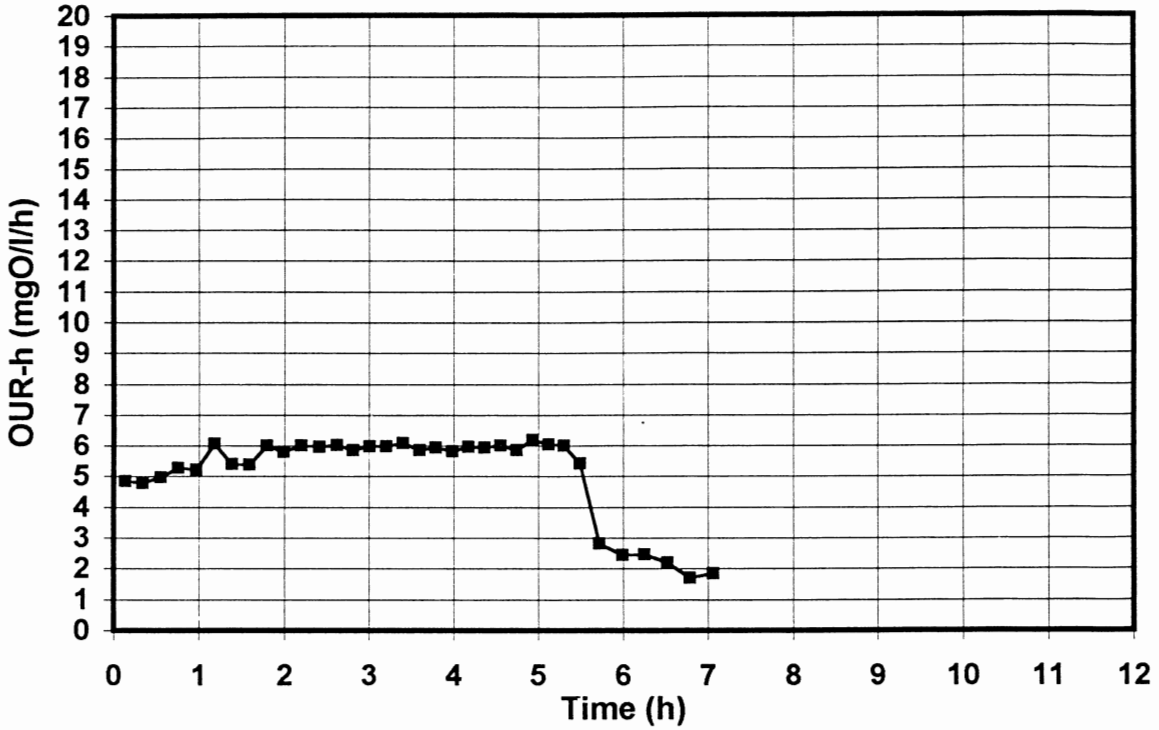
ℓn(OUR-h) graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ) batch test F8 , 28-03 , waste water batch no.22



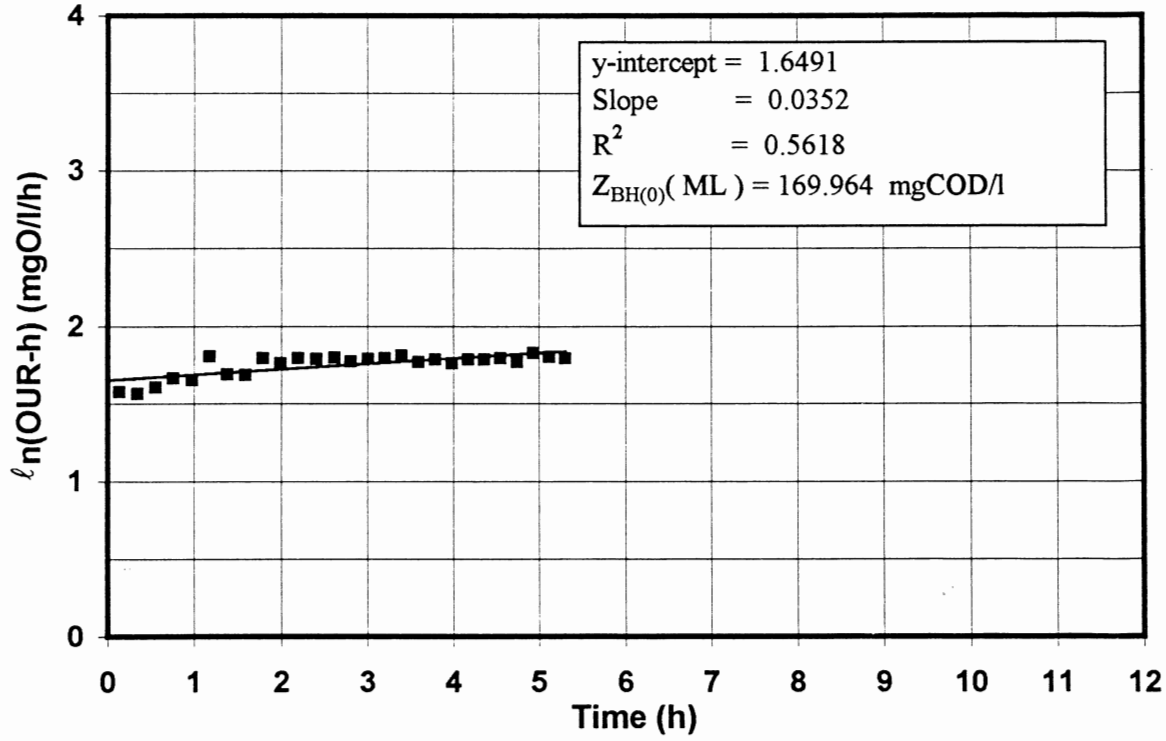
OUR Graph for filtered wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test F9 , 29-03a , wastewater batch no. 22



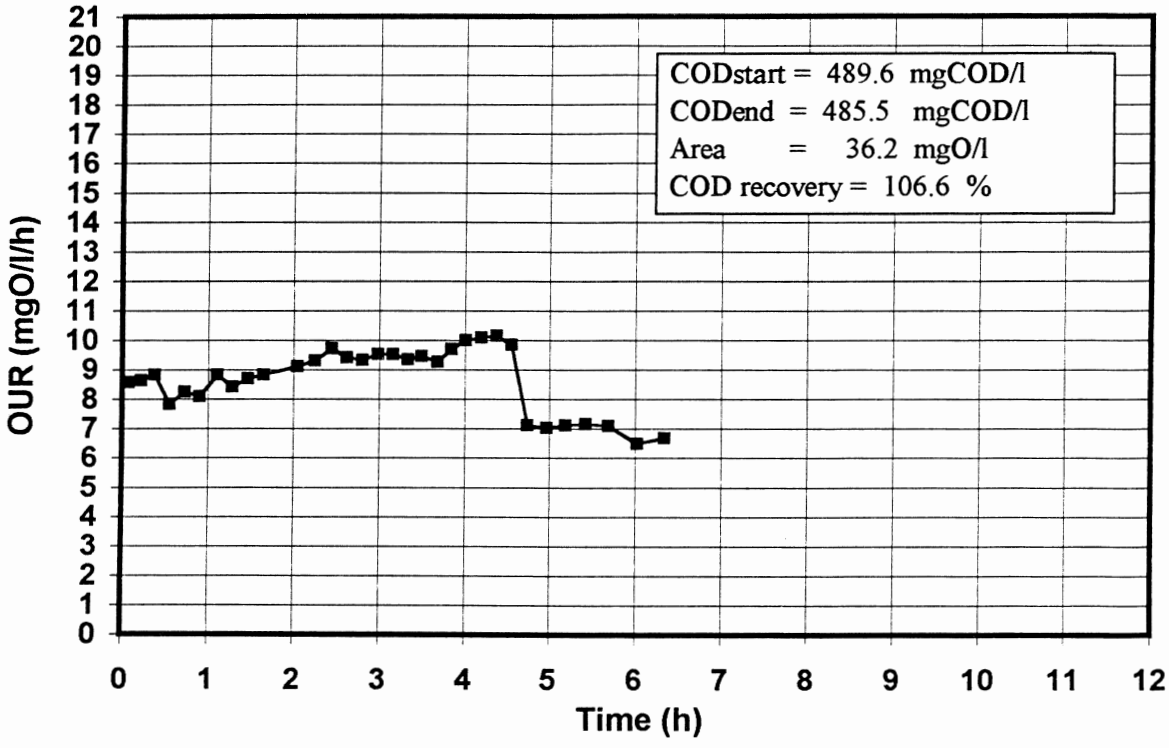
NO_3 Graph for filtered wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test F9 , 29-03a , wastewater batch no. 22



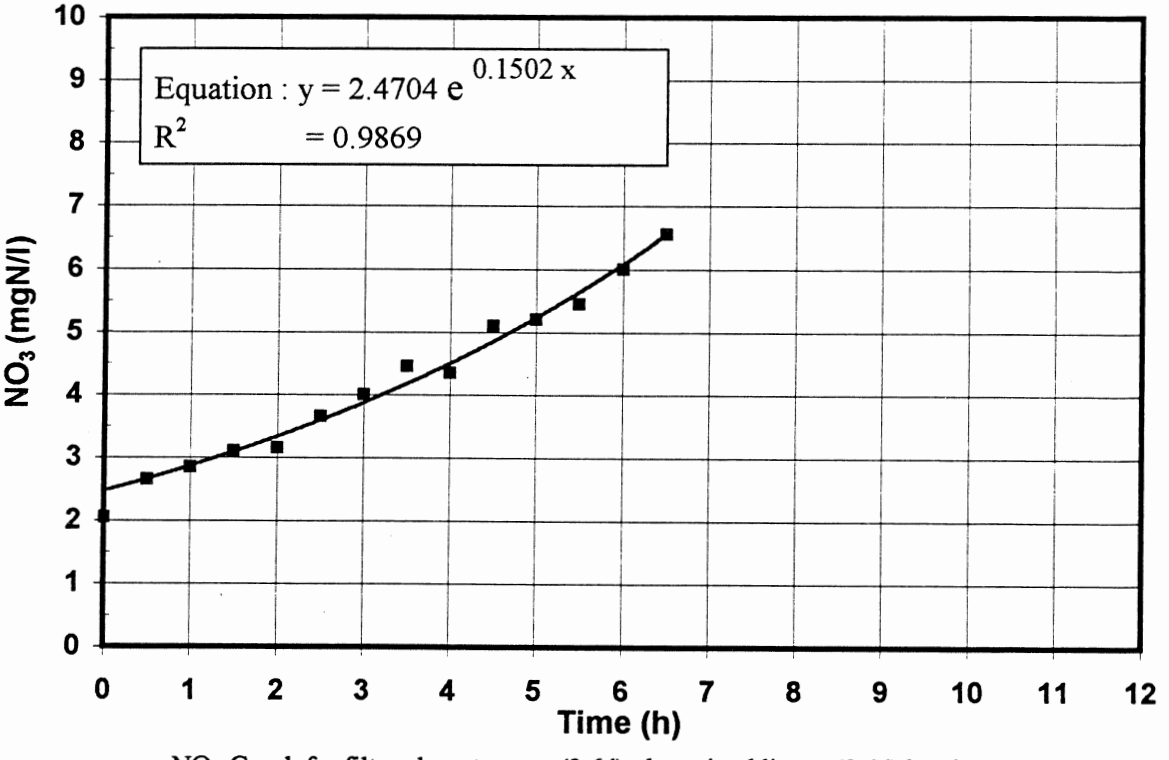
OUR-h Graph for filtered wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test F9 , 29-03a , wastewater batch no. 22



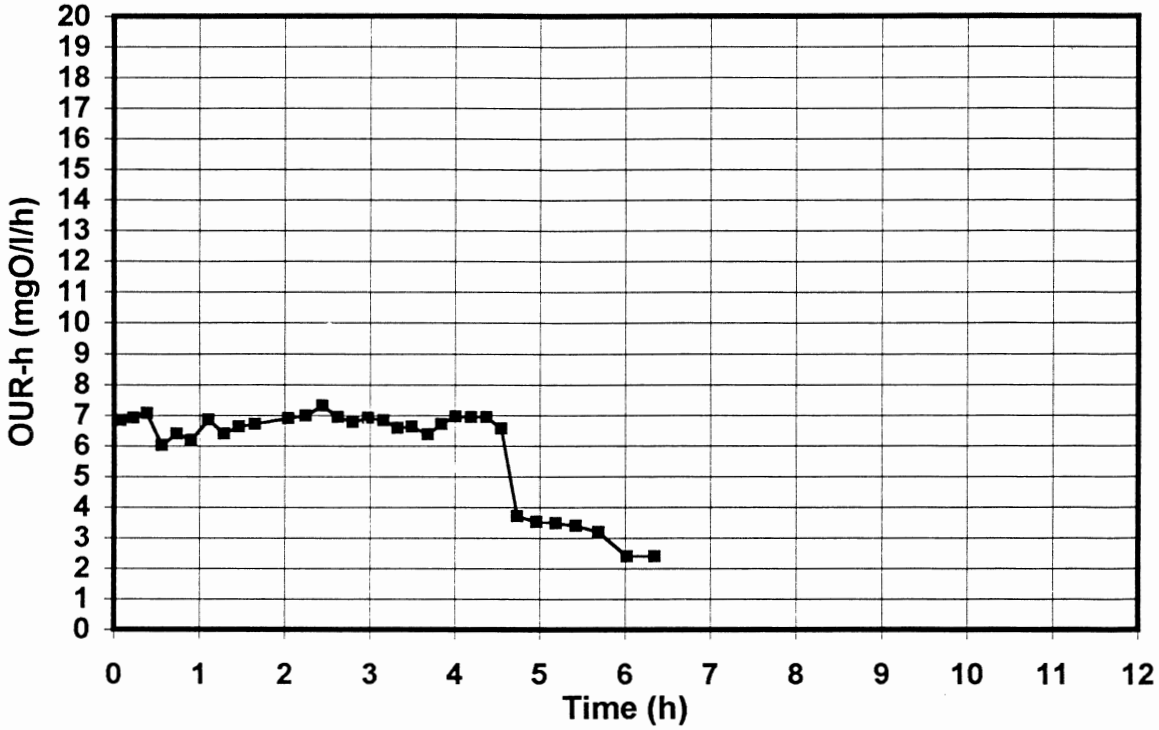
ℓn(OUR-h) graph for filtered wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test F9 , 29-03a , waste water batch no.22



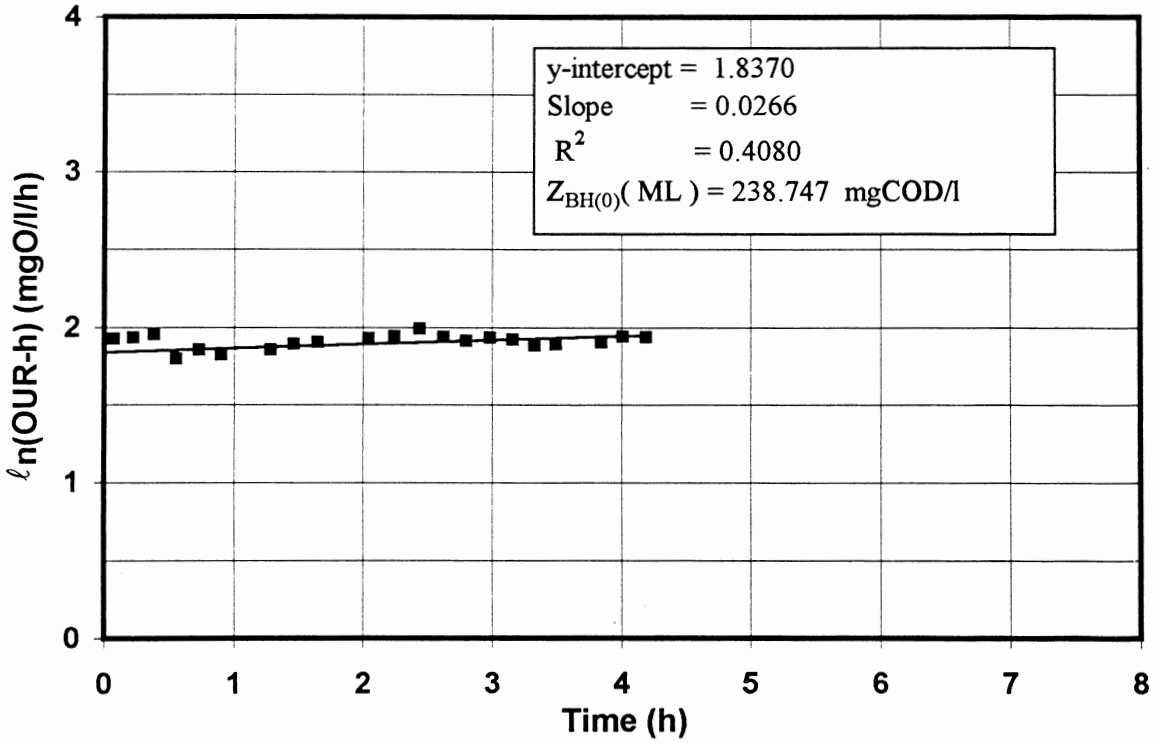
OUR Graph for filtered wastewater (2.6ℓ) plus mixed liquor (0.4ℓ) batch test F10 , 29-03b, wastewater batch no. 22



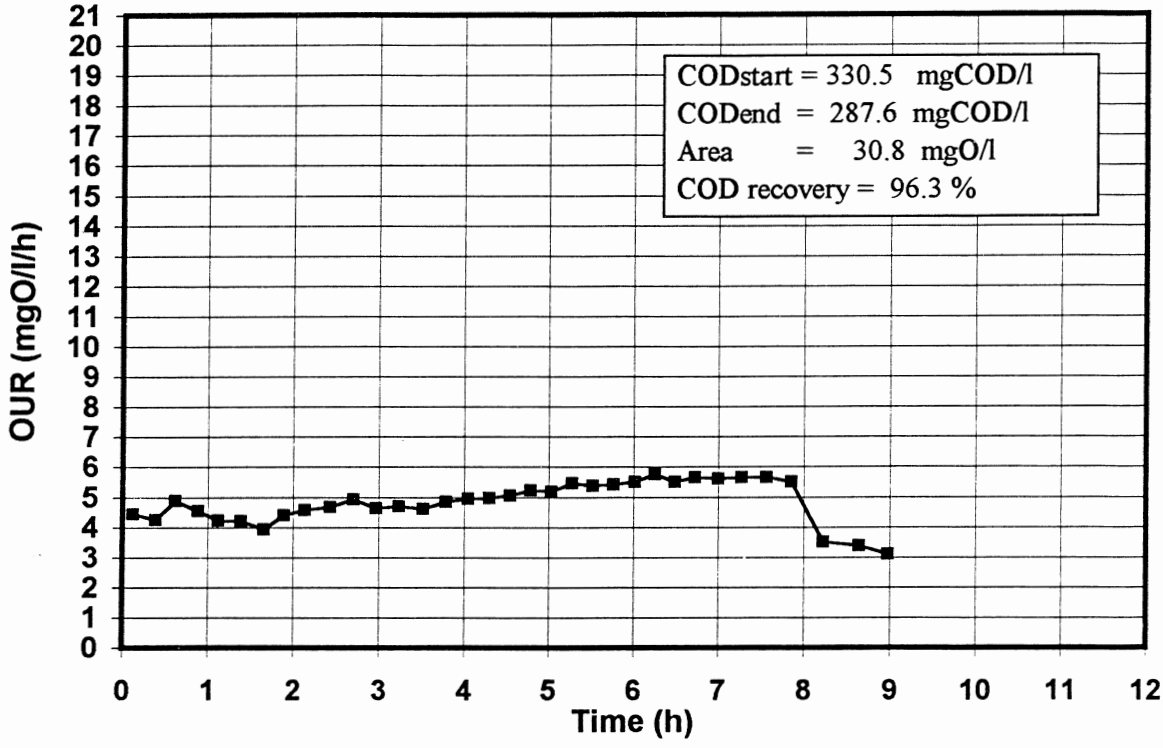
NO_3 Graph for filtered wastewater (2.6ℓ) plus mixed liquor (0.4ℓ) batch test F10 , 29-03b, wastewater batch no. 22



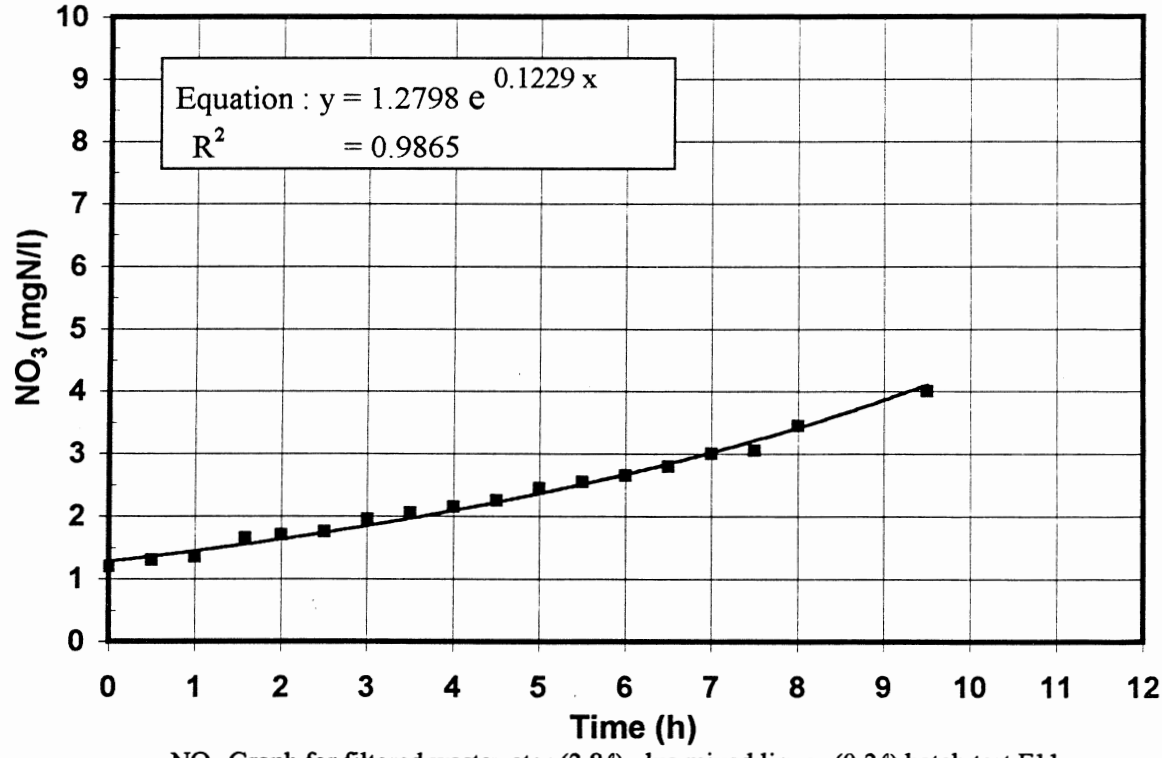
OUR-h Graph for filtered wastewater (2.6ℓ) plus mixed liquor (0.4ℓ) batch test F10 , 29-03b , wastewater batch no. 22



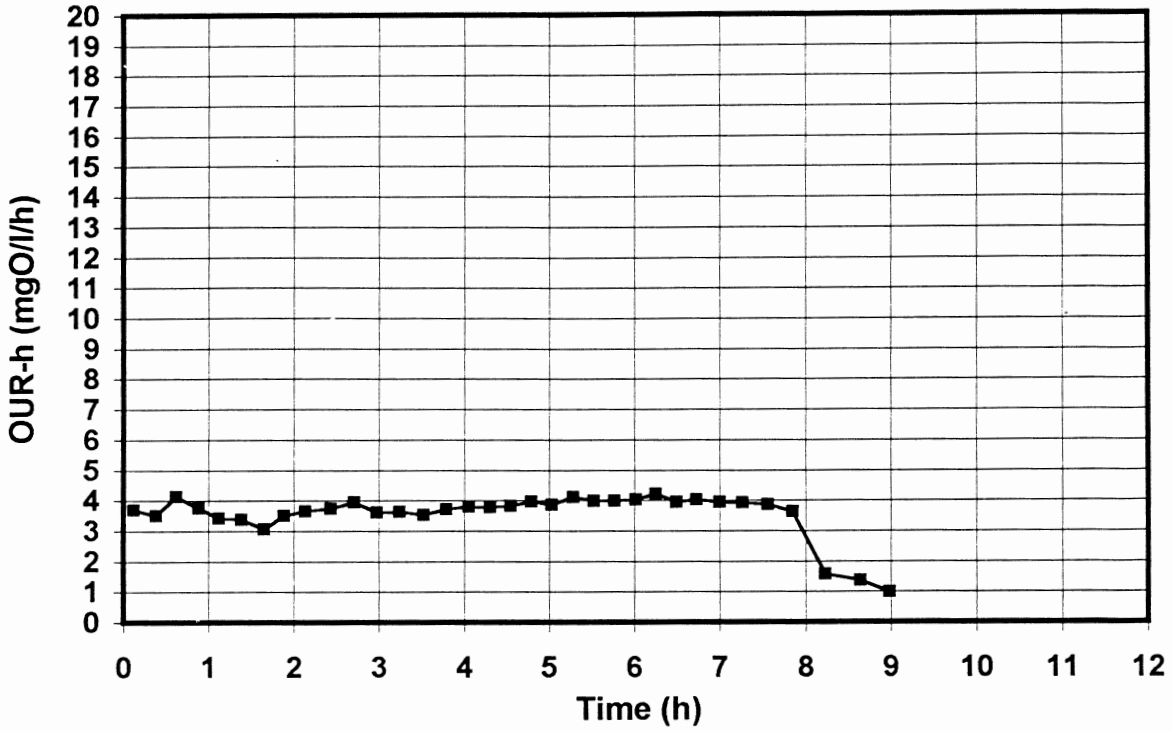
ℓn(OUR-h) graph for filtered wastewater (2.6ℓ) plus mixed liquor (0.4ℓ) batch test F10 , 29-03b, waste water batch no.22



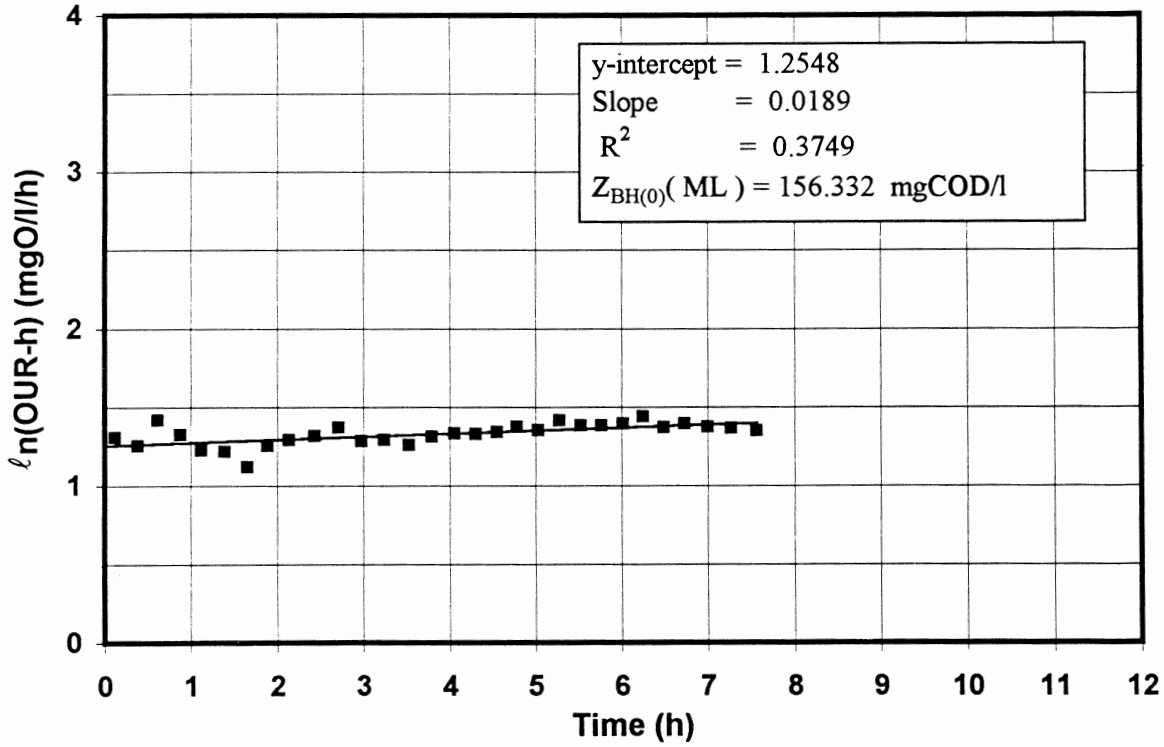
OUR Graph for filtered wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test F11 , 30-03a , wastewater batch no. 22



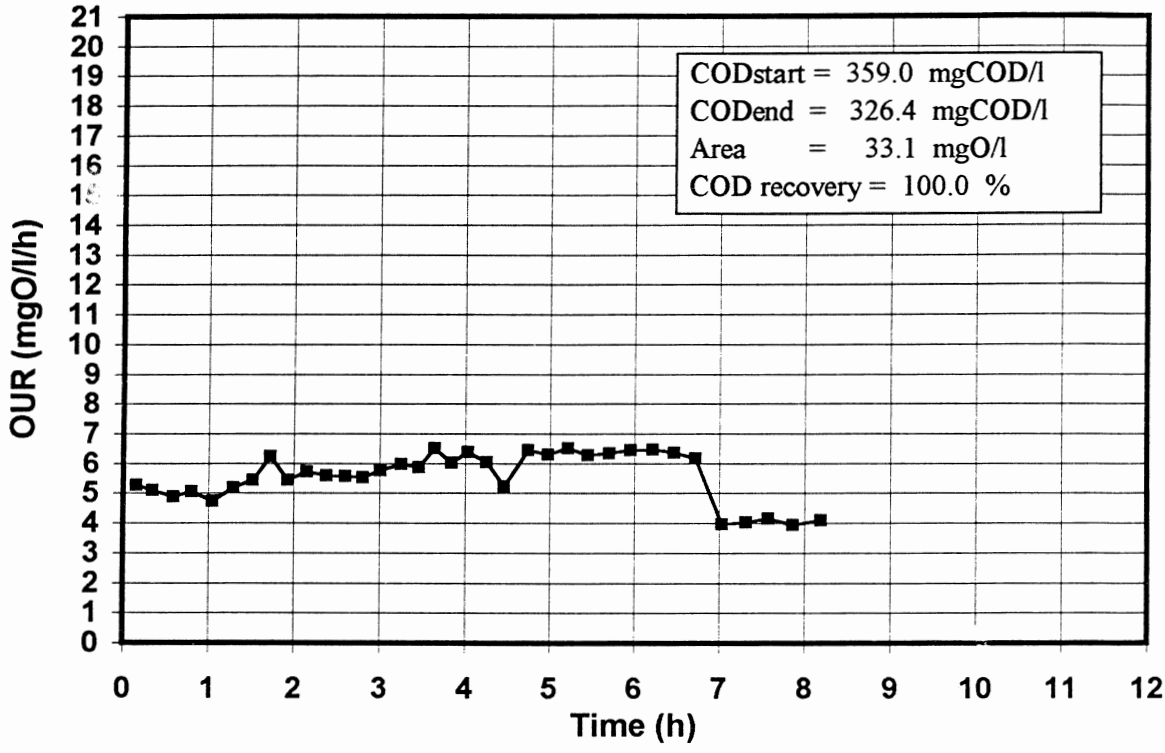
NO_3 Graph for filtered wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test F11 , 30-03a , wastewater batch no. 22



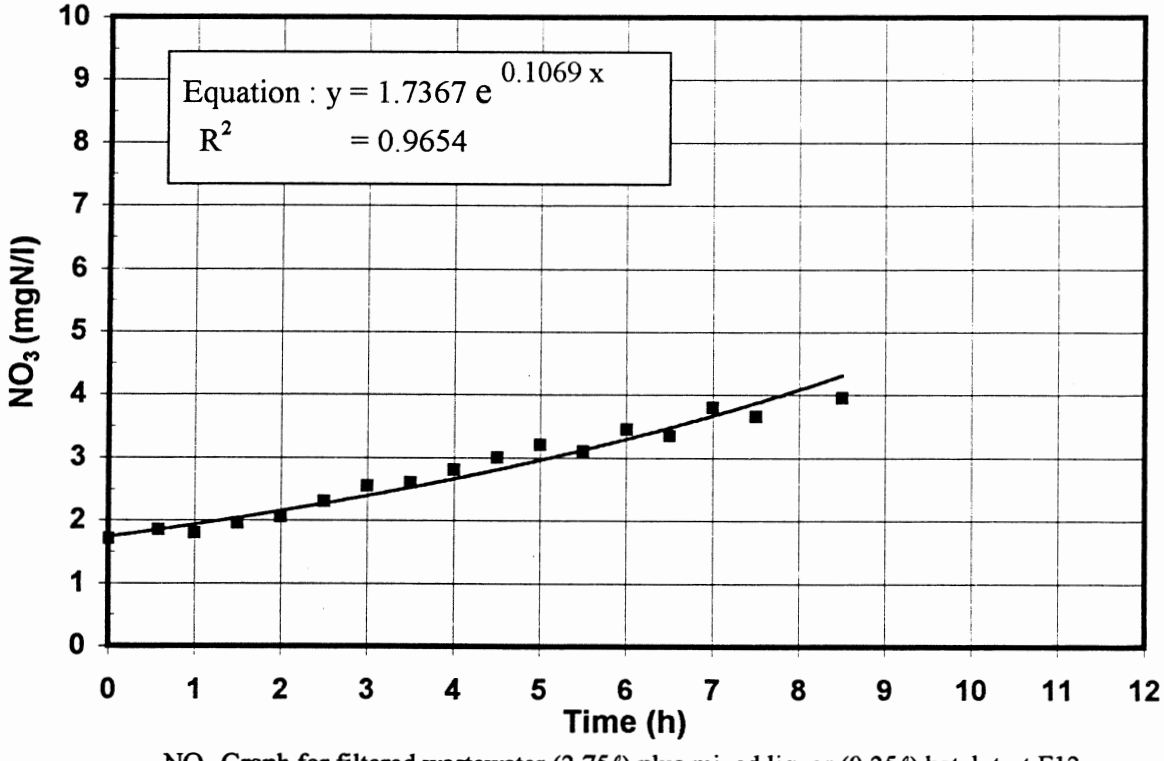
OUR-h Graph for filtered wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test F11 , 30-03a , wastewater batch no. 22



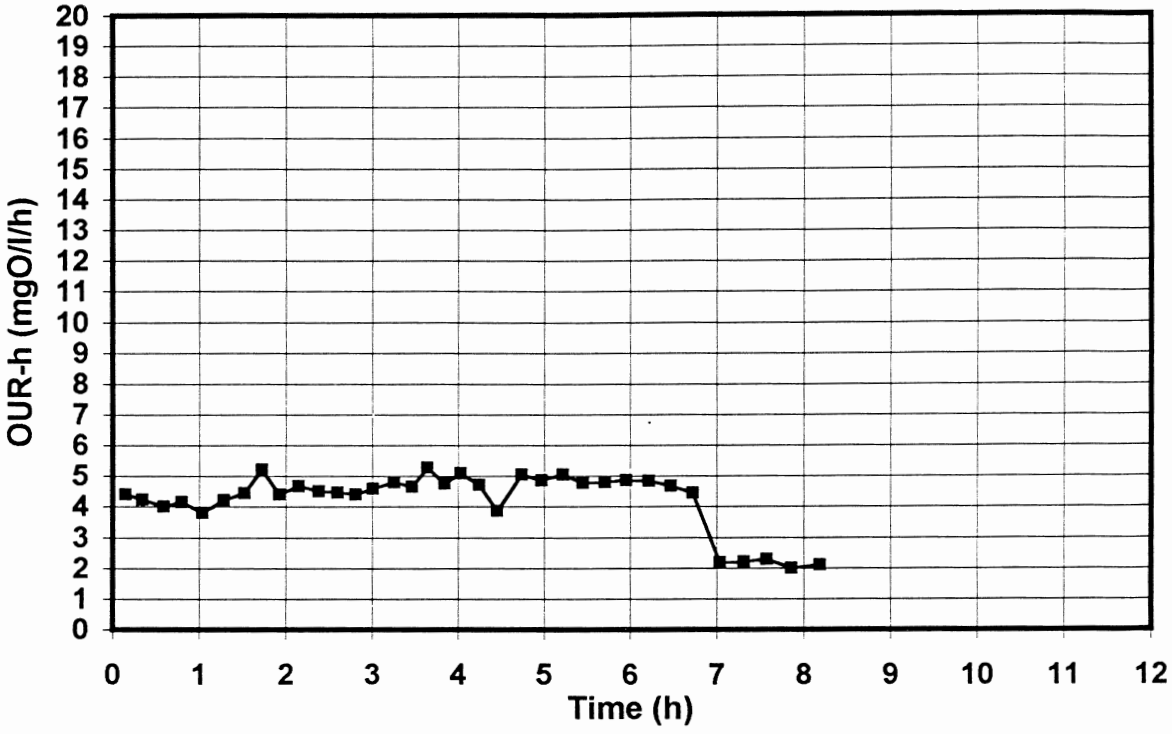
ℓn(OUR-h) graph for filtered wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test F11 , 30-03a, waste water batch no.22



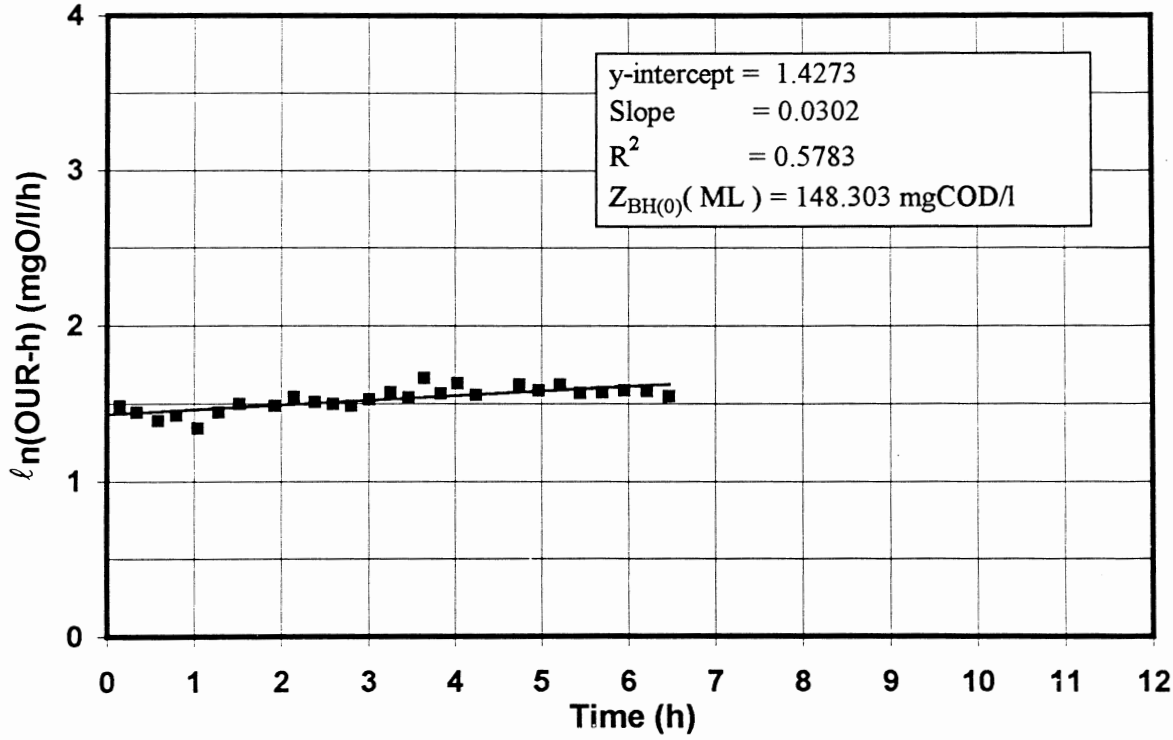
OUR Graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ) batch test F12, 30-03b , wastewater batch no. 22



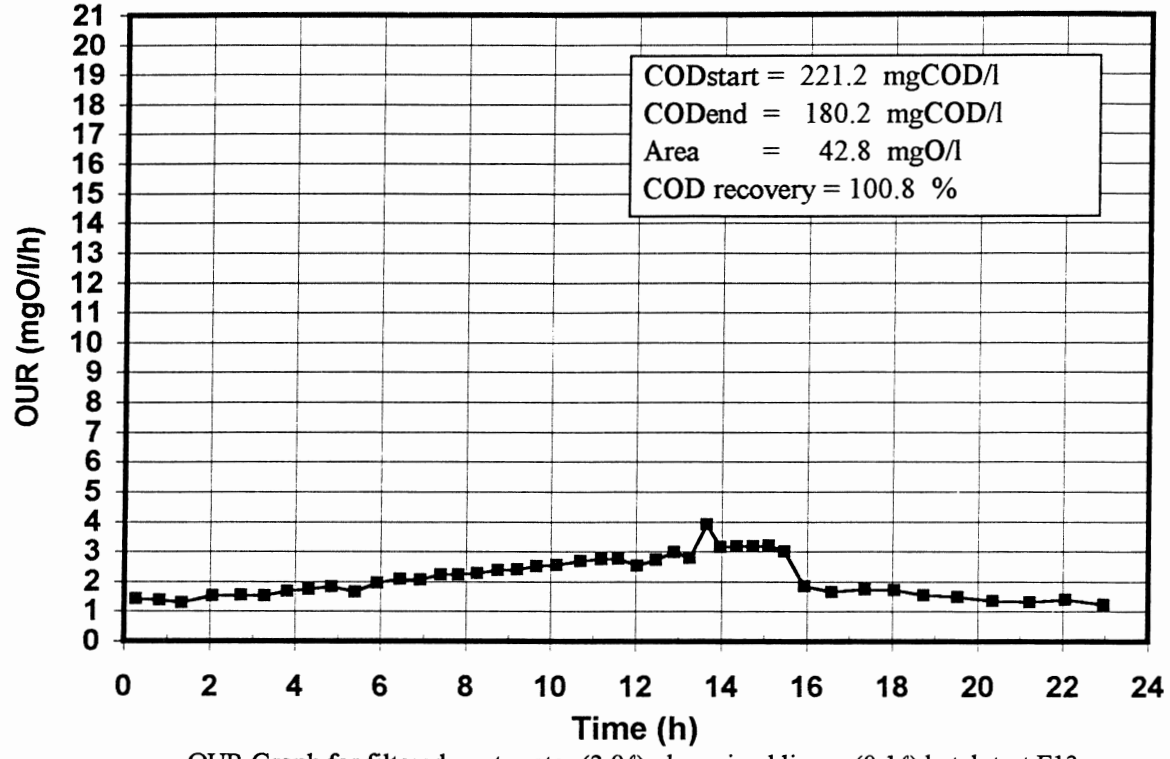
NO_3 Graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ) batch test F12 , 30-03b , wastewater batch no. 22



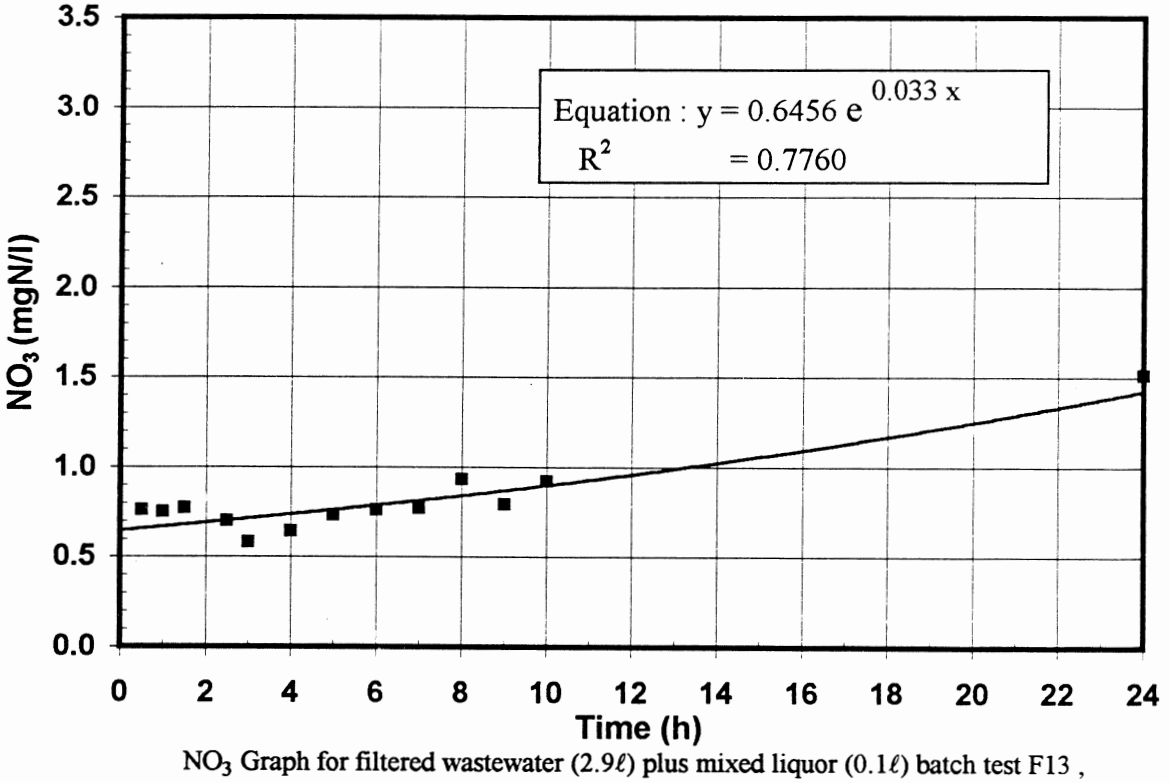
OUR-h Graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25) batch test F12 , 30-03b , wastewater batch no. 22



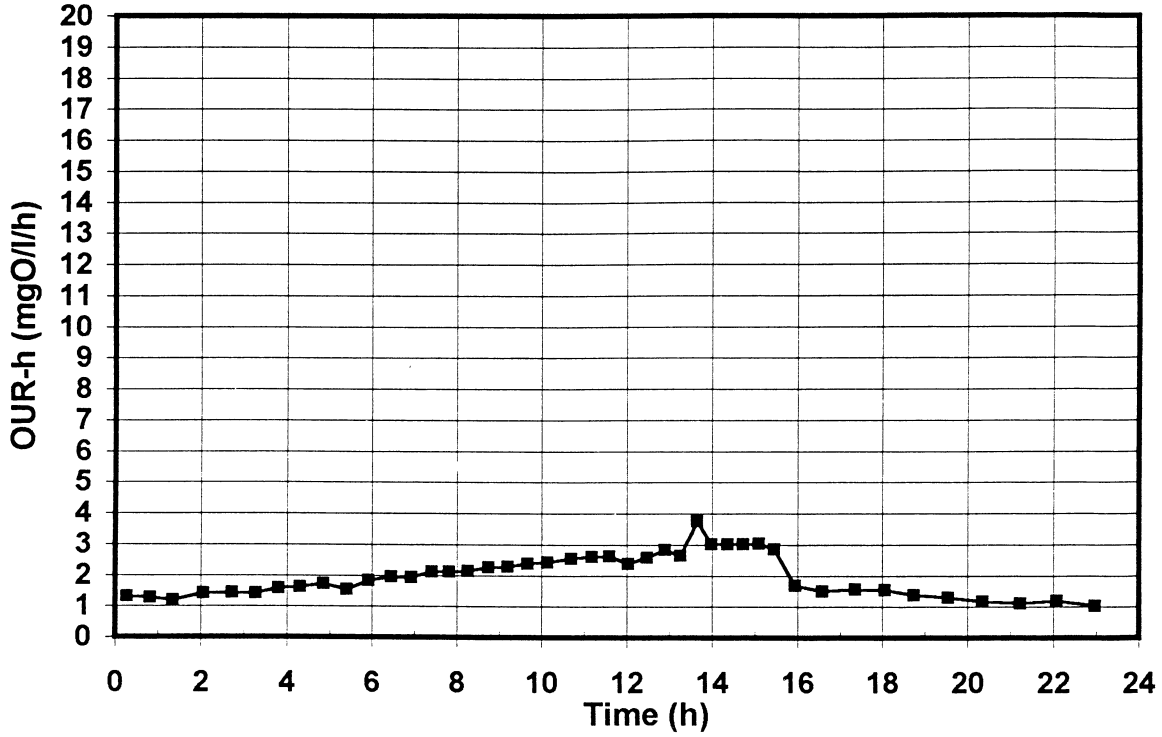
ℓn(OUR-h) graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ) batch test F12, 30-03b , waste water batch no.22



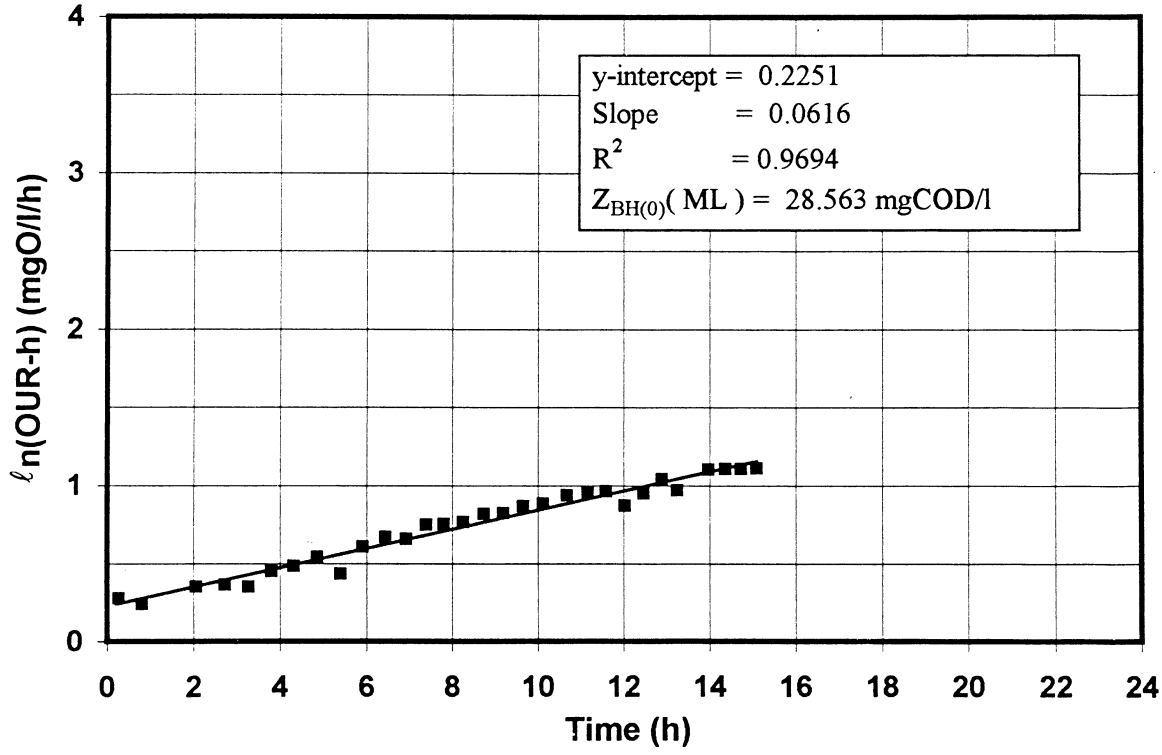
OUR Graph for filtered wastewater (2.9ℓ) plus mixed liquor (0.1ℓ) batch test F13, 04-04a , wastewater batch no. 22



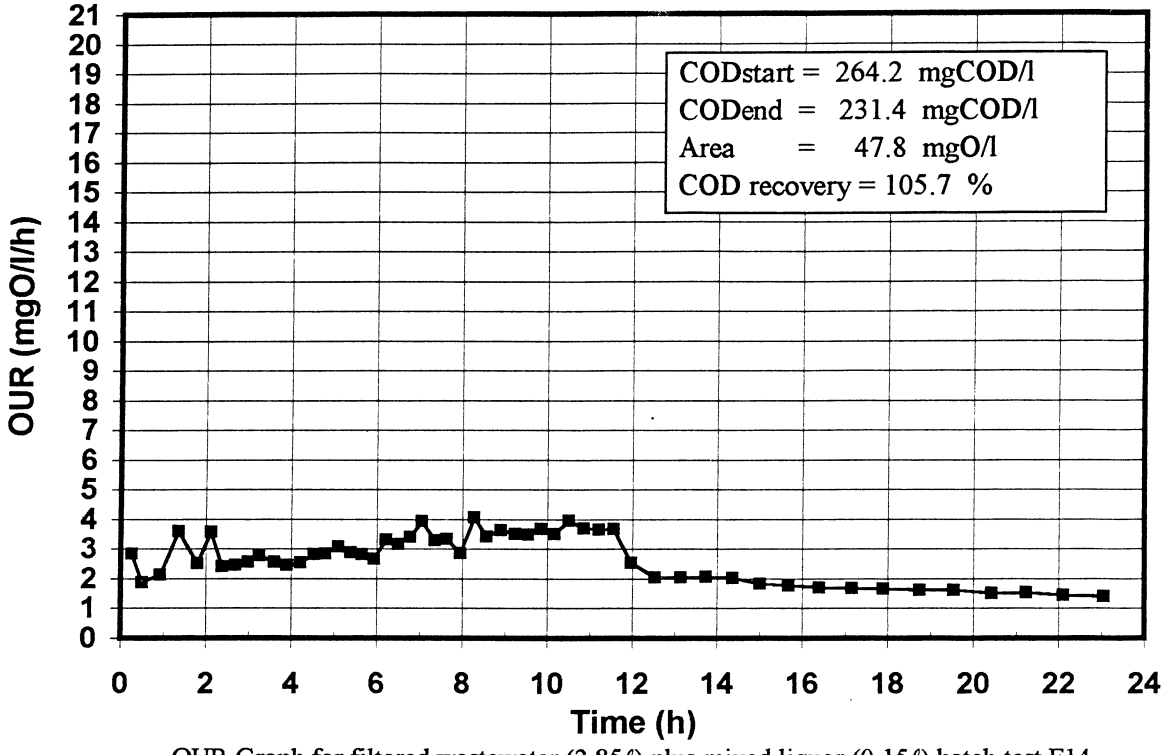
NO_3 Graph for filtered wastewater (2.9ℓ) plus mixed liquor (0.1ℓ) batch test F13 , 04-04 a, wastewater batch no. 22



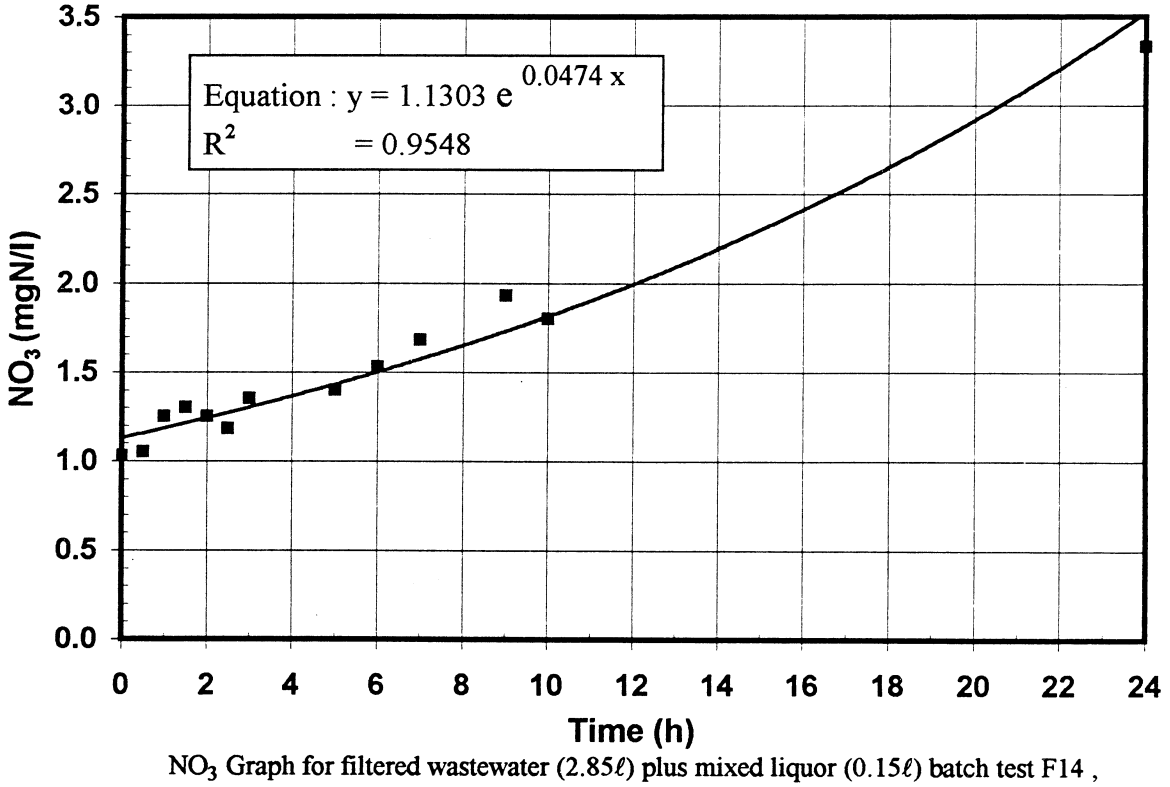
OUR-h Graph for filtered wastewater (2.9ℓ) plus mixed liquor (0.1ℓ) batch test F13 , 04-04 a, wastewater batch no. 22



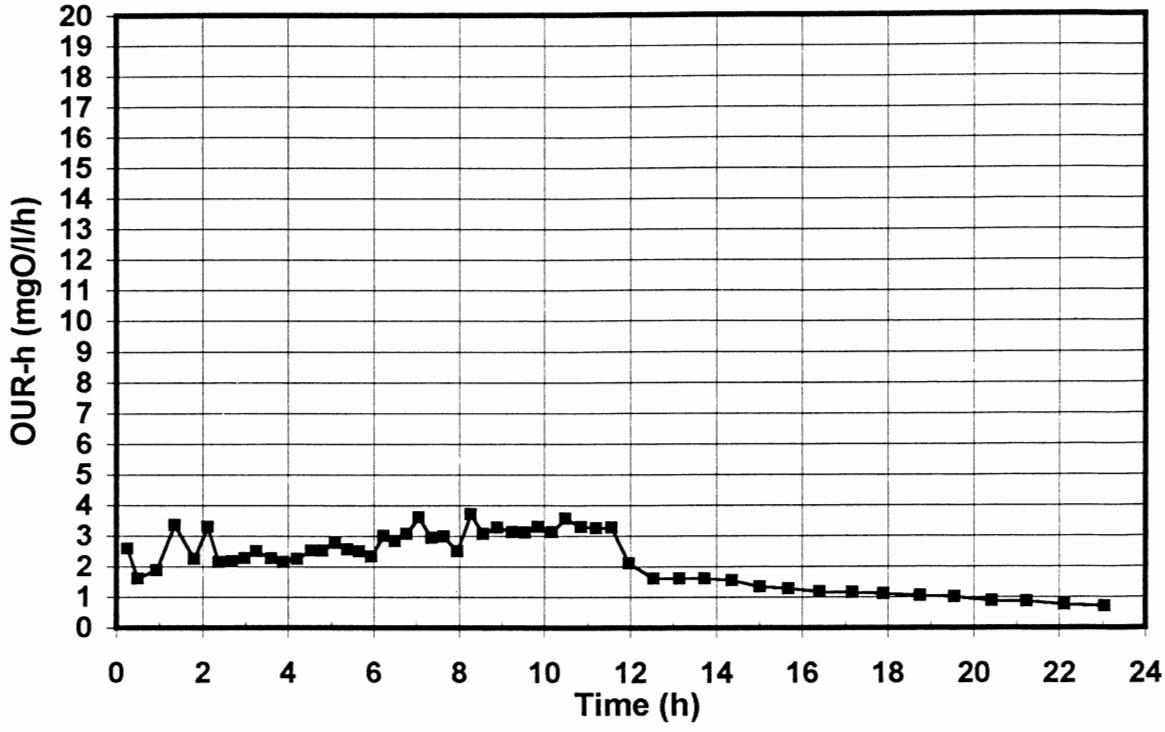
ℓn(OUR-h) graph for filtered wastewater (2.9ℓ) plus mixed liquor (0.1ℓ) batch test F13 , 04-04 a, waste water batch no.22



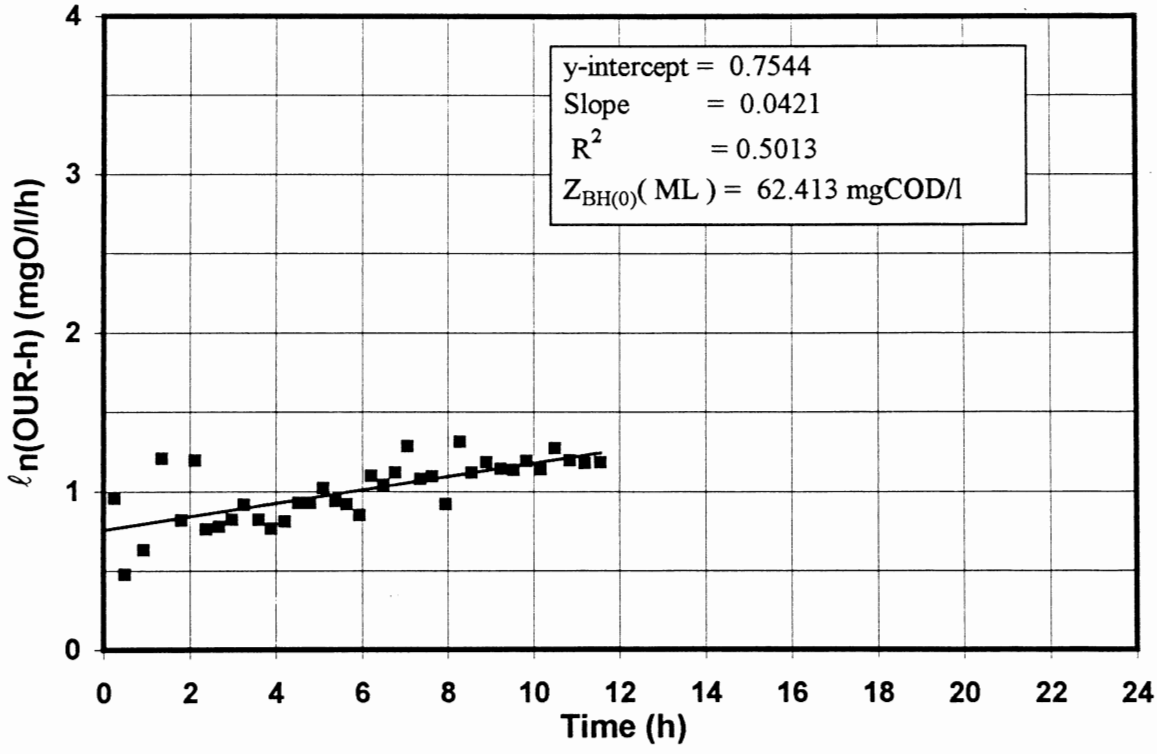
OUR Graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ) batch test F14 , 04-04 b, wastewater batch no. 22



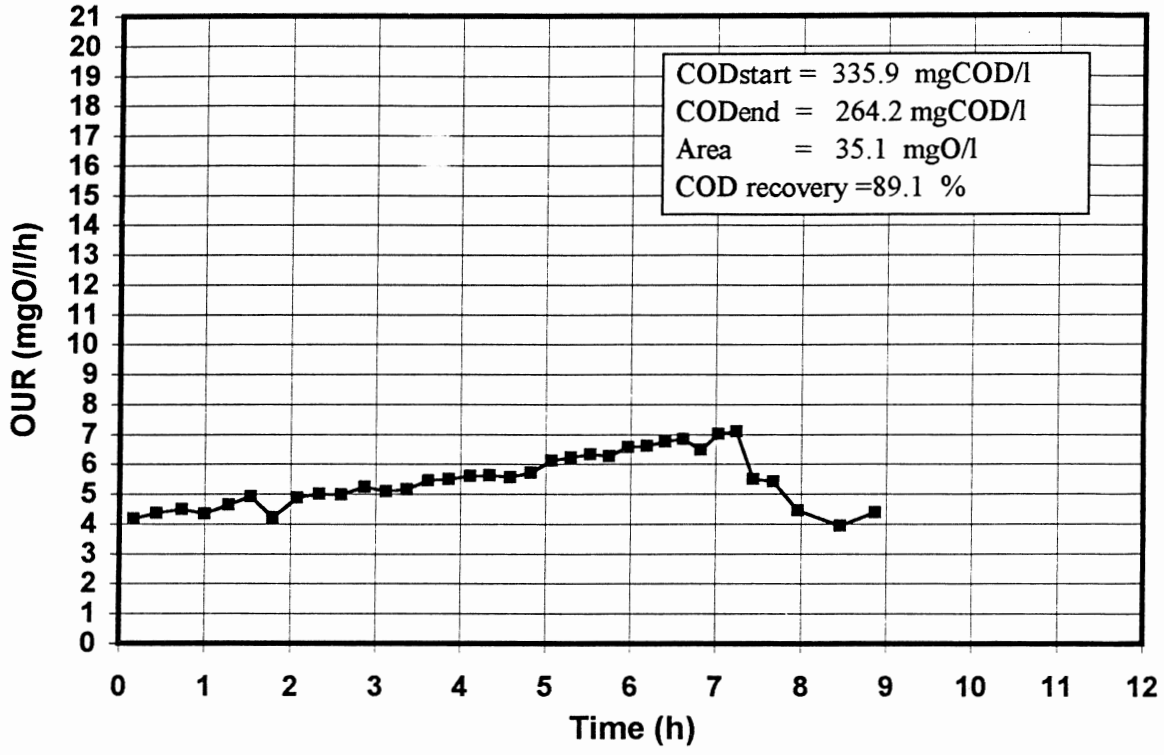
NO_3 Graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ) batch test F14 , 04-04 b, wastewater batch no. 22



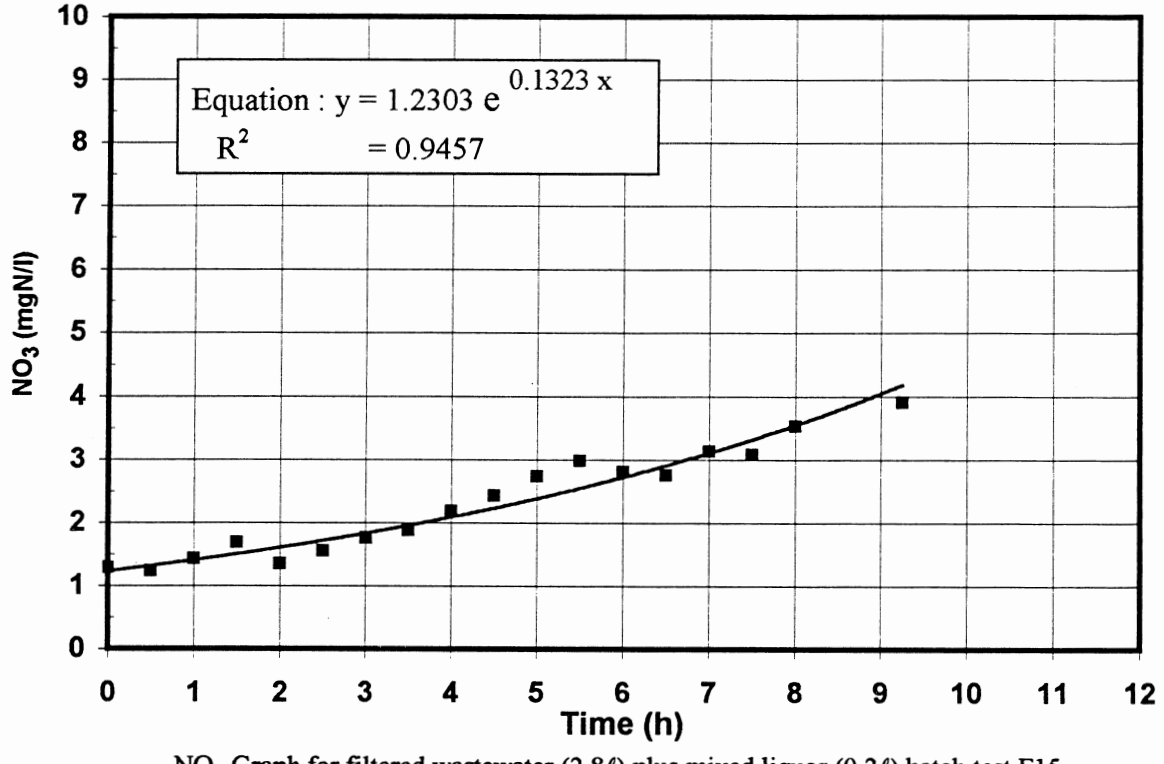
OUR-h Graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ) batch test F14 , 04-04 b, wastewater batch no. 22



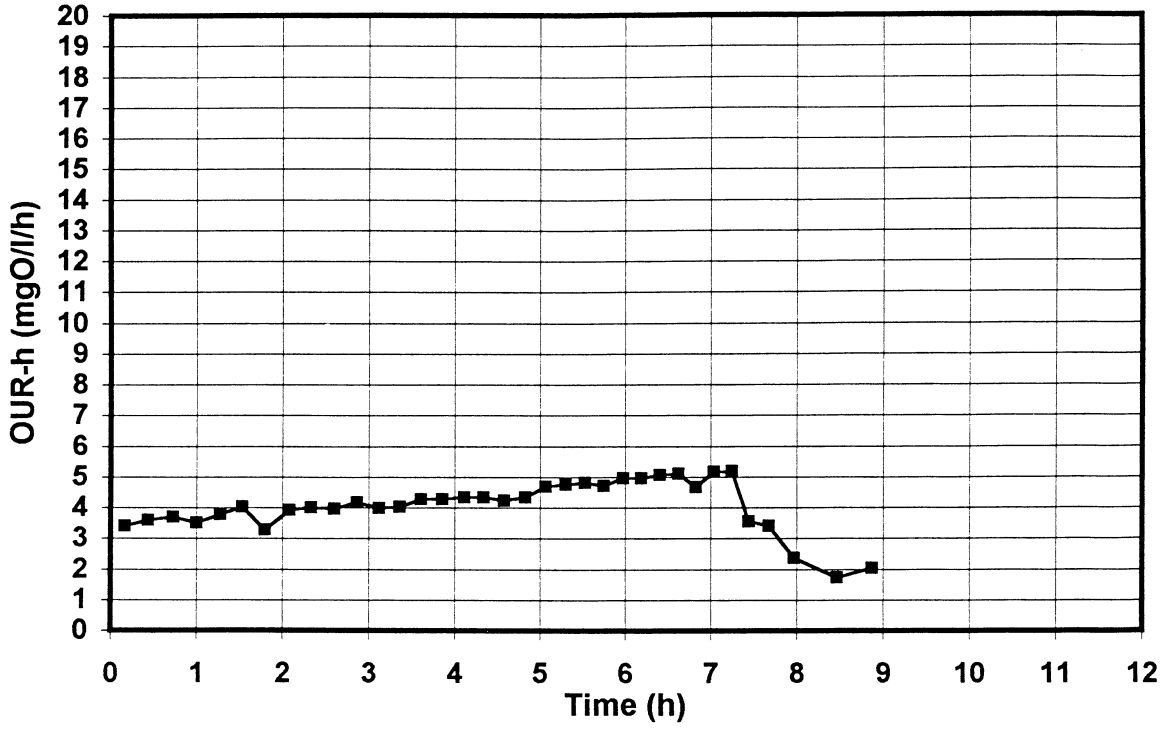
ℓn(OUR-h) for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ) batch test F14 , 04-04 b, waste water batch no.22



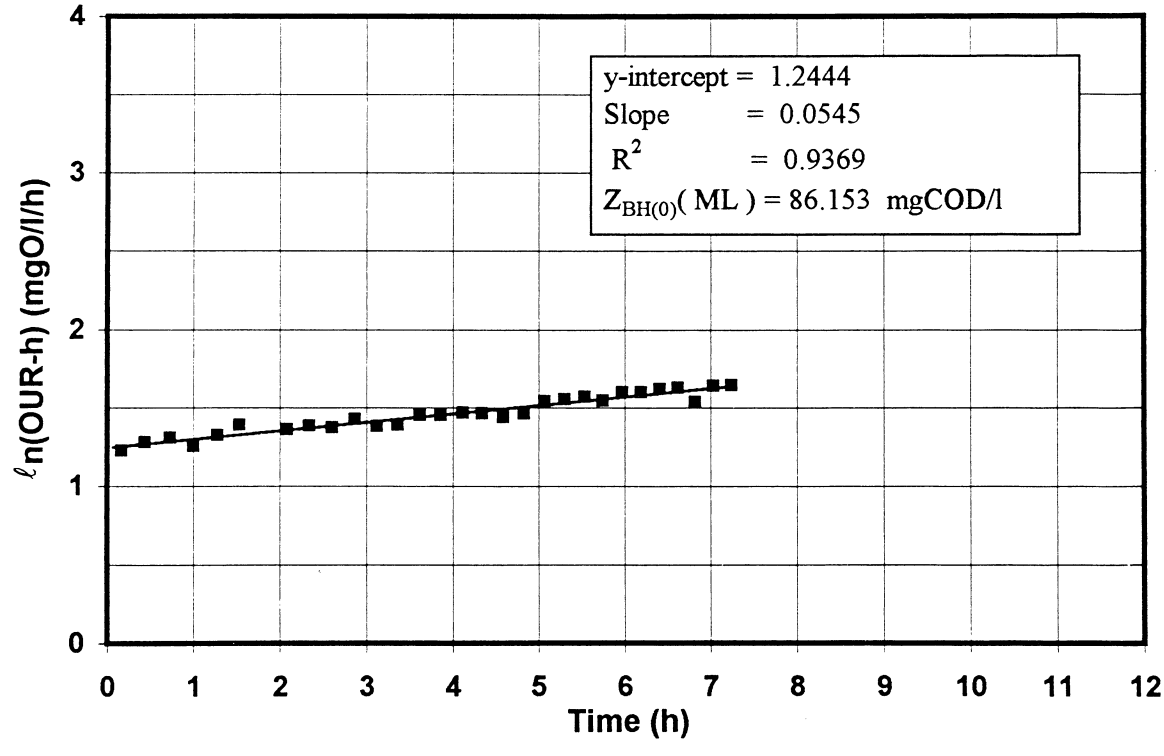
OUR Graph for filtered wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test F15 , 10-04a , wastewater batch no. 23



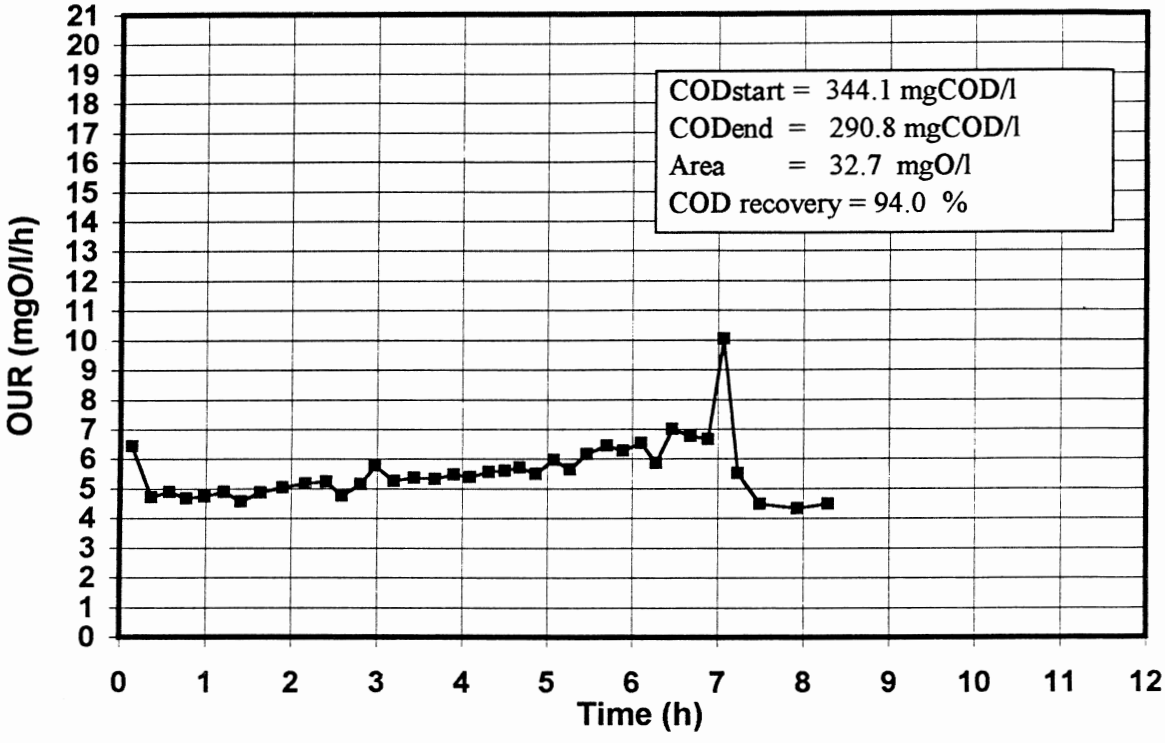
NO_3 Graph for filtered wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test F15 , 10-04a , wastewater batch no. 23



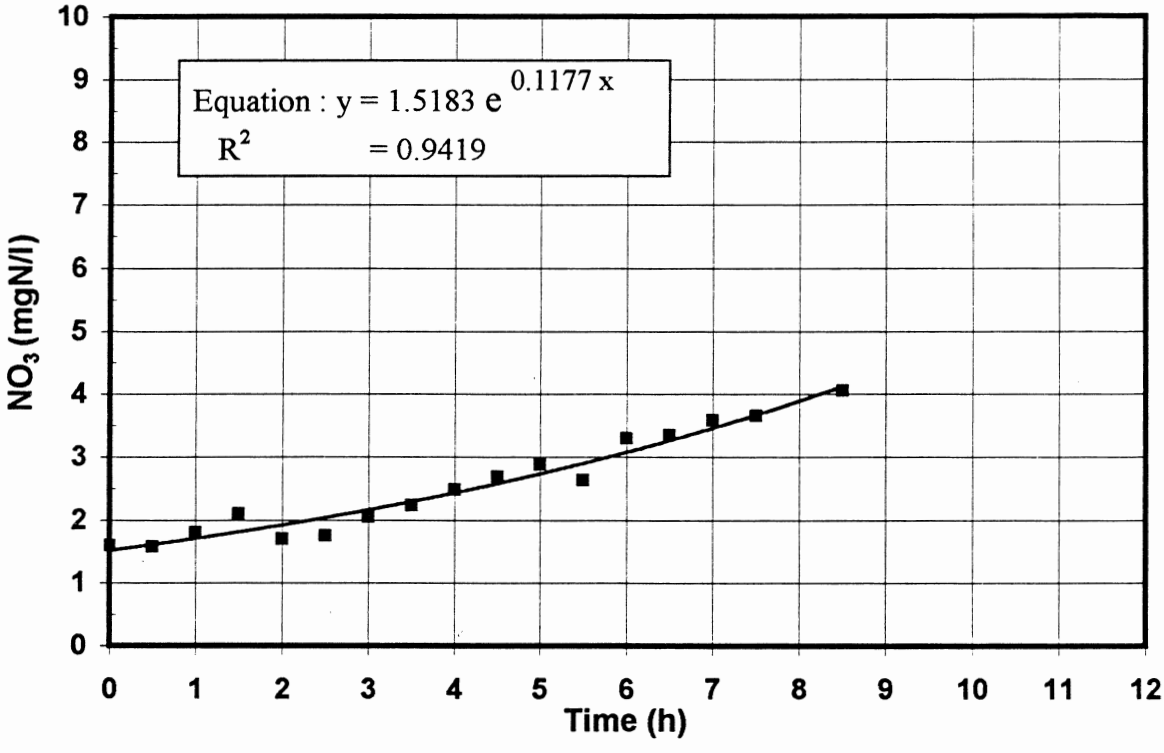
OUR-h Graph for filtered wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test F15 , 10-04 a, wastewater batch no. 23



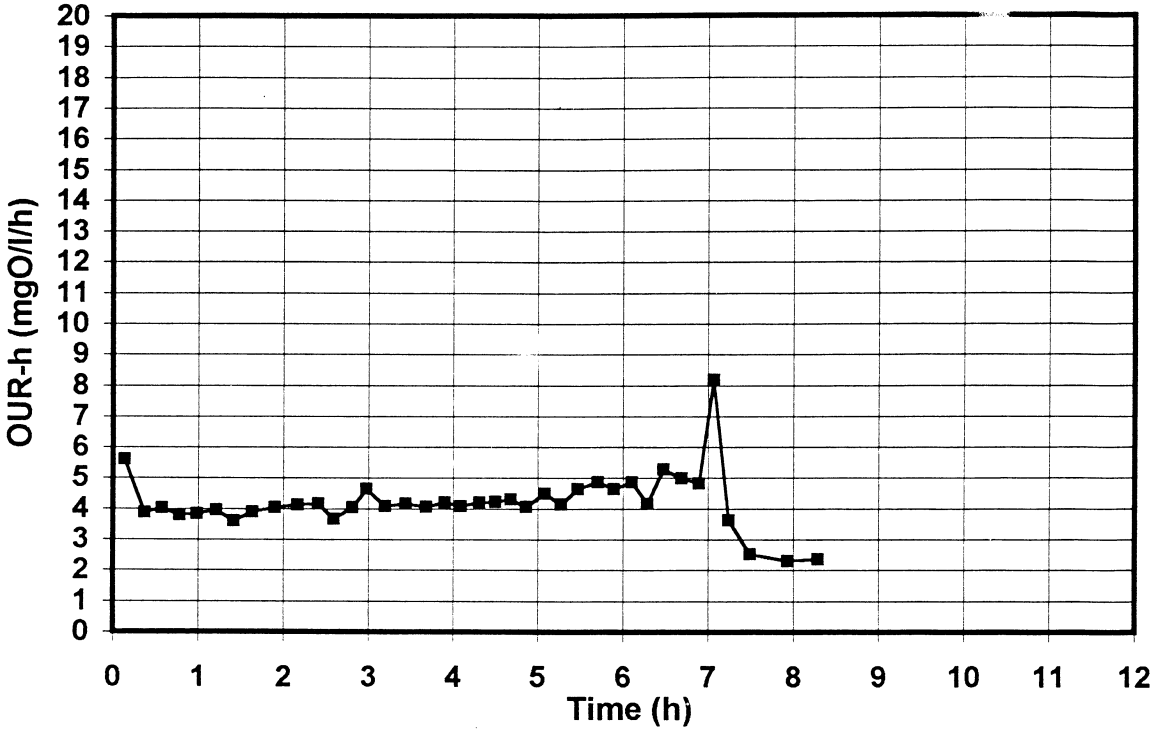
ℓn(OUR-h) for filtered wastewater (2.8ℓ) plus mixed liquor (0.2ℓ) batch test F15 , 10-04a , waste water batch no.23



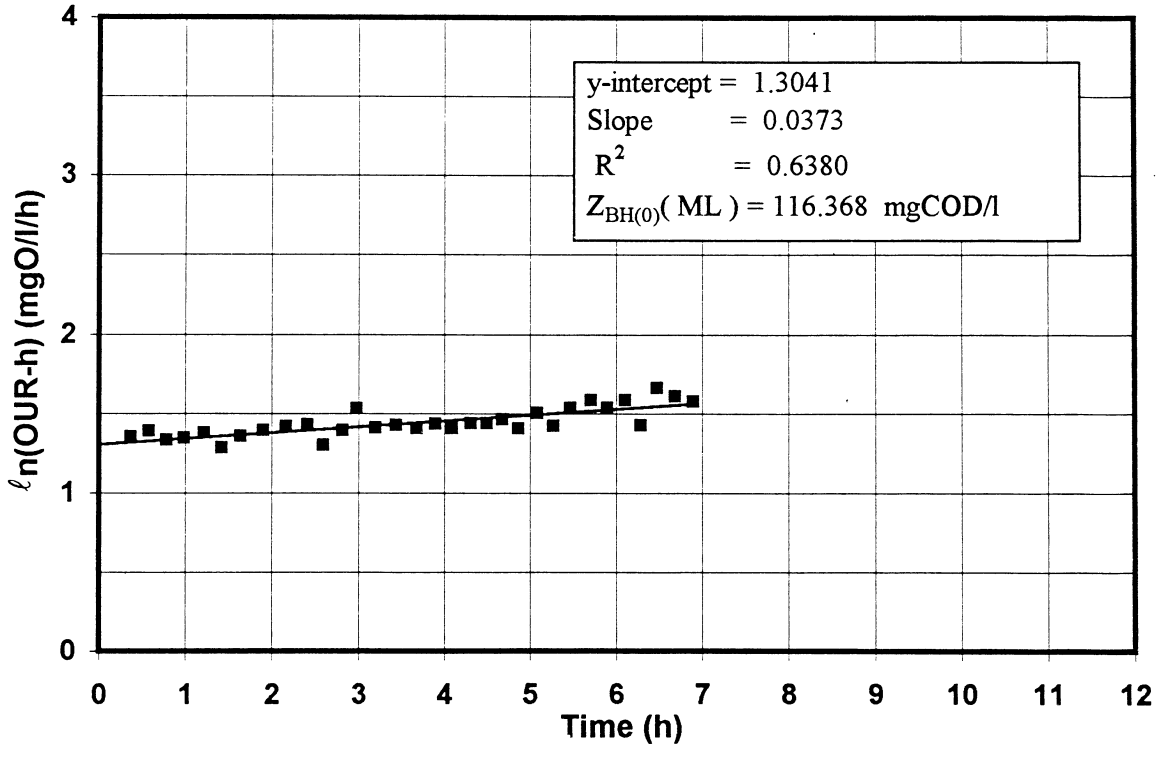
OUR Graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ) batch test F16 , 10-04b , wastewater batch no. 23



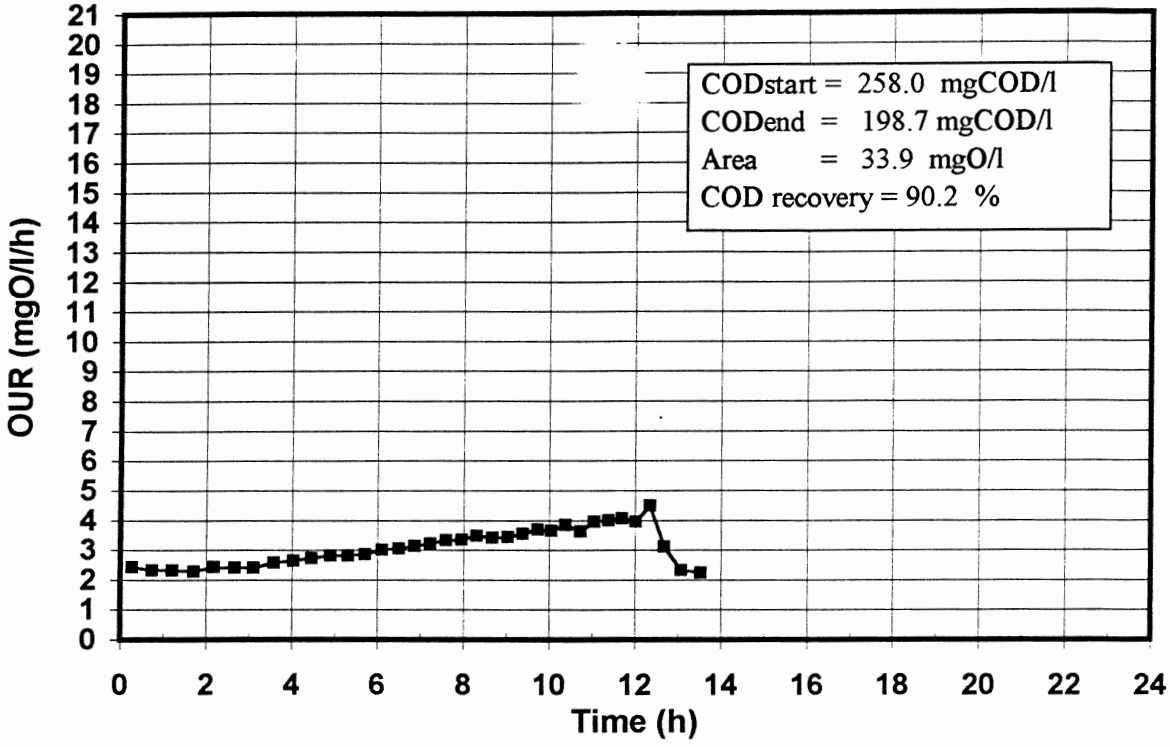
NO_3 Graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ) batch test F16 , 10-04b , wastewater batch no. 23



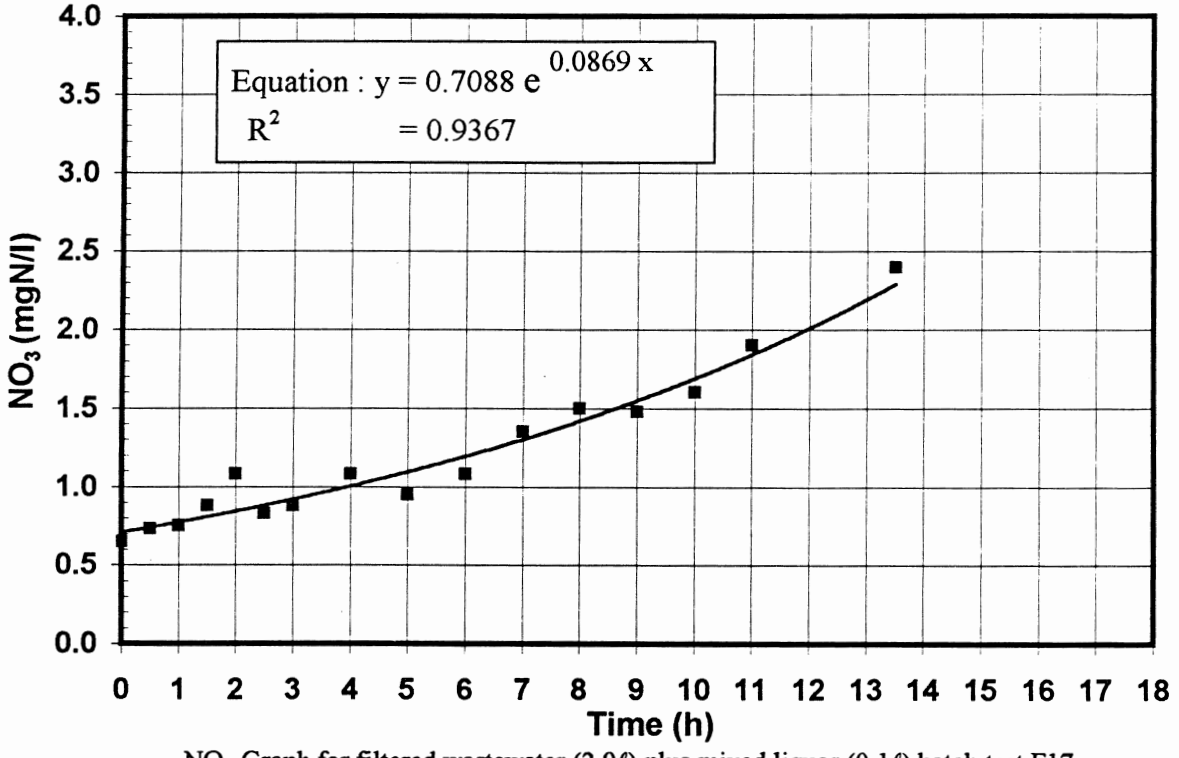
OUR-h plot for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ) batch test F16, 10-04b , wastewater batch no. 23



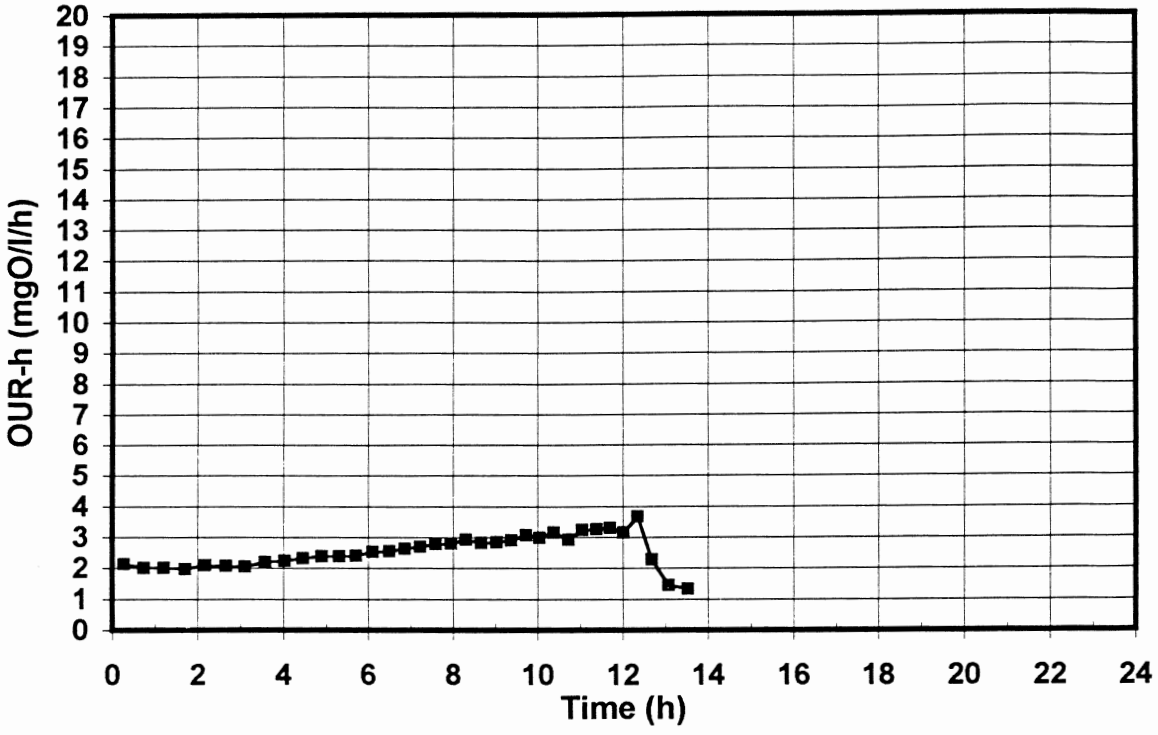
ℓn(OUR-h) for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ) batch test F16 , 10-04b , waste water batch no.23



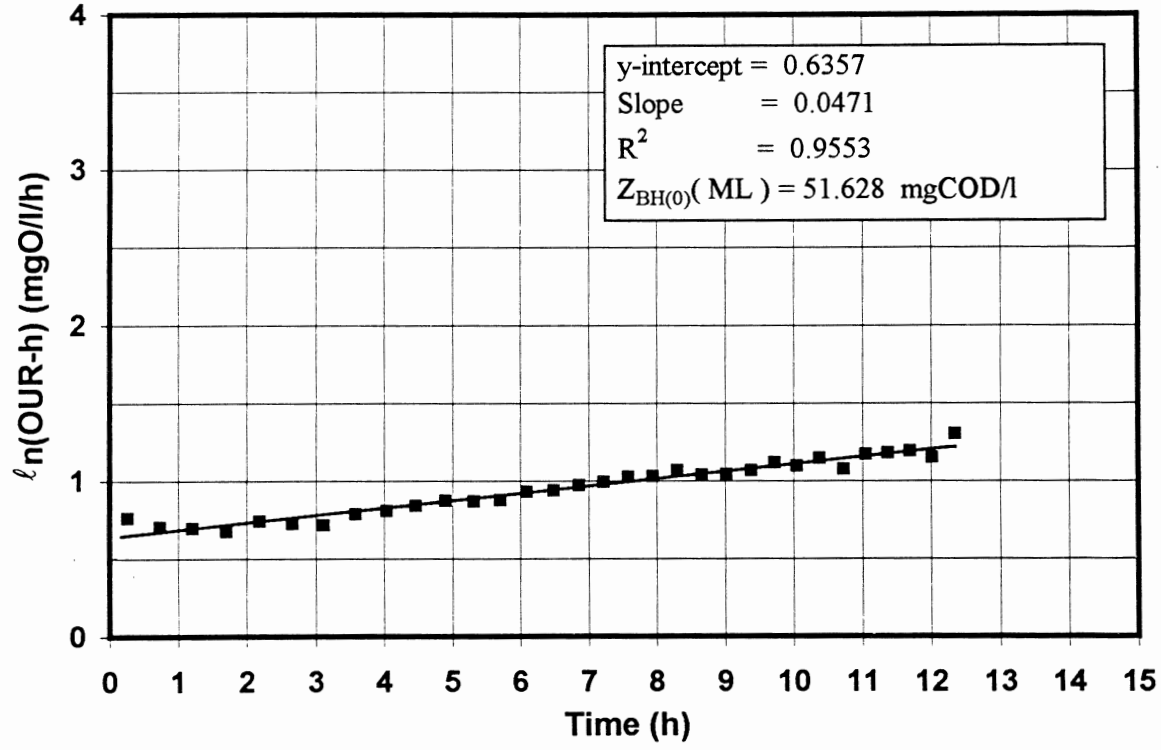
OUR Graph for filtered wastewater (2.9ℓ) plus mixed liquor (0.1ℓ) batch test F17 , 11-04 a, wastewater batch no. 23



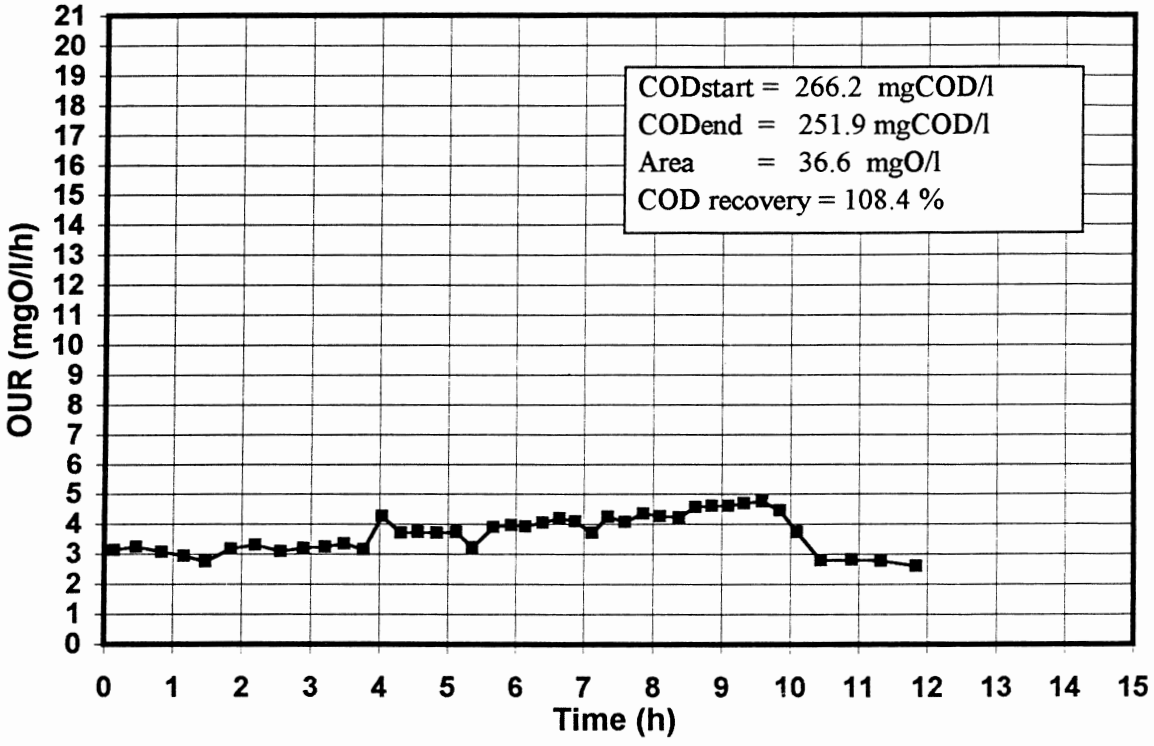
NO_3 Graph for filtered wastewater (2.9ℓ) plus mixed liquor (0.1ℓ) batch test F17 , 11-04a, wastewater batch no. 23



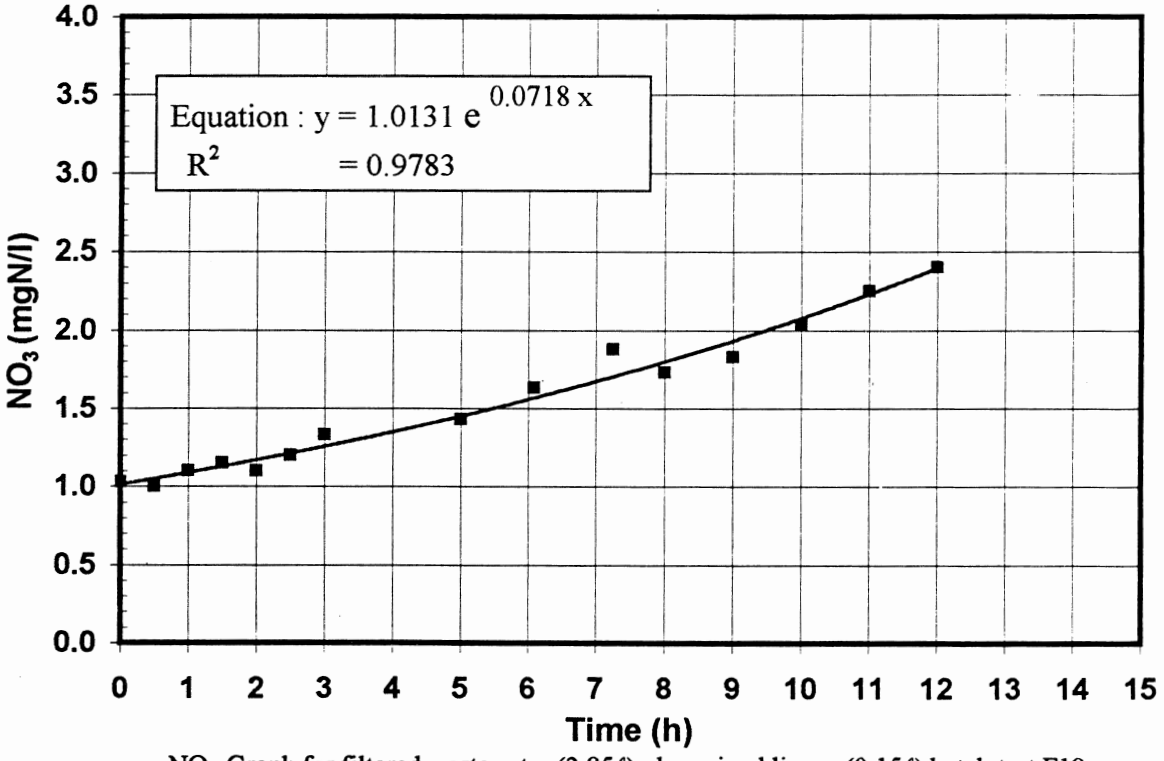
OUR-h plot for filtered wastewater (2.9ℓ) plus mixed liquor (0.1ℓ) batch test F17 , 11-04a, wastewater batch no. 23



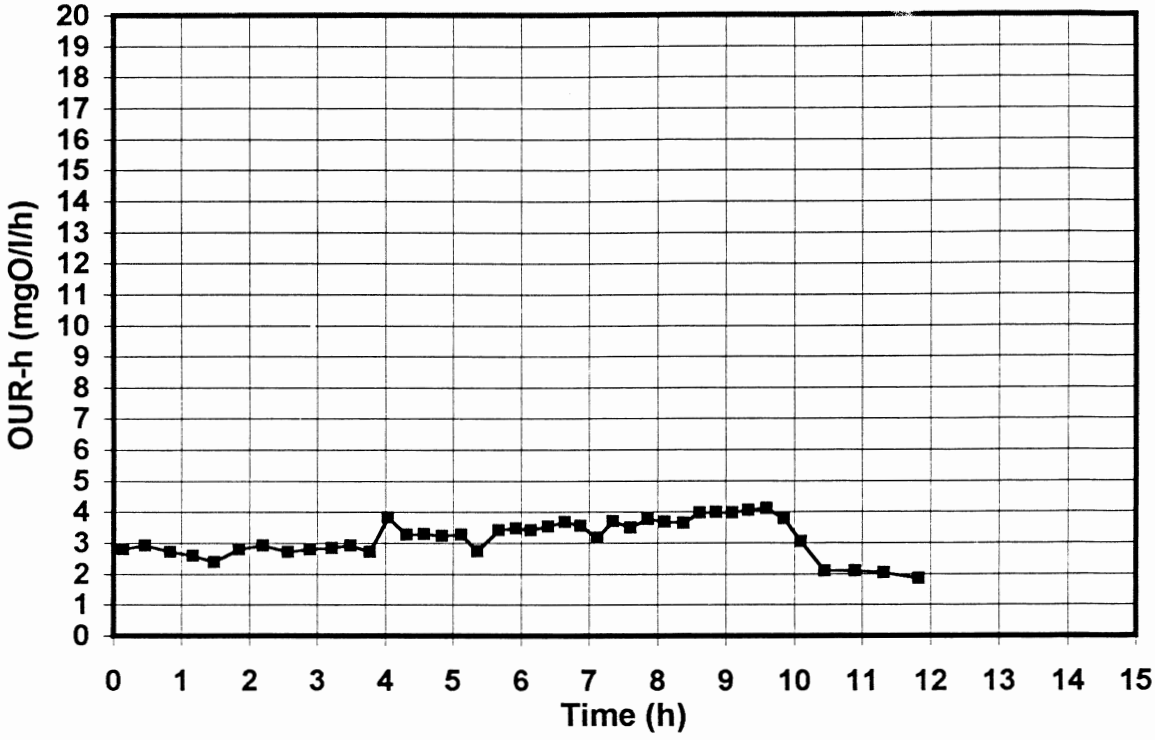
ℓn(OUR-h) for filtered wastewater (2.9ℓ) plus mixed liquor (0.1ℓ) batch test F17 , 11-04a , waste water batch no.23



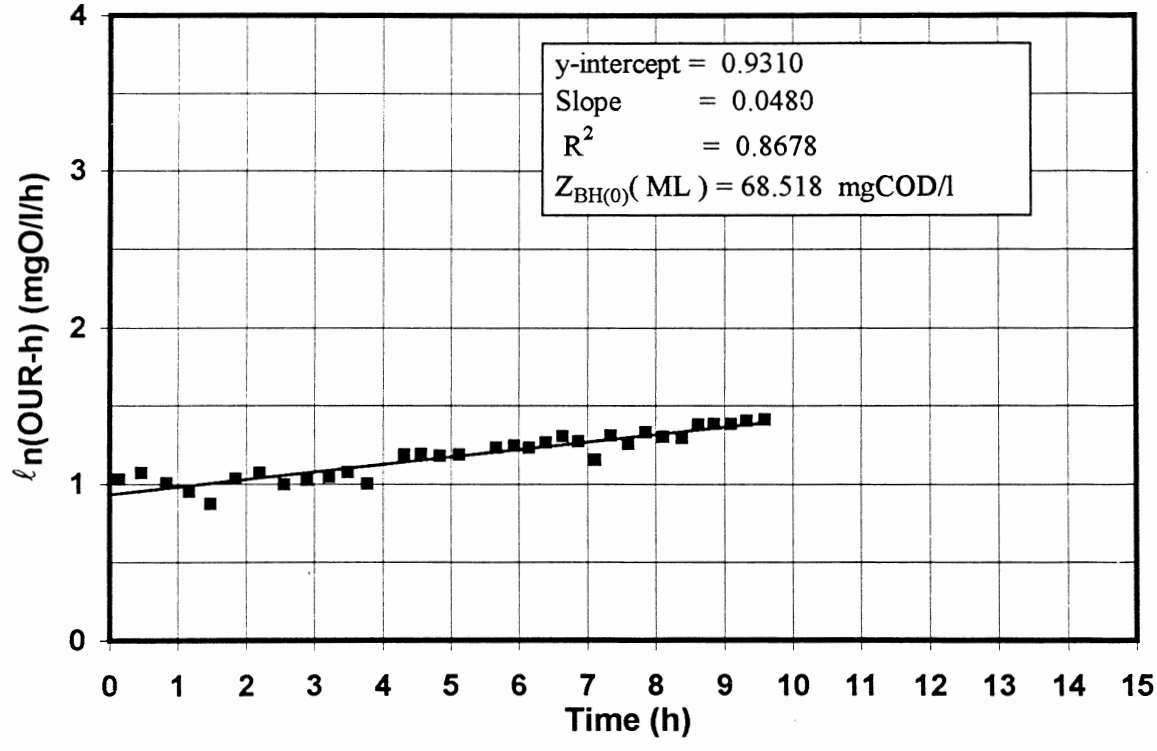
OUR Graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ) batch test F18, 11-04b, wastewater batch no. 23



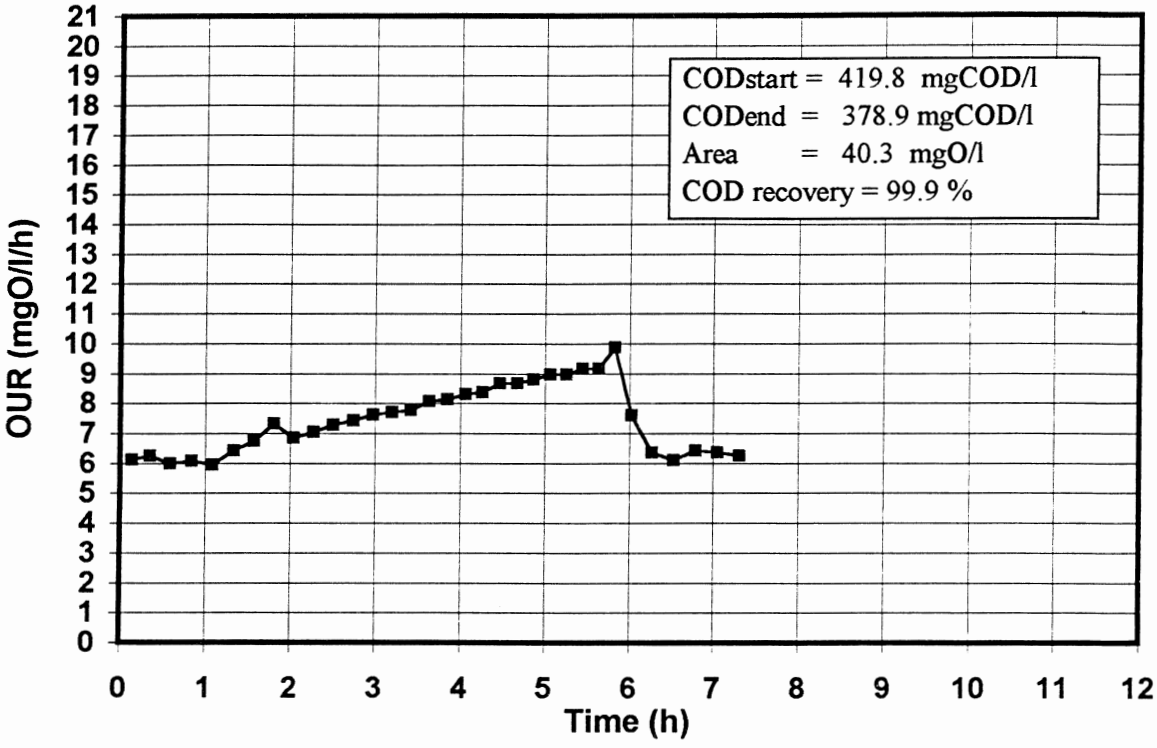
NO_3 Graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ) batch test F18, 11-04b , wastewater batch no. 23



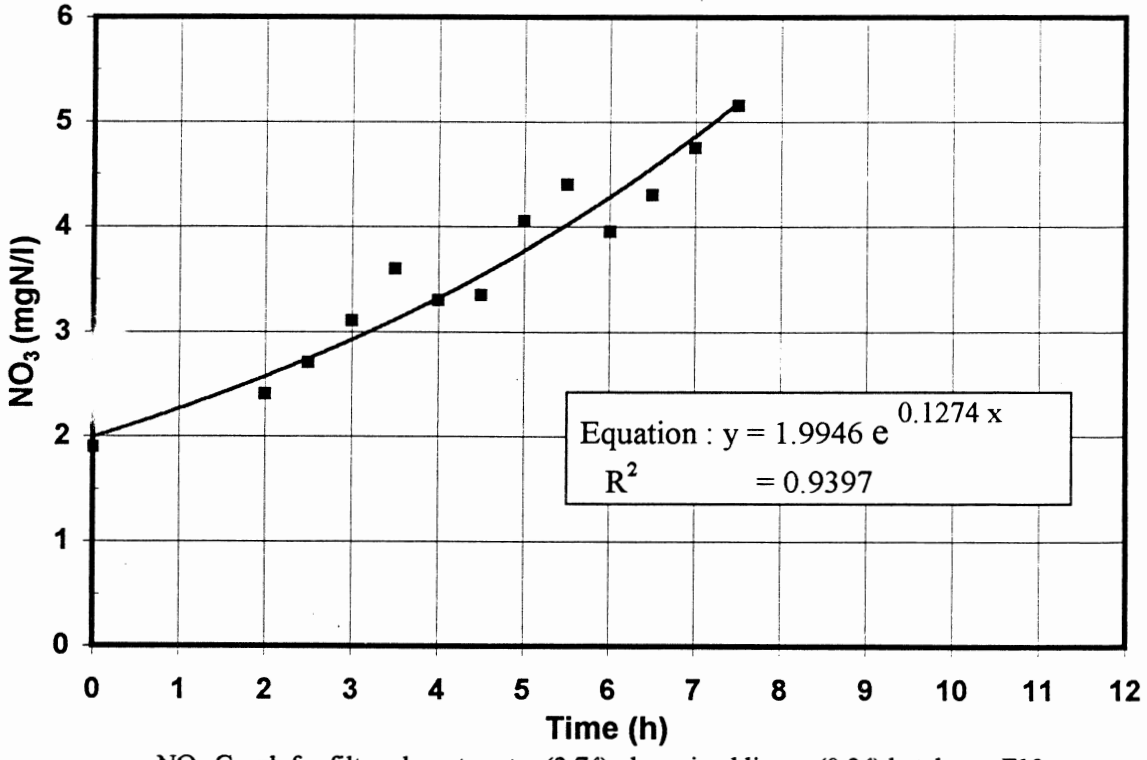
OUR-h plot for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ) batch test F18 , 11-04b, wastewater batch no. 23



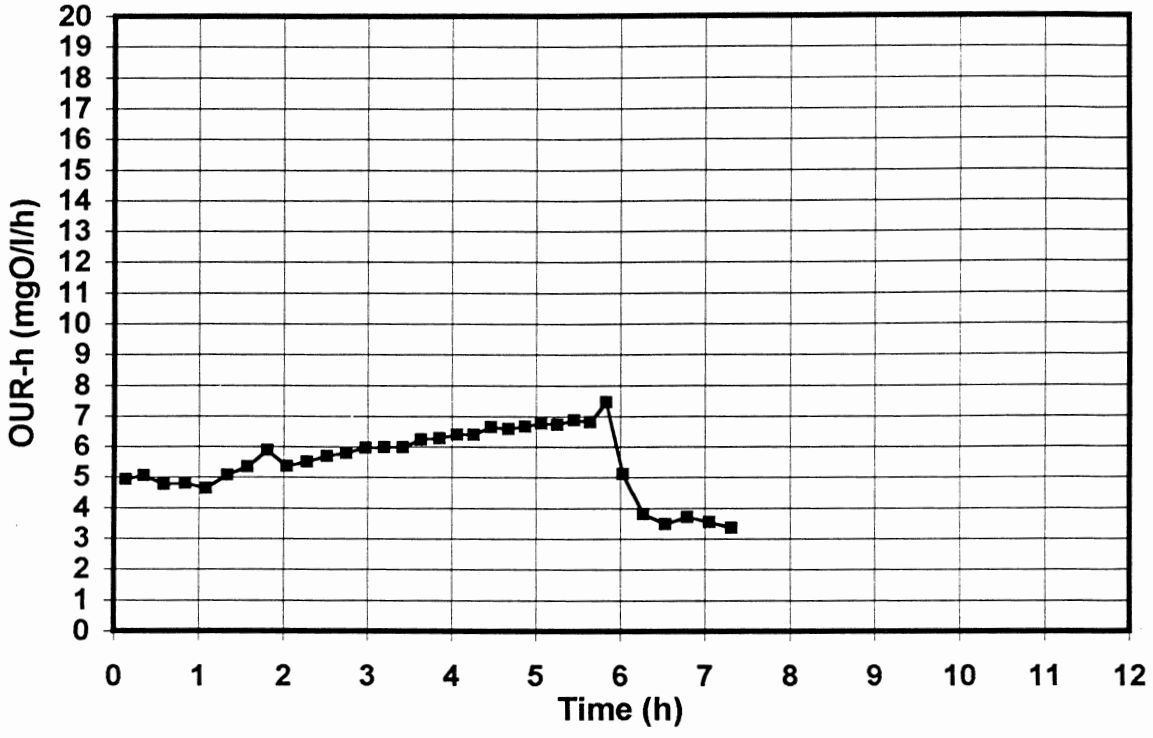
ℓn(OUR-h) for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ) batch test F18 , 11-04b , waste water batch no.23



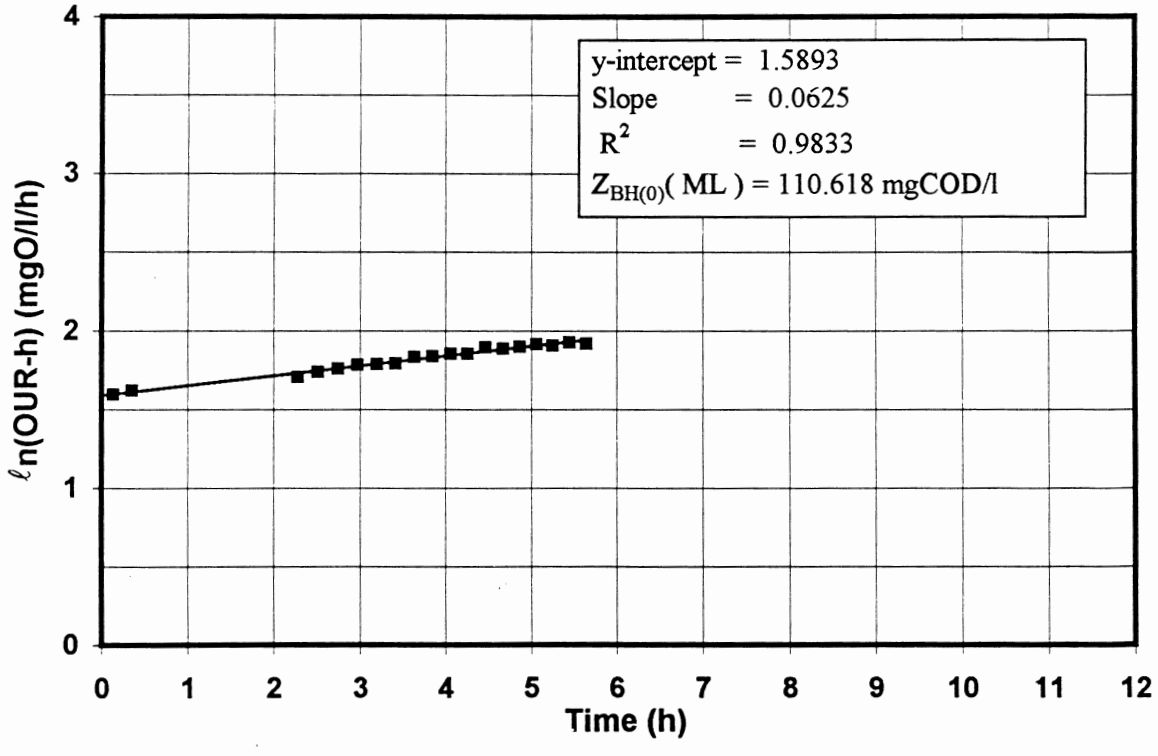
OUR Graph for filtered wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test F19 , 12-04a , wastewater batch no. 23



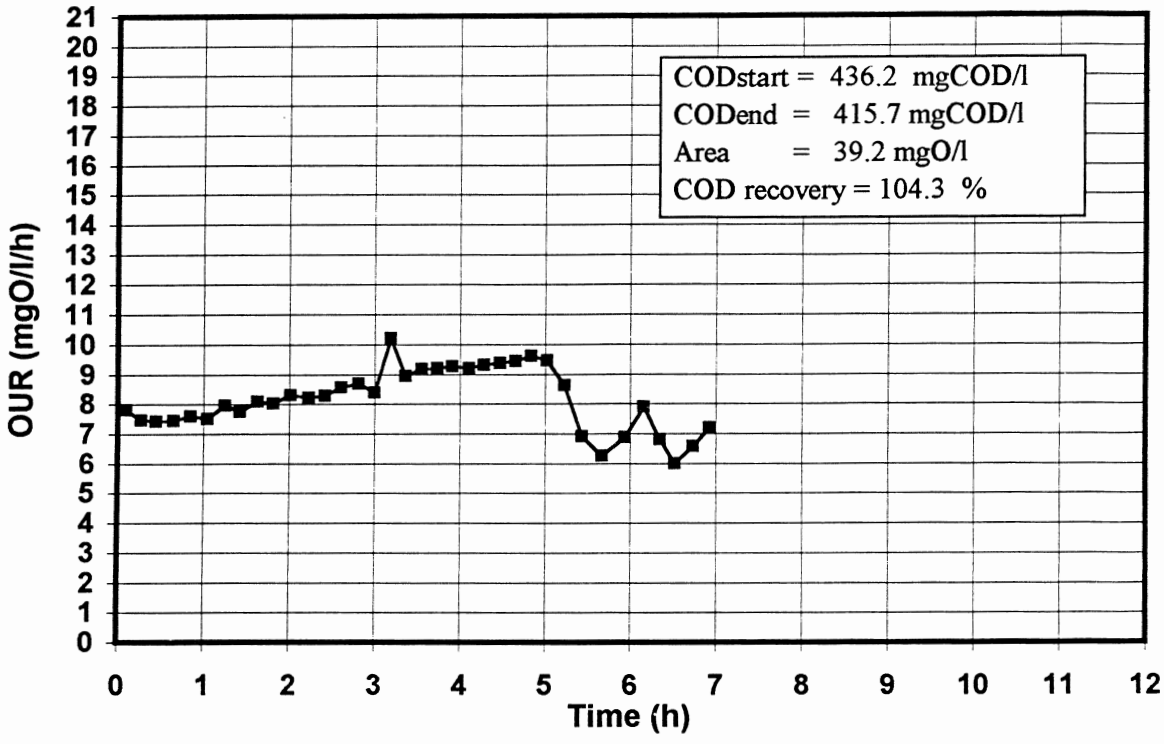
NO_3 Graph for filtered wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test F19 , 12-04a , wastewater batch no. 23



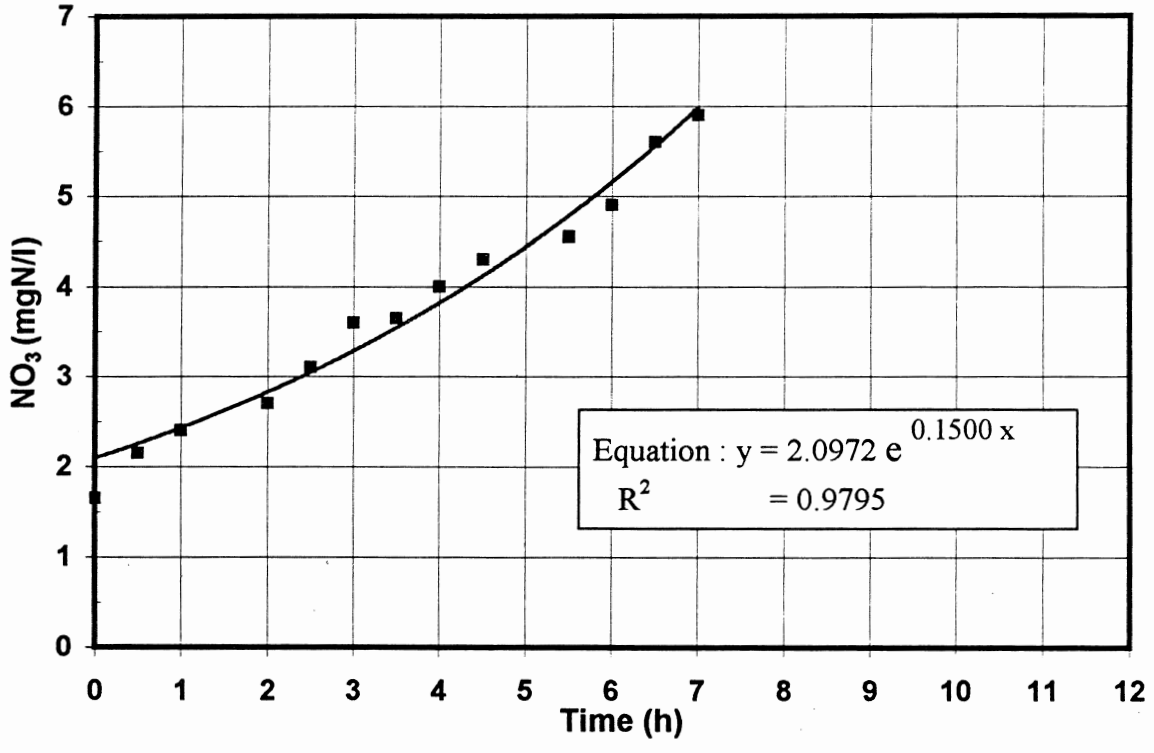
OUR-h plot for filtered wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test F19 , 12-04a , wastewater batch no. 23



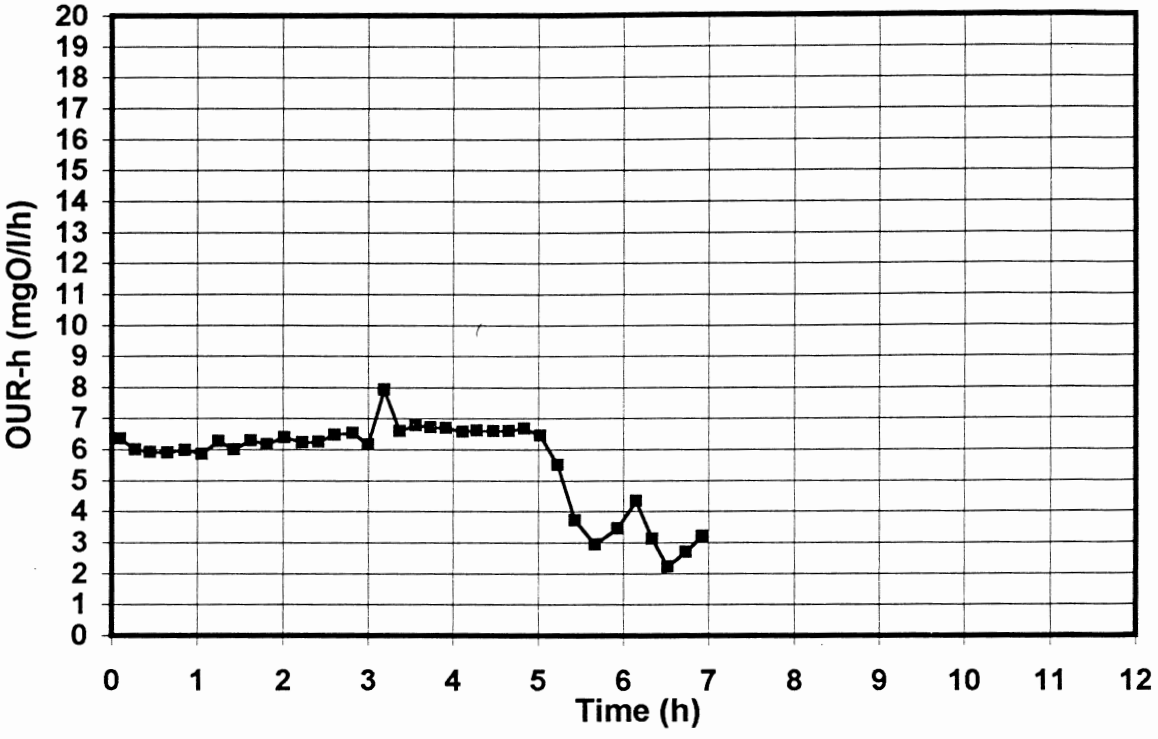
ℓn(OUR-h) for filtered wastewater (2.7ℓ) plus mixed liquor (0.3ℓ) batch test F19 , 12-04a , waste water batch no.23



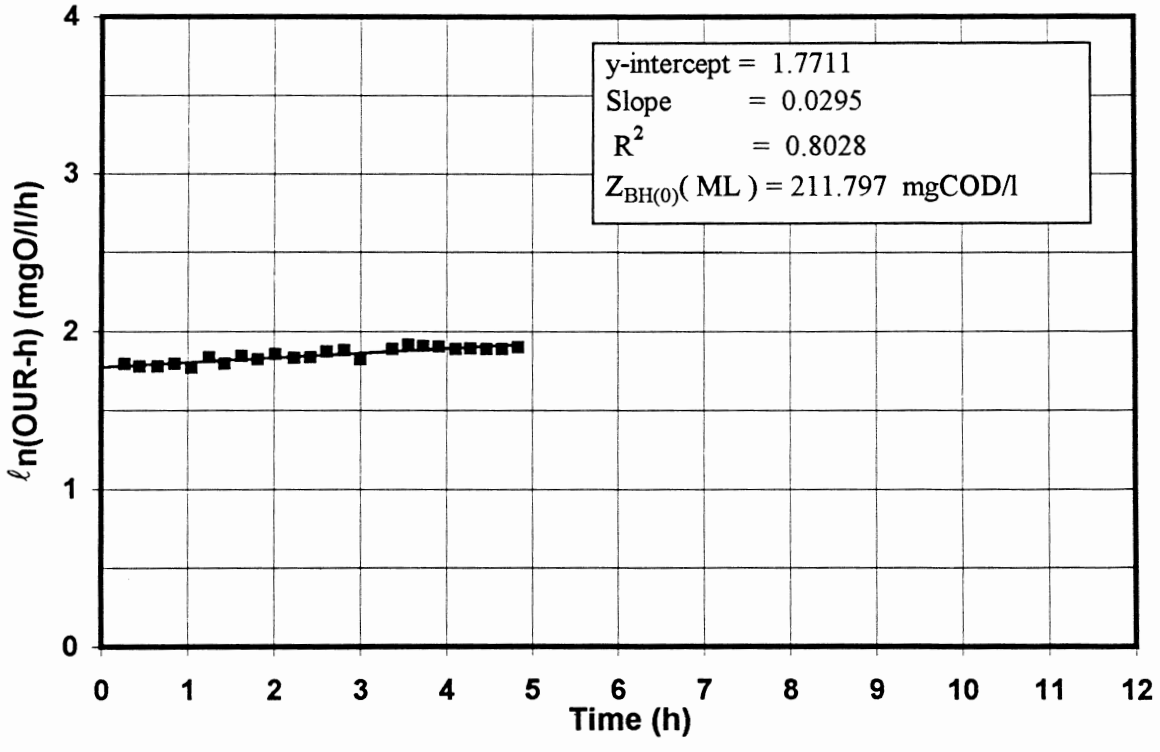
OUR Graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ) batch test F20 , 12-04b , wastewater batch no. 23



NO_3 Graph r filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ) batch test F20 , 12-04b , wastewater batch no. 23



OUR-h plot for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ) batch test F20 , 12-04b , wastewater batch no. 23



ℓn(OUR-h) for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ) batch test F20 , 12-04b , waste water batch no.23

APPENDIX C

COMPREHENSIVE DATA FOR THE PARENT LABORATORY-SCALE NITRIFICATION / DENITRIFICATION ACTIVATED SLUDGE SYSTEM

TABLE OF CONTENTS

Table C1 Summary of statistical data for the various wastewater batches, listing the mean, standard deviation of the sample and the number of samples tested, for the influent, unfiltered effluent and mixed liquor TKN; the nitrate the measured in the aerobic and anoxic reactors and effluent nitrate .

Table C2 Summary of statistical data for the various wastewater batches, listing the mean, standard deviation of the sample and the number of samples tested, for the influent, unfiltered effluent and mixed liquor COD concentrations; the OUR and the VSS measured in the aerobic reactor.

Table C3 Daily influent and effluent COD and TKN together with nitrate results for the parent laboratory-scale activated sludge system.

Table C4 Daily mixed liquor VSS, COD, TKN and OUR together with pH results for the parent laboratory-scale activated sludge system.

Table C5 Summary of the parent system N and COD mass balances; wastewater fractions f,sus and f,sup; mixed liquor parameters fcv and fn; TKN/COD ratio's for the various wastewater batches and comparison with the TKN/COD ratio's measured by Söteman (2000) .

Fig. C1 – C5 Graphical representations of daily COD, TKN, nitrate, VSS, OUR and DSVI measurements for the parent laboratory-scale activated sludge system.

Table C1 : Summary of the parent system TKN and Nitrate statistical data for the various wastewater batches.

Sew. Batch No.	TKN (mgN/l)									NITRATES (mgN/l)								
	INFLUENT			UNFILT. EFFLUENT			MIXED LIQUOR			ANOXIC			AEROBIC			EFFLUENT		
	Mean	SSD	No. of tests	Mean	SSD	No. of tests	Mean	SSD	No. of tests	Mean	SSD	No. of tests	Mean	SSD	No. of tests	Mean	SSD	No. of tests
1	51	1	3	5,7	0,3	2	202	22	3	5,7	1,0	4	6,8	1,5	4	12,3	1,7	4
2	48	1	6	5,2	1,2	7	206	14	7	1,9	1,3	10	7,2	1,7	10	8,7	2,1	10
3	45	2	10	4,6	1,1	9	220	5	10	0,9	0,5	10	8,4	0,9	10	8,9	1,1	10
4	62	2	11	7,2	3,0	10	226	25	11	10,6	0,5	5	19,0	2,4	12	21,8	2,5	11
5	62	4	8	5,5	1,4	9	245	8	8	8,5	2,1	9	17,4	1,0	8	19,0	1,3	9
6a	30	2	5	3,9	0,4	4	247	20	5	0,5	0,3	5	3,2	0,5	5	3,0	0,9	5
6b	55	1	6	3,3	1,9	6	227	7	7	10,6	2,0	7	18,0	2,5	7	20,8	2,1	7
7	53	1	8	5,2	1,4	7	231	9	8	10,6	1,6	8	17,9	1,7	7	19,1	1,9	8
8	64	5	2	5,4	2,3	3	262	6	3	10,8	7,5	3	21,8	9,7	3	23,8	5,3	3
9	63	3	5	5,4	0,8	4	246	14	5	12,9	3,4	6	24,0	4,5	6	27,4	2,7	6
10	51	6	5	5,7	2,7	5	262	28	4	5,0	2,5	6	14,1	3,9	6	15,5	3,5	6
11	46	1	8	3,7	0,2	7	248	9	8	1,1	0,7	8	6,7	1,3	9	8,1	1,5	9
12	47	2	6	4,0	0,9	6	248	33	5	2,2	1,5	6	7,8	2,9	6	10,2	2,4	6
13	47	1	7	4,1	0,3	7	243	6	8	3,7	0,5	8	9,8	0,8	8	11,3	0,9	8
14	48	1	4	3,7	0,4	4	226	7	4	5,5	1,2	4	11,7	0,8	4	13,2	0,5	4
17	60	2	10	6,1	0,6	10	198	7	8	19,7	1,6	10	29,0	2,5	10	28,5	2,5	10
18	44	4	8	5,3	0,5	8	230	7	8	4,1	0,9	7	10,6	1,7	9	11,5	1,2	8
19	46	1	9	4,3	0,4	8	231	7	8	5,5	2,6	9	13,8	2,8	9	15,4	2,6	9
20	39	1	6	5,2	0,5	7	219	4	7	1,8	2,1	5	7,1	3,9	7	9,2	2,4	8
21	50	3	8	4,0	0,9	8	250	8	7	10,3	2,1	9	20,6	2,0	9	22,9	2,4	9
22	45	2	6	5,0	0,6	6	219	11	6	8,1	1,1	7	15,7	1,2	7	18,1	0,8	6
23	51	5	7	6,2	1,9	7	216	26	7	7,8	1,1	7	14,9	0,9	7	16,7	1,1	7

Table C2 : Summary of the parent system COD, OUR and VSS statistical data for the various wastewater batches.

Sew. Batch No.	COD (mgCOD/l)									OUR (mgO/l/h)			VSS (mgVSS/l)		
	INFLUENT			UNFILT. EFFLUENT			MIXED LIQUOR								
	Mean	SSD	No. of tests	Mean	SSD	No. of tests	Mean	SSD	No. of tests	Mean	SSD	No. of tests	Mean	SSD	No. of tests
1	488	16	4	49	12	4	2333	170	4	26,5	3,8	4	1752	69	4
2	503	31	10	53	10	9	2879	532	10	29,2	3,0	9	2011	305	10
3	501	21	10	70	15	10	3358	164	10	29,0	2,0	8	2350	110	10
4	491	20	12	74	14	12	3294	209	11	31,8	3,7	12	2273	80	10
5	497	11	8	83	30	9	3522	114	9	34,8	3,5	8	2444	66	9
6a	526	16	5	44	19	5	3678	107	5	23,0	0,8	5	2481	73	5
6b	547	69	7	78	23	6	2679	176	7	34,6	1,4	7	2386	68	7
7	473	27	8	75	25	8	3443	232	8	33,3	1,3	8	2319	62	8
8	507	12	2	71	35	3	3771	154	3	38,4	0,0	2	2541	69	3
9	540	43	6	81	23	6	3458	210	6	38,1	0,1	2	2407	138	6
10	457	22	6	62	16	6	3831	366	6	32,7	0,0	1	2545	251	6
11	488	29	9	52	12	9	3471	230	9	33,5	0,7	3	2419	111	9
12	500	42	6	43	9	6	3372	121	6	33,5	1,9	6	2293	70	6
13	540	25	8	59	14	7	3681	148	8	34,8	0,7	7	2469	83	8
14	517	25	4	61	12	4	3403	185	4	37,4	2,1	4	2227	163	4
17	489	19	10	89	4	8	2916	134	10	42,8	3,8	10	2016	73	9
18	526	37	9	73	10	9	3502	118	9	36,1	1,7	8	2387	59	8
19	498	22	8	60	7	9	3513	103	9	35,4	1,2	8	2400	90	8
20	500	10	8	83	8	8	3418	109	8	30,3	1,4	8	2202	84	8
21	510	39	9	69	7	8	3664	92	7	39,4	1,6	8	2457	74	8
22	493	28	7	79	17	7	3427	170	7	35,2	2,5	7	2138	135	7
23	530	30	6	80	15	7	3116	263	7	34,1	1,3	7	2043	145	7

Table C3 : Daily COD , TKN and Nitrates concentration results for the parent laboratory-scale MLE activated sludge system.

Sew. Batch No.	Dates of Test	Day No.	COD (mgCOD/l)			TKN (mgN/l)			NITRATES (mgN/l)		
			Unfilt. Infl.	Unfilt. Effl.	Filt. Effl.	Unfilt. Infl	Unfilt. Effl.	Filt. Effl.	Anoxic	Aerobic	Effl.
1	01/06/1999	1	498	36	28	51	5.9	2.8	6.5	9.0	14.5
	02/06/1999	2	466	42	37	51	5.5	4.3	6.5	6.8	10.7
	03/06/1999	3	491	61	40	–	–	–	4.3	5.7	11.4
	04/06/1999	4	500	59	54	52	–	–	5.7	5.8	12.5
MEAN			488	49	40	51	5.7	3.6	5.7	6.8	12.3
Std. Deviation			16	12	10	1	0.3	1.1	1.0	1.5	1.7
2	07/06/1999	7	502	61	24	47	7.1	3.8	3.5	5.0	11.1
	08/06/1999	8	495	70	61	47	6.3	5.3	2.9	5.9	10.0
	09/06/1999	9	447	63	51	50	5.7	3.9	1.6	9.8	10.6
	10/06/1999	10	488	43	26	48	4.1	3.9	2.3	8.3	9.7
	11/06/1999	11	506	45	36	46	4.3	3.9	2.6	10.2	11.6
	14/06/1999	14	531	43	30	49	3.9	3.4	0.5	7.0	6.3
	15/06/1999	15	547	57	38	–	–	–	0.1	6.3	6.8
	16/06/1999	16	533	47	34	–	–	–	0.3	7.0	7.0
	17/06/1999	17	516	49	49	–	–	–	3.4	6.0	6.4
	18/06/1999	18	466	85 *	46	54 *	4.8	4.9	1.4	6.5	7.5
MEAN			503	53	40	48	5.2	4.2	1.9	7.2	8.7
Std. Deviation			31	10	12	1	1.2	0.7	1.3	1.7	2.1
3	21/06/1999	21	499	85	39	43	5.6	3.6	1.3	7.8	7.8
	22/06/1999	22	516	89	56	45	3.5	3.5	1.6	8.9	9.6
	23/06/1999	23	500	67	17 *	46	4.8	3.8	1.1	8.3	10.2
	24/06/1999	24	499	93	41	49	2.8	7.1	0.2	7.7	7.9
	25/06/1999	25	487	58	41	46	4.6	4.2	1.0	8.1	8.4
	30/06/1999	30	512	68	46	46	4.2	2.8	0.3	7.1	8.7
	01/07/1999	31	541	66	44	45	–	2.7	0.1	7.8	7.6
	02/07/1999	32	469	45	35	42	–	–	0.9	9.7	8.2
	05/07/1999	35	473	63	51	46	6.0	5.6	0.6	9.7	10.6
	06/07/1999	36	516	67	41	46	5.5	–	1.4	8.8	9.9
MEAN			501	70	44	45	4.6	4.2	0.9	8.4	8.9
Std. Deviation			21	15	6	2	1.1	1.5	0.5	0.9	1.1
4	09/07/1999	39	514	80	45	60	25.6 *	16 *	–	19.4	14.6 *
	10/07/1999	40	494	69	55	60	6.4	5	–	15.0	19.7
	12/07/1999	42	461	93	27	61	11.8	9.2	–	19.2	20.2
	13/07/1999	43	477	84	41	52 *	10.6	9	–	17.0	20.6
	14/07/1999	44	514	76	37	59	11.3	8.7	–	16.4	19.2
	15/07/1999	45	498	73	42	62	5.0	4.5	–	22.3	25.3
	16/07/1999	46	500	97	16	66	7.4	4.5	–	20.4	20.1
	19/07/1999	49	522	70	29	66	4.1	3.4	10.1	17.8	20.4
	20/07/1999	50	471	47	39	60	5.3	4.2	10.8	17.2	20.8
	21/07/1999	51	476	56	44	65	5.2	2.8	11.2	20.1	23.6
	22/07/1999	52	468	81	48	64	4.6	3.2	11.0	20.2	23.6
	23/07/1999	53	502	60	16	62	–	3.1	10.0	22.7	26.8
MEAN			491	74	37	62	7.2	5.2	10.6	19.0	21.8
Std. Deviation			20	14	12	2	3.0	2.5	0.5	2.4	2.5

* test rejected as an outlier at 95% confidence interval

– testing procedure yielded inconclusive results

Table C3 (cont.) : Daily COD , TKN and Nitrates concentration results for the parent laboratory-scale MLE activated sludge system.

Sew. Batch No.	Dates of Test	Day No.	COD (mgCOD/l)			TKN (mgN/l)			NITRATES (mgN/l)		
			Unfilt. Infl.	Unfilt. Effl.	Filt. Effl.	Unfilt. Infl.	Unfilt. Effl.	Filt. Effl.	Anoxic	Aerobic	Effl.
5	26/07/1999	56	540 *	105	36	65	5.3	3.9	6.6	17.7	17.2
	27/07/1999	57	488	89	40	66	7.4	4.8	4.6	11.4 *	18.8
	28/07/1999	58	512	40	40	58	7.3	4.8	9.3	18.4	17.1
	29/07/1999	59	500	93	36	65	4.8	3.9	9.1	18.1	19.7
	30/07/1999	60	506	58	35	53	3.6	2.1	10.7	16.7	20.1
	02/08/1999	63	485	90	41	63	5.0	2.8	7.2	15.9	19.4
	03/08/1999	64	487	130	47	65	5.6	3.5	8.1	18.7	21.2
	04/08/1999	65	512	43	27	67	3.8	3.5	11.1	16.3	19.0
	06/08/1999	67	489	96	31	61	7.0	3.9	9.4	17.0	18.4
MEAN			497	83	37	62	5.5	3.7	8.5	17.4	19.0
Std. Deviation			11	30	6	4	1.4	0.9	2.1	1.0	1.3
6a	09/08/1999	70	541	37	20	32	4.3	–	0.3	4.1	4.1
	10/08/1999	71	541	78	45	31	–	2.4	0.8	2.9	3.7
	11/08/1999	72	502	39	31	28	4.2	–	0.7	3.0	2.8
	12/08/1999	73	522	33	20	29	3.6	1.4	0.3	3.3	2.3
	13/08/1999	74	526	35	23	29	3.5	2.2	0.2	2.7	2.0
MEAN			526	44	28	30	3.9	2.0	0.5	3.2	3.0
Std. Deviation			16	19	11	2	0.4	0.5	0.3	0.5	0.9
6b	20/08/1999	81	617	258 *	37	57	2.5	2	9.2	18.4	20.7
	21/08/1999	82	576	98	57	56	–	–	8.3	15.4	18.7
	23/08/1999	84	643	91	37	62 *	6.3	6.7	11.8	17.9	20.5
	24/08/1999	85	453	66	37	56	1.8	1	13.6	22.7	22.7
	25/08/1999	86	488	58	29	54	3.2	1.3	12.6	19.6	23.8
	26/08/1999	87	531	49	49	54	1.1	0.4	9.5	16.0	21.5
	27/08/1999	88	519	105	52	54	4.6	2.8	9.4	16.2	18.0
MEAN			547	78	43	55	3.3	2.4	10.6	18.0	20.8
Std. Deviation			69	23	10	1	1.9	2.3	2.0	2.5	2.1
7	30/08/1999	91	490	91	33	55	5.0	1.8	7.7	17.5	17.4
	31/08/1999	92	483	87	37	53	4.8	2.7	8.7	16.7	17.8
	01/09/1999	93	486	111	54	53	7.4	1.7	11.6	16.6	18.8
	02/09/1999	94	507	62	25	51	–	1.3	10.3	18.3	18.3
	03/09/1999	95	490	86	41	52	6.7	2.1	11.9	18.5	20.1
	07/09/1999	99	443	35	43	52	5.5	2.2	10.6	15.8	16.9
	08/09/1999	100	456	49	41	53	3.9	2.5	12.3	18.1	22.3
	10/09/1999	102	431	76	55	55	3.4	2.5	11.3	21.3	21.3
MEAN			473	74	41	53	5.2	2.1	10.6	17.9	19.1
Std. Deviation			27	25	10	1	1.4	0.5	1.6	1.7	1.9
8	21/09/1999	113	499	53	49	60	4.5	3.5	16.0	26.4	27.3
	22/09/1999	114	515	111	41	67	8.0	6.6	2.1	10.6	17.6
	23/09/1999	115	–	49	45	–	3.6	3.4	14.2	28.4	26.4
MEAN			507	71	45	64	5.4	4.5	10.8	21.8	23.8
Std. Deviation			12	34	4	5	2.3	1.8	7.5	9.7	5.3

* test rejected as an outlier at 95% confidence interval

– testing procedure yielded inconclusive results

Table C3 (cont.) : Daily COD , TKN and Nitrates concentration results for the parent laboratory-scale MLE activated sludge system.

Sew. Batch No.	Dates of Test	Day No.	COD (mgCOD/l)			TKN (mgN/l)			NITRATES (mgN/l)		
			Unfilt. Infl.	Unfilt. Effl.	Filt. Effl.	Unfilt. Infl.	Unfilt. Effl.	Filt. Effl.	Anoxic	Aerobic	Effl.
9	29/09/1999	121	620	57	49	67	–	3.6	15.4	25.4	28.6
	30/09/1999	122	527	60	60	65	5.5	5	17.4	30.0	30.4
	01/10/1999	123	519	66	45	–	–	–	14.6	22.4	30.0
	06/10/1999	128	510	88	–	59	6.3	3.8	8.8	16.4	24.6
	07/10/1999	129	504	107	98	60	4.3	3.2	11.0	24.4	24.4
	08/10/1999	130	557	107	90	62	5.6	3.8	10.0	25.2	26.2
MEAN			540	81	68	63	5.4	3.9	12.9	24.0	27.4
Std. Deviation			43	23	24	3	0.8	0.7	3.4	4.5	2.7
10	13/10/1999	135	480	62	44	60	9.9	4.9	3.6	12.8	12.8
	14/10/1999	136	478	53	53	52	4.5	2.7	0.6	8.2	10.2
	20/10/1999	142	447	92	80	46	5.5	3.5	7.2	20.0	19.8
	21/10/1999	143	451	61	61	48	6.0	2.3	6.0	14.4	16.8
	22/10/1999	144	461	61	–	48	2.7	2.5	6.2	15.8	18.2
	26/10/1999	148	422	43	43	–	–	–	6.4	13.4	15.4
MEAN			457	62	56	51	5.7	3.2	5.0	14.1	15.5
Std. Deviation			22	16	15	6	2.7	1.1	2.5	3.9	3.5
11	28/10/1999	150	483	71	35	46	3.9	2.1	0.2	6.4	10.4
	29/10/1999	151	512	43	31	47	3.4	2.7	0.6	5.8	8.6
	01/11/1999	154	543	61	47	46	3.8	–	2.0	8.0	8.4
	02/11/1999	155	490	41	41	46	3.6	2.8	0.5	5.2	6.0
	03/11/1999	156	494	41	41	45	3.6	3.5 *	0.7	5.5	6.2
	04/11/1999	157	467	49	45	45	2.5 *	2.1	1.4	6.8	7.1
	05/11/1999	158	496	39	35	42 *	3.6	2.4	1.1	7.8	8.4
	08/11/1999	161	463	55	47	44	3.9	2.2	2.1	5.9	8.5
	09/11/1999	162	442	66	53	45	–	2.1	3.6 *	8.8	9.6
MEAN			488	52	42	46	3.7	2.3	1.1	6.7	8.1
Std. Deviation			29	12	7	1	0.2	0.3	0.7	1.3	1.5
12	12/11/1999	165	510	41	33	47	2.4	2.1	0.4	5.1	6.4
	15/11/1999	168	466	32	24	46	3.4	–	1.8	8.0	9.2
	16/11/1999	169	440	32	32	45	4.9	3.2	2.8	9.0	9.6
	17/11/1999	170	546	49	49	49	4.2	3.8	3.1	3.5	11.0
	18/11/1999	171	544	53	–	50	4.8	2.2	0.7	10.6	12.1
	19/11/1999	172	493	49	45	47	4.2	3.1	4.2	10.6	13.1
MEAN			500	43	37	47	4.0	2.9	2.2	7.8	10.2
Std. Deviation			42	9	10	2	0.9	0.7	1.5	2.9	2.4
13	24/11/1999	177	548	74	46	45	6.6 *	3.8	4.7	10.3	11.3
	25/11/1999	178	494	77	72	48	4.3	3.8	3.9	10.6	11.7
	26/11/1999	179	542	151 *	60	48	3.9	3.1	3.6	10.4	13.0
	29/11/1999	182	577	58	41	49	4.1	2.7	4.2	10.8	11.4
	30/11/1999	183	546	41	29	48	3.6	2.8	3.1	9.4	10.6
	01/12/1999	184	544	51	47	48	4.3	2.9	3.3	9.2	10.8
	02/12/1999	185	55	63	–	47	3.9	3.2	3.4	8.8	11.6
	03/12/1999	186	519	45	–	41 *	4.3	–	3.5	8.9	10.2
MEAN			540	59	49	47	4.1	3.2	3.7	9.8	11.3
Std. Deviation			25	14	15	1	0.3	0.4	0.5	0.8	0.9

* test rejected as an outlier at 95% confidence interval

– testing procedure yielded inconclusive results

Table C3 (cont.) : Daily COD , TKN and Nitrates concentration results for the parent laboratory-scale MLE activated sludge system.

Sew. Batch No.	Dates of Test	Day No.	COD (mgCOD/l)			TKN (mgN/l)			NITRATES (mgN/l)		
			Unfilt. Infl.	Unfilt. Effl.	Filt. Effl.	Unfilt. Infl.	Unfilt. Effl.	Filt. Effl.	Anoxic	Aerobic	Effl.
14	07/12/1999	190	517	57	49	47	4.1	3.5	4.2	10.4	12.7
	08/12/1999	191	531	63	49	47	3.2	2.9	5.6	12.1	13.8
	09/12/1999	192	482	48	46	46	3.5	3.5	7.0	12.1	12.9
	10/12/1999	193	538	77	46	50	4.1	2.9	5.3	12.1	13.5
MEAN			517	61	47	48	3.7	3.2	5.5	11.7	13.2
Std. Deviation			25	12	2	1	0.4	0.3	1.2	0.8	0.5
17	17/01/2000	231	467	84	31	57	6.2	3.9	22.0	28.2	27.4
	18/01/2000	232	465	88	33	57	6.3	4.5 *	22.0	29.4	24.8
	19/01/2000	233	485	86	47	57	5.9	3.1	19.8	31.8	31.4
	20/01/2000	234	461	90	33	59	7.1	–	19.2	24.8	30.0
	21/01/2000	235	508	84	49	62	6.7	3.6	20.0	32.4	30.6
	24/01/2000	238	516	90	58	61	5.5	3.2	19.0	26.8	24.6
	25/01/2000	239	496	93	60	64	6.3	3.6	18.0	30.0	28.0
	26/01/2000	240	505	56 *	48	60	5.9	3.4	20.8	29.2	29.2
	27/01/2000	241	488	66 *	33	60	6.3	3.8	17.8	26.0	27.8
	28/01/2000	242	498	93	41	62	5.2	3.2	18.0	31.0	31.4
MEAN			489	89	43	60	6.1	3.5	19.7	29.0	28.5
Std. Deviation			19	4	11	2	0.6	0.3	1.6	2.5	2.5
18	01/02/2000	246	540	64	41	38	4.9	2.9	5.0	8.0	9.8
	02/02/2000	247	583	81	66	49	4.8	2.9	2.8	8.6	9.6
	03/02/2000	248	511	64	60	43	4.9	2.7	3.0	9.6	11.5
	04/02/2000	249	481	79	48	39	5.3	3.6	4.3	11.3	12.1
	07/02/2000	252	571	60	37	47	5.6	3.6	7.3 *	13.2	12.1
	08/02/2000	253	530	88	57	46	5.5	2.9	4.3	9.8	11.6
	09/02/2000	254	473	82	39	42	5.3	–	11.6 *	11.4	4.6 *
	10/02/2000	255	508	73	39	–	–	–	4.3	12.2	12.3
	11/02/2000	256	541	69	55	45	6.3	2.9	5.0	11.6	13.1
MEAN			526	73	49	44	5.3	3.1	4.1	10.6	11.5
Std. Deviation			37	10	11	4	0.5	0.4	0.9	1.7	1.2
19	15/02/2000	260	479	59	41	45	4.1	2.7	4.0	10.8	11.7
	16/02/2000	261	492	59	53	45	4.6	2.9	4.4	11.4	12.7
	17/02/2000	262	530	63	53	46	3.9	–	3.0	11.3	13.1
	18/02/2000	263	520	57	43	48	3.8	2.5	4.1	11.9	14.0
	21/02/2000	266	575 *	61	35	48	4.2	2.8	8.5	17.8	18.6
	22/02/2000	267	520	49	29	46	4.2	2.7	6.3	14.8	17.6
	23/02/2000	268	471	55	41	47	4.5	3.2	8.2	18.1	18.2
	24/02/2000	269	479	74	59	45	7.0 *	2.1	9.2	14.9	17.3
	25/02/2000	270	492	66	43	48	4.9	2.7	2.1	13.5	15.6
MEAN			498	60	44	46	4.3	2.7	5.5	13.8	15.4
Std. Deviation			22	7	10	1	0.4	0.3	2.6	2.8	2.6

* test rejected as an outlier at 95% confidence interval

– testing procedure yielded inconclusive results

Table C3 (cont.) : Daily COD , TKN and Nitrates concentration results for the parent laboratory-scale MLE activated sludge system.

Sew. Batch No.	Dates of Test	Day No.	COD (mgCOD/l)			TKN (mgN/l)			NITRATES (mgN/l)		
			Unfilt. Infl.	Unfilt. Effl.	Filt. Effl.	Unfilt. Infl.	Unfilt. Effl.	Filt. Effl.	Anoxic	Aerobic	Effl.
20	28/02/2000	273	504	51 *	39	38	4.8	3.5	5.4	12.6	13.6
	29/02/2000	274	500	94	47	41	5.7	3.4	0.8	8.7	11.6
	01/03/2000	275	514	82	47	39	5.7	2.9	2.20	10.0	9.1
	02/03/2000	276	483	74	51	38	5.2	3.6	0.6	–	7.9
	03/03/2000	277	508	89	57	40	4.9	3.1	–	5.5	7.2
	06/03/2000	280	493	72	47	–	5.5	3.5	–	5.1	9.7
	07/03/2000	281	508	87	47	–	4.3	3.1	0.2	7.3	7.4
	08/03/2000	282	491	85	45	39	8.7 *	–	–	0.4	6.7
MEAN			500	83	47	39	5.2	3.3	1.8	7.1	9.2
Std. Deviation			10	8	5	1	0.5	0.3	2.1	3.9	2.4
21	14/03/2000	288	558	68	53	50	2.7	2.5	9.8	20.2	26.2
	15/03/2000	289	507	64	35	48	4.5	2.8	8.3	19.2	22.8
	16/03/2000	290	468	65	39	47	4.8	–	7.9	18.1	20.3
	17/03/2000	291	462	79	55	50	4.8	2.5	7.5	17.6	19.9
	20/03/2000	294	566	93 *	57	56	3.9	3.6	11.6	23.0	25.4
	21/03/2000	295	531	81	57	–	–	2.8	13.0	23.6	25.6
	22/03/2000	296	535	68	39	50	2.5	2.4	10.0	21.0	22.3
	23/03/2000	297	473	66	53	50	4.1	2.5	11.2	21.0	23.4
	24/03/2000	298	493	64	49	51	4.6	4.1	13.2	21.6	20.6
MEAN			510	69	49	50	4.0	2.9	10.3	20.6	22.9
Std. Deviation			39	7	9	3	0.9	0.6	2.1	2.0	2.4
22	27/03/2000	301	533	62	56	47	4.2	2.9	6.1	14.6	12.2 *
	28/03/2000	302	461	68	51	44	5.2	2.2	8.6	16.9	17.4
	29/03/2000	303	473	74	64	41	4.9	2.5	8.2	16.7	18.6
	30/03/2000	304	492	104	84	45	4.6	–	7.4	14.2	18.0
	31/03/2000	305	488	65	53	–	–	–	7.8	15.8	17.2
	03/04/2000	308	528	100	65	44	5.0	2.9	8.8	14.8	18.2
	04/04/2000	309	477	78	49	47	5.9	3.4	9.6	17.2	19.4
MEAN			493	79	60	45	5.0	2.8	8.1	15.7	18.1
Std. Deviation			28	17	12	2	0.6	0.4	1.1	1.2	0.8
23	07/04/2000	312	533	100	43	48	4.1	1.1	7.4	14.0	17.6
	10/04/2000	315	584	92	55	47	4.2	1.7	7.0	15.6	18.0
	11/04/2000	316	514	90	45	48	6.0	3.2	7.0	14.4	15.4
	12/04/2000	317	492	55	35	49	5.9	3.2	9.0	14.8	15.2
	13/04/2000	318	526	78	63	48	5.9	–	6.4	13.8	16.0
	17/04/2000	322	533	74	47	55	8.7	5.3	8.4	15.6	17.2
	18/04/2000	323	391 *	72	57	60	8.7	5.3	9.2	16.2	17.2
MEAN			530	80	49	51	6.2	3.3	7.8	14.9	16.7
Std. Deviation			30	15	10	5	1.9	1.8	1.1	0.9	1.1

* test rejected as an outlier at 95% confidence interval

– testing procedure yielded inconclusive results

Table C4 : Daily MLTSS, MLVSS, MLCOD, MLTKN, OUR, DSVI and pH results for the parent laboratory-scale MLE activated sludge system.

Sew. Batch No.	Dates of Test	Day No.	MIXED LIQUOR					AEROBIC	
			MLTSS (mgTSS/l)	MLVSS (mgVSS/l)	COD (mgCOD/l)	TKN (mgN/l)	DSVI (ml/g)	OUR (mgO/l/h)	pH
1	01/06/1999	1	1978	1786	2145	193	126	23.72	–
	02/06/1999	2	1986	1802	2388	185	114	24.72	–
	03/06/1999	3	1858	1650	2257	–	132	25.43	–
	04/06/1999	4	1982	1768	2540	227	116	32.12	–
MEAN			1951	1752	2333	202	122	26.50	–
Std. Deviation			62	69	170	22	8	3.81	–
2	07/06/1999	7	2104	1874	3125	210	105	30.80	–
	08/06/1999	8	2034	1806	2660	209	108	24.54	–
	09/06/1999	9	2278	2054	2834	217	101	32.03	7.60 *
	10/06/1999	10	2086	1884	2328	192	105	26.24	7.15
	11/06/1999	11	1882	1660	2247	197	109	32.10	6.99
	14/06/1999	14	1792	1568	2145	190	120	24.96	7.07
	15/06/1999	15	2502	2092	3046	–	128	30.29	7.04
	16/06/1999	16	2732	2390	3299	–	125	30.79	7.04
	17/06/1999	17	2744	2404	3360	–	120	–	7.04
	18/06/1999	18	2742	2380	3750	230	124	30.79	6.94
MEAN			2290	2011	2879	206	114	29.17	7.04
Std. Deviation			366	305	532	14	10	3.03	0.07
3	21/06/1999	21	2510	2226	3046	221	104	–	–
	22/06/1999	22	2686	2330	3419	214	103	22.40 *	6.98
	23/06/1999	23	2576	2454	3253	210	105	30.08	6.84
	24/06/1999	24	2886	2542	3419	216	94	27.09	6.86
	25/06/1999	25	2740	2404	3336	223	88	30.10	–
	30/06/1999	30	2638	2312	3626	221	91	31.96	–
	01/07/1999	31	2696	2368	3564	227	93	29.59	7.05
	02/07/1999	32	2516	2156	3346	223	95	27.95	6.77
	05/07/1999	35	2734	2394	3305	221	84	29.63	6.78
	06/07/1999	36	2658	2314	3264	225	87	25.41	–
MEAN			2664	2350	3358	220	94	28.97	6.88
Std. Deviation			113	110	164	5	7	2.05	0.11
4	09/07/1999	39	2428	2188	3529	263	91	27.47	6.25
	10/07/1999	40	2586	2304	3672	262	93	29.46	6.17
	12/07/1999	42	2550	2330	3187	204	82	31.36	5.79
	13/07/1999	43	2724	2286	3207	–	73	38.63	5.63
	14/07/1999	44	2400	2192	3084	210	92	32.85	5.62
	15/07/1999	45	2448	2236	3281	207	94	26.50	7.04
	16/07/1999	46	2108 *	1902 *	2570 *	185	100	30.61	–
	19/07/1999	49	2570	2318	3330	217	101	36.25	7.31
	20/07/1999	50	2608	2342	3269	218	111	30.72	7.64
	21/07/1999	51	2822	2562 *	3105	228	99	36.07	7.53
	22/07/1999	52	2666	2394	3548	249	113	29.26	7.57
	23/07/1999	53	2424	2144	3024	242	136 *	32.23	7.33
MEAN			2566	2273	3294	226	95	31.79	6.72
Std. Deviation			136	80	209	25	11	3.66	0.83

* test rejected as an outlier at 95% confidence interval

– testing procedure yielded inconclusive results

Table C4 (cont.) : Daily MLTSS, MLVSS, MLCOD, MLTKN, OUR, DSVI and pH results for the parent laboratory-scale MLE activated sludge system.

Sew. Batch No.	Dates of Test	Day No.	MIXED LIQUOR					AEROBIC	
			MLTSS (mgTSS/l)	MLVSS (mgVSS/l)	COD (mgCOD/l)	TKN (mgN/l)	DSVI (ml/g)	OUR (mgO/l/h)	pH
5	26/07/1999	56	2586	2366	3468	242	131	36.42	–
	27/07/1999	57	2652	2380	3387	230	136	–	7.50
	28/07/1999	58	2722	2432	3669	–	132	35.77	7.47
	29/07/1999	59	2762	2524	3669	246	135	37.32	–
	30/07/1999	60	2854	2546	3598	260	126	29.57	–
	02/08/1999	63	2836	2488	3372	244	120	37.09	7.61
	03/08/1999	64	2780	2470	3598	246	119	39.16	7.53
	04/08/1999	65	2704	2402	3475	245	111	31.05	7.46
	06/08/1999	67	2686	2384	3461	244	115	31.86	7.61
MEAN			2731	2444	3522	245	125	34.78	7.53
Std. Deviation			86	66	114	8	9	3.47	0.07
6a	09/08/1999	70	2926	2570	3768	273	109	21.86	7.68
	10/08/1999	71	2740	2402	3727	259	117	23.04	–
	11/08/1999	72	2884	2534	3543	238	118	23.52	7.76
	12/08/1999	73	2750	2416	3768	242	120	22.55	7.84
	13/08/1999	74	2812	2482	3584	221	117	23.89	7.68
MEAN			2822	2481	3678	247	116	22.97	7.74
Std. Deviation			82	73	107	20	4	0.80	0.08
6b	20/08/1999	81	2630	2326	3597	220	137	35.76	7.10
	21/08/1999	82	2820	2510	3720	220	128	36.87	7.09
	23/08/1999	84	2736	2426	3378	225	121	32.63	6.85
	24/08/1999	85	2636	2344	3584	223	129	33.36	6.98
	25/08/1999	86	2738	2406	3749	235	131	33.94	7.04
	26/08/1999	87	2658	2376	3914	228	139	34.73	7.32
	27/08/1999	88	2624	2316	3811	237	122	34.61	7.25
MEAN			2692	2386	3679	227	130	34.56	7.09
Std. Deviation			74	68	176	7	7	1.44	0.16
7	30/08/1999	91	2648	2332	3626	238	98	35.25	7.65
	31/08/1999	92	2748	2420	3626	231	95	31.06	7.51
	01/09/1999	93	2642	2330	3626	227	95	33.68	7.53
	02/09/1999	94	2668	2348	3447	225	90	32.54	7.52
	03/09/1999	95	2572	2246	3612	213	101	33.58	7.58
	07/09/1999	99	2666	2330	3386	241	94	34.83	7.50
	08/09/1999	100	2650	2326	2996	239	91	32.67	7.56
	10/09/1999	102	2534	2218	3230	232	104	32.65	7.42
MEAN			2641	2319	3443	231	96	33.28	7.53
Std. Deviation			65	62	232	9	5	1.35	0.07
8	21/09/1999	113	2950	2576	3638	256	105	38.40	7.23
	22/09/1999	114	2964	2586	3940	262	108	–	7.49
	23/09/1999	115	2818	2462	3735	267	117	38.35	7.40
MEAN			2911	2541	3771	262	110	38.37	7.37
Std. Deviation			81	69	154	6	6	0.03	0.13

* test rejected as an outlier at 95% confidence interval

– testing procedure yielded inconclusive results

Table C4 (cont.) : Daily MLTSS, MLVSS, MLCOD, MLTKN, OUR, DSVI and pH results for the parent laboratory-scale MLE activated sludge system.

Sew. Batch No.	Dates of Test	Day No.	MIXED LIQUOR					AEROBIC	
			MLTSS (mgTSS/l)	MLVSS (mgVSS/l)	COD (mgCOD/l)	TKN (mgN/l)	DSVI (ml/g)	OUR (mgO/l/h)	pH
9	29/09/1999	121	2790	2434	3529	258	104	–	7.37
	30/09/1999	122	2750	2404	3345	246	113	38.10	7.33
	01/10/1999	123	2566	2246	3406	–	117	–	–
	06/10/1999	128	2600	2264	3133	221	123	–	7.32
	07/10/1999	129	2840	2476	3604	251	123	38.17	7.59
	08/10/1999	130	2982	2616	3727	252	117	–	7.45
MEAN			2755	2407	3458	246	116	38.14	7.41
Std. Deviation			155	138	210	14	7	0.05	0.11
10	13/10/1999	135	2798	2420	4055	281	150	32.68	7.56
	14/10/1999	136	2534	2190	3262	221	170	–	7.61
	20/10/1999	142	3272	2844	4141	280	125	–	7.69
	21/10/1999	143	3226	2826	4162	–	130	–	7.55
	22/10/1999	144	2892	2504	3835	266	137	–	7.50
	26/10/1999	148	2766	2488	3529	–	145	–	7.50
MEAN			2915	2545	3831	262	143	32.68	7.57
Std. Deviation			285	251	366	28	16	–	0.07
11	28/10/1999	150	2718	2328	3407	248	129	–	7.55
	29/10/1999	151	2820	2450	3611	262	135 *	–	7.66
	01/11/1999	154	3024	2628	3876	–	126	–	7.74
	02/11/1999	155	2888	2522	3672	255	125	–	–
	03/11/1999	156	2828	2472	3590	249	124	–	7.56
	04/11/1999	157	2760	2402	3359	253	130	33.08	7.53
	05/11/1999	158	2636	2324	3256	237	125	33.11	7.59
	08/11/1999	161	2738	2366	3154	238	124	–	7.61
	09/11/1999	162	2634	2278	3318	241	125	34.25	7.45
MEAN			2783	2419	3471	248	127	33.48	7.59
Std. Deviation			124	111	230	9	4	0.67	0.09
12	12/11/1999	165	2670	2318	3400	235	127	31.46	7.55
	15/11/1999	168	2496	2184	3245	–	128	31.68	7.56
	16/11/1999	169	2568	2236	3204	237	132	32.20	7.54
	17/11/1999	170	2690	2370	3488	237	126	35.07	7.51
	18/11/1999	171	2664	2312	3488	225	128	35.72	7.47
	19/11/1999	172	2698	2340	3407	307	122	34.69	7.53
MEAN			2631	2293	3372	248	127	33.47	7.53
Std. Deviation			81	70	121	33	3	1.90	0.03
13	24/11/1999	177	2732	2360	3516	232	117	34.07	7.58
	25/11/1999	178	2844	2454	3909	246	116	35.33	7.52
	26/11/1999	179	2770	2384	3743	238	119	35.66	7.56
	29/11/1999	182	2994	2578	3764	245	117	34.55	7.59
	30/11/1999	183	2872	2476	3805	251	118	34.02	–
	01/12/1999	184	2874	2488	3659	244	118	34.28	7.40 *
	02/12/1999	185	2986	2588	3536	248	114	38.29 *	7.59
	03/12/1999	186	2812	2422	3516	239	117	35.34	7.57
MEAN			2861	2469	3681	243	117	34.75	7.57
Std. Deviation			94	83	148	6	2	0.68	0.03

* test rejected as an outlier at 95% confidence interval

– testing procedure yielded inconclusive results

Table C4 (cont.) : D MLTSS, MLVSS, MLCOD, MLTKN, OUR, DSVI and pH results for the parent laboratory-scale MLE activated sludge sy m.

Sew. Batch No.	Dates of Test	Day No.	MIXED LIQUOR					AEROBIC	
			MLTSS (mgTSS/l)	MLVSS (mgVSS/l)	COD (mgCOD/l)	TKN (mgN/l)	DSVI (ml/g)	OUR (mgO/l/h)	pH
14	07/12/1999	190	2764	2394	3577	232	119	35.80	7.70
	08/12/1999	191	2490	2158	3189	232	129	35.98	7.59
	09/12/1999	192	2706	2324	3309	220	122	37.53	7.60
	10/12/1999	193	2372	2032	3536	220	135	40.22	7.56
MEAN			2583	2227	3403	226	126	37.38	7.61
Std. Deviation			184	163	185	7	7	2.05	0.06
17	17/01/2000	231	2316	2012	2920	–	69	45.03	7.42
	18/01/2000	232	2316	2058	2981	200	69	43.20	7.38
	19/01/2000	233	2278	1992	2981	–	75	42.09	7.31
	20/01/2000	234	2186	1906	2673	190	73	50.04	7.41
	21/01/2000	235	2224	1940	2714	190	72	46.40	7.42
	24/01/2000	238	2270	1960	2858	189	75	40.85	7.27
	25/01/2000	239	2424	2086	3081	209	74	43.85	7.52
	26/01/2000	240	2350	2056	2999	202	81	39.27	7.37
	27/01/2000	241	2426	2130	3040	203	91	39.37	7.40
	28/01/2000	242	2632 *	2292 *	2916	204	87	37.18	7.38
MEAN			2310	2016	2916	198	77	42.73	7.39
Std. Deviation			82	73	134	7	7	3.83	0.07
18	01/02/2000	246	2448 *	2118 *	3267	204 *	94	28.94 *	7.76 *
	02/02/2000	247	2686	2342	3474	216	89	38.12	7.61
	03/02/2000	248	2710	2334	3681	224	96	36.95	7.59
	04/02/2000	249	2710	2326	3536	228	92	33.55	7.48
	07/02/2000	252	2856	2448	3557	232	88	37.87	7.50
	08/02/2000	253	2784	2418	3611	235	86	37.13	7.46
	09/02/2000	254	2746	2380	3427	228	91	35.08	7.54
	10/02/2000	255	2732	2358	3468	239	84	35.66	7.56
	11/02/2000	256	2886	2488	3495	234	87	34.20	7.45
MEAN			2764	2387	3502	230	90	36.07	7.52
Std. Deviation			73	59	118	7	4	1.70	0.06
19	15/02/2000	260	2648	2302	3495	–	94	31.05 *	7.56
	16/02/2000	261	2616	2264	3434	237	103 *	33.54	7.54
	17/02/2000	262	2798	2412	3698	237	93	35.03	7.53
	18/02/2000	263	2890	2530	3604	235	90	36.58	7.44
	21/02/2000	266	2820	2478	3523	239	82	35.12	7.48
	22/02/2000	267	2736	2394	3441	227	88	37.41	7.42
	23/02/2000	268	2648	–	3359	224	83	35.61	7.48
	24/02/2000	269	2662	2358	3482	224	90	34.66	7.43
	25/02/2000	270	2788	2458	3584	225	86	35.54	7.42
MEAN			2734	2400	3513	231	88	35.43	7.48
Std. Deviation			95	90	103	7	5	1.18	0.05

* test rejected as an outlier at 95% confidence interval

– testing procedure yielded inconclusive results

Table C4 (cont.) : Daily MLTSS, MLVSS, MLCOD, MLTKN, OUR, DSVI and pH results for the parent laboratory-scale MLE activated sludge system.

Sew. Batch No.	Dates of Test	Day No.	MIXED LIQUOR					AEROBIC	
			MLTSS (mgTSS/l)	MLVSS (mgVSS/l)	COD (mgCOD/l)	TKN (mgN/l)	DSVI (ml/g)	OUR (mgO/l/h)	pH
20	28/02/2000	273	2642	2286	3297	221	91	28.63	7.53
	29/02/2000	274	2662	2332	3502	218	79	31.76	7.52
	01/03/2000	275	2600	2246	3277	225	81	30.11	7.58
	02/03/2000	276	2566	2222	3359	217	94	28.26	7.65
	03/03/2000	277	2504	2170	3568	213	100	29.58	7.61
	06/03/2000	280	2428	2104	3356	220	95	30.24	7.66
	07/03/2000	281	2490	2146	3483	221	88	32.40	7.86
	08/03/2000	282	2442	2106	3505	239 *	94	31.05	7.85
MEAN			2542	2202	3418	219	90	30.25	7.66
Std. Deviation			89	84	109	3.9	7	1.44	0.13
21	14/03/2000	288	2850	2470	3755	253	95	41.77	7.54
	15/03/2000	289	2798	2424	3694	246	93	41.98	7.55
	16/03/2000	290	2696	2332	3508	300 *	96	38.88	7.60
	17/03/2000	291	2984	2550	3772	265	87	38.17	7.67
	20/03/2000	294	2378 *	2068 *	3184 *	216 *	88	33.79 *	–
	21/03/2000	295	2850	2446	4015 *	251	77	38.82	7.54
	22/03/2000	296	2936	2512	3680	251	75	39.02	7.46
	23/03/2000	297	2782	2394	3598	239	72	39.05	7.46
	24/03/2000	298	2970	2530	3639	244	67	37.86	7.45
MEAN			2858	2457	3664	250	83	39.44	7.53
Std. Deviation			100	74	92	8	11	1.56	0.08
22	27/03/2000	301	2656	2318	3639	234	68	37.24	7.50
	28/03/2000	302	2554	2208	3475	218	67	39.43	7.39
	29/03/2000	303	2542	2188	3577	220	59	34.70	7.41
	30/03/2000	304	2474	2132	3509	228	61	34.93	7.48
	31/03/2000	305	2534	2202	3284	–	59	34.67	7.46
	03/04/2000	308	2288	1986	3346	203	57	32.39	7.50
	04/04/2000	309	2272	1932	3162	213	53	32.88	7.46
MEAN			2474	2138	3427	219	60	35.18	7.46
Std. Deviation			143	135	170	11	5	2.45	0.04
23	07/04/2000	312	2274	1986	3072	196	53	33.06	7.41
	10/04/2000	315	2130	1856	2888	183	56	34.60	7.58
	11/04/2000	316	2454	2096	3113	207	53	34.61	7.56
	12/04/2000	317	2190	1864	2683	210	55	33.17	7.42
	13/04/2000	318	2562	2228	3338	217	51	33.65	7.51
	17/04/2000	322	2512	2122	3297	244	56	33.28	7.60
	18/04/2000	323	2482	2152	3420	256	56	36.61	7.56
MEAN			2372	2043	3116	216	54	34.14	7.52
Std. Deviation			171	145	263	26	2	1.26	0.08

* test rejected as an outlier at 95% confidence interval

– testing procedure yielded inconclusive results

Table C5: Summary of parent system N and COD mass balances, wastewater fractions and mixed liquor parameters. Summary of wastewater TKN/COD ratio compared to Söteman's measurements and revised N mass balances based on Söteman's TKN/COD ratio's.

Sewage Batch No.	MASS BALANCE (%)		WASTEWATER FRACTIONS		MIXED LIQUOR		INFLUENT TKN/COD evaluation		
	N	COD	Unbio. Soluble ($f_{s,us}$) (mgCOD/mgCOD)	Unbio. Particulate ($f_{s,up}$) (mgCOD/mgCOD)	COD/VSS (f_{CV}) (mgCOD/mgVSS)	TKN/VSS (f_N) (mgN/mgVSS)	Measured TKN/COD ratio	Söteman's TKN/COD ratio	Revised N mass balance
1			0.0820	-0.0007*	1.33*	0.115*			
2			0.0795	0.0408	1.43	0.102			
3			0.0878	0.1164	1.43	0.094			
4	93	80	0.0754	0.1049	1.45	0.099	0.126	0.114	
5	91	90	0.0744	0.1346	1.44	0.100	0.125	0.112	
6a	90	77	0.0532*	0.1050	1.48	0.100	0.057		
6b	90	82	0.0786	0.0784	1.54	0.095	0.101	0.105	
7	91	92	0.0867	0.1380	1.48	0.100	0.112	0.113	
8	104	89	0.0888	0.1503	1.48	0.103	0.126	0.126	
9	109	79	0.1259*	0.1076	1.44	0.102	0.117	0.112	
10	113	95	0.1225*	0.2221*	1.51	0.103	0.111	0.101	
11	90	94	0.0861	0.1434	1.43	0.103	0.094	0.091	
12	92	87	0.0740	0.0999	1.47	0.108	0.094	0.125	
13	93	88	0.0907	0.1053	1.49	0.098	0.087	0.093	
14	90	93	0.0909	0.0777	1.53	0.100	0.093	0.090	
17	89	97	0.0879	0.0573	1.45	0.098	0.123	0.125	
18	101	93	0.0932	0.1027	1.47	0.096	0.084	0.086	
19	113	90	0.0884	0.1300	1.46	0.096	0.092	0.101	104%
20	106	90	0.0920	0.0882	1.55	0.099	0.078	0.081	116%
21	124	90	0.0961	0.1330	1.49	0.102	0.098	0.099	124%
22	113	92	0.1217*	0.0934	1.60*	0.102	0.091	0.096	108%
23	96	83	0.0925	0.0312	1.53	0.106	0.096	0.091	102%
MEAN			0.0853	0.1029	1.48	0.100			
Std. Deviation			0.0079	0.0333	0.04	.0.004			

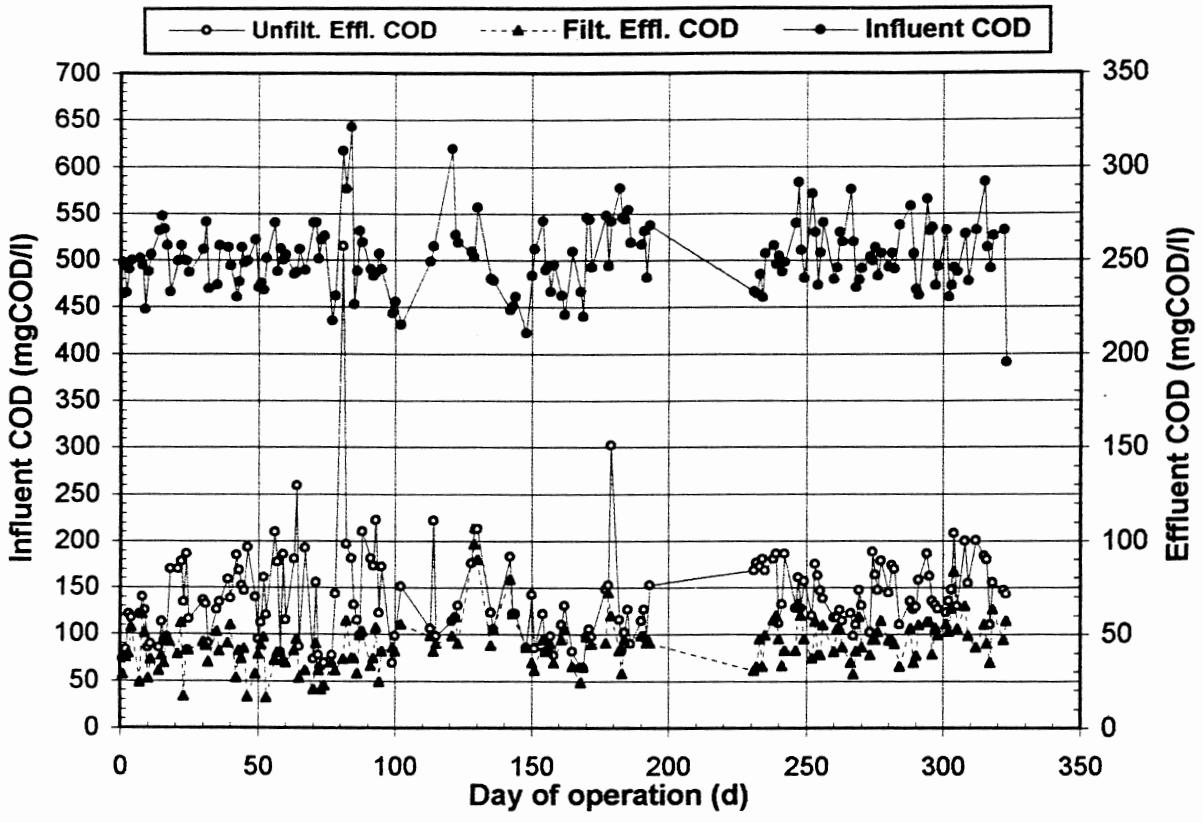


Figure C1 : Graphical representation of the daily influent,unfiltered effluent and filtered effluent COD concentrations for the parent laboratory-scale activated sludge system.

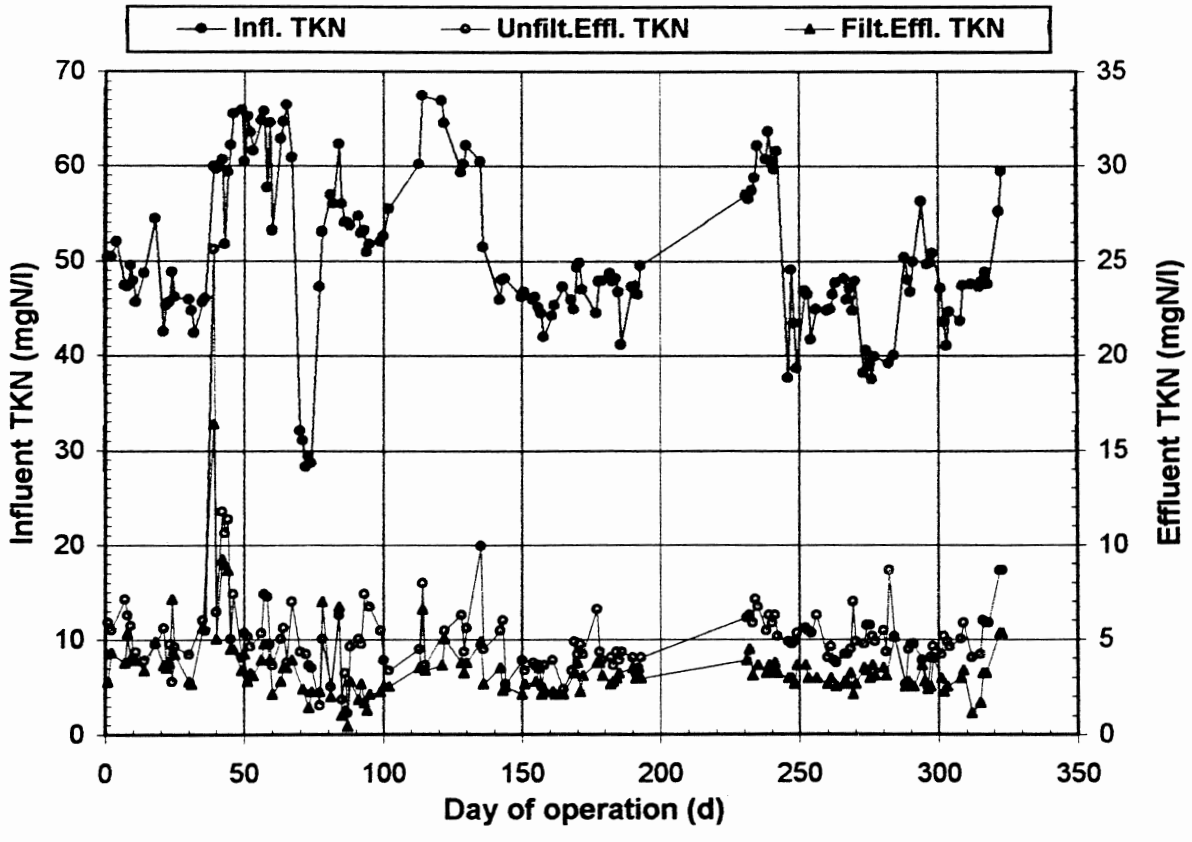


Figure C2 : Graphical representation of the daily influent, unfiltered effluent and filtered effluent TKN concentrations for the parent laboratory-scale activated sludge system.

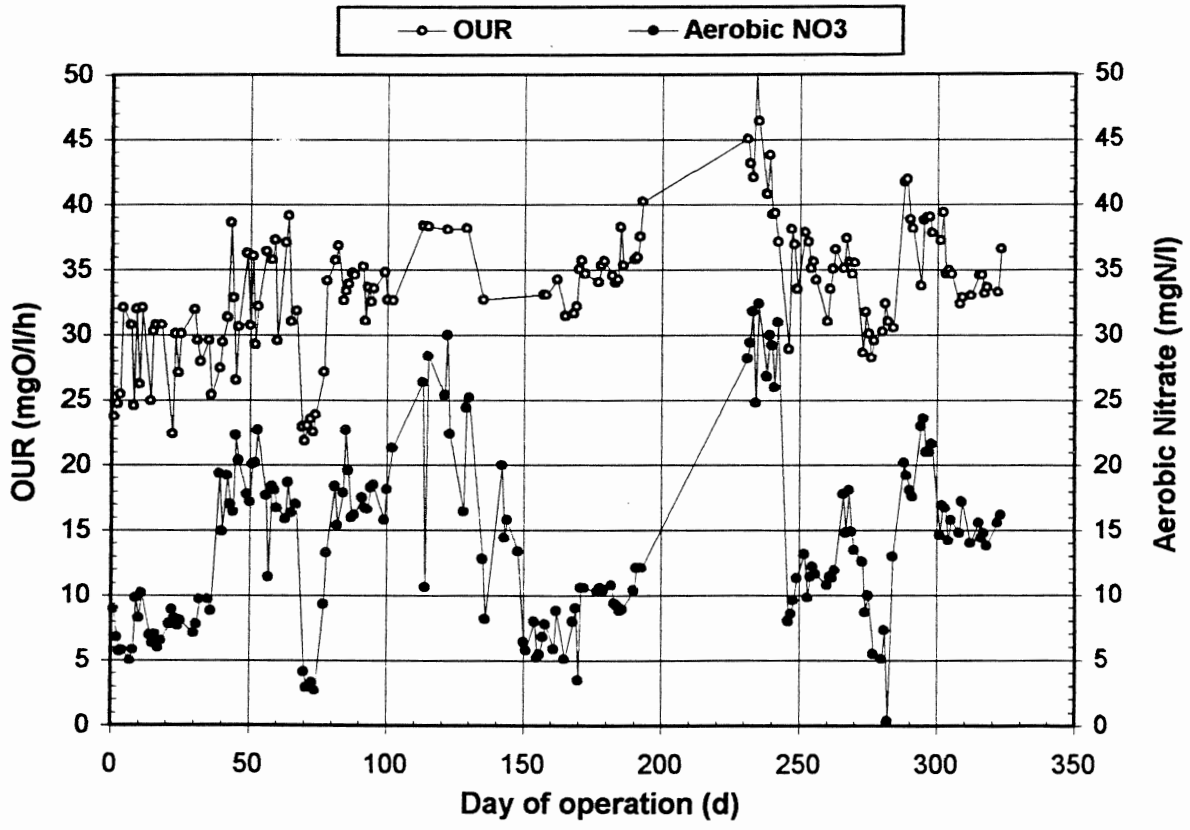


Figure C3 : Graphical representation of the daily oxygen utilization rate (OUR) and nitrate (NO_3) concentration for the aerobic reactor of the parent laboratory-scale activated sludge system.

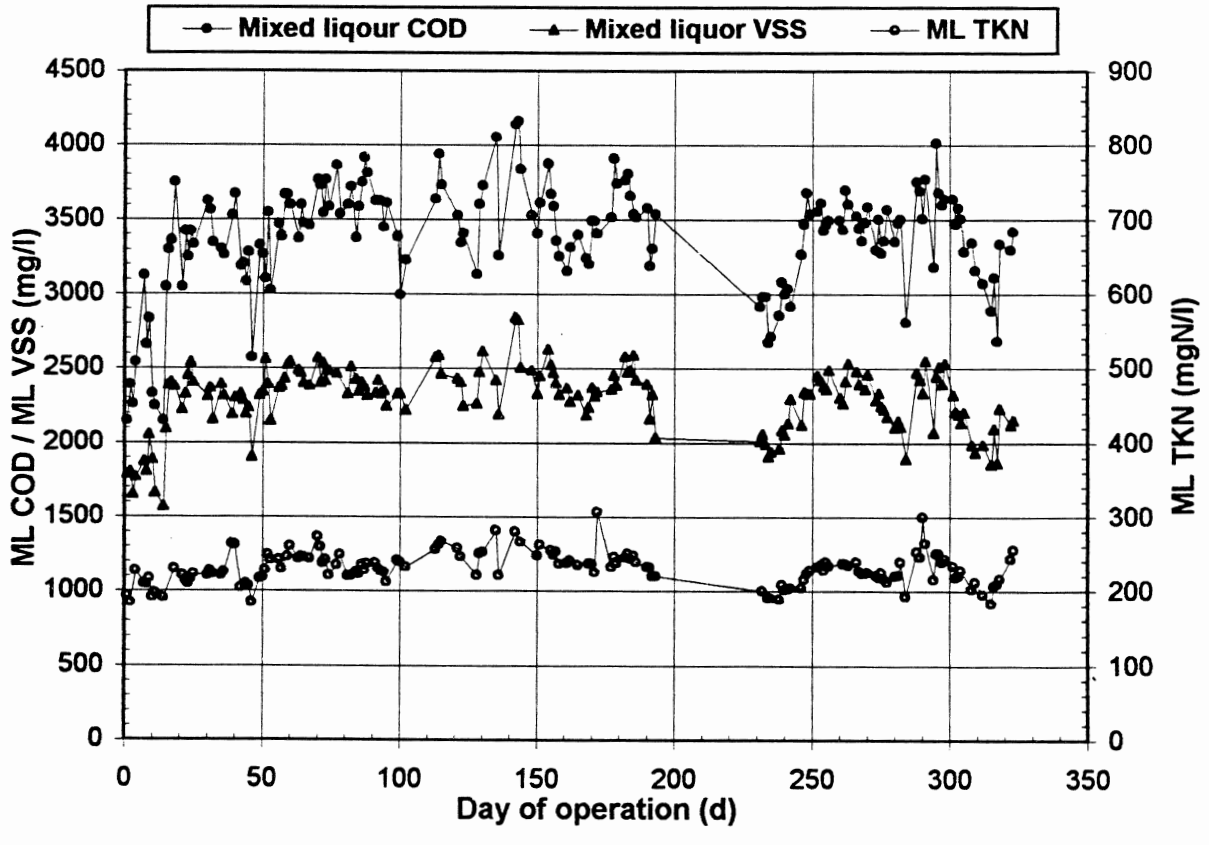


Figure C4 : Graphical representation of the daily mixed liqour VSS, COD and TKN concentrations for the parent laboratory-scale activated sludge system.

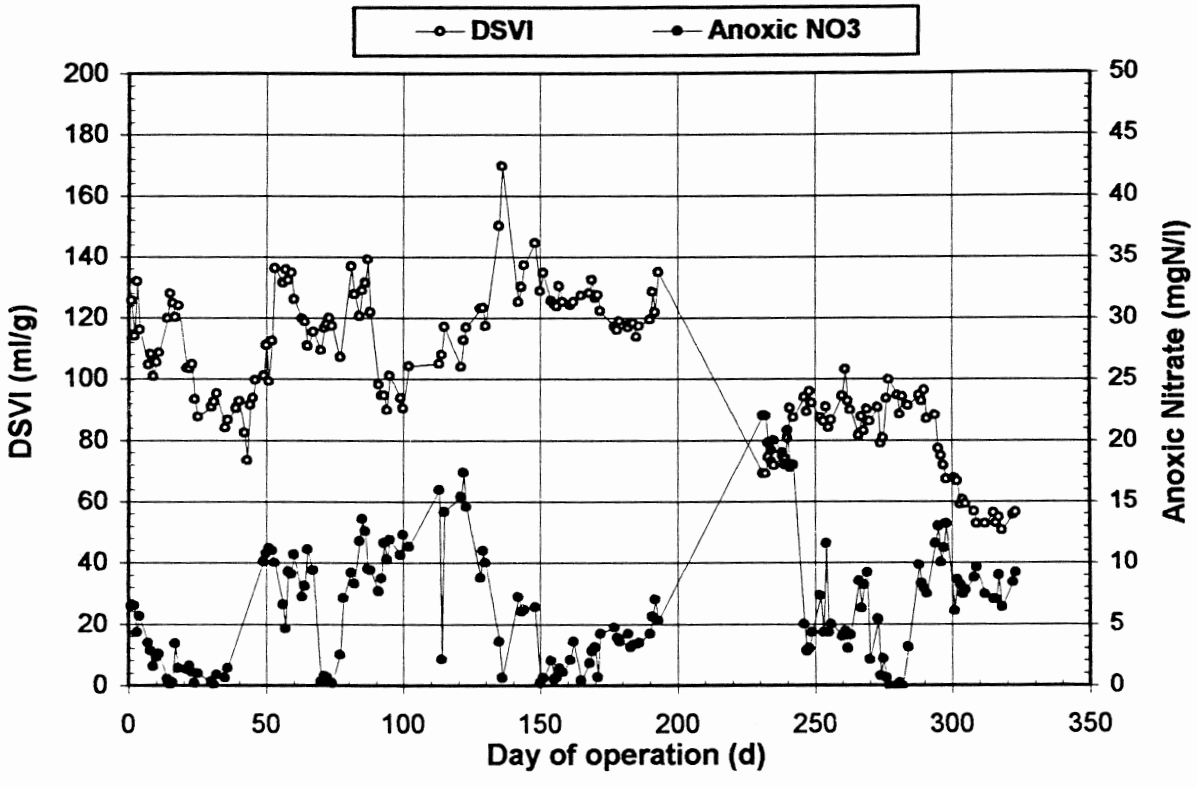


Figure C5 : Graphical representation of the daily Diluted Sludge Volume Index (DSVI) and anoxic nitrate (NO_3) concentration for the parent laboratory-scale activated sludge system.

APPENDIX D

EXTENSION TO THE MODIFIED BATCH TEST INVESTIGATION:

COMPREHENSIVE DATA FOR THE PARENT LABORATORY-SCALE IFFD SYSTEM

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Table D1 Summary of statistical data for the various wastewater batches, listing the mean, standard deviation and the number of samples tested, for the influent, unfiltered effluent and mixed liquor COD and TKN; the mixed liquor VSS; area under the daily OUR-time plot and effluent nitrate.

Table D2 Summary of the daily influent and effluent COD and TKN measurements together with effluent nitrite and nitrate results for the parent IFFD system.

Table D3 Summary of the daily mixed liquor TSS, VSS, COD, TKN and area under the daily OUR-time plot together with pH results for the parent IFFD system.

Table D4 Summary of the parent IFFD system N and COD mass balances, wastewater fractions f,s_{us} and f,s_{up} and mixed liquor parameters f_{cv} and f_N.

Appendix D5 Graphical representations of daily COD, TKN, nitrate, VSS and OUR measurements for the parent laboratory-scale activated sludge system. The daily OUR-time responses are also given.

Appendix D6 Calculation procedure for the construction of the expected IFFD system OUR response.

Table D1 (a) : Summary of the parent laboratory-scale intermittently fed fill and draw (IFFD) system COD statistical data for the various wastewater batches.

Sew. Batch No.	COD (mgCOD/l)								
	INFLUENT			UNFILT. EFFLUENT			MIXED LIQUOR		
	Mean	SSD	No. of tests	Mean	SSD	No. of tests	Mean	SSD	No. of tests
24	1021	41	9	116	21	9	3839	176	9
25	999	38	13	90	8	13	4094	260	13
27	1026	85	7	90	20	7	4134	147	7
28	785	18	3	67	12	3	3974	38	3

Table D1 (b) : Summary of the parent laboratory-scale intermittently fed fill and draw (IFFD) system TKN statistical data for the various wastewater batches.

Sew. Batch No.	TKN (mgN/l)								
	INFLUENT			UNFILT. EFFLUENT			MIXED LIQUOR		
	Mean	SSD	No. of tests	Mean	SSD	No. of tests	Mean	SSD	No. of tests
24	89	2	9	8.0	1.2	9	253	12	9
25	85	3	10	3.4	1.4	10	260	11	10
27	121	6	7	1.5	0.5	7	266	4	6
28	126	2	3	2.7	1.2	3	252	9	3

Table D1 (c) : Summary of the parent laboratory-scale intermittently fed fill and draw (IFFD) system VSS, Area under the daily OUR - time plot and Effluent nitrate statistical data for the various wastewater batches.

Sew. Batch no.	VSS (mgVSS/ℓ)			OUR Area (mgO/ℓ)			EFFLUENT NITRATE (mgN/ℓ)		
	Mean	SSD	No. of tests	Mean	SSD	No. of tests	Mean	SSD	No. of tests
24	2632	184	9	777.6	14.0	9	58.1	2.5	9
25	2779	73	13	737.5	27.6	12	52.7	2.2	13
27	2713	103	7	1030.0	105.7	5	94.6	6.7	7
28	2709	109	3	1153.6	14.1	3	99.2	7.3	2

Table D2 : Daily COD , TKN and Nitrates concentration results for the parent laboratory-scale intermittently fed fill and draw (IFFD) system.

Sew. Batch No.	Dates of Test	Day No.	COD (mgCOD/l)			TKN (mgN/l)			Effluent NITRATE (mgN/l)	Effluent NITRITE (mgN/l)
			Unfilt. Infl.	Unfilt. Effl.	Filt. Effl.	Unfilt. Infl	Unfilt. Effl.	Filt. Effl.		
24	26/04/2000	1	1069	218 *	94	88	27.3 *	16.9 *	46.5 *	0.0
	27/04/2000	2	1011	152	100	90	17.6 *	7.7 *	59.0	0.0
	28/04/2000	3	1094	137	81	91	8.3	2.8	60.0	0.0
	29/04/2000	4	1036	113	63	90	8.8	3.6	59.0	0.0
	30/04/2000	5	1014	123	75	88	9.7	3.2	56.0	0.0
	01/05/2000	6	990	105	73	85	8.1	3.6	58.5	0.0
	02/05/2000	7	974	105	67	88	8.3	3.8	62.0	0.0
	03/05/2000	8	1026	107	79	90	6.2	2.5	54.5	0.0
	04/05/2000	9	974	87	59	90	7.0	4.6	55.5	0.0
MEAN			1021	116	77	89	8.0	3.5	58.1	0.0
Std. Deviation			41	21	14	2	1.2	0.7	2.5	0.0
25	08/05/2000	13	1072	98	74	88	4.8	1.1	55.5	0.0
	09/05/2000	14	1036	88	78	89	1.5	–	54.5	0.0
	10/05/2000	15	1012	76	64	85	2.1	0.8	56.0	0.0
	11/05/2000	16	1000	78	78	81	2.2	1	53.5	0.0
	12/05/2000	17	994	91	83	84	3.8	3.1	49.5	0.0
	15/05/2000	20	982	105	77	82	5.6	1.3	50.0	0.0
	16/05/2000	21	1034	114 *	101 *	81	4.5	2.4	53.5	0.0
	17/05/2000	22	961	87	67	–	–	–	55.0	0.0
	18/05/2000	23	931	85	63	–	–	–	51.0	0.0
	19/05/2000	24	1004	89	61	–	–	–	50.5	0.0
	22/05/2000	27	1008	87	69	86	2.7	1.7	53.0	0.0
	23/05/2000	28	959	93	65	84	4.5	2	52.0	0.0
	24/05/2000	29	894 *	100	79	85	2.8	1.8	51.0	0.0
MEAN			999	90	72	85	3.4	1.7	52.7	0.0
Std. Deviation			38	8	8	3	1.4	0.7	2.2	0.0
27	09/06/2000	45	1092	127	96	129	1.4	0.6	103.0	0.0
	10/06/2000	46	1157	104	92	129	1.5	1.1	103.5	0.0
	13/06/2000	49	976	74	62	114	1.7	0.7	93.5	0.0
	15/06/2000	51	1073	93	87	120	1.1	0.8	93.5	0.0
	16/06/2000	52	1004	77	56	120	0.7	0.7	85.0	0.0
	17/06/2000	53	968	71	67	119	1.5	1.1	93.5	0.0
	19/06/2000	55	912	83	79	115	2.4	1	90.0	0.0
MEAN			1026	90	77	121	1.5	0.9	94.6	0.0
Std. Deviation			85	20	16	6	0.5	0.2	6.7	0.0
28	21/06/2000	12.50	802	77	56	127	2.1	0	108	0.0
	22/06/2000	12.50	766	54	48	126	2.0	0	94	0.0
	23/06/2000	12.50	787	71	55	124	4.1	2	96	0.0
MEAN			785	67	53	126	2.7	0.7	99.2	0.0
Std. Deviation			18	12	4	2	1.2	1.1	7.3	0.0

* test rejected as an outlier at 95% confidence interval

– testing procedure yielded inconclusive results

Table D3 : Daily MLTSS, MLVSS, MLCOD, MLTKN, OUR, DSVI and pH results for the parent laboratory-scale intermittently fed fill and draw (IFFD)system.

Sew. Batch No.	Dates of Test	Day No.	MIXED LIQUOR					OUR AREA (mgO/l)	min. pH
			MLTSS (mgTSS/l)	MLVSS (mgVSS/l)	COD (mgCOD/l)	TKN (mgN/l)	DSVI (ml/g)		
24	26/04/2000	1	2732	2438	3598	235	51	666.8 *	4.35
	27/04/2000	2	2580	2280	3619	242	58	779.1	6.59
	28/04/2000	3	2940	2560	4035	246	54	765.3	7.26
	29/04/2000	4	3004	2628	3625	251	47	804.2	7.12
	30/04/2000	5	3154	2782	3858	262	48	779.9	7.08
	01/05/2000	6	3136	2748	4000	263	51	770.0	7.12
	02/05/2000	7	3050	2618	3959	266	56	789.6	7.04
	03/05/2000	8	3232	2792	3899	246	53	761.8	7.24
	04/05/2000	9	3268	2838	3959	267	52	770.9	7.19
MEAN			3011	2632	3839	253	52	777.6	6.78
Std. Deviation			229	184	176	12	4	14.0	0.93
25	08/05/2000	13	3358	2888	4180	276	54	738.8	7.14
	09/05/2000	14	3300	2898	4260	269	61	764.3	7.19
	10/05/2000	15	3210	2820	4240	262	62	735.7	7.30
	11/05/2000	16	3254	2846	4180	273	65	697.8	7.23
	12/05/2000	17	3208	2796	4279	265	66	721.4	7.19
	15/05/2000	20	3048	2670	4218	252	62	775.3	7.17
	16/05/2000	21	3148	2744	4299	249	60	728.1	7.13
	17/05/2000	22	3196	2746	3792	–	60	713.2	7.13
	18/05/2000	23	3176	2776	3901	–	54	719.7	7.12
	19/05/2000	24	3148	2744	3861	–	57	–	7.34
	22/05/2000	27	2906	2528 *	4023	249	65	780.9	7.13
	23/05/2000	28	3110	2698	3983	244	64	816.3 *	7.18
	24/05/2000	29	3116	2716	4003	260	67	857.8 *	7.19
MEAN			3168	2779	4094	260	61	737.5	7.19
Std. Deviation			114	73	174	11	4	27.6	0.07
27	09/06/2000	45	3128	2762	4377	270	58	1044	6.32
	10/06/2000	46	3170	2818	4177	265	60	1195	6.03
	13/06/2000	49	2854 *	2512	3936	259	77	1023	7.00
	15/06/2000	51	3210	2802	4254	267	75	908	7.14
	16/06/2000	52	3066	2688	4052	269	75	–	6.88
	17/06/2000	53	3130	2680	4052	267	80	–	6.90
	19/06/2000	55	3156	2730	4092	–	76	980	7.44
MEAN			3143	2713	4134	266	71	1030.0	6.82
Std. Deviation			48	103	147	4	9	105.7	0.48
28	21/06/2000	57	3056	2718	3951	260	75	1138.8	5.69
	22/06/2000	58	3150	2814	3951	252	76	1155.2	6.50
	23/06/2000	59	3002	2596	4018	242	77	1166.8	7.03
MEAN			3069	2709	3974	252	76	1153.6	6.41
Std. Deviation			75	109	38	9	1	14.1	0.67

* test rejected as an outlier at 95% confidence interval

– testing procedure yielded inconclusive results

Table D4 : Summary of parent laboratory-scale intermittently fed fill and draw (IFFD) system N and COD mass balances, wastewater fractions and mixed liquor parameters.

Sewage Batch No.	MASS BALANCE (%)		WASTEWATER FRACTIONS		MIXED LIQUOR	
	N	COD	Unbiodegr. Soluble ($f_{S,us}$) (mgCOD / mgCOD)	Unbiodegr. Particulate ($f_{S,up}$) (mgCOD / mgCOD)	COD/VSS (f_{CV}) (mgCOD / mgVSS)	TKN/VSS (f_N) (mgN / mgVSS)
24	102	98	0.0754	0.1598	1.46	0.096
25	96	99	0.0721	0.2009	1.47	0.094
27	101	106	0.0750	0.1735	1.52	0.098
28	97	108	0.0675	0.1945	1.47	0.093
MEAN			0.0725	0.1822	1.48	0.095
Std. Deviation			0.0037	0.0190	0.03	0.002

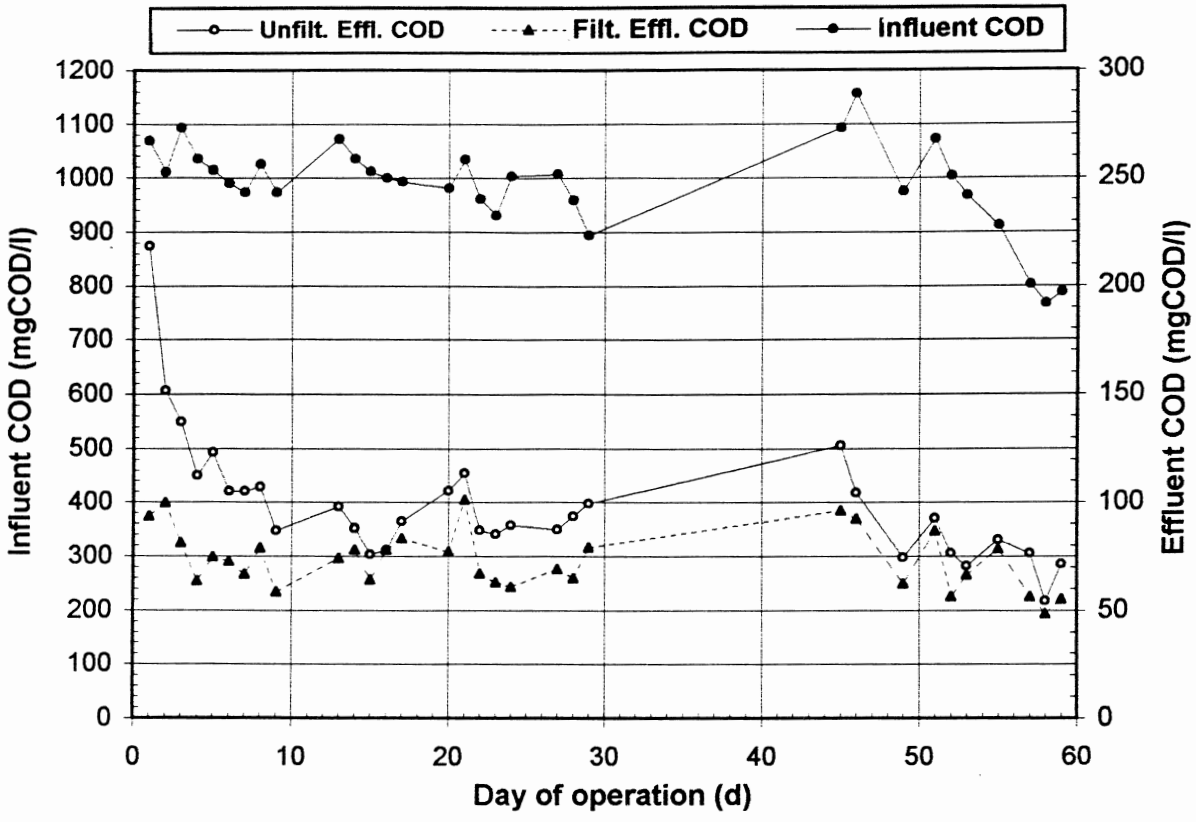


Figure D5.1: Graphical representation of the daily influent , unfiltered effluent and filtered effluentCOD concentrations for the parent laboratory-scale IFFD system.

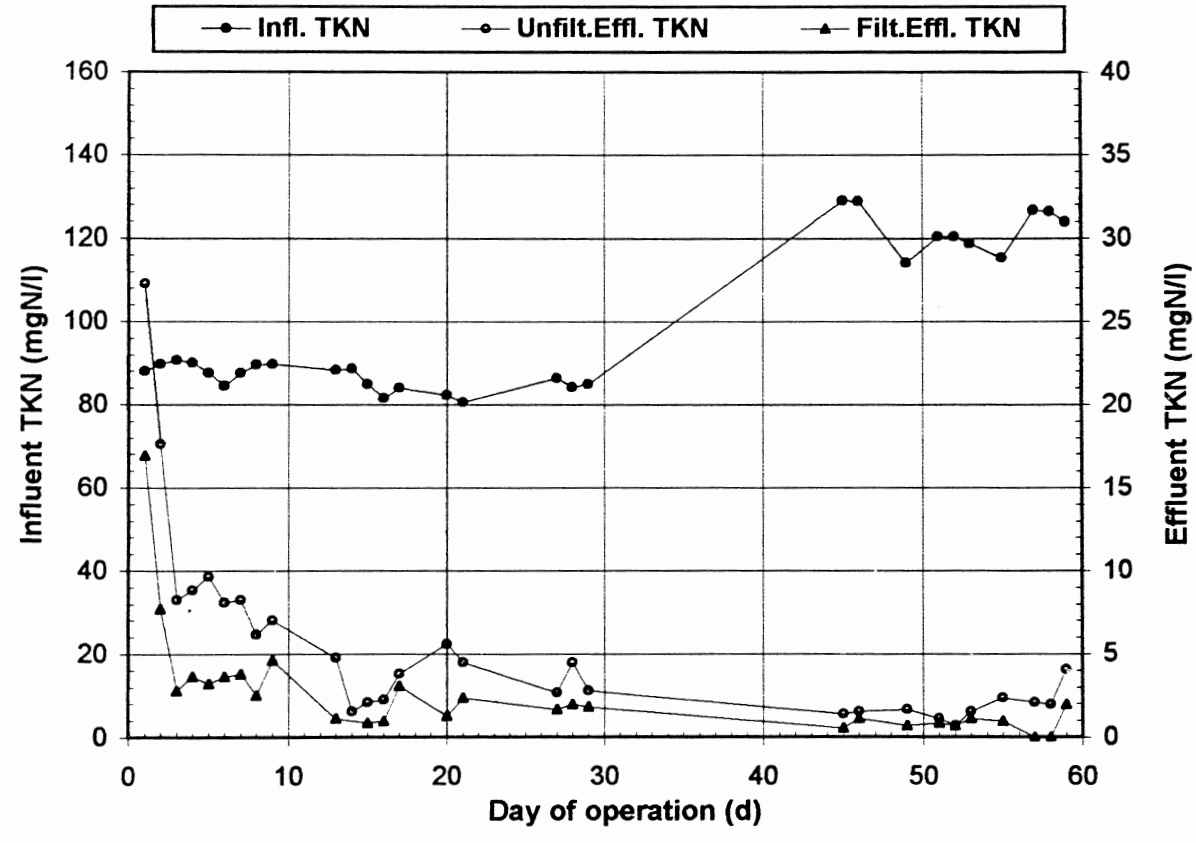


Figure D5.2: Graphical representation of the daily influent, unfiltered effluent and filtered effluent TKN concentrations for the parent laboratory-scale IFFD system.

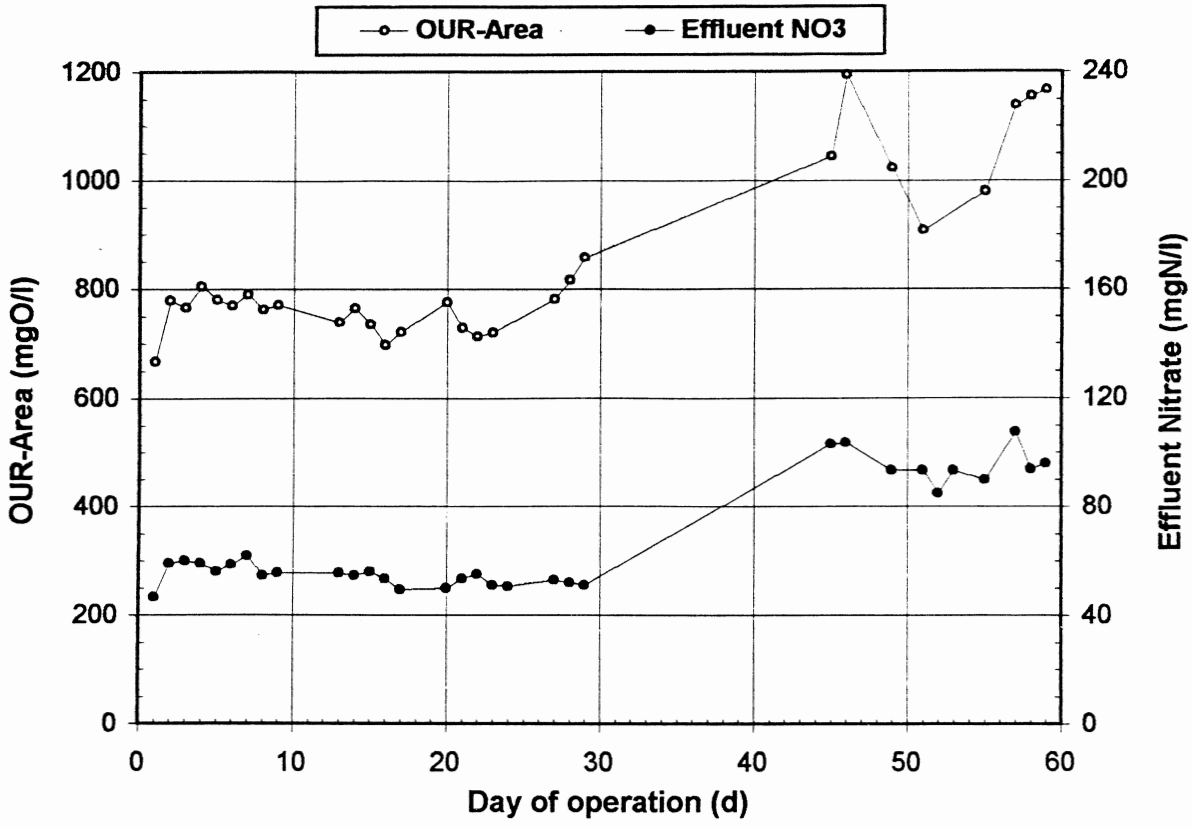


Figure D5.3: Graphical representation of the area measured under the daily OUR - time plots and the effluent nitrate (NO_3) concentration for the parent laboratory-scale IFFD system.

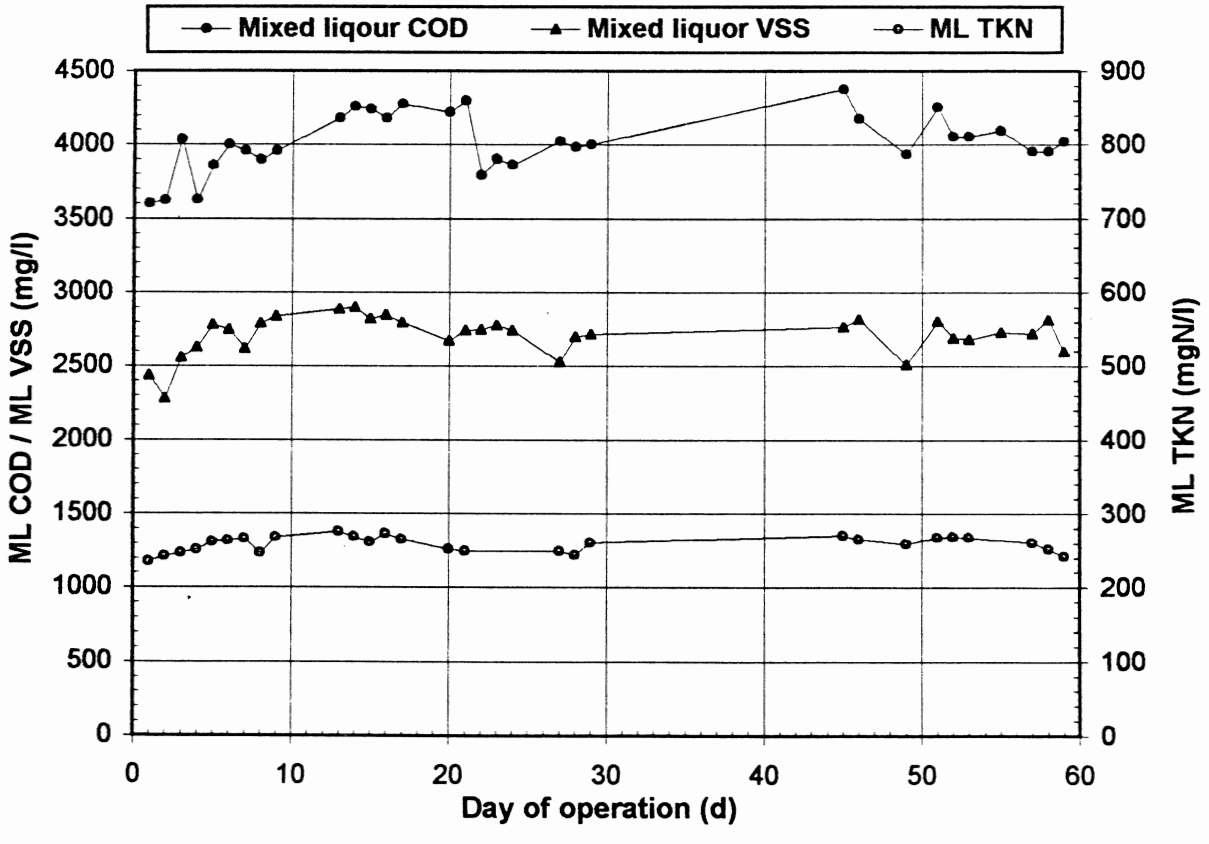


Figure D5.4: Graphical representation of the daily mixed liqour VSS, COD and TKN concentrations for the parent laboratory-scale IFFD system.

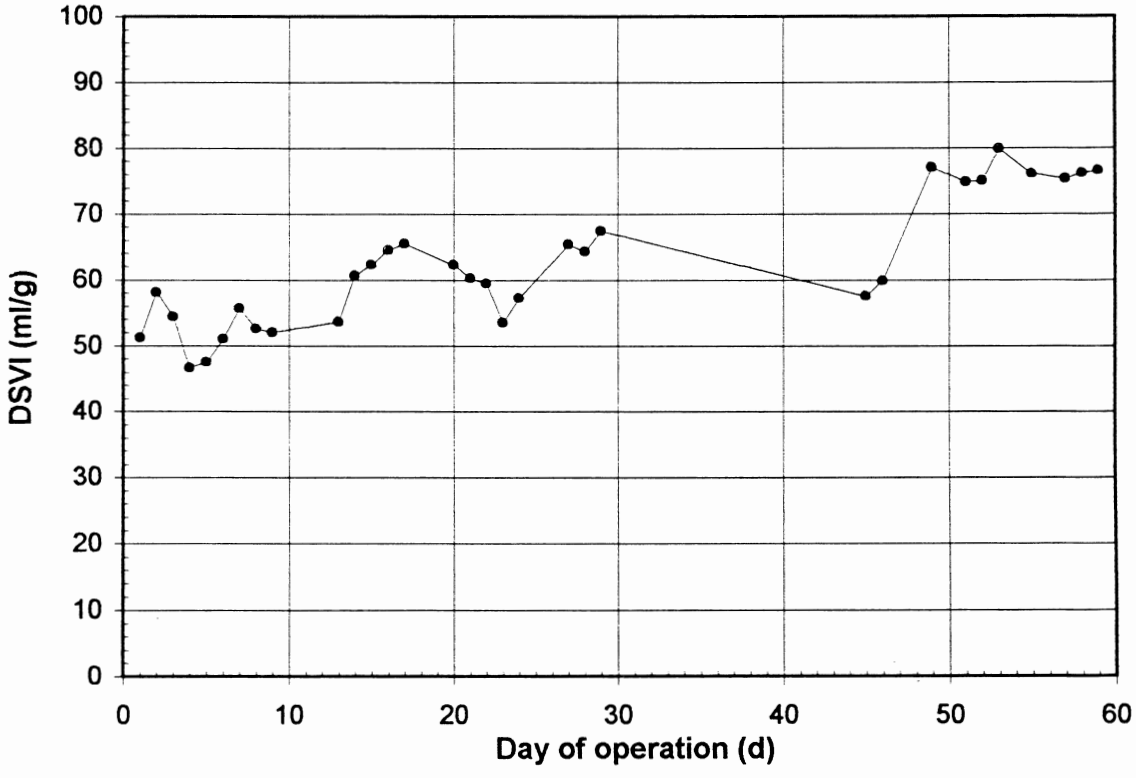
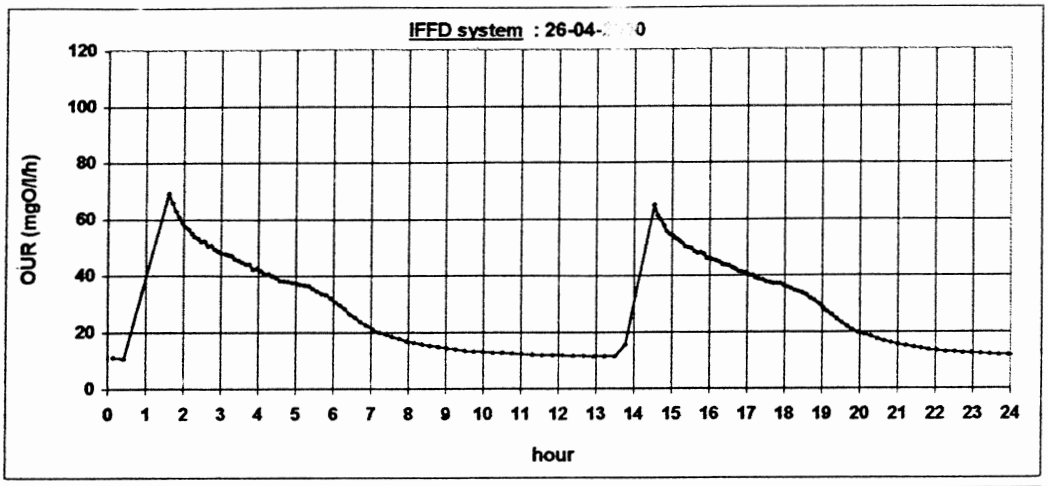
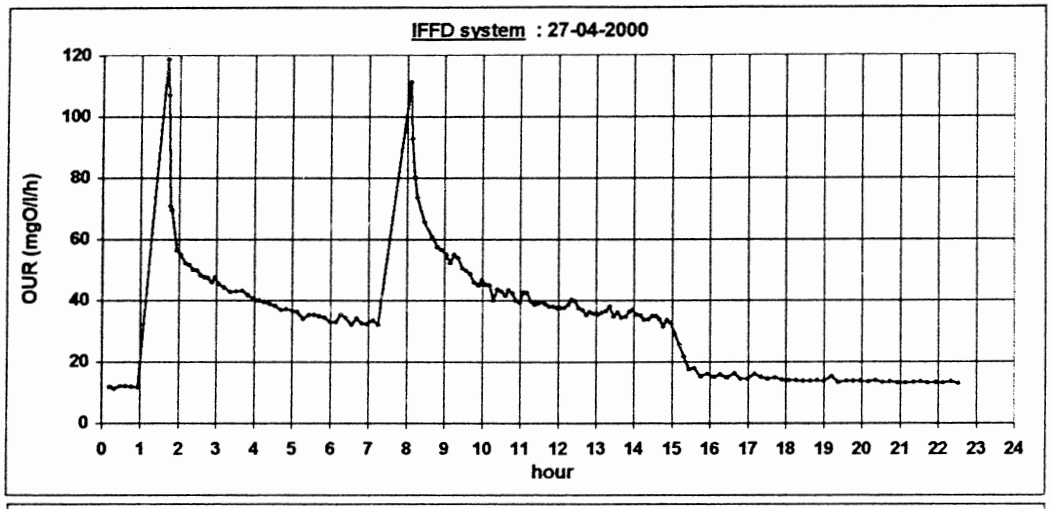
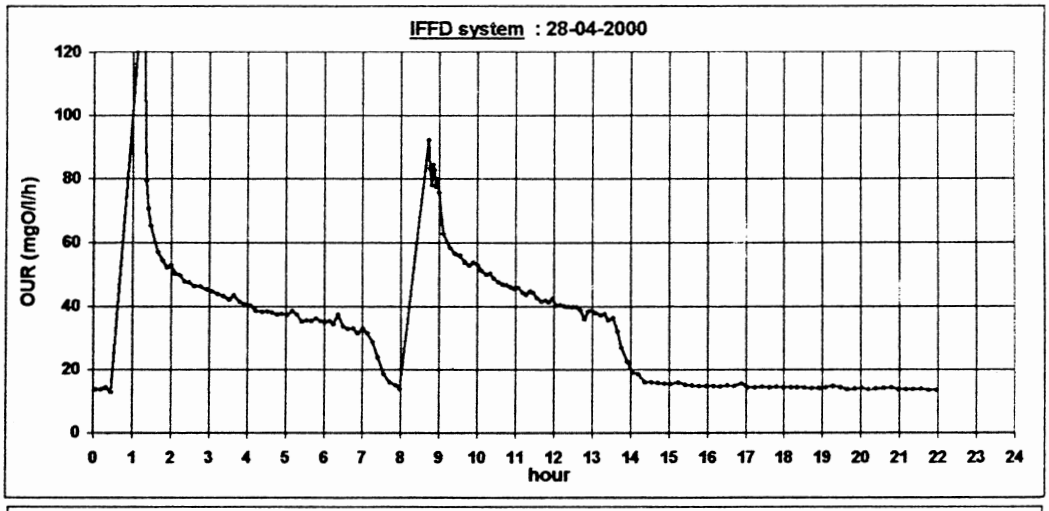
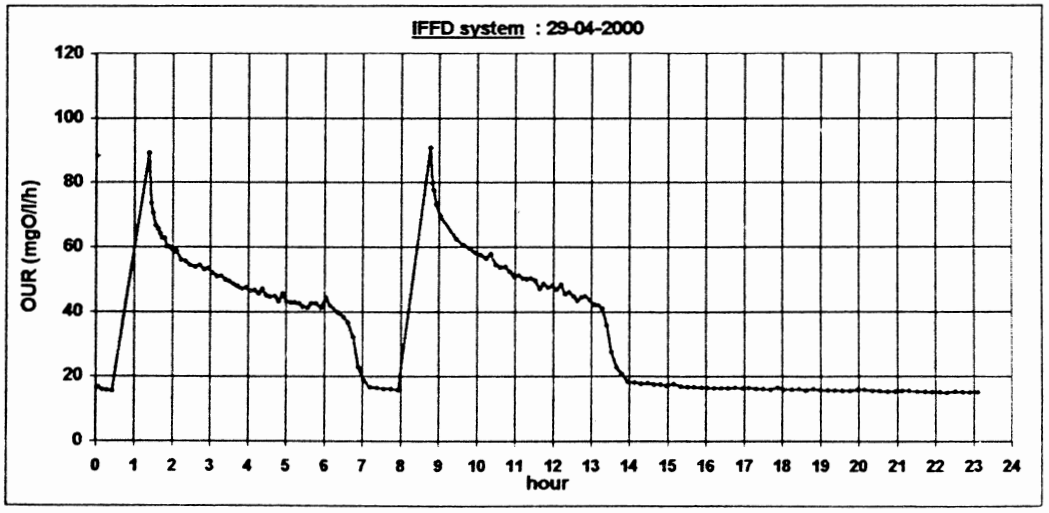


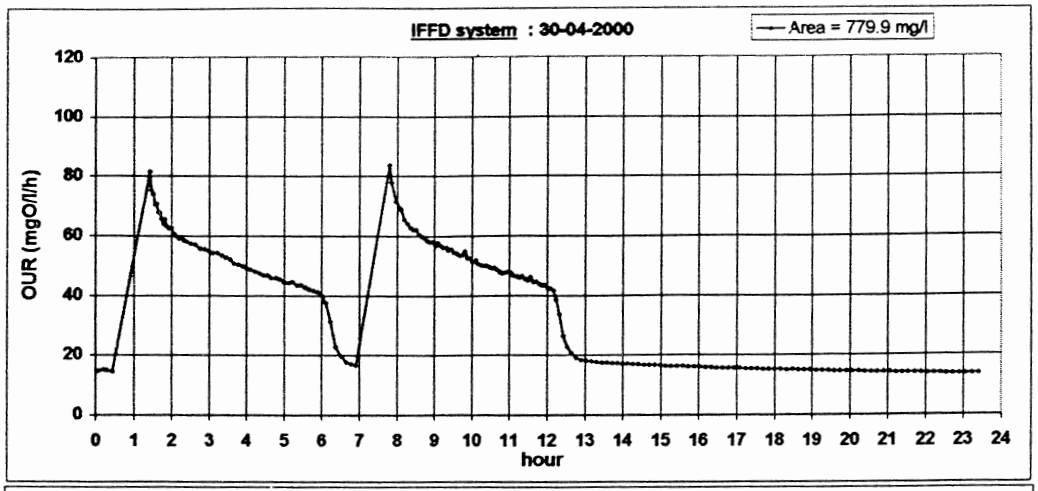
Figure D5.5: Graphical representation of the daily Diluted Sludge Volume Index (DSVI) variation for the parent laboratory-scale IFFD system.

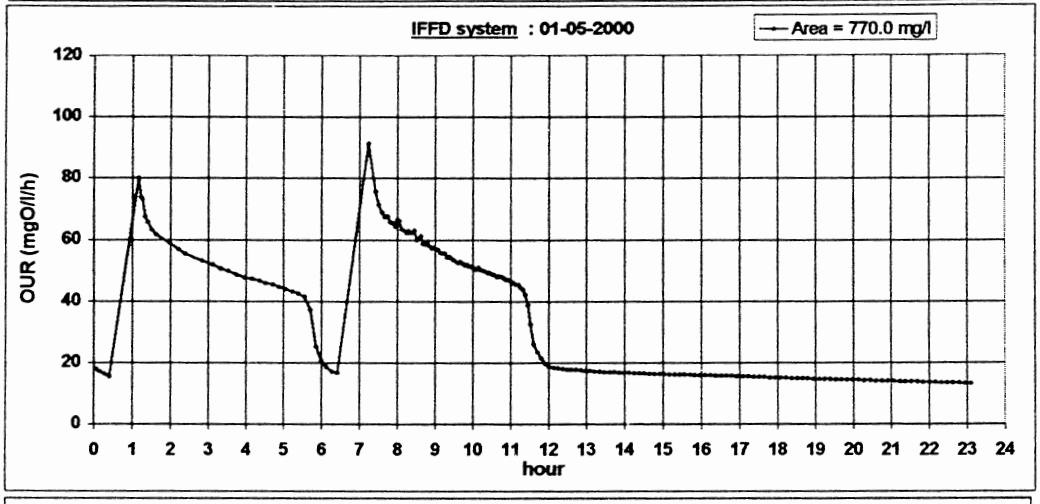

IFFD system : 26-04-2000
OUR (mgO/l/h)
hour

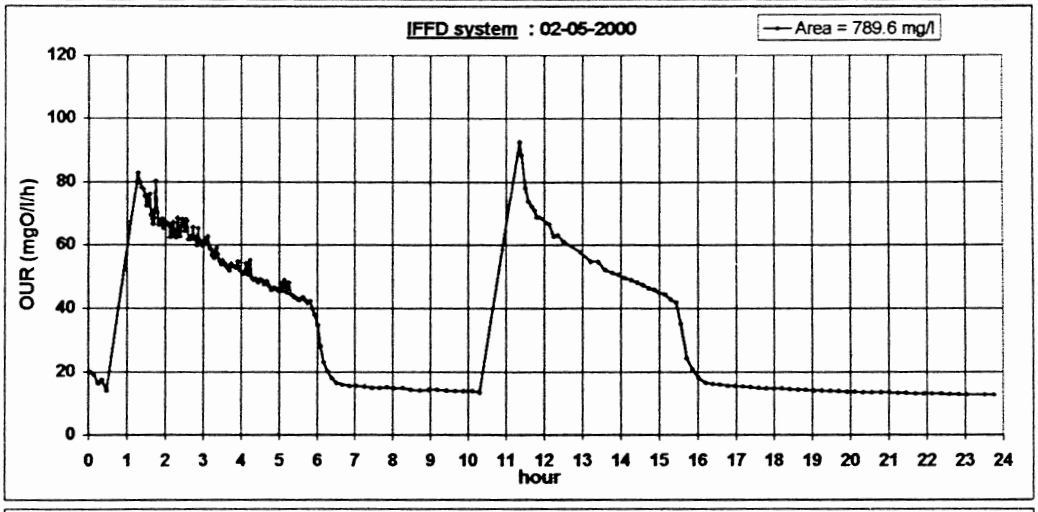

IFFD system : 27-04-2000
OUR (mgO/l/h)
hour

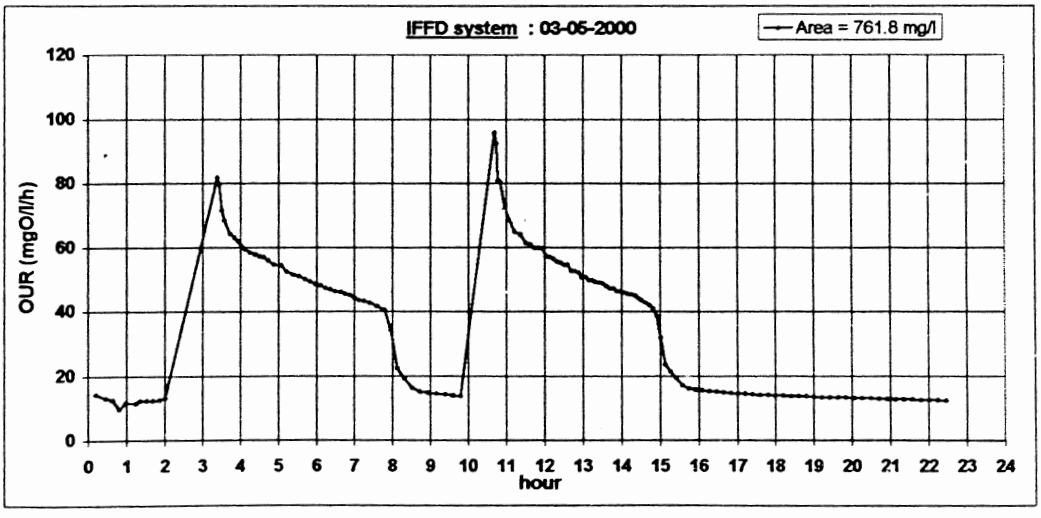

IFFD system : 28-04-2000
OUR (mgO/l/h)
hour

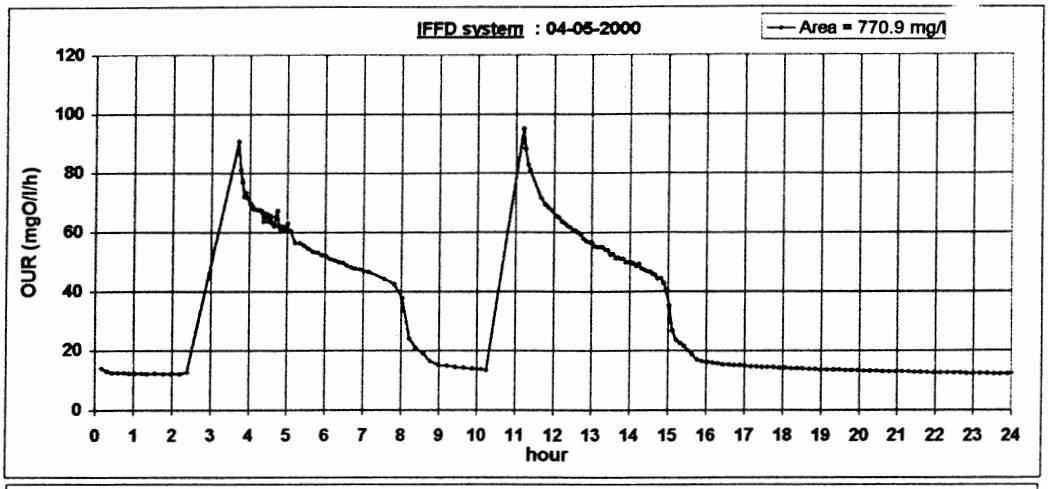

IFFD system : 29-04-2000
OUR (mgO/l/h)
hour

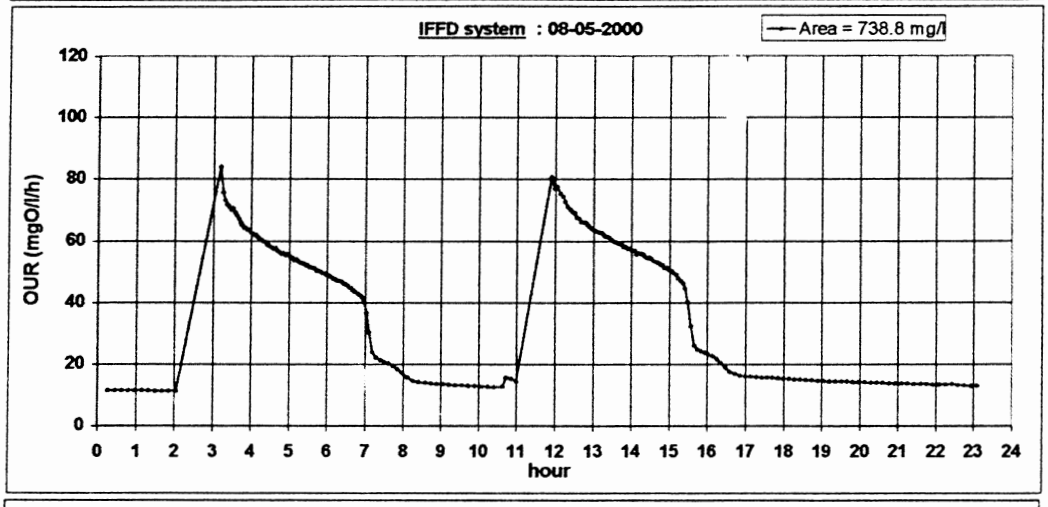

IFFD system : 30-04-2000
Area = 779.9 mg/l
OUR (mgO/l/h)
hour

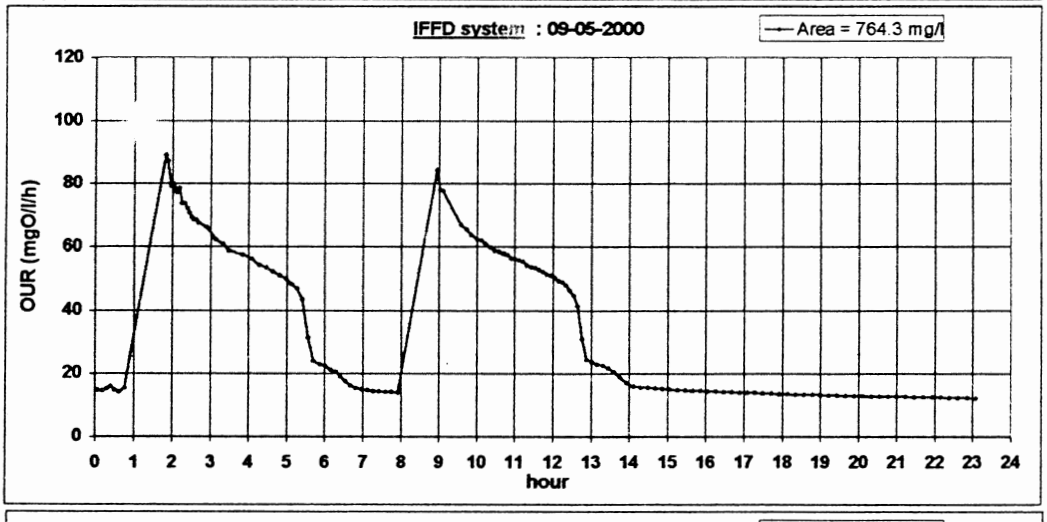

IFFD system : 01-05-2000
Area = 770.0 mg/l
OUR (mgO/l/h)
hour

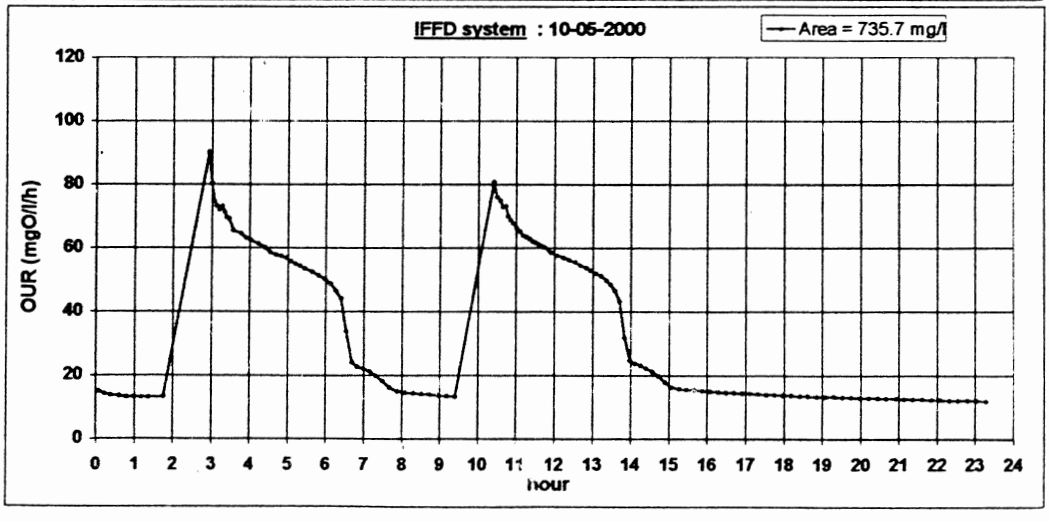

IFFD system : 02-05-2000
Area = 789.6 mg/l
OUR (mgO/l/h)
hour

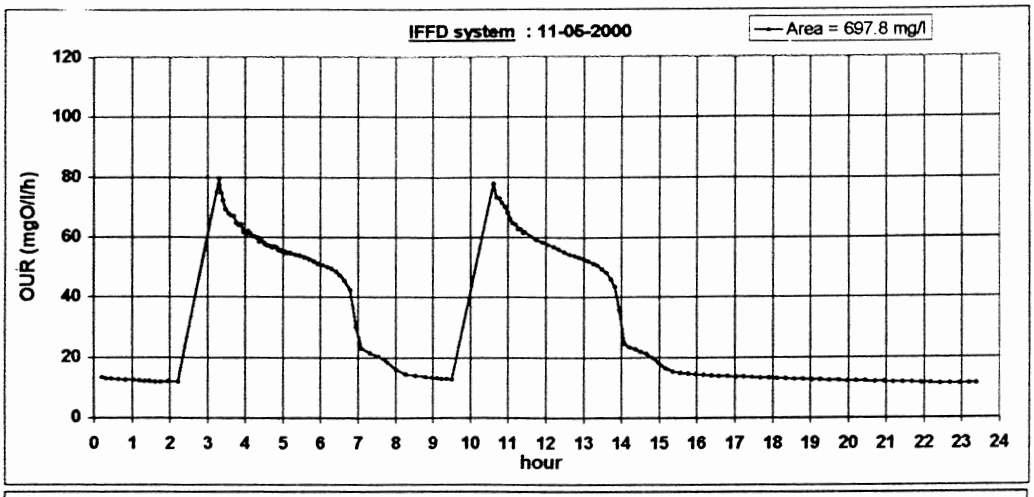

IFFD system : 03-05-2000
Area = 761.8 mg/l
OUR (mgO/l/h)
hour

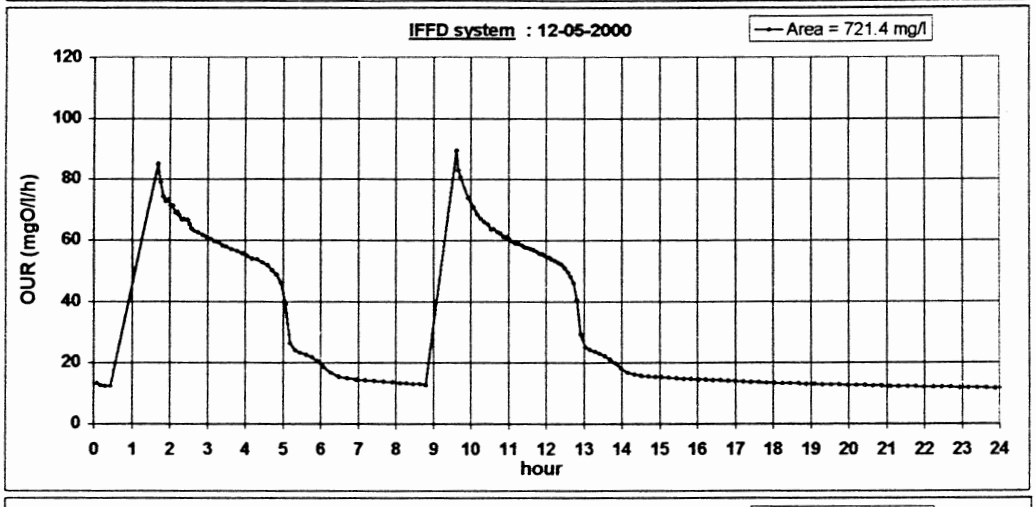

IFFD system : 04-05-2000
Area = 770.9 mg/l
OUR (mgO/l/h)
hour

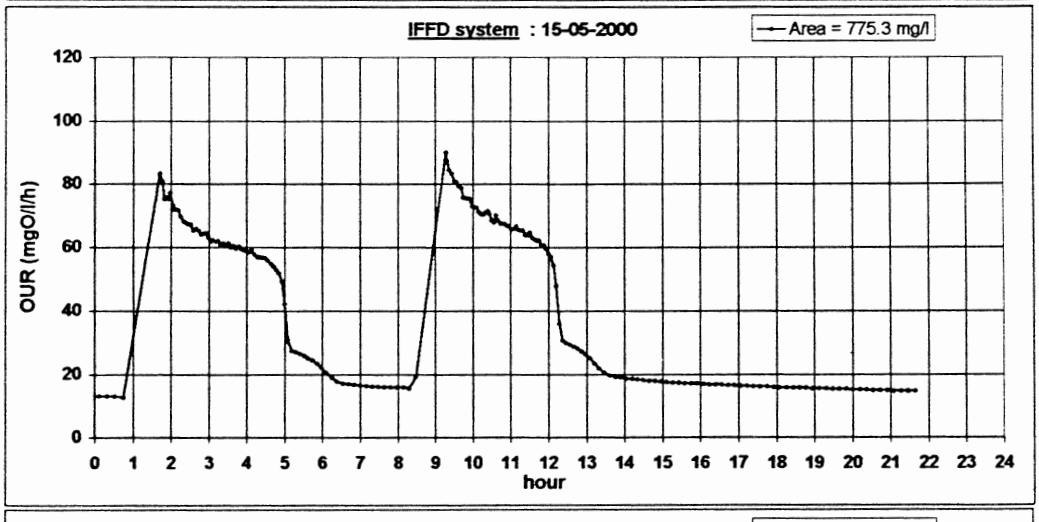

IFFD system : 08-05-2000
Area = 738.8 mg/l
OUR (mgO/l/h)
hour

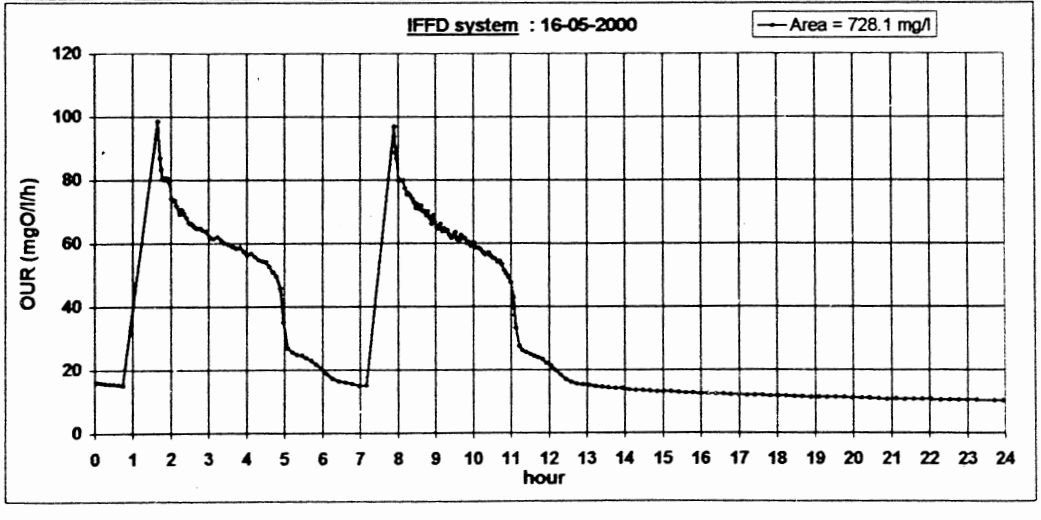

IFFD system : 09-05-2000
Area = 764.3 mg/l
OUR (mgO/l/h)
hour

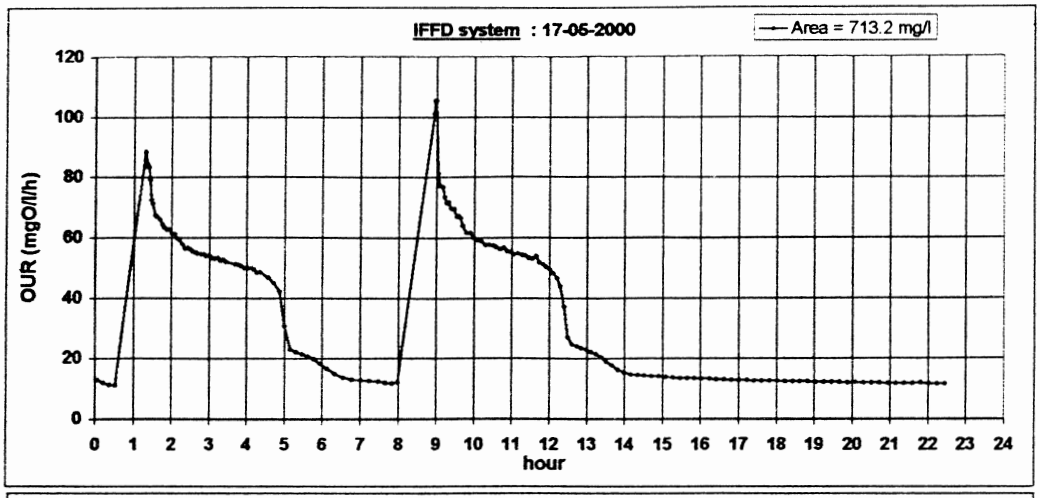

IFFD system : 10-05-2000
Area = 735.7 mg/l
OUR (mgO/l/h)
hour

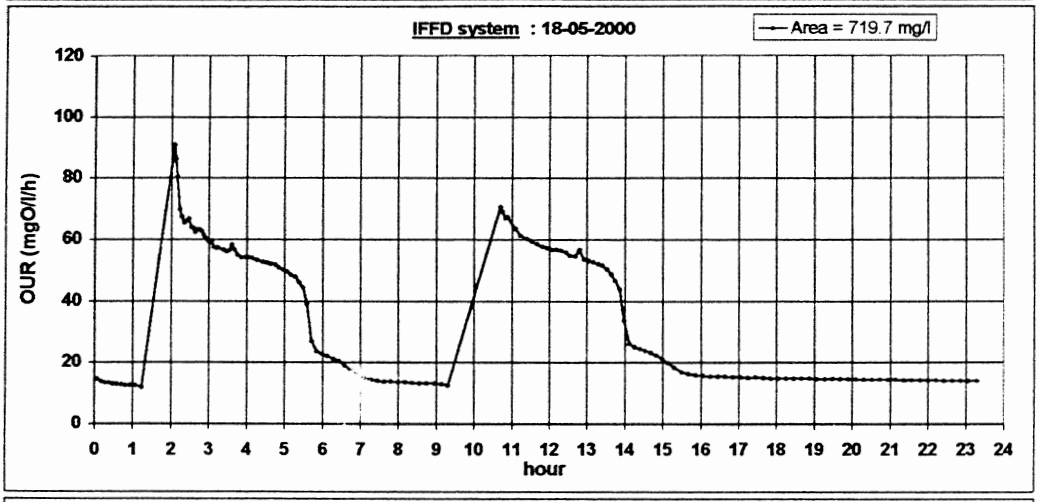

IFFD system : 11-05-2000
Area = 697.8 mg/l
OUR (mgO/l/h)
hour

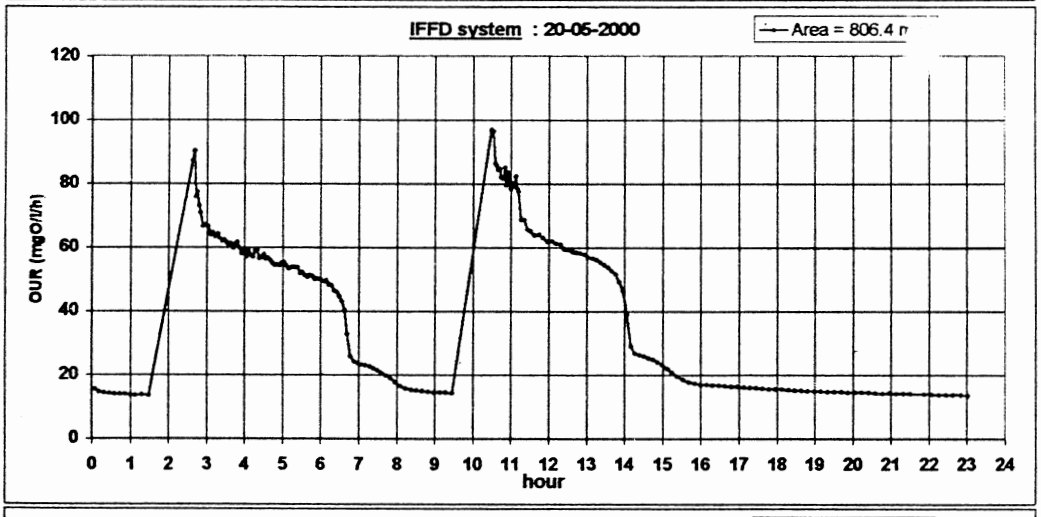

IFFD system : 12-05-2000
Area = 721.4 mg/l
OUR (mgO/l/h)
hour

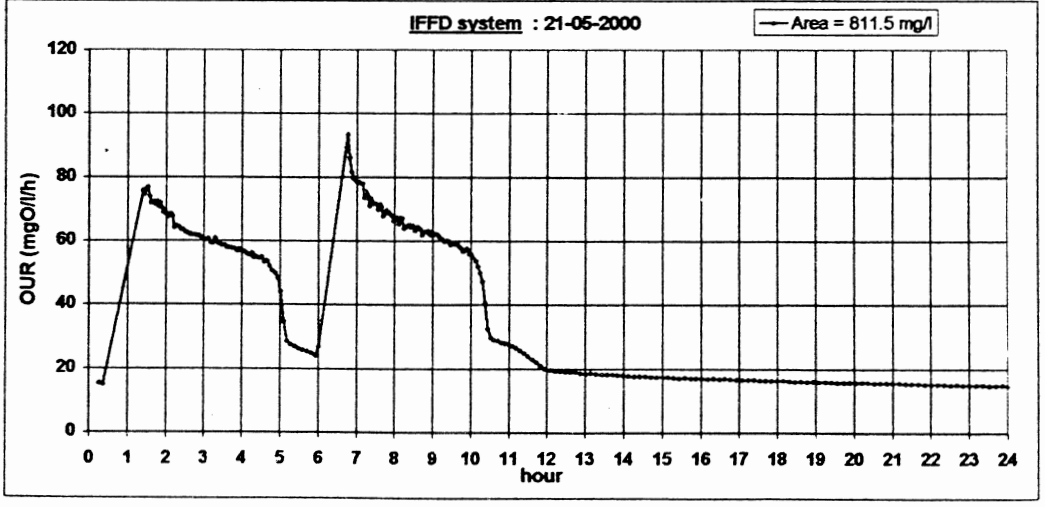

IFFD system : 15-05-2000
Area = 775.3 mg/l
OUR (mgO/l/h)
hour

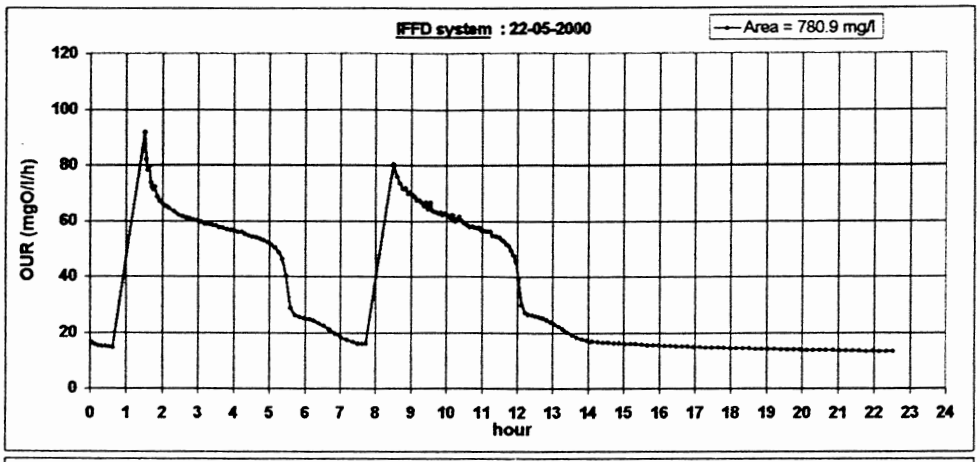

IFFD system : 16-05-2000
Area = 728.1 mg/l
OUR (mgO/l/h)
hour

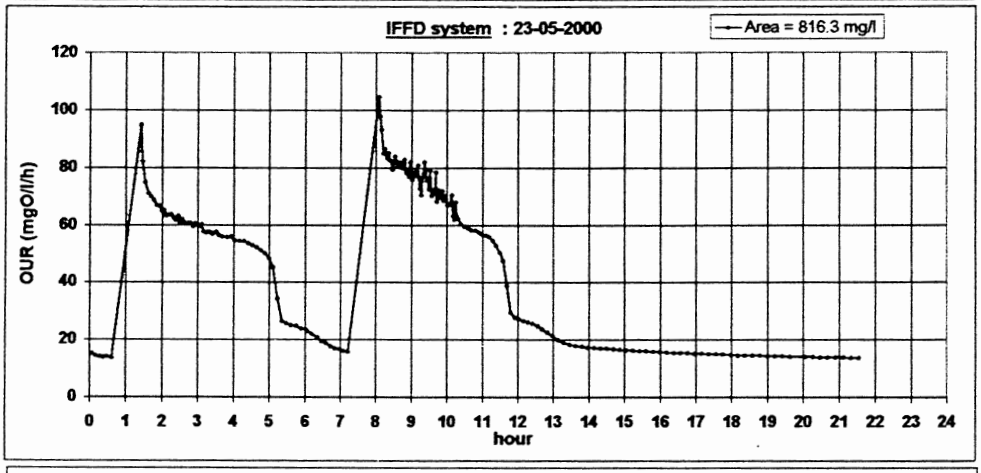

IFFD system : 17-05-2000
Area = 713.2 mg/l
OUR (mgO/l/h)
hour

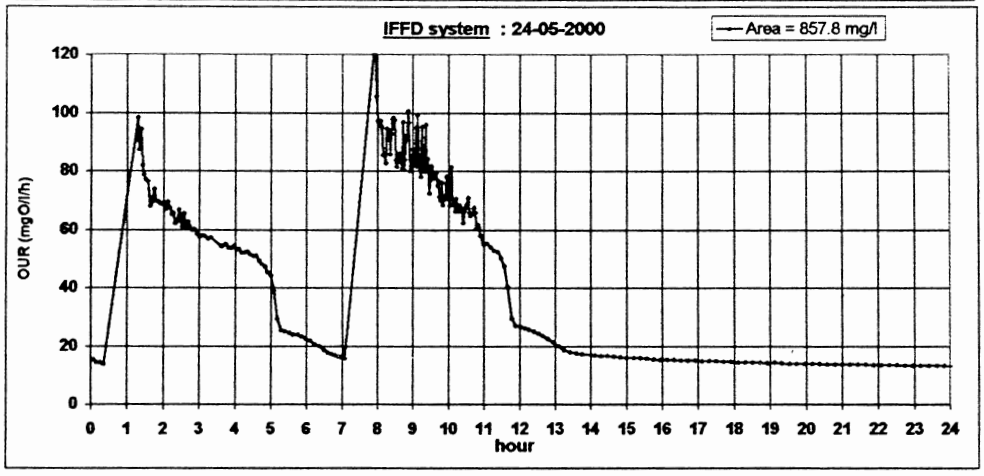

IFFD system : 18-05-2000
Area = 719.7 mg/l
OUR (mgO/l/h)
hour

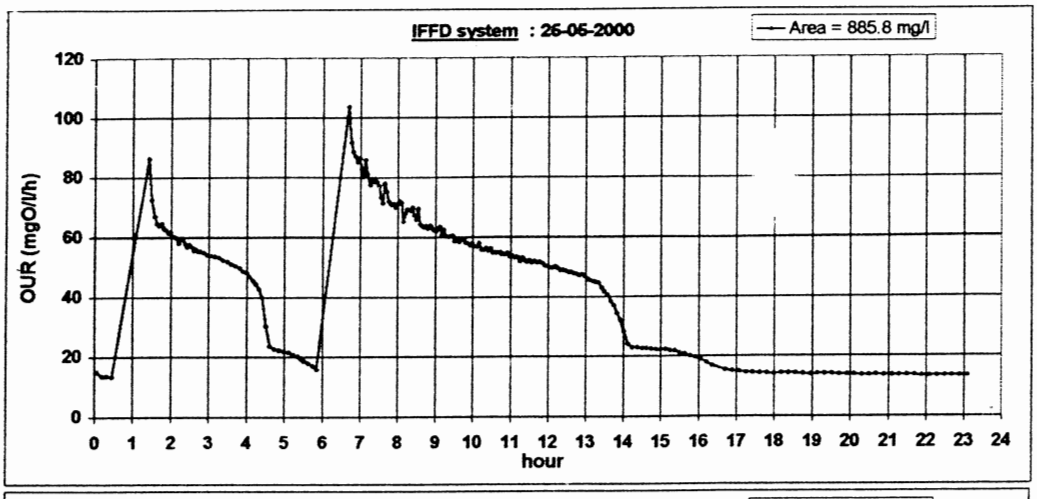

IFFD system : 20-05-2000
Area = 806.4 m
OUR (mgO/l/h)
hour

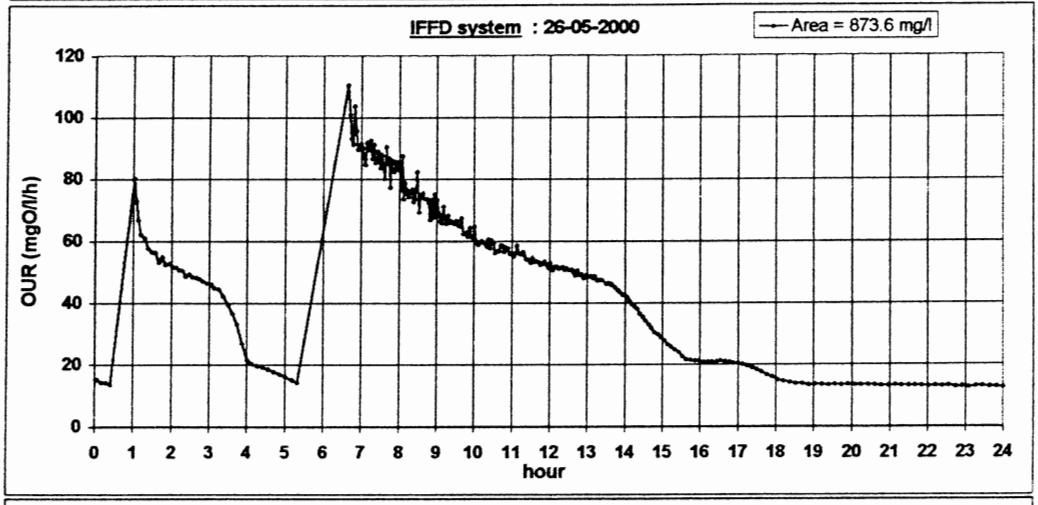

IFFD system : 21-05-2000
Area = 811.5 mg/l
OUR (mgO/l/h)
hour

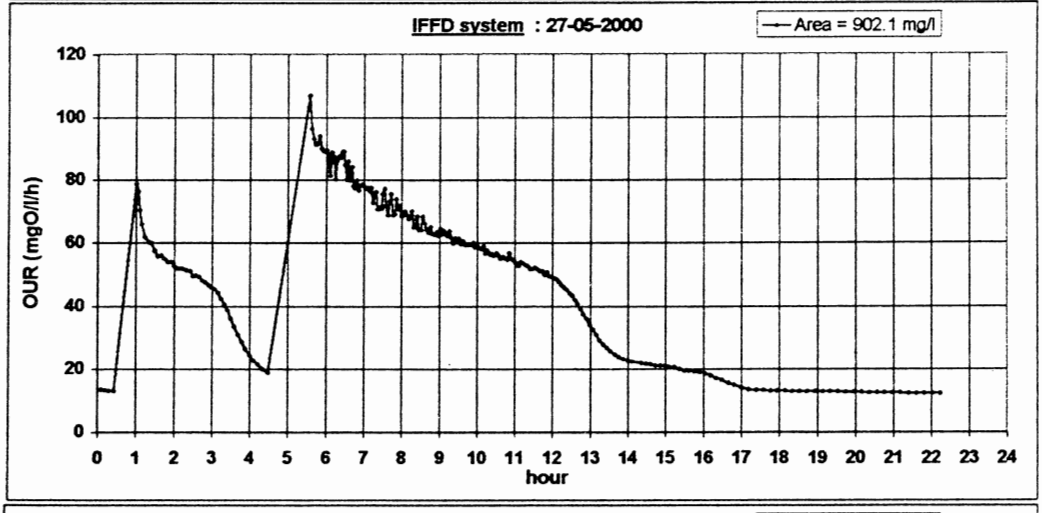

IFFD system : 22-05-2000
Area = 780.9 mg/l
OUR (mgO/l/h)
hour

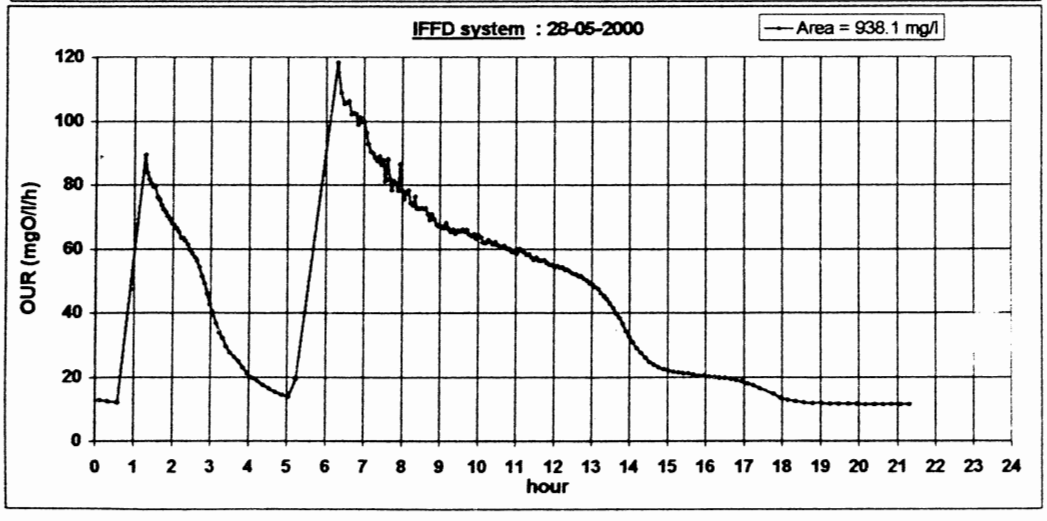

IFFD system : 23-05-2000
Area = 816.3 mg/l
OUR (mgO/l/h)
hour

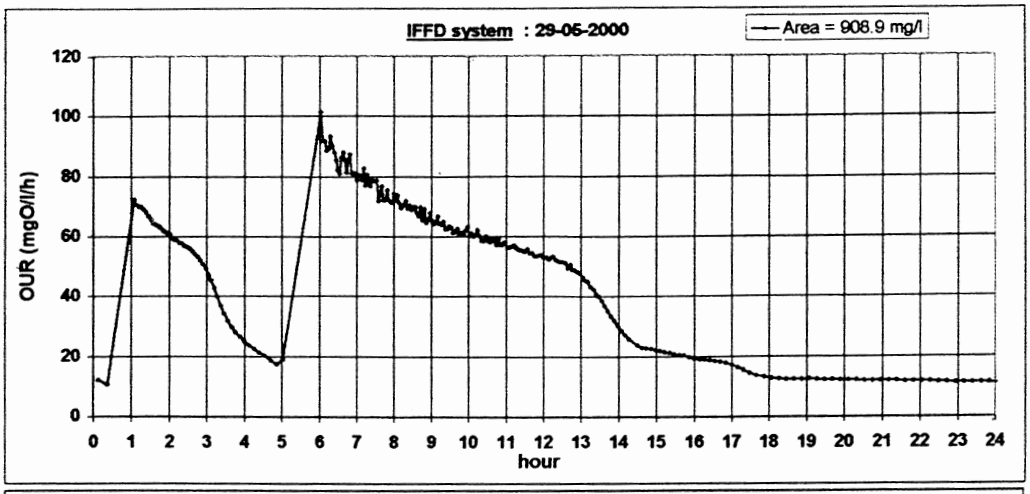

IFFD system : 24-05-2000
Area = 857.8 mg/l
OUR (mgO/l/h)
hour

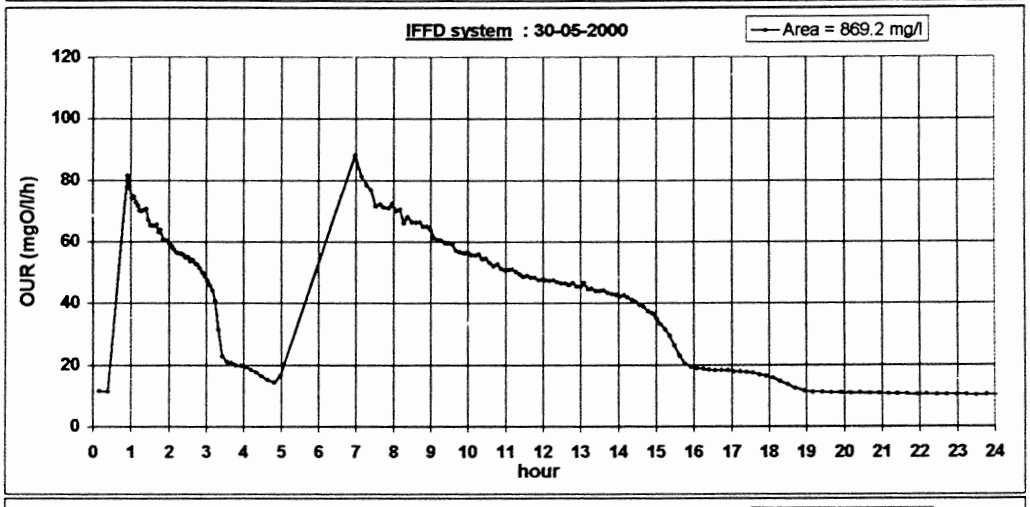

IFFD system : 26-05-2000
Area = 885.8 mg/l
OUR (mgO/l/h)
hour

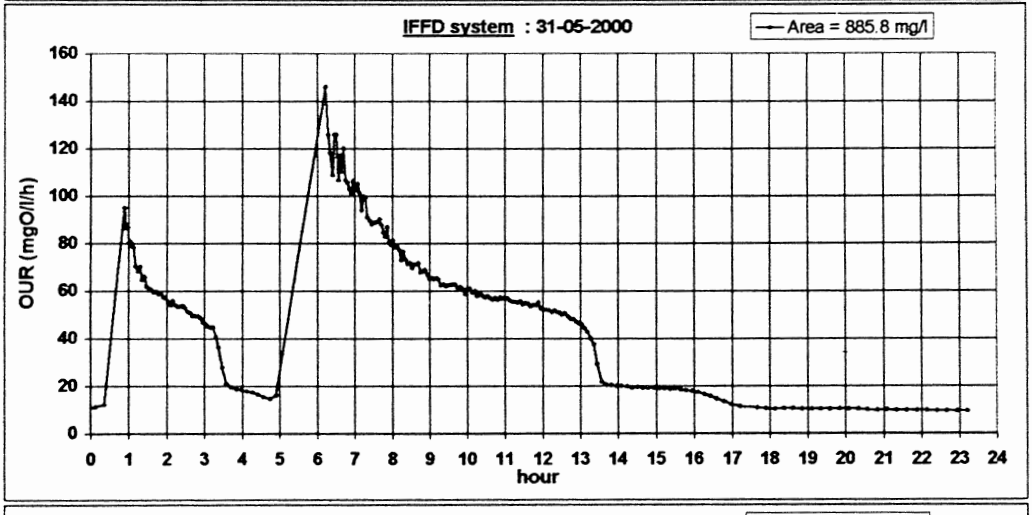

IFFD system : 26-05-2000
Area = 873.6 mg/l
OUR (mgO/l/h)
hour

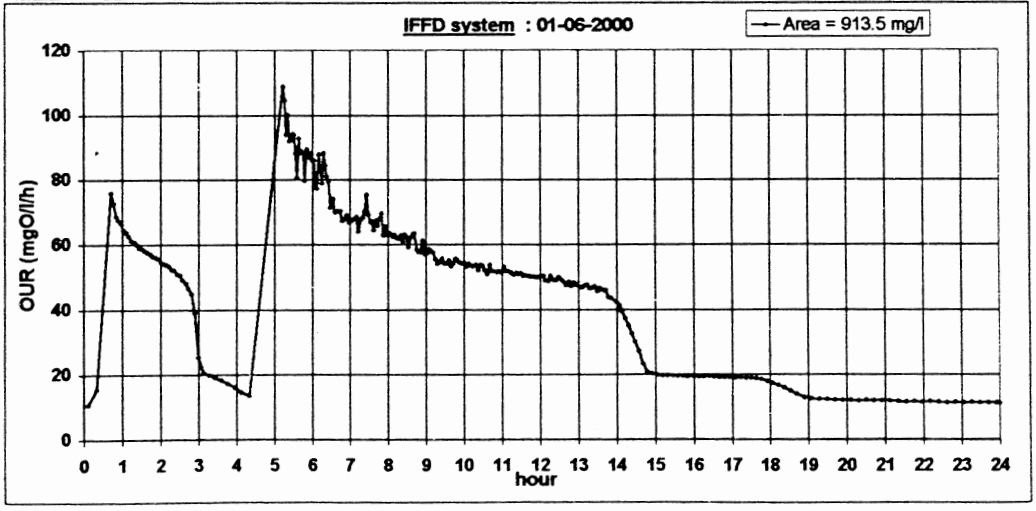

IFFD system : 27-05-2000
Area = 902.1 mg/l
OUR (mgO/l/h)
hour

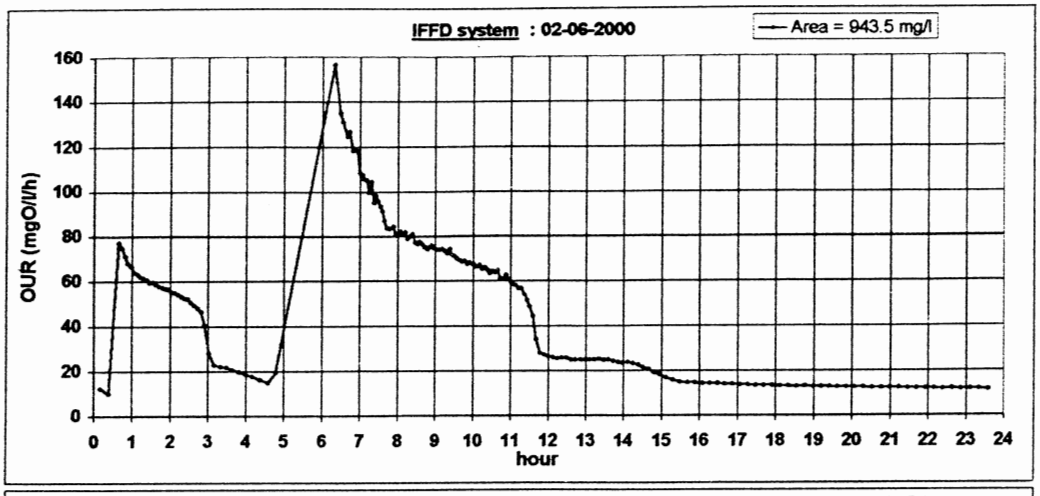

IFFD system : 28-05-2000
Area = 938.1 mg/l
OUR (mgO/l/h)
hour

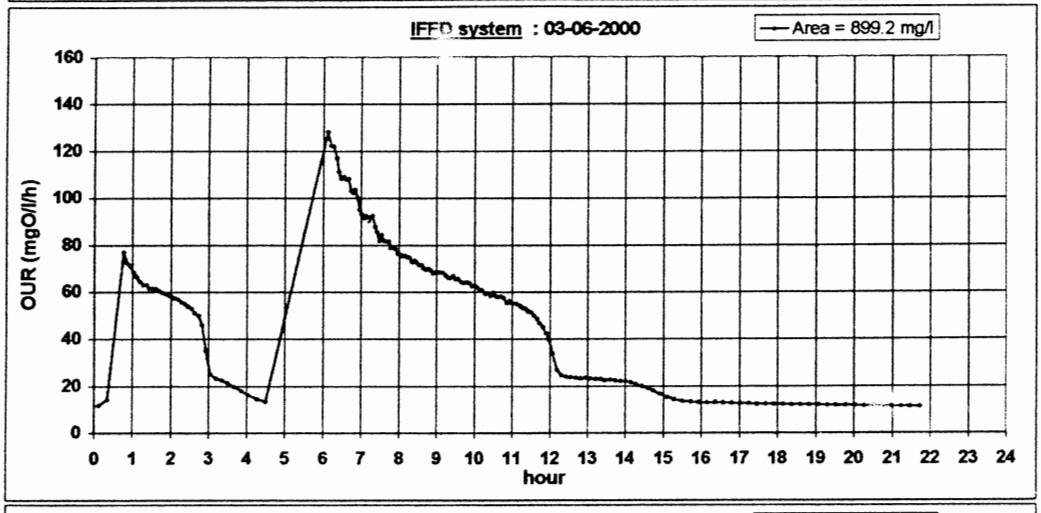

IFFD system : 29-05-2000
Area = 908.9 mg/l
OUR (mgO/l/h)
hour

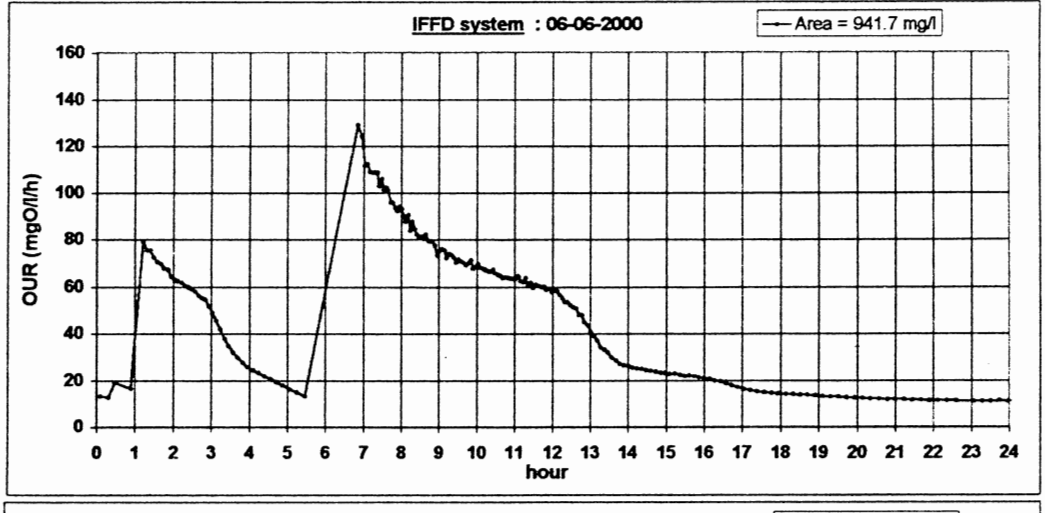

IFFD system : 30-05-2000
Area = 869.2 mg/l
OUR (mgO/l/h)
hour

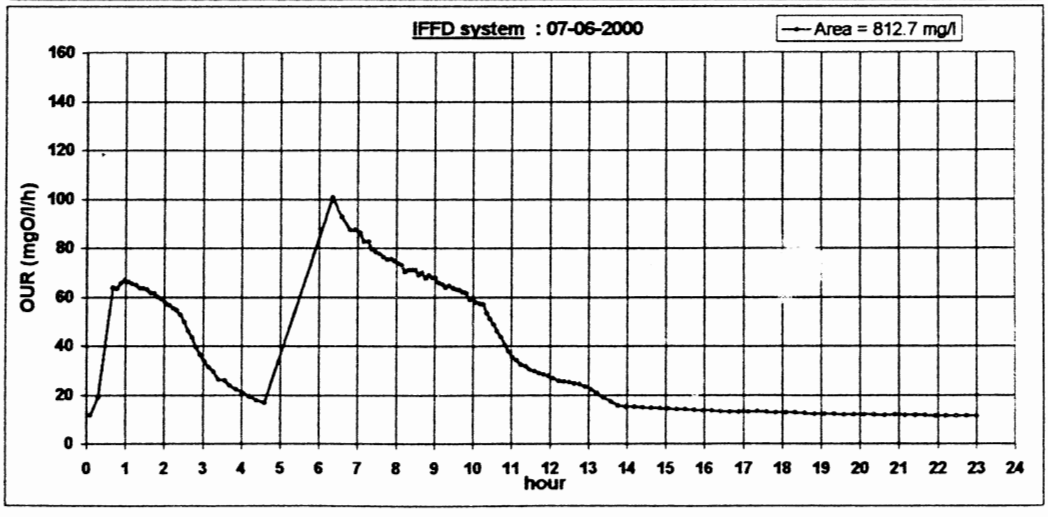

IFFD system : 31-05-2000
Area = 885.8 mg/l
OUR (mgO/l/h)
hour

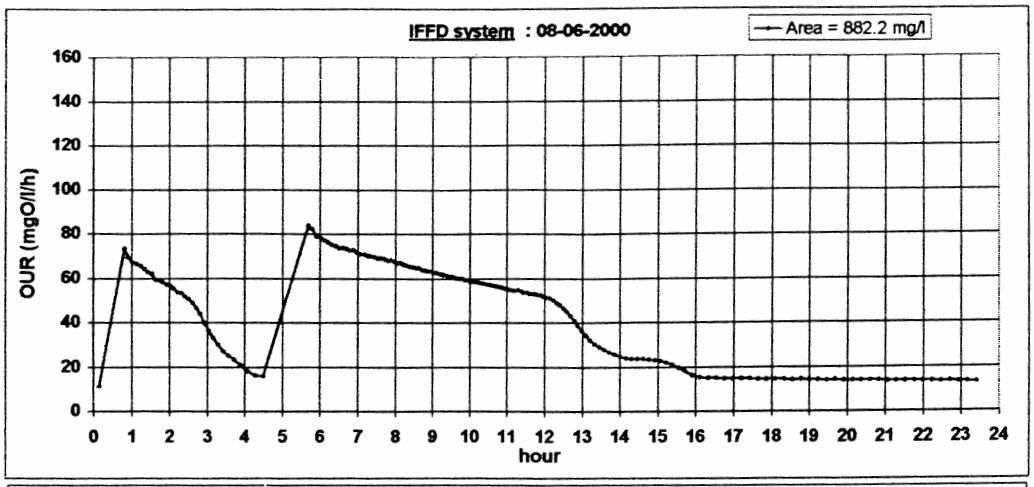

IFFD system : 01-06-2000
Area = 913.5 mg/l
OUR (mgO/l/h)
hour

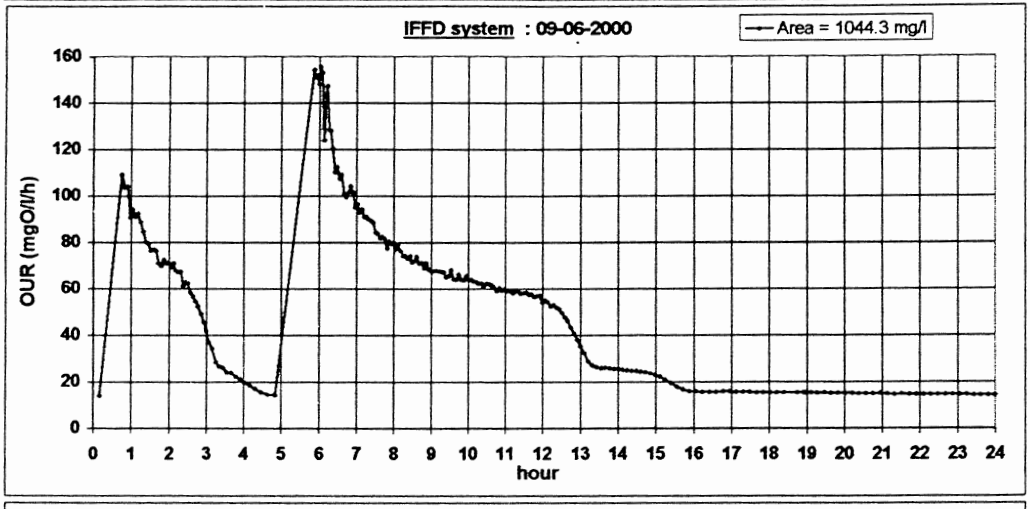

IFFD system : 02-06-2000
Area = 943.5 mg/l
OUR (mgO/l/h)
hour

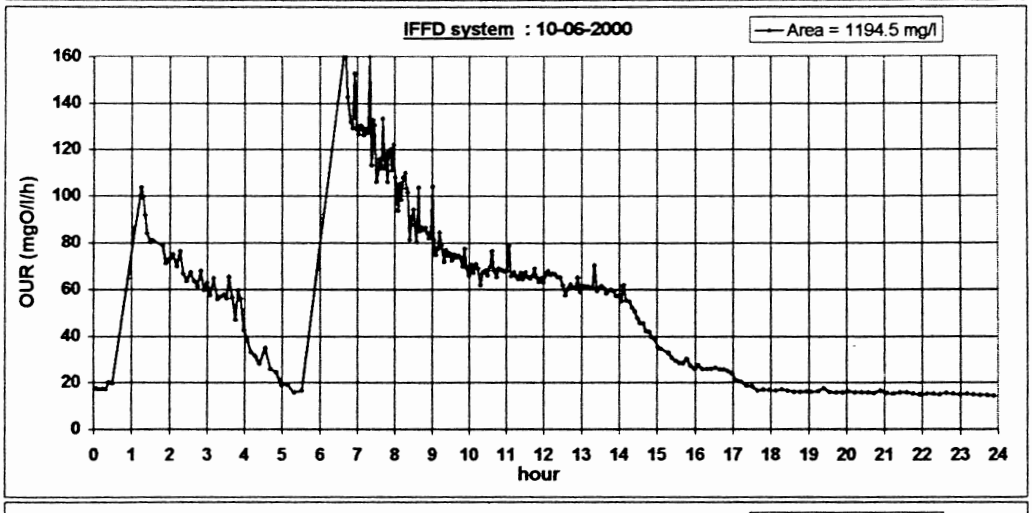

IFFD system : 03-06-2000
Area = 899.2 mg/l
OUR (mgO/l/h)
hour

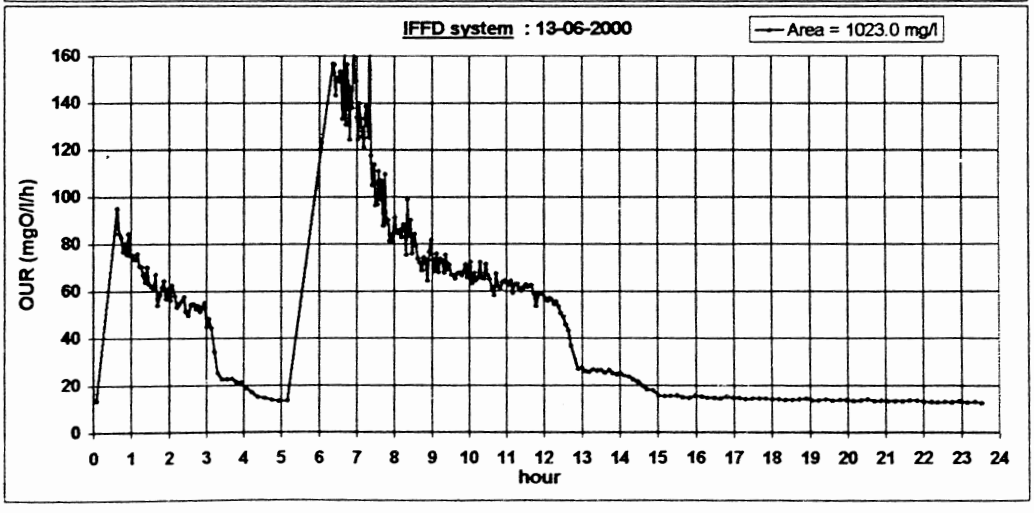

IFFD system : 06-06-2000
Area = 941.7 mg/l
OUR (mgO/l/h)
hour

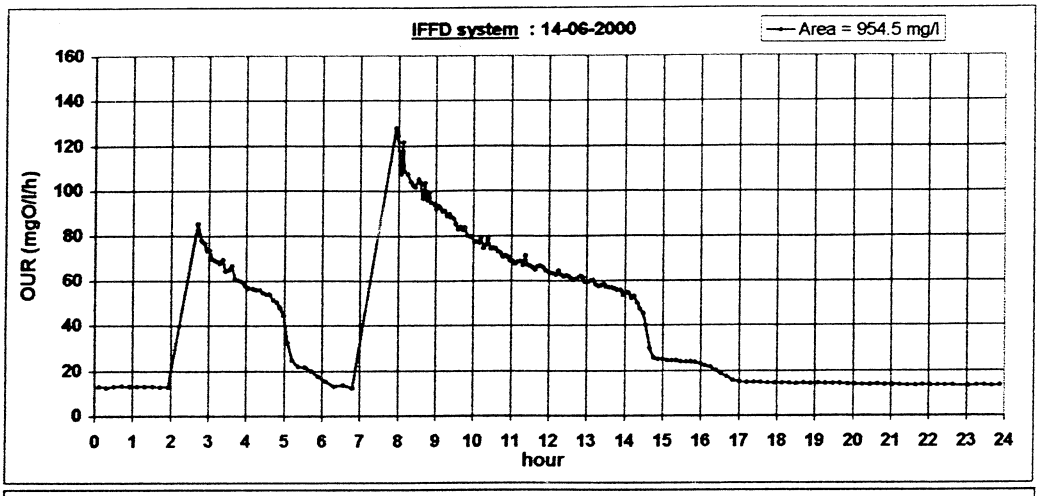

IFFD system : 07-06-2000
Area = 812.7 mg/l
OUR (mgO/l/h)
hour

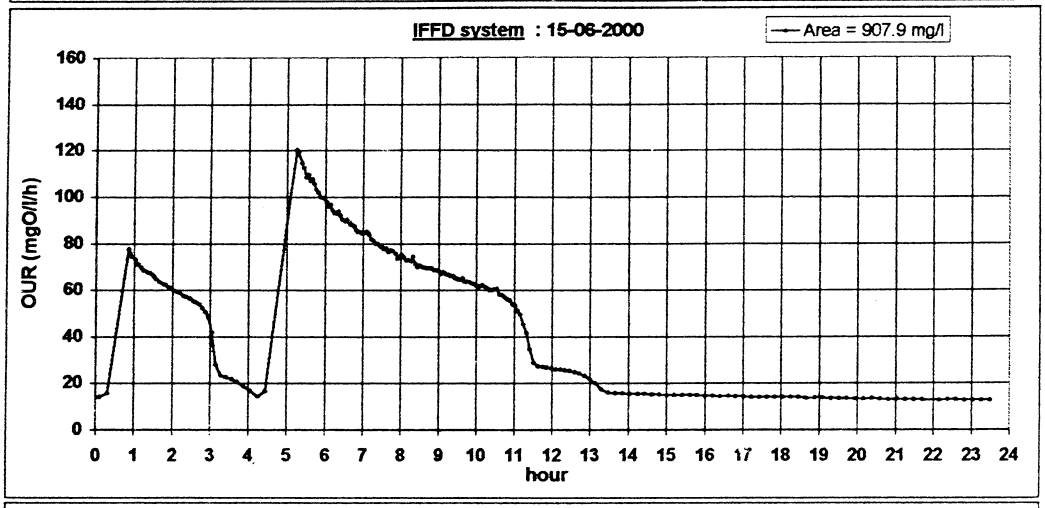

IFFD system : 08-06-2000
Area = 882.2 mg/l
OUR (mgO/l/h)
hour

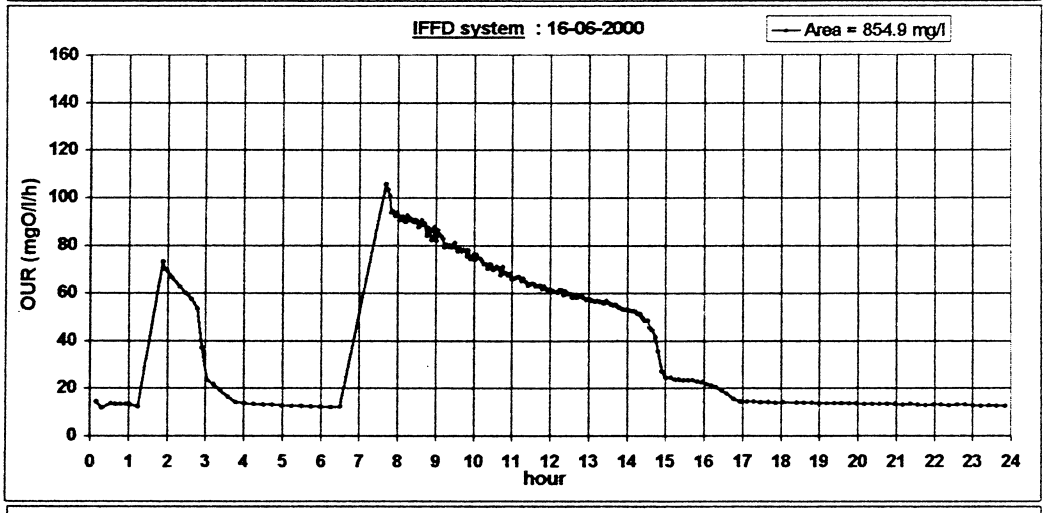

IFFD system : 09-06-2000
Area = 1044.3 mg/l
OUR (mgO/l/h)
hour

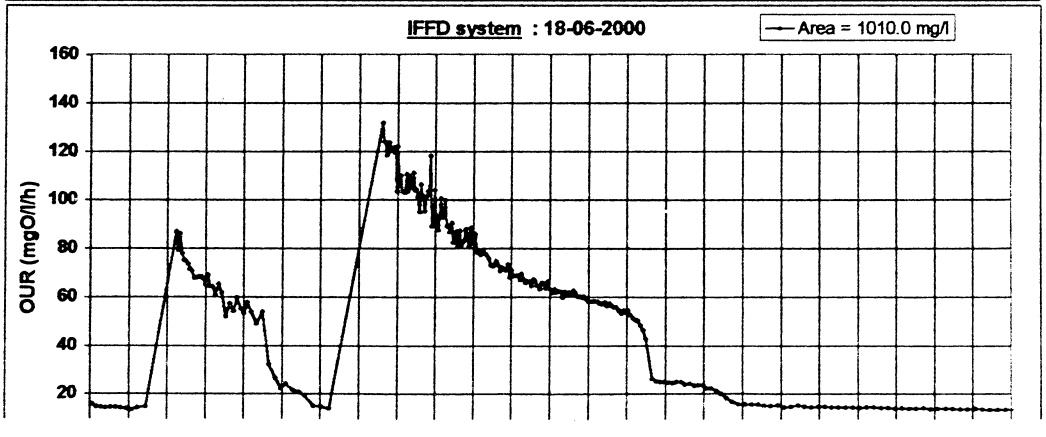

IFFD system : 10-06-2000
Area = 1194.5 mg/l
OUR (mgO/l/h)
hour

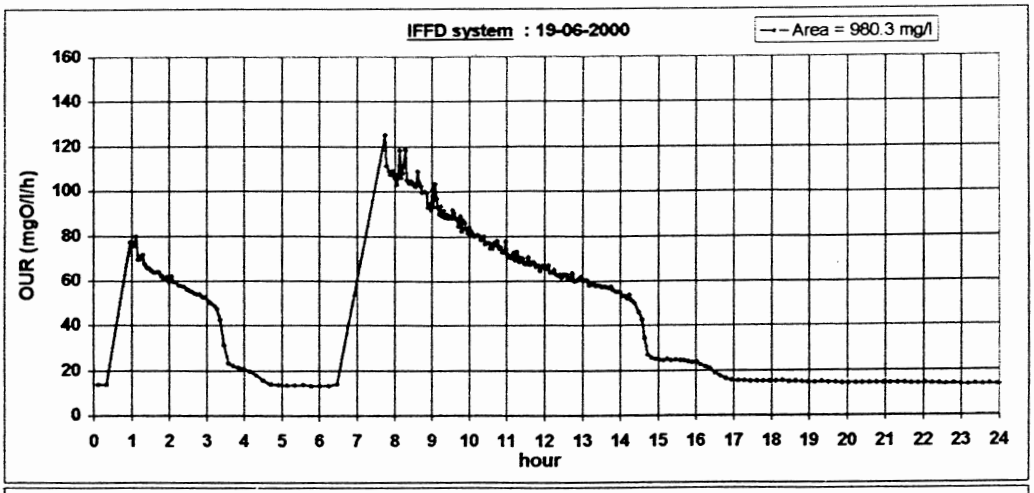

IFFD system : 13-06-2000
Area = 1023.0 mg/l
OUR (mgO/l/h)
hour

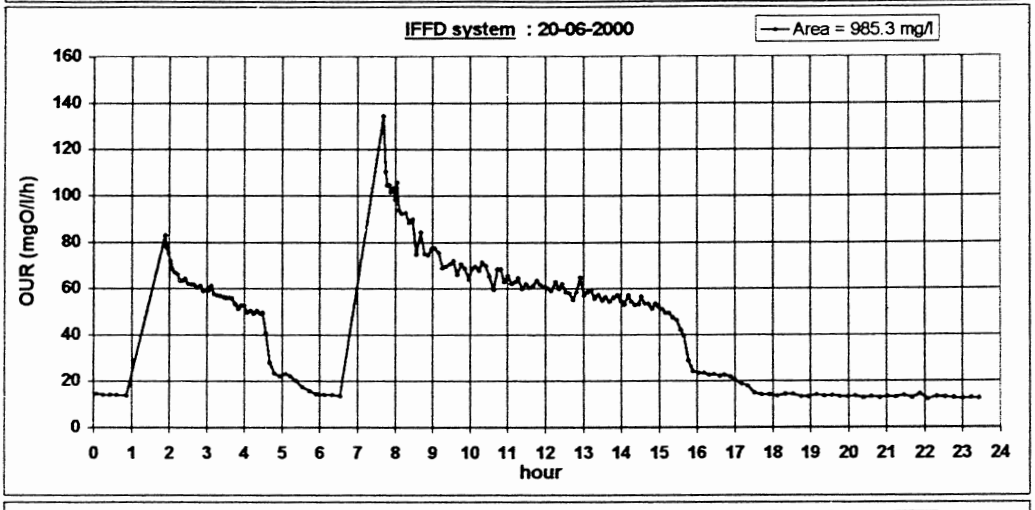

IFFD system : 14-06-2000
Area = 954.5 mg/l
OUR (mgO/l/h)
hour

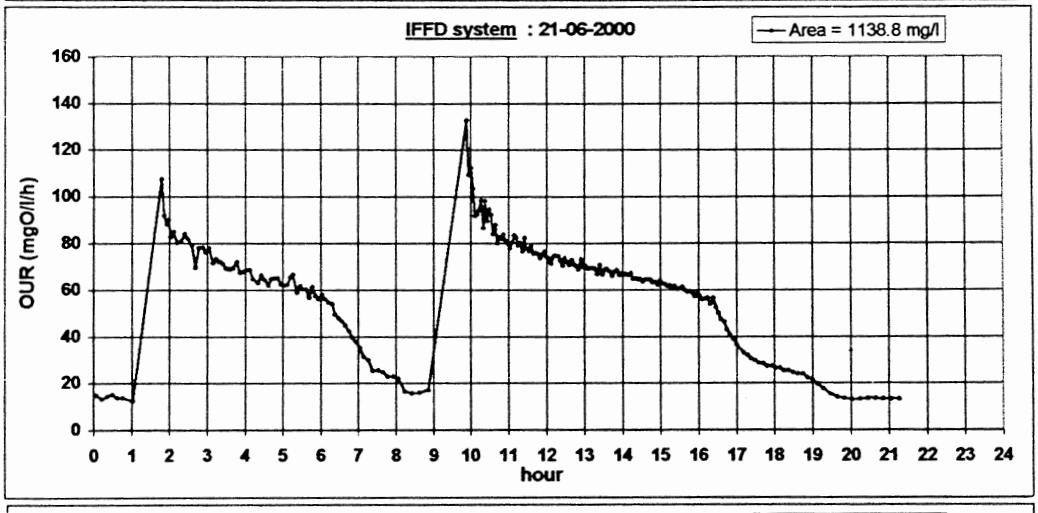

IFFD system : 15-06-2000
Area = 907.9 mg/l
OUR (mgO/l/h)
hour

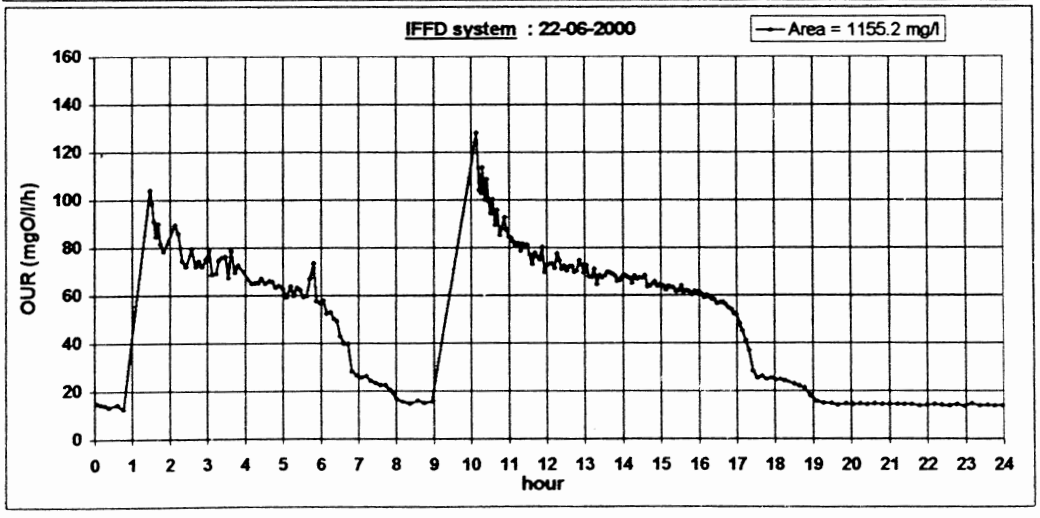

IFFD system : 16-06-2000
Area = 854.9 mg/l
OUR (mgO/l/h)
hour

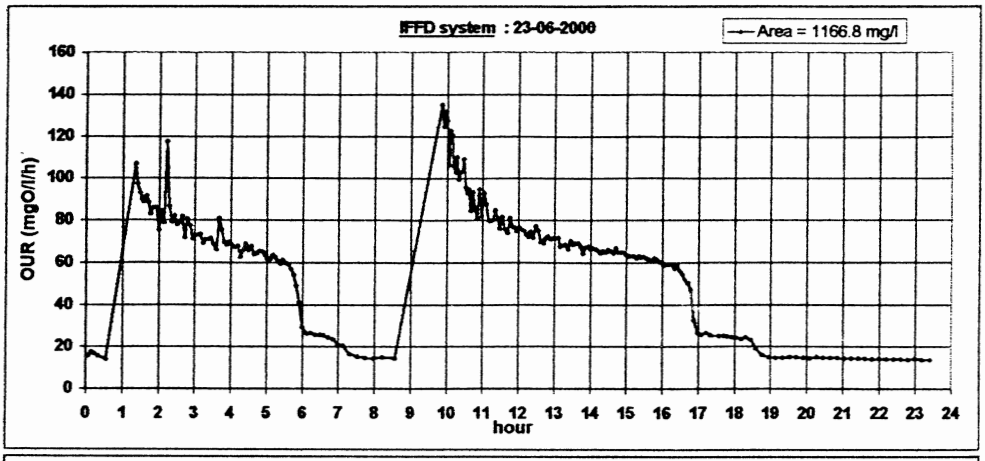

IFFD system : 18-06-2000
Area = 1010.0 mg/l
OUR (mgO/l/h)

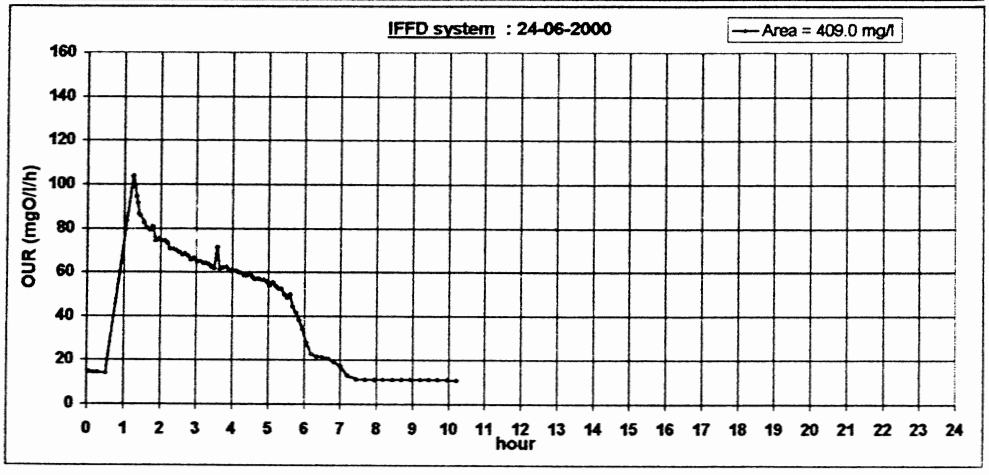

IFFD system : 19-06-2000
Area = 980.3 mg/l
OUR (mgO/l/h)
hour


IFFD system : 20-06-2000
Area = 985.3 mg/l
OUR (mgO/l/h)
hour


IFFD system : 21-06-2000
Area = 1138.8 mg/l
OUR (mgO/l/h)
hour


IFFD system : 22-06-2000
Area = 1155.2 mg/l
OUR (mgO/l/h)
hour


IFFD system : 23-06-2000
Area = 1166.8 mg/l
OUR (mgO/l/h)
hour


IFFD system : 24-06-2000
Area = 409.0 mg/l
OUR (mgO/l/h)
hour

APPENDIX D6

CONSTRUCTION OF THE EXPECTED OUR RESPONSE

The OUR response shown in Fig 6.2 (Chapter 6) was obtained during the steady state period when the IFFD parent system was receiving sewage batch 25. From the steady state results for sewage batch 25, values for the following parameters were calculated and are relevant to the construction of the *expected* OUR curve:

- From the $f_{S,us}$ and $f_{S,up}$ values (Table 6.4), the mean influent COD and mixed liquor VSS concentration (Table 6.3) for sewage batch 25, the theoretical heterotrophic active biomass concentration in the parent system was calculated as Z_{BH} = 1423 mgCOD/ℓ.

- From the mass balance calculations (see section 6.6.2, Chapter 6) for sewage batch 25, the following values for the mass of oxygen demand were obtained:

  - Mass of Total oxygen demand MOt = 7375 mgO/d
  - Mass of carbonaceous oxygen demand MOc = 4968 mgO/d
  - Mass of nitrification oxygen demand MOc = 2408 mgO/d

Oxygen demand due to endogenous respiration (Plateau 4)

From Fig 6.2 the mean endogenous respiration oxygen demand was taken as:

$$OUR_{(ENDOG.)} = 13 \text{ mgO}/\ell/\text{h}$$

In terms of the daily oxygen demand : $MOc_{(ENDOG.)} = 13 \text{ mgO}/\ell/\text{h} \cdot 10\ell \cdot 24\text{h}$

$$MOc_{(ENDOG.)} = 3120 \text{ mgO/d}$$

Oxygen demand due to SBCOD utilization (Plateau 3)

From Fig 6.2 the mean oxygen demand due to SBCOD utilization was taken as:

$$OUR_{(SBCOD)} = 13 \text{ mgO}/\ell/\text{h}$$

From Fig 6.2 it is evident that the SBCOD was depleted after ± 4.8 hours and in terms of the daily oxygen demand : $MOc_{(SBCOD)} = 13 \text{ mgO}/\ell/\text{h} \cdot 10\ell \cdot 4.8\text{h} \cdot 2$ feeding cycles

$$MOc_{(SBCOD)} = 1248 \text{ mgO/d}$$

Oxygen demand due to nitrification (Plateau 2)

From the COD mass balance, the daily oxygen demand due to nitrification was calculated as MOn = 2408 mgO/d, or in terms of each feeding cycle:

$$MOn = 1204 \text{ mgO/cycle}$$

In Fig 6.2 nitrification ceased after ± 3.8 hours, hence OUR_N = 1204 mgO / (3.8h • 10ℓ)

$$OUR_N = 32 \text{ mgO/ℓ/h}$$

Oxygen demand due to RBCOD utilization (Plateau 1)

Because the experimental OUR response in Fig 6.2 did not display the characteristic IFFD system OUR response for RBCOD utilization (described in section 6.7, Chapter 6), the relevant parameters had to be estimated as follows:

From the batch tests conducted on the IFFD system mixed liquor samples (see Chapter 7) the mean maximum specific growth rate on RBCOD (μ_H) was calculated as μ_H = 1.35/d. In terms of the UCT kinetic model, the simplified formulation for the OUR due to the RBCOD utilization is given by Eq (3.5):

$$OUR_{(RBCOD)} = \frac{1-Y_{ZH}}{Y_{ZH}} (\mu_H) Z_{BH} / 24 \qquad (mgO/ℓ/h)$$

Substituting Y_{ZH} = 0.666 mgCOD/mgCOD, μ_H = 1.35/d and Z_{BH} = 1423 mgCOD/ℓ yields:

$$OUR_{(RBCOD)} = 40 \text{ mgO/ℓ/h}$$

The oxygen demand due to RBCOD utilization [$MOc_{(RBCOD)}$] was calculated from the COD mass balance for sewage batch 25:

$$MOc_{(RBCOD)} = MOc_{(TOTAL)} - MOc_{(ENDO.)} - MOc_{(SBCOD)}$$

$$MOc_{(RBCOD)} = 4967 - 3120 - 1248 = 600 \text{ mgO/d}$$

For each feeding cycle, per ℓ reactor volume : $MOc_{(RBCOD)}$ = (600 / 2 cycles) / 10ℓ

$$MOc_{(RBCOD)} = 30 \text{ mgO/ℓ}$$

The time required for RBCOD depletion is expressed in terms of the calculated oxygen utilization rate of 40 mgO/ℓ/h :

$$\underline{\text{time}} = (30 \text{ mgO/ℓ}) / (40 \text{ mgO/ℓ/h}) = \underline{0.75 \text{ h}}$$

Determination of K_{MP}

Knowing the oxygen demand for SBCOD utilization from the observed OUR response in Fig 6.2, enabled an estimate for the value of K_{MP} to be determined through substituting $Y_{ZH} = 0.666$ mgCOD/mgCOD, $Z_{BH} = 1423$ mgCOD/ℓ and $OUR_{(SBCOD)} = 40$ mgO/ℓ/h into Eq (3.5):

$$OUR_{(SBCOD)} = \frac{1-Y_{ZH}}{Y_{ZH}} (K_{MP}) Z_{BH} / 24 \qquad (mgO/\ell/h)$$

Solving for K_{MP} yields $\boxed{K_{MP} = 0.44 /d}$

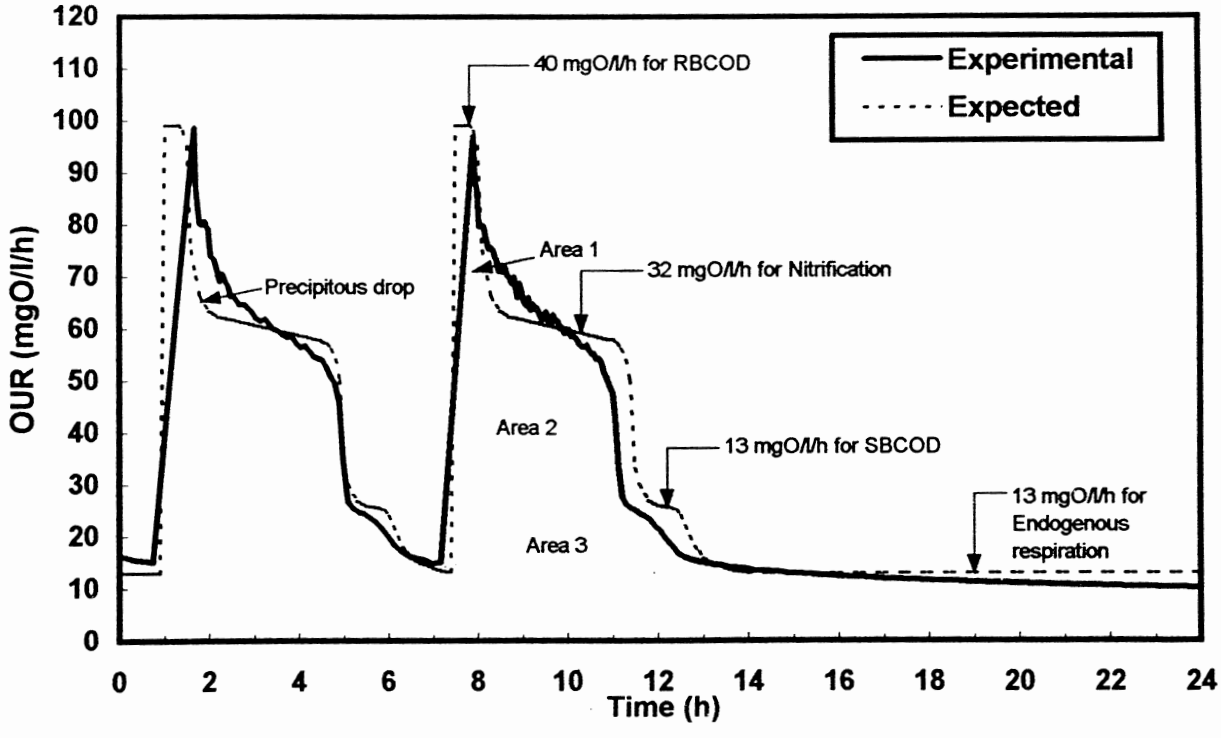


Figure 6.2 : Typical OUR response (16-05-2000) from the intermittently fed fill and draw (IFFD system during a 24 hour period when the system was fed twice daily with equal volumes (5ℓ) of wastewater. Also shown is the theoretically predicted OUR respons

APPENDIX E

EXTENSION TO MODIFIED BATCH TEST INVESTIGATION:

OUR vs TIME PLOTS FOR MODIFIED BATCH TESTS CONDUCTED WITH FILTERED WASTEWATER PLUS IFFD SYSTEM MIXED LIQUOR SAMPLES

TABLE OF CONTENTS

Table E1 — Summary of the data recorded for modified batch tests, conducted with a mixture of filtered wastewater and IFFD system mixed liquor. Mixed liquor sample volumes, calculated COD recovery, maximum specific growth rates and measured heterotrophic active biomass.

Appendix E1 — OUR versus time and ln(OUR) versus time profiles for modified batch tests E1 - E18. The nitrate concentration versus time profiles recorded during each batch test are also given.

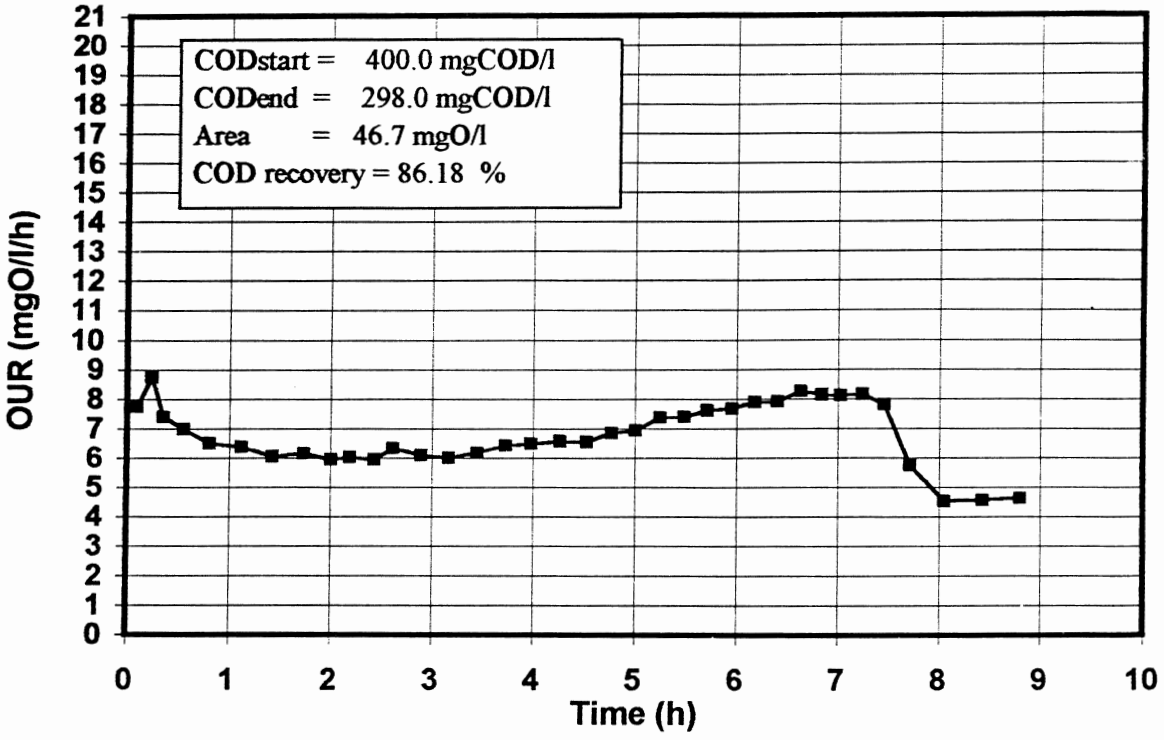
Appendix E2 — OUR versus time profiles for batch tests conducted on 23-05-2000 with a mixture of unfiltered *effluent* and IFFD system mixed liquor samples.

Extension to modified batch test investigation : IFFD system

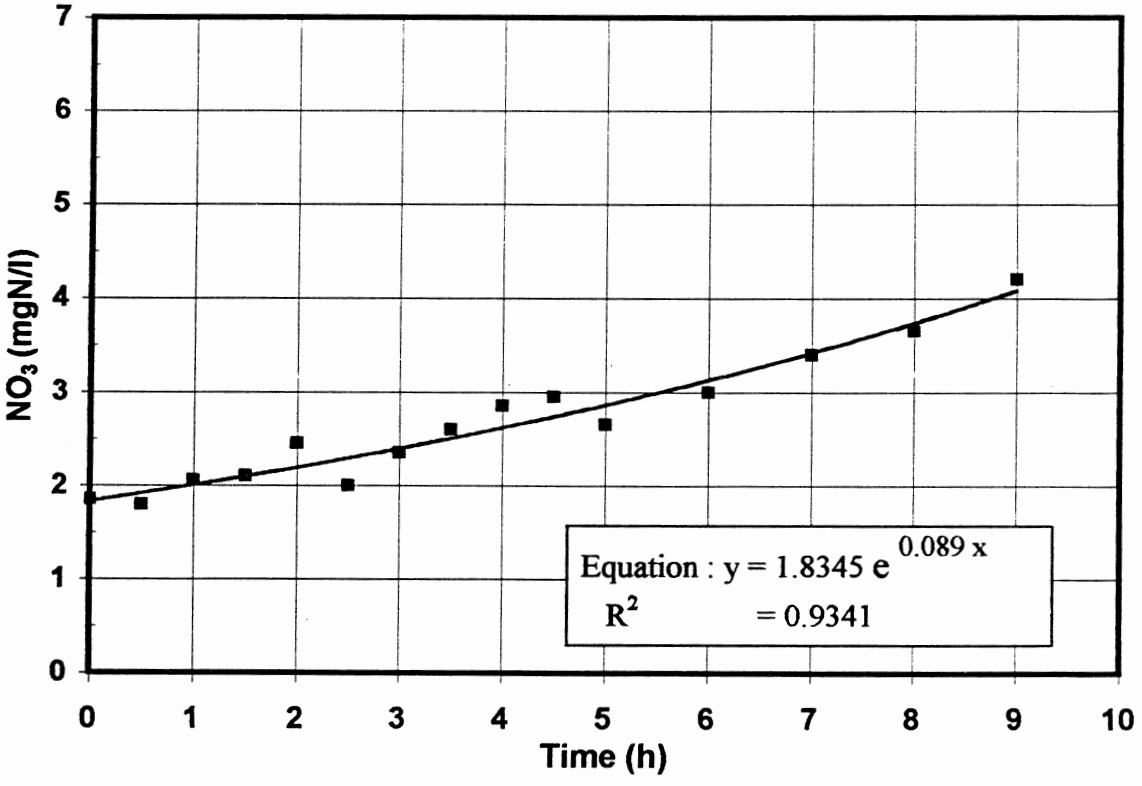
Table E1 : Summarized data for modified batch tests with filtered wastewater and mixed liquor from the intermittently fed fill and draw (IFFD) parent system; mixed liquor volume, COD recovery, maximum specific growth rates and heterotrophic active biomass.

Sew. batch no.	Batch Test No.	Date of test	M L vol.	COD (mgCOD/l)		Area mgO/l	% COD Recov	Linear regression data			Max. specific growth rates (/d)		$Z_{BH(0)}$ (ML) mgCOD/l
				Start	End			y-int.	slope	R^2	K_{MP}	μ_H	
25	E1	09-05a	0.100	400.0	298.0	46.7	86.2	1.4089	0.0740	0.9218	1.01	1.39	81.720
	E2	09-05b	0.150	408.0	352.0	43.2	96.9	1.6129	0.0476	0.4298	0.81	0.95	136.241
	E3	10-05a	0.075	374.0	294.0	46.2	91.0	1.1700	0.0829	0.9762	1.13	1.48	59.087
	E4	10-05b	0.125	372.0	318.0	46.5	98.0	1.6015	0.0516	0.8772	0.86	1.00	127.739
	E5	12-05a	0.150	442.1	381.3	62.9	100.5	2.2087	0.0430	0.9405	0.81	0.84	263.727 *
	E6	12-05b	0.175	444.1	387.4	54.0	99.4	2.2429	0.0014	0.0024	0.31	0.34	689.773 *
	E7	16-05a	0.075	399.5	265.7	69.5	83.9	1.1172	0.0817	0.9234	1.42	1.16	56.673 *
	E8	16-05b	0.125	391.4	338.7	58.7	101.5	1.3822	0.0357	0.6037	0.73	0.74	129.092 *
	E9	17-05a	0.100	400.3	260.1	50.8	77.7	1.6615	0.0680	0.9761	1.11	1.15	111.931 *
	E10	17-05b	0.150	418.6	298.7	73.3	88.9	1.6632	0.0587	0.7219	0.99	1.04	124.457 *
	E11	19-05a	0.125	434.8	290.6	99.6	89.7	1.9331	0.0463	0.9089	0.82	0.91	191.041
	E12	19-05b	0.175	434.9	317.0	96.2	95.0	1.9314	0.0375	0.6343	0.76	0.76	217.216
27	E13	16-06a	0.025	288.3	151.2	54.7	71.4	0.2880	0.0682	0.7722	0.58	1.68	28.283
	E14	16-06b	0.050	322.6	219.7	45.6	82.2	0.6357	0.0571	0.9433	0.54	1.45	45.402
	E15	19-06a	0.075	314.5	215.7	57.3	86.8	1.3501	0.1341	0.9645	1.16	2.68	48.089
	E16	19-06b	0.100	340.7	246.0	62.4	90.5	1.3339	0.1566	0.8685	1.61	2.77	41.489
28	E17	21-06a	0.025	237.9	169.3	41.8	88.7	1.1820	0.0849	0.7396	0.66	2.00	58.720
	E18	21-06b	0.050	284.3	229.8	49.3	98.2	0.8009	0.1026	0.8726	1.06	2.02	34.584
			MEAN				90.4			0.7821	0.91	1.35	
			Std Deviation				8.2			0.2463	0.32	0.66	

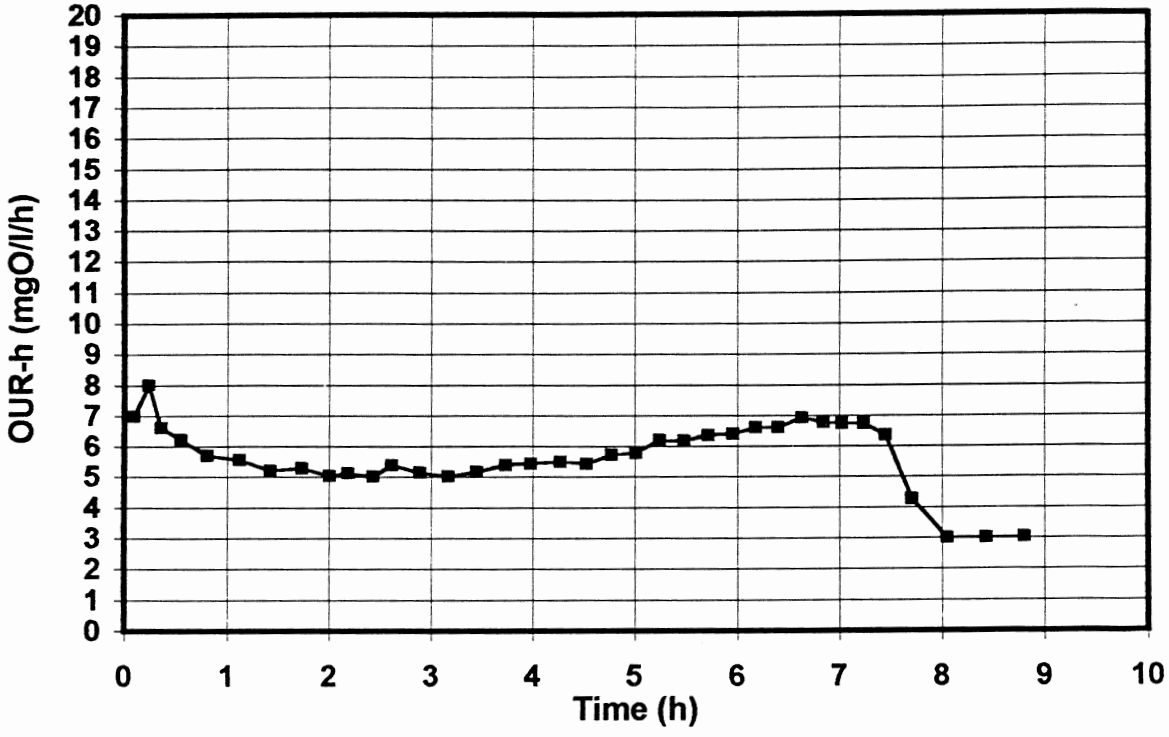
* Batch tests conducted on ML samples drawn from IFFD system after the system was fed.



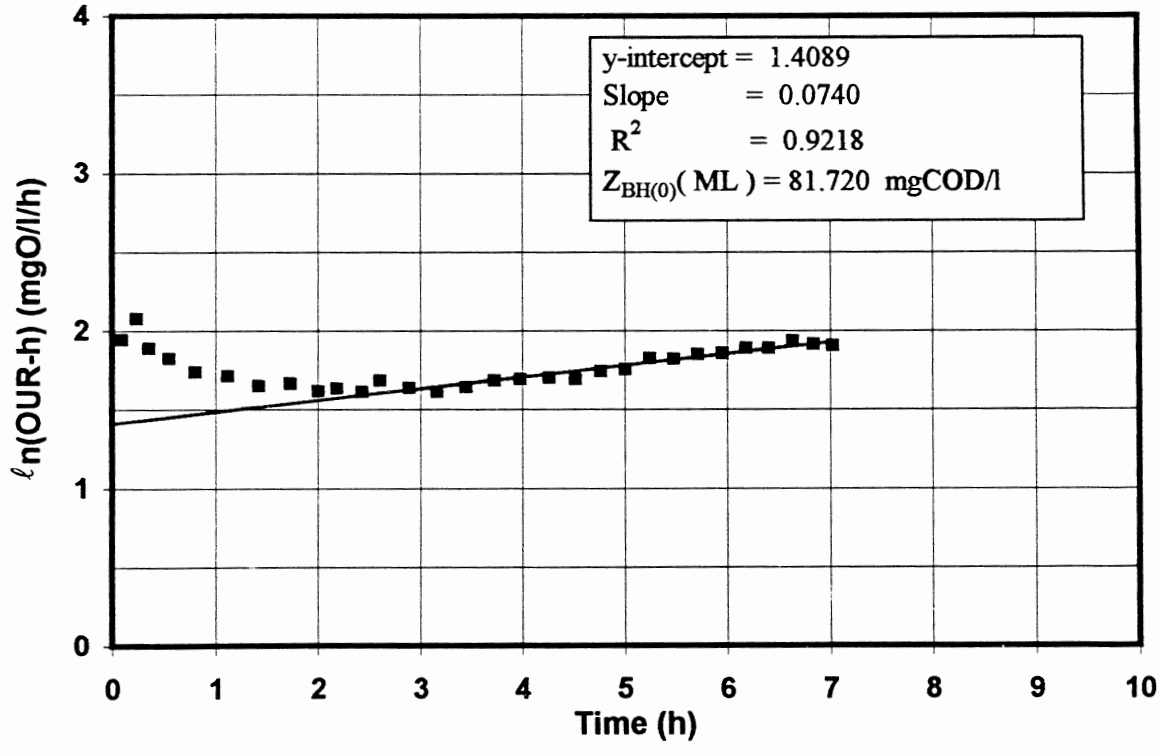
OUR Graph for filtered wastewater (2.9ℓ) plus IFFD system mixed liquor (0.1ℓ)
batch test E1 , 09-05a , wastewater batch no. 25



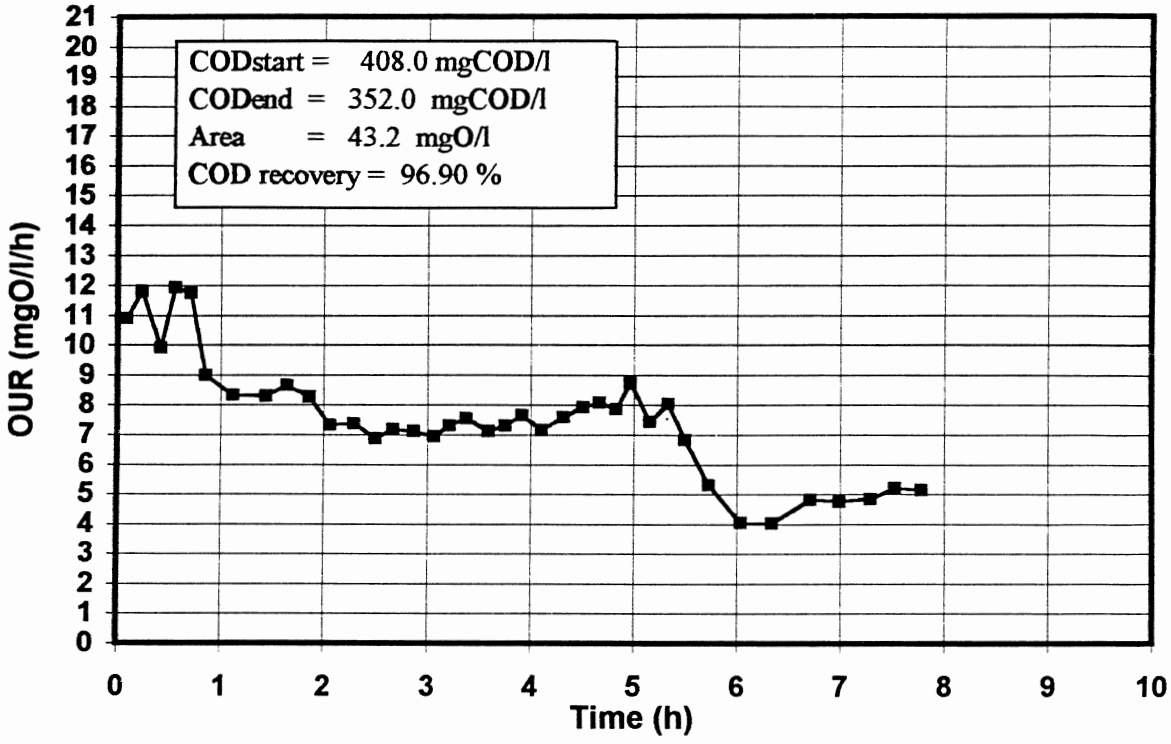
NO_3 Graph for filtered wastewater (2.9ℓ) plus IFFD system mixed liquor (0.1ℓ)
batch test E1 , 09-05a , wastewater batch no. 25



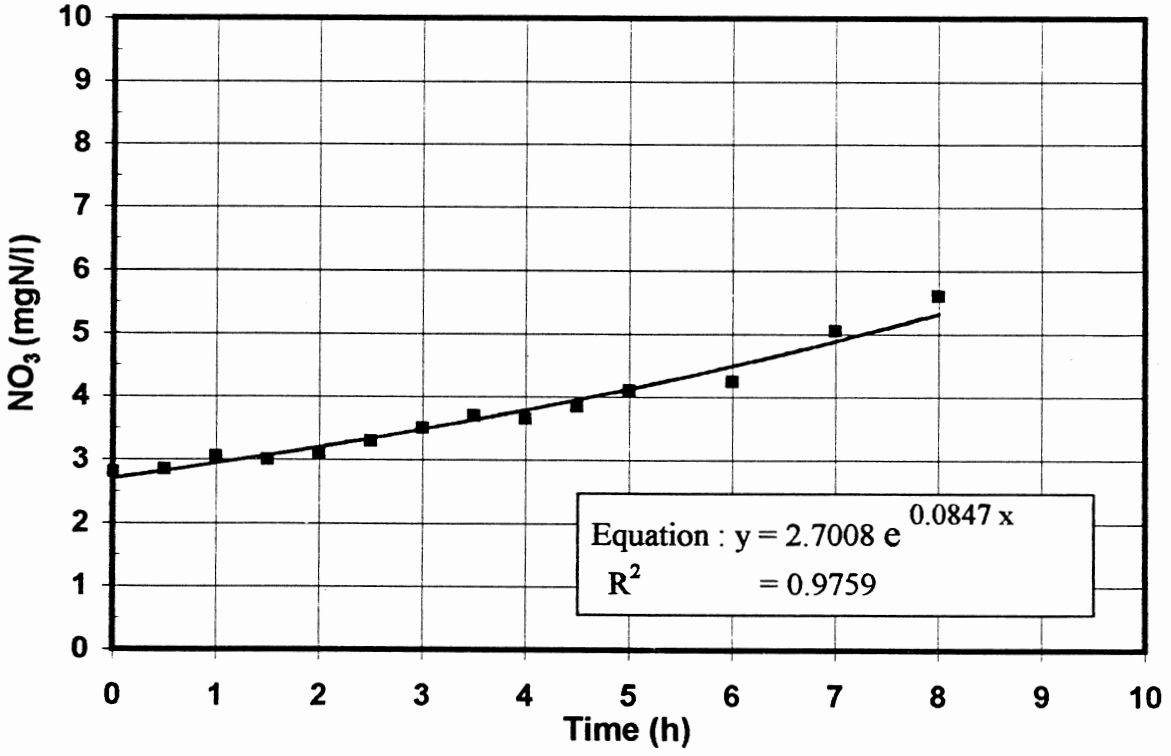
OUR-h Graph for filtered wastewater (2.9ℓ) plus IFFD system mixed liquor (0.1ℓ)
batch test E1 , 09-05a , wastewater batch no. 25



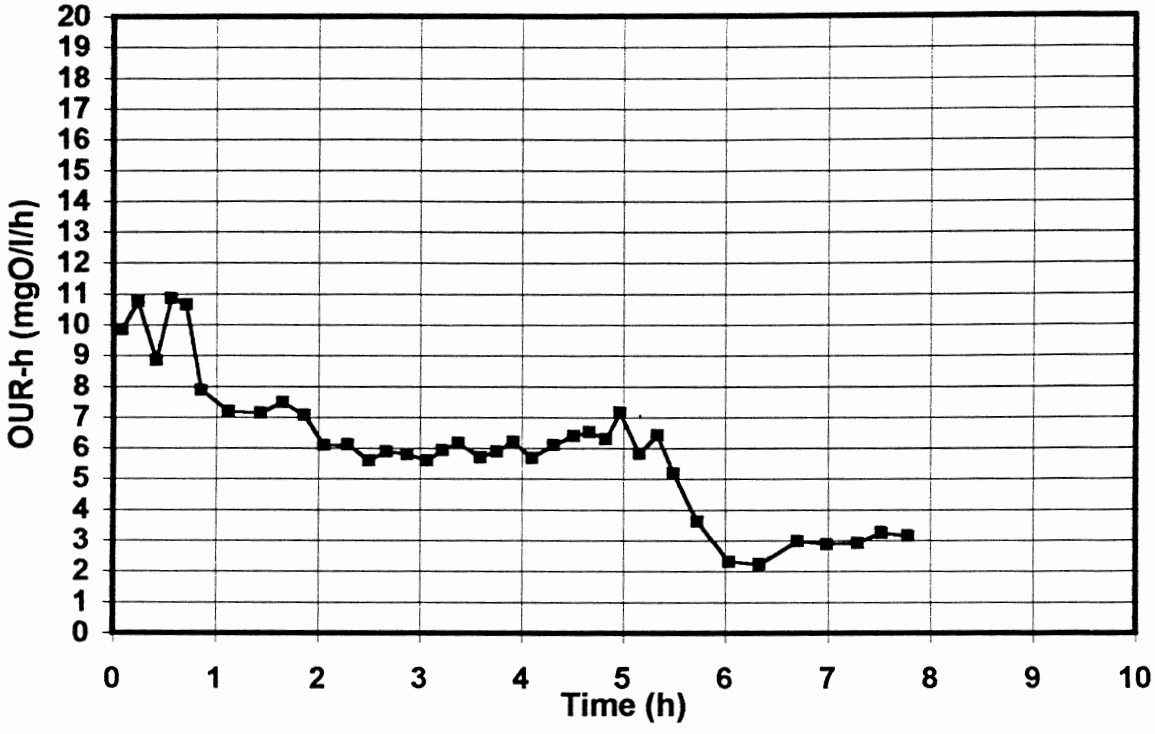
ℓn(OUR-h) graph for filtered wastewater (2.9ℓ) plus IFFD system mixed liquor (0.1ℓ)
batch test E1 , 09-05a , waste water batch no.25



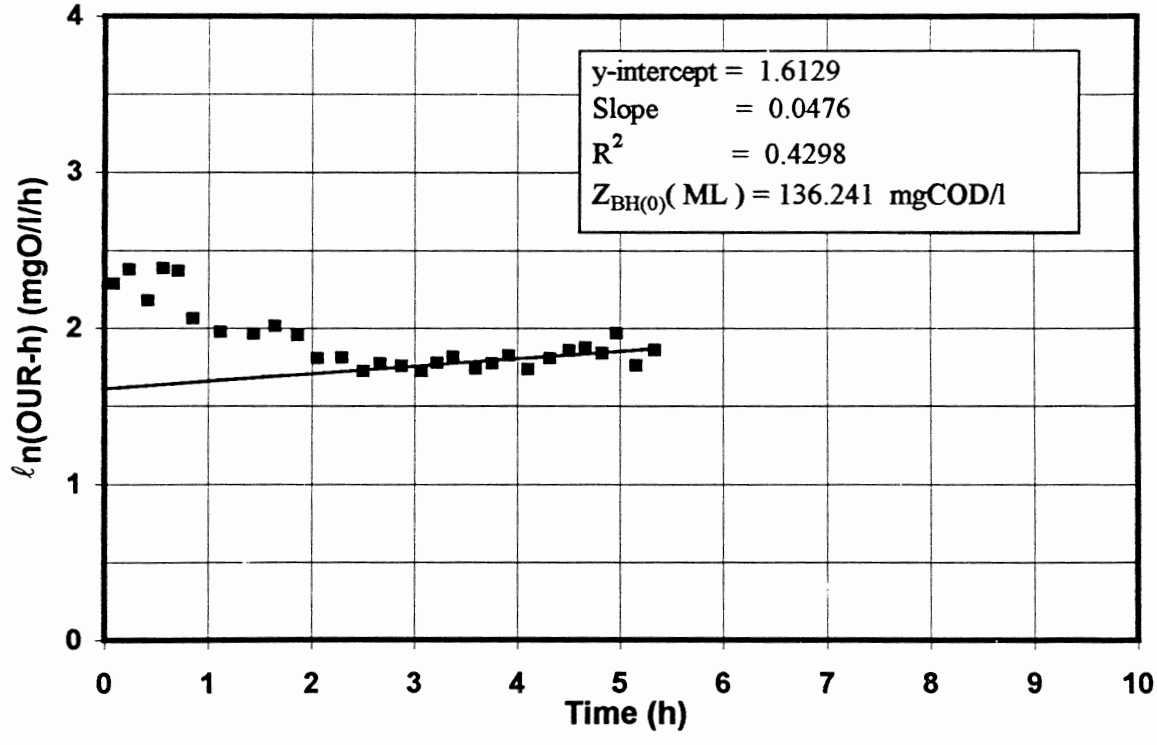
OUR Graph for filtered wastewater (2.85ℓ) plus IFFD system mixed liquor (0.15ℓ)
batch test E2 , 09-05b , wastewater batch no. 25



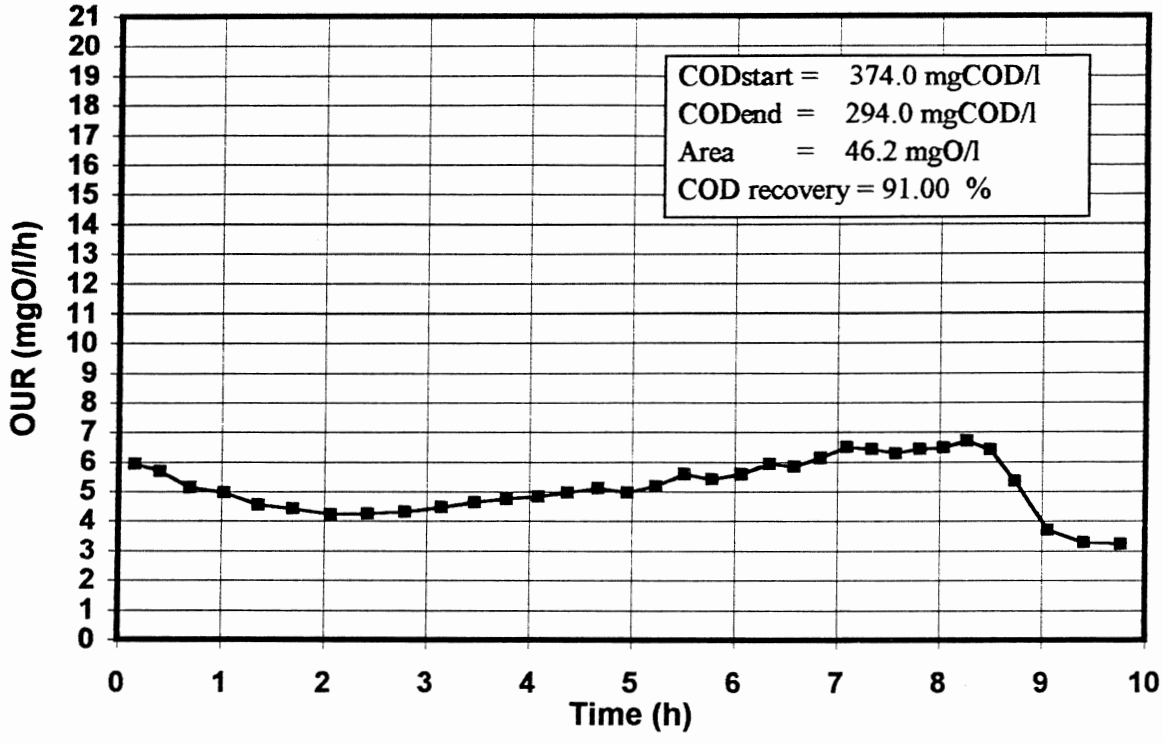
NO_3 Graph for filtered wastewater (2.85ℓ) plus IFFD system mixed liquor (0.15ℓ)
batch test E2 , 09-05b , wastewater batch no. 25



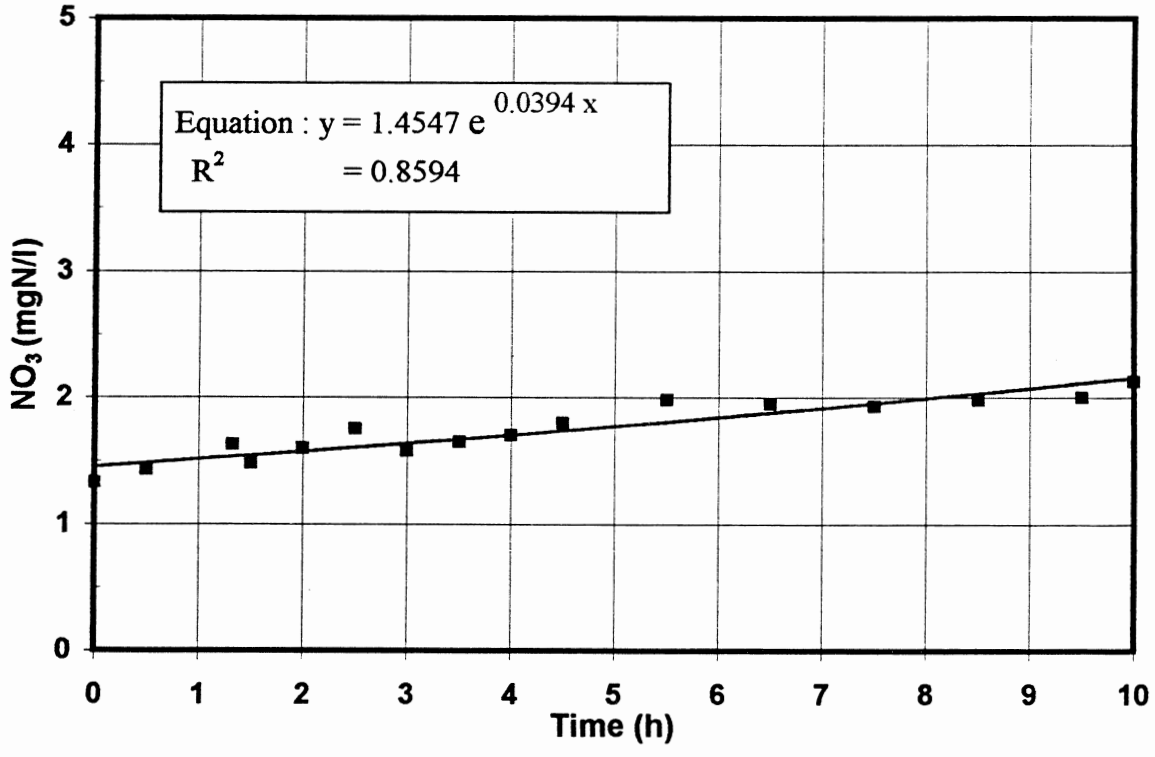
OUR-h Graph for filtered wastewater (2.85ℓ) plus IFFD system mixed liquor (0.15ℓ)
batch test E2 , 09-05b , wastewater batch no. 25



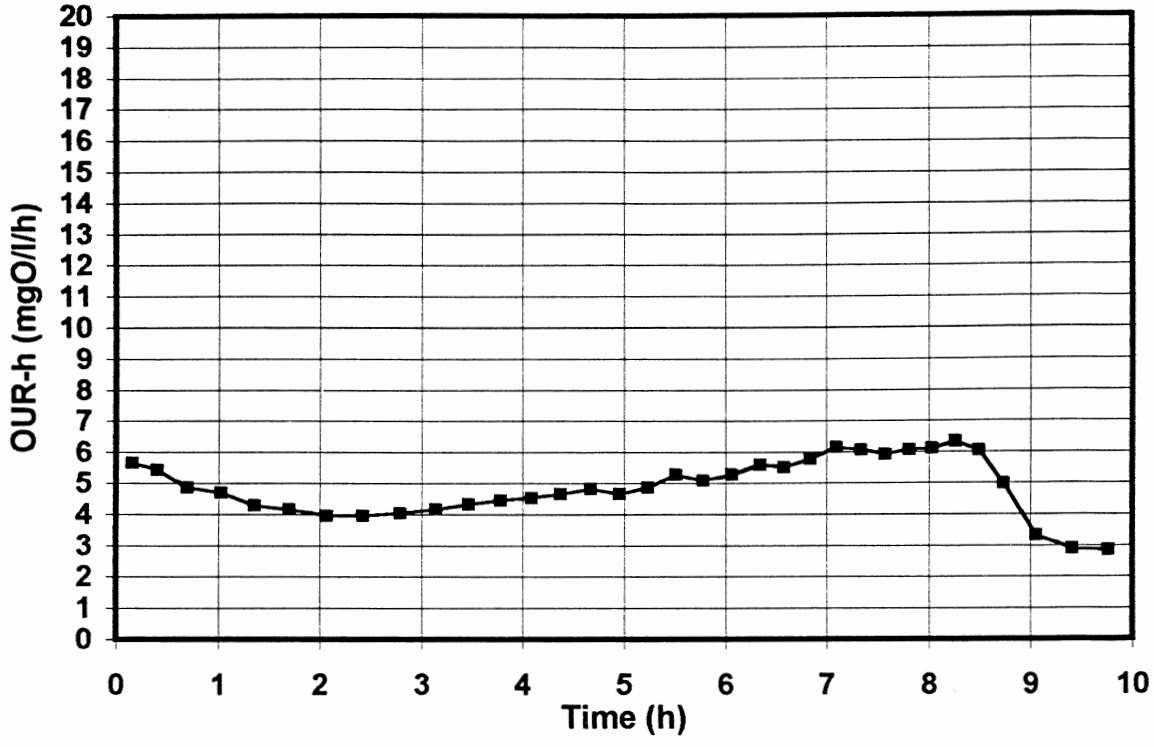
ℓn(OUR-h) graph for filtered wastewater (2.85ℓ) plus IFFD system mixed liquor (0.15ℓ)
batch test E2 , 09-05b , waste water batch no.25



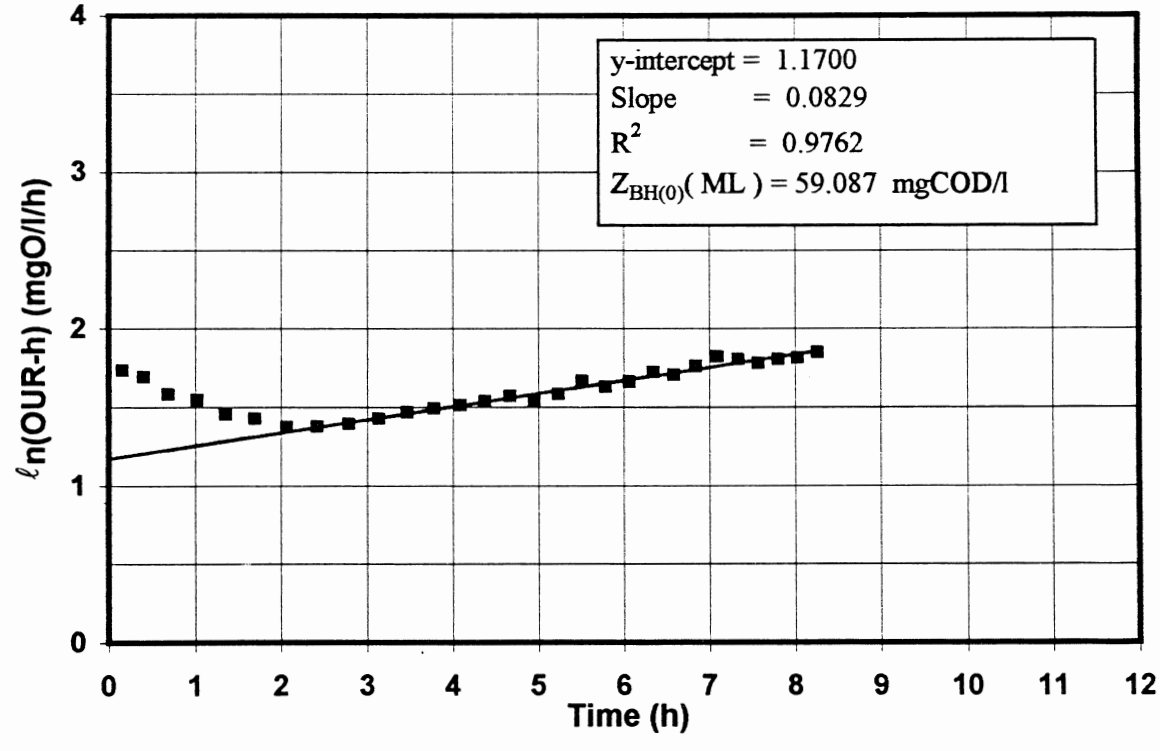
OUR Graph for filtered wastewater (2.925ℓ) plus IFFD system mixed liquor (0.075ℓ)
batch test E3 , 10-05a , wastewater batch no. 25



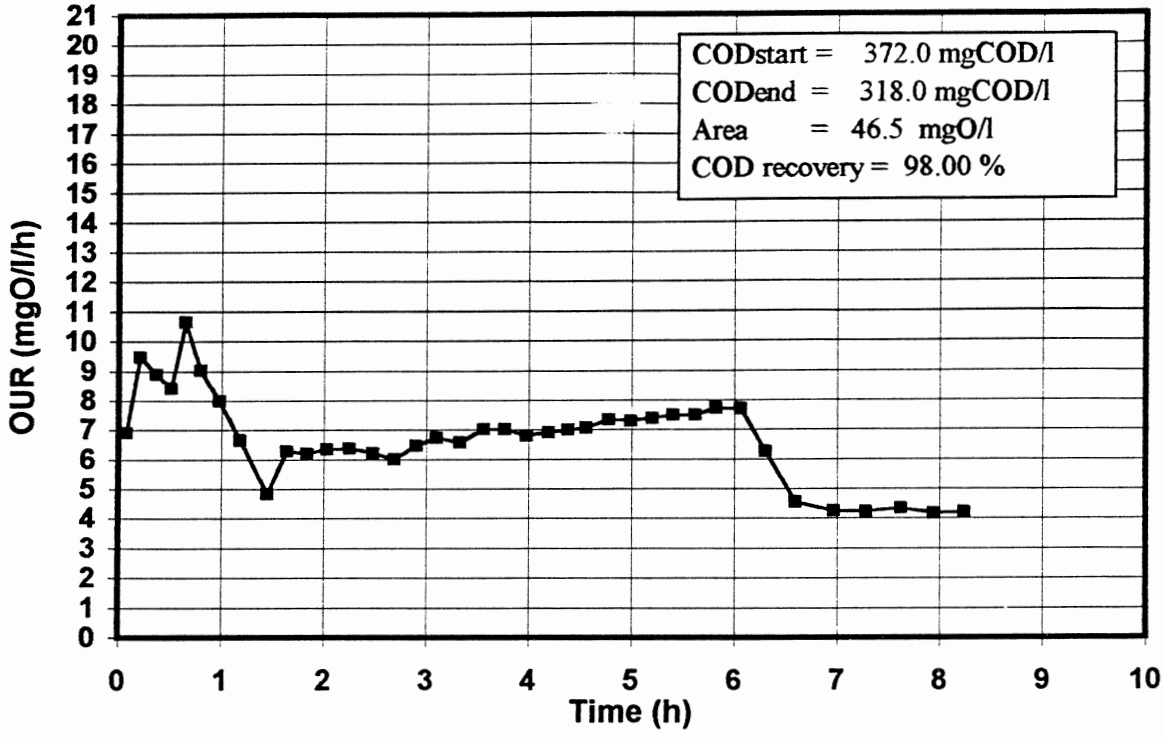
NO_3 Graph for filtered wastewater (2.925ℓ) plus IFFD system mixed liquor (0.075ℓ)
batch test E3 , 10-05a , wastewater batch no. 25



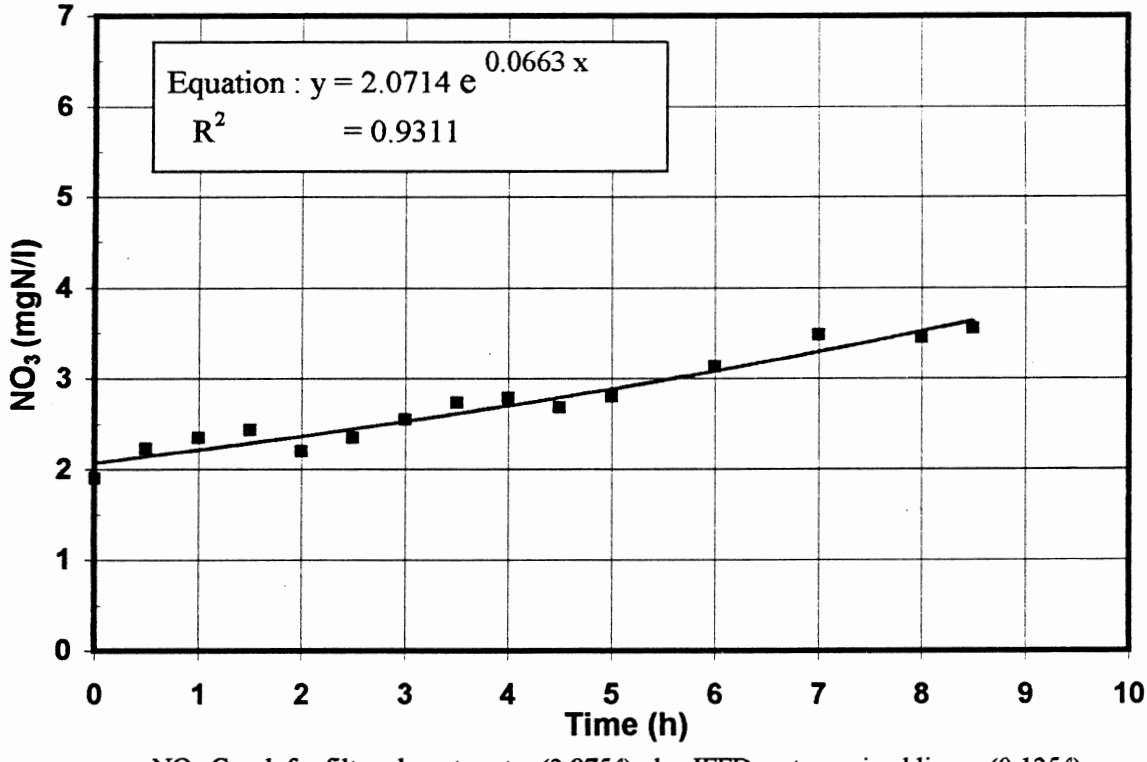
OUR-h Graph for filtered wastewater (2.925ℓ) plus IFFD system mixed liquor (0.075ℓ)
batch test E3 , 10-05a , wastewater batch no. 25



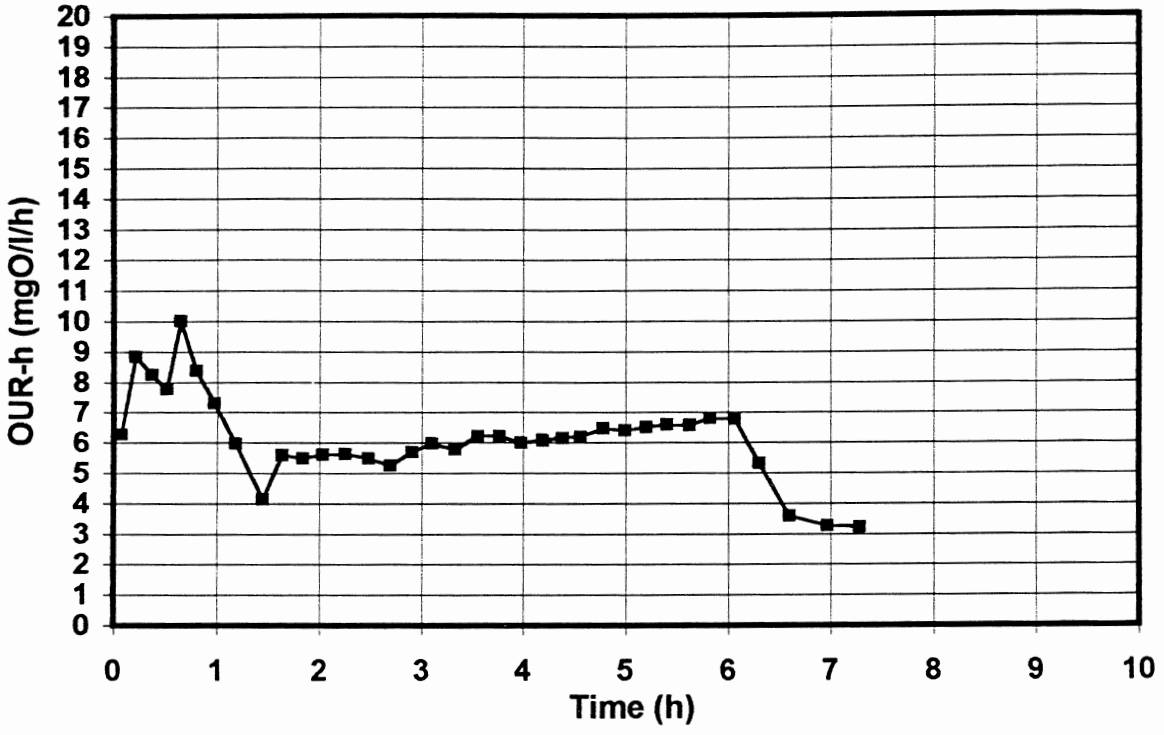
ℓn(OUR-h) for filtered wastewater (2.925ℓ) plus IFFD system mixed liquor (0.075ℓ)
batch test E3 , 10-05a , waste water batch no.25



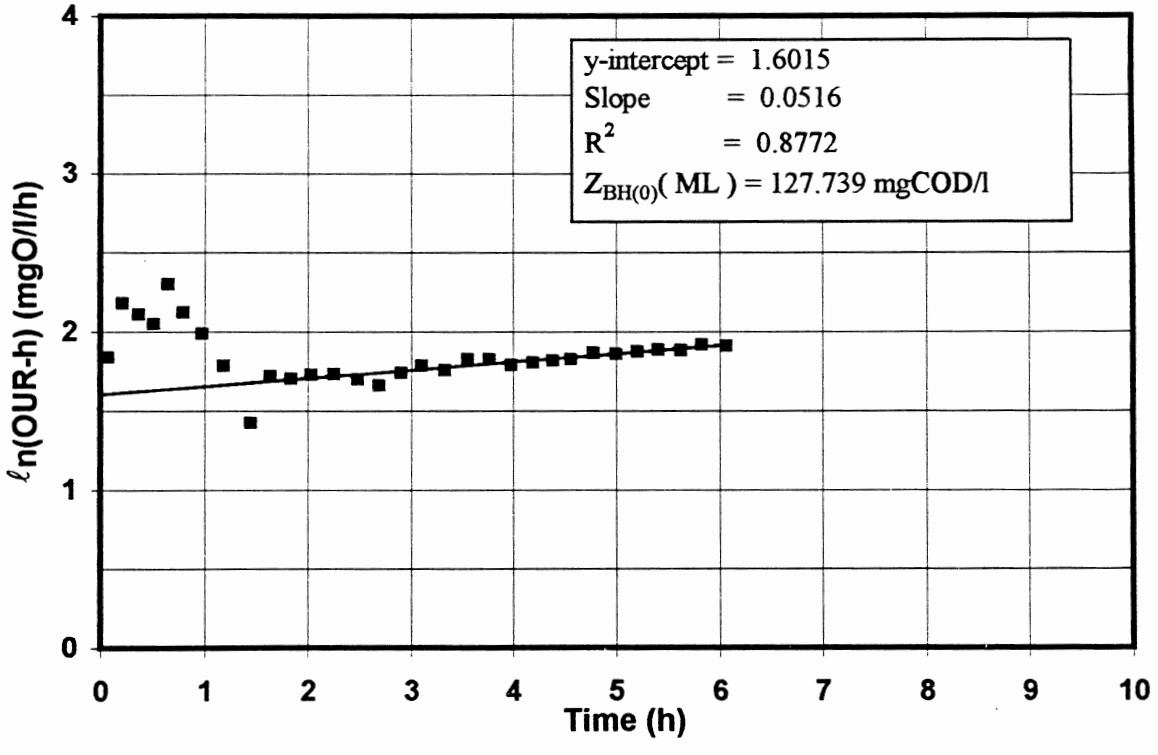
OUR Graph for filtered wastewater (2.875ℓ) plus IFFD system mixed liquor (0.125ℓ)
batch test E4 , 10-05b , wastewater batch no. 25



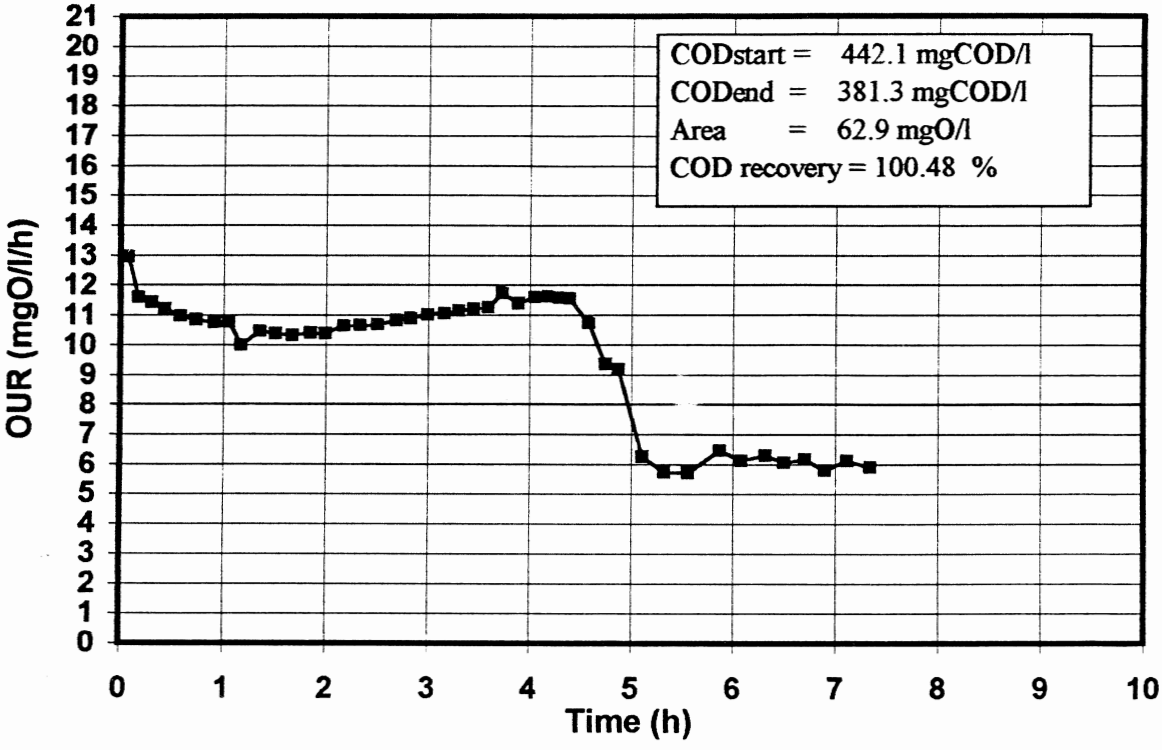
NO_3 Graph for filtered wastewater (2.875ℓ) plus IFFD system mixed liquor (0.125ℓ)
batch test E4 , 10-05b , wastewater batch no. 25



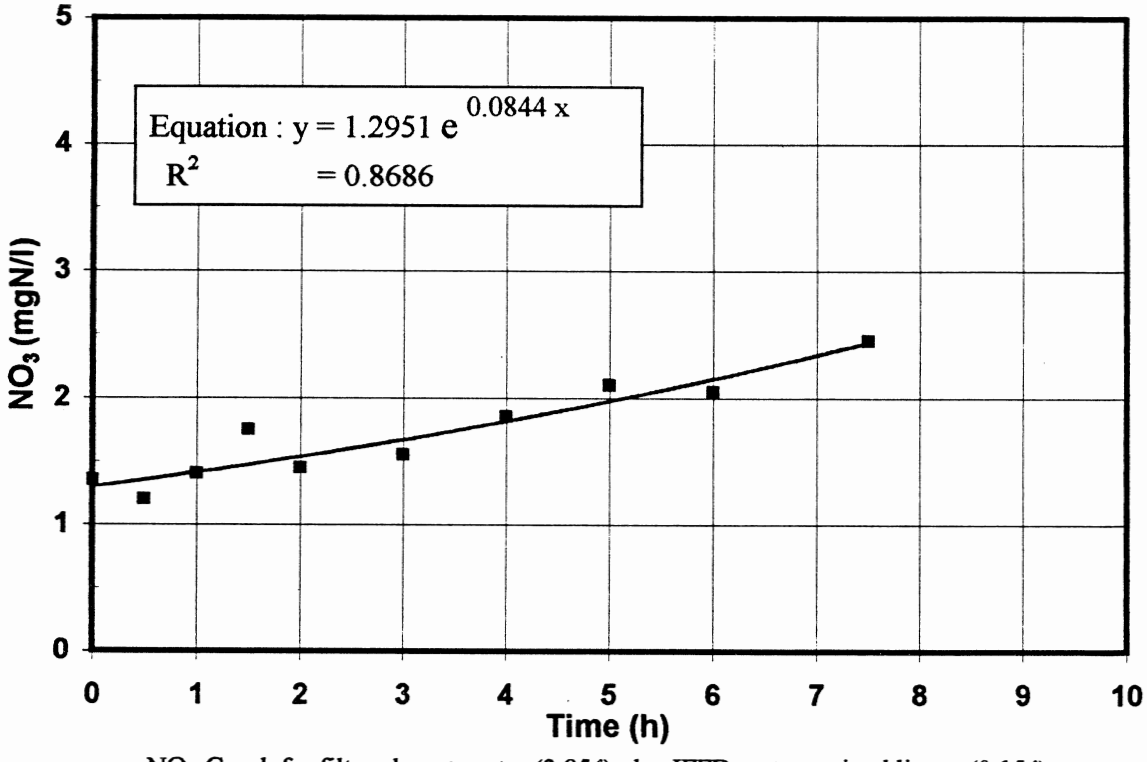
OUR-h Graph for filtered wastewater (2.875ℓ) plus IFFD system mixed liquor (0.125ℓ)
batch test E4 , 10-05b , wastewater batch no. 25



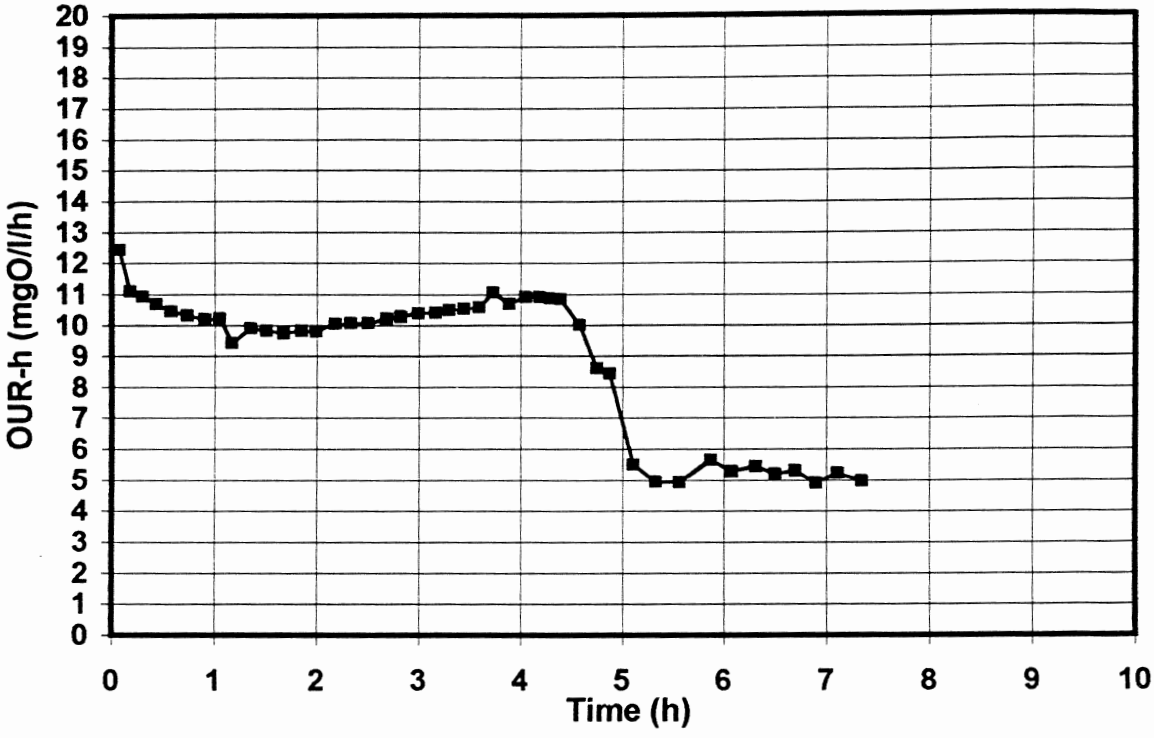
ℓn(OUR-h) for filtered wastewater (2.875ℓ) plus IFFD system mixed liquor (0.125ℓ)
batch test E4 , 10-05b , waste water batch no.25



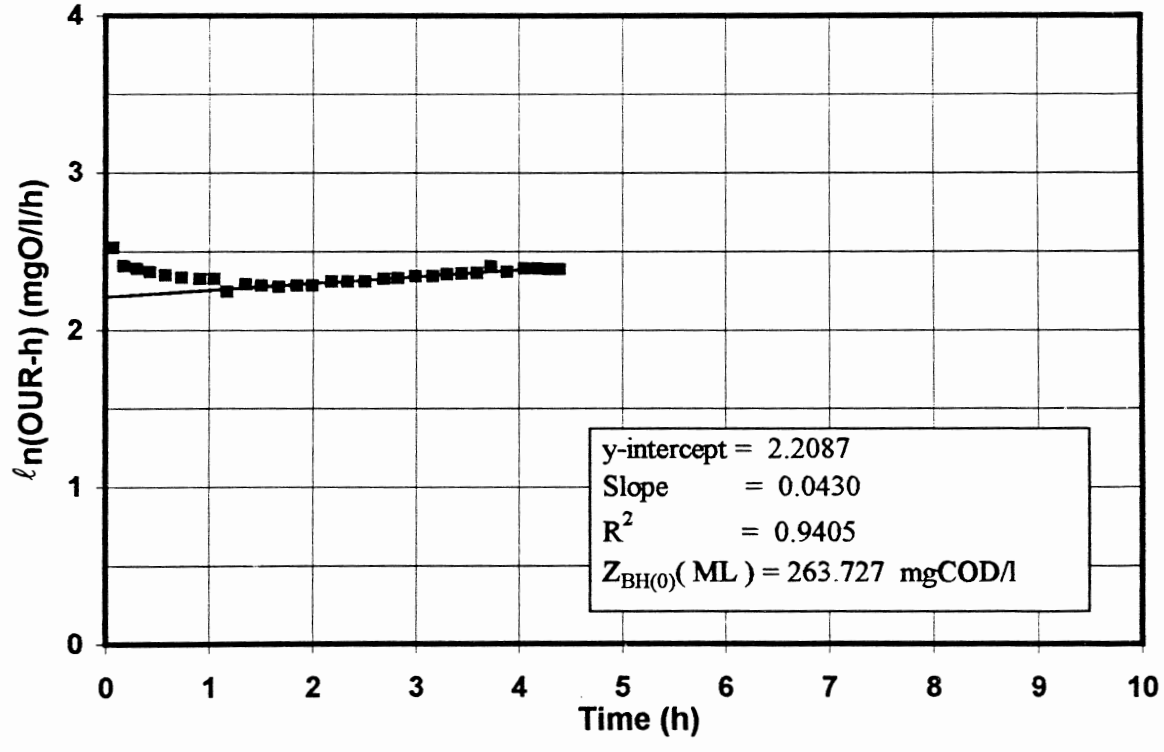
OUR Graph for filtered wastewater (2.85ℓ) plus IFFD system mixed liquor (0.15ℓ)
batch test E5 , 12-05a , wastewater batch no. 25



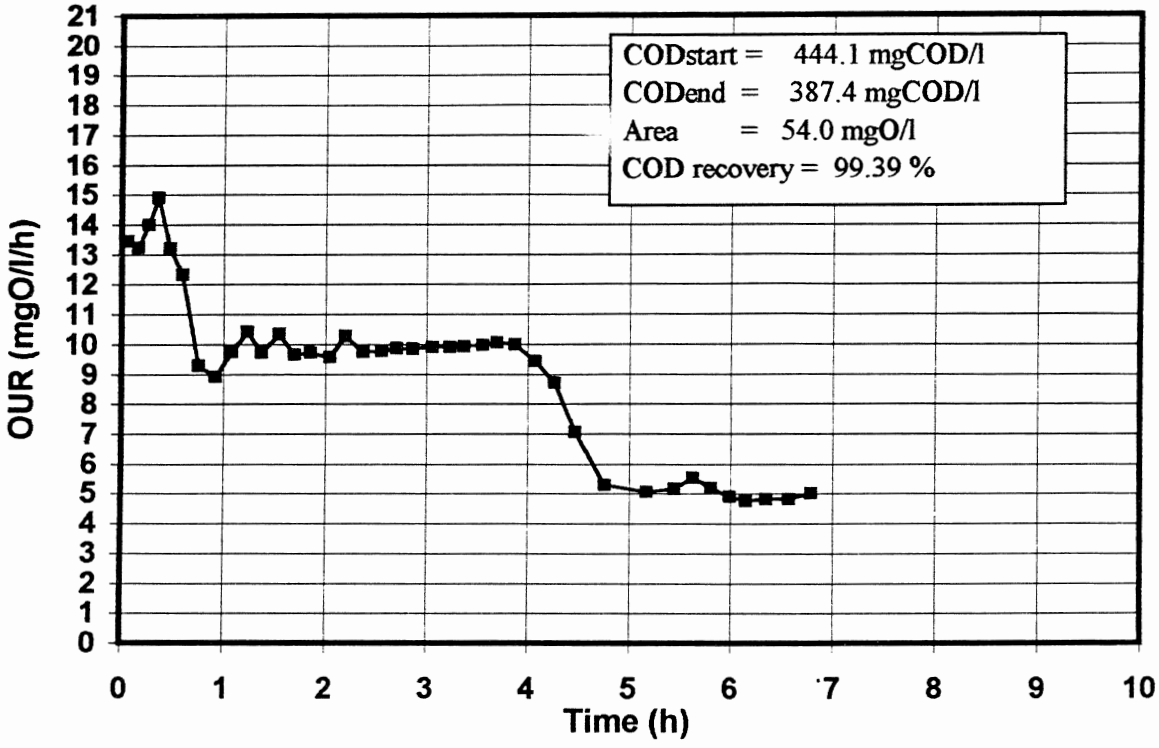
NO_3 Graph for filtered wastewater (2.85ℓ) plus IFFD system mixed liquor (0.15ℓ)
batch test E5 , 12-05a , wastewater batch no. 25



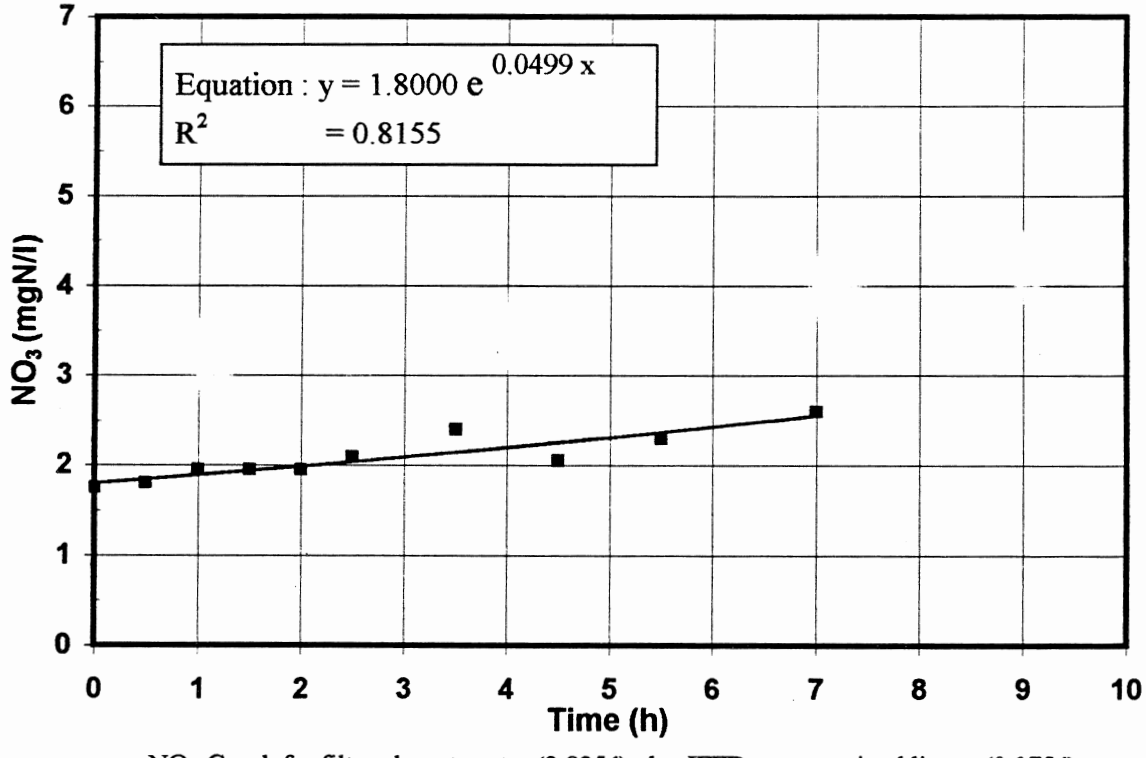
OUR-h Graph for filtered wastewater (2.85ℓ) plus IFFD system mixed liquor (0.15ℓ)
batch test E5 , 12-05a , wastewater batch no. 25



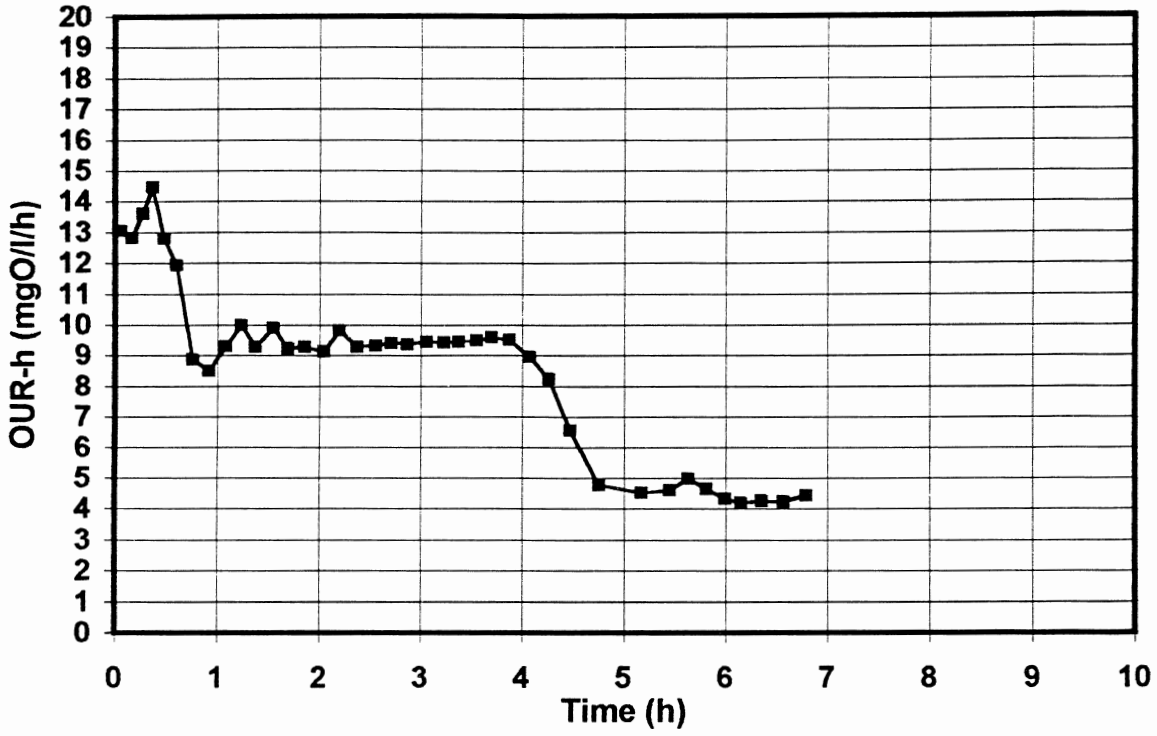
ℓn(OUR-h) graph for filtered wastewater (2.85ℓ) plus IFFD system mixed liquor (0.15ℓ)
batch test E5 , 12-05a , waste water batch no.25



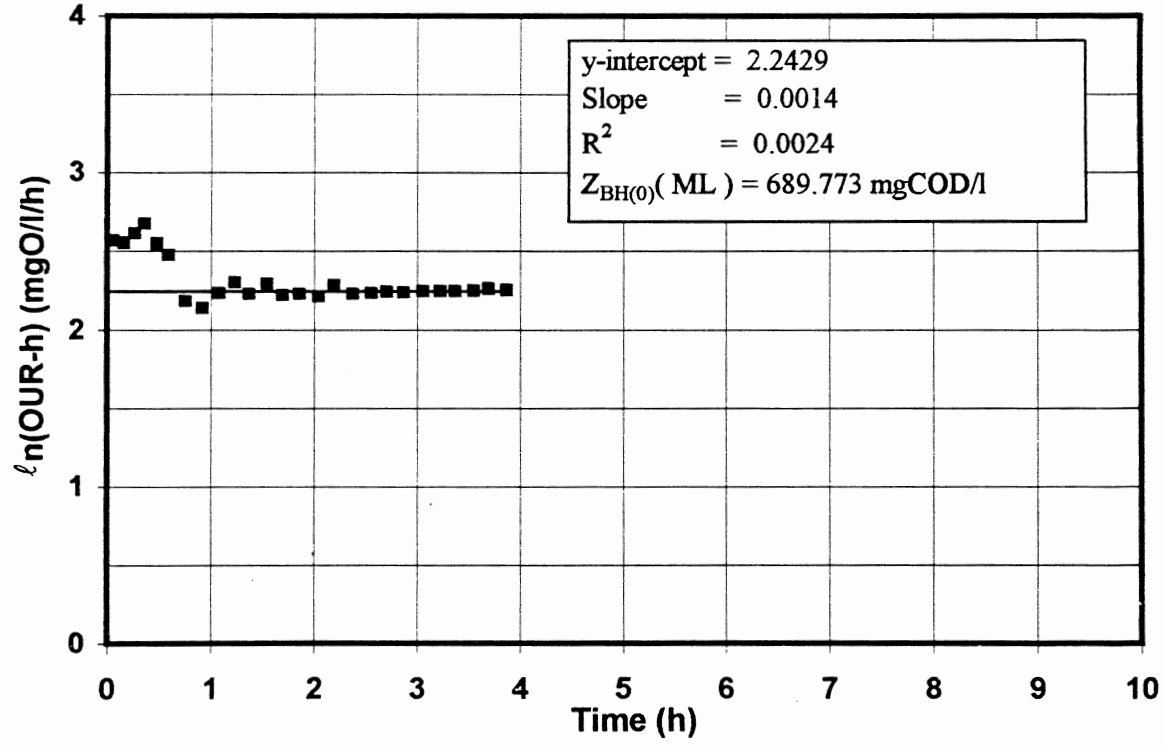
OUR Graph for filtered wastewater (2.825ℓ) plus IFFD system mixed liquor (0.175ℓ)
batch test E6 , 12-05b , wastewater batch no. 25



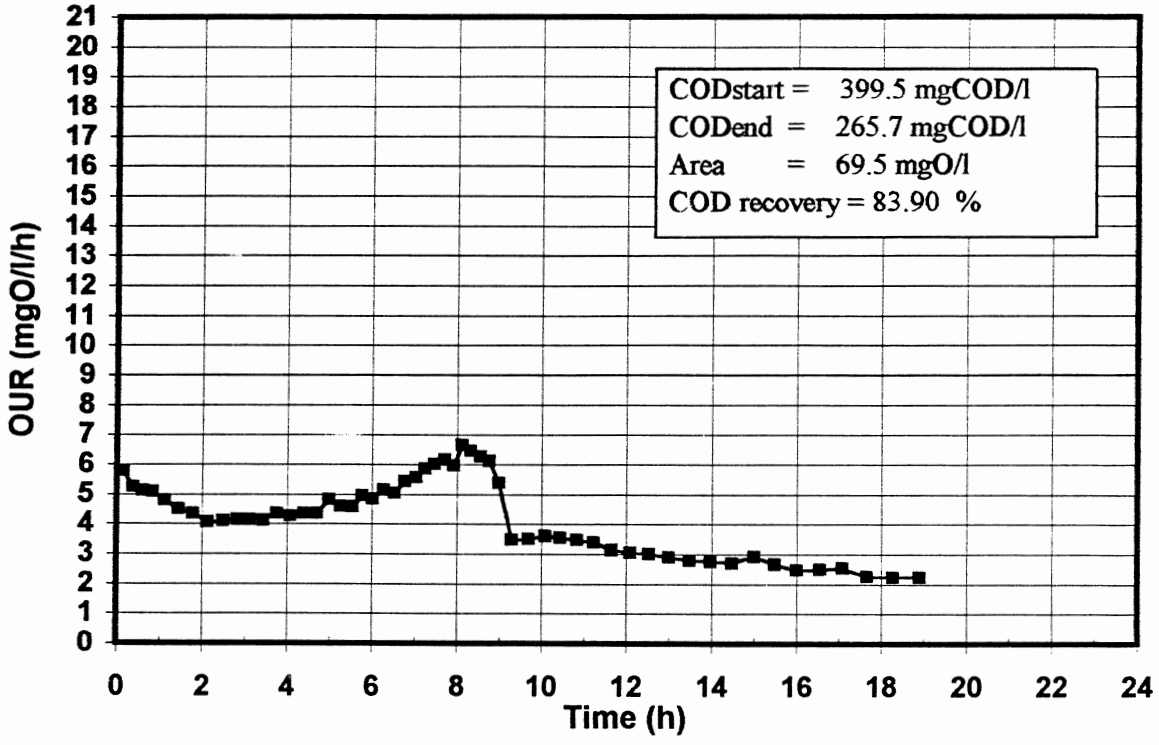
NO_3 Graph for filtered wastewater (2.825ℓ) plus IFFD system mixed liquor (0.175ℓ)
batch test E6 , 12-05b , wastewater batch no. 25



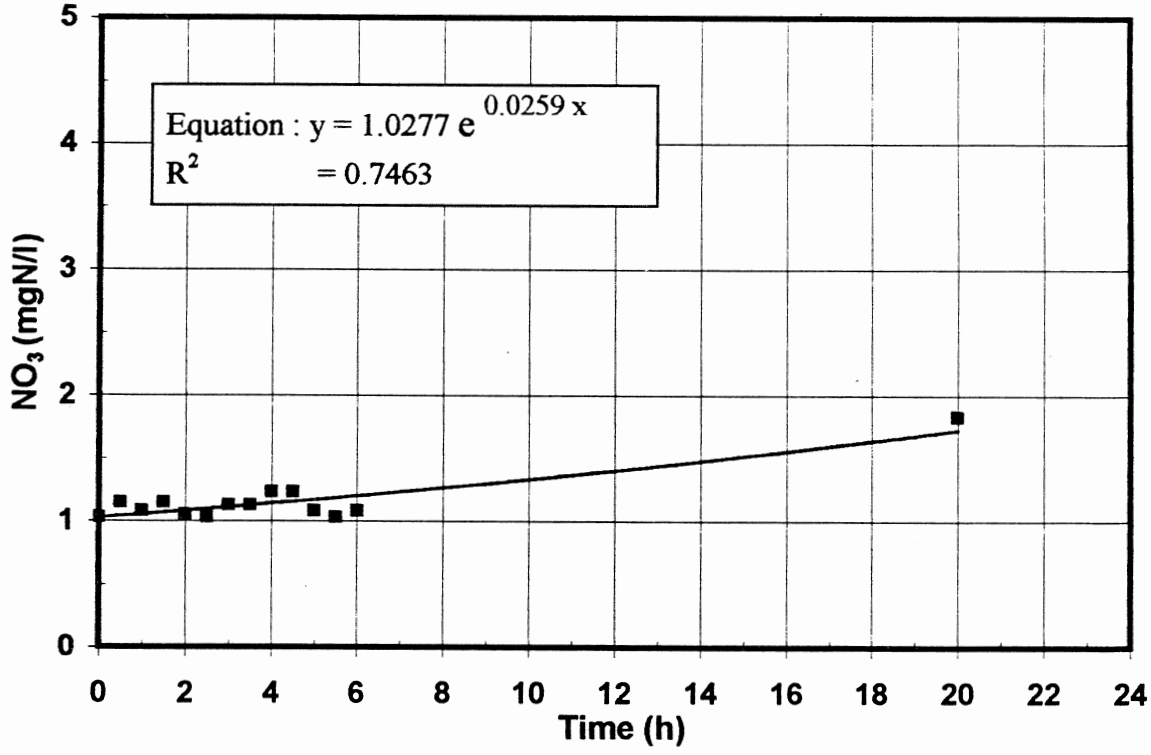
OUR-h Graph for filtered wastewater (2.825ℓ) plus IFFD system mixed liquor (0.175ℓ)
batch test E6 , 12-05b , wastewater batch no. 25



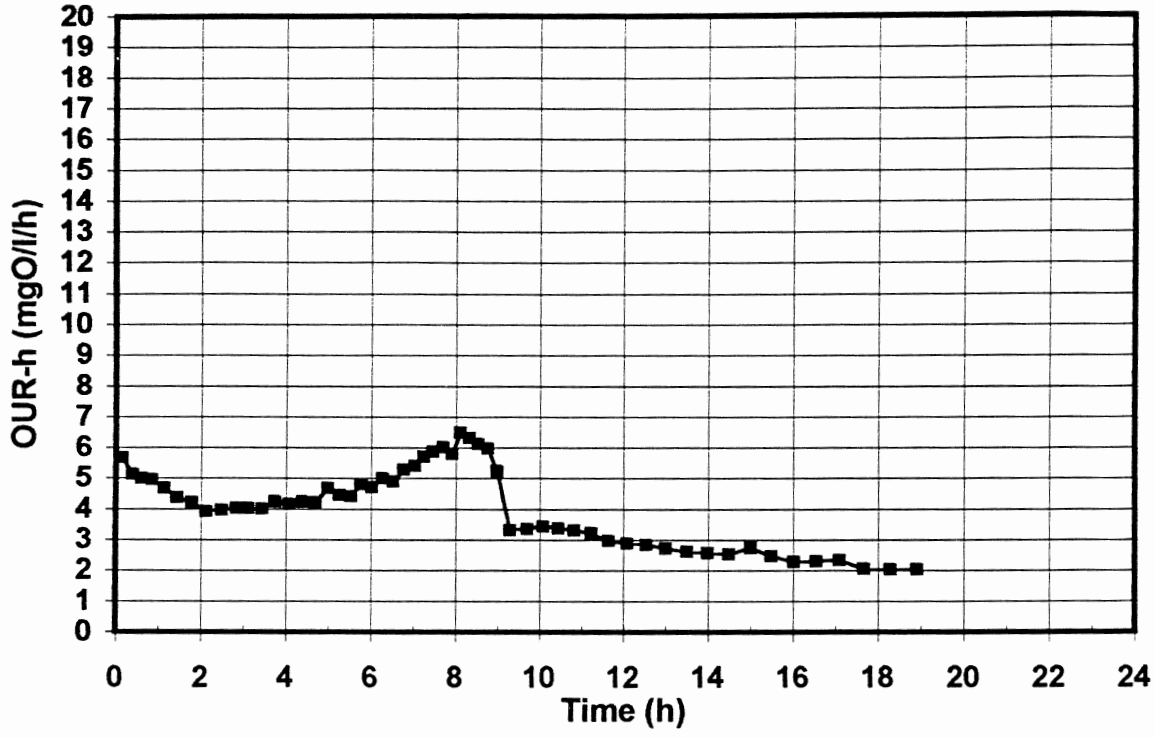
ℓn(OUR-h) for filtered wastewater (2.825ℓ) plus IFFD system mixed liquor (0.175ℓ)
batch test E6 , 12-05b , waste water batch no.25



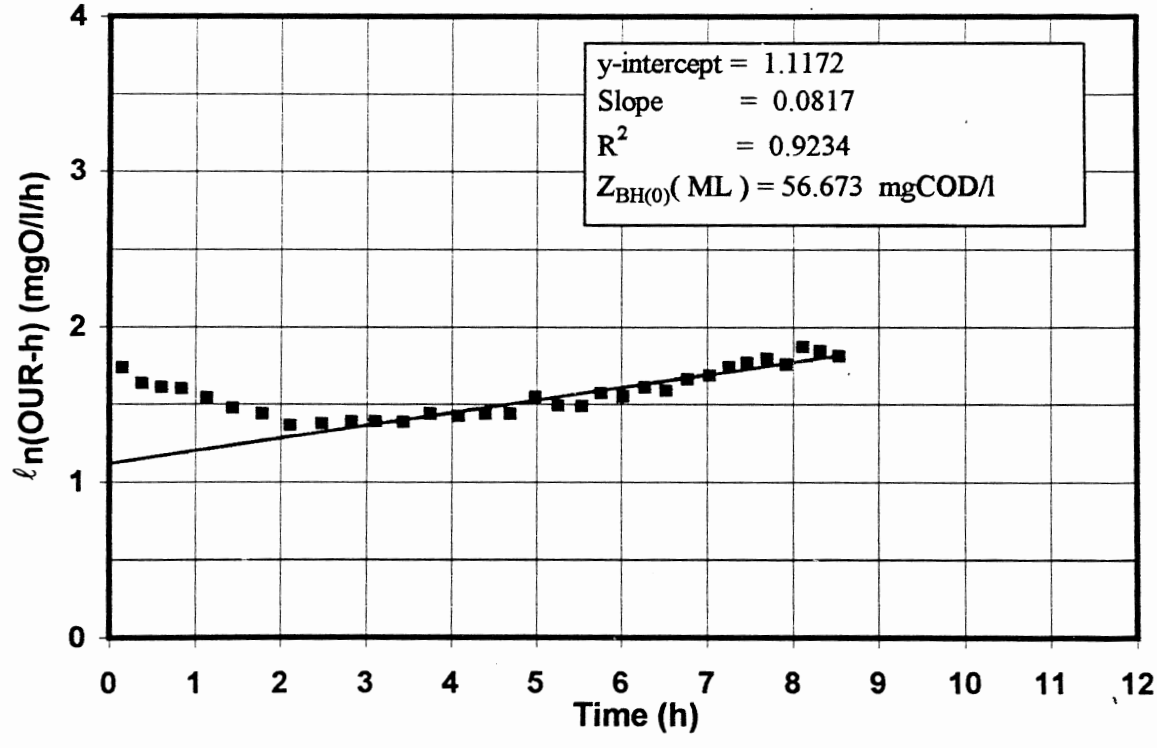
OUR Graph for filtered wastewater (2.925ℓ) plus IFFD system mixed liquor (0.075ℓ)
batch test E7 , 16-05a , wastewater batch no. 25



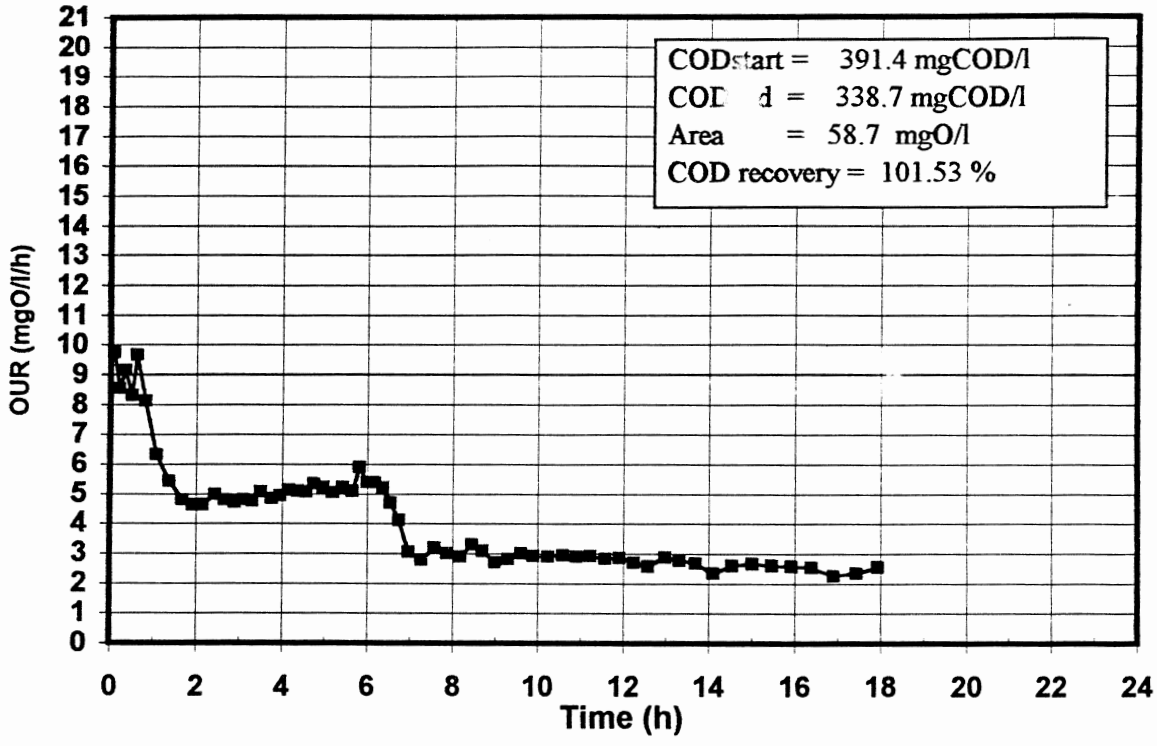
NO_3 Graph for filtered wastewater (2.925ℓ) plus IFFD system mixed liquor (0.075ℓ)
batch test E7 , 16-05a , wastewater batch no. 25



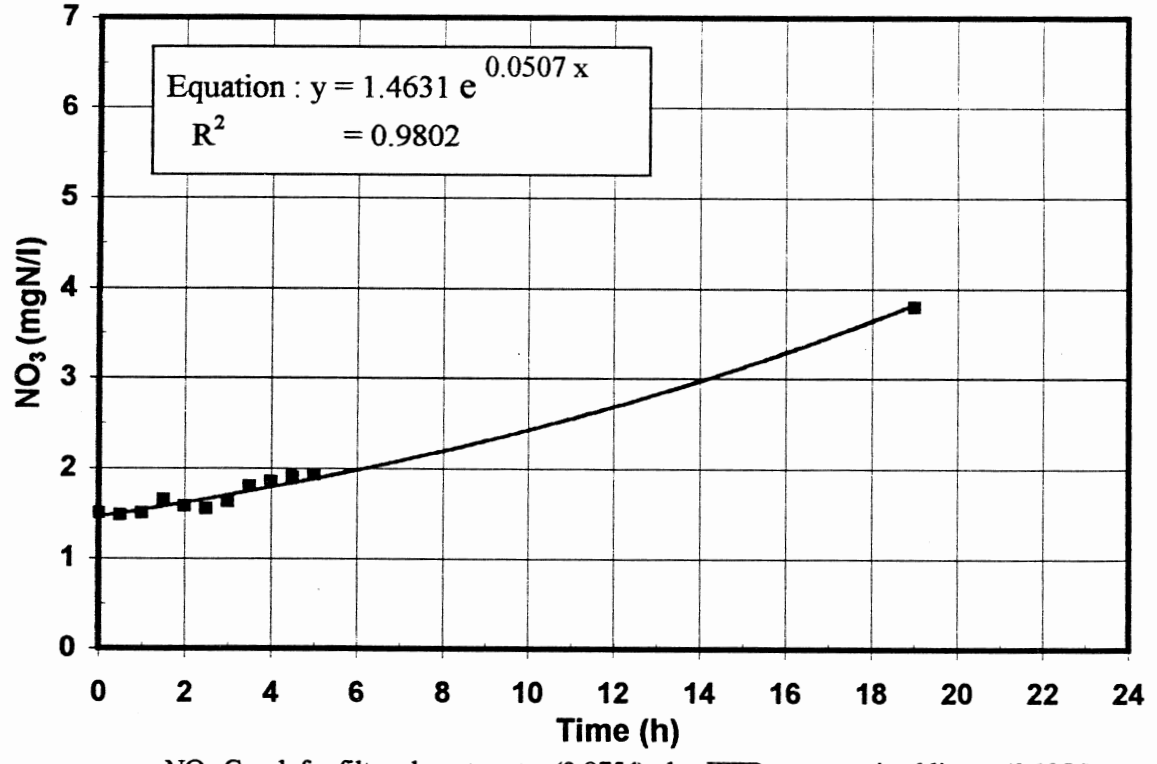
OUR-h Graph for filtered wastewater (2.925ℓ) plus IFFD system mixed liquor (0.075ℓ)
batch test E7 , 16-05a , wastewater batch no. 25



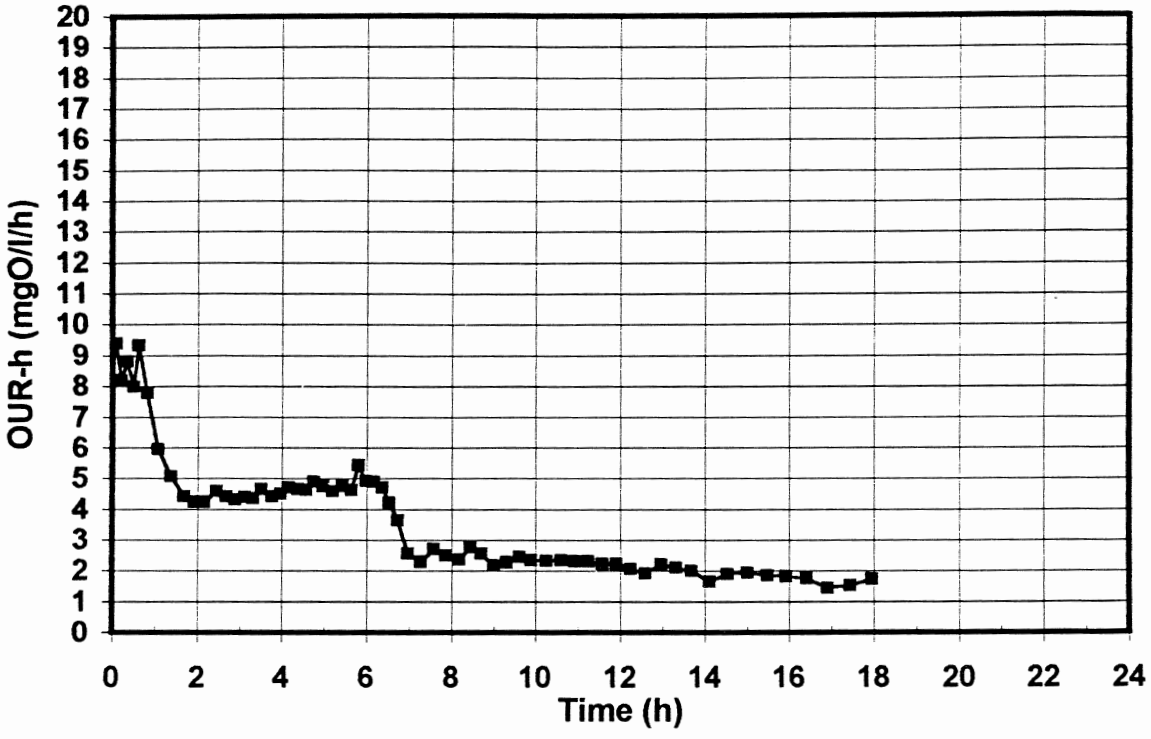
ℓn(OUR-h) for filtered wastewater (2.925ℓ) plus IFFD system mixed liquor (0.075ℓ)
batch test E7 , 16-05a , waste water batch no.25



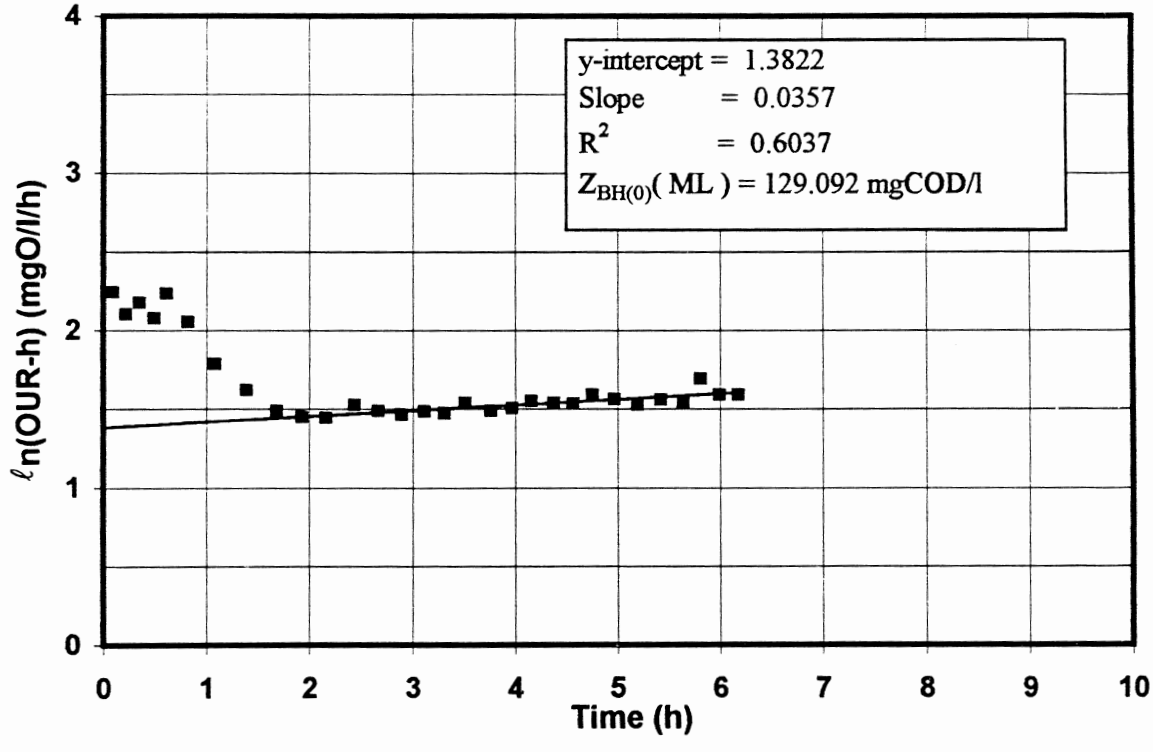
OUR Graph for filtered wastewater (2.875ℓ) plus IFFD system mixed liquor (0.125ℓ)
batch test E8 , 16-05b , wastewater batch no. 25



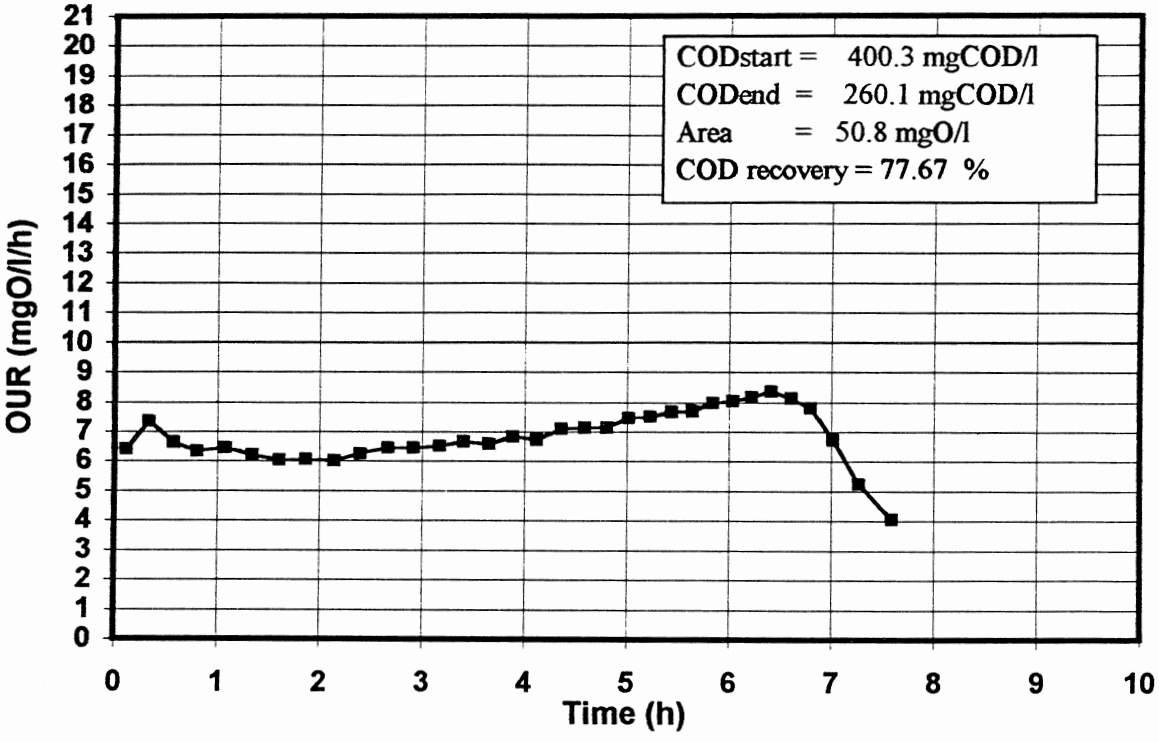
NO_3 Graph for filtered wastewater (2.875ℓ) plus IFFD system mixed liquor (0.125ℓ)
batch test E8 , 16-05b , wastewater batch no. 25



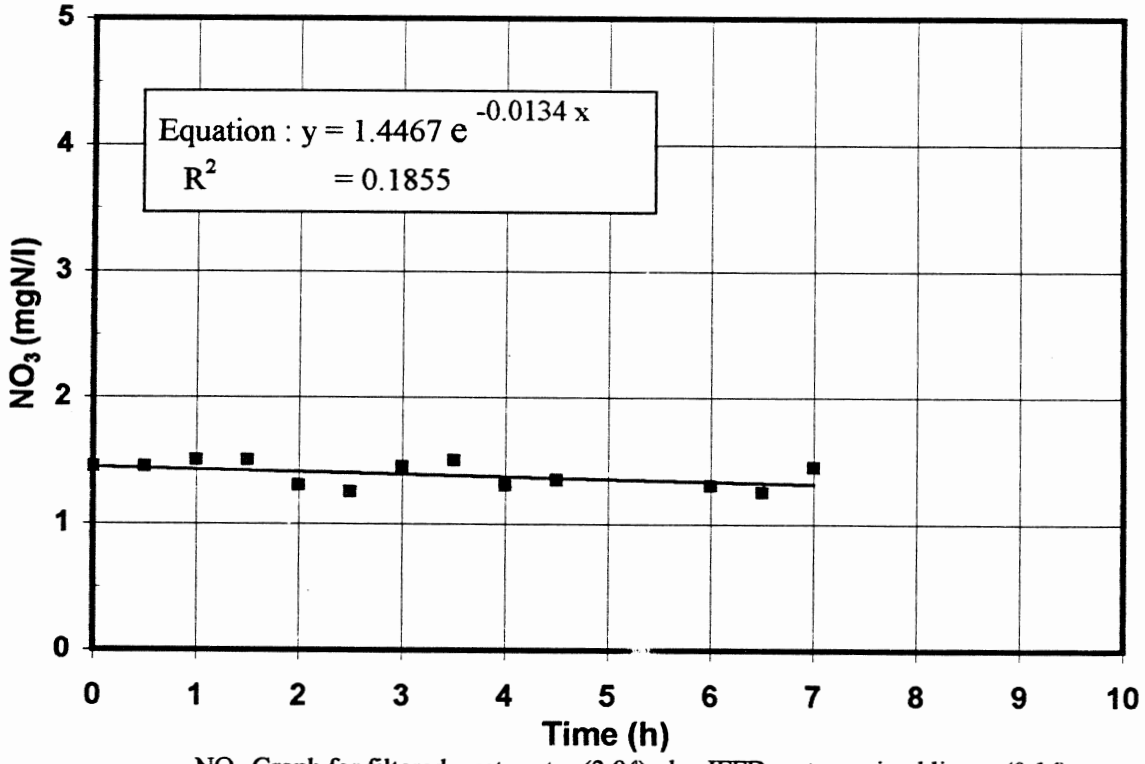
OUR-h Graph for filtered wastewater (2.875ℓ) plus IFFD system mixed liquor (0.125ℓ)
batch test E8 , 16-05b , wastewater batch no. 25



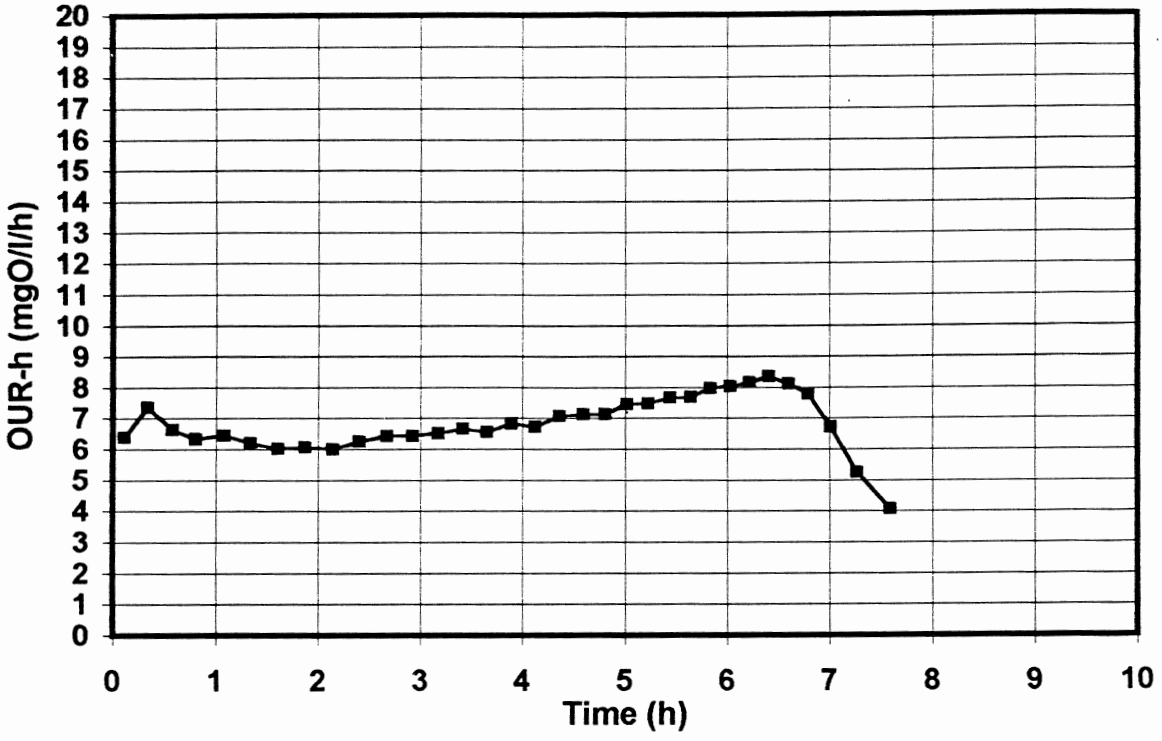
ℓn(OUR-h) for filtered wastewater (2.875ℓ) plus IFFD system mixed liquor (0.125ℓ)
batch test E8 , 16-05b , waste water batch no.25



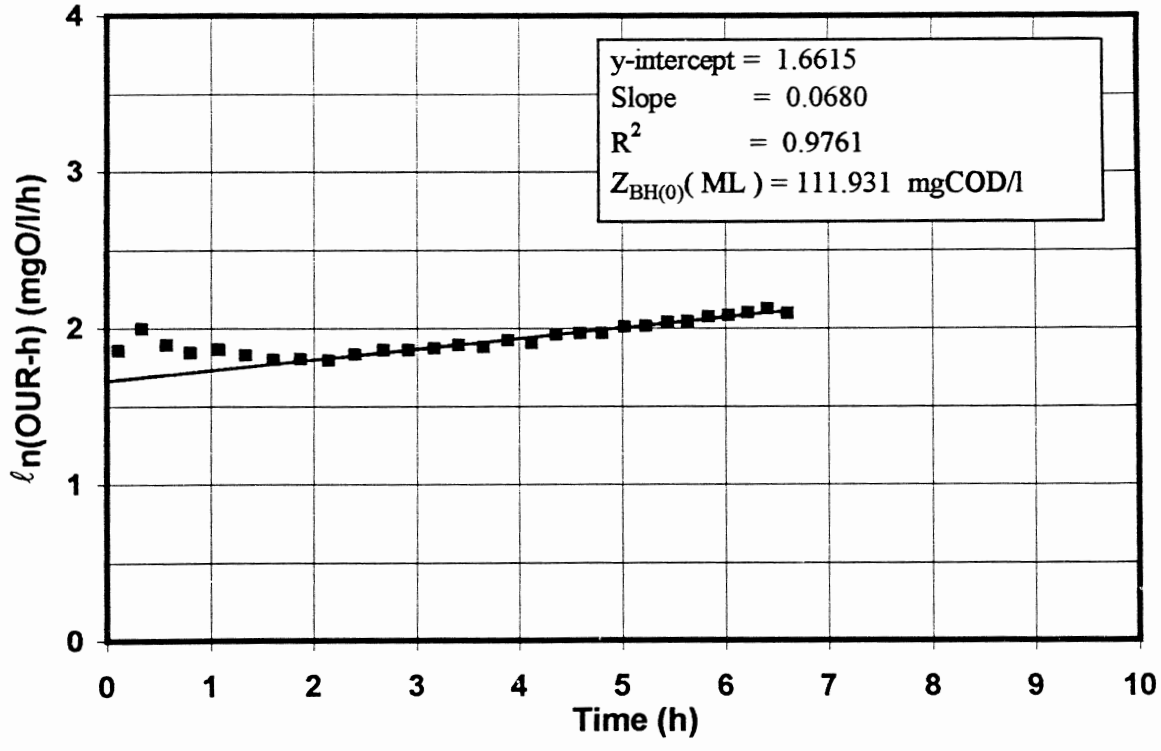
OUR Graph for filtered wastewater (2.9ℓ) plus IFFD system mixed liquor (0.1ℓ)
batch test E9 , 17-05a , wastewater batch no. 25



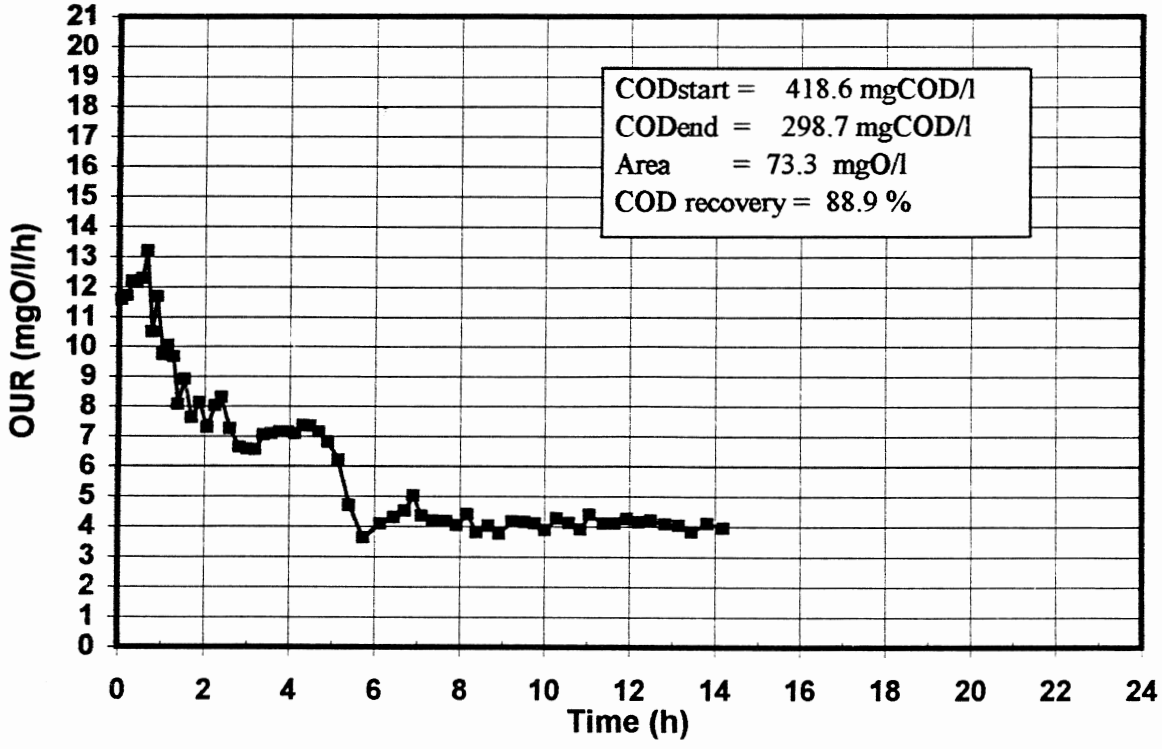
NO_3 Graph for filtered wastewater (2.9ℓ) plus IFFD system mixed liquor (0.1ℓ)
batch test E9 , 17-05a , wastewater batch no. 25



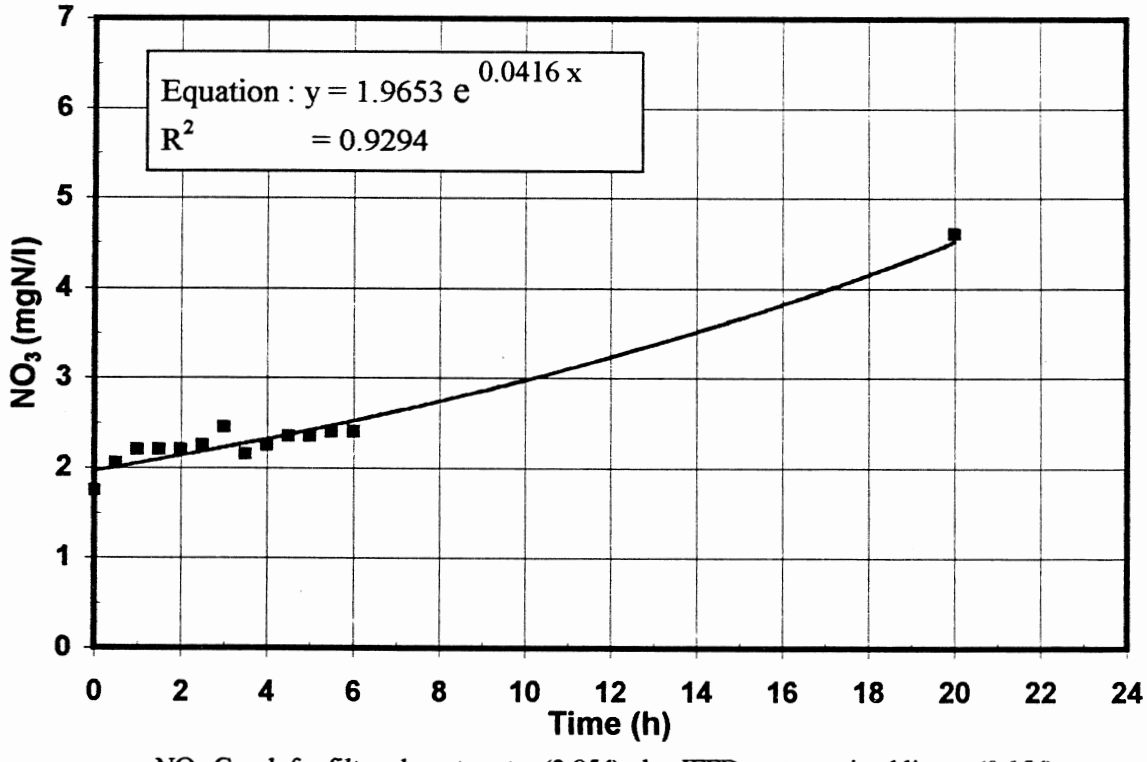
OUR-h Graph for filtered wastewater (2.9ℓ) plus IFFD system mixed liquor (0.1ℓ)
batch test E9 , 17-05a , wastewater batch no. 25



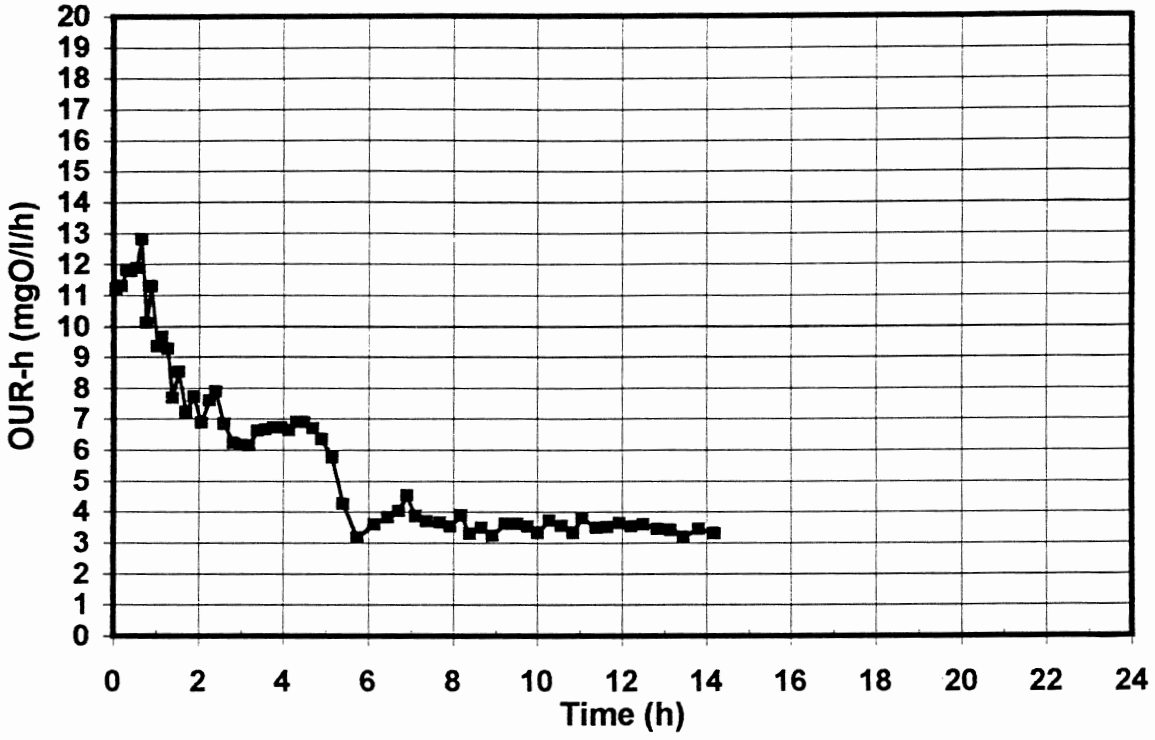
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batch test E9 , 17-05a , waste water batch no.25



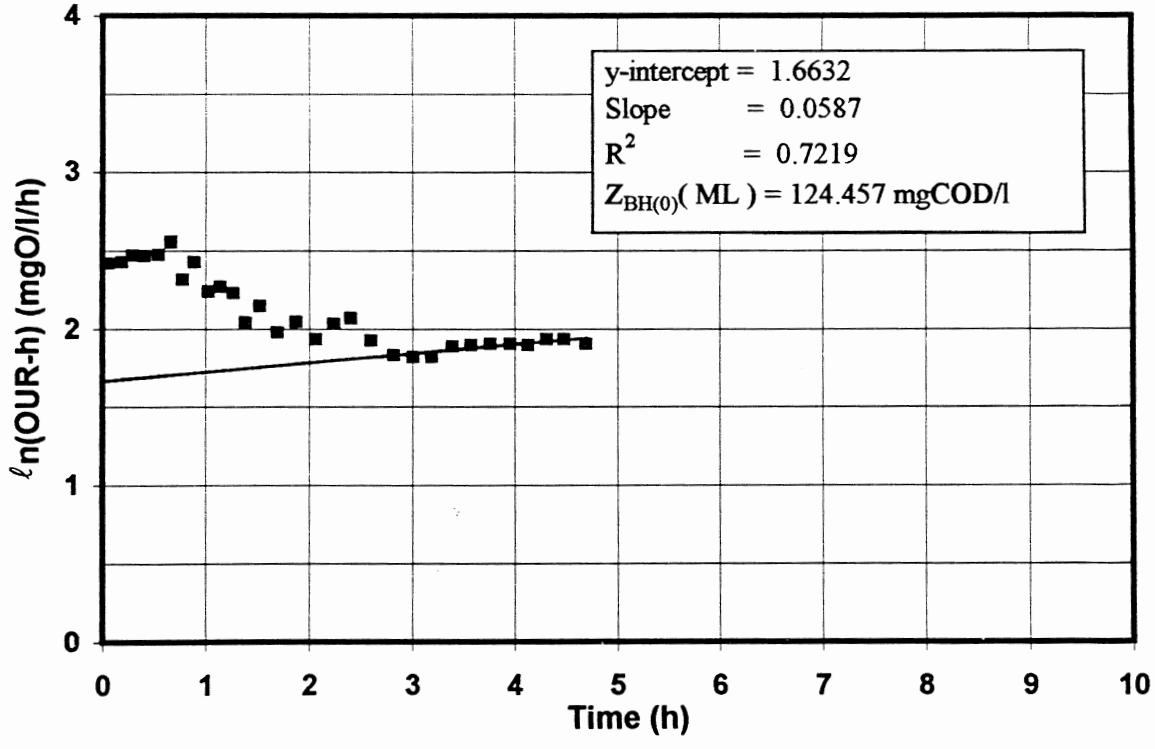
OUR Graph for filtered wastewater (2.85ℓ) plus IFFD system mixed liquor (0.15ℓ)
batch test E10 , 17-05b , wastewater batch no. 25



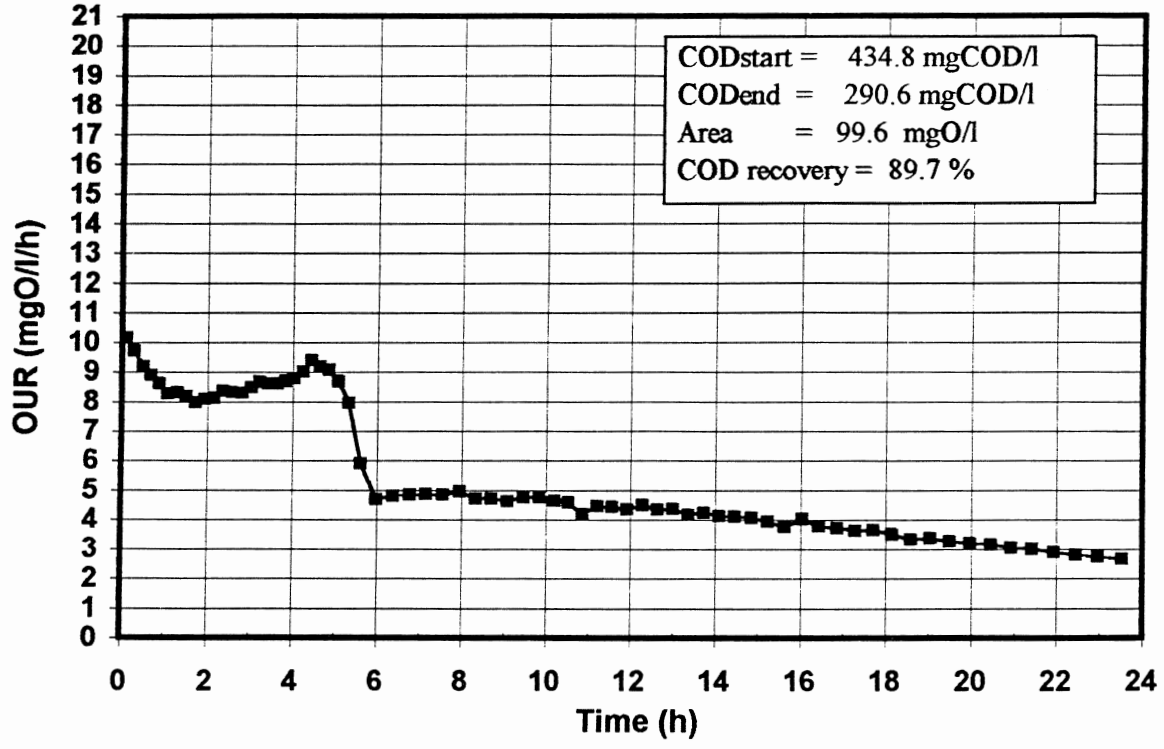
NO_3 Graph for filtered wastewater (2.85ℓ) plus IFFD system mixed liquor (0.15ℓ)
batch test E10 , 17-05b , wastewater batch no. 25



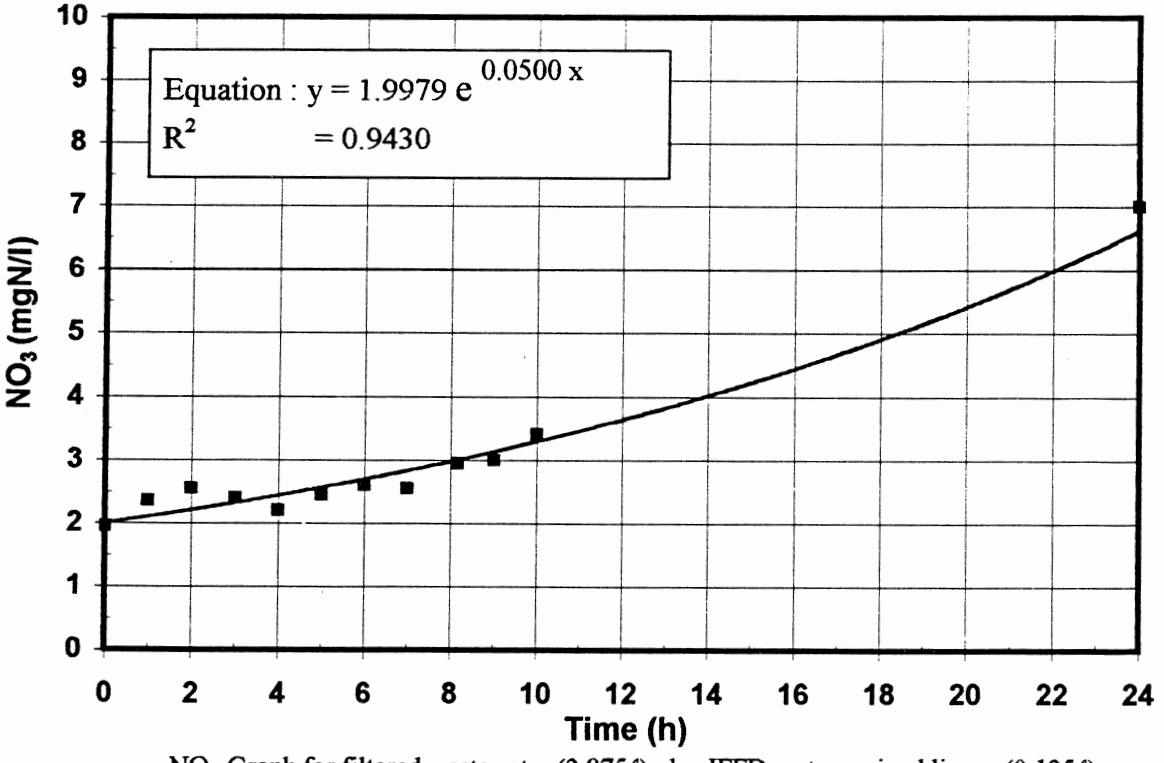
OUR-h Graph for filtered wastewater (2.85ℓ) plus IFFD system mixed liquor (0.15ℓ)
batch test E10 , 17-05b , wastewater batch no. 25



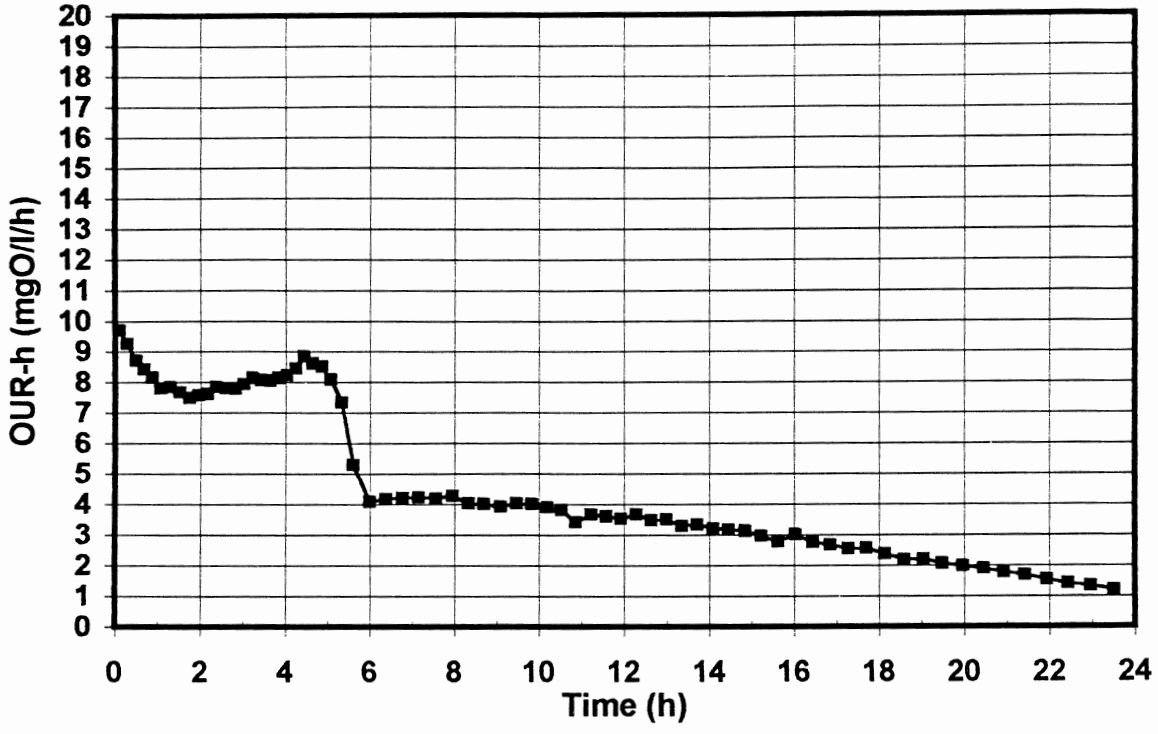
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batch test E10 , 17-05b , waste water batch no.25



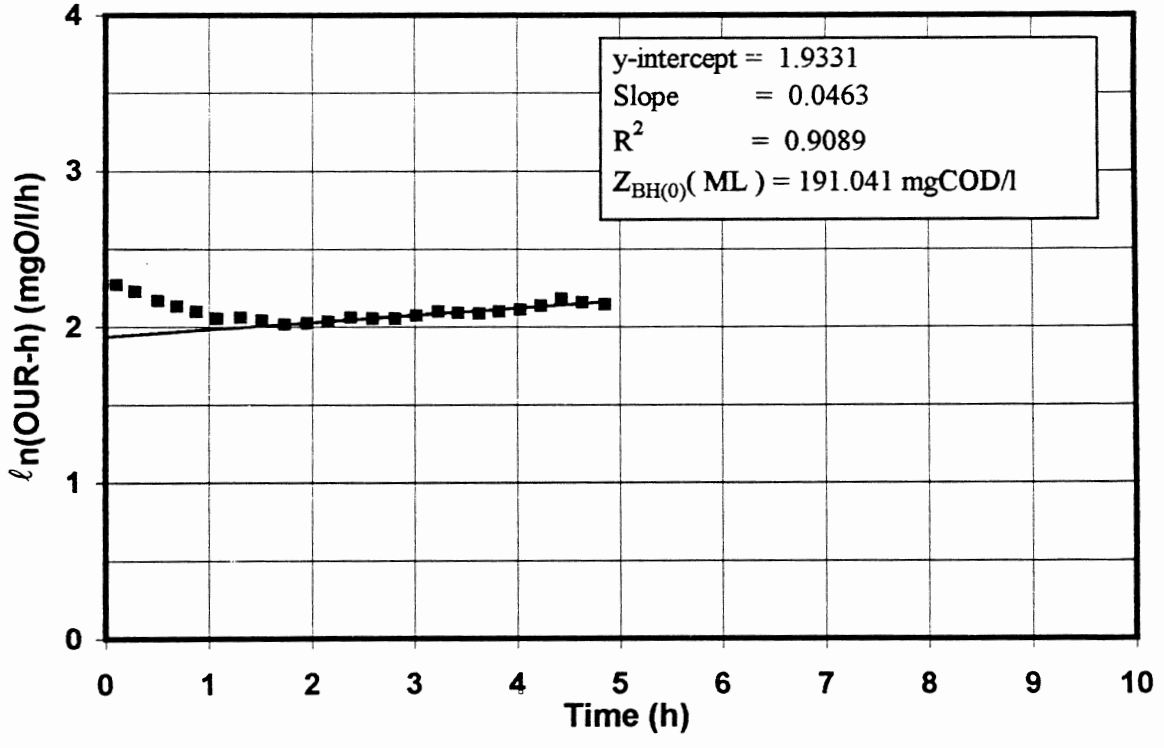
OUR Graph for filtered wastewater (2.875ℓ) plus IFFD system mixed liquor (0.125ℓ)
batch test E11 , 19-05a , wastewater batch no. 25



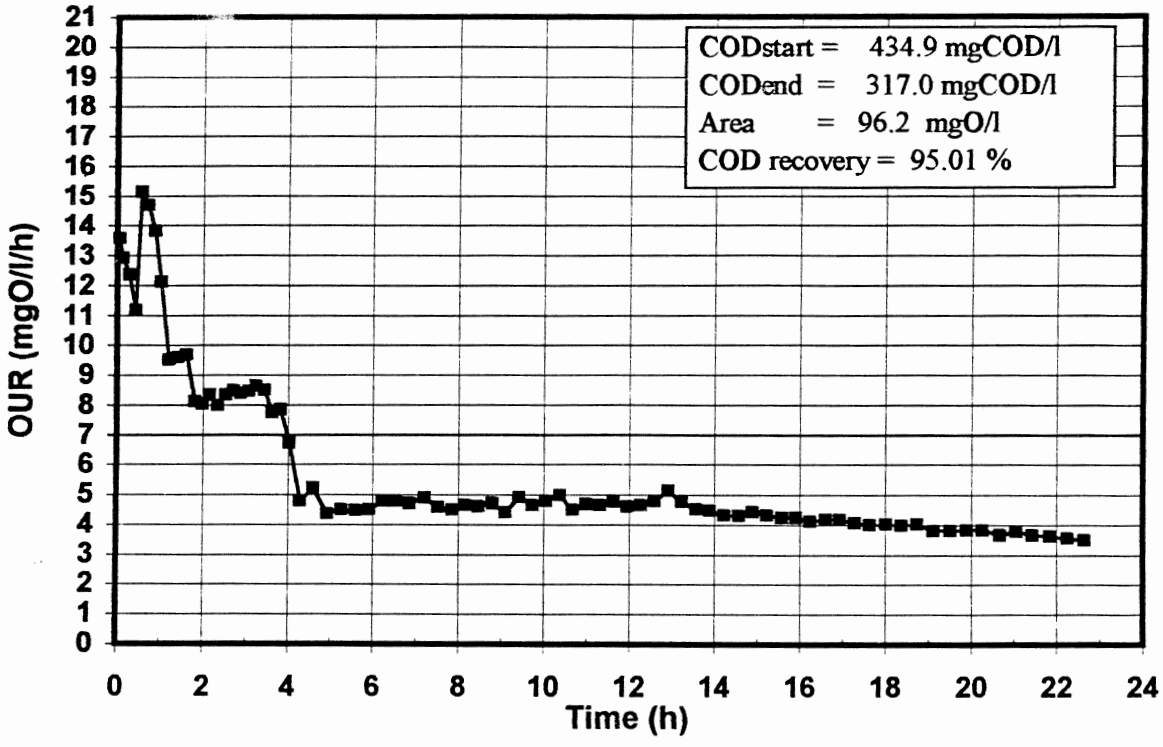
NO_3 Graph for filtered wastewater (2.875ℓ) plus IFFD system mixed liquor (0.125ℓ)
batch test E11 , 19-05a , wastewater batch no. 25



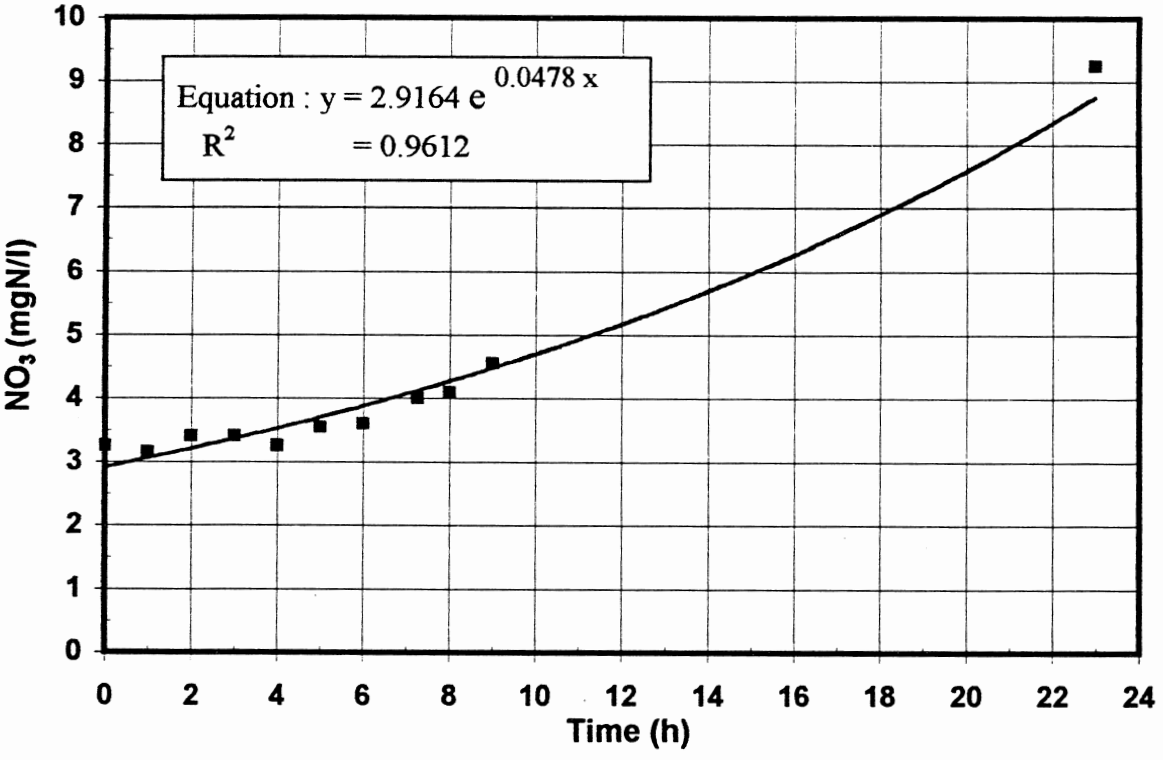
OUR-h Graph for filtered wastewater (2.875ℓ) plus IFFD system mixed liquor (0.125ℓ)
batch test E11 , 19-05a , wastewater batch no. 25



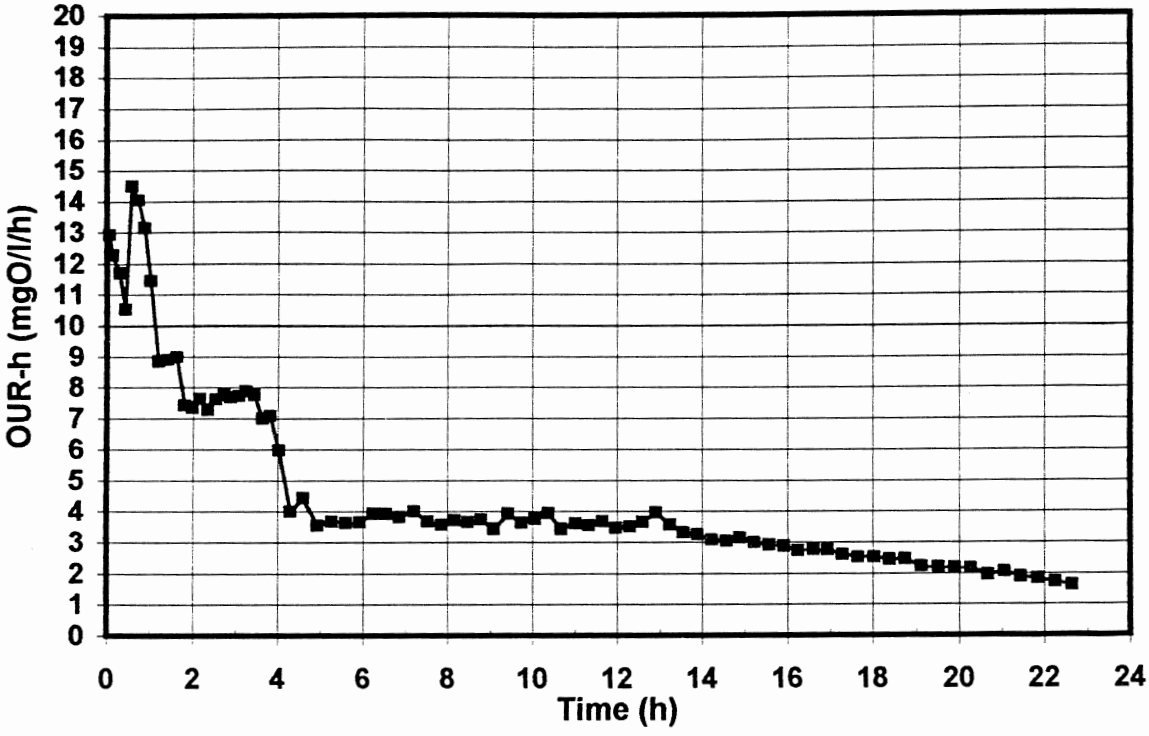
ℓn(OUR-h) for filtered wastewater (2.875ℓ) plus IFFD system mixed liquor (0.125ℓ)
batch test E11 , 19-05a , waste water batch no.25



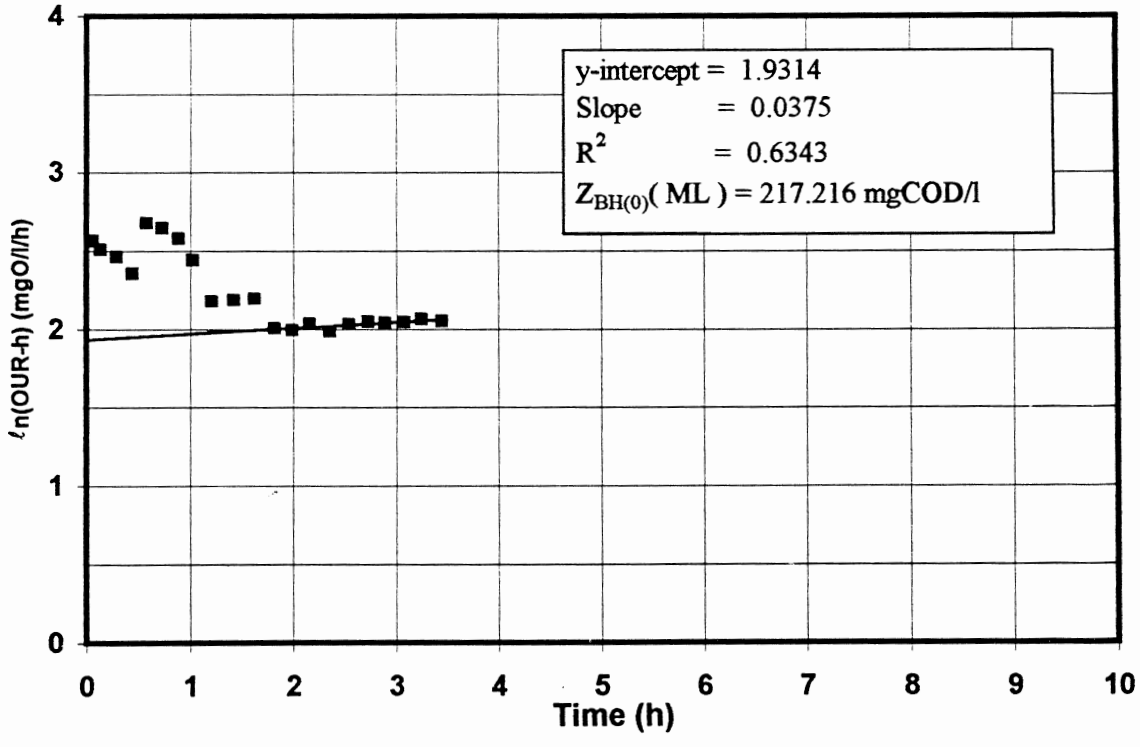
OUR Graph for filtered wastewater (2.825ℓ) plus IFFD system mixed liquor (0.175ℓ)
batch test E12 , 19-05b , wastewater batch no. 25



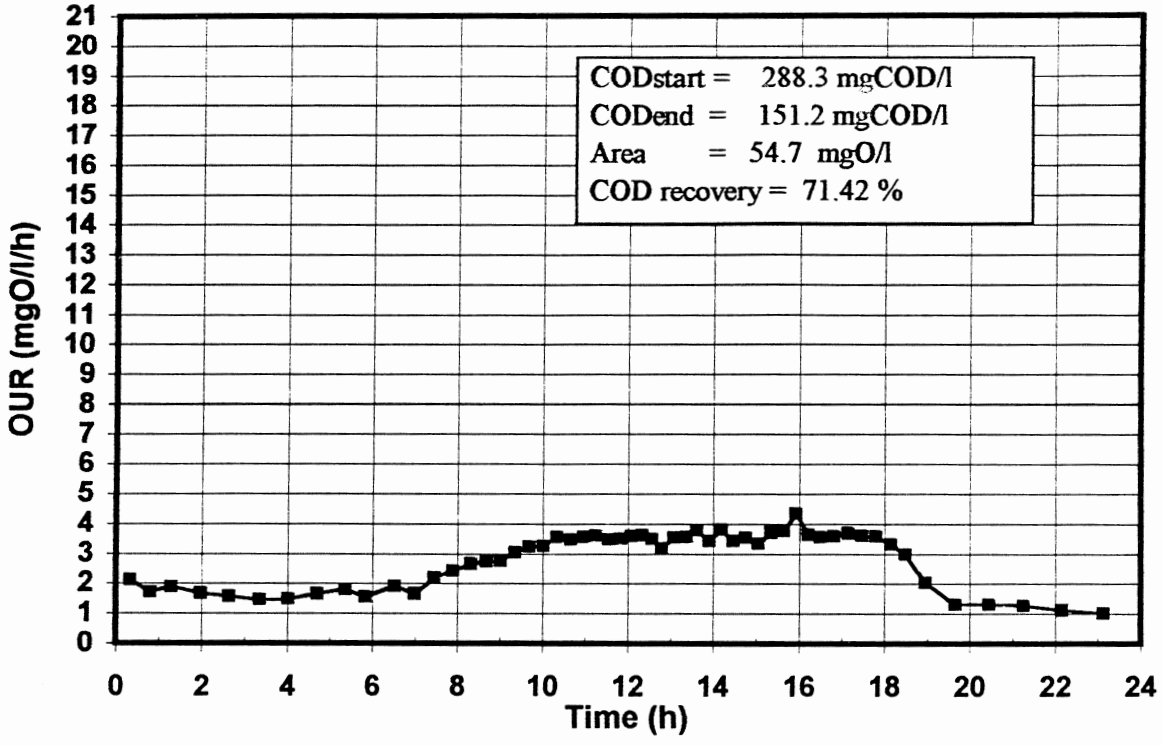
NO_3 Graph for filtered wastewater (2.825ℓ) plus IFFD system mixed liquor (0.175ℓ)
batch test E12 , 19-05b , wastewater batch no. 25



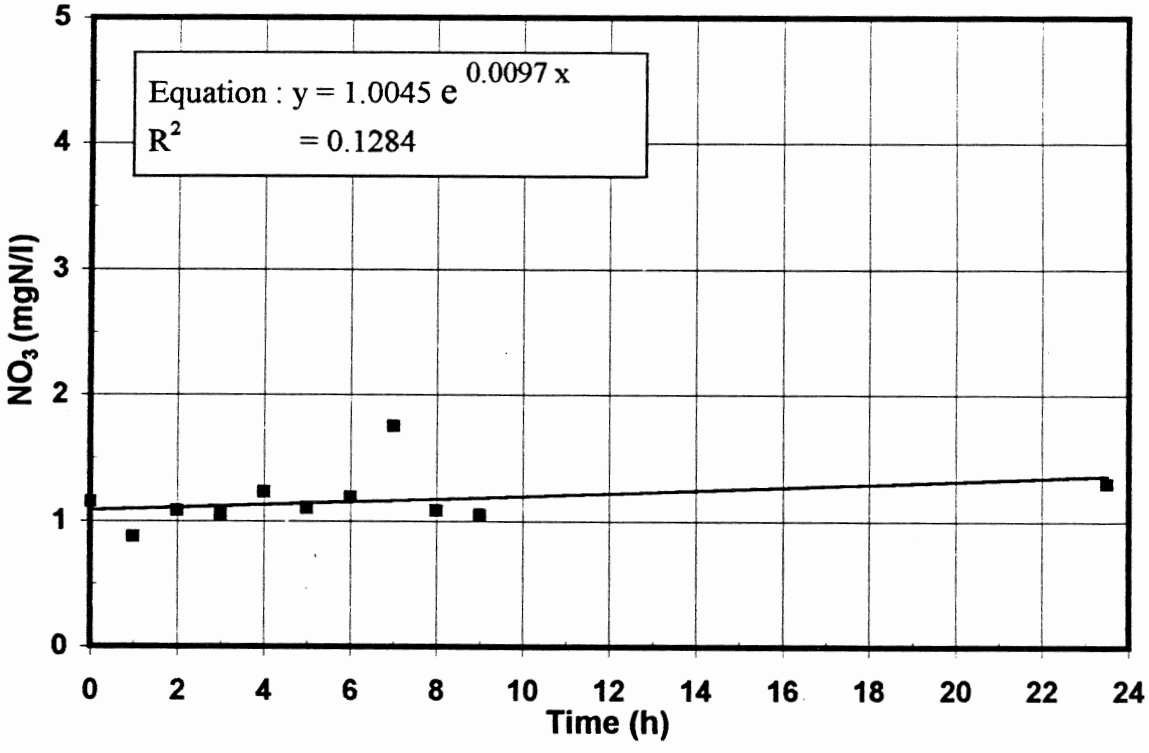
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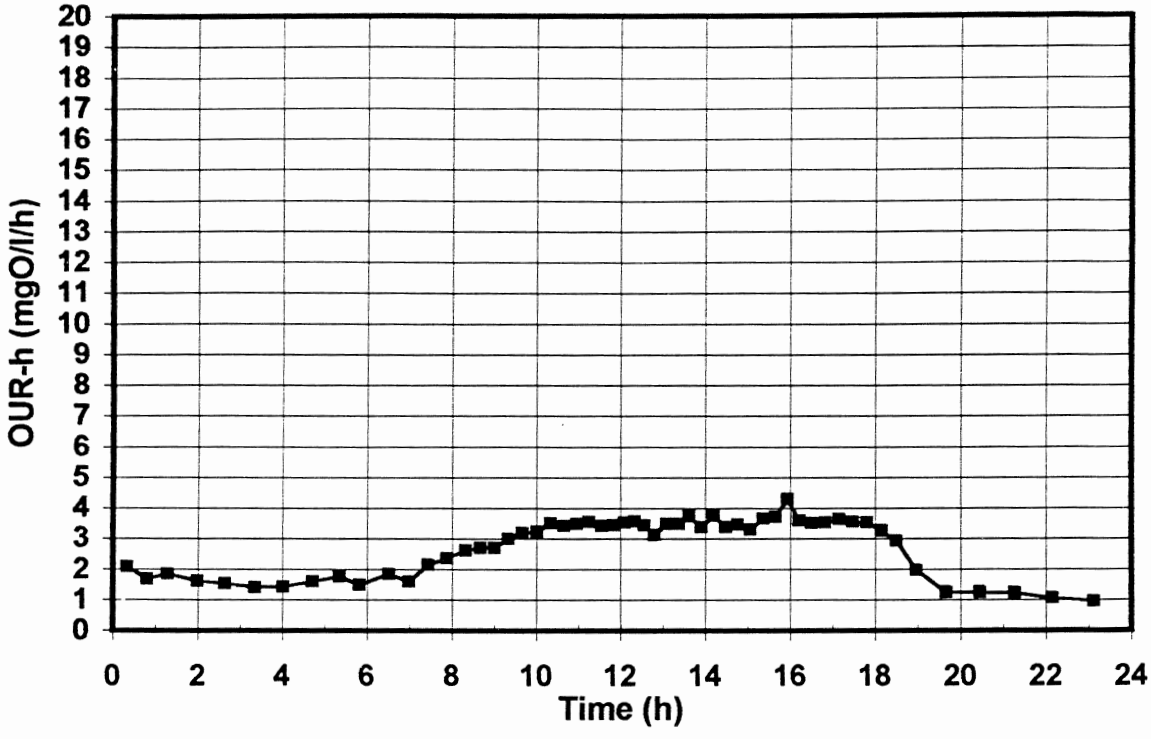
ℓn(OUR-h) for filtered wastewater (2.825ℓ) plus IFFD system mixed liquor (0.175ℓ)
batch test E12 , 19-05b , waste water batch no.25



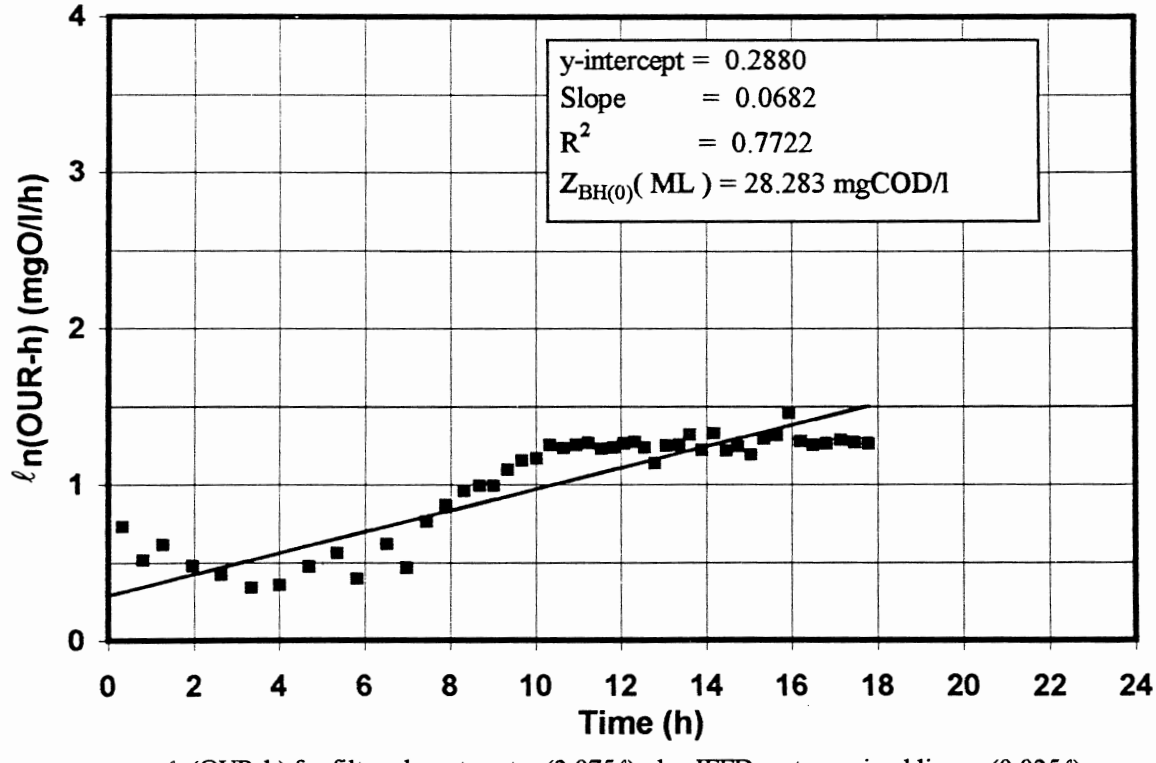
OUR Graph for filtered wastewater (2.975ℓ) plus IFFD system mixed liquor (0.025ℓ)
batch test E13 , 16-06a , wastewater batch no. 27



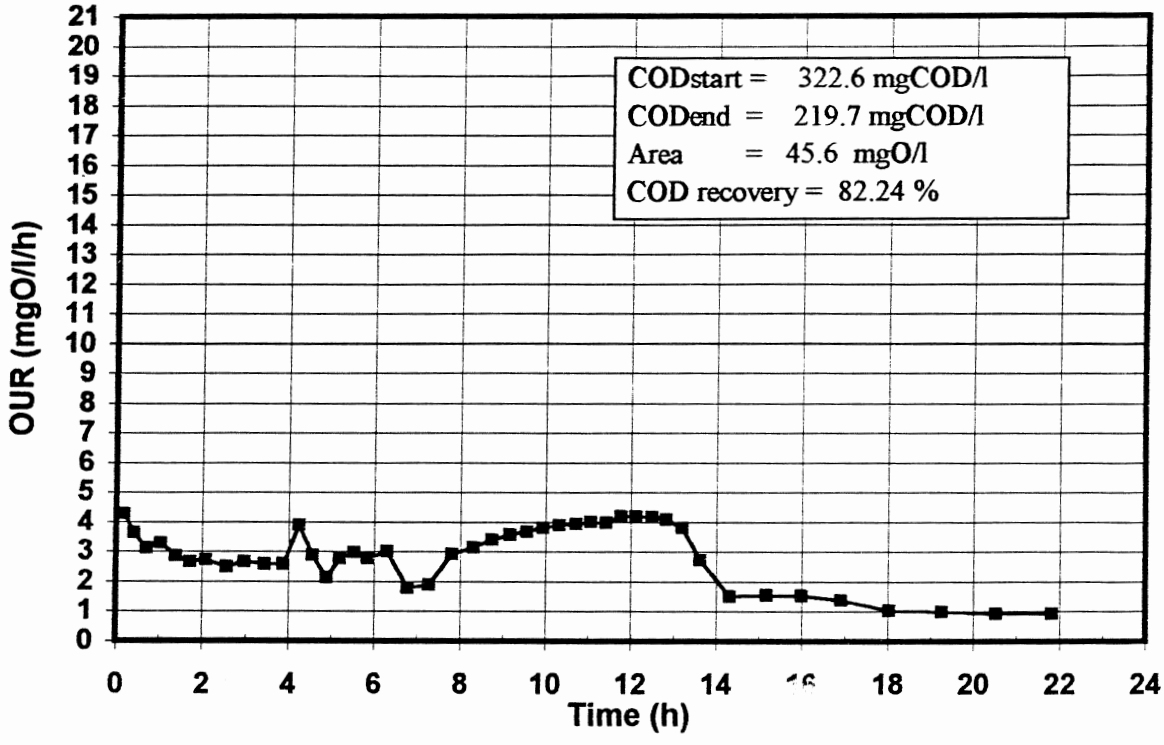
NO_3 Graph for filtered wastewater (2.975ℓ) plus IFFD system mixed liquor (0.025ℓ)
batch test E13 , 16-06a , wastewater batch no. 27



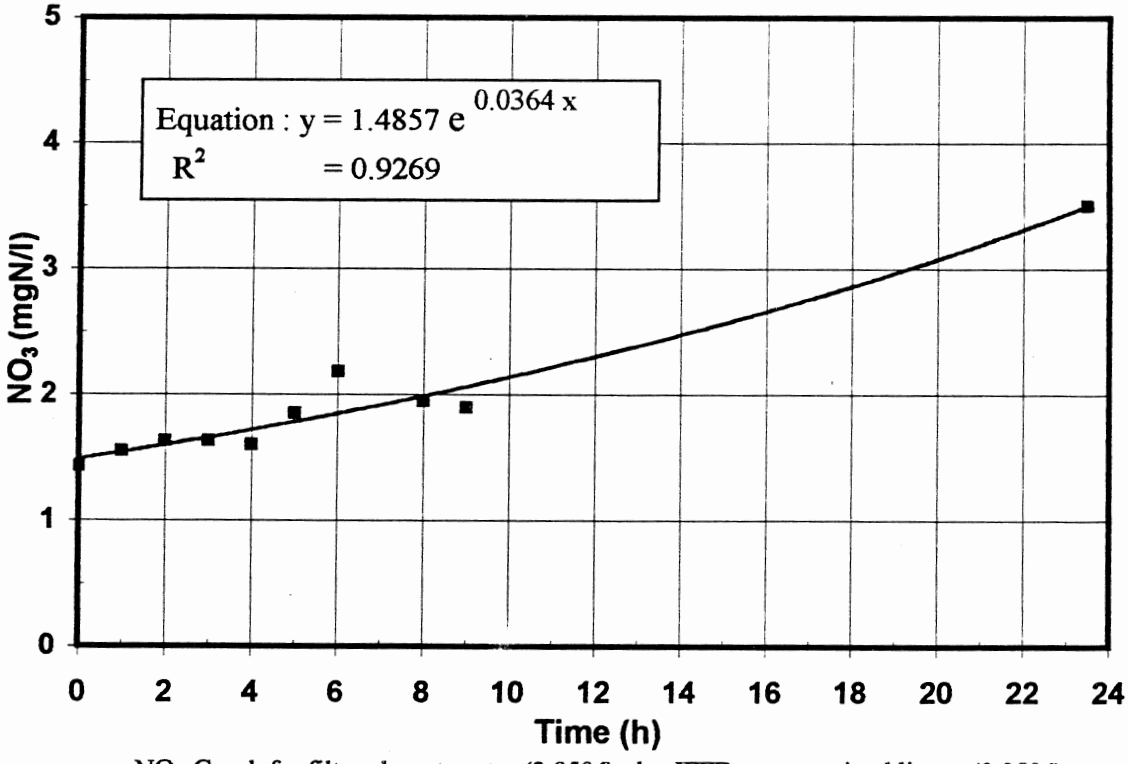
OUR-h Graph for filtered wastewater (2.975ℓ) plus IFFD system mixed liquor (0.025ℓ)
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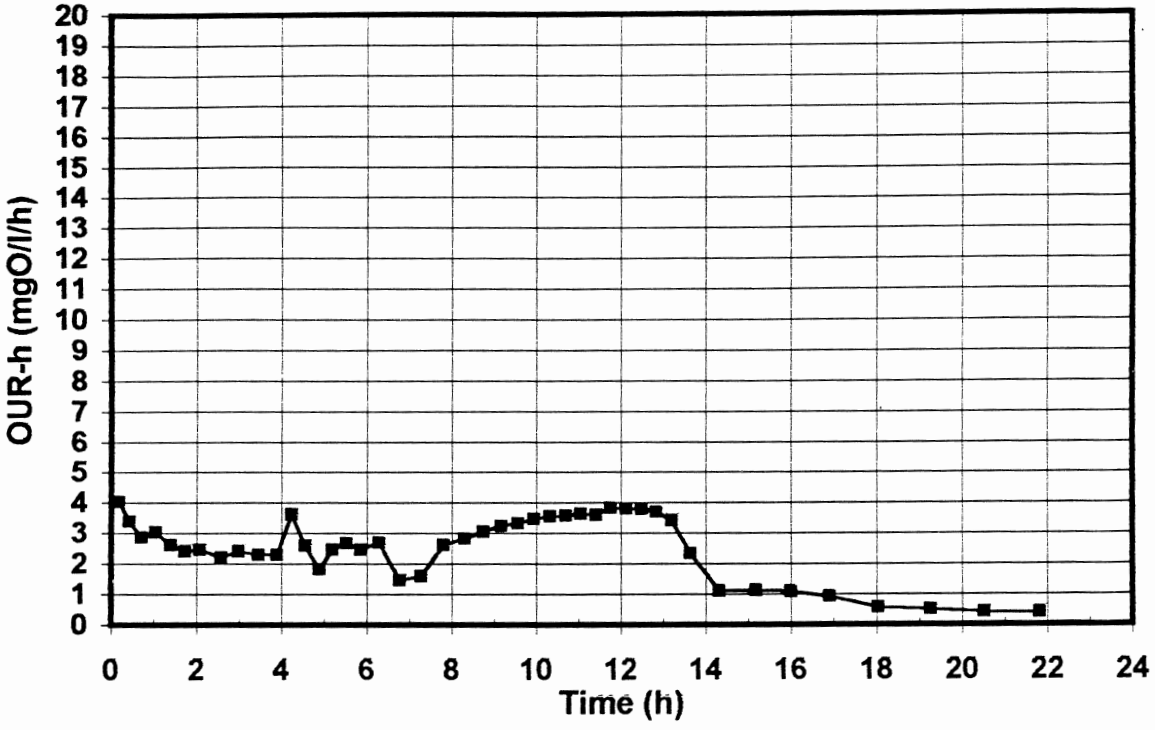
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batch test E13 , 16-06a , waste water batch no.27



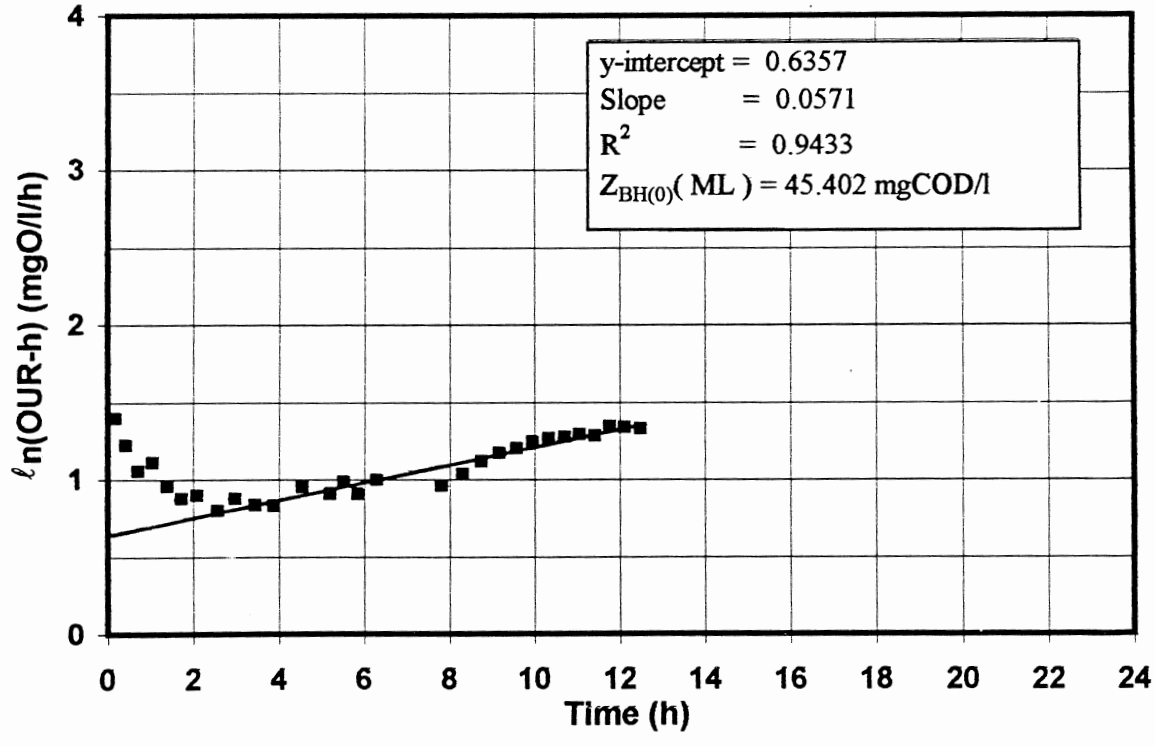
OUR Graph for filtered wastewater (2.950ℓ) plus IFFD system mixed liquor (0.050ℓ)
batch test E14 , 16-06b , wastewater batch no. 27



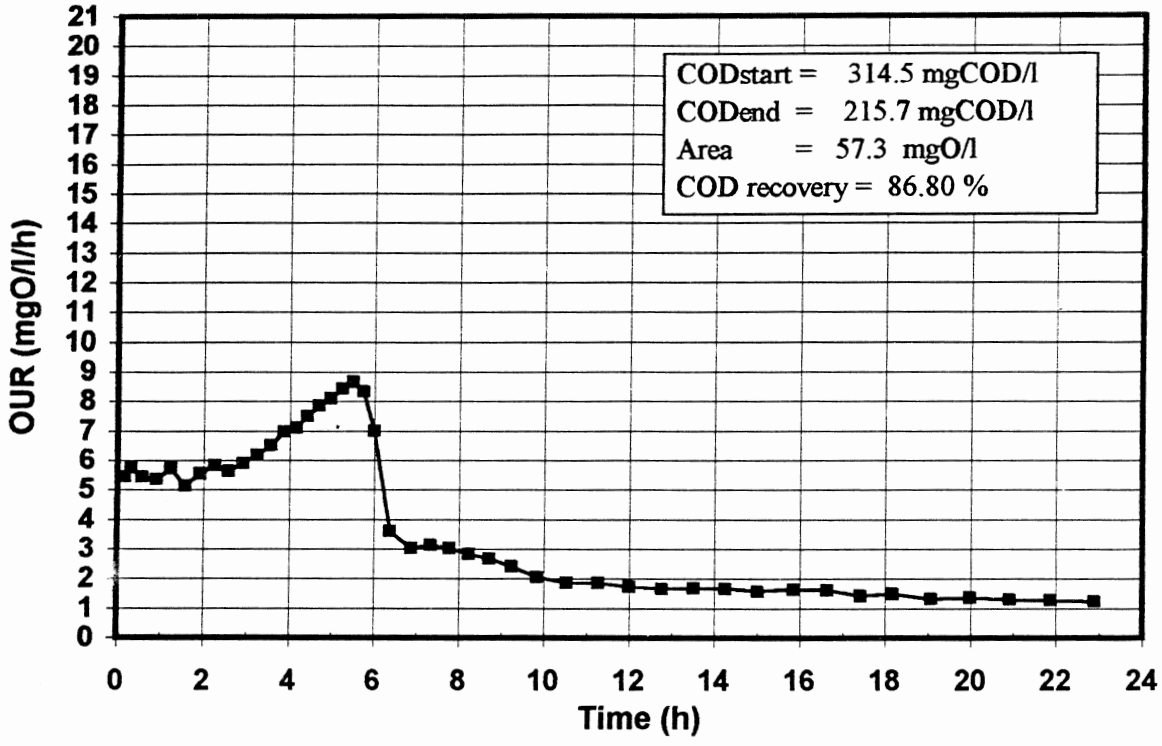
NO_3 Graph for filtered wastewater (2.950ℓ) plus IFFD system mixed liquor (0.050ℓ)
batch test E14 , 16-06b, wastewater batch no. 27



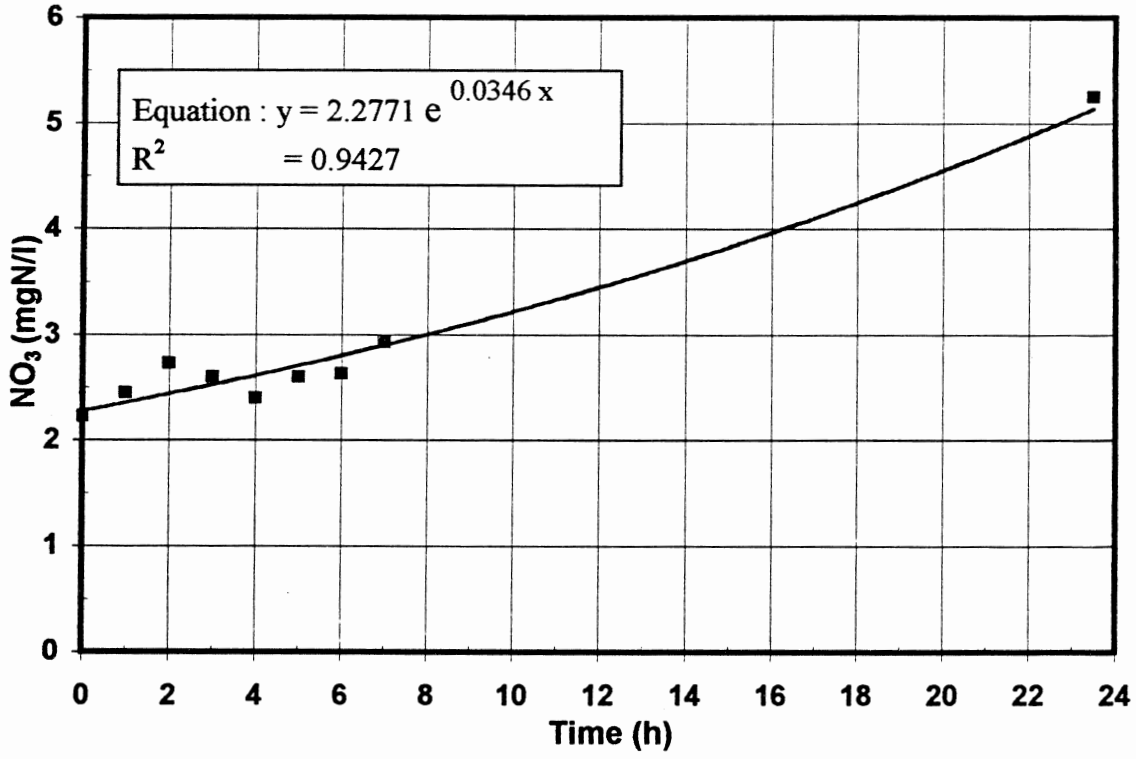
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batch test E14 , 16-06b , wastewater batch no. 27



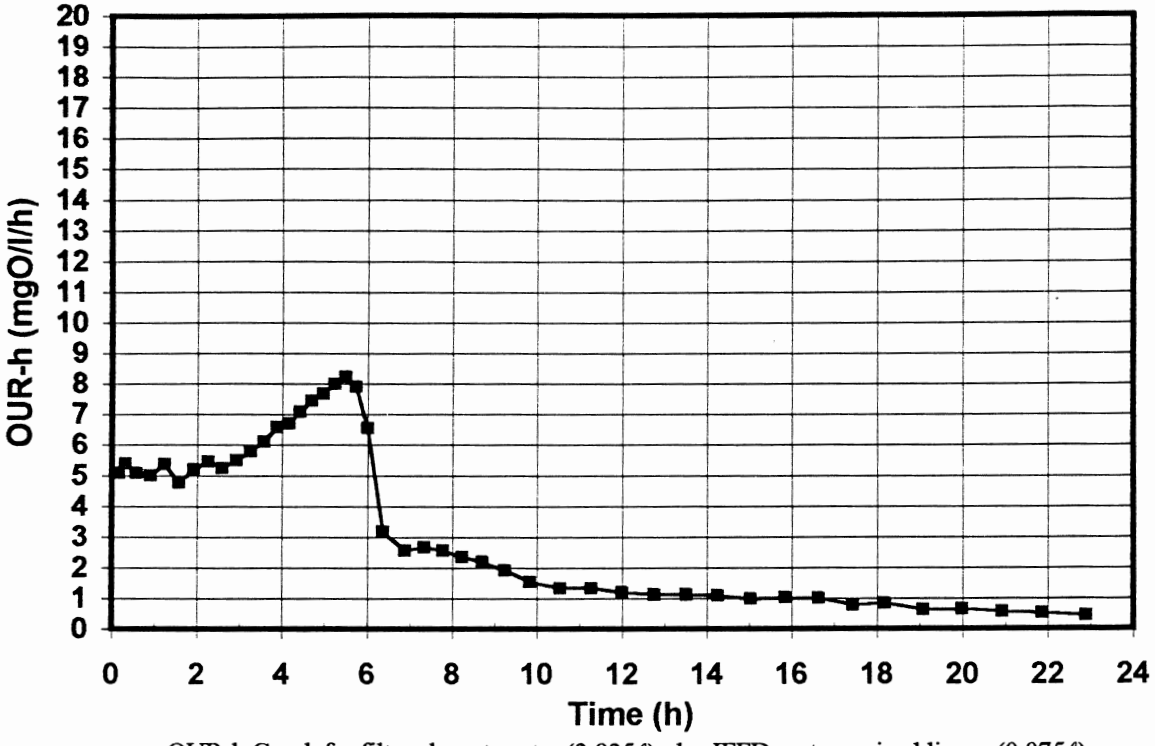
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batch test E14 , 16-06b , waste water batch no.27



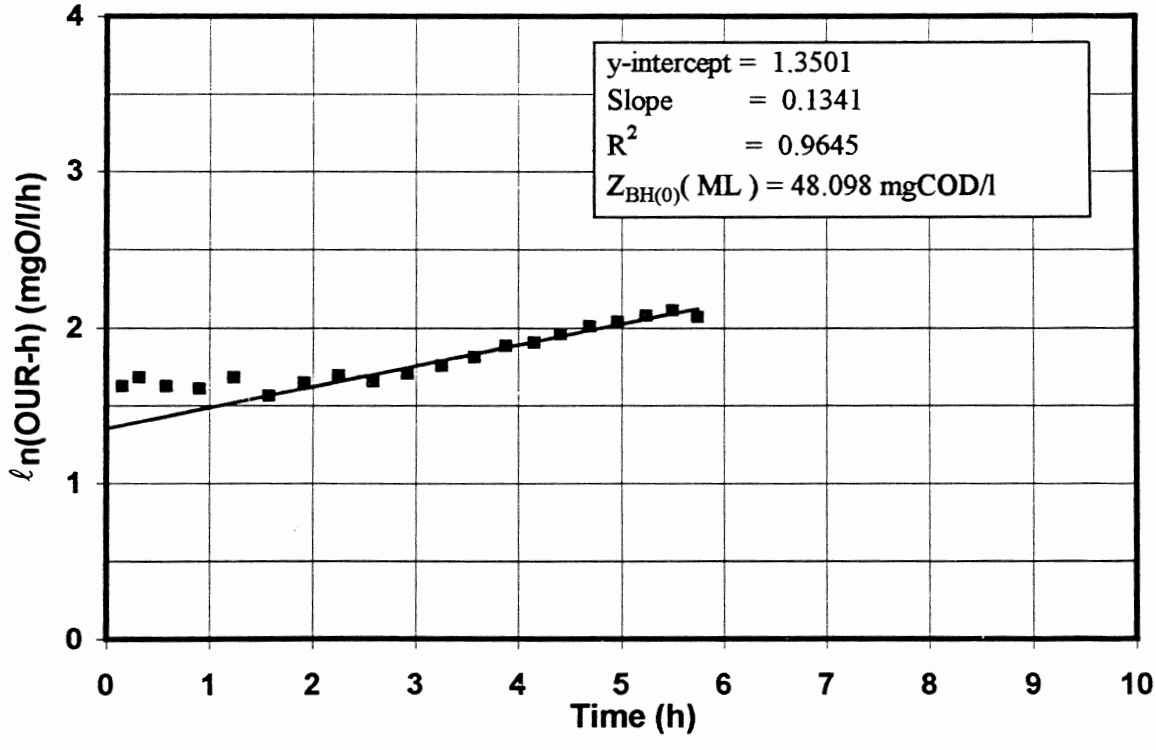
OUR Graph for filtered wastewater (2.925ℓ) plus IFFD system mixed liquor (0.075ℓ)
batch test E15 , 19-06a , wastewater batch no. 27



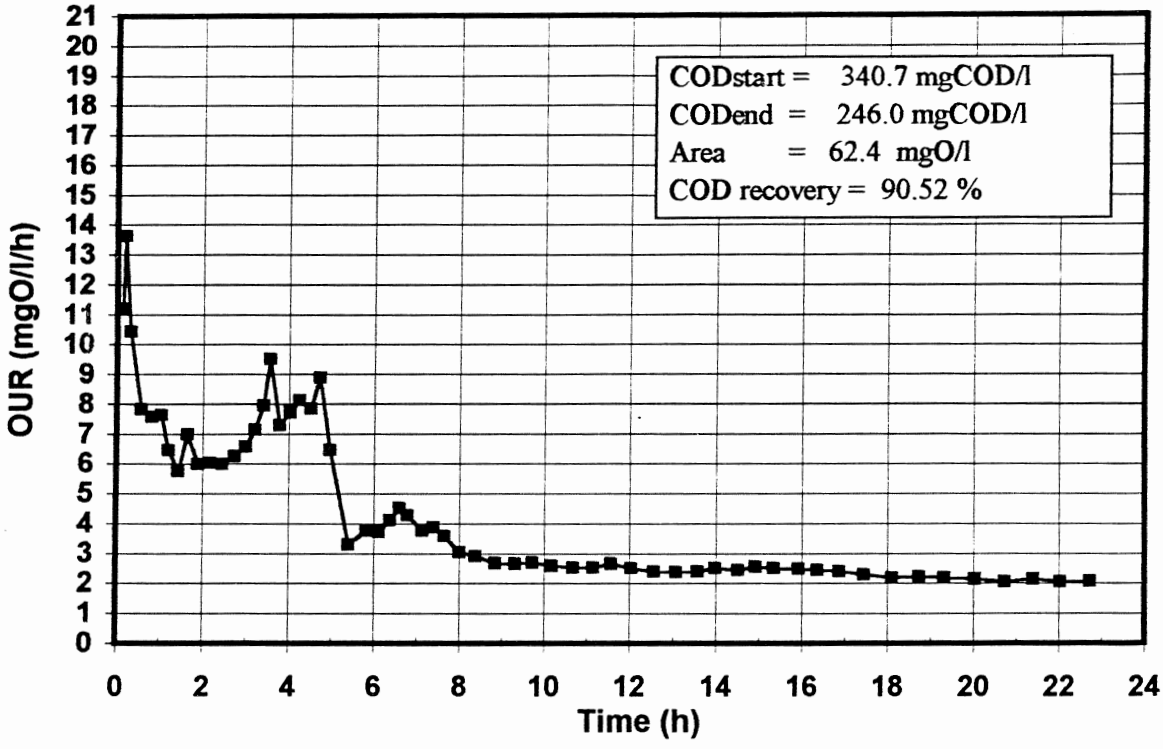
NO_3 Graph for red wastewater (2.925ℓ) plus IFFD system mixed liquor (0.075ℓ)
batch test E15 , 19-06a , wastewater batch no. 27



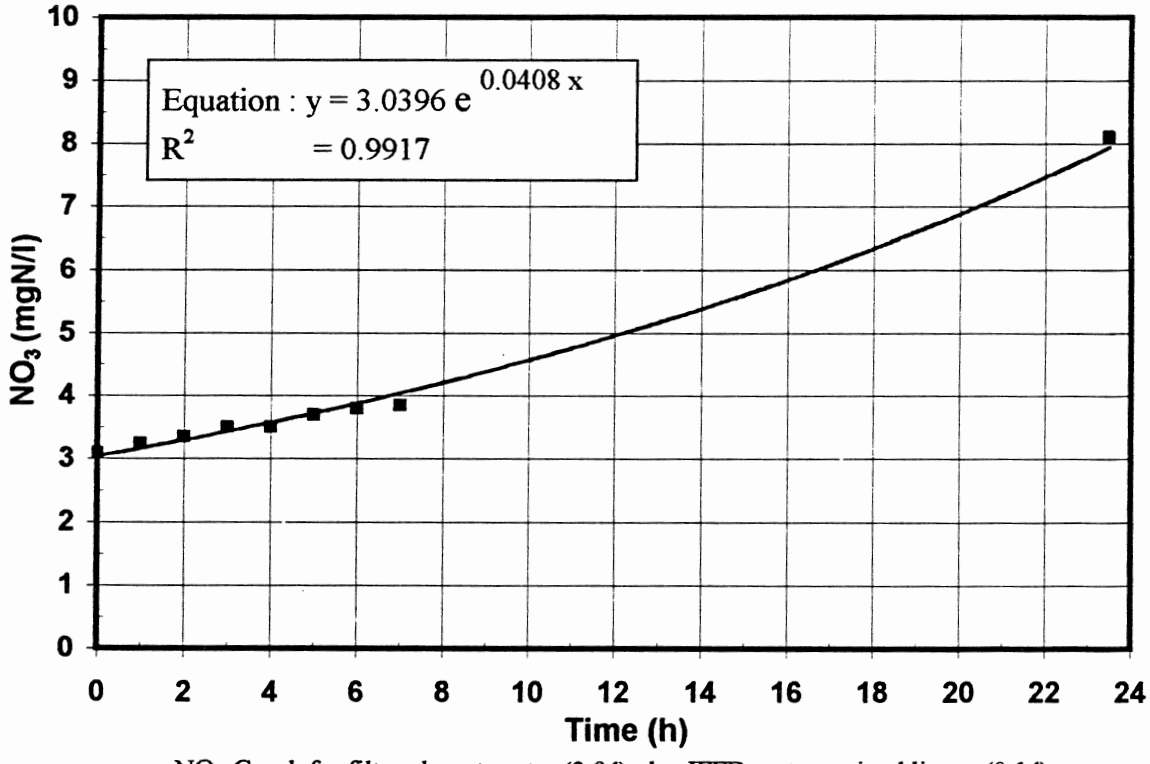
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batch test E15 , 19-06a , wastewater batch no. 27



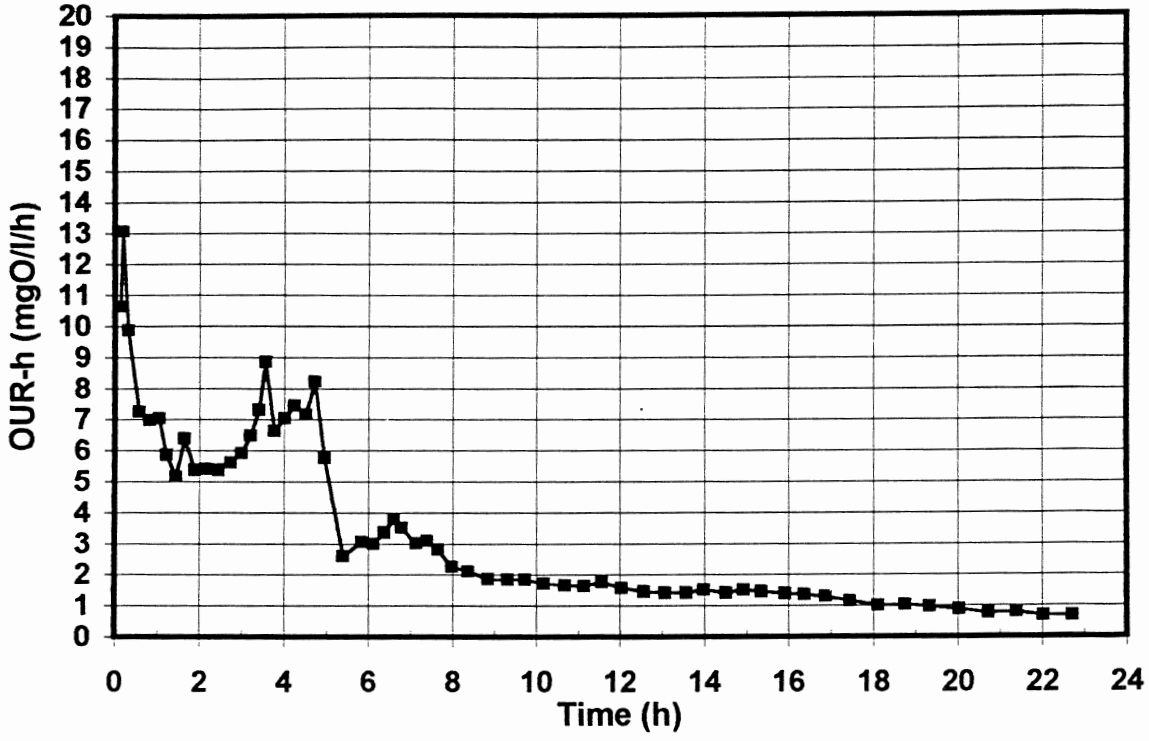
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batch test E15 , 19-06a , waste water batch no.27



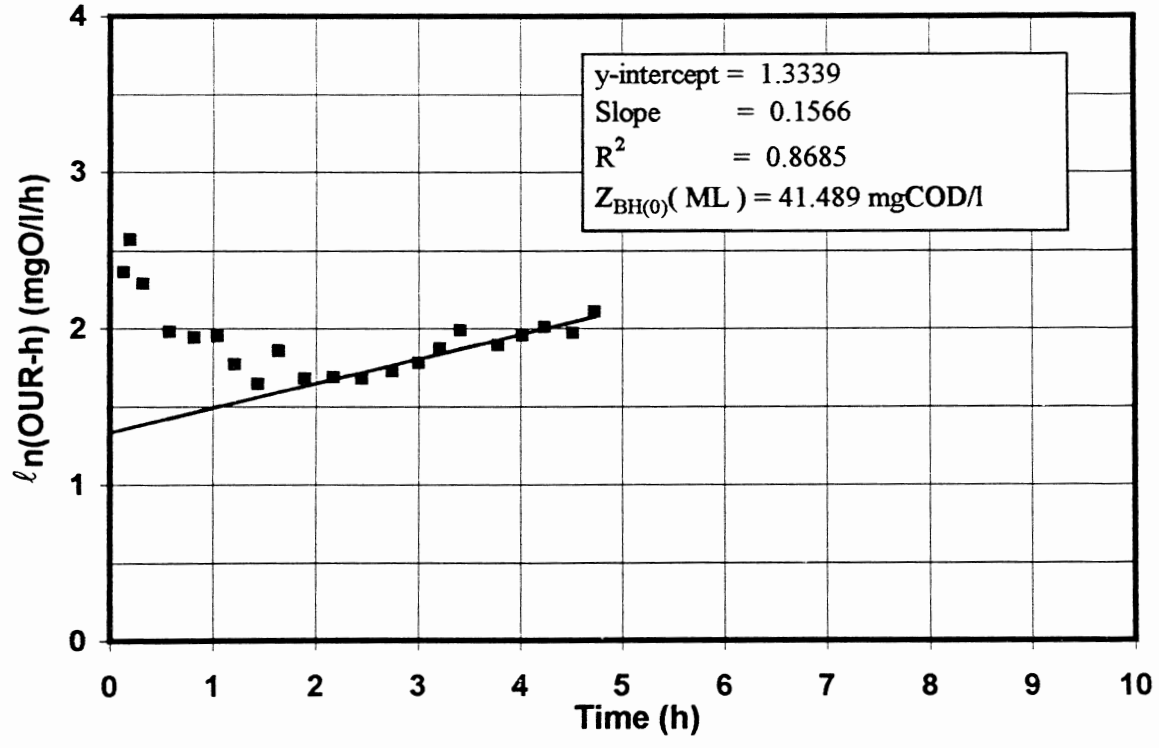
OUR Graph for filtered wastewater (2.9ℓ) plus IFFD system mixed liquor (0.1ℓ)
batch test E16 , 19-06b , wastewater batch no. 27



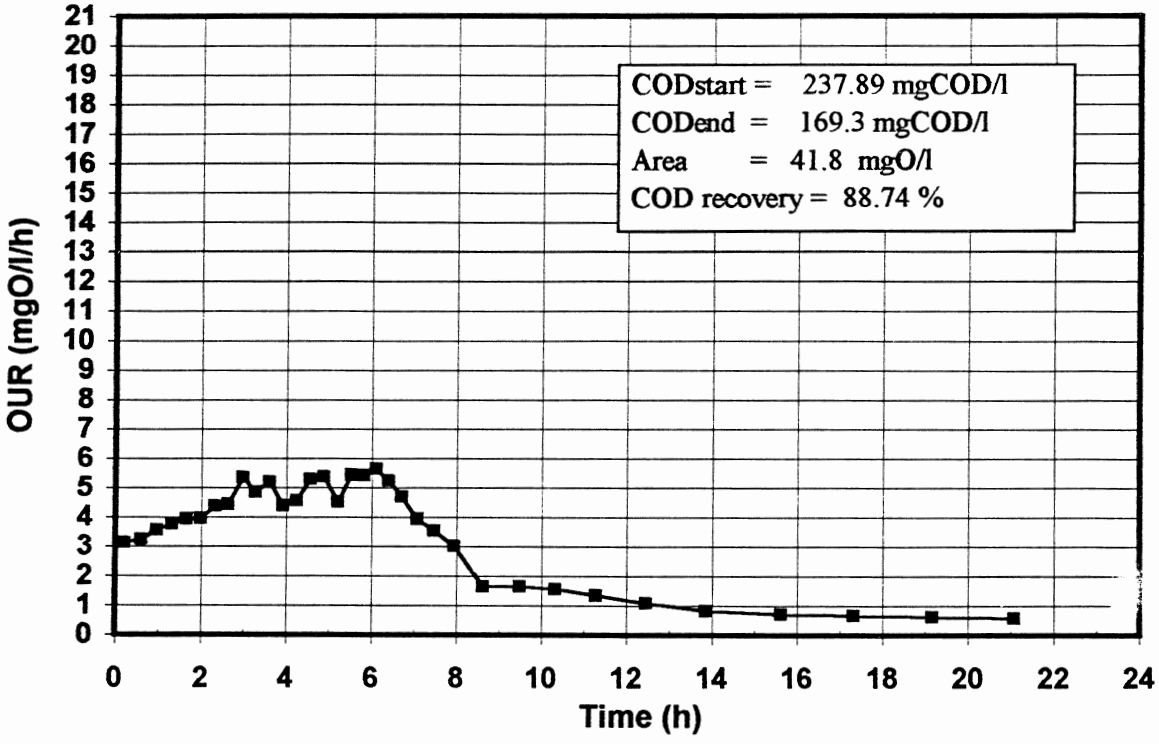
NO_3 Graph for filtered wastewater (2.9ℓ) plus IFFD system mixed liquor (0.1ℓ)
batch test E16 , 19-06b , wastewater batch no. 27



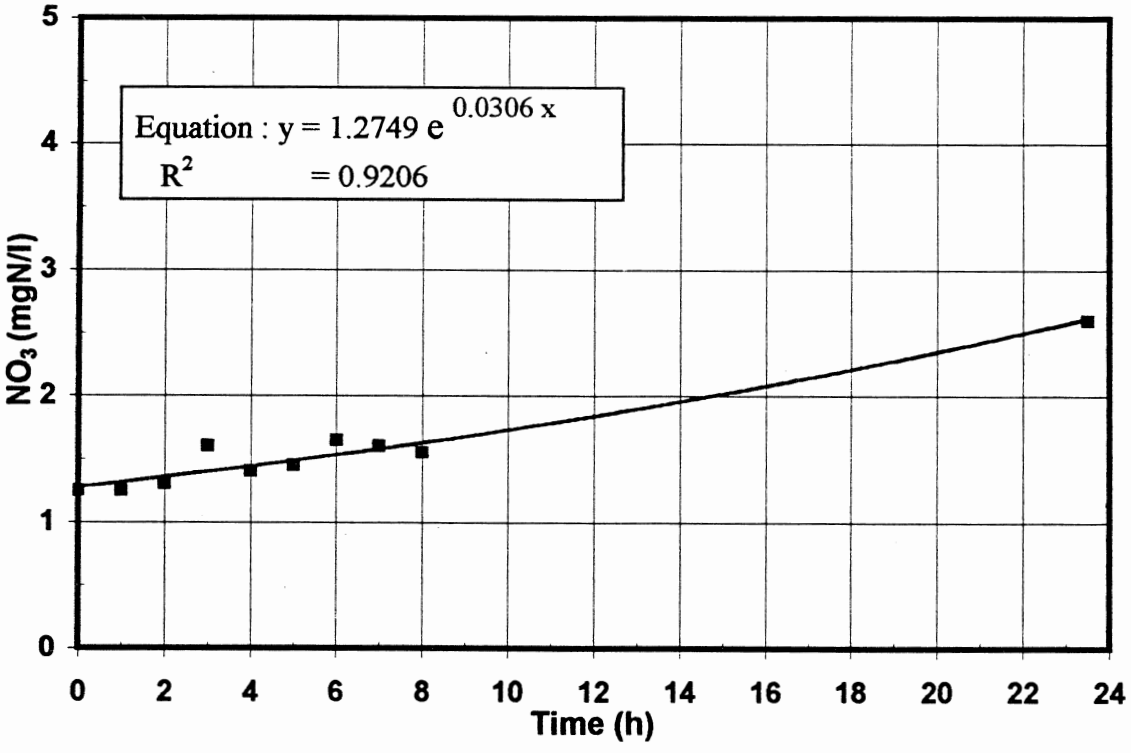
OUR-h Graph for filtered wastewater (2.9ℓ) plus IFFD system mixed liquor (0.1ℓ)
batch test E16 , 19-06b , wastewater batch no. 27



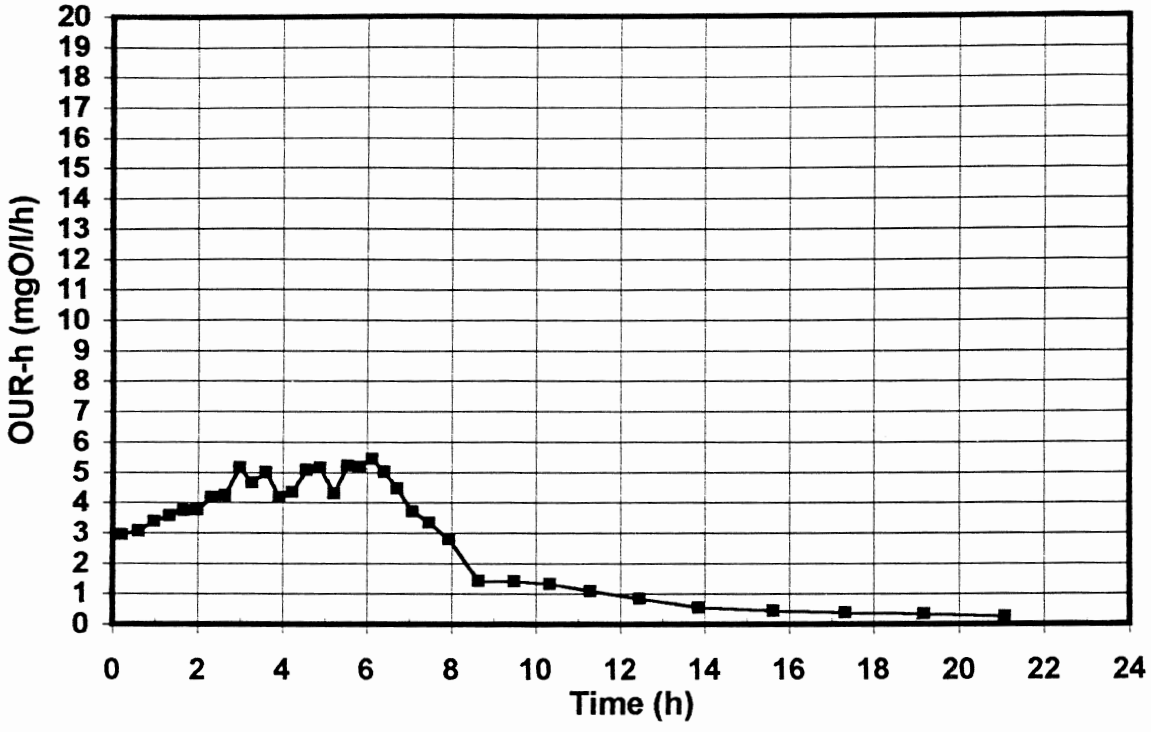
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batch test E16 , 19-06b , waste water batch no.27



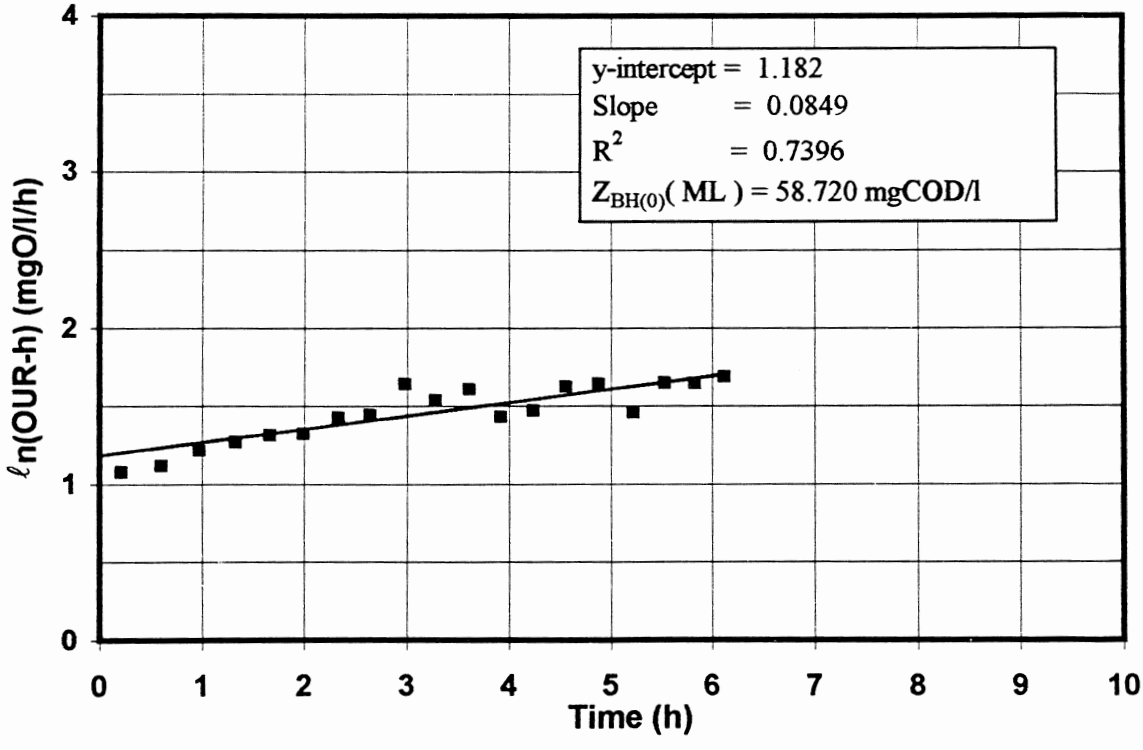
OUR Graph for filtered wastewater (2.975ℓ) plus IFFD system mixed liquor (0.025ℓ)
batch test E17 , 21-06a , wastewater batch no. 28



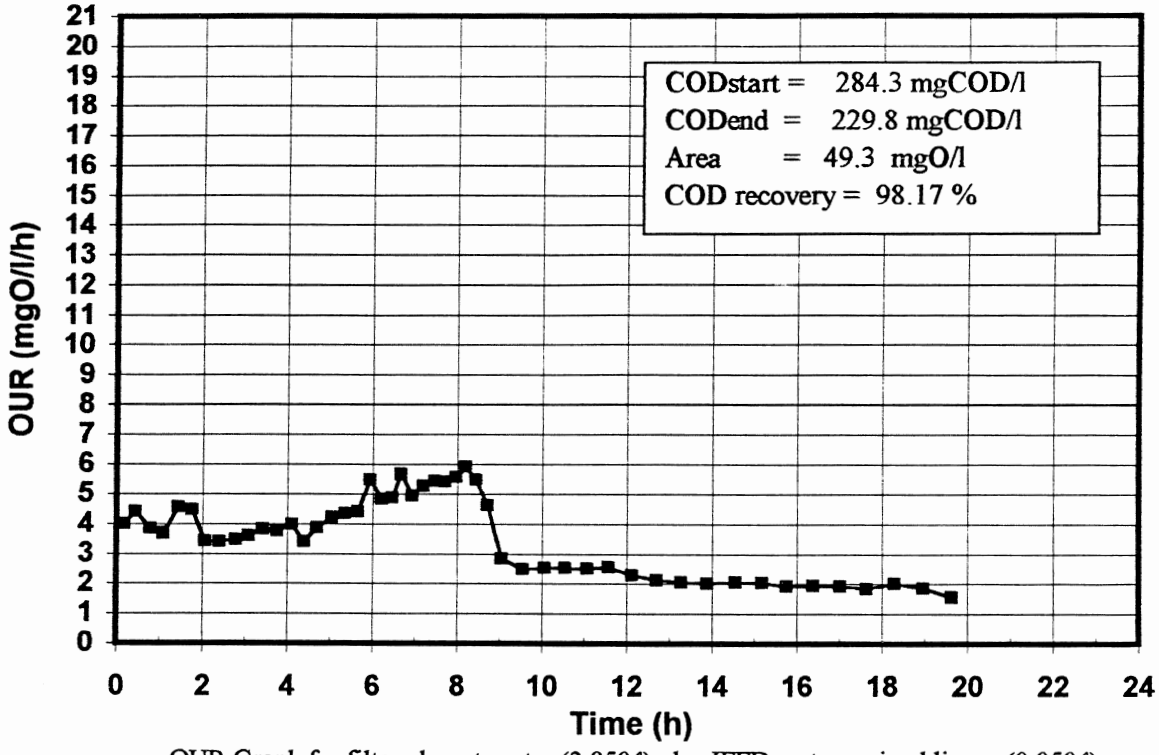
NO_3 Graph for filtered wastewater (2.975ℓ) plus IFFD system mixed liquor (0.025ℓ)
batch test E17 , 21-06a , wastewater batch no. 28



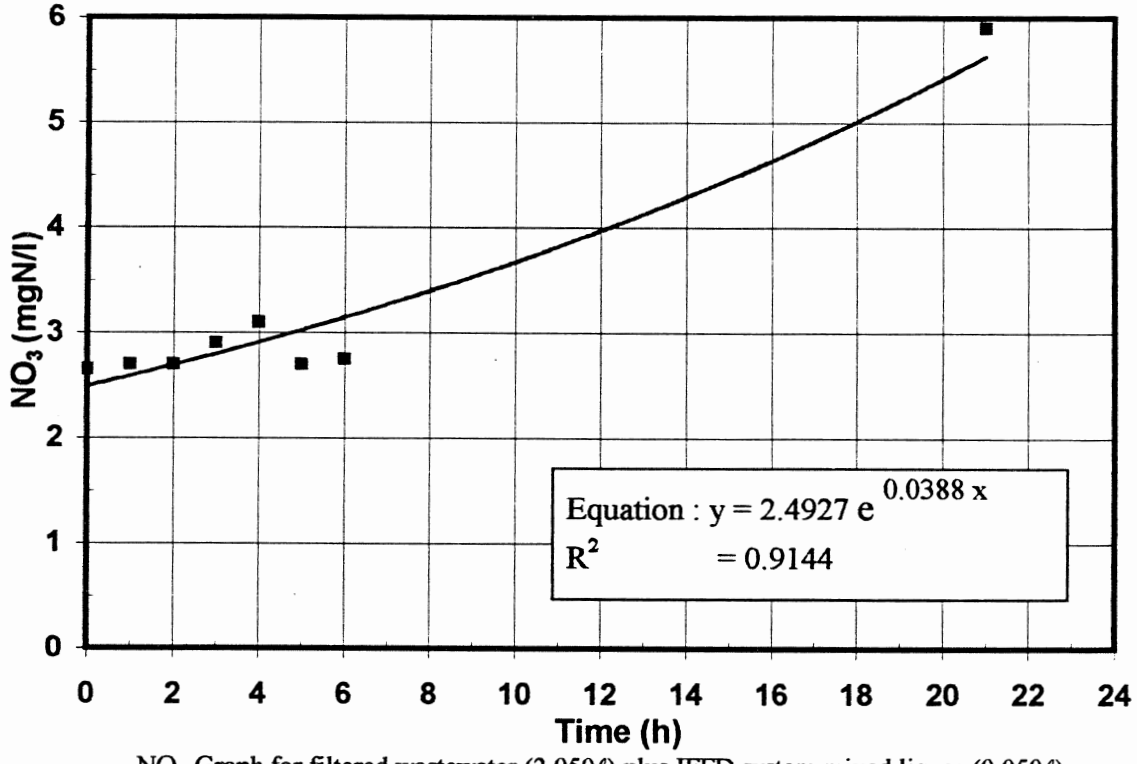
OUR-h Graph for filtered wastewater (2.975ℓ) plus IFFD system mixed liquor (0.025ℓ)
batch test E17 , 21-06a , wastewater batch no. 28



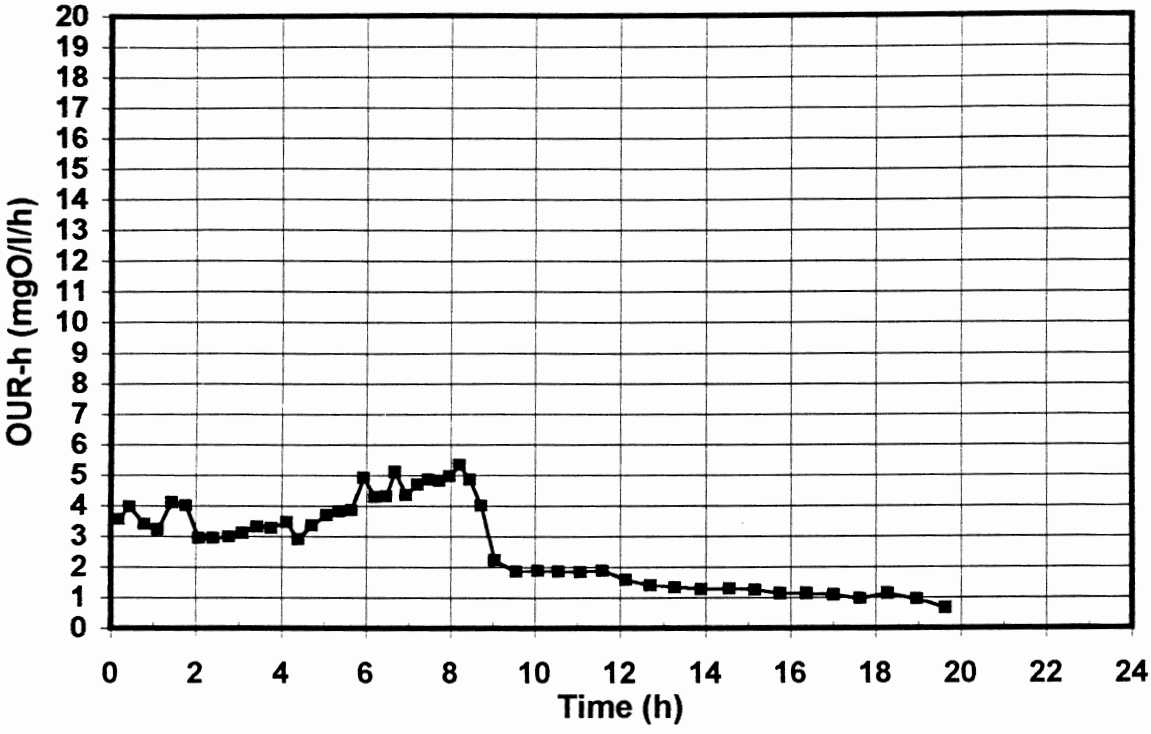
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batch test E17 , 21-06a , waste water batch no.28



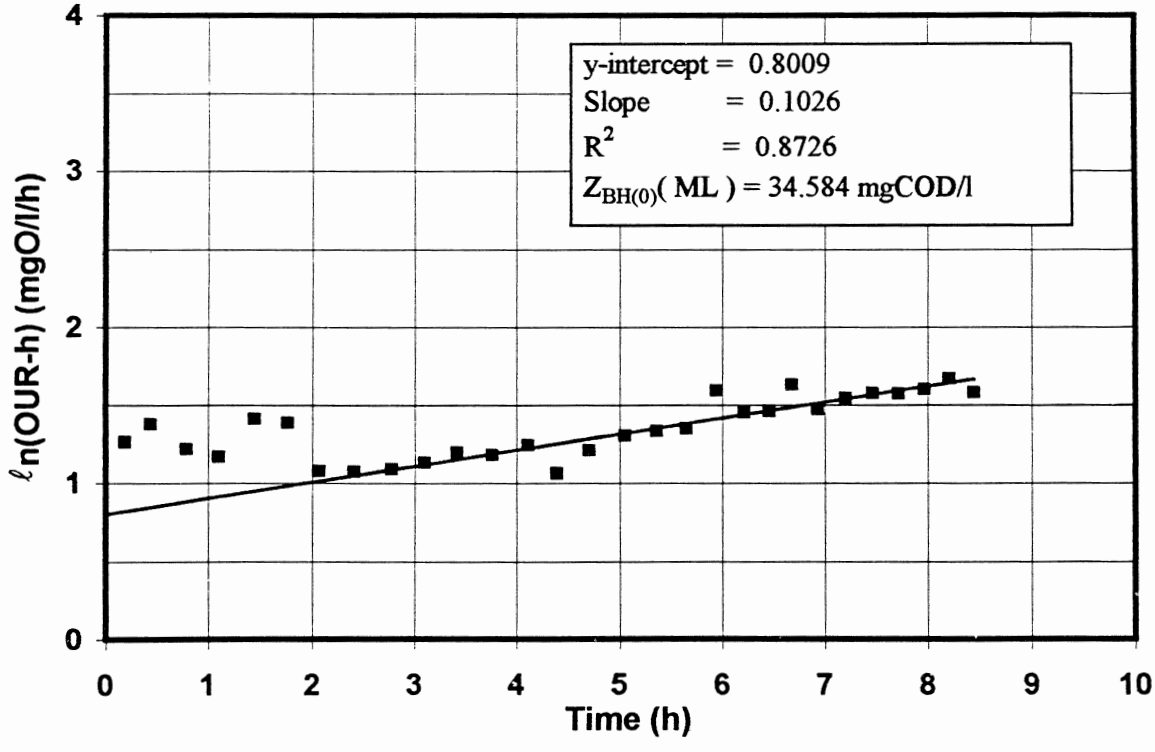
OUR Graph for filtered wastewater (2.950ℓ) plus IFFD system mixed liquor (0.050ℓ)
batch test E18 , 21-06b , wastewater batch no. 28



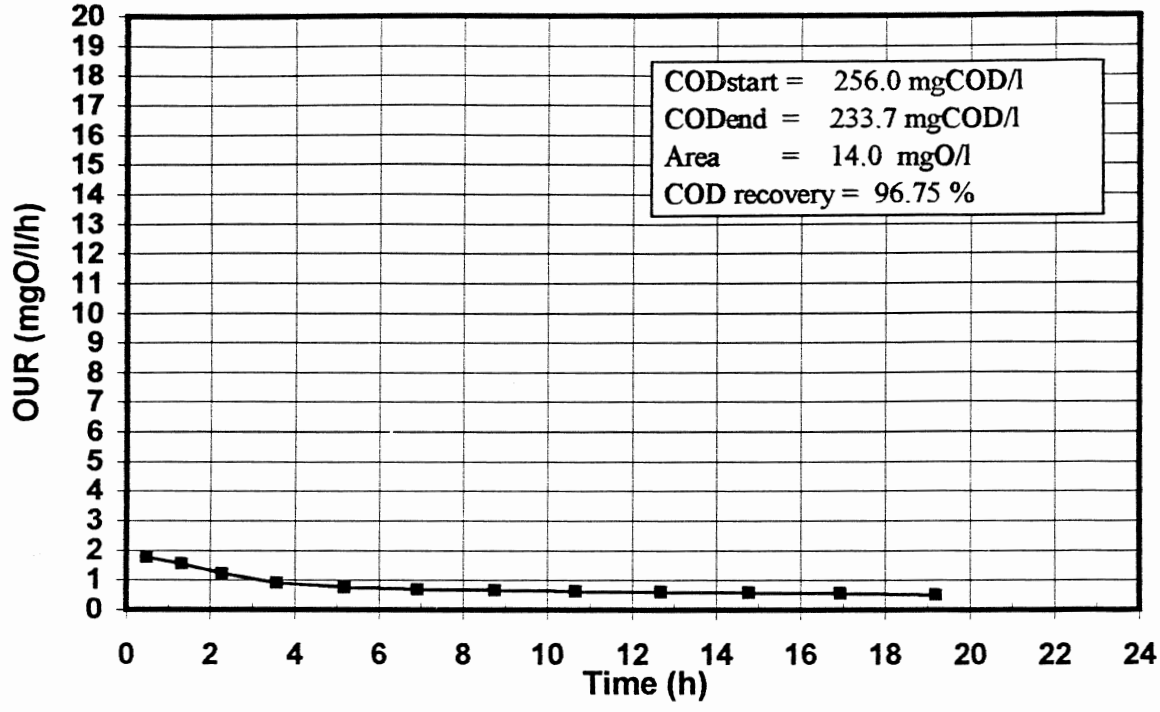
NO_3 Graph for filtered wastewater (2.950ℓ) plus IFFD system mixed liquor (0.050ℓ)
batch test E18 , 21-06b , wastewater batch no. 28



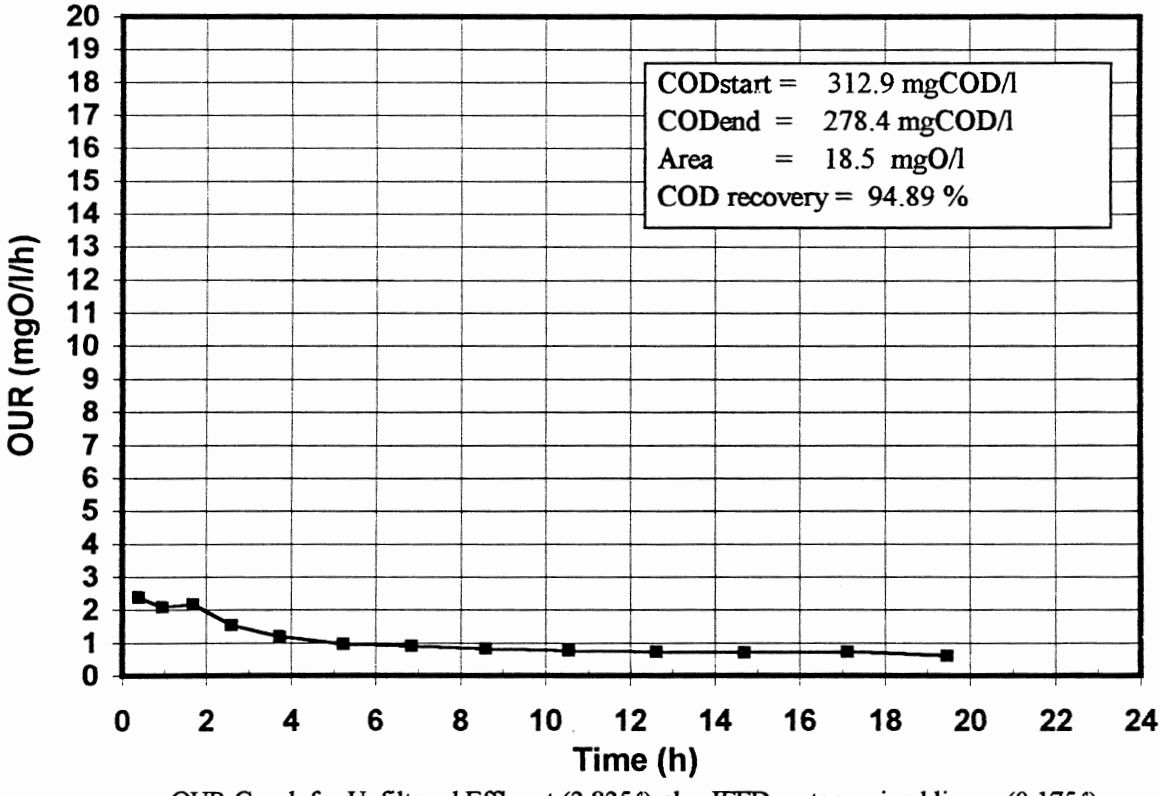
OUR-h Graph for filtered wastewater (2.950ℓ) plus IFFD system mixed liquor (0.050ℓ)
batch test E18 , 21-06b , wastewater batch no. 28



ℓn(OUR-h) for filtered wastewater (2.950ℓ) plus IFFD system mixed liquor (0.050ℓ)
batch test E18 , 21-06b , waste water batch no.28



OUR Graph for Unfiltered Effluent (2.875ℓ) plus IFFD system mixed liquor (0.125ℓ)
batch test , 23-05a , wastewater batch no. 25



OUR Graph for Unfiltered Effluent (2.825ℓ) plus IFFD system mixed liquor (0.175ℓ)
batch test , 23-05b , wastewater batch no. 25

APPENDIX F

CONSTRUCTION AND INTERPRETATION OF STATISTICAL PLOTS FOR DATA ANALYSIS

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F.5 ILLUSTRATION BY AN EXAMPLE

Fig F1 Example of statistical probability plot for a number of heterotrophic active biomass (mgCOD/ℓ) derived from a batch test.

CONSTRUCTION AND INTERPRETATION OF STATISTICAL PLOT FOR DATA ANALYSIS

F.1 INTRODUCTION

Data from different tests could not be compared directly on a daily basis because of the variability in results from all the tests, due to variations in multitude of factors that influence the data. Therefore a graphical approach was used to evaluate the data (Velz, 1950), to interpret the trends and compare the results between two test methods.

For a particular batch of wastewater, the data obtained from the different test methods were statistically analysed using a graphical procedure, to determine the mean, sample standard deviation, and standard deviation of the mean for the data set. This information then could be used to evaluate whether the difference between the means from two data sets is statistically significant at a selected confidence level, or not.

F.2 CONSTRUCTION OF STATISTICAL PLOT

The experimental data is plotted using the procedure below:

- Arrange the data (n in number) in order of ascending magnitude.
- Assign a serial number "m" to each of the values (1, 2, 3, 4 .....n)
- Compute the y-axis plotting the position of each serial value, as the probability equal to or less than from the expression [m/(n + 1)]. The x-axis plotting position is the actual value for the data.
- The probability curve is linearized and plotted; for this investigation the transformed rank probability method (Scientific Tables, 1975) was used to linearize the probability curve, see Fig. F1. Alternatively, probability paper can be used on which the y-axis has been linearized.

F.3 INTERPRETATION OF THE STATISTICAL PLOT

The data plotted can give an indication of whether the data is normally distributed or not:

- If a straight line can be fitted to the plot it indicates that the data have a normal distribution.
- If a straight line can not be fitted to the plot, the data are not normally distributed.

If the data are normally distributed it indicates that a multitude of factors have each had an independent small influence on the measurements; if the data are not normally distributed it indicates that one factor has had a dominating influence.

From the above, provided a straight line can be fitted to he distribution (i.e. the data are normally distributed), it is possible to determine graphically (refer to Fig. F1):

- The mean of the data plotted - this is determined as the x-value where the straight line of the distribution intercepts a vertical line extended from y = 5.

- The standard deviation of the sample, which provides a measure of the variation of the data - this is the difference between the mean (i.e. the x-value that gives y = 5) and the x-value that gives y = 4 (or y = 6).

F.4 TEST FOR STATISTICAL SIGNIFICANCE OF THE DIFFERENCES BETWEEN TWO MEAN VALUES

Visual comparison of two data (or data sets) is a common method of appraisal, to determine whether they differ. However, observed differences or similarities may not be significant as these may arise solely by chance. Statistics defines the expected variations due to chance, to determine whether the observed differences between two data have arisen by chance alone or are significant. In the graphical method, by plotting of two or more series of data on the same probability plot, a quick visual appraisal of similarities and differences can be obtained. To test whether the visual differences in the two series of data are statistically significant, a mathematical significant test is done as follows:

- Plot the two or more distributions to test for normality as described above.

- If normal, obtain the mean (m) and the sample standard deviation (σ) of each series.

- Compute standard deviation of each mean:
  SD(mean) = $(\sigma/\sqrt{n})$
  where n = number of data points.

- Compute the standard deviation of the difference between the two means:
  SD(difference) = $\sqrt{\{(\text{SD mean1})^2 = (\text{SD mean2})^2\}}$

- Compute the absolute value (i.e. positive) of the difference between the two means.
  mean(difference) = $|\ \text{mean1} - \text{mean2}\ |$

- Decide upon a confidence level for the test for significance, 95% certainty or 99% or any other level desired.

- Apply the test for statistical significance of the difference.

For example, if 95% is selected as the confidence level, subtract from the difference between the two means [SD(difference)], i.e. [mean(difference) – 2 • SD(difference)] - if positive number is obtained it can be concluded that the difference between the two means is statistically significant at the selected level of confidence; if a negative value is obtained, then the difference between the two means was by chance alone, and it can be concluded that the apparent difference between the two means is **NOT** statistically significant.

F.5 ILLUSTRATION BY AN EXAMPLE

An example plot is given in Fig. F1.

The mean of a set of values from an experiment is read off from the statistical graph as the value of x that gives y = 5, in this case:

from the graph the mean = 18 mgCOD/ℓ

The standard deviation of a set of values is calculated from the difference between the x-value that gives y = 5 and the x-value that gives y = 6, OR, from the difference between the x-value that gives y = 5 and the x-value that gives y = 4, as shown in Fig. F1, i.e. from graph:

the x-value at y = 6 = 22,6 mgCOD/ℓ
the x-value at y = 4 = 13,4 mgCOD/ℓ
∴ the standard deviation (σ) = 22.6 – 18 **OR** 18 – 13.4 = 4.6 mgCOD/ℓ

The standard deviation of the mean is the standard deviation divided by the square root of the number of values in the data set. In this case:-

number data in set (n) = 12
∴ SD mean = $4.6/\sqrt{(12)}$ = 1.33 mgCOD/ℓ

Say a second set of 10 data is analysed as above to give:

mean = 16 mgCOD/ℓ
then standard deviation (σ) = 5.1 mgCOD/ℓ

Standard deviation of the mean is calculated:

SD mean = $5.1/\sqrt{(10)}$ = 1.61 mgCOD/ℓ

Now, comparing the data from the two sets:

SD(difference) $= \sqrt{\{1.33^2 + 1.61^2\}}$

$= 2.09$ mgCOD/ℓ

mean(difference) $= |18 - 16| = 2$ mgCOD/ℓ

Selecting a 95% confidence interval:

$$\text{Test} = \text{mean(difference)} - 2 \cdot \text{SD(difference)}$$
$$= 2 - 2 \cdot 2.09$$
$$= \mathbf{-2.18}$$

Since the resultant value is negative, it can be concluded that the two means are not significantly different at the 95% confidence interval.

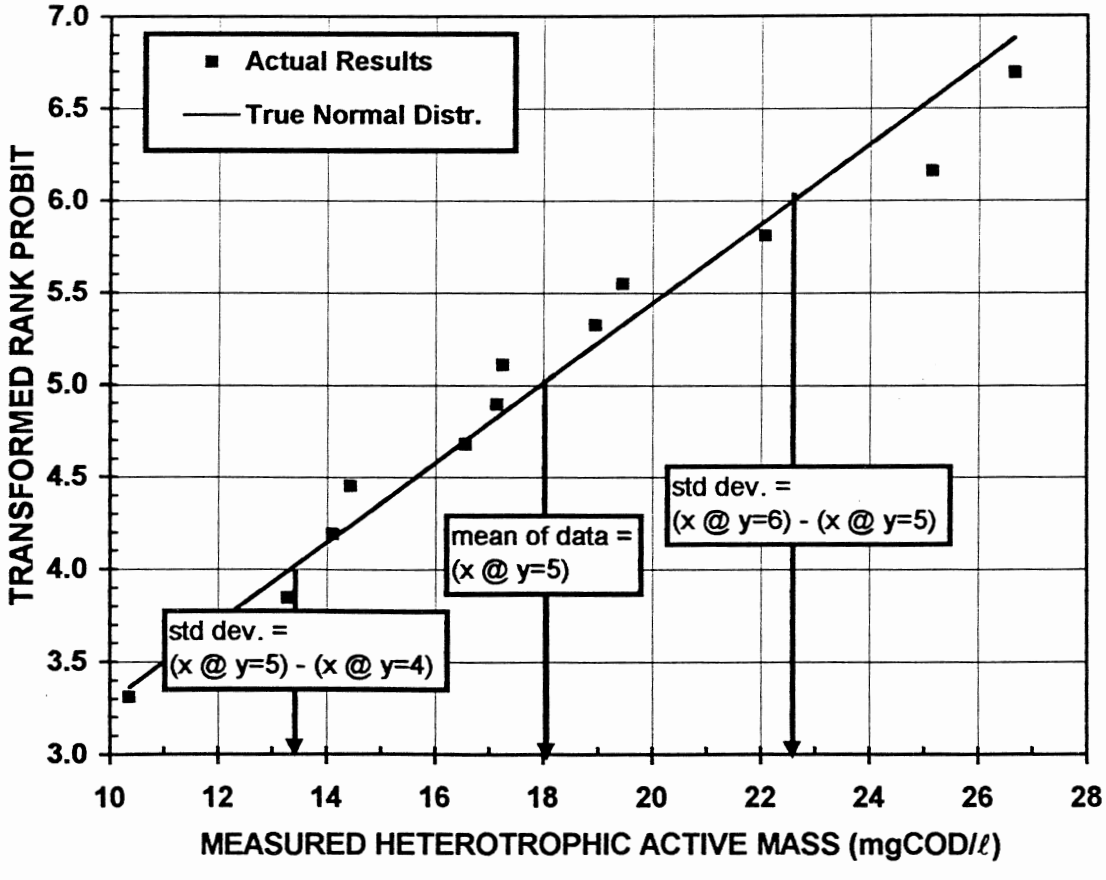


Figure F 1 : Example of a statistical probability plot for a number of measured heterotrophic active biomass derived from the batch test.