EVALUATION OF BATCH TEST FOR MEASUREMENT OF ACTIVE BIOMASS IN ACTIVATED SLUDGE MIXED LIQUOR

by

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DECLARATION BY CANDIDATE

I, AMAL OODHAY BEEHARRY, 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 OHO 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 OHO 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, Z_{BH} 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 Z_{BH}.

This research project investigates the measurement of OHO 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 OHO ACTIVE BIOMASS

Kappelar and Gujer (1992) describe a simple batch test to quantify OHO 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 OHO active biomass concentration. Wentzel *et al.* (1995) and Mbewe *et al.* (1995) modified and extended this method for application to the characterization of municipal wastewaters. Ubisi *et al.* (1997a,b) further extended this simple batch test method 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. They could provide no explanation for the inconsistency in results.

The batch test method of Ubisi *et al.* (1997a,b) was further investigated by Cronje *et al.* (2000) to attempt to identify possible cause(s) for the inconsistency noted by Wentzel *et al.* (1998). Initially, they applied the batch test method of Ubisi *et al.* (1997a,b) to mixed liquor samples drawn from a well-defined parent laboratory-scale anoxic/aerobic activated sludge system operated at 10d sludge age. In comparing the measured OHO active biomass with theoretical values, Cronje *et al.* (2000) found that the correlation was poor, and remarkably similar to that obtained by Wentzel *et al.* (1998) on mixed liquor samples drawn from their parent system at 20d sludge age. From a detailed examination of the batch test procedure and data, Cronje *et al.* (2000) attributed the poor correlation between the OHO active biomass concentration measured in the batch tests and the theoretical values predicted via the steady state design model to two main factors:

(i) 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 biomass partially masked the OUR response of the OHO active biomass from the mixed liquor. Accordingly, a potential source of error in the batch test procedure 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 of Ubisi *et al.* (1997a,b) that nitrification in the batch test with wastewater and mixed liquor gives rise to a linear increase in the nitrate concentration with time, proved to be unduly simplified, and was better represented by an exponential increase. Thus, in the 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 OHO active biomass (OUR_H), another potential source of error was introduced.

To overcome the deficiencies identified above, Cronje *et al.* (2000) proposed two main modifications to the batch test procedure of Ubisi *et al.* (1997a,b):

- Physically remove the OHO active biomass from the wastewater: This was achieved through flocculation of the wastewater with aluminium sulphate followed by filtration. Batch tests demonstrated no observable biological activity in the *flocculated-filtered* wastewater, indicating that all OHO active biomass had been successfully removed.

- Use exponential fits to the nitrate concentration – time profiles to determine nitrification OURs, as opposed to a linear increase.

The modifications proposed above greatly simplified the batch test procedure – since the *flocculated-filtered* wastewater does not contain OHO active biomass, a parallel batch test no longer needs to be conducted to determine the wastewater OHO active biomass, which in the "old" batch test method was subtracted from the mixed liquor + wastewater OHO active biomass to give the mixed liquor OHO active biomass.

Cronje *et al.* (2000) evaluated this modified batch test method by drawing mixed liquor samples from a well-defined parent laboratory-scale anoxic/aerobic activated sludge system operated at 10d sludge age. From a comparison of the OHO active biomass concentrations measured in the batch test with theoretical values, a close agreement was found. Cronje *et al.* (2000) concluded that the modified batch test holds merit, but requires further investigation.

RESEARCH OBJECTIVES

The development of the modified 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 parameter. The modified batch test method developed by Cronje *et al.* (2000) has shown considerable promise as an independent means to quantify the

hypothesized concentration of OHO active biomass present in an activated sludge system. However, the method does require more extensive evaluation; ***in this research project, the modified batch test procedure of Cronje et al. (2000) will be further investigated.*** To achieve this aim, two primary objectives for the research project have been identified:

(1) Evaluate the reliability of the modified batch test method, by comparing the OHO active biomass concentrations measured in the batch test on samples drawn from a well-defined parent anoxic/aerobic activated sludge system, with the theoretical values predicted by the steady state design model. Good correspondence between the theoretical and measured values would provide substantive direct evidence supporting both the steady state design model and the modified experimental method.

(2) In addressing the main aim above, it was decided to run and operate a parallel parent laboratory-scale anoxic/aerobic activated sludge system having a different OHO active biomass fraction of the mixed liquor. To change the OHO active biomass fraction of the mixed liquor, a known concentration of macerated toilet paper solution was dosed to this system. Toilet paper is mainly constituted of wood pulp, which is composed of 75% cellulose and 25% lignin. These two organic components are believed to be largely unbiodegradable in the activated sludge system. Accordingly, toilet paper should contribute significantly to the inert sludge mass in the laboratory-scale anoxic/aerobic activated sludge system, thereby significantly increasing the MLOSS concentration in the system, and reducing the OHO active biomass fraction of the MLOSS. This would provide the opportunity to evaluate the ability of the modified batch test procedure to detect the decreased active biomass fraction.

RESEARCH APPROACH

The research approach adopted was to operate and monitor two well-defined and controlled continuously fed parent activated sludge systems in parallel. The *control* activated sludge system provided the mixed liquor samples for measuring the OHO active biomass to address objective (1) above. To address objective (2), the *experimental* activated sludge system provided the mixed liquor samples for measuring the OHO active biomass.

EVALUATION OF THE MODIFIED BATCH TEST METHOD

The batch test method of Cronje *et al.* (2000) was evaluated by conducting batch tests on:

- Control anoxic/aerobic parent system
- Experimental anoxic/aerobic parent system
- Control fully aerobic parent system

Control anoxic/aerobic parent system

To further investigate the modified batch test procedure, the first objective of this research project was to operate and maintain a *control* parent laboratory-scale nitrification / denitrification activated sludge system identical to that of Cronje *et al.* (2000) which would have the same mixed liquor characteristics. This system would serve as a source of mixed liquor for the batch tests.

System operation and monitoring

Initially, the *control* system layout constituted a Modified Ludzack-Ettinger (MLE) configuration and consisted of an anoxic reactor of 2.5ℓ volume (25% of the total system volume), an aerobic reactor of 7.5ℓ volume (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 total system volume was 10ℓ. As the experimental investigation proceeded, denitrification performance deteriorated. Thus, it was decided to slightly modify the original Modified Ludzack-Ettinger (MLE) configuration by having a larger anoxic mass fraction of 3.3ℓ volume (33% of the total system volume) and a reduced aerobic mass fraction of 6.7ℓ volume (67% of the total system volume), with the total system volume remaining fixed at 10ℓ. During the latter part of the investigation, severe bulking problems arose. Hence, to improve the sludge settleability, it was decided to change the laboratory-scale *Modified Ludzack-Ettinger (MLE)* system to a completely mixed *fully aerobic* system of 8ℓ volume.

The influent for the parent laboratory-scale activated sludge system was raw (unsettled) sewage 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, before both the course and fine screens and before grit removal and the primary sedimentation tanks. The sewage batch was brought to the laboratory and stored in 400ℓ stainless steel tanks in a cold room at 4°C for 10 to 14 days. For the parent system, the sewage was drawn daily from the storage tanks after thorough mixing and diluted with tap water to give an influent feed total COD ~ 10 000 mgCOD/d. System operation procedures detailed by Ekama *et al.* (1986) were followed: Daily monitoring included influent COD, TKN; all reactors nitrate + nitrite; aerobic reactor TSS, VSS, COD and TKN; effluent COD, TKN, nitrate + nitrite (Standard Methods, 1985).

The *control* parent system was operated for 417 days and received 26 batches of sewage; 22 sewage batches served as feed to the *MLE* activated sludge system and the last 4 sewage batches served as feed to the completely mixed *fully aerobic* activated sludge system. Each sewage 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 OHO active biomass fraction of the mixed liquor organic suspended solids (f_{av}).
- The theoretical OHO active biomass concentration in the steady state system bioreactor.

Parent system results

From the results on the *control* parent system:

- N mass balances were consistent and were generally in the range 90 to 110%. Sewage batches that gave mass balances falling outside this range were No. 3, 4, 8A, 10, 11, 12, 16 and 22. Batch tests were conducted only during Sewage Batch No. 22. The batch test data collected during this sewage batch was included where appropriate, and analysed, but it was noted that the data should be interpreted with caution.

- Generally COD mass balances were poor, with 15 of 26 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 = 1.42 mgCOD/mgVSS (sample standard deviation = 0.05) and TKN/VSS = 0.086 mgN/mgVSS (sample standard deviation = 0.008). These values are close to 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 OHO active biomass. Accordingly, the lower limit for the COD mass balance was set at 80%. On this basis, only Sewage Batches No. 7 and 11 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 0.050 (sample standard deviation = 0.014). This value is lower than the $f_{S,us}$ values obtained by both Ubisi *et al.* (1997a,b), $f_{S,us}$ = 0.095 and Cronje *et al.* (2000), $f_{S,us}$ = 0.085 for the same Mitchell's Plain raw wastewater. Of interest is the fact that both Ubisi *et al.* (1997a,b) and Cronje *et al.* (2000) were feeding a COD concentration of 500 ± 50 mgCOD/ℓ to their parent systems. In this experimental investigation, the same feed concentration of 500 ± 50 mgCOD/ℓ was fed to the parent system for the first 107 days, whereafter, the feed concentration was increased to 750 ± 75 mgCOD/ℓ and this increased COD concentration was fed to the parent system till closure (day 417). Thus, despite that the $f_{S,us}$ value would be expected to be the same, given that the influent wastewater being treated was the same, the higher COD concentration gave a lower $f_{S,us}$. The lower $f_{S,us}$ value is however, in the range of accepted values of 0.04 – 0.10 mgCOD/mgCOD for municipal raw wastewaters in South Africa (WRC, 1984).

- The wastewater mean unbiodegradable particulate COD fraction ($f_{S,up}$) was determined to be 0.161 (sample standard deviation = 0.037). This value compares favourably with that observed by Ubisi *et al.* (1997a,b), $f_{S,up}$ = 0.120, Cronje *et al.* (2000), $f_{S,up}$ = 0.103 for the same Mitchell's Plain raw wastewater, and conforms to the accepted range of 0.07 – 0.20 mgCOD/mgCOD for municipal raw wastewaters in South Africa (WRC, 1984). This indicates that the value obtained for $f_{S,up}$ is reasonable.

Batch tests on control parent system

To determine if the modified batch tests conducted in accordance with the procedures of Cronje *et al.* (2000) would yield the same consistent results, the modified batch test method was evaluated by applying the batch test to mixed liquor samples drawn from the well-defined and controlled *control* parent laboratory-scale nitrification / denitrification activated sludge system, identical to that of Cronje *et al.* (2000).

In evaluating the modified batch test method, a total of 18 modified batch tests were conducted. From analysis of the batch test results, the following were concluded:

- In interpreting the nitrate and nitrite concentrations with time observed in their batch tests, both Ubisi *et al.* (1997a,b) and Cronje *et al.* (2000) found that the nitrite concentrations were very low, and hence could be neglected. However, in this investigation nitrite concentrations were found to be significant compared to nitrate concentrations, and hence need to be taken into account to determine OUR_N. This arises because the oxygen requirement to nitrify ammonia-N to nitrite is lower than that for nitrification of ammonia-N to nitrate.

- In the batch tests with wastewater and mixed liquor conducted by Ubisi *et al.* (1997a,b), they observed that nitrification in these batch tests caused a linear increase in the nitrate concentration with time. Cronje *et al.* (2000) observed that the generation of nitrate in the batch reactor was better represented by an exponential increase. In this experimental investigation, it was observed that the nitrate/nitrite concentrations could be represented by either a linear or an exponential increase. To select the best type of fit for a particular batch test, this was done by visually checking which of the linear or exponential lines best fitted the data, and confirming the best-fit line by doing a regression analysis and noting the correlation coefficient. A reasonable correlation coefficient ($R^2 > 0.90$) implies that the selected best-fit line gives a good approximation of the experimental data. For the various batch tests, both linear and exponential fits were used. Thus, selecting the type of fit is not general, but must be based on the data for a particular batch test.

- The modified batch tests done using mixed liquor drawn from the *MLE control* activated sludge system yielded good %COD recoveries, with only 2 out of 18 batch tests (No. 3 and 7) yielding %COD recoveries < 90 %. Statistical analysis indicated that these poor COD mass balances may have arisen from random effects and accordingly these batch tests data were not rejected. The mean %COD recovery was

97.8 % with sample standard deviation of 6.9 %. The good %COD recoveries lend credibility to the reliability of the measurements and the batch test procedure.

- Comparing the measured and the theoretical OHO active biomass concentrations, it would appear that there is reasonably close correspondance between theoretical and measured OHO active biomass concentrations; the "serial dilutions" of mixed liquor give an almost linear decrease in OHO active biomass concentration. However, the values plot virtually parallel to the 45° line (i.e. 1:1 correspondance). This implies that there is a constant (i.e. independent of volume of mixed liquor added) difference between the measured and theoretical values – when the measured OHO active biomass concentration is zero, the theoretical OHO active biomass concentration in the batch test is approximately 25 mgCOD/ℓ. No explanation for this deviation was apparent.

- Although some correlation does exist between the theoretical and measured OHO active biomass concentrations for the range of mixed liquor volumes used in the batch tests, individual data points tend to exhibit some variation from the appropriate correlation line. This variation can be attributed to the sensitivity of the measured OHO active biomass concentration, to the slope of the ℓn (OUR_H) – time plot. Even the smallest change in the slope (magnitude ~ 0.05) can result in marked variations in the OHO active biomass concentration values. This would suggest that a number of batch tests need to be conducted to establish a reasonable estimate for OHO active biomass concentration.

Experimental anoxic/aerobic parent system

As mentioned above, to address the second objective of this research project, it was decided to run and operate a parallel parent laboratory-scale anoxic/aerobic activated sludge system, termed the *experimental* system, having a different OHO active biomass fraction of the mixed liquor. Both the *control* and *experimental* parent systems were set-up and operated identically, but the *experimental* system additionally received a known mass of toilet paper. It was envisaged that the toilet paper would be largely unbiodegradable, and hence the *experimental* system would have a mixed liquor OHO active biomass fraction that would deviate significantly from the parallel *control* system. The ability of the batch test procedure of Cronje *et al.* (2000) to correctly detect this difference in OHO active biomass would be evaluated.

System operation and monitoring

The *experimental* parent laboratory-scale was identical in set-up to the *control* system. The main changes to system configuration and operation were identical to those made to the *control* system, described above, thus, three main configurations were used. The procedures followed for the wastewater collection and storage was similar to the *control* system, as above. For the wastewater feed, the procedures followed for the feed preparation were identical to those for the *control* system, described above; however, additionally toilet paper was dosed to the *experimental* system. Prior to the addition of

toilet paper, the system was run for 2 sludge ages to ensure steady state conditions in the *experimental* system.

From the literature review on the composition and biodegradation of toilet paper, it was initially thought that the toilet paper would contain a high unbiodegradable particulate fraction ($f_{S,up}$) which would contribute significantly to the mixed liquor inert component in the *experimental* activated sludge system. Thus, it was decided to add a dose of ≈ 2 000 mgCOD/d as toilet paper solution to the influent wastewater feed of ≈ 10 000 mgCOD/d. A stock solution of toilet paper of 20g/ℓ was made by macerating 20g of toilet paper into a litre of distilled water. A known volume of the stock toilet paper solution was macerated in a liquidizer with some diluted raw influent sewage and was added to the total feed volume. The total COD load per day on the *experimental* activated sludge system was ≈ 12 000 mgCOD/d. System operation procedures detailed by Ekama *et al.* (1986) were followed: Daily monitoring included influent COD, TKN; all reactors nitrate + nitrite; aerobic reactor TSS, VSS, COD and TKN; effluent COD, TKN, nitrate + nitrite (Standard Methods, 1985).

The *experimental* system was operated for 382 days in total and received 24 batches of sewage. Each sewage 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 OHO active biomass fraction of the mixed liquor organic suspended solids (f_{av}).
- The theoretical OHO active biomass concentration in the steady state system bioreactor.

Parent system results

From the results on the *experimental* parent system:

- N mass balances were consistent and were generally in the range 90 to 110%. Only 6 out of 19 sewage batches gave mass balances falling outside this range, with 3 only marginally outside the range. No batch tests were conducted during these sewage batches.

- Generally COD mass balances were reasonable, with 7 out of 19 sewage batches giving mass balances < 90%. Of these, 3 sewage batches had COD mass balances only marginally less than 90%. No batch tests were conducted during any of these sewage batches.

- The mixed liquor organic solids were determined by three independent tests – VSS, COD and TKN. Mean ratios for these measurements gave COD/VSS = 1.39

mgCOD/mgVSS (sample standard deviation = 0.06) and TKN/VSS = 0.078 mgN/mgVSS (sample standard deviation = 0.004). These values are lower than the values measured for the *control* parent system (1.42 mgCOD/mgVSS and 0.086 mgN/mgVSS respectively) and the accepted standard values of 1.48 mgCOD/mgVSS and 0.10 mgN/mgVSS respectively (WRC, 1984). More than likely, the lower values were caused by the toilet paper dose.

- The original amount of toilet paper solution added (which provided an additional dose of 2 000 mgCOD/d) did not cause a significant change in the OHO active biomass fraction of the mixed liquor organic solids of the *experimental* system compared to the *control* system. The unbiodegradable soluble ($f_{S,us}$) and particulate ($f_{S,up}$) fractions of the toilet paper were determined to be 0.035 mgCOD/mgCOD and 0.309 mgCOD/mgCOD respectively; these values are reasonably close to the values determined for the wastewater itself, 0.050 mgCOD/mgCOD and 0.161 mgCOD/mgCOD respectively. This implies that the toilet paper was 65.6 % biodegradable and hence did not increase the inert fraction of the mixed liquor significantly and thus the concentration of OHO active biomass fraction of the mixed liquor would not be expected to decrease significantly. Larger doses of toilet paper could not be used, as these led to blockages of pipes between reactors, resulting in reactor overflows. Thus, the objective of dosing toilet paper to significantly change the OHO biomass fraction of the mixed liquor and evaluating the ability of the batch test to detect this change could not be achieved.

Batch tests on experimental parent system

To further evaluate the reliability of the modified batch test procedure and its application to anoxic/aerobic activated sludge systems, subjected to decreased OHO active biomass fractions, a total number of 18 modified batch tests were conducted using mixed liquor drawn from the *MLE experimental* activated sludge system and were done in parallel to the batch tests on the *control* system.

From analysis of the batch test results, the following were concluded:

- In general, good %COD recoveries were achieved with only one batch test (No. 32) yielding %COD recovery < 90 %. The %COD recovery for Batch Test No. 32 was marginally < 90 % (89.0 %); however, statistical analysis indicated that this COD mass balance arose from random effects and accordingly this batch test data was not rejected. The mean %COD recovery was 95.9 % with sample standard deviation of 5.2 %. The good %COD recoveries lend credibility to the reliability of the measurements and the batch test procedure.

- Comparing the measured and the theoretical OHO active biomass concentrations, the correlations show remarkable similarity to those obtained for the *control* system – there is a close correlation but the values plot parallel to the 45° line. Again, this implies that there is a constant difference between measured and theoretical OHO

active biomass concentrations; as for the *control* system, this difference is approximately 25 mgCOD/ℓ.

- Although some correlation does exist between the theoretical and measured OHO active biomass concentrations for the range of mixed liquor volumes used in the batch tests, individual data points tend to exhibit some variation from the appropriate correlation line. This variation can be attributed to the sensitivity of the measured OHO active biomass concentration (as explained above).

Comparison between OHO active biomass between the control and experimental systems

One of the common tasks was to perform batch tests, identical in procedure to those conducted by Cronje *et al.* (2000), using mixed liquor samples drawn from both the *control* and *experimental* parent laboratory-scale anoxic/aerobic activated sludge system. From a comparison of the OHO active biomasses between the two systems, it would be possible to evaluate whether the batch test successfully detects any change in OHO active biomass fraction of the mixed liquor in the parent systems.

From a comparison of the results, it was apparent that the data for the *control* and *experimental* systems are remarkably similar: Both data sets plot on a line parallel to the 1:1 correspondance (45°) line – as noted above, this implies that there is a constant (i.e. independent of volume of mixed liquor added) difference between measured and theoretical values, of about 25 mgCOD/ℓ. No explanation for this difference could be found. That the two data sets are similar would indicate that the batch test has correctly detected the change in OHO active biomass fraction due to the toilet paper added to the *experimental* system: The effect of the toilet paper is taken into account automatically in calculating the theoretical OHO active biomass concentration.

Thus, the original objectives of this investigation were achieved. However, since the toilet paper proved largely biodegradable, its effect was not as marked as was hoped. Hence, it was decided to increase the dosage of toilet paper to the *experimental* activated sludge system (to further increase the contribution of inert sludge mass in the system, thereby achieving a larger increase in the MLOSS concentration in the system, and a more significant reduction of the OHO active biomass fraction of the MLOSS). Unfortunately, in practice it proved not possible to operate the laboratory-scale *experimental* activated sludge system with the higher toilet paper dose; the toilet paper caused frequent blockages of pipes between reactors which caused reactor overflows.

Effect of aluminium sulphate on batch test results

The preparation of the wastewater for the modified batch tests incorporated flocculating and filtering the raw wastewater to remove all the particulate material: Aluminium sulphate was chosen for the flocculation step. During the course of the experimental investigation, it was thought that the use of aluminium sulphate as a flocculant possibly removed a large fraction of the available phosphorus required for the growth of the OHO

active biomass. If true, this would have a direct impact on the OHO active biomass concentration measured in the batch tests since the growth of OHOs would be restricted by non-availability of phosphorus. It was thought that this possibly caused the deviation in correlation between theoretical and measured values from the 1:1 line.

To evaluate this possibility, the soluble ortho-P concentration of both the *raw* and the *flocculated-filtered* wastewaters were measured on a number of occasions. The soluble ortho-P concentration averaged 12 mgP/ℓ in the *raw* wastewater and 1.6 mgP/ℓ in the *flocculated-filtered* wastewater. Thus, it appeared that, phosphorus could be the limiting factor in the growth of OHO active biomass, which may have caused the deviation between measured and theoretical OHO active biomass concentrations noted above. Accordingly, it was deemed necessary to further investigate this aspect.

Modified batch tests using mixed liquor drawn from the *control* activated sludge system only were run in parallel for Sewage Batches No. 21, 22 and 26; to the one batch test *flocculated-filtered* wastewater plus mixed liquor were added and to the other, *flocculated-filtered* wastewater plus mixed liquor plus 5 mℓ of stock potassium hydrogen phosphate (K_2HPO_4, stock at 33.68 g/ℓ) were added per ℓ of wastewater (10 mgP/ℓ *batch reactor*). It must be emphasized that 6 batch tests were conducted during Sewage Batches No. 21 and 22 using mixed liquor drawn from the *MLE control* activated sludge system and another 6 batch tests were conducted during Sewage Batch No. 26 using mixed liquor drawn from the *fully aerobic control* activated sludge system.

- The N mass balance for the *parent* system for Sewage Batch No. 22 was < 90 %, hence the batch test results conducted during this sewage batch should be rejected for further analysis, but were included where appropriate, and analysed.

- In general, good %COD recoveries were achieved with only three batch tests (No. 61, 63 and 65) yielding %COD recoveries < 90 %. Statistical analysis indicated that these COD mass balance may have arisen from random effects and accordingly these batch tests data were not rejected. The mean %COD recovery was 93.9 % with sample standard deviation of 5.5 %. The good %COD recoveries lend credibility to the reliability of the measurements and the batch test procedure.

For Sewage Batch No. 21, the addition of P caused the measured OHO active biomass concentration to increase. For Sewage Batch No. 22, again addition of P caused a significant increase in the measured OHO active biomass concentration. However, for Sewage Batch No. 26, either no significant change was observed, or a slight decrease in OHO active biomass concentration with P addition.

From the results above, it was noted that the effect of adding P to the batch test was inconsistent, and not entirely conclusive. For some sewage batches, the effect was negligible, while for others adding P caused an increase or decrease in the OHO active biomass concentration. Thus, it appears that the effect of adding P may be dependent on the particular sewage batch used in the batch test, possibly depending on the P concentration available after flocculation and filtration. With the clarity of hindsight, P

should have been supplemented to all subsequent batch tests when this became apparent, but at the time from the results on Sewage Batch No. 26, it was thought that the effect of P addition was negligible, so this was not done. Clearly, this aspect deserves further attention. However, for the results for Sewage Batch No. 26, it is evident that P limitation was not the cause for the significant deviation between measured and theoretical values observed for the *fully aerobic* parent system below.

Control fully aerobic system

Throughout the experimental investigation, bulking in the parent systems was a continual problem. Whenever bulking manifested itself, a short-term remedy to mitigate its effects was to dose aluminium sulphate to the aerobic reactor of the MLE activated sludge system. However, during the final stages of the experimental investigation, to try to permanently cure bulking, it was decided to modify the *MLE* activated sludge system to a *fully aerobic* system (single aerobic reactor and secondary settling tank, i.e. the anoxic reactor was removed).

A total number of 24 modified batch tests (including 6 batch test where the effect of phosphate addition was monitored) were conducted using mixed liquor drawn from the *fully aerobic control* activated sludge system. The sewage batches during which batch tests were conducted on the *fully aerobic control* system were Sewage Batches No. 23A, 23B, 24, 25 and 26. Sewage Batch No. 23 is divided into 23A and 23B, because the system configuration was changed from *MLE* to *fully aerobic* in the middle of Sewage Batch No. 23.

- The fact that the COD and N mass balances for the parent system were good during these sewage batches lends credibility to the measurements done on the parent system.

- In general, good %COD recoveries were achieved with only three batch tests (No. 61, 63 and 65) yielding %COD recoveries < 90 %. Statistical analysis indicated that these COD mass balance may have arisen from random effects and accordingly these batch tests data were not rejected for further analysis. The mean %COD recovery was 93.9 % with sample standard deviation of 3.8 %. The good %COD recoveries lend credibility to the reliability of the measurements and the batch test procedure.

The batch tests done using mixed liquor drawn from the *MLE* activated sludge system during Sewage Batch No. 23A show a reasonable agreement between the measured and the theoretical OHO active biomass concentrations. However, the batch tests performed using mixed liquor drawn from the *fully aerobic* activated sludge system during all other sewage batches show a very poor agreement between the measured and the theoretical OHO active biomass concentrations: There is a close correlation between the theoretical and measured values, but the theoretical values are approximately 3 to 4 times those measured. The fact that the COD and N mass balances both on the parent system and for the batch tests were good during all these sewage batches lends credibility to the measurements.

Comparing the data obtained with the mixed liquor drawn from the *fully aerobic* system with that from the *MLE anoxic/aerobic* system, the trends are completely different: For the *anoxic/aerobic* system mixed liquor, there is a close correlation between measured and theoretical values, but with a constant difference between the actual values (i.e. the values fall on a line parallel to the 1:1 correlation line); for the *fully aerobic* system mixed liquor, the measured values are about $\frac{1}{3}$ to $\frac{1}{4}$ the theoretical values [i.e. the values fall on a line that passes through the (0,0) origin, but which has a reduced slope]. In seeking an explanation for this difference in response, the data collected during Sewage Batch No. 23 is of interest: For the batch test conducted during Sewage Batch No. 23A, the system was operated as an *MLE* and the batch test data falls close to or higher than the 1:1 correlation line. The system was then changed to *fully aerobic*, and shortly thereafter batch tests were conducted. With each successive set of batch tests, the measured OHO active biomass concentration decreased, to reach the trend line for the *fully aerobic* system apparent for the batch tests that followed. This would suggest that changing from the *anoxic/aerobic* to *aerobic* configuration caused a significant change in the behaviour of the mixed liquor. Such a change in population dynamics is to be expected. However, why the population did not re-establish to the theoretical values after 3 sludge ages of operation is not clear: It would be expected that with time the data should return to 1:1 correlation line – this clearly did not happen.

CONCLUSIONS

In closure, (i) the remarkable similarity in the correlation between theoretical and measured OHO active biomass concentrations for mixed liquor drawn from the *control* and *experimental MLE* systems, (ii) the linearity of results with "serial" dilutions, and (iii) the consistent progressive change in behaviour detected by the batch test in changing from the *MLE* to *fully aerobic* configurations all indicate that the batch test method is a valuable tool for examining activated sludge system behaviour. However, the lack of a 1:1 correlation between theoretical and measured values requires further investigation. In this regard, the possibility of P limitation due to aluminium sulphate flocculation of the wastewater should be examined more closely.

RECOMMENDATIONS

From this investigation the following recommendations can be made:

- Dosing toilet paper to significantly change the OHO active biomass fraction of the mixed liquor in the activated sludge system was not successful. On the one hand, toilet paper is more biodegradable than expected, and thus did not exert the anticipated influence on OHO active biomass fraction of the mixed liquor; on the other hand, with increased doses of toilet paper it became very difficult to operate and maintain steady state conditions in the activated sludge system. As an alternative, to significantly change the OHO active biomass fraction of the mixed liquor, the

modified batch test method needs to be tested on mixed liquor drawn from parent systems at different sludge ages, say at 10 and 20 days.

- The effect of adding P to the batch test was inconsistent, and not entirely conclusive. For some sewage batches, the effect is negligible, while for others adding P caused an increase or decrease in the OHO active biomass concentration. Thus, it appears that the effect of adding P may be dependent on the particular sewage batch used in the batch test, possibly depending on the P concentration available after flocculation and filtration. Clearly, this aspect deserves further attention and investigation.

- The results from this research project demonstrate the behavioural differences between sludges originating from different parent systems and the influence of the behavioural differences on the batch test results. Changing from the *anoxic/aerobic* to *aerobic* configuration caused a significant change in the behaviour of the mixed liquor. Such a change in population dynamics is to be expected. However, why the population did not re-establish to the theoretical values after 3 sludge ages of operation is not clear. This warrants further investigation.

- Quantifying OHO 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 OHO 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. Exploration of ways to integrate the engineering and technology paradigm with the microbiological and biochemistry paradigm should receive attention.

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. Measurement of this parameter within the engineering paradigm by means of the batch test procedure described here and within the microbiological and biochemical paradigms by means of the newly developed analytical techniques can initiate links and overlap between the two paradigm sets.

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LIST OF SYMBOLS

AA	Anoxic-aerobic
AO	Acridine orange
AODC	Acridine orange direct count
AOE	Acridine orange epifluorescence
ATP	Adenosine triphosphate
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:N	Carbon to nitrogen ratio
°C	Degrees celsius
COD	Chemical Oxygen Demand
COD_{FE}	Total filtered 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
DAPI	Diamidino-2-phenylindole
DNA	Deoxyribonucleic acid
DO	Dissolved Oxygen (mgO/ℓ)
DSVI	Diluted sludge volume index (mℓ/gTSS)
e^-	Electron
e.g.	For example
EDTA	Ethylene diamine tetra acetic acid
f^*	Endogenous residue fraction, endogenous respiration theory
f_E^*	Endogenous residue fraction, endogenous respiration theory
FDA	Fluorescein diacetate
FISH	Fluorescent *in situ* hybridization
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
INT	2-(*p*-codophenyl)-5-phenyl tetrazolium chloride
ISS	Inorganic suspended solids (mgISS/ℓ)
K_{SH}	OHO half saturation rate on RBCOD (mgCOD/ℓ)
K_{SP}	OHO half saturation rate on SBCOD (mgCOD/ℓ)
K_{MP}	OHO maximum specific growth rate on SBCOD (/d)
ℓ	Litre
LR	Loading rate
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)
MN_c	Mass of nitrate generated (mgN/d)
MN_d	Mass of nitrate denitrified (mgN/d)
MN_{ne}	Mass of nitrate in the effluent (mgN/ℓ)
MN_{nw}	Mass of nitrate in the waste sludge (mgN/ℓ)
MN_{te}	Mass of effluent TKN (mgN/ℓ)
MN_{ti}	Mass of influent TKN (mgN/ℓ)
MN_w	Mass of TKN in the waste sludge (mgN/ℓ)
MO_c	Mass of carbonaceous oxygen demand (mgO/d)
MO_d	Equivalent mass of oxygen demand for denitrification (mgO/d)
MO_n	Mass of oxygen demand for nitrification (mgO/d)
MO_t	Mass of total oxygen demand (mgO/d)
MS_{te}	Mass of total filtered effluent COD concentration (mgCOD/d)
MS_{ti}	Mass of total unfiltered influent COD concentration (mgCOD/d)
MX_{BA}	Mass of AO active biomass (mgVSS)
MX_{BH}	Mass of OHO active biomass (mgVSS)
MX_E	Mass of endogenous material (mgVSS)
MX_I	Mass of inert material (mgVSS)
MXsvw	Mass of COD wasted (mgCOD/d)
MX_V	Mass of volatile suspended solids (mgVSS)
N	Nitrogen
NaCl	Sodium chloride
NAD	Nicotinamide adenine dinucleotide (oxidized)
NADH	Nicotinamide adenine dinucleotide (reduced)
N_2	Nitrogen gas
NH_3	Ammonia (mgN)
NH_4^+	Ammonium (mgN)

N_{ne}	Nitrate 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)
N_{te}	TKN concentration in the effluent (mgN/ℓ)
O_2	Oxygen
OD	Optical density
OHO	Ordinary heterotrophic organism
OUR	Oxygen utilization rate (mgO/ℓ/h)
$OUR_{H(t)}$	OHO active biomass oxygen utilization rate at time t (mgO/ℓ/h)
$OUR_{M(t)}$	OUR measured at time t (mgO/ℓ/h)
$OUR_{N(t)}$	AO oxygen utilization rate at time t (mgO/ℓ/h)
OUR_{NO2}	OUR for NO_2^- nitrification (mgO/ℓ/h)
OUR_{NO3}	OUR for NO_3^- nitrification (mgO/ℓ/h)
P	Phosphorus
PAO	Phosphate accumulating organism
polyP	Polyphosphate
PVC	Poly vinyl chloride
Q or Qi	Influent flow (ℓ/d)
RBCOD;S_{bsi}	Readily biodegradable COD (mgCOD/ℓ)
rDNA	Ribosomal deoxyribonucleic acid
RNA	Ribonucleic acid
rRNA	Ribosomal Ribonucleic acid
R_S	Sludge age (d)
s	Second
SBCOD;S_{bpi}	Slowly biodegradable COD (mgCOD/ℓ)
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)
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

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	AO maximum specific growth rate (/d)
μ_H	OHO 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}	AO active biomass concentration (mgVSS/ℓ)
X_{BH}	OHO 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}	OHO active biomass yield, COD units (mgCOD/mgCOD)
Y_H^*	OHO active biomass yield, VSS units (mgVSS/mgCOD)
Y_{ZA}	AO biomass yield (mgCOD/mgN)
Z_{BH}	OHO active biomass concentration (mgCOD/ℓ)
$Z_{BH(0)}$	OHO active biomass conc. at the start of the batch test (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, to a large degree, on a common conceptualization of the processes acting in the system. In terms of this conceptualization, in the bioreactor of the non-nitrifying aerobic activated sludge system (Ubisi *et al.*, 1997; Cronje *et al.*, 2000), 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. The OHO active biomass arises from synthesis of living OHOs on biodegradable organic substrates and is "lost" via endogenous respiration/death processes; in the activated sludge system the mixed liquor component performs the biodegradation processes of COD removal and denitrification. The AO active biomass arises from synthesis of AOs in the nitrification of ammonia to nitrate under aerobic conditions and is "lost" via endogenous respiration/death processes. The endogenous residue is generated from the unbiodegradable portion of the OHO and AO active biomasses that are lost in the endogenous respiration/death process. The inert material arises from the influent wastewater unbiodegradable particulate organics which, on entry into the bioreactor, are enmeshed in the MLOSS. 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 (6) 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. To avoid the complication of the PAOs, ***in this research project only the aerobic and anoxic/aerobic systems will be considered;*** this effectively reduces the MLOSS components to the first four above.

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 active biomass 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 has cast a measure of uncertainty on the entire framework within which the models have been developed and is a 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 Ubisi *et al.* (1997a,b) and Wentzel *et al.* (1998) to quantify the OHO active biomass concentration of mixed liquor samples drawn from aerobic and anoxic/aerobic activated sludge systems. In essence, in this modified method two aerobic batch tests are run in parallel; to the one batch test only unsettled wastewater is added and to the other, a mixture of wastewater and mixed liquor. Taking due account of nitrification, the difference in OUR response with time in the two batch tests can be used to derive an estimate of the OHO active biomass concentration due to the added mixed liquor. Wentzel *et al.* (1998) evaluated this batch test method, by applying it 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.* (1998) were not able to provide an explanation for this inconsistency, so the uncertainty around the active biomass concept largely remained.

Cronje *et al.* (2000) further evaluated the batch test method, by applying the method to mixed liquor samples drawn from a well-defined parent laboratory-scale activated sludge system operated at 10d sludge age. They found that the correlation between measured and theoretical active biomass was poor, and remarkably similar to that obtained by Wentzel *et al.* (1998) on mixed liquor samples drawn from their parent system at 20d sludge age. This prompted a detailed examination of the batch test method. Two sources of potential error in the method were identified:

(i) It was observed that the OHO active biomass present in the wastewater exhibited a growth rate that was much faster than that of the OHO active biomass present in the mixed liquor drawn from the parent laboratory-scale activated sludge system. This caused that in the batch tests conducted with a mixture of wastewater and mixed liquor, the wastewater OHO active biomass partially masked the OUR response of the OHO active biomass 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 of Ubisi *et al.* (1997a,b) that nitrification in the batch test with wastewater and mixed liquor caused 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 a linear increase with time. Thus, in the batch test method 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 OHO active biomass (OUR_H), another potential source of error is introduced.

To eliminate the potential errors above, Cronje *et al.* (2000) proposed the following modifications to the batch test method:

- Physically remove the OHO active biomass from the wastewater: This was achieved through flocculation of the wastewater with aluminium sulphate followed by filtration. Batch tests on the *flocculated-filtered* wastewater demonstrated no observable biological activity, indicating that all the OHO active biomass had been successfully removed.

- In the calculation of the oxygen demand due to nitrification (OUR_N) (Antoniou *et al.*, 1990; Sözen *et al.*, 1996), it was assumed that the nitrate concentration – time profile follows an exponentially increasing trend, as opposed to a linear increase.

The modifications above proposed by Cronje *et al.* (2000) greatly simplified the batch test procedure – since the *flocculated-filtered* wastewater does not contain OHO active biomass, a parallel wastewater only batch test no longer needs to be conducted to

determine the wastewater OHO active biomass, which in the Ubisi *et al.* (1997a,b) method was subtracted from the mixed liquor + wastewater OHO active biomass, to give the mixed liquor OHO active biomass.

Cronje *et al.* (2000) compared the results from the modified batch test with theoretical values for OHO active biomass concentrations from the steady state design model (WRC, 1984). From this comparison they concluded that the results obtained showed good agreement, see Fig. 1.1. Also, there was remarkable agreement between the theoretical OHO active biomass concentration in the parent system and the mean of the measured OHO active biomass values *projected* to the parent system. However, they noted that the individually measured OHO active biomass values were prone to significant variation. They attributed this to mainly the sensitivity of the measured OHO 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 OHO active biomass values.

The good correlation that Cronje *et al.* (2000) found between the theoretical and measured values was remarkable, considering the sensitivity of the analysis. The results appeared to substantiate the modified batch test method as a reliable means of quantifying the OHO active biomass. However, the modified method does require more extensive evaluation.

In this research project, ***the principle aim is to further investigate the modified batch test method of Cronje et al. (2000).***

1.2 RESEARCH OBJECTIVES

The development of the modified 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 parameter. The modified batch test method developed by Cronje *et al.* (2000) has shown considerable promise as an independent means to quantify the hypothesized concentration of OHO active biomass present in an activated sludge system.

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

(1) Evaluate the reliability of the modified batch test method, by comparing the measured OHO active biomass concentrations with the theoretical values predicted by the steady state design model. Good correspondence between the theoretical and measured values would provide substantive direct evidence supporting the steady state design model and the modified experimental method.

To achieve the objective above, the specific tasks identified are:

- Operate and maintain a *control* parent laboratory-scale anoxic/aerobic activated sludge system, identical to that of Cronje *et al.* (2000), which would have the same mixed liquor characteristics.

- Perform batch tests identical in procedure to those conducted by Cronje *et al.* (2000), using mixed liquor samples drawn from the *control* parent laboratory-scale anoxic/aerobic activated sludge 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 anoxic/aerobic activated sludge systems.

(2) In addressing the main aim above, it was decided to run and operate a parallel parent laboratory-scale anoxic/aerobic activated sludge system having a different OHO active biomass fraction of the mixed liquor. To change the OHO active biomass fraction of the mixed liquor, a known concentration of macerated toilet paper solution was dosed to this system. Toilet paper is mainly constituted of wood pulp, which is composed of 75% cellulose and 25% lignin. These two organic components are believed to be largely unbiodegradable in the activated sludge system. Accordingly, toilet paper should contribute significantly to the inert sludge mass in the laboratory-scale anoxic/aerobic activated sludge system, thereby significantly increasing the MLOSS concentration in the system, and reducing the OHO active biomass fraction of the MLOSS. This would also provide the opportunity to evaluate the ability of the modified batch test procedure to detect the decreased active biomass fraction. As this constituted an independent investigation, the specific tasks are:

- Operate and maintain an *experimental* parent laboratory-scale anoxic/aerobic activated sludge system, identical to that of the *control* activated sludge system above, which would have the same mixed liquor characteristics initially and receiving the same daily mass of COD as the system described in (1) above, but with the addition of toilet paper solution subsequently in order to decrease the OHO active biomass fraction of the mixed liquor.

- From a comparison of the steady state results of the *control* and *experimental* parent systems, determine the biodegradability of the toilet paper, and hence its effect on the components making up the MLOSS, to derive a theoretical value of OHO active biomass.

- Perform batch tests identical in procedure to those described in (1) above, using mixed liquor samples drawn from the *experimental* parent laboratory-scale anoxic/aerobic activated sludge 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 anoxic/aerobic activated sludge systems, subjected to decreased OHO active biomass fractions.

1.3 RESEARCH APPROACH

The research approach adopted was to operate and monitor two well-defined and controlled continuously fed parent activated sludge systems (Chapters 3 and 4). The *control* activated sludge system provided the mixed liquor samples for measuring the OHO active biomass to address objective (1) above, see Chapter 5. To address objective (2), the *experimental* activated sludge system provided the mixed liquor samples for measuring the OHO active biomass.

1.4 CLOSURE

The principle objective identified for this research project is to evaluate the modified batch test procedure proposed by Cronje *et al.* (2000) to quantify the OHO active biomass concentration of the mixed liquor drawn from aerobic and anoxic/aerobic activated sludge systems. Should the values measured in the batch test agree with the theoretical values derived from the steady state design and kinetic simulation models, this will provide users of the models with greater surety in model application, and substantiate the batch test method.

However, the batch test method and interpretation of the data derived from it remains firmly rooted within the engineering and technology paradigm; in other words, the interpretation of the data is in terms of the concepts embodied within the models.

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.

Thus, measurement of OHO active biomass can form a starting point to build bridges between the two paradigm sets. If in this research project the OHO active biomass parameter can be successfully quantified within the engineering and technology 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. Further, the microbiological and biochemical information could provide independent confirmation of the OHO active biomass concept. This integration between the two paradigms can then be extended to other active biomasses.

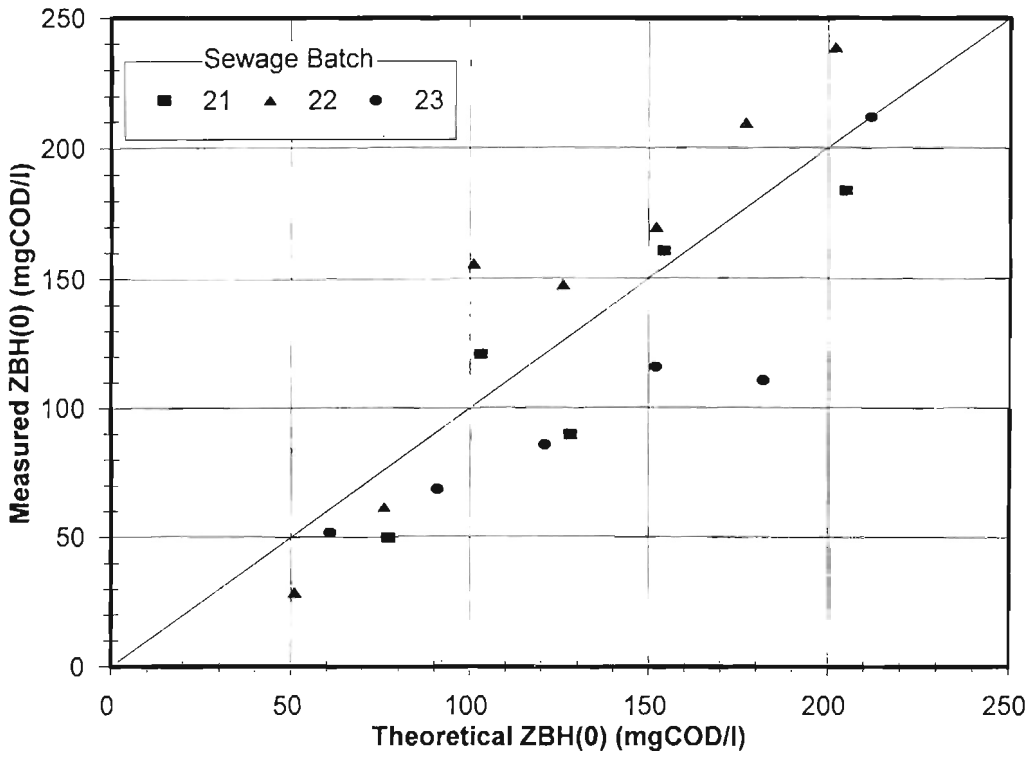


Figure 1.1: Modified batch test results: Graph of measured versus theoretical OHO active biomass, $Z_{BH(0)}$, for the various sewage batches with the parent laboratory-scale system operated at 10 days sludge age.

CHAPTER 2

EXISTING METHODS FOR QUANTIFYING OHO ACTIVE BIOMASS

2.1 INTRODUCTION

In Chapter 1, the central role in the steady state design procedures and kinetic simulation models of the mixed liquor organic suspended solids (MLOSS) component ordinary heterotrophic organism (OHO) active biomass has been highlighted. Further, the need for accurate quantification of this mixed liquor component has been demonstrated. To quantify OHO active biomass, the batch test method of Ubisi *et al.* (1997a,b), as modified by Cronje *et al.* (2000), was identified as a simple procedure that holds considerable promise, and this research project focuses on evaluating this batch test procedure. However, in addition to the batch test, a number of other methods described in the literature may be suitable to quantify OHO active biomass. Also, in Chapter 1 measurement of active biomass has been identified as a potential means to facilitate links and overlap between the engineering (design) and technology (implementation and operation) and microbiological and biochemical paradigms. Accordingly, in this Chapter, existing methods or methods with potential to quantify the OHO active biomass in activated sludge or similar systems from both the engineering and technology and microbiological and biochemical paradigms will be reviewed, to identify their strengths and weaknesses. Included in this review is a more detailed description of the batch test method.

2.2 MEASUREMENT METHODS

A variety of methods (both direct and indirect) have been developed to attempt to experimentally quantify the parameters loosely termed "biomass". However, as will become evident in the review, the "biomass" parameter does not necessarily relate directly to the OHO active biomass in the steady state design procedures and kinetic simulation models for activated sludge and similar systems. This deficiency limits possible application of a number of the methods.

2.2.1 Weight

Weight has been widely used as a measure of biomass, either by direct measurement or by the use of indirect measurements such as optical density/turbidity.

2.2.1.1 Direct measurement

The dry weight per unit of volume is readily obtained by separating the solid materials from the liquid and then drying at 105°C and weighing in a tared container; the dry weight is termed total suspended solids (TSS), Standard Methods (1985). Also, the volatile or organic solids weight can be obtained by combusting the dried sample at 600°C; the mass that combusts is termed the volatile suspended solids (VSS), Standard Methods (1985). Alternatively, the COD of the solid material can be measured (Standard Methods, 1985). These methods are widely used in practice to quantify the mixed liquor in the activated sludge system.

2.2.1.2 Optical density (OD)

A simple technique proposed to measure biomass is to use optical density. The optical density (OD) of a growth culture is measured with a spectrophotometer at 450 nm (Jensen *et al.*, 1988; Jørgensen *et al.*, 1992). In parallel, samples of the growth culture are centrifuged at 4 000 rpm for 10 minutes. The sediment/pellet is dried for 24 hours at 105°C, then weighed to determine the growth culture dry weight. A calibration curve to determine the conversion of OD to dry weight is made (a conversion factor of 250 ng/mℓ per absorbance unit is typically obtained). Absorbance of the sample to be quantified is measured and then converted to dry weight using the calibration curves. The dry weight is used as an approximation of biomass.

2.2.1.3 Summary

For activated sludge mixed liquor the weight determined with these methods will include all three organic components, i.e. active, endogenous and inert (see Chapter 1), and the dry weight (TSS) will additionally include an inorganic component. Thus, these types of tests will not be capable of isolating OHO active biomass.

2.2.2 Total cell count

The number of cells in a population can be measured by counting under the microscope, a method called the direct microscopic count (Brock and Madigan, 1988). Two kinds of count are done, either on samples dried on slides or on samples in liquid. With liquid counts, special counting chambers are used consisting of a slide with a grid marked on the surface, the volume above each grid being precisely measured. The number of cells per grid is counted under the microscope, this giving the number of cells per chamber volume.

Direct microscopic counting has a number of limitations: (1) The method is tedious, (2) living cells are not distinguished from dead, or inert/endogenous material, (3) small cells are difficult to see under the microscope and probably are missed, (4) precision is difficult to achieve, and (5) with the flocs from activated sludge it is difficult to separate out individual organisms. Thus, the method is not suitable to quantify OHO active biomass.

2.2.3 Viable cell count

In the total cell count described above, one limitation identified was that both living and dead cells are counted. To distinguish the living cells, viable cell counting methods have been developed. A viable cell may be defined as one that is able to divide and produce off-spring, i.e. replicate. The most usual way to perform a viable count is to determine the number of cells in the sample capable of forming colonies on a "suitable" medium. For this reason, the viable cell count also has been called the plate or colony count. Measurements of the number of cells capable of replication can be correlated to the weight of biomass. The viable count and the relation between viable numbers of cells and the weight of the biomass has been used as a basis for estimating the OHO active biomass in biological wastewater treatment systems (Gaudy and Gaudy, 1980; Droste and Sanchez, 1983).

Several techniques have been used in estimating the viable count, but the most common are: (1) colony count using solid media, (2) membrane filter and (3) null-point dilution in liquid medium.

2.2.3.1 Colony count using solid media

In this method, a solid medium is used to determine the number of cells capable of forming colonies. The three main types of colony count using solid media are: (1) Pour plate, (2) spread plate and (3) spot plating. The methods differ principally in how the medium is inoculated with the sample: In pour plating, the sample is mixed with melted agar medium and then allowed to cool, in spread plating the sample is spread evenly over the surface of a solid agar plate, while in spot plating a micropipette is used to add a very small discrete volume to a solid agar plate. The pour plate method is perhaps the most common plating method. In all the plating methods, the number of colonies that develop on the plate must not be too large. Thus, to obtain the correct colony numbers, the sample usually must be prediluted. Several ten-fold (serial) dilutions are commonly used.

The assumption made in all the solid media methods is that each visible colony grows from a single cell. Therefore, if cells are flocculated they must be thoroughly dispersed before conducting the test. For activated sludge system mixed liquor, often it is difficult to disperse the cells without influencing their viability. Thus, to retain viability less harsh dispersal methods are used, and the counts are expressed as the number of colony forming units, not as viable cells. Spread and spot plating usually have some advantage over pour plating because (i) agar plates contaminated during the pouring of agar can be discarded, and this eliminates counting errors, (ii) in pour plating the organism must be able to withstand the temperature of the melted medium, and (iii) because all colonies will be in the same plane in spread and spot plating, counting is easier. The spread and spot plating methods also have been found to be more serviceable (Gaudy *et al.*, 1963).

In all plating techniques, the number of colonies obtained on the plate will depend not only on the innoculum sample size, but also on the suitability of the culture medium, the incubation conditions and the length of incubation. Despite these limitations, and the

others listed above, these methods have been widely used; although for the activated sludge system, the plating methods have been more commonly used for organism identification than for viable cell counts.

2.2.3.2 Filter membrane

In this method a sample is poured over a membrane filter, preferably marked with grids to facilitate counting of colonies that develop. The filter paper then is placed on an absorbent pad containing nutrients, the pad being of such thickness that the paper will take up approximately 2 mℓ of the nutrient solution. The nutrient in the pad diffuses to the cells on the filter. The filter paper may also be placed on an agar plate. Since large volumes can be passed through the filter, the method can be used for dilute suspensions. Although this method offers some advantages over the solid media plating methods described above (e.g. easier to apply), because it also relies on colony growth on a selected medium it experiences a number of the same limitations. Furthermore, the method has inherent increased costs associated with it (Gaudy and Gaudy, 1980).

2.2.3.3 Null-point dilution in liquid medium

The basis of this method is to determine the dilution factor for a sample that no longer will provide sufficient seed of microorganisms to permit growth in fresh liquid media, i.e. the sample is diluted serially and the presence/absence of microorganisms determined. Any convenient quantitative measurement of growth can be used to detect the presence/absence of organisms in the liquid medium, e.g. gas formation has been used in a standard test for coliform organisms (Gaudy and Gaudy, 1980). However, because the measurement method is preselected, certain organisms may be excluded. The viable count in the original sample is estimated from the dilution and presence/absence results using the appropriate probability theory (Gaudy and Gaudy, 1980). The method usually is applied to estimate the concentration of a specific organism type, and is applicable to samples that contain very few organisms; both these factors limit possible application to determine the OHO active biomass in activated sludge mixed liquor.

2.2.3.4 Summary

All the tests to detect viable cells described above rely on the ability of the organisms to replicate (plating and membrane techniques) or exhibit a specific metabolic activity (null-point dilution) in an artificial medium. This will cause the tests to be selective – only those organisms with the ability to replicate/metabolize on the artificial substrates will be included. For example, it has been estimated that less than 10% of the organisms present in activated sludge mixed liquor will be cultured on the agar plates used as a standard in the plating techniques (Cloete and Steyn, 1988). Furthermore, dispersion of the cells in the activated sludge floc (a requirement in the tests) is difficult without reducing cell viability. These factors limit possible application of this type of test to determine activated sludge mixed liquor OHO active biomass.

2.2.4 Epifluorescence microscopy

To overcome the problems associated with culturing organisms to determine viable numbers (described above), techniques have been developed to count viable cells by microscopic examination of samples. The total cell count using microscopy has been described earlier; the principle deficiency identified with this method was that it could not distinguish living cells from dead. To get around this problem, the organisms can be stained with any one of a variety of fluorescent dyes specific for living cells, and the fluorescing cells counted under the microscope; the method is termed cell epifluorescence microscopy. Fluorescent dyes are used as they aid in microscopic counting. As an alterative to cell counts, biomass volume can be determined (Andreottola *et al.*, 2001). Both the cell count and biomass volume can be converted to biomass by using conversion factors such as weight of carbon per cell or cell volume (typically 310fg μm^{-3}, Fry, 1990). Converting from the derived weight of C to a dry weight or a COD requires an assumed cell formulation (Andreottola *et al.*, 2001).

2.2.4.1 Acridine orange (AO) direct count

Acridine orange (AO) is a fluorescent dye that is commonly used; this dye stains any organism containing DNA (i.e. any living organism); cells staining green with AO are generally viable – AO binds to nucleic acids, with the resultant RNA-AO complexes fluorescing orange-red while DNA-AO complexes fluoresce green (Porter and Feig, 1980).

Briefly the method is: The samples are diluted with phosphate buffer to give a bacterial count of about 100 bacterial cells per microscopic field. A sample (0.1 mℓ) is placed in a filter tower, and 1 mℓ of 0.1% acridine orange (AO) added. The sample is incubated for 2 minutes and 3 to 5 mℓ of 0.1M phosphate buffer added and the sample filtered. The damp filter is placed on a drop of immersion oil on a glass-slide. Immersion oil and a cover slip are then added on top of the filter. The sample is examined at 1 000X (oil immersion) magnification and the number of green fluorescing bacteria counted to determine active biomass. The AO method is mainly used in water treatment (Albat *et al.*, 1986), but Bitton *et al.* (1993) have used the method for total bacterial counts in samples of non-chlorinated activated sludge effluent.

The method has a number of deficiencies for application to activated sludge mixed liquor: (i) The method yields inconsistent cell fluorescence; the fluorescence does not differentiate microbial cells on the basis of metabolic activity or viability (APHA *et al.*, 1989; ASTM, 1985), (ii) the method is tedious, (iii) with the organisms in activated sludge mixed liquor binding in flocs, counting of individual cells is difficult – dispersion of the cells is a problem, as discussed earlier, and (iv) the colour of fluorescence depends on the moisture content of the filter paper; Bitton *et al.* (1993) found that addition of moisture to filter papers could change some cells fluorescence from orange-red to green.

2.2.4.2 DAPI direct count

The technique is similar to the AO direct count above, but with the fluorescent stain 4',6-diamidino-2-phenylindole (DAPI).

2.2.4.3 SYBR-Green I direct count

Another epifluorescence microscopy technique is that with the flourescent dye SYBR-Green I which offers a brighter flourescent signal (Andreottola *et al.*, 2001). However, this stain permeates all cells, both viable and dead.

2.2.4.4 Combination stains

Andreottola *et al.* (2001) have proposed using a combination of two flourescent stains to distinguish dead and viable organisms: SYBR-Green I is used to stain both dead and viable cells, while Propidium is used to stain dead cells only, with viable cells given by the difference.

2.2.4.5 Summary

Epifluorescence microscopy has a number of potential deficiencies for application to activated sludge mixed liquor: (i) The method can yield inconsistent cell fluorescence, (ii) the method is complex, expensive and tedious, (iii) with the organisms in activated sludge mixed liquor binding in flocs, counting of individual cells is difficult – dispersion of the cells is a problem, as discussed earlier, (iv) small volumes are used which may not be representative of the "true" sample, (v) converting from cell or cell biovolume to cell dry weight or COD requires assumed conversion factors, and (vi) it is not clear how inert material in the mixed liquor is taken into account.

2.2.5 Flow cytometry

As an alternative to epiflourescent staining and direct microscopy counting above, Andreottola *et al.* (2001) used flow cytometry to "count" cells stained with a combination of flourescent stains. The advantage of the method is that large numbers of cells can be counted in a short period (1 000 cells/s). However, the method still retains the difficulties associated with flourescent stains above.

2.2.6 Measurement of biochemical compounds

Due to the difficulties associated with the counting of organisms (directly via microscopy or indirectly via plating), various methods have been developed to measure quantitatively key compounds in organism's biochemical pathways and to relate these in some manner to organism mass. The two most commonly measured compounds are adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NAD).

2.2.6.1 Adenosine triphosphate (ATP) measurement

In this method the amount of adenosine triphosphate (ATP) is used as the indicator of microbial biomass. The ATP has the advantage of being a non-conservative constituent of the living cell which is directly related to the energy-growth process (Postage and Hunter, 1962; Holm-Hansen and Booth, 1966; Chappelle and Levin, 1968; Weddle *et al.*, 1971; Jensen *et al.*, 1988 and Jørgensen *et al.*, 1992). ATP is an energy-carrier molecule in microorganisms, has a rapid turnover and is lost very rapidly from dead or dormant organisms. In addition, its concentration remains relatively constant and independent of the growth rate in living cells (Franzen and Binkley, 1961; Forrest, 1965; D'Eustachio and Levin, 1967; Weddle and Jenkin, 1971). Hence, the total amount of ATP measured should provide an estimate of the number of living active microorganisms; one μg of ATP is equivalent to about 250 μg of carbon in living organisms. In the test, a sample is treated to extract ATP, and the ATP of the extract is measured. A number of sensitive methods are available for measuring ATP (Brock and Madigan, 1988). The most common method involves the measurement of light produced in the luciferin – luciferase reaction: Luciferin and luciferase are obtained from firefly lanterns and the amount of light produced when the enzyme, luciferase, acts on the substrate, luciferin, is proportional to the amount of ATP present. Thus, the ATP extracted from the sample is mixed with luciferin and luciferase and the light emmission in the reaction is measured using a scintillation spectrophotometer. The light emission is proportional to the ATP present, so that from the light emission the ATP can be determined from a calibration curve. Accepting a constant ATP per unit biomass, the biomass concentration then can be calculated.

Nelson and Lawrence (1980) applied the ATP measurement method to mixed liquor drawn from a laboratory-scale completely mixed fill and draw activated sludge system receiving a synthetic wastewater. The biological solids retention time (= sludge age, R_s) in the system was varied from 0.5 to 12 days. They found that the microbial viability (measured via the ATP) of the activated sludge mixed liquor volatile suspended solids (MLVSS) exhibits a functional relationship with R_s: Expressed as % viability of the MLVSS, it is close to 100% at low values of R_s and decreases to an approximate constant value at high R_s values; this type of behaviour is typical of the activated sludge system (WRC, 1984). Nelson and Lawrence (1980), confirmed from their study that the ATP pool level for a 100% viable culture of activated sludge is in reasonable agreement with many previously reported results for pure cultures of bacteria, and that the viable percentage of MLVSS varied with the value of R_s in a manner similar to the variations described by Postage and Hunter (1962), Weddle and Jenkins (1971) and Upadhyaya and Eckenfelder (1975). In activated sludge studies using domestic sewage as substrate, Weddle and Jenkins (1971) reported a lower viable percentage (10-20%) than found in the Nelson and Lawrence (1980) study (40-50%) at the larger values of R_s which are typical of normal process operation. Nelson and Lawrence (1980) proposed that the lower viable percentages reported in the studies treating domestic sewages are due to accumulation of non-biodegradable MLVSS which are originally present in the influent wastewater, i.e. the inert material accumulating from the unbiodegradable particulates in the influent.

Summary

The ATP measurement method requires sophisticated equipment and analytical techniques which are not widely available. This will cause the method to be unsuitable for general routine application. Furthermore, because ATP turns over rapidly in metabolizing cells, the levels of ATP in a single cell can vary depending upon the conditions that the cell is subjected to, e.g. concentrations of substrate, oxygen. For example, under starvation conditions the ATP levels reduce to low values (Brock and Madigan, 1988). Since the method is based on the assumption that the ATP level per unit organism remains constant (to convert ATP to biomass concentrations), the ATP may not be a good measure of biomass, but may rather be a measure of a combination of organism activity and biomass. However, the method does appear to hold promise and has been shown to correlate to the engineering concepts of activated sludge behaviour.

2.2.6.2 Nicotinamide Adenine Dinucleotide (NADH)

This test is very similar to that for ATP, with nicotinamide adenine dinucleotide (NADH) being measured instead. NADH is the electron and proton carrier molecule in organisms and its metabolism is an indicator of metabolic activity. The NADH measurement is based upon the principle that NADH, which is found in all living cells, fluoresces at 460 nm when radiated with light at 340 nm (Armiger *et al.*, 1986; 1993), the intensity of fluorescence being proportional to the NADH present. Measurement of NADH has been proposed as a method to control biological nutrient removal plant (BNR) processes (Armiger *et al.*, 1990b; 1991; Yang *et al.*, 1991). The environmental conditions of the activated sludge determine the metabolic pathways by which NADH is constantly recycled from the oxidised to the reduced form. Specifically, in BNR processes the reduction state of the activated sludge varies as the mixed culture flows from the anaerobic zone to the anoxic zone to the aerobic zone. The biological activity in each zone is defined as the reduction state of the activated sludge times the viable cell population. By constantly measuring the fluorescence from the NADH, it is possible to monitor changes in the biological activity of the activated sludge system.

Summary

NADH measurement has potential more as an indicator of biological activity than as a method to quantify OHO active biomass.

2.2.7 Measurement of biochemical reactions

This group of tests involves measurement of the "activity" of key biochemical reactions, by monitoring the changes in substrates or products of the selected reaction. Two examples of this type of test are given below.

2.2.7.1 Fluorescein diacetate (FDA) hydrolysis

In this method the ability of the mixed liquor to hydrolyse flourescein diacetate (FDA) is monitored. FDA can be quantified by measuring light adsorbance at 450 nm. Specific volumes (50 mℓ) of mixed liquor are centrifuged at 5 000 rpm for 5 minutes (Jensen *et al.*, 1988; Jørgensen *et al.*, 1992). The pellet is resuspended in 5 mℓ $NaHPO_4$ buffer and homogenised for two minutes by heavy stirring (Jensen *et al.*, 1988). A 4.5 mℓ volume of the resuspension is placed in a 10 mℓ flask containing 0.1 mℓ EDTA and 0.4 mℓ of a solution of protein synthesis inhibitors. A 25-μl volume of FDA solution is added, the flask incubated on a rotating axis for 45 minutes at room temperature. After incubation the reaction is terminated by transferring to 3 mℓ of acetone (Schnürer and Roswall, 1982). The mix is then vortexed and centrifuged at 5 000 rpm for 5 minutes. The absorbance of the supernatant at 450 nm is measured with a spectrophotometer, the adsorbance quantifying the FDA. Autoclaved samples are treated in the same way to serve as blanks, the difference in adsorbance between the samples and blanks quantifying the FDA that has been hydrolysed. The FDA hydrolysis results are converted, using a conversion factor of 10, to determine the biomass in the sample. Thus, this method assumes that the FDA hydrolysis per unit of viable organisms is essentially constant.

Summary

As a method for OHO active biomass measurement, this technique has serious deficiencies as the values obtained are generally higher than suspended solids measurements; the opposite is expected, as it has been found that not all bacteria are able to hydrolyse FDA (Leach, 1981; Chrzanowski *et al.*, 1984).

2.2.7.2 Dehydrogenase enzyme activity

This method measures the activity of the dehydrogenase enzyme using fluorescence microscopy. It is based on the principle that the electron transport system of respiring organisms reduce 2-(*p*-codophenyl)-5-phenyl tetrazolium chloride (INT) to INT-formazan (Zimmermann *et al.*, 1978; Droste and Sanchez, 1983). Respiring bacteria deposit accumulated INT-formazan as optically dense, dark red intracellular spots which can be examined by light microscopy; the amount of INT-formazan deposited corresponding to the intensity of the respiration. By combining formazan detection with acridine orange (AO) epifluorescence microscopy (see above), a method is then obtained which allows discrimination of bacteria from detritus, and differentiation between respiring and non-respiring cells (dehydrogenase enzyme activity) (Droste and Sanchez, 1983).

Summary

Although the method allows the determination of the total and active (cells with formazan) number of bacteria from the same sample, the method, however, fails to differentiate between OHOs and AOs. Also, the method fails to distinguish formazan deposits in small bacteria due to interference from the structure (pore openings) of the

filter paper on which the microorganisms are collected (Droste and Sanchez, 1983) and the method is thus conservative. Furthermore, since acridine orange epifluorescence is required, the problems detailed above for this method apply here also. Thus, the method cannot be used for routine OHO active biomass quantification.

2.2.8 Determination of deoxyribonucleic acid (DNA) content

In this method, deoxyribonucleic acid (DNA), which constitutes the genetic material of organisms, is extracted from the activated sludge mixed liquor and the amount extracted is measured. This is used to derive an estimate for the number of active organisms present; it is assumed that each organism has a constant known amount of DNA (Weddle *et al.*, 1971).

In using measured cellular constituents (e.g. Protein, carbohydrates, ATP, DNA) to calculate active biomass, a requirement is that the quantity of the constituent per unit active biomass remains constant. However, this may not be true in activated sludge mixed liquor because, (1) some of the measured components are not exclusively found in the biomass, and (2) the nutritional conditions of the activated sludge micro-organisms are not constant; depending on the sludge loading rate (SLR) micro-organisms contain different amounts of storage polymers (Liesbekind and Dohmann, 1994). Although the nutritional condition of the activated sludge may differ, the genome size (i.e. DNA) probably does not; thus a proportionality factor between DNA and the number of microorganisms present can be found. Microorganism genomes contain (with some exceptions) approximately 4 to 5×10^6 base pairs (bp) (Liesbekind and Dohmann, 1994); for example *E.coli* has 4.35×10^6 bp (Schlegel, 1985). Since activated sludge does not represent a pure culture, but is a bioceonosis of several hundreds or thousands of different microorganisms species, an average genome size of 4.5×10^6 bp per microorganism can be assumed.

The DNA method relies on reliable extraction of the DNA. However, extraction of the DNA is not without problems: Iron has a significant effect on the amount of acid extractable DNA; Hall and Axelrod (1977) showed that in pure cultures of *Asperigillus nidulans* trace quantities of cellular ferric iron (5.6 mg/ℓ) inhibited complete DNA extraction with perchloric acid at 70°C. Iron is a common component of activated sludge, sometimes reaching concentration levels as high as 40 mg/ℓ. Temperature and the technique of washing with EDTA solution also have a significant effect on the measured DNA content. For these and other reasons, the conventional method of biomass determination using DNA (Thomanetz, 1982, Obst and Holzatel-Pschorr, 1988), can only detect up to half the actual biomass DNA present in most activated sludge systems (Raebel and Schliert, 1980), depending on the presence/absence of substances and conditions that inhibit DNA extraction.

Despite the DNA extraction problems, in a general study on biomass characterization of activated sludge, Thomanetz (1982) described and tested 17 methods for living biomass estimation and biomass activity determination and found that the best method to

determine living biomass is via the determination of the DNA content, because the method was comparatively simple, quick and reproducible.

Liesbekind and Dohmann (1994) applied the method to activated sludge mixed liquor using acid extraction of DNA, quantitative determination of the deoxyribose sugar by a colour reaction with diphenylamine, calibration of the colour reaction with standard DNA, and mathematical conversion of the measured DNA into biomass and found that the conventional DNA method is strongly affected by unknown activated sludge constituents and in particular iron. They found that washing the sludges with EDTA first improved DNA extraction, but concluded that there still is no surety as to whether all the DNA is successfully extracted.

Summary

The method, described in detail by Liesbekind and Dohmann (1994) is complicated, tedious and requires sophisticated equipment to extract DNA. Furthermore, the extraction of all DNA is problematic and depends on the presence/absence of substances and conditions that inhibit its extraction – there is uncertainty on whether all the DNA is extracted from activated sludge mixed liquid. Also, the conversion of the measured DNA to the OHO active biomass parameter used in the steady state design and kinetic simulation models is unclear. Thus this method is not practical for general routine application.

2.2.9 Molecular identification of activated sludge bacteria using rRNA/DNA

This method seeks to identify bacteria by detecting nucleic acid sequences common to the targeted bacteria. The most common nucleic acid sequences targeted are ribosomal RNA (rRNA). Ribosomal RNAs (rRNA) are selected because they possess qualities that cause them to be suitable for discerning evolutionary relationships between bacteria: rRNAs are ancient molecules, functionally constant, universally distributed and moderately well conserved across broad phylogenetic distances. They are also readily purified from organisms without the use of cloning procedures (Brock and Madigan, 1988). There are three rRNA molecules, which in procaryotes have sizes 5S, 16S and 23S. The small size of 5S rRNA (~ 120 nucleotides) limits the information contained in the molecule, and so limits its use. However, the large rRNAs, 16S and 23S (containing approximately 1 500 and 3 000 nucleotides respectively) contain several regions of highly conserved sequence useful for proper sequence alignment, yet have sufficient sequence variability in other regions to show phylogenetic diversity. Of the two large rRNAs, 16S RNA is more experimentally manageable than 23S RNA, and so has been used extensively (it has been termed small subunit, SSU, rRNA).

Exploiting the above properties of rRNA, a number of techniques have been developed for bacterial identification, and to estimate proportions of specific or functional groups of bacteria in a sample. It is not the intention here to provide an exhaustive review of these techniques, but rather to provide a very simplified overview of some of these:

(1) **rRNA sequence analysis**

This technique involves sequencing the 16S rRNA. A number of methods are used to do this. For example: The rRNA is extracted from the bacteria of interest. A small DNA oligonucleotide primer (15 – 20 nucleotides in length) complementary in base sequence to some highly conserved section of the 16S rRNA molecule, is added. The enzyme reverse transcriptase (adds to the primer nucleotides which are complimentary to the rRNA) is added with 32P – labelled deoxyadenosine triphosphate and the other unlabelled deoxyribonucleotides. The mixture then is divided into four portions, and to each a small amount of different 2', 3' dideoxynucleotide is added. The enzyme reverse transcriptase will read the rRNA and make a DNA copy interrupted at various points by the incorporation of the dideoxynucleotide. The fragments are then sequenced by electrophoresis and autoradiography. From knowledge of the complementary DNA sequence, the sequence of the original 16S rRNA can be deduced. Once the sequence is known, it can be compared to known sequences of known bacteria, and the sample bacteria identified or placed in the correct phylogenetic group.

(2) **rDNA gene sequencing**

The principle is the same as for the rRNA sequence analysis, except that the DNA gene coding for the 16S rRNA is sequenced. Also, instead of using the enzyme reverse transcriptase to make a complimentary copy of the nucleotide sequence, the enzyme polymerase is used to make an identical copy.

(3) **In situ hybridization**

In this technique an oligonucleotide compliment (called a probe) is manufactured for a specific bacterial 16S rRNA sequence. On being combined with the sample, the oligonucleotide probe will hybridize with its compliment rRNA sequence. On hybridization, the paired oligonucleotide can be caused to fluoresce and this fluorescence can be viewed under a microscope. The technique is known as fluorescent *in situ* hybridization (FISH). By careful selection of the oligonucleotide probe, the probe can be hybridized to any desired target sequence. Since some areas of the rRNA sequence are common to specific species, while others are common to genus, sub groups, groups subphyla, etc., specific bacteria or functional groups can be identified. Also, by comparative tests the proportion of a specific bacteria or group relative to other groups (e.g. proportion of a species relative to a genus) can be determined.

The rRNA/DNA based methods are gaining increasing popularity for application to activated sludge mixed liquor. For example, Blackall (1994) applied rDNA gene sequencing to investigate filamentous bacteria in the stable dark viscous foam on the activated sludge aeration basin surfaces, and found that the diversity of the filamentous organisms in the foam increased with time. Similar studies were carried out on *Nocardia amarae* and *Nocardia pinensis* (now reclassified as *Gordona amarae* and *Skermania pinensis* respectively), both prominent foaming filaments in Australia. Genomine DNA was isolated from strains of *N.amarae* and *N.pinensis*. The 16S rDNA was amplified by the polymerize chain reaction and sequenced using an automated DNA sequencing

machine. The sequences were compared and regions that could be exploited for oligonucleotide probes highlighted. Regions in the evolutionary conserved 16S rDNA gene were highlighted as possible contenders for an oligonucleotide probe for *in situ* identification and quantification of these bacteria in activated sludge plants. Good yields of unsheared, genomic DNA were obtained with all bacterial strains studied; sequences of 16S rDNA of *N.pinensis* strains were identical, whilst those for *N.amarae* varied in a couple of positions (Blackall, 1994).

Using FISH, Wagner *et al.* (1994) compared the results from *in situ* rRNA oligonucleotide probes with those from culturing samples on nutrient rich media and found large discrepancies. They ascribed these discrepancies to the selectivity of the media and culture conditions. They successfully developed probes for *Acinetobacter*, and found that the probe results indicated that these organisms were present in BEPR systems at significantly lower levels than indicated by culturing techniques. Further, they demonstrated the application of probes to study the filamentous organism *Sphaerotilus natans*. They concluded that oligonucleotide probes will provide a tool that will greatly enhance knowledge of the ecology and phylogeny of wastewater organisms.

Summary

For the rRNA/DNA based methods, these are complex and analytically tedious requiring sophisticated equipment and considerable expertise. At present, the methods appear more suited for bacteria identification and the study of particular organism species or groups, than for quantification of total heterotrophic active biomass in terms of the total mass in the activated sludge system. However, the methods appear to hold promise to provide quantitative data on active biomass, and this requires further investigation.

2.2.10 Batch test method

Kappelar and Gujer (1992) describe a simple batch test to quantify OHO 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 OHO active biomass concentration. 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 OHO 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 OHO 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 OHO active biomass during these periods.

Ubisi *et al.* (1997a,b) extended this simple batch test method 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. They could provide no explanation for the inconsistency in results.

The batch test method of Ubisi *et al.* (1997a,b) was further investigated by Cronje *et al.* (2000) to attempt to identify possible cause(s) for the inconsistency noted by Wentzel *et al.* (1998). Initially, they applied the batch test method of Ubisi *et al.* (1997a,b) to mixed liquor samples drawn from a well-defined parent laboratory-scale anoxic/aerobic activated sludge system operated at 10d sludge age, with one minor modification: To ensure that both parallel batch tests had equal concentrations of wastewater, the same volume of wastewater was added to the batch tests, and the total volumes made up to 3ℓ by adding the mixed liquor sample to the one batch test and an equivalent volume of effluent from the parent activated sludge system to the other. This allowed Cronje *et al.* (2000) to directly compare the OUR responses in the two batch tests. In comparing the measured OHO active biomass with theoretical values, Cronje *et al.* (2000) found that the correlation was poor (Fig. 2.1), and remarkably similar to that obtained by Wentzel *et al.* (1998) on mixed liquor samples drawn from their parent system at 20d sludge age (Fig. 2.2). From a detailed examination of the batch test procedure and data, Cronje *et al.* (2000) attributed the poor correlation between the OHO active biomass concentration measured in the batch tests and the theoretical values predicted via the steady state design model to two main factors:

(i) Being able to directly compare the OUR responses from the two parallel batch tests, they observed that the OHO present in the wastewater exhibited a growth rate that was much faster than the growth rate exhibited by the OHOs present in the mixed liquor drawn from 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 biomass partially masked the OUR response of the OHO active biomass from the mixed liquor. Accordingly, a potential source of error in the batch test procedure 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 of Ubisi *et al.* (1997a,b) that nitrification in the batch test with wastewater and mixed liquor gives rise to 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), Cronje *et al.* (2000) 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 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 OHO active biomass (OUR_H), another potential source of error is introduced.

To overcome the deficiencies identified above, Cronje *et al.* (2000) proposed two main modifications to the batch test procedure of Ubisi *et al.* (1997a,b):

- Physically remove the OHO active biomass from the wastewater: This was achieved through flocculation of the wastewater with aluminium sulphate followed by filtration. Batch tests demonstrated no observable biological activity in the *flocculated-filtered* wastewater, indicating that all OHO active biomass had been successfully removed.

- Use exponential fits to the nitrate concentration – time profiles to determine nitrification OURs, as opposed to a linear increase.

The modifications proposed above greatly simplified the batch test procedure – since the *flocculated-filtered* wastewater does not contain OHO active biomass, a parallel batch test no longer needs to be conducted to determine the wastewater OHO active biomass, which in the "old" batch test method was subtracted from the mixed liquor + wastewater OHO active biomass to give the mixed liquor OHO active biomass.

Cronje *et al.* (2000) assessed the modified batch test procedure by applying the method to mixed liquor drawn from a well-defined parent laboratory-scale MLE activated sludge system operated at 10d sludge age, and compared the measured results to those predicted theoretically, see Fig. 1.1. This comparison demonstrated that the correlation between measured and theoretical OHO active biomasses was good. This indicates that the batch test method holds potential as a valuable tool that can be used to provide greater insight into the activated sludge system. However, the method does require more extensive

evaluation; ***in this research project, the modified batch test procedure of Cronje et al. (2000) will be further investigated.***

Summary

The experimental procedure for the modified batch test proposed by Cronje *et al.* (2000) is relatively simple and does not require sophisticated equipment. It would appear that the method can be readily applied to quantify the OHO active biomass in activated sludge mixed liquor samples. Such an application, however, still requires extensive evaluation.

2.3 CLOSURE

In this Chapter a number of experimental methods to quantify OHO active biomass have been reviewed. The vast majority of these methods find their origin in the microbiological and biochemical sciences, in the detailed study of pure cultures. For most of the tests, their application to activated sludge mixed liquor has been limited. For those that have been applied to activated sludge mixed liquor, or have potential for application, some possible deficiencies have been identified; in general, for the simpler tests, these give estimates that are too approximate to provide meaningful results and for the more rigorous tests, these may be too elaborate for routine use requiring sophisticated equipment and experimental techniques. Of this group of analytical methods, probably epiflourescent microscopy/flow cytometry, ATP analysis and the new molecular techniques appear to be the most suitable for measurement of OHO active biomass. However, these methods provide estimates for active (viable) biomass that are not directly related to the OHO active biomass parameter in the steady state design and kinetic simulation models; integration of the estimates from these tests with the design and modelling theory is an area that requires investigation.

Within the engineering and technology paradigm, the batch test method for quantifying OHO active biomass of Kappelar and Gujer (1992) as modified and extended by Wentzel *et al.* (1995), Mbewe *et al.* (1995), Ubisi *et al.* (1997a,b) and Cronje *et al.* (2000) is relatively simple and does not require sophisticated equipment.

With the modified batch test method of Cronje *et al.* (2000), estimates for OHO active biomass are obtained that are directly related to this parameter in the modelling theory. Furthermore, the correlation between measured and theoretical OHO active biomass values appear good. Consequently, this test appears to hold promise for application – in this research project this test method will be further evaluated and developed. Should the method provide OHO active biomass estimations that correlate closely with the activated sludge theory, this will provide users of the activated sludge simulation models with greater surety in application. Further, if the method proves reliable it will provide a future opportunity to integrate results from the microbiological/biochemical analytical methods with the modelling theory, by comparison of the data obtained from the different test methods.

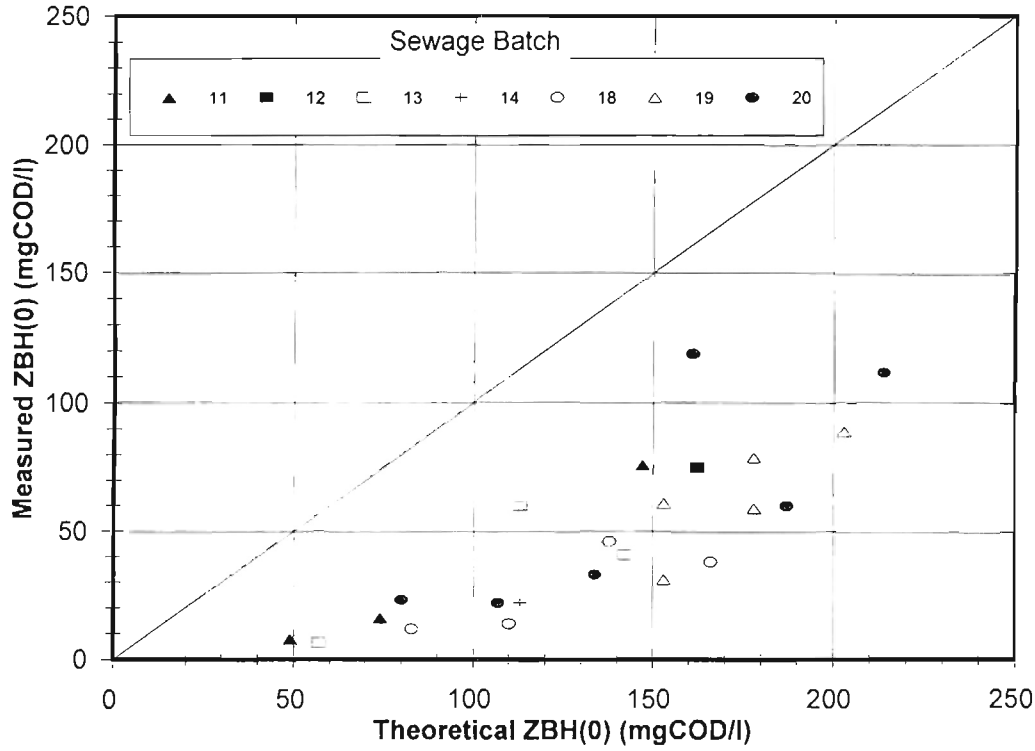


Figure 2.1: Graph of measured versus theoretical OHO active biomass, $Z_{BH(0)}$, for the various sewage batches with the parent laboratory-scale system operated at 10 days sludge age.
[Cronje *et al.* (2000)]

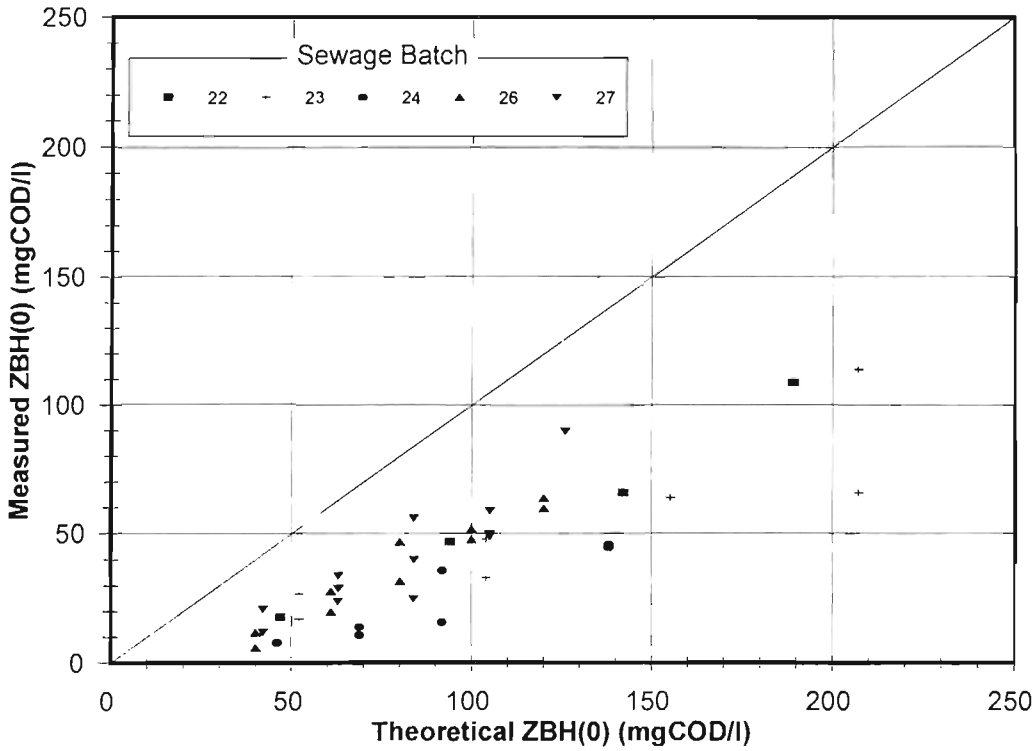


Figure 2.2: Graph of measured versus theoretical OHO active biomass, $Z_{BH(0)}$, for the various sewage batches with the parent laboratory-scale system operated at 20 days sludge age.
[Wentzel *et al.* (1998)]

CHAPTER 3

THE PARENT CONTROL LABORATORY-SCALE NITRIFICATION / DENITRIFICATION ACTIVATED SLUDGE SYSTEM

3.1 INTRODUCTION

As described in Chapters 1 and 2, the results from the modified batch tests conducted by Cronje *et al.* (2000) on samples harvested from a nitrification / denitrification laboratory-scale activated sludge system operated at 10 days sludge age, showed remarkably good agreement between the measured and theoretical OHO active biomass concentration. However, the method does require more extensive evaluation; ***the principle aim in this research project is to extensively evaluate the modified batch test procedure of Cronje et al. (2000).***

To further investigate the modified batch test procedure, the first objective of this research project was to operate and maintain a *control* parent laboratory-scale nitrification / denitrification activated sludge system identical to that of Cronje *et al.* (2000) which would have the same mixed liquor characteristics. This system would serve as a source of mixed liquor for the batch tests.

To further evaluate the reliability of the modified batch test method, the second objective was to run and operate an *experimental* parent laboratory-scale anoxic/aerobic activated sludge system in parallel to the *control* system above, but having a different OHO active biomass fraction of the mixed liquor. Thus, this system would serve as a source of mixed liquor for the batch tests, with a different OHO active biomass fraction. The ability of the modified batch test to correctly detect this change in OHO active biomass fraction would be evaluated. To change the OHO active biomass fraction of the mixed liquor, in this research project the *experimental* system was set-up and operated identically to the *control*, but additionally a known concentration of macerated toilet paper solution was dosed to the *experimental* system. Toilet paper is mainly constituted of wood pulp. Pulp is composed of 75% cellulose and 25% lignin and these components are believed to be largely unbiodegradable. This should cause the toilet paper to contribute a significant proportion of inert sludge mass to the *experimental* laboratory-scale anoxic/aerobic activated sludge system mixed liquor, thus decreasing the fraction of OHO active biomass in the mixed liquor.

This Chapter describes the configuration and operation of the parent *control* laboratory-scale system, and details the response of the system. In Chapter 4, the *experimental* system is described.

3.2 CONTROL SYSTEM LAYOUT

The *control* parent laboratory-scale system is shown schematically in Figs. 3.1 and 3.2. The system was operated for 417 days in total. The main changes to system configuration and operation are summarised in Table 3.2. The system had three main configurations, which are described below.

3.2.1 Configuration 1

Initially, the system layout constituted a Modified Ludzack-Ettinger (MLE) configuration and consisted of an anoxic reactor of 2.5ℓ volume (25% of the total system volume), an aerobic reactor of 7.5ℓ volume (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 total system volume was 10ℓ.

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, at the end of which was an air-stone terminating 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 layout of the system is shown in Fig. 3.1.

After operating the Modified Ludzack-Ettinger (MLE) activated sludge system for the first sewage batch, severe bulking[1] problems arose soon afterwards (the DSVI increased rapidly from 135 mℓ/g to 553 mℓ/g in 13 days) and significant solids were lost to the effluent daily (this is clear from the measured high average unfiltered effluent COD value of 144 mgCOD/ℓ for Sewage Batch No. 2). Lee *et al.* (1983) showed that at DSVI's above 150 mℓ/g, the filamentous organisms commence to dominate the behaviour of the settling sludge. As a rough guide therefore, a bulking sludge can be accepted as one having a DSVI > 150 mℓ/g (Ekama and Marais, 1984).

Filamentous organism identification showed that the predominant filament species present was *Sphaerotilus natans* which were very common, followed by *Microthrix parvicella* and *Thiothrix sp.* According to Jenkins *et al.* (1984), *S. natans* sorts into the low DO filament group, *M. parvicella* into a low F/M type filament and *Thiothrix sp.* into the septic sewage or nutrient deficient groups. Although the specific cause for bulking could not be ascertained, it was hypothesized that *S. natans* proliferation in the laboratory-scale activated sludge system was caused by seeding from *S. natans* attached growth on the influent feed line walls. Regular and thorough cleaning of the influent feed

[1] Bulking due to excessive growth of filamentous organisms causes deterioration in the mixed liquor settleability. The filament lengths extending into the bulk liquid form web-like structures which cause either the floc structure itself to be diffuse or bridging between the flocs.

lines could have eliminated the *S. natans* bulking problem (Gabb *et al.*, 1989a). However, since the proliferation of the above filamentous organisms occurred at the onset of the experimental investigation, no control strategy against these organisms were taken to remedy the situation. Instead, the contents of the reactor were drained and the system was reseeded with mixed liquor from the Mitchell's Plain Treatment Plant in Cape Town (South Africa).

3.2.2 Configuration 2

As the experimental investigation proceeded, it was observed that denitrification was poor. Thus, it was decided to slightly modify the original Modified Ludzack-Ettinger (MLE) configuration (from day 107 to day 379) by having a larger anoxic mass fraction of 3.3ℓ volume (33% of the total system volume) and a reduced aerobic mass fraction of 6.7ℓ volume (67% of the total system volume), with the total system volume remaining fixed at 10ℓ. At the same time, the opening of the anoxic reactor was closed by means of a cork to limit surface exchange of oxygen.

After 304 days of operation, the problem of bulking re-emerged; the DSVI value increased steadily from 107 mℓ/g to 333 mℓ/g in a few weeks. Filamentous organism identification revealed that the poor settleability was caused by a proliferation of the filament *Microthrix parvicella*; they were very common to abundantly present in the laboratory-scale activated sludge system. A short-term remedy was to dose aluminium sulphate [Aℓ(SO$_4$) • 15H$_2$O, stock at 133g/ℓ] to control its proliferation. A starting dose of 50 mℓ was added on the first day to the aerobic reactor of the activated sludge system, thereafter 5 mℓ of aluminium sulphate was dosed daily for the next 47 days, until the DSVI decreased to 177 mℓ/g. While successful, the problem with non-specific control methods is that they treat temporarily the symptoms of bulking, but do not constitute a permanent cure (Casey *et al.*, 1995) – immediately after dosing of aluminium sulphate ceased, the filaments started to regrow and the DSVI increased steadily to 208 mℓ/g in 2 days. The dominant species were again identified as *Microthrix parvicella*.

3.2.3 Configuration 3

With specific bulking control, the causes for the proliferation of the filaments are sought. Casey *et al.* (1995) concluded that by eliminating these through wastewater characteristic or system modification, the bulking problems caused by specific filamentous organism types are cured permanently. They also concluded that low F/M filament bulking sludges containing amongst others the filamentous organism *Microthrix parvicella*, from full-scale or laboratory-scale activated sludge systems invariably ceased bulking within a short space of time under fully aerobic conditions. Hence, to improve the sludge settleability, it was decided to change the laboratory-scale *Modified Ludzack-Ettinger* (MLE) system to a completely mixed *fully aerobic* system.

The completely mixed *fully aerobic* system layout consisted of a single biological reactor and a secondary settling tank in series, with an underflow recycle from the settling tank to the biological reactor of 1:1 with respect to the influent flow, see Fig. 3.3. Accordingly,

this system configuration was used for the rest of the experimental investigation. The total system volume was however reduced to 8ℓ. This was done by mixing the sludge from both the anoxic and aerobic reactors of the MLE system, settling the mixture and decanting off 2ℓ of the supernatant.

After a week of operation, sludge settleability did not ameliorate that much in the completely mixed *fully aerobic* system and it was decided to continue dosing 5 mℓ of aluminium sulphate daily and this was carried on till the end of the experimental investigation (day 417). The DSVI by then had decreased to 73 mℓ/g.

3.3 WASTEWATER COLLECTION AND STORAGE

The influent for the parent laboratory-scale activated sludge system was raw (unsettled) sewage 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, before both the course and fine screens and before grit removal and the primary sedimentation tanks. The sewage batch was brought to the laboratory and stored in 400ℓ 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 sulphide 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, which is required for subsequent dilution (see below) (COD of the undiluted sewage ranged from 1 000 to 1 500 mgCOD/ℓ).

3.4 FEED PREPARATION

3.4.1 Configuration 1

The total COD concentration which served as feed to the parent laboratory-scale activated sludge system for Configuration 1 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 20ℓ 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 and to give the required daily feed volume of 20ℓ. Thus, the total COD load per day on the MLE activated sludge system was ± 10 000 mgCOD/d.

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 1ℓ of distilled water. By adding 100mℓ of the buffer solution to the 20ℓ diluted sewage an increase in alkalinity of 200 mg/ℓ (as $CaCO_3$) was achieved. After thorough mixing, samples were drawn for influent analysis.

3.4.2 Configuration 2

As mentioned in Section 3.2.2 above, the main motivation behind modifying the configuration of the original MLE system was the poor denitrification that was achieved. The denitrification potential[2] is directly proportional to the influent readily biodegradable COD (RBCOD) concentration (Ekama and Marais, 1984). Thus, to improve the denitrification potential, the total COD concentration which served as feed to the parent laboratory-scale activated sludge system for Configuration 2 was increased to 750 ± 75 mgCOD/ℓ. However, to maintain the original characteristics of the activated sludge system, the total COD load per day on the MLE activated sludge system was kept at ± 10 000 mgCOD/d, so the new feed volume was reduced to 13.3ℓ. The buffer solution added to the 13.3ℓ diluted sewage was adjusted to about 70 mℓ to provide the same alkalinity of 200 mg/ℓ (as $CaCO_3$).

3.4.3 Configuration 3

The total COD concentration which served as feed to the parent laboratory-scale completely mixed *fully aerobic* system for Configuration 3 was the same as for Configuration 2, at 750 ± 75 mgCOD/ℓ, and the feed volume was also the same, at 13.3ℓ.

3.5 FEEDING THE SYSTEM

After thoroughly mixing the diluted sewage above, the influent feed for the parent activated sludge system over the next 24h was drawn (20ℓ for Configuration 1 and 13.3ℓ for Configurations 2 and 3), and placed in an upright PVC bucket which had a stirrer driven at 10rpm, to keep the contents in the feed 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 feed 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 swapped. 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.

[2] The denitrification potential of a process is the maximum mass of nitrate per unit influent flow that the process as designed can denitrify.

The calibration of the feed pump was checked daily through ensuring that the feed bucket was empty at exactly the same time (say 14h00) every day. The feeding rate was increased or decreased by adjusting the pumping frequency.

3.6 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 mixed liquor in the anoxic reactor was maintained at 2.5ℓ and at 3.3ℓ for Configurations 1 and 2 respectively and that in the aerobic reactor at 7.5ℓ, 6.7ℓ and 8ℓ for Configurations 1, 2 and 3 respectively by controlling the outlet water level in the reactors. The sludge age (R_s) was controlled hydraulically at 10 days, by wasting 1ℓ of mixed liquor daily from the aerobic reactor for Configurations 1 and 2 and 0.8ℓ of mixed liquor daily from the aerobic reactor for Configuration 3 (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, the mixed liquor rediluted to the correct volume with effluent and the appropriate volume required to avoid over-wasting was returned to the system. The activated sludge system was operated in a temperature-controlled room at 20°C. In the bioreactor, pH was controlled at 7.5 (±0.2).

The dissolved oxygen (DO) concentration was controlled 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.

3.7 SAMPLING AND MEASUREMENTS

3.7.1 Configurations 1 and 2

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 out and stored at 4°C for analysis the following day. Experience showed that inaccuracies were common 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. To do this, the following sampling sequence was adopted:

- First the two 50 mℓ samples for nitrate/nitrite analysis 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 mercuric chloride solution (8.6 g $HgCl_2$ /ℓ, Standard Methods, 1985) to each sample and were stored at 4°C for not more than 7 days before conducting the nitrate/nitrite analysis.

- After storing the nitrate/nitrite 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 below and above the water surface back into the mixed liquor; sludge particles were splashed out of solution and deposited above the surface water level of the aerobic reactor wall as a result of intermittent aeration.

- After brushing, the system was allowed to operate normally for ± 30 minutes to allow sludge particles to be distributed evenly throughout the system.

- 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 in a 1:10 ratio 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 3.1:

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 acclimatisation 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 downloaded from the DO meter to a PC 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, the mean OUR determined from the remaining data and recorded as the OUR for the day. The number of OUR readings ranged between 120 and 150 per day.

Table 3.1: Daily tests conducted on parent MLE activated sludge 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

3.7.2 Configuration 3

The sampling and measurements done on the completely mixed fully aerobic system was similar to that described in Section 3.7.1 above, except that no anoxic samples needed to be taken or analysed since the anoxic reactor was not present.

3.8 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 OHO 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 26 batches of sewage were fed to the parent system; 22 sewage batches served as feed to the *MLE* activated sludge system and the last 4 sewage batches served as feed to the completely mixed *fully aerobic* activated sludge system. These sewage batches and the dates they were used as feed are listed in Table 3.2. Also indicated are the sewage batches during which mixed liquor samples were drawn from the system for batch tests.

Table 3.2: Details of the parent *control* laboratory-scale activated sludge system, sewage batch number, sewage feed dates, days of operation and batch tests conducted.

Sewage Batch No.	Date of tests (2000/2001)	Days of operation (d)	Batch Test	Parent System
1	28 Apr – 7 May	day 1 – 10	No	25% Anoxic 75% Aerobic MLE
2	8 May – 19 May	day 11 – 22	No	
3	2 Jun – 14 Jun	day 36 – 48	No	
4	15 Jun – 22 Jun	day 49 – 56	No	
5	23 Jun – 10 Jul	day 57 – 74	No	
6	11 Jul – 26 Jul	day 75 – 90	No	
7	27 Jul – 02 Aug	day 91 – 97	No	
8	03 Aug – 17 Aug	day 98 – 112	No	
9	18 Aug – 03 Sep	day 113 – 129	No	33% Anoxic 67% Aerobic MLE
10	04 Sep – 21 Sep	day 130 – 147	No	
11	22 Sep – 6 Oct	day 148 – 162	No	
12	07 Oct – 19 Oct	day 163 – 175	No	
13	20 Oct – 3 Nov	day 176 – 190	No	
14	04 Nov – 17 Nov	day 191 – 204	No	
15	18 Nov – 29 Nov	day 205 – 216	No	
16	30 Nov – 09 Dec	day 217 – 226	No	
17	09 Feb – 15 Feb	day 288 – 294	No	
18	16 Feb – 11 Mar	day 295 – 318	Yes	
19	12 Mar – 23 Mar	day 319 – 330	Yes	
20	24 Mar – 08 Apr	day 331 –346	Yes	
21	09 Apr – 22 Apr	day 347 – 360	Yes	
22	23 Apr – 08 May	day 361 – 376	Yes	
23	09 May – 20 May	day 377 – 388	Yes	100% Aerobic Fully Aerobic
24	21 May – 03 Jun	day 389 – 402	Yes	
25	04 Jun – 13 Jun	day 403 – 412	Yes	
26	14 Jun – 18 Jun	day 413 – 417	Yes	

3.9 RESULTS

3.9.1 Steady state periods

Daily results for the parent *control* activated sludge system are listed in Appendix B, Tables B3 and B4. Each sewage batch was accepted as a steady state period. The daily data for each sewage 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 sewage 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 3.3 (a and b).

3.9.2 N and COD 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 and the fully aerobic systems, using the averaged data from Sewage Batch No. 5 and Sewage Batch No. 24 respectively as examples, are given below. The description is followed by an analysis of the results obtained for the various sewage batches.

3.9.2a Nitrogen (N) mass balance: Calculation method

(1) MLE activated sludge system

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:

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

$$MNti = MNte + MNne + MNd + MNw \qquad \text{(mg N/d)} \qquad (3.1)$$

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 primarily in the anoxic reactor, except in a few batches of sewage where a small amount of denitrification (about 1-2 mgN/ℓ) was also observed in the settling tank. This was taken into account in the N mass balance for these particular batches of sewage. For the anoxic reactor:

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

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

where

Qi = influent flow rate (20 ℓ/d)
a = a –recycle ratio = 2
s = s –recycle ratio = 1

$NO_{3(AE)}$ = NO_3 concentration in the aerobic reactor *(ie. 15.2 mgN/ℓ for S. Batch No. 5)*
$NO_{3(FE)}$ = NO_3 concentration of the filtered effluent *(ie. 17.2 mg N/ℓ for S. Batch No. 5)*
$NO_{3(AN)}$ = NO_3 concentration in the anoxic reactor *(ie. 7.0 mgN/ℓ for S. Batch No. 5)*

For Sewage Batch No. 5: MNd = [(15.2•20•2) + (17.2•20•1)] – [(7.0•20•(1+2+1)]
MNd = 392 mgN/d

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

For Sewage Batch No. 5: MNte = Nte • Qi = 2.4 • 20 = 48 mgN/d

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

For Sewage Batch No. 5: MNne = 17.2 • 20 = 344 mgN/d

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

For Sewage Batch No. 5: MNw = (N_{ML} – Nte) • Qw = (255 –2.4) • 1.0 = 252.6 mgN/d

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

For Sewage Batch No. 5: MNti = 48.8 • 20 = 976 mgN/d

By substituting the masses calculated above into Eq. (3.1), the **N mass balance** is given by

$$Nbal\ (\%) = 100 \cdot (MNte + MNne + MNd + MNw) / MNti \qquad (3.2)$$

For Sewage Batch No. 5: Nbal (%) = 100 • (48+344+392+252.6) / 976
Nbal (%) = 106.2 %

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

(2) Fully Aerobic activated sludge system

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, and the mass of TKN abstracted through the waste sludge:

mass of influent TKN	=	mass of effluent TKN	+	mass of effluent NO_3	+	mass of TKN wasted

$$MNti = MNte + MNne + MNw \quad \text{(mg N/d)} \qquad (3.1)$$

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

For Sewage Batch No. 24: MNte = Nte • Qi = 7.1 • 13.3 = 94.4 mgN/d

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

For Sewage Batch No. 24: MNne = 30.0 • 13.3 = 399 mgN/d

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

For Sewage Batch No. 24: $MNw = (N_{ML} - Nte) \cdot Qw = (257.6 - 7.1) \cdot 0.8 = 200.4$ mgN/d

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

For Sewage Batch No. 24: MNti = 57.2 • 13.3 = 760.8 mgN/d

By substituting the masses calculated above into Eq. (3.1), the **N mass balance** is given by

$$Nbal\ (\%) = 100 \cdot (MNte + MNne + MNw) / MNti \qquad (3.2)$$

For Sewage Batch No. 24: Nbal (%) = 100 • (94.4+399+200.4) / 760.8
Nbal (%) = 91.2 %

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 3.3(a) and are shown graphically in Fig. 3.4. The results can be commented on as follows.

- For Sewage Batches No. 3 and 4, the mass balances were 82% and 116% respectively. This considerable variability was attributed to the fact that the activated sludge system had just been reseeded with mixed liquor from the Mitchell's Plain Treatment Plant in Cape Town, South Africa (after the severe bulking problems experienced in Sewage Batch No. 2, see Section 3.2.1) and it was not yet at steady state. The problem was resolved from Sewage Batch No. 5 onwards where the N mass balances showed improvement.

- For Sewage Batch No. 8A the N mass balance was 83%. Analysis of the experimental data showed that denitrification was poor. To improve denitrification, the system configuration was modified to incorporate a larger anoxic zone (Configuration 1 to 2) and the N mass balance improved to 95% for the remainder of the sewage batch, No. 8B.

- For Sewage Batch No. 11 the N mass balance was particularly poor (76%). Here the problem appeared to be related to the particular sewage batch used as influent. The average unfiltered TKN values were higher than expected and denitrification was also quite low. This sewage batch should be rejected for further analysis.

- N mass balance for Sewage Batches No. 10, 12, 16 and 22 were marginally less than 90% (87%, 88%, 86% and 86% respectively). No assignable cause could be identified for these mass balances, but probably lies in the nitrate/nitrite and/or influent TKN measurements. Since the N mass balances are only marginally outside the acceptable range, the data for these sewage batches will be retained, but with due caution exercised in interpreting the data. In any event, batch tests were only conducted during Sewage Batch No. 22, see Table 3.2.

- Generally, acceptable N mass balances could be achieved without undue difficulty.

3.9.2b COD mass balance: Calculation method

(1) <u>MLE activated sludge system</u>

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

$$MSti = MSte + MOc + MOd + MXsvw \quad \text{(mgCOD/d)} \quad (3.3)$$

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

For Sewage Batch No. 5: MSte = 42 • 20 = 840 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):

$$24 \cdot OUR \cdot V_{AE} = MOt = MOc + MOn \quad \text{(mgO/d)} \quad (3.4)$$

where

V_{AE} = volume of the aerobic reactor (7.5 ℓ)
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):

$$MOn = 4.57 \cdot MNc \quad \text{(mgO/d)} \quad (3.5)$$

where

4.57 = mass in mg of oxygen utilized per mg NH_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):

$$MNc = MNd + MNne \quad \text{(mgN/d)} \quad (3.6)$$

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

For Sewage Batch No. 5: MNc = 392 + 344 = 736 mgN/d

The carbonaceous oxygen demand (MOc) was calculated by substituting Eq. (3.5) into Eq. (3.4) and solving for MOc:

$$MOc = (24 \cdot OUR \cdot V_{AE}) - (4.57 \cdot MNc) \quad \text{(mgO/d)} \quad (3.7)$$

For Sewage Batch No. 5: MOc = (24•36.22•7.5) – (4.57•736)
MOc = 3156.1 mgO/d

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

$$MOd = 2.86 \bullet MNd \qquad (mgO/d) \qquad (3.8)$$

where 2.86 = oxygen equivalent of nitrate as electron acceptor ($mgO/mgNO_3$-N)

For Sewage Batch No. 5: $MOd = 2.86 \bullet 392$

$MOd = 1121.1\ mgO/d$

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

$$MX_{SVW} = (COD_{ML} - Ste) \bullet Qw \qquad (mgCOD/d) \qquad (3.9)$$

For Sewage Batch No. 5: $MX_{SVW} = (4344 - 42) \bullet 1.0$

$MX_{SVW} = 4302\ mgCOD/d$

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

For Sewage Batch No. 5: $MSti = 500 \bullet 20 = 10000\ mgCOD/d$

By substituting the masses calculated above into Eq. (3.3), the **COD mass balance** is given by:

$$COD\ bal(\%) = 100 \bullet (MSte + MOc + MOd + MX_{SVW}) / MSti \qquad (3.10)$$

For Sewage Batch No. 5: $COD\ bal(\%) = 100*(840 + 3156.1 + 1121.1 + 4302) / 10000$

$COD\ bal(\%) = 94.2\%$

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

(2) Fully Aerobic activated sludge system

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 and COD mass of the waste sludge:

mass of influent COD	=	mass of effluent COD	+	mass of carbonaceous oxygen demand	+	mass of COD wasted

$$MSti = MSte + MOc + MXsvw \qquad (mgCOD/d) \qquad (3.3)$$

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

For Sewage Batch No. 24: MSte = 51.5 • 13.3 = 685.0 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):

$$24 \cdot OUR \cdot V_{AE} = MOt = MOc + MOn \quad \text{(mgO/d)} \quad (3.4)$$

where

V_{AE} = volume of the aerobic reactor (8.0 ℓ)
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)

$$MOn = 4.57 \cdot MNc \quad \text{(mgO/d)} \quad (3.5)$$

where

4.57 = mass in mg of oxygen utilized per mg NH_4-N nitrified.

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

$$MNc = MNne \quad \text{(mgN/d)} \quad (3.6)$$

MNne was determined from the N mass balance calculations above:

For Sewage Batch No. 24: MNc = 399 mgN/d

The carbonaceous oxygen demand (MOc) was calculated by substituting Eq. (3.5) into Eq. (3.4) and solving for MOc:

$$MOc = (24 \cdot OUR \cdot V_{AE}) - (4.57 \cdot MNc) \quad \text{(mgO/d)} \quad (3.7)$$

For Sewage Batch No. 24: MOc = (24•43.03•8.0) – (4.57•399)
MOc = 6438.3 mgO/d

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

$$MX_{SVW} = (COD_{ML} - Ste) \bullet Qw \qquad \text{(mgCOD/d)} \qquad (3.9)$$

For Sewage Batch No. 24: $MX_{SVW} = (5083.4 - 51.5) \bullet 0.8$
$MX_{SVW} = 4025.5$ *mgCOD/d*

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

For Sewage Batch No. 24: $MSti = 797.9 \bullet 13.3 = 10612$ *mgCOD/d*

By substituting the masses calculated above into Eq. (3.3), the **COD mass balance** is given by:

$$\text{COD bal}(\%) = 100 \bullet (MSte + MOc + MX_{SVW}) / MSti \qquad (3.10)$$

For Sewage Batch No. 24: $COD\ bal(\%) = 100*(685 + 6438.3 + 4025.5) / 10612$
$COD\ bal(\%) = 105.1\%$

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

Table 3.3 (a): Parent system steady state data; for each sewage batch (steady state period, see Table 3.2) the data have been averaged and the means, sample standard deviations (SSD) and number of tests are listed.

Sewage Batch No.	TKN (mgN/ℓ)									NITRATES (mgN/ℓ)								
	INFLUENT			UNFIL.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	42	2	7	4.6	2.8	7	194	10	7	14.8	1.6	7	20.0	2.1	7	21.9	2.0	7
2	46	2	10	6.0	1.2	10	160	23	10	13.5	2.1	10	19.4	1.8	10	21.8	3.8	10
3	61	2	8	5.0	1.1	8	265	5	8	12.8	1.6	8	18.0	0.9	8	23.8	1.8	8
4	59	1	6	2.1	0.9	6	294	12	6	12.7	1.5	6	25.7	0.8	6	25.8	1.9	6
5	49	3	6	2.4	1.0	6	255	20	6	7.0	1.3	6	15.2	3.1	6	17.2	3.1	6
6	46	3	12	3.6	1.2	12	224	9	12	9.6	3.0	12	17.0	3.0	12	17.5	1.7	12
7	63	2	4	3.5	0.3	4	215	24	4	24.5	0.7	4	36.0	1.2	4	36.8	1.8	4
8A	74	3	7	3.6	0.8	7	216	12	7	22.7	1.7	7	24.9	1.5	7	34.2	1.1	7
8B	106	0	4	4.4	2.3	4	227	7	4	16.6	2.4	4	36.7	2.6	4	36.3	1.9	4
9	97	1	11	5.1	0.9	11	222	13	11	3.6	2.2	11	21.7	1.0	11	21.9	1.8	11
10	74	8	13	3.7	1.6	13	199	23	13	0.3	0.2	13	12.1	0.9	13	11.3	0.9	13
11	81	3	10	8.3	1.2	10	238	16	10	1.6	1.7	10	10.4	2.6	10	11.0	1.4	10
12	93	5	10	6.7	0.8	10	256	17	10	5.6	2.1	10	19.4	2.4	10	20.3	1.9	10
13	73	6	11	5.1	1.2	11	255	10	11	0.3	0.1	11	11.5	1.1	11	11.5	1.3	11
14	79	2	9	6.2	0.8	9	227	20	9	7.7	1.3	9	19.6	1.7	9	20.7	2.2	9
15	64	3	10	6.8	1.2	10	230	13	10	2.4	1.5	10	11.1	2.0	10	11.1	1.3	10
16	73	2	9	7.9	0.9	9	253	7	9	1.6	0.6	9	10.6	1.5	9	10.9	1.6	9
17	78	6	5	8.2	1.5	5	213	30	5	2.2	0.4	5	14.3	1.0	5	15.5	1.7	5
18	63	3	8	8.1	0.7	8	208	14	8	1.6	0.4	8	9.2	1.1	8	8.6	0.9	8
19	85	4	8	6.2	1.8	8	243	14	8	4.8	1.9	8	17.3	1.5	8	15.2	1.2	8
20	70	4	8	5.1	0.9	8	239	7	8	5.4	3.5	8	14.6	5.2	8	12.6	3.0	8
21	70	3	9	5.6	1.3	9	241	11	9	5.7	1.4	9	14.7	1.4	9	11.5	1.8	9
22	73	2	10	8.3	0.6	10	224	16	10	3.3	1.5	10	11.5	2.1	10	9.1	1.3	10
23A	86	5	3	5.5	1.4	3	252	11	3	24.8	0.3	3	36.4	0.3	3	35.6	1.1	3
23B	80	3	5	6.1	1.4	5	257	11	5			5	60.4	3.1	5	58.8	4.7	5
24	57	6	6	7.1	1.6	6	258	21	6			6	28.8	3.9	6	30.0	6.0	6
25	104	2	5	4.3	0.4	5	277	7	5			5	69.3	3.0	5	73.1	1.6	5
26	72	5	5	4.5	0.5	5	250	6	5			5	50.0	1.0	5	52.6	0.3	5

Table 3.3 (b): Parent system steady state data; for each sewage batch (steady state period, see Table 3.2) the data have been averaged and the means, sample standard deviations (SSD) and number of tests are listed.

Sewage 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	495	35	7	62	17	7	3309	229	7	37.8	0.8	7	2320	133	7
2	514	20	10	144	81	10	2706	221	10	37.8	1.5	10	1878	255	10
3	525	51	8	51	5	8	4242	173	8	37.0	0.6	8	3405	491	8
4	494	9	6	41	5	6	5137	267	6	41.5	2.3	6	3528	145	6
5	500	10	6	42	5	6	4344	117	6	36.2	1.4	6	3044	77	6
6	498	70	12	40	12	12	3538	257	12	32.7	2.7	12	2622	186	12
7	420	19	4	30	8	4	3058	131	4	35.5	0 7	4	2348	88	4
8A	509	18	7	43	11	7	3378	208	7	41.7	2.0	7	2480	177	7
8B	701	39	4	52	11	4	3533	214	4	43.7	4.3	4	2638	270	4
9	744	85	11	52	16	11	3411	255	11	39.0	2.5	11	2584	291	11
10	756	46	13	54	10	13	3452	147	13	34.5	2.5	13	2464	169	13
11	730	27	10	61	11	10	3070	166	10	31.2	2.2	10	2548	237	10
12	751	71	10	49	20	10	3769	274	10	36.9	1.8	10	2651	185	10
13	766	67	11	43	11	11	4089	213	11	34.8	4.0	11	2622	74	11
14	735	23	9	44	7	9	3413	354	9	38.3	0.9	9	2408	77	9
15	728	48	10	48	18	10	3722	105	10	34.6	1.9	10	2483	132	10
16	766	22	9	48	7	9	3764	95	9	38.2	1.1	9	2700	126	9
17	759	92	5	57	13	5	3335	276	5	42.4	4.1	5	2406	127	5
18	655	60	8	36	14	8	3119	301	8	37.5	1.4	8	2409	138	8
19	728	37	8	52	10	8	4073	260	8	41.6	0.9	8	3042	80	8
20	741	56	8	65	14	8	3936	158	8	40.9	2.3	8	3760	106	8
21	774	30	9	40	10	9	3908	91	9	36.4	1.3	9	2890	164	9
22	749	37	10	46	18	10	3862	216	10	37.1	2.6	10	2736	143	10
23A	795	23	3	57	7	3	4410	128	3	41.4	0.9	3	3111	88	3
23B	785	30	5	66	26	5	4429	66	5	47.9	1.3	5	3220	69	5
24	798	16	6	52	21	6	5083	125	6	43.0	2.1	6	3625	160	6
25	815	25	5	54	12	5	5313	64	5	43.7	0.9	5	3726	128	5
26	787	29	5	31	13	5	5112	173	5	45.9	1.6	5	3526	72	5

Table 3.4: Steady state N and COD mass balances, wastewater fractions and mixed liquor parameters for parent system. Data calculated from data in Table 3.3. (*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	93	93	0.071	0.103	1.44	0.085
2	92	98	0.097*	0.011*	1.43	0.085
3	82	90	0.065	0.233	1.25*	0.078
4	116	103	0.053	0.349*	1.42	0.081
5	106	94	0.058	0.237	1.44	0.085
6	98	83	0.051	0.141	1.40	0.089
7	98	75	0.070	0.170	1.35	0.094
8A	83	80	0.062	0.111	1.40	0.089
8B	95	86	0.062	0.180	1.42	0.091
9	97	80	0.052	0.134	1.46	0.095
10	87	82	0.049	0.114	1.48	0.085
11	76	78	0.061	0.124	1.46	0.113*
12	88	83	0.049	0.156	1.44	0.098
13	93	87	0.043	0.157	1.57*	0.098
14	92	84	0.044	0.115	1.44	0.096
15	90	90	0.046	0.148	1.53	0.095
16	86	91	0.048	0.151	1.42	0.096
17	109	89	0.050	0.119	1.37	0.088
18	93	98	0.043	0.171	1.31	0.087
19	94	99	0.066	0.249	1.35	0.080
20	96	100	0.081	0.198	1.44	0.087
21	92	86	0.040	0.170	1.38	0.085
22	86	93	0.038	0.168	1.45	0.084
23A	98	86	0.044	0.210	1.42	0.081
23B	101	96	0.041	0.111	1.38	0.080
24	91	105	0.026	0.172	1.40	0.071
25	90	100	0.022	0.165	1.43	0.074
26	101	96	0.026	0.159	1.45	0.071
MEAN			**0.050**	**0.161**	**1.42**	**0.086**
Std. Deviation			**0.014**	**0.037**	**0.05**	**0.008**

COD mass balances: Analysis of results

The results of the COD mass balances calculated for each sewage batch are listed in Table 3.4 and are shown graphically in Fig. 3.5. The results can be commented on as follows:

- In general, the COD mass balances were poor; only 15 out of 26 sewage batches (No. 1, 2, 3, 4, 5, 15, 16, 18, 19, 20, 22, 23B, 24, 25 and 26) gave mass balances in the range of 90% - 110%.

- Also, the COD mass balances showed more variation than the N mass balances.

- Sewage Batch No. 11 had a particularly poor COD mass balance (78%); a similarly poor N mass balance was obtained and the sewage batch has already been rejected on the basis of the N mass balance (see section 3.9.2a).

- For the sewage batches with poor COD mass balances, batch tests were only conducted during Sewage Batch No. 21 and 23A.

The data and analytical techniques were examined to determine if the source for the low COD mass balances could be determined:

- Investigations showed that the COD measurement techniques were 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 OHO active biomass active fraction (see Chapter 5).

- 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 3.4. Statistical plots for these ratio's were constructed, see Figs. (3.6, 3.7) and (3.8, 3.9) respectively (see Appendix D for interpretation of statistical plots). From the statistical plots it is evident that the data are normally distributed; this indicates that (i) an infinite number of parameters had an influence on the measurements (ii) each influence was small, and (iii) no single factor has had a dominating influence on the measurements. The means for COD/VSS and TKN/VSS were 1.42 mgCOD/mgVSS and 0.086 mgN/mgVSS respectively, with sample standard deviations 0.05 and 0.008 respectively. These values are close 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 only, Sewage Batches No. 7 and 11 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 No. 7 and 11 were rejected for further analysis. This does not impact on the batch test data analysis since batch tests were not conducted during these periods.

From the analysis of the N mass balances, sewage batches which yielded mass balances outside the 90% – 110% range should be rejected for further analysis. These were Sewage Batches No. 3, 4, 8A, 10, 11, 12, 16 and 22. The specific causes for the poor N mass balances for these sewage batches are discussed in Section 3.9.2a above and those rejected for further analysis were No. 3, 4, 8A and 11.

In any event, batch tests were only conducted during Sewage Batch No. 22. For this sewage batch it will be noted that the data should be interpreted with caution.

3.9.3 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:

$$f_{S,us} = S_{te} / S_{ti} \qquad (3.11)$$

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 3.3b), the $f_{S,us}$ was calculated using Eq.(3.11), and the values are listed in Table 3.4. The $f_{S,us}$ data from all the sewage batches were analysed using a statistical plot, see Fig. 3.10 (for interpretation of the statistical plot, see Appendix D). Following the procedures above, one outlier was identified, $f_{S,us}$ = 0.097, see Fig. 3.10. Rejecting this point, the data are replotted in Fig. 3.11. The data are normally distributed, giving for Mitchell's Plain raw wastewater a mean $f_{S,us}$ = 0.050 and sample standard deviation of 0.014. This value is lower than the $f_{S,us}$ values obtained by both Ubisi *et al.* (1997a,b), $f_{S,us}$ = 0.095 and

Cronje *et al.* (2000), $f_{S,us}$ = 0.085 for the same Mitchell's Plain raw wastewater. Of interest is the fact that both Ubisi *et al.* (1997a,b) and Cronje *et al.* (2000) were feeding a COD concentration of 500 ± 50 mgCOD/ℓ to their parent systems. In this experimental investigation, the same feed concentration of 500 ± 50 mgCOD/ℓ was fed to the parent system for the first 107 days, whereafter, the feed concentration was increased to 750 ± 75 mgCOD/ℓ and this increased COD concentration was fed to the parent system till closure (day 417). Thus, despite that the $f_{S,us}$ value would be expected to be the same, given that the influent wastewater being treated was the same, the higher COD concentration gave a lower $f_{S,us}$. The lower $f_{S,us}$ value is, however, in the range of accepted values of 0.04 – 0.10 mgCOD/mgCOD for municipal raw wastewaters in South Africa (WRC, 1984).

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 3.3b) and the calculated $f_{S,us}$ (Table 3.4), the $f_{S,up}$ was calculated using the following equation (Ekama *et al.*, 1986):

$$MX_V = \frac{MS_{ti}(1 - f_{S,us} - f_{S,up})\, Y_H^* R_S}{(1 + b_H^* R_S)} (1 + f^* b_H^* R_S) + f_{S,up}\, MS_{ti}\, R_S / f_{cv} \qquad (3.12)$$

MX_V = total volatile solids mass (mgVSS)
= $X_V \bullet V_P$
X_V = MLVSS concentration (mgVSS/ℓ)
= measured value (Table 3.3b)
V_P = system volume
= 10ℓ for Configurations 1 and 2 and 8ℓ for Configuration 3
Y_H^* = OHO 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 20°C
f^* = endogenous residue fraction
= 0.20
f_{cv} = COD/VSS ratio of mixed liquor (mgCOD/mgVSS)
= 1.42 mgCOD/mgVSS
MS_{ti} = total influent COD mass fed per day (mgCOD/d)
= Qi • Sti
Qi = influent flow rate
= 20 ℓ/d for Configuration 1 and 13.3 ℓ/d for Configurations 2 and 3.
S_{ti} = influent COD concentration (mgCOD/ℓ)
= measured value (Table 3.3)

For each batch of sewage tested, successive values for $f_{S,up}$ were substituted into Eq. (3.12); 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 3.4.

- The $f_{S,up}$ data for all the wastewater batches were analyzed using a statistical plot, see Fig. 3.12. Two outliers were identified, $f_{S,up}$ = 0.011 and 0.349, see Fig. 3.12 (the high value of 0.349 reflects the non-steady state of the activated sludge system during the reseeding start-up period, Sewage Batch No. 4). Rejecting these points, the data are shown plotted in Fig. 3.13. The data are normally distributed, giving for Mitchell's Plain raw wastewater a mean $f_{S,up}$ = 0.161 with sample standard deviation of 0.037. This $f_{S,up}$ value compares favourably with that observed by Ubisi *et al.* (1997a,b), $f_{S,up}$ = 0.120, Cronje *et al.* (2000), $f_{S,up}$ = 0.103 for the same Mitchell's Plain raw wastewater, and conforms to the accepted range of 0.07 – 0.20 mgCOD/mgCOD for municipal raw wastewaters in South Africa (WRC, 1984). This indicates that the value obtained for $f_{S,up}$ is reasonable.

3.9.4 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). As explained in Section 3.2 above, on a number of different occasions, the DSVI increased to values above 150 mℓ/g (indicating a bulking sludge): Microscopic identification implicated the filamentous organism *Microthrix parvicella*. 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 Fig. 3.14, the primary anoxic reactor nitrate concentration is shown plotted on the same graph as the DSVI versus day of operation.

In general, the DSVI and the anoxic reactor nitrate concentration behaviour tends to conform to the hypothesis of Casey *et al.* (1994a,b): 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*.

3.9.5 Operational problems

Occasional pipeline blockages, caused by lumps of sludge which settled in the outlet pipelines of the reactors, resulted in sludge losses from overflowing reactors. During the day following a sludge spillage, the reactors were drained and the mixed liquor was screened through a 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.

Sludge losses could well account for the poor mass balances observed in a few of the sewage batches. To prevent pipeline blockages and consequent sludge losses, towards the latter part of the experimental investigation an improved version of Y-connectors were used at the outflow of the aerobic reactor into the secondary settling tank. This improved sludge recycle considerably. Inconsequential sludge overflows were experienced after this measure was introduced.

When the parent laboratory-scale system was receiving Sewage Batches No. 17 and 18, the laboratory's air-conditioning system failed and resulted in higher ambient temperatures (22°C to 30°C) than the controlled 20°C. From the data in Table 3.3(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. For Sewage Batch No. 17, this did not have an impact on the batch test data analysis since no batch tests were conducted during this period. However, batch tests were conducted during Sewage Batch No. 18 and the increased temperature would have an effect, since it influences calculation of $f_{S,up}$ via Eq. (3.12) and hence calculation of OHO active biomass fraction of the mixed liquor which is used to derive the theoretical OHO active biomass concentration in the batch test (see Chapter 5). To take account of this effect, the temperature dependent constants in Eq. (3.12) were adjusted to the actual temperature via the *Arhennius* equation set out above.

3.10 CLOSURE

The parent system was operated for 417 days and received 26 batches of raw municipal wastewater from the Mitchell's Plain Treatment Plant in Cape Town. From the results obtained from the system:

- N mass balances were consistent and were generally in the range 90 to 110%. Sewage batches that gave mass balances falling outside this range were No. 3, 4, 8A, 10, 11, 12, 16 and 22. Batch tests were conducted on Sewage Batch No. 22. 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 interpreted with caution.

- Generally COD mass balances were poor, with 15 of 26 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 = 1.42 mgCOD/mgVSS (sample standard deviation = 0.05) and TKN/VSS = 0.086 mgN/mgVSS (sample standard deviation = 0.008). These values are close to 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 OHO active biomass. Accordingly, the lower limit for the COD mass balance was set at 80%. On this basis, only Sewage

Batches No. 7 and 11 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 0.050 (sample standard deviation = 0.014). This value is lower than the $f_{S,us}$ values obtained by both Ubisi *et al.* (1997a,b), $f_{S,us}$ = 0.095 and Cronje *et al.* (2000), $f_{S,us}$ = 0.085 for the same Mitchell's Plain raw wastewater. Of interest is the fact that both Ubisi *et al.* (1997a,b) and Cronje *et al.* (2000) were feeding a COD concentration of 500 ± 50 mgCOD/ℓ to their parent systems. In this experimental investigation, the same feed concentration of 500 ± 50 mgCOD/ℓ was fed to the parent system for the first 107 days, whereafter, the feed concentration was increased to 750 ± 75 mgCOD/ℓ and this increased COD concentration was fed to the parent system till closure (day 417). Thus, despite that the $f_{S,us}$ value would be expected to the same, given that the influent wastewater being treated was the same, the higher COD concentration gave a lower $f_{S,us}$. The lower $f_{S,us}$ value is, however, in the range of accepted values of 0.04 – 0.10 mgCOD/mgCOD for municipal raw wastewaters in South Africa (WRC, 1984).

- The wastewater mean unbiodegradable particulate COD fraction ($f_{S,up}$) was determined to be 0.161 (sample standard deviation = 0.037). This value compares favourably with that observed by Ubisi *et al.* (1997a,b), $f_{S,up}$ = 0.120, Cronje *et al.* (2000), $f_{S,up}$ = 0.103 for the same Mitchell's Plain raw wastewater, and conforms to the accepted range of 0.07 – 0.20 mgCOD/mgCOD for municipal raw wastewaters in South Africa (WRC, 1984). This indicates that the value obtained for $f_{S,up}$ is reasonable.

- Minor sludge losses occurred during Sewage Batches No. 9, 10, 11, 12 and 13 but did neither 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 Batches No. 17 and 18; 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), and did impact on the batch test data analysis for Sewage Batch No. 18, since batch tests were conducted during this period. To take account of the increased temperature, the temperature effect was included in the formulation to calculate $f_{S,up}$ [Eq. (3.12)], and hence in the OHO active biomass fraction estimate (see Chapter 5).

Figure 3.1: Physical layout of parent laboratory-scale Modified Ludzack-Ettinger (MLE) anoxic/aerobic activated sludge system.

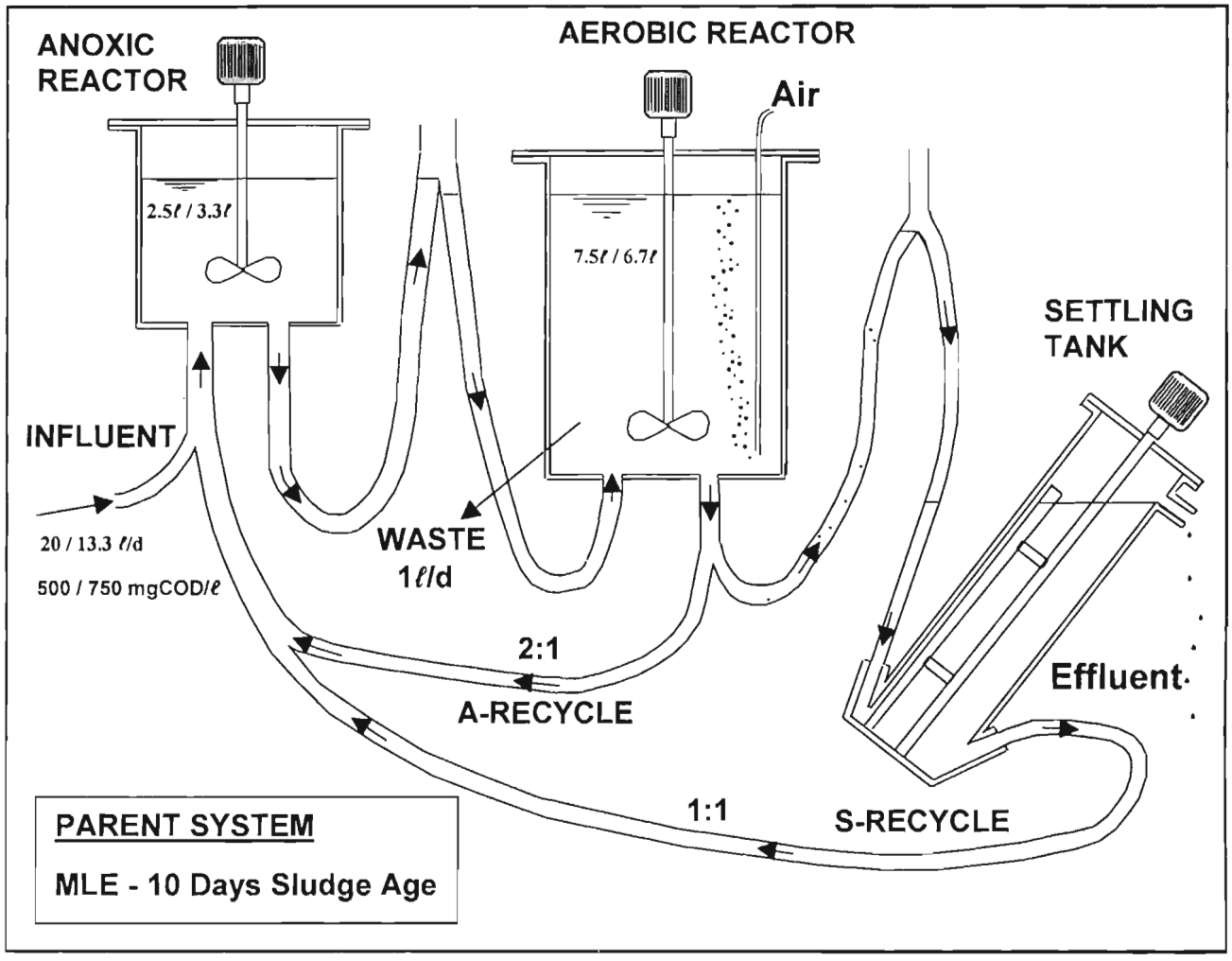


Figure 3.2: Schematic layout and operational data for the laboratory-scale Modified Ludzack-Ettinger (MLE) anoxic/aerobic activated sludge system.

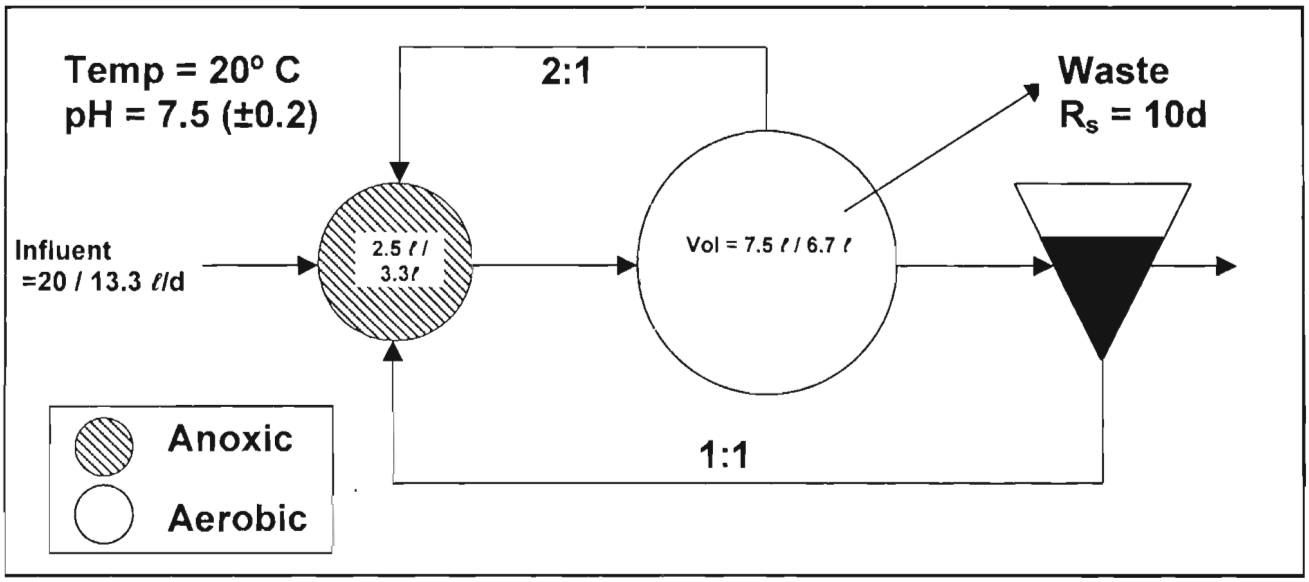
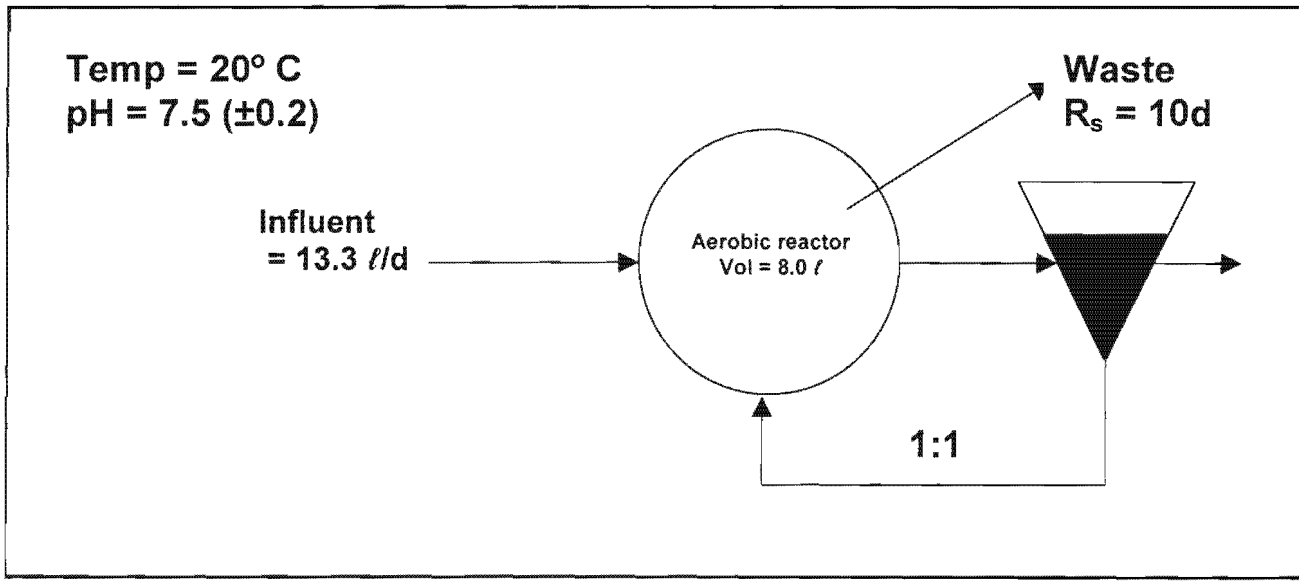


Figure 3.3: Schematic layout and operational data for the parent laboratory-scale completely mixed Fully Aerobic activated sludge system.



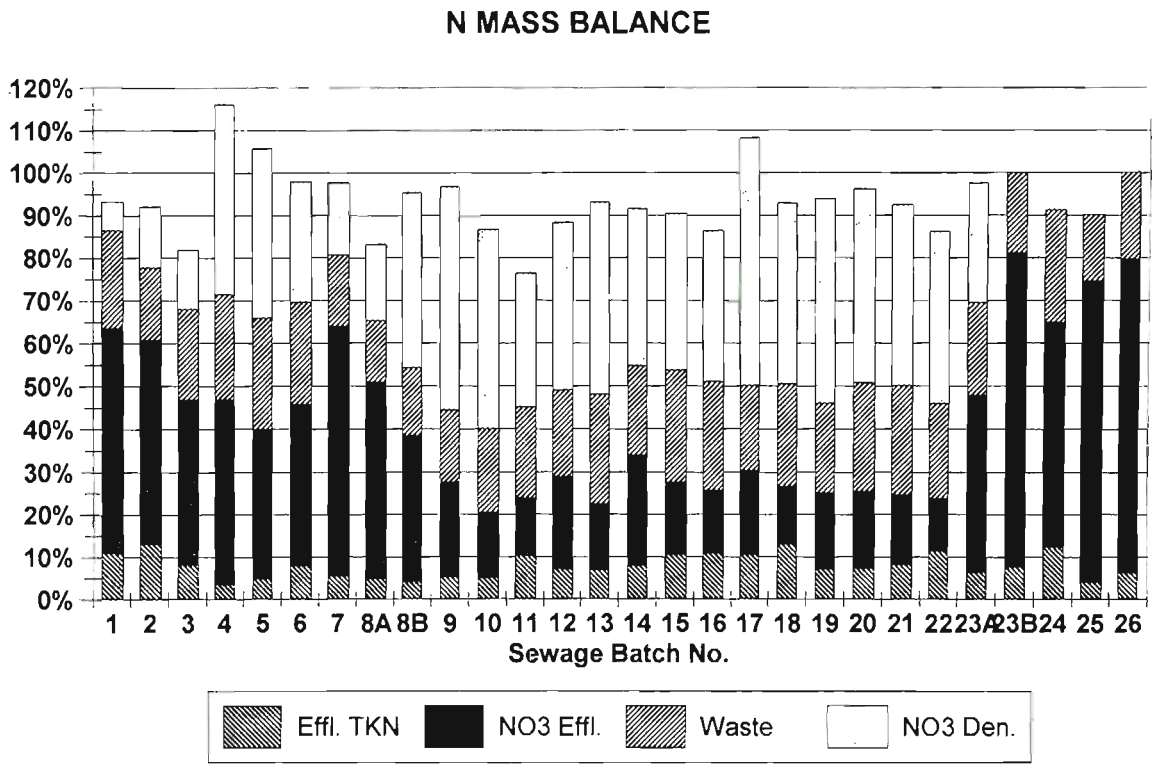


Figure 3.4: Graphical representation of the percentage N mass balance for the various sewage batches. Percentages are also shown for N for sludge production, effluent TKN, effluent NO3, and NO3 for denitrification.

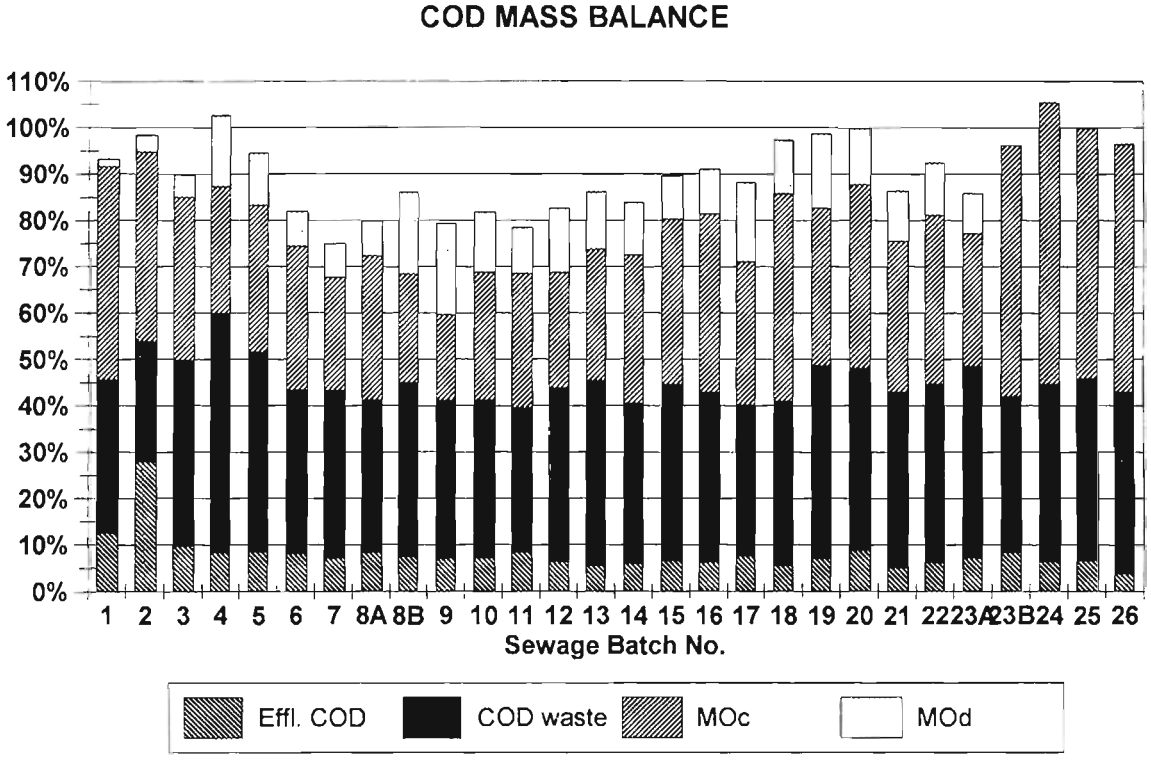


Figure 3.5: Graphical representation of the percentage COD mass balance for the various sewage batches. Percentages are also shown for waste sludge COD, unfiltered effluent COD, carbonaceous oxygen demand and oxygen required for nitrification.

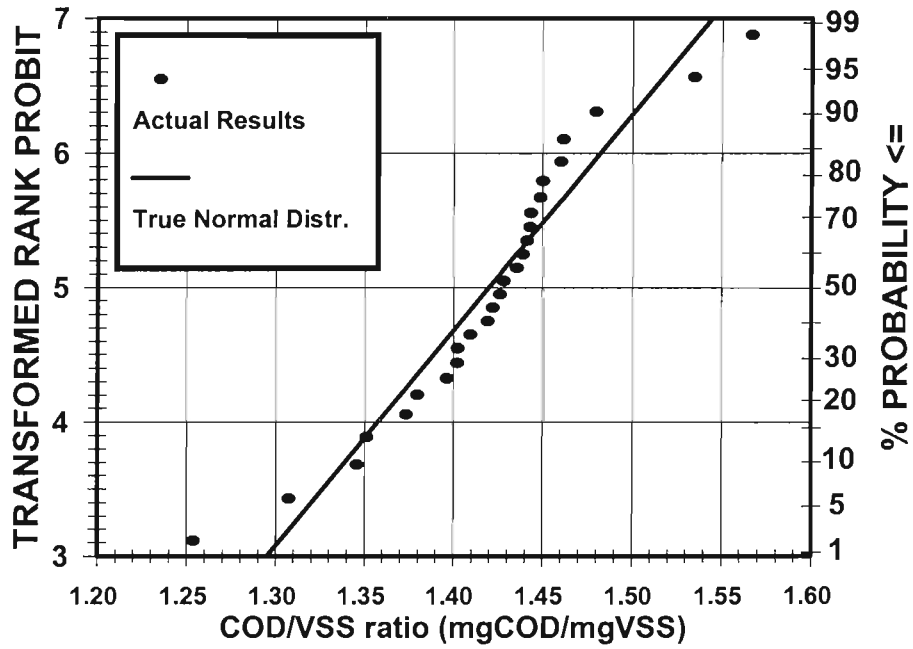


Figure 3.6: Statistical plot for the COD/VSS ratio for the parent control laboratory-scale activated sludge system.

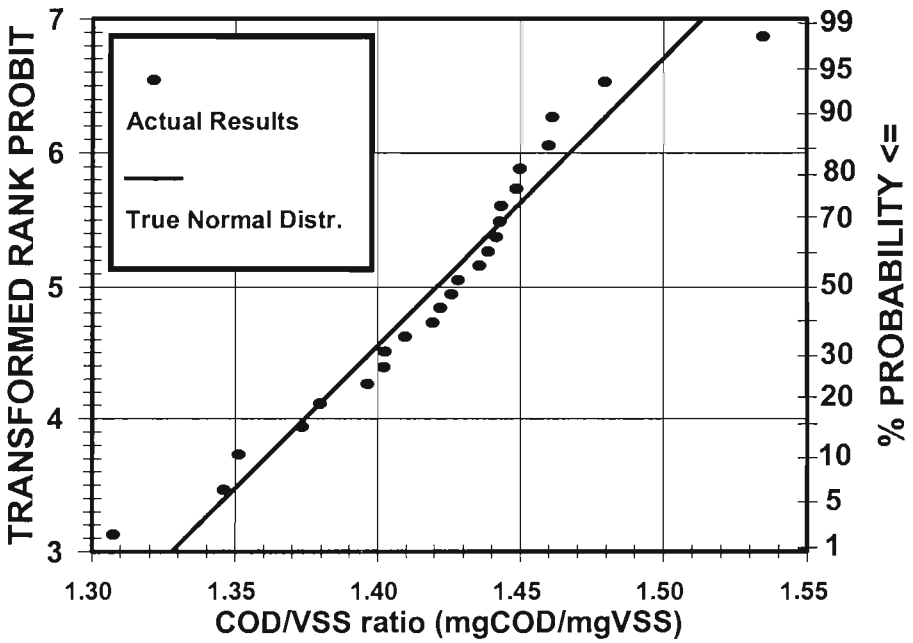


Figure 3.7: Statistical plot for the COD/VSS ratio for the parent control laboratory-scale activated sludge system: * outliers rejected.

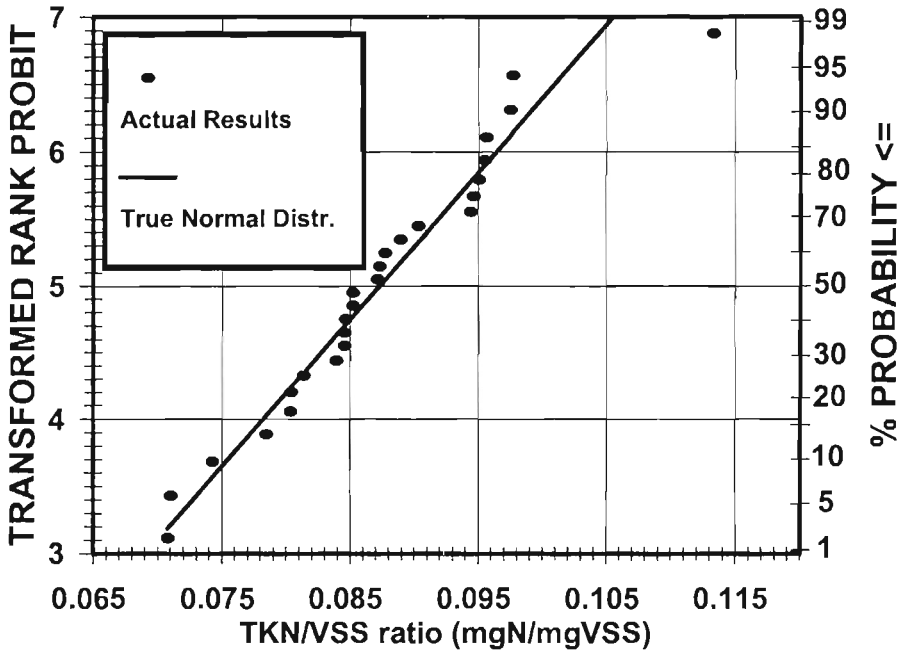


Figure 3.8: Statistical plot for the TKN/VSS ratio for the parent control laboratory-scale activated sludge system.

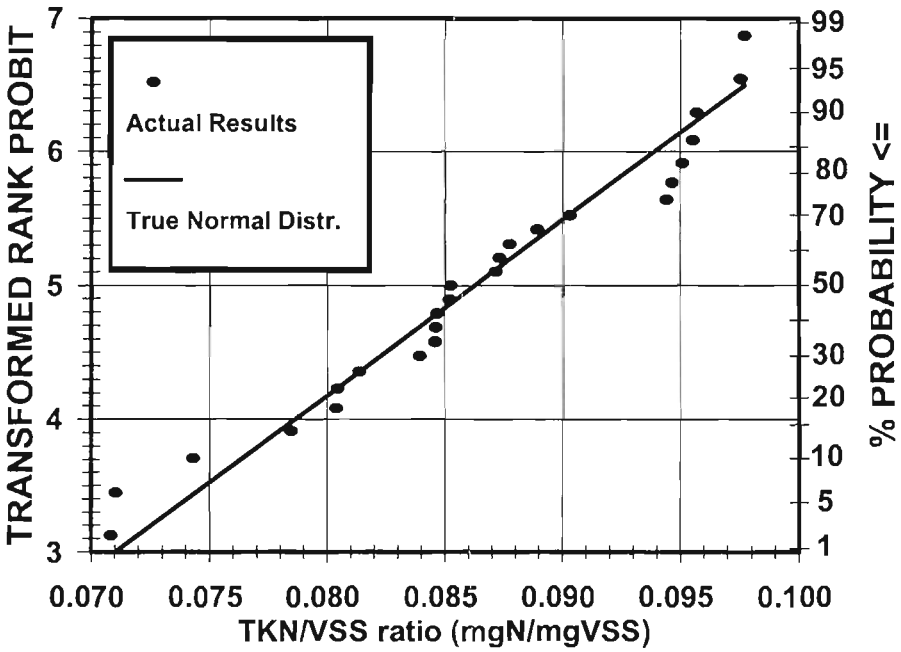


Figure 3.9: Statistical plot for the TKN/VSS ratio for the parent control laboratory-scale activated sludge system: * outliers rejected.

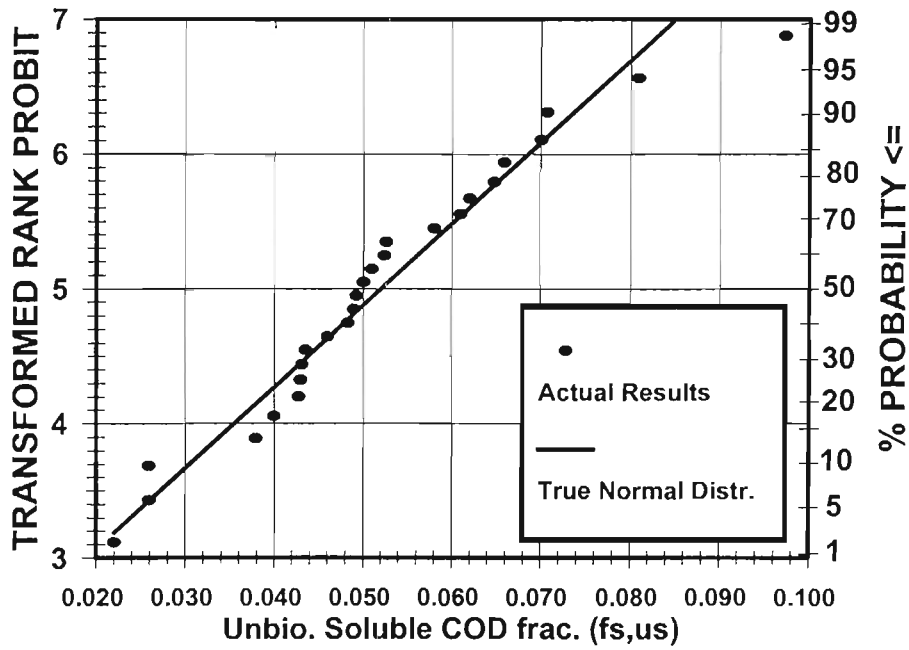


Figure 3.10: Statistical plot for unbiodegradable soluble ($f_{S,us}$) fraction for the parent control laboratory-scale activated sludge system fed with municipal wastewater from Mitchell's Plain Treatment Plant (Cape Town, South Africa).

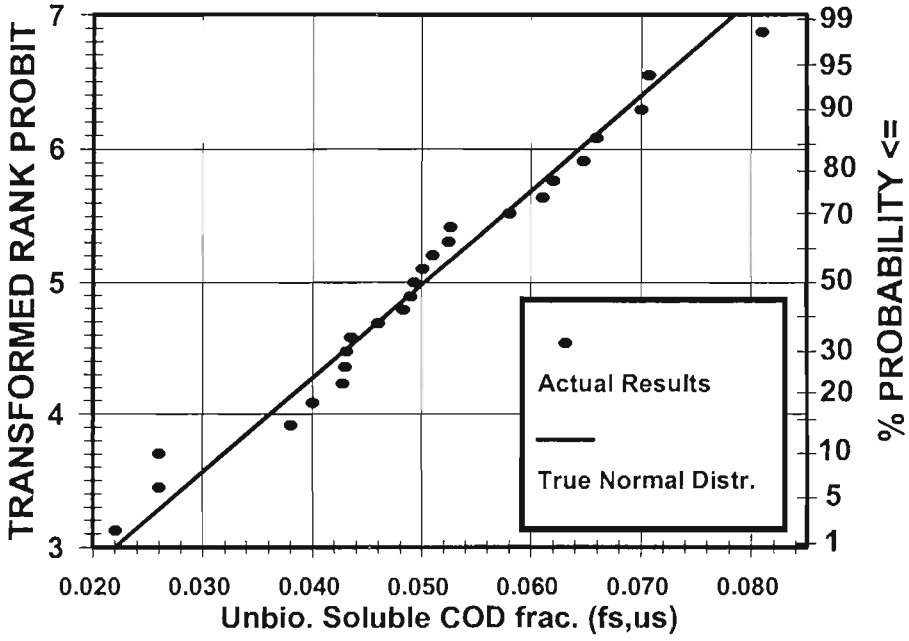


Figure 3.11: Statistical plot for unbiodegradable soluble ($f_{S,us}$) fraction for the parent control laboratory-scale activated sludge system fed with municipal wastewater from Mitchell's Plain Treatment Plant (Cape Town, South Africa): * outliers rejected.

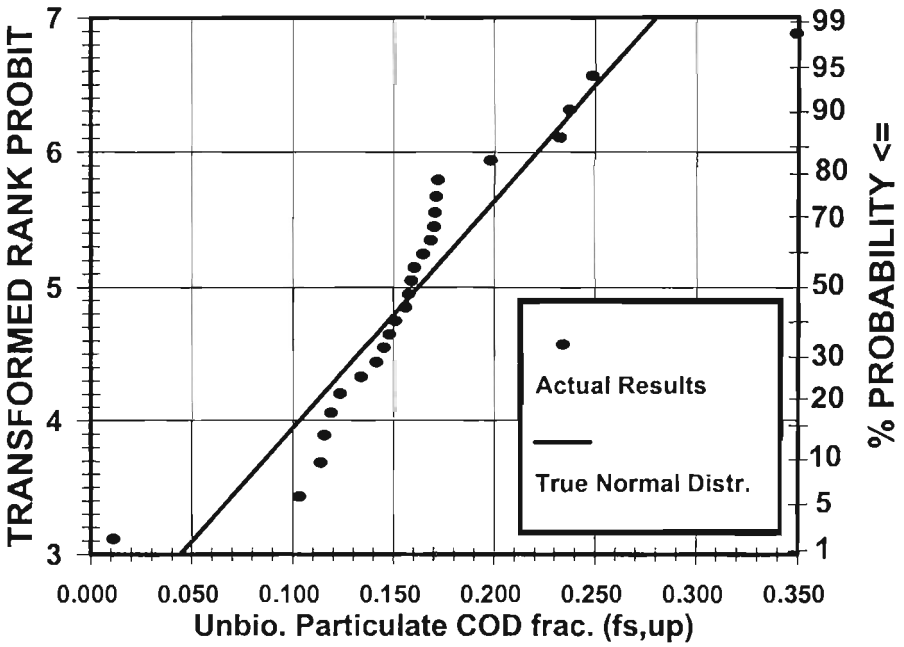


Figure 3.12: Statistical plot for unbiodegradable particulate ($f_{S,up}$) fraction for the parent control laboratory-scale activated sludge system fed with municipal wastewater from Mitchell's Plain Treatment Plant (Cape Town, South Africa).

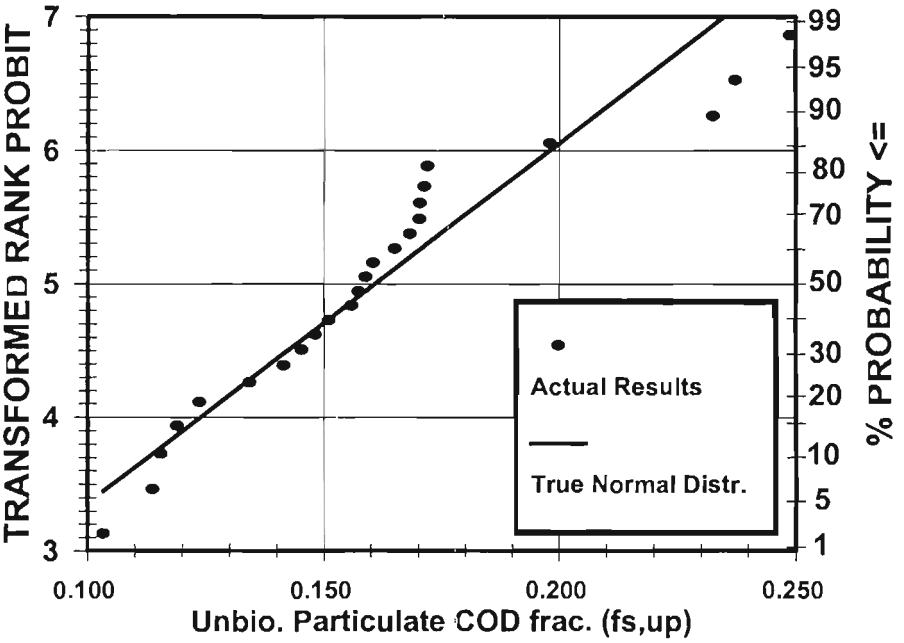


Figure 3.12: Statistical plot for unbiodegradable particulate ($f_{S,up}$) fraction for the parent control laboratory-scale activated sludge system fed with municipal wastewater from Mitchell's Plain Treatment Plant (Cape Town, South Africa): * outliers rejected.

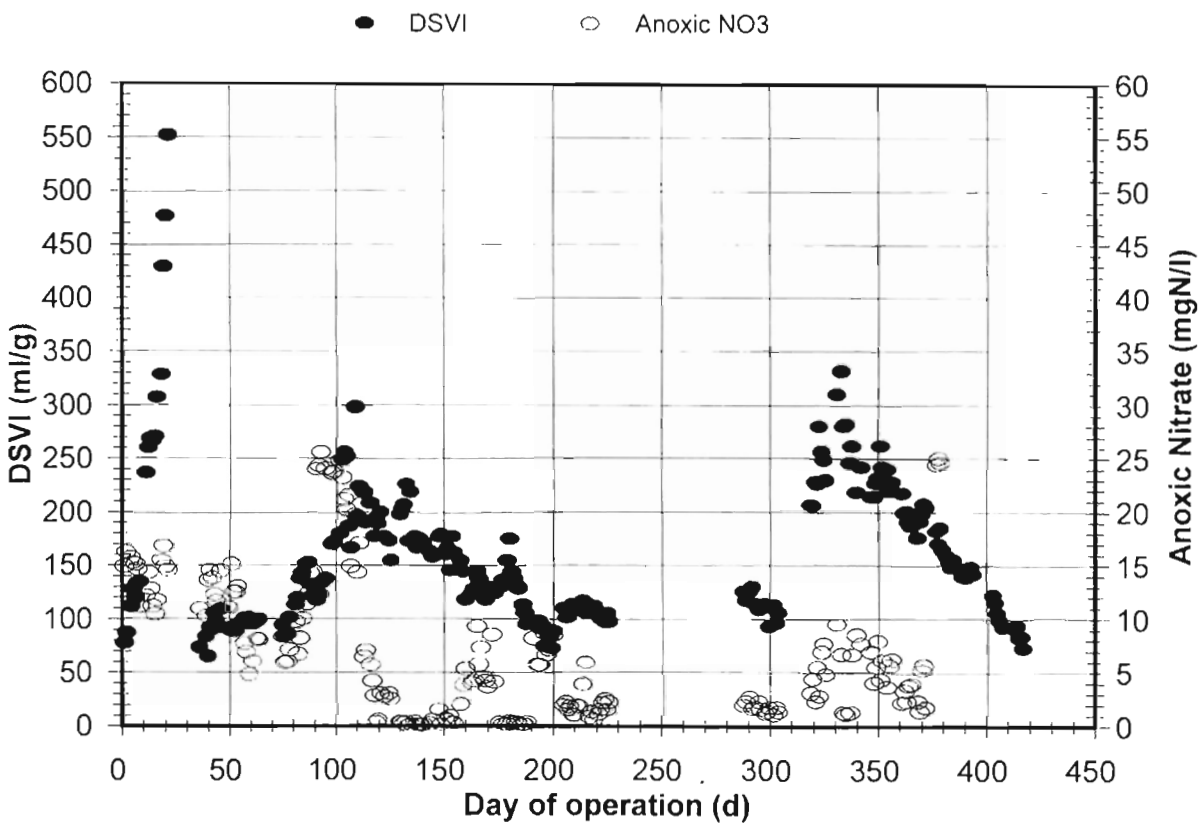


Figure 3.14: Graphical representation of the daily Diluted Sludge Volume Index (DSVI) and anoxic nitrate (NO_3) concentration for the parent control laboratory-scale activated sludge system.

CHAPTER 4

THE PARENT EXPERIMENTAL LABORATORY-SCALE NITRIFICATION / DENITRIFICATION ACTIVATED SLUDGE SYSTEM

4.1 INTRODUCTION

As described in Chapters 1 and 2, the results from the modified batch tests conducted by Cronje *et al.* (2000) on samples harvested from a nitrification / denitrification laboratory-scale activated sludge system operated at 10 days sludge age, showed remarkably good agreement between the measured and theoretical OHO active biomass concentration. However, the method does require more extensive evaluation; ***the principle aim in this research project is to extensively evaluate the modified batch test procedure of Cronje et al. (2000).***

To address this aim, two parent laboratory-scale nitrification / denitrification systems were run in parallel, both at 10 days sludge age. These systems would provide the source mixed liquor used to evaluate the batch test method. As described in Chapter 3, the two parent systems would be set-up and operated identically, but the second parent system, termed the *experimental* system, would additionally receive a known mass of toilet paper. It was envisaged that the toilet paper would be largely unbiodegradable, and hence the *experimental* system would have a mixed liquor OHO active biomass fraction that would deviate significantly from the parallel *control* system. The ability of the batch test procedure of Cronje *et al.* (2000) to correctly detect this difference in OHO active biomass would be evaluated.

The set-up, operation and steady state data for the parent *control* system have been detailed in Chapter 3. In this Chapter similarly the parent *experimental* system will be described: Firstly, a review of the literature on toilet paper will be presented; intriguing aspects of toilet paper biodegradation by organisms is then dealt with; the configuration of the parent *experimental* laboratory-scale nitrification / denitrification activated sludge system and its response then follows and finally, the results, analysis and effects of toilet paper on the activated sludge system are given.

4.2 GENERAL BACKGROUND ON PAPER

All paper, card, fibre board and similar products are made from the basic raw material of vegetable fibres, which are first "beaten up" and then mixed with water. The water is then drained off, and the fibres which are left behind are pressed and dried. The individual fibres, which are up to a few millimetres in length, consist of cellulose, a strong and almost transparent plant material.

When a wet mass of these cellulose fibres is allowed to dry they adhere to each other, and it is this bonding which enables these fibres to be used to make a sheet of paper. The processes of extracting and blending the fibres, and then forming them into a sheet of paper with the necessary properties, constitute the art of the paper-maker.

Two developments in the art of paper-making lie at opposite extremes: From paper, research has devised building board strong enough for the wall of a room and tissue so soft that it can be used next to the skin of a baby. As a result, two rapidly growing industries have been added to the already wide field of paper manufacture. Tissues have come into use in place of the traditional fabric handkerchiefs, towels and similar articles. Paper tissue also finds a wide application in the home, in industry, in the catering trade and in the medical and hospital field – wherever cleanliness and a freedom from laundering costs are important considerations. Toilet paper is, in effect, a specific form of tissue paper.

Of the different kinds of papers mentioned above, only toilet paper is of interest within the context of the current research project.

4.3 TOILET PAPER

4.3.1 Composition of toilet paper

Toilet paper is primarily made of 25% raw wood pulp and 75% recycled paper (Barlows, Nampak, personal communication). Recycled paper generally consists of 5% pulp and the balance is water (Val, Sappi, personal communication). Pulp consists of 75% cellulose and 25% lignin. From the above, it is evident that toilet paper consists mainly of cellulose and lignin.

4.3.2 Cellulose

Cellulose is the main structural material of plant life, being the major compound of thick, rigid plant cell walls; wood is about 50% cellulose, and cotton nearly pure cellulose. Its chemical composition has been elucidated by experiments involving hydrolysis (Hart and Schuetz, 1972). Complete hydrolysis of cellulose gives the monosaccharide D-glucose. Partial hydrolysis gives the disaccharide, cellobiose.

X-Ray examination of cellulose has disclosed that it consists of linear chains made up of cellobiose units in which the oxygen ring alternate forwards and backwards (Hart and Schuetz, 1972). A diagrammatic representation of a cellobiose unit is shown in Fig. 4.1. From Fig. 4.1, the cellobiose unit consists of two glucose molecules joined by β (1 $\rightarrow$ 4) linkages. A number of cellobiose units are linked together to form the cellulose chain, approximately 1800 to 3000 glucose units per molecule. Cellulose fibres consist of bundles of such chains held together by hydrogen bonds between hydroxyls on adjacent chains; the hydrogen bonds cause that most solvents have little effect on cellulose. In the cell walls of plants, densely packed cellulose fibrils surround the cell in regular parallel

arrays, often in criss cross layers. These fibrils are "cemented" together by a matrix of hemicellulose, pectin and extensin (Lehninger, 1975). Hemicelluloses are polymers of pentoses, particularly polymers of D-xylose in β (1 → 4) linkages with side chains of arabinose and other saccharides. Pectin is a polymer of methyl D-galacturonate. Extensin is a complex glycoprotein (carbohydrate groups attached to polypeptide chains), and is attached covalently to the cellulose fibrils; it is rich in hydroxyproline residues and also contains many side chains with arabinose and galactose residues. The cell walls of higher plants can be compared to reinforced concrete, with the cellulose fibrils corresponding to the steel rods, and the matrix material to the concrete. These walls are capable of withstanding enormous weights and stresses.

Most animals do not contain the enzymes necessary for hydrolysing β-glucosidic linkages, as well as a number of microorganisms. Thus, cellulose is not readily degradable. Wood pulp contains cellulose fibres as above, and additionally a high molecular weight polymeric substance called lignin (Hart and Schuetz, 1972).

4.3.3 Lignin

Lignin is a major component of wood and confers structural rigidity to the cellulosic walls of woody plants. The chemical nature of lignin is known largely from studies of its biosynthesis (Sarkanen and Ludwig, 1971), work pioneered by Freudenberg and his co-workers between about 1930 and 1965. Lignin is an amorphous, three-dimensional, aromatic polymer composed of oxyphenylpropae units. It is formed at the sites of lignification in plants by enzyme-mediated polymerisation of three substituted cinnamyl alcohols: p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (Fig. 4.2). Lignin binds the cellulose fibres in wood pulp and constitutes 25% of the pulp. Lignin molecules are insoluble, too large to pass through the cell walls and too heterogeneous to be disassembled with specific enzymes (Kirk and Farrell, 1987) and thus are resistant to decay. Further, lignin resists biological decay because the C:N ratio in lignin is so high that even if the enzymes could digest it, very few organisms could subsist on lignin without going nutrient deficient (Robinson, 1990).

4.3.4 Biodegradation

Since paper and toilet paper are made from wood pulp, they contain significant cellulose and lignin and although paper and especially toilet paper is a component of domestic sewage, little or no research has been done regarding its biodegradation in the activated sludge system. However, some studies on their degradation in soils have been done. A study on the biodegradability of paper board under aerobic soil exposure conditions was undertaken by Andrady *et al.* (1992), who found that both hemi-cellulose and lignin can undergo biodegradation, although the latter degrades at a very slow rate. They also found that because cellulose is insoluble in water, the enzymatic processes occur outside the microbial cell via enzymes secreted by the organisms into the bulk solution.

The sequential and coorperative action of the enzymes endocellulase, exocellulase and β-glucosidase is believed to bring about the biodegradative hydrolysis of cellulose

(Andrady *et al.*, 1992). A small fraction of lignin is susceptible to biodegradation, but its utilisation generally requires the availability of cellulose as co-substrate. Lignin breakdown products contribute to the soil's pool of organic material, as humic and fulvic acid components (Andrady *et al.*, 1992). Paper takes long to degrade; this is not surprising since paper being fibrous, has a functional cellulose type cell wall as well as lignin (Whitehouse, 1990).

From the brief review on toilet paper above, it is evident that the main organic materials present are cellulose and lignin, and that these are very difficult to biodegrade. Accordingly, it can be expected that in an activated sludge system, toilet paper should make a significant contribution to the unbiodegradable particulate fraction of the mixed liquor. Thus, by dosing toilet paper to the *experimental* system, the OHO active biomass fraction of the mixed liquor should decrease. The set-up, operation and data from a parent system receiving a toilet paper dose are described below.

4.4 EXPERIMENTAL SYSTEM LAYOUT

The *experimental* parent laboratory-scale was identical in set-up to the *control* system shown schematically in Figs. 3.1 and 3.2. The system was operated for 382 days in total. The main changes to system configuration and operation were identical to those made to the *control* system, see Table 4.2. Thus, three main configurations can be identified, and are described below.

4.4.1 Configuration 1

For Configuration 1, the *experimental* system layout was similar to the *control* laboratory-scale activated sludge system as described in Chapter 3, Section 3.2.1.

4.4.2 Configuration 2

As the experimental investigation proceeded, denitrification performance deteriorated in the *experimental* system (same phenomenon was observed in the *control* system). The same approach as that applied to the *control* system was adopted, in that the original Modified Ludzack-Ettinger (MLE) configuration was slightly modified (from day 107 to day 379) by increasing the anoxic mass fraction to 3.3ℓ volume (33% of the total system volume) and reducing the aerobic mass fraction to 6.7ℓ volume (67% of the total system volume), with the total system volume remaining fixed at 10ℓ. At the same time, the opening of the anoxic reactor used for sample extraction was closed by means of a cork to limit surface exchange of oxygen. Similarly to the *control* system, the *experimental* system experienced severe sludge bulking problems starting after day 304, and therefore aluminium sulphate was dosed to the system. A starting dose of 50 mℓ from the stock solution was added on the first day to the aerobic reactor of the activated sludge system, thereafter 5 mℓ of aluminium sulphate was dosed daily for the next 24 days, until the DSVI decreased to 119 mℓ/g when the dose was terminated.

4.4.3 Configuration 3

The *control* laboratory-scale activated sludge system was converted from the *Modified Ludzack-Ettinger* (MLE) configuration to a completely mixed *fully aerobic* system (on day 380) because of continuous bulking problems (see Chapter 3, Section 3.2.3). The same procedure was followed with the *experimental* system to keep the two systems' layout and operation identical. This was done despite the fact that the *experimental* system was not bulking. Since the *experimental* system was not bulking, aluminium sulphate was not dosed to this system, in contrast to the *control* system, see Chapter 3, Section 3.2.3.

The layout of the completely mixed *fully aerobic experimental* system layout is described in Chapter 3 Section 3.2.3.

4.5 WASTEWATER COLLECTION AND STORAGE

The procedures followed for the wastewater collection and storage is described in Chapter 3, Section 3.3

4.6 FEED PREPARATION

4.6.1 Configuration 1

For the wastewater feed, the procedures followed for the feed preparation were identical to those for the *control* system, described in Chapter 3, Section 3.4.1. However, additionally toilet paper was dosed to the *experimental* system. The addition of toilet paper solution to the *experimental* system started from Sewage Batch No. 6. Prior to the addition of toilet paper, the system was run for 2 sludge ages to ensure steady state conditions in the *experimental* activated sludge system. Some preliminary tests were also done on the toilet paper solution to characterise it (for characterisation of toilet paper see Section 4.11 below).

From the literature review on the composition and biodegradation of toilet paper (Section 4.3 above), it was initially thought that the toilet paper would contain a high unbiodegradable particulate fraction ($f_{S,up}$) which would contribute significantly to the mixed liquor inert component in the *experimental* activated sludge system. Thus, it was decided to add a dose of 100 mgCOD/ℓ *influent* as toilet paper solution to the influent wastewater feed of 500 ± 50 mgCOD/ℓ *influent*. A stock solution of toilet paper of 20g/ℓ was made by macerating 20g of toilet paper into a litre of distilled water. A known volume of the stock toilet paper solution (100 mℓ – equivalent to 100 mgCOD/ℓ *influent*) was macerated in a liquidizer with some diluted raw influent sewage and the total feed volume used was 20.1 ℓ. The toilet paper solution would provide ≈ 2 000 mgCOD/d, so the total COD load per day on the *experimental* activated sludge system would be ≈ 12 000 mgCOD/d.

4.6.2 Configuration 2

The procedure followed for the wastewater feed preparation is the same as described in Chapter 3, Section 3.4.2 for the *control* system. To maintain the COD dosage of toilet paper solution at ≈ 2 000 mgCOD/d, the daily dose of toilet paper solution to the *experimental* system was increased to 150 mgCOD/ℓ *influent* (150 mℓ of toilet paper solution) when the feed volume was decreased to 13.3ℓ. The total feed volume was thus 13.45ℓ.

From Sewage Batch No. 21 onwards (day 347), it was decided to double the daily dosage of toilet paper solution (see Section 4.13 below for reasons) thus increasing the toilet paper COD load to ≈ 4 000 mgCOD/d – thus, 300 mℓ of toilet paper solution was added to the *experimental* activated sludge system. The total feed volume was 13.6ℓ.

4.6.3 Configuration 3

The procedure followed for the wastewater feed preparation is the same as described in Chapter 3, Section 3.4.3 for the *control* system. Varying doses of toilet paper solution were added to the *experimental* system (see Section 4.10.2 below), to provide ≈ 2 000 – 3 000 mgCOD/d.

4.7 FEEDING THE SYSTEM

The procedure followed for feeding the *experimental* system was the same as for the *control* system, described in Chapter 3, Section 3.5.

4.8 SYSTEM MAINTENANCE AND OPERATION

The procedures followed for the *experimental* system were the same as for the *control* system, detailed in Chapter 3, Section 3.6.

4.9 SAMPLING AND MEASUREMENTS

The sampling and measurements procedures followed for the *experimental* system were the same as for the *control* system, described in Chapter 3, Section 3.7.

4.10 PARENT SYSTEM CONDITIONS

4.10.1 Sludge settleability

On two separate occasions, the *experimental* system experienced bulking. A bulking sludge can be accepted as one having a DSVI > 150 mℓ/g (Ekama and Marais, 1984). The

first case of bulking was reported in Sewage Batch No. 8B (DSVI = 182 mℓ/g) with the settleability deteriorating further in Sewage Batch No. 9 (DSVI = 221 mℓ/g); the second case of bulking occurred during Sewage Batch No. 19 (DSVI = 202 mℓ/g). On both occasions, the system recovered fully within one sludge age and filamentous organism identification indicated the principle organism causing bulking to be *Microthrix parvicella*, which was common to very common.

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). As mentioned above, on two different occasions, the DSVI increased to values above 150 mℓ/g (indicating a bulking sludge): Microscopic identification implicated the filamentous organism *Microthrix parvicella*. 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 Fig. 4.3, the primary anoxic reactor nitrate concentration is shown plotted on the same graph as the DSVI versus day of operation.

In general, the DSVI and the anoxic reactor nitrate concentration behaviour tends to conform to the hypothesis of Casey *et al.* (1994a,b): 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*.

4.10.2 The consequence of increased toilet paper dosage

It is clear from Table 4.1, that no batch tests were conducted after Sewage Batch No. 20. The underlying reason was that when the toilet paper dosage was doubled (from Sewage Batch No. 21, day 347), the *experimental* system became prone to blockages of the pipes connecting the reactors which caused frequent mixed liquor spillages: Overflows occurred on every second day and the resultant mixed liquor losses caused that steady state operation could not be achieved. After three subsequent sludge ages, the toilet paper dosage was stopped to restabilise the system; when this was done, the blockages stopped. Subsequently, it was decided to dose a lower load of toilet paper of 225 mℓ toilet paper solution (225 mgCOD/ℓ *influent* i.e. 3 000 mgCOD/d). Once again, reactor overflows and mixed liquor losses were very common. Accordingly, from Sewage Batch No. 24 it was decided to revert to the original dose of 150 mℓ of toilet paper solution (i.e. 2 000 mgCOD/d). This resolved the blockages and overflow problems, and the daily testing and analysis of samples were recommenced from Sewage Batch No. 25. For Sewage Batch No. 25, although the N mass balance was reasonable (109%), a poor COD mass balance (71%) was obtained; this was attributed to the fact that the system would require at least 2 sludge ages to reach steady state. Since it had become evident from an analysis of the results that the toilet paper was significantly more biodegradable than expected (see Section 4.12.3 below), and thus would not exert the anticipated influence on OHO active biomass, and that higher toilet paper doses could not be achieved, the *experimental* system was terminated.

Table 4.1: Details of the parent *experimental* laboratory-scale activated sludge system, sewage batch number, sewage feed dates, days of operation and batch tests conducted.

Sewage Batch No.	Date of tests (2000/2001)	Days of operation (d)	Batch Test	Parent System
3	2 Jun – 14 Jun	day 36 – 48	No	
4	15 Jun – 22 Jun	day 49 – 56	No	
5	23 Jun – 10 Jul	day 57 – 74	No	25% Anoxic 75% Aerobic MLE
6	11 Jul – 26 Jul	day 75 – 90	No	
7	27 Jul – 02 Aug	day 91 – 97	No	
8	03 Aug – 17 Aug	day 98 – 112	No	
9	18 Aug – 03 Sep	day 113 – 129	No	
10	04 Sep – 21 Sep	day 130 – 147	No	
11	22 Sep – 6 Oct	day 148 – 162	No	
12	07 Oct – 19 Oct	day 163 – 175	No	
13	20 Oct – 3 Nov	day 176 – 190	No	
14	04 Nov – 17 Nov	day 191 – 204	No	
15	18 Nov – 29 Nov	day 205 – 216	No	33% Anoxic 67% Aerobic MLE
16	30 Nov – 09 Dec	day 217 – 226	No	
17	09 Feb – 15 Feb	day 288 – 294	No	
18	16 Feb – 11 Mar	day 295 – 318	Yes	
19	12 Mar – 23 Mar	day 319 – 330	Yes	
20	24 Mar – 08 Apr	day 331 –346	Yes	
21	09 Apr – 22 Apr	day 347 – 360	No	
22	23 Apr – 08 May	day 361 – 376	No	
23	09 May – 20 May	day 377 – 388	No	
24	21 May – 03 Jun	day 389 – 402	No	100% Aerobic Fully Aerobic
25	04 Jun – 13 Jun	day 403 – 412	No	
26	14 Jun – 18 Jun	day 413 – 417	No	

4.11 CHARACTERISTICS OF TOILET PAPER

To chemically characterize the toilet paper, analytical tests were done on the toilet paper stock solution [stock at 20 g/ℓ], and included COD, TKN, FSA, Total Phosphate, TSS, VSS and ISS. Each of these tests were repeated three times to confirm whether the tests results were reliable; this was done because the various samples that were used for the tests were not homogeneous since they contained lumps of toilet paper (these could not be dissolved into solution), making the sampling procedure (such as pipetting an exact volume into a flask) difficult. All the independent test results were reasonably close (within 10%), so a mean value for the COD, TKN, Total Phosphates TSS, VSS and ISS of the toilet paper was determined. The average values are given in Table 4.2 below.

Table 4.2: Mean values for the characterisation of toilet paper solution fed to the parent *experimental* laboratory-scale activated sludge system.

Parameter	Mean value
COD	20 000 mgCOD/ℓ
TKN	6 mgN/ℓ
FSA	5 mgN/ℓ
Total Phosphate	2 mgP/ℓ
TSS	17 000 mgTSS/ℓ
VSS	16 800 mgVSS/ℓ
ISS	200 mgISS/ℓ

It can be inferred from the above table that the TKN, FSA and Total Phosphate concentrations of toilet paper are very low and consequently can be neglected. The toilet paper does however contain significant COD, TSS, VSS and ISS which will contribute to the mixed liquor in the *experimental* activated sludge system.

4.12 RESULTS

4.12.1 Steady state periods

Daily results for the parent *experimental* activated sludge system are listed in Appendix C, Tables C3 and C4. Each sewage batch was accepted as a steady state period. The daily data for each sewage 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 sewage 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 4.3 (a and b).

4.12.2 N and COD mass balances

The procedure followed to calculate the N and COD mass balances was as described in Chapter 3, Section 3.9.2 for the *control* system. The data can be considered acceptable if the mass balances fall in the range of 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 4.3(a) and are shown graphically in Fig. 4.4. The results can be commented on as follows.

- For Sewage Batch No. 8A, the N mass balance was 86%. Analysis of the experimental data showed that denitrification was poor. To improve denitrification, the system configuration was modified to incorporate a larger anoxic zone and the N mass balance improved to 95% for the remainder of the sewage batch, No. 8B.

- N mass balance for Sewage Batches No. 5, 10 and 14 were marginally outside the accepted range of 90 – 110% (113%, 86% 88% respectively). No assignable cause could be identified for these mass balances, but probably lies in the nitrate/nitrite and/or influent TKN measurements. Since the N mass balances are only marginally outside the acceptable range, the data for these sewage batches will be retained, but with due caution exercised in interpreting the data. In any event, no batch tests were conducted during these sewage batches, see Table 4.1.

- For Sewage Batches No. 11 and 12, the N mass balances were particularly poor (68% and 78 % respectively). Here the problem appeared to be related to that particular sewage batch; similar poor N mass balances were obtained for the *control* system. These sewage batches should be rejected for further analysis.

- Generally, acceptable N mass balances could be achieved without undue difficulty.

Table 4.3(a): Parent *experimental* system steady state data; for each sewage batch (steady state period, see Table 4.1) the data have been averaged and the means, sample standard deviations (SSD) and number of tests are listed.

Sewage Batch No.	TKN (mgN/ℓ)									NITRATES (mgN/ℓ)								
	INFLUENT			UNFIL. 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
3	61	2	8	4.8	1.2	8	303	10	8	12.5	1.2	8	20.7	1.1	8	22.6	1.5	8
4	59	1	6	2.4	1.1	6	318	19	6	11.6	0.7	6	20.3	1.0	6	23.4	2.3	6
5	49	3	6	3.1	0.9	6	265	19	6	9.0	1.7	6	17.4	2.0	6	20.1	2.9	6
6	46	2	12	4.5	1.3	12	231	13	12	9.5	2.0	12	16.4	1.8	12	16.8	1.4	12
7	61	4	4	6.4	5.1	4	214	2	4	22.1	0.5	4	32.0	1.2	4	33.9	0.4	4
8A	74	1	7	6.2	1.8	7	226	7	7	20.1	1.4	7	30.6	2.0	7	32.6	0.8	7
8B	105	5	4	7.2	1.6	4	225	5	4	12.4	2.1	4	30.4	2.3	4	32.2	2.1	4
9	95	10	11	11.0	9.9	11	234	15	11	0.5	0.6	11	15.5	0.7	11	14.8	1.7	11
10	71	8	13	4.8	2.5	13	198	44	13	0.1	0.0	13	10.4	2.3	13	10.5	1.4	13
11	81	4	10	8.0	1.7	10	248	38	10	0.4	0.7	10	7.7	1.5	10	7.9	1.0	10
12	91	5	10	8.5	2.1	10	239	27	10	0.5	0.4	10	12.0	1.1	10	11.7	0.5	10
13	73	7	11	6.8	2.8	11	281	12	11	0.1	0.0	11	9.3	1.0	11	9.6	1.1	11
14	79	2	9	6.3	0.9	9	270	6	9	8.2	1.5	9	17.2	1.0	9	20.9	2.2	9
15	63	3	10	6.6	1.2	10	294	8	10	2.5	0.6	10	10.6	0.9	10	9.7	0.9	10
16	75	1	9	8.0	0.9	9	302	10	9	2.2	0.5	9	11.3	1.4	9	12.0	1.4	9
17	75	8	5	8.2	2.1	5	240	21	5	2.3	1.4	5	12.0	0.8	5	13.7	1.4	5
18	63	3	8	9.2	1.8	8	227	13	8	0.7	0.1	8	7.7	1.7	8	8.0	0.7	8
19	83	5	8	7.8	0.9	8	266	15	8	2.3	1.2	8	15.8	1.6	8	14.8	1.6	8
20	71	2	8	6.6	1.4	8	247	13	8	3.2	1.1	8	11.3	2.5	8	15.5	2.5	8

Table 4.3(b): Parent *experimental* system steady state data; for each sewage batch (steady state period, see Table 4.1) the data have been averaged and the means, sample standard deviations (SSD) and number of tests are listed.

Sewage 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
3	525	51	8	56	7	8	4381	122	8	40.2	1.5	8	3045	78	8
4	494	9	6	39	7	6	5399	233	6	41.3	2.5	6	3745	169	6
5	500	10	6	39	7	6	4362	198	6	36.3	1.9	6	3122	86	6
6	515	68	12	58	11	12	3771	152	12	35.1	2.1	12	2792	165	12
7	483	20	4	68	60	4	3673	104	4	43.2	0.5	4	2805	89	4
8A	609	25	7	74	36	7	4126	201	7	45.4	2.1	7	3115	155	7
8B	827	39	4	72	29	4	3838	106	4	46.2	4.3	4	2934	69	4
9	886	81	11	56	10	11	4178	121	11	45.0	3.3	11	3085	136	11
10	1045	38	13	62	10	13	3944	488	13	42.8	4.2	13	2689	378	13
11	981	43	10	83	28	10	4347	929	10	44.3	5.4	10	2807	552	10
12	1070	87	10	98	32	10	4265	453	10	48.4	4.1	10	3033	355	10
13	1012	86	11	63	20	11	5268	299	11	43.9	4.8	11	3653	156	11
14	935	30	9	48	11	9	4827	106	9	44.4	1.8	9	3493	82	9
15	963	37	10	46	14	10	5200	87	10	40.7	1.6	10	3966	113	10
16	992	13	9	64	10	9	5335	174	9	43.0	0.7	9	3861	93	9
17	957	107	5	69	37	5	4657	193	5	44.7	6.0	5	3147	107	5
18	799	62	8	66	26	8	4011	251	8	41.8	2.4	8	3103	178	8
19	807	34	8	66	12	8	4720	268	8	49.0	4.4	8	3482	72	8
20	904	23	8	81	19	8	4778	289	8	42.6	1 1	8	3359	92	8

Table 4.4: Steady state N and COD mass balances, wastewater fractions and mixed liquor parameters for parent system. Data calculated from data in Table 4.3. (*indicate batches rejected as outliers at 95% confidence interval).

Sewage Batch No.	MASS BALANCE (%)		Wastewater and Toilet Paper 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)
			$f_{S,us}$ - ww+toiletpaper	$f_{S,us}$ - Toilet Paper only	$f_{S,up}$ - ww+toiletpaper	$f_{S,up}$ - Toilet Paper only		
3	92	98	0.061		0.213		1.44	0.099*
4	99	110	0.054		0.389*		1.44	0.085
5	113	91	0.054		0.244		1.40	0.085
6	97	91	0.045	-0.107*	0.154	0.545	1.35	0.083
7	99	97	0.032	-0.219*	0.178	0.234	1.31	0.076
8A[x]	86	85	0.056	0.032	0.127	0.214	1.32	0.073
8B[x]	95	84	0.045	-0.045*	0.140	-0.072*	1.31	0.077
9	91	87	0.048	0.030	0.139	0.169	1.35	0.076
10[x]	86	74	0.040	0.019	0.011	-0.253*	1.47	0.074
11[x]	68	90	0.052	0.030	0.065	-0.100*	1.55*	0.088
12[x]	78	83	0.043	0.030	0.050	-0.195*	1.41	0.079
13	90	90	0.037	0.018	0.166	0.200	1.44	0.077
14	88	87	0.042	0.037	0.176	0.405	1.38	0.077
15	93	88	0.032	-0.011*	0.212	0.414	1.31	0.074
16	91	90	0.042	0.024	0.199	0.367	1.38	0.078
17	99	90	0.048	0.043	0.147	0.260	1.48	0.076
18	94	99	0.047	0.064	0.198	0.325	1.29	0.073
19	107	107	0.076*	0.169*	0.275	0.529	1.36	0.076
20	110	90	0.076*	0.058	0.194	0.185	1.42	0.074
MEAN			**0.045**	**0.035**	**0.162**	**0.309**	**1.39**	**0.078**
Std. Deviation			**0.008**	**0.015**	**0.066**	**0.141**	**0.06**	**0.004**

[x] Sewage batches rejected on COD and/or N mass balances.

COD mass balances: Analysis of results

The results of the COD mass balances calculated for each sewage batch are listed in Table 4.4 and are shown graphically in Fig. 4.5. The results can be commented on as follows:

- In general, the COD mass balances were reasonable; 12 out of 19 sewage batches, (No. 3, 4, 5, 6, 7, 11, 13, 16, 17, 18, 19 and 20) gave mass balances in the range of 90 to 110%, while 7 out of 19 sewage batches (No. 8A, 8B, 9, 10, 12, 14 and 15) fell outside this range.

- Sewage Batch No. 10 had a particularly poor COD mass balance (74%) and should be rejected for further analysis.

- Sewage Batches No. 9, 14 and 15 had COD mass balances only marginally below the 90% lower limit (87%, 87% and 88% respectively), and hence were retained.

- The sewage batches during which batch tests were conducted (No. 18, 19 and 20) all had mass balances that fell within the acceptable range.

The data indicated that reasonable COD mass balances were achieved, particularly for the sewage batches during which batch tests were conducted. To evaluate the effect of the toilet paper on the mixed liquor organic solids, measurements on these were examined in more detail:

- Three independent measurements were made on the mixed liquor organic solids, volatile suspended solids (VSS), COD and TKN. The ratios of COD/VSS and TKN/VSS for the parent system mixed liquor were calculated for each sewage batch, see Table 4.4. Statistical plots for these ratio's were constructed, see Figs. (4.6, 4.7) and (4.8, 4.9) respectively (see Appendix D for interpretation of statistical plots). From the statistical plots it is evident that the data are normally distributed; this indicates that (i) an infinite number of parameters had an influence on the measurements (ii) each influence was small, and (iii) no single factor has had a dominating influence on the measurements. The means for COD/VSS and TKN/VSS were 1.39 mgCOD/mgVSS and 0.078 mgN/mgVSS respectively, with sample standard deviations 0.06 and 0.004 respectively. These values are lower than the values measured for the *control* parent system (1.42 mgCOD/mgVSS and 0.086 mgN/mgVSS respectively) and the accepted standard values of 1.48 mgCOD/mgVSS and 0.10 mgN/mgVSS respectively (WRC, 1984). This effect probably is due to the toilet paper dosed to the system.

Batches rejected for further analysis:

From the analysis of the COD mass balances, Sewage Batches No. 8A, 8B, 10 and 12 should be 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 No. 8A, 11 and 12 should be rejected. The specific causes for the poor N mass balances for these sewage batches are discussed in Section 4.12.2 above.

Thus, sewage batches that should be rejected are 8A, 8B, 10, 11 and 12. The data, however, will be retained for further analysis, but will be marked as rejected. No batch tests were conducted during these sewage batches.

4.12.3 Determination of unbiodegradable soluble and particulate fractions

From the steady state data on the *experimental* parent system, for each sewage batch, the unbiodegradable soluble and particulate fractions of the influent COD + toilet paper solution ($f_{S,us\,-\,WW+TP}$ and $f_{S,up\,-\,WW+TP}$ respectively) were determined using the methods of Ekama *et al.* (1986). The procedure is detailed in Chapter 3, Section 3.9.3.

The unbiodegradable soluble and particulate fractions of the wastewater ($f_{S,us\,-\,WW}$ and $f_{S,up\,-\,WW}$ respectively) are available from the *control* parent system, see Chapter 3, Section 3.9.3. To calculate the unbiodegradable soluble and particulate fractions of the toilet paper only ($f_{S,us\,-\,TP\ only}$ and $f_{S,up\,-\,TP\ only}$ respectively), the equivalent concentration for the S_{us} and S_{up} in the *experimental* system (i.e. wastewater + toilet paper, $S_{us\,-\,WW+TP}$ and the $S_{up\,-\,WW+TP}$) and the *control* system (i.e. wastewater only, $S_{us\,-\,WW}$ and $S_{up\,-\,WW}$) for each sewage batch were first calculated. From these, by difference the $S_{us\,-\,TP\ only}$ and the $S_{up\,-\,TP\ only}$ were calculated, taking into account the volumes of wastewater (V_{WW}) and toilet paper (V_{TP}) added and the $f_{S,us\,-\,TP\ only}$ and $f_{S,up\,-\,TP\ only}$ were found, as follows:

$$S_{us:\,TP\ only} = S_{us:\,(WW\,+\,TP)} - \left[S_{us:\,(WW\ only)} \cdot \frac{V_{ww}}{V_{ww} + V_{TP}} \right]$$

$$f_{S,\,us:\,TP\ only} = \frac{S_{us:\,TP}}{S_{ti:\,TP}\ \{= S_{ti:\,(WW\,+\,TP)} - S_{ti,\,(WW)}\}}$$

$$S_{up:\,TP\ only} = S_{up:\,(WW\,+\,TP)} - \left[S_{up:\,(WW\ only)} \cdot \frac{V_{ww}}{V_{ww} + V_{TP}} \right]$$

$$f_{S,\,up\ \,TP\ only} = \frac{S_{up:\,TP}}{S_{ti.\,TP}\ \{= S_{ti:\,(WW\,+\,TP)} - S_{ti:\,(WW)}\}}$$

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

- The $f_{S,us}$ values for {Wastewater + Toilet Paper} and for {Toilet Paper only} are listed in Table 4.4. The $f_{S,us}$ data for all the sewage batches were analysed using a statistical plot, see Figs. 4.10 and 4.12 respectively (for interpretation of the statistical plot, see Appendix D). Outliers were identified and rejected and the data are replotted in Figs. 4.11 and 4.13 respectively. The data are normally distributed, giving for the *experimental* system a mean $f_{S,us}$ for {Wastewater + Toilet Paper} of 0.045 and sample standard deviation of 0.008. The $f_{S,us}$ for {Toilet Paper only} is 0.035 with a sample standard deviation of 0.015.

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

- The $f_{S,up}$ values for {Wastewater + Toilet Paper} and for {Toilet Paper only} are listed in Table 4.4. The $f_{S,up}$ data for all the wastewater batches were analysed using a statistical plot, see Figs. 4.14 and 4.16. Outliers for $f_{S,up}$ {Wastewater + Toilet Paper} were identified and rejected and the data are shown replotted in Fig. 4.15. The data are normally distributed, giving for the *experimental* system a mean $f_{S,up}$ for {Wastewater + Toilet Paper} of 0.162 and sample standard deviation of 0.066. The $f_{S,up}$ for {Toilet Paper only} is 0.309 with a sample standard deviation of 0.141.

Table 4.5: Parent systems (*control & experimental*) steady state data. The data have been averaged and the means and no. of tests are listed.

Sewage Batch No.	COD (AVERAGES) (mgCOD/ℓ)									OUR (mgO/ℓ/h)			VSS (mgVSS/ℓ)		
	INFLUENT			UNFILT. EFFLUENT			MIXED LIQUOR								
	Control	Expt	No. of Tests	Control	Expt	No. of tests	Control	Expt	No. of tests	Control	Expt	No. of tests	Control	Expt	No. of Tests
1	495		7	62		7	3309		7	37.8			2320		7
2	514		10	144		10	2706		10	37.8			1878		10
3	525	525	8	51	56	8	4242	4381	8	37.0	40.2	8	3405	3045	8
4	494	494	6	41	39	6	5137	5399	6	41.5	41.3	6	3528	3745	6
5	500	500	6	42	39	6	4344	4362	6	36.2	36.3	6	3044	3122	6
6	498	515	12	40	58	12	3538	3771	12	32.7	35.1	12	2622	2792	12
7	420	483	4	30	68	4	3058	3673	4	35.5	43.2	4	2348	2805	4
8A	509	609	7	43	74	7	3378	4126	7	41.7	45.4	7	2480	3115	7
8B	701	827	4	52	72	4	3533	3838	4	43.7	46.2	4	2638	2934	4
9	744	886	11	52	56	11	3411	4178	11	39.0	45.0	11	2584	3085	11
10	756	1045	13	54	62	13	3452	3944	13	34.5	42.8	13	2464	2689	13
11	730	981	10	61	83	10	3070	4347	10	31.2	44.3	10	2548	2807	10
12	751	1070	10	49	98	10	3769	4265	10	36.9	48.4	10	2651	3033	10
13	766	1012	11	43	63	11	4089	5268	11	34.8	43.9	11	2622	3653	11
14	735	935	9	44	48	9	3413	4827	9	38.3	44.4	9	2408	3493	9
15	728	963	10	48	46	10	3722	5200	10	34.6	40.7	10	2483	3966	10
16	766	992	9	48	64	9	3764	5335	9	38.2	43.0	9	2700	3861	9
17	759	957	5	57	69	5	3335	4657	5	42.4	44.7	5	2406	3147	5
18	655	799	8	36	66	8	3119	4011	8	37.5	41.8	8	2409	3103	8
19	728	807	8	52	66	8	4073	4720	8	41.6	49.0	8	3042	3482	8
20	741	904	8	65	81	8	3936	4778	8	40.9	42.6	8	3760	3359	8

After operating the *experimental* activated sludge system for 15 sewage batches (Sewage Batch No. 6 to Sewage Batch No. 20) and obtaining sufficient reliable data (272 days) on toilet paper biodegradation, the results were analyzed and it could be concluded that the unbiodegradable particulate fraction of the toilet paper was about 31% ($f_{S,up}$ = 0.309, see Section 4.12.3). In fact, this does not differ that much from the unbiodegradable particulate fraction of sewage ($f_{S,up}$ = 0.161), as determined in Chapter 3, Table 3.4.

This indicated that the initial proposal that the toilet paper would contribute a high unbiodegradable particulate fraction ($f_{S,up}$) to the *experimental* activated sludge system was not true. This was attributed to the fact that the active organisms were degrading the toilet paper, in the process consuming oxygen: From Table 4.5, it is clear that the average rate of oxygen consumption is higher in the *experimental* system by about 2 to 13 mgO/ℓ/h for the various sewage batches compared to the *control* system. Hence, in terms of the original objectives (objective 2, see Section 4.1), the original amount of toilet paper solution added (which provided an additional dose of 2 000 mgCOD/d) did not cause a significant change in the active fraction of the VSS of the *experimental* system compared to the *control* system: This implies that the toilet paper did not cause a sufficient increase in inert material to the *experimental* system, thus the concentration of OHO active biomass fraction of the mixed liquor would not be expected to decrease significantly (this assumption was confirmed by the batch test results, see Chapter 5).

Therefore, from Sewage Batch No. 21 (day 347), it was decided to double the dosage of toilet paper to 4 000 mg COD/d and observe the response of the system. On the first day, the system was given a dose of 15 000 mgCOD (as toilet paper solution) in one batch to hasten steady state, and the subsequent doses were 4 000 mgCOD/d. When this was done, the *experimental* system became difficult to operate, with frequent blockages of pipes connecting reactors so that reactor overflows occurred on every second day, making it difficult to maintain steady state. After three subsequent sludge ages, the toilet paper dosage was stopped to restabilise the system. This caused the blockages to stop, whereupon a dose of 3 000 mgCOD/d as toilet paper solution was recommenced. Once again, reactor overflows became common. Accordingly, the original dose of 2 000 mgCOD/d as toilet paper solution was reverted to from Sewage Batch No. 24, and the daily testing and analysis of samples were restarted from Sewage Batch No. 25. Although the N mass balance was reasonable (109%), a poor COD mass balance (71%) was obtained for Sewage Batch No. 25; this was attributed to the fact that the system needed at least 2 sludge ages to reach steady state. Since it was evident that the toilet paper was more biodegradable than initially surmised, and would not achieve the desired effect of substantially decreasing the OHO active biomass fraction of the mixed liquor, this investigation was terminated.

4.14 CLOSURE

The parent *experimental* system was operated for 382 days and received 24 batches of raw municipal wastewater from the Mitchell's Plain Treatment Plant in Cape Town. From the results obtained from the system:

- N mass balances were consistent and were generally in the range 90 – 110%. Sewage batches that gave mass balances falling outside this range were No. 5, 8A, 10, 11, 12, and 14, with No. 5, 10 and 14 only marginally outside the range. No batch tests were conducted on these sewage batches.

- Generally COD mass balances were reasonable, with 7 out of 19 sewage batches giving mass balances < 90%. Of these, 3 sewage batches had COD mass balances only marginally less than 90%. No batch tests were conducted during any of these sewage batches.

- The mixed liquor organic solids were determined by three independent tests – VSS, COD and TKN. Mean ratios for these measurements gave COD/VSS = 1.39 mgCOD/mgVSS (sample standard deviation = 0.06) and TKN/VSS = 0.078 mgN/mgVSS (sample standard deviation = 0.004). These values are lower than the values measured for the *control* parent system (1.42 mgCOD/mgVSS and 0.086 mgN/mgVSS respectively) and the accepted standard values of 1.48 mgCOD/mgVSS and 0.10 mgN/mgVSS respectively (WRC, 1984). More likely, the lower values were caused by the toilet paper dose.

- Operational problems were experienced when the parent system was receiving Sewage Batches No. 17 and 18; 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), and did impact on the batch test data analysis for Sewage Batch No. 18, since batch tests were conducted during this period. To take account of the increased temperature, the temperature effect was included in the formulation to calculate $f_{S,up}$ [Eq. (3.12)], and hence in the OHO active biomass fraction estimate (see Chapter 5).

- The original amount of toilet paper solution added (which provided an additional dose of 2 000 mgCOD/d) did not cause a significant change in the OHO active biomass fraction of the mixed liquor organic solids of the *experimental* system compared to the *control* system. The unbiodegradable soluble ($f_{S,us}$) and particulate ($f_{S,up}$) fractions of the toilet paper were determined to be 0.035 mgCOD/mgCOD and 0.309 mgCOD/mgCOD respectively; these values are reasonably close to the values determined for the wastewater itself, 0.050 mgCOD/mgCOD and 0.161 mgCOD/mgCOD respectively, see Chapter 3. This implies that the toilet paper was 65.6% biodegradable and hence did not increase the inert fraction of the mixed liquor significantly and thus the concentration of OHO active biomass fraction of the mixed liquor would not be expected to decrease significantly (this assumption was confirmed by the batch test results, see Chapter 5). Larger doses of toilet paper could

not be used, as these led to blockages of pipes between reactors, resulting in reactor overflows. Thus, the objective of dosing toilet paper to significantly change the OHO biomass fraction of the mixed liquor and evaluating the ability of the batch test to detect this change could not be achieved.

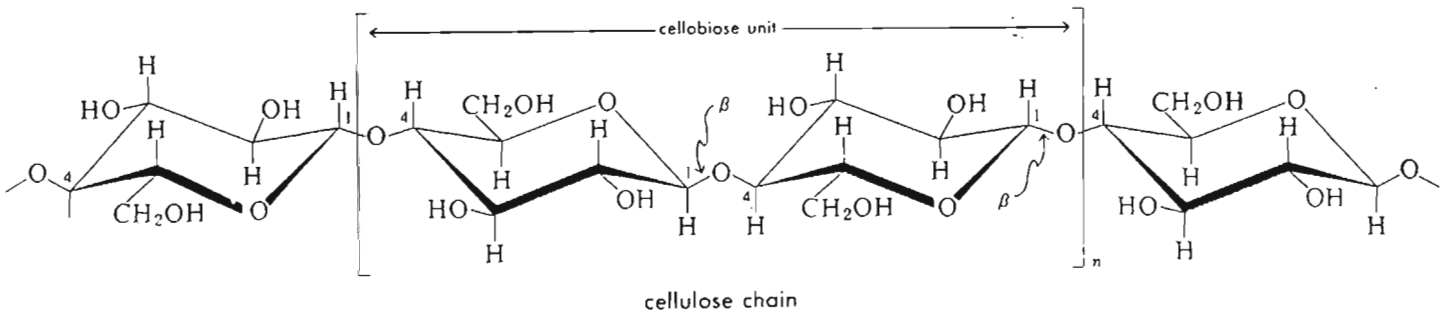


Figure 4.1: Partial structure of a cellulose molecule showing the β-linkage of glucose units.

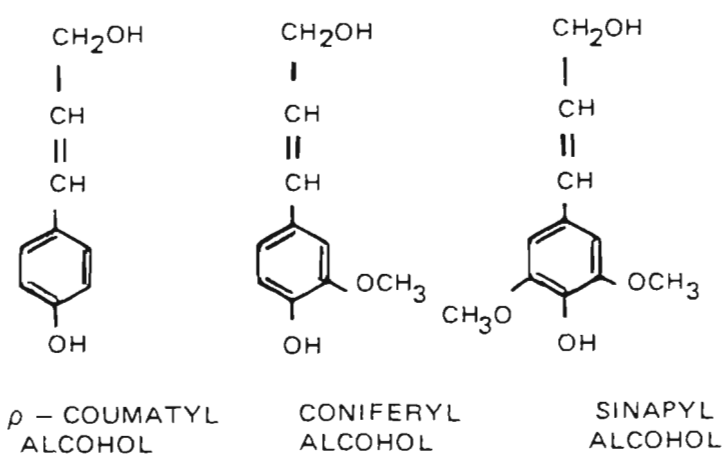


Figure 4.2: The three primary monomeric precursors of lignin.

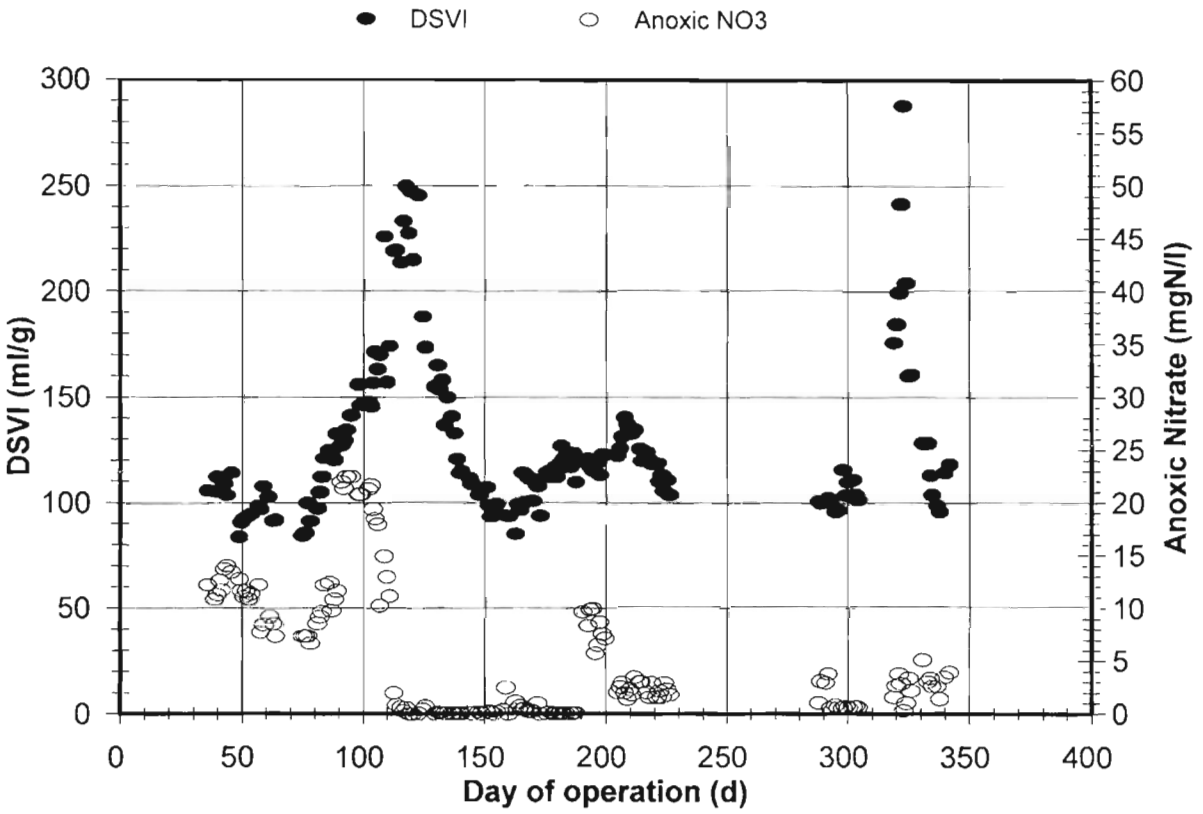


Figure 4.3: Graphical representation of the daily Diluted Sludge Volume Index (DSVI) and anoxic nitrate (NO_3) concentration for the parent experimental laboratory-scale activated sludge system.

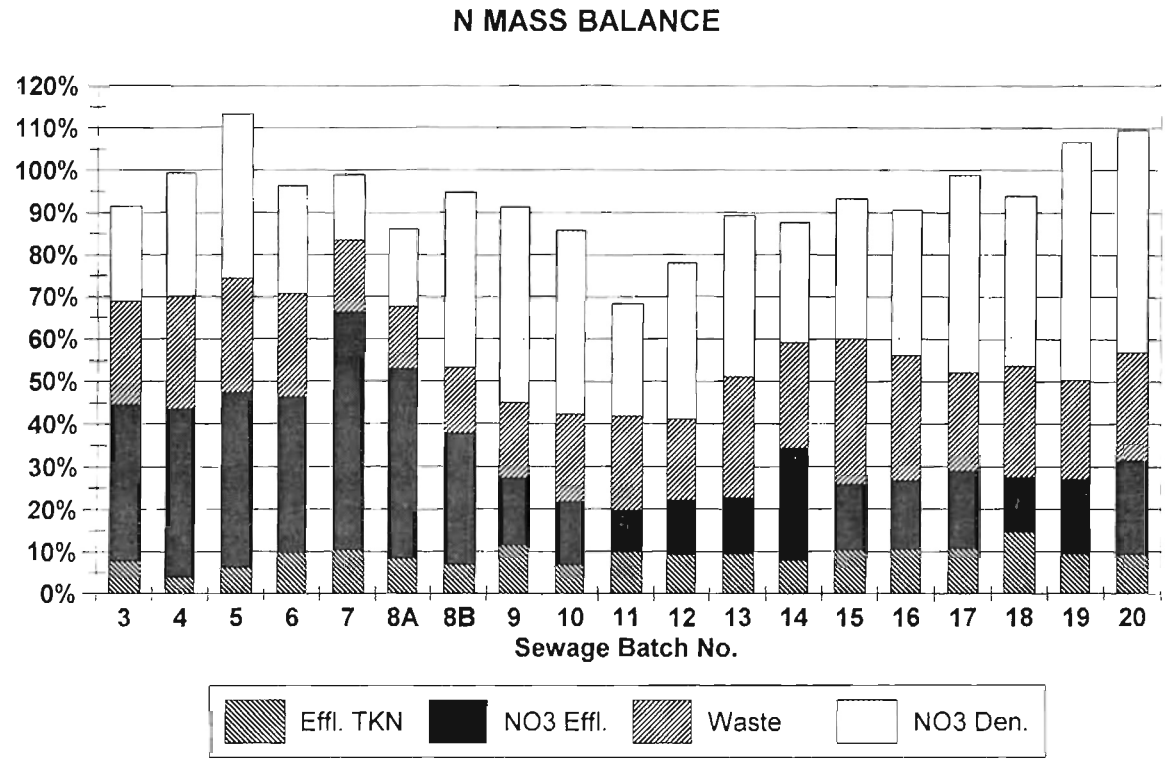


Figure 4.4: Graphical representation of the percentage N mass balance for the various sewage batches. Percentages are also shown for N for sludge production, effluent TKN, effluent NO3, and NO3 for denitrification.

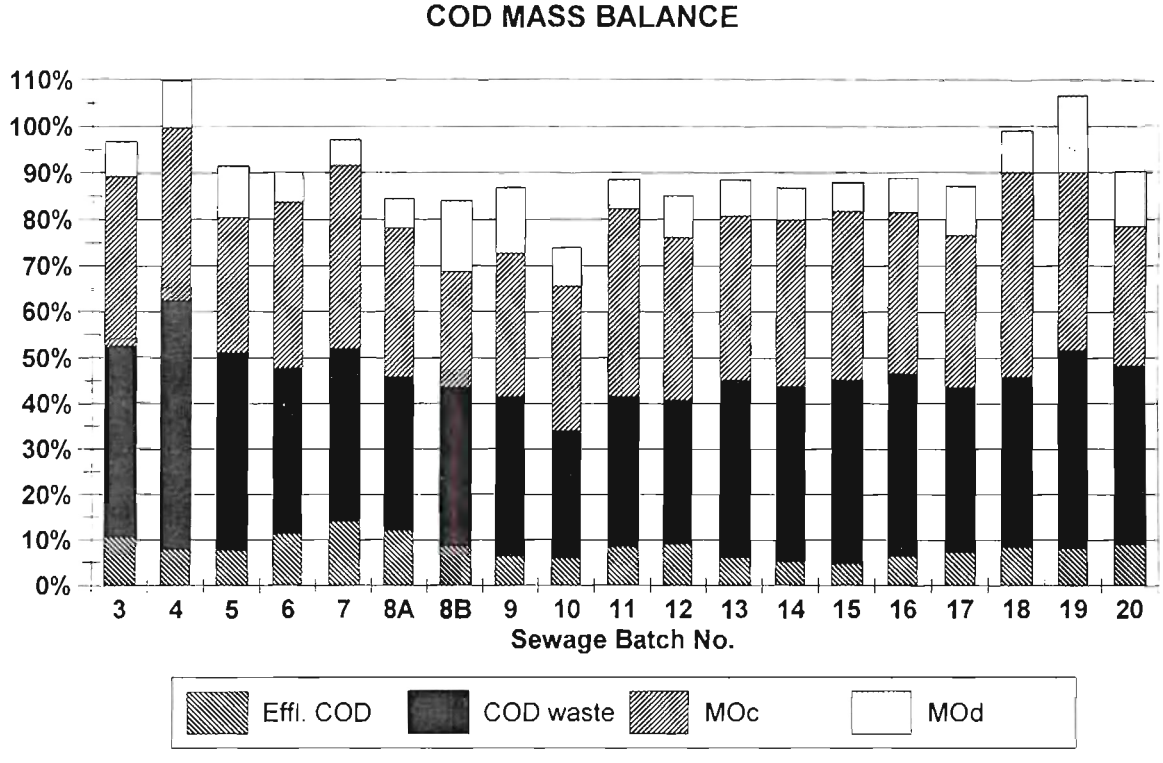


Figure 4.5: Graphical representation of the percentage COD mass balance for the various sewage batches. Percentages are also shown for waste sludge COD, unfiltered effluent COD, carbonaceous oxygen demand and oxygen required for nitrification.

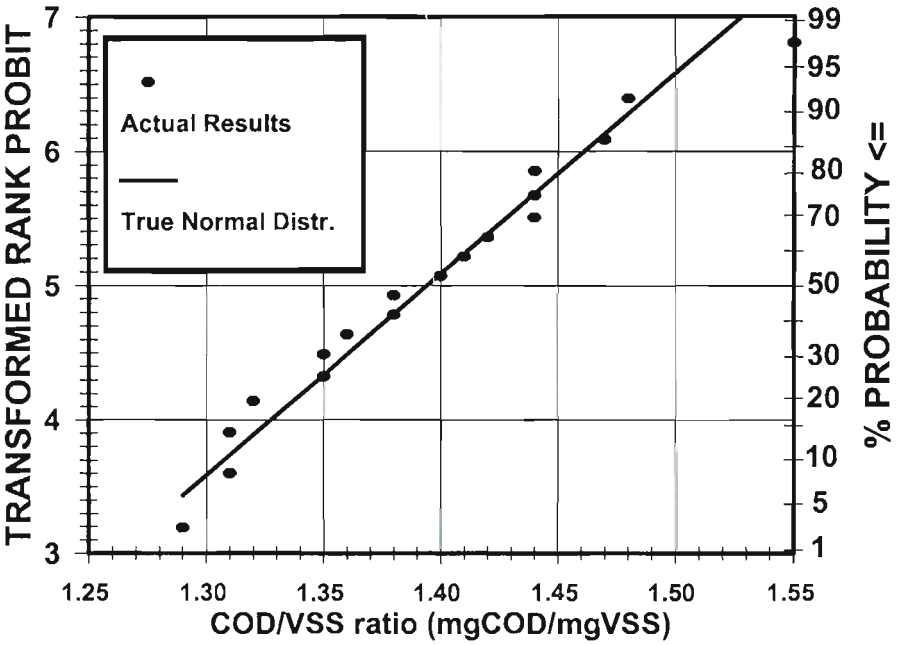


Figure 4.6: Statistical plot for the COD/VSS ratio for the parent experimental laboratory-scale activated sludge system.

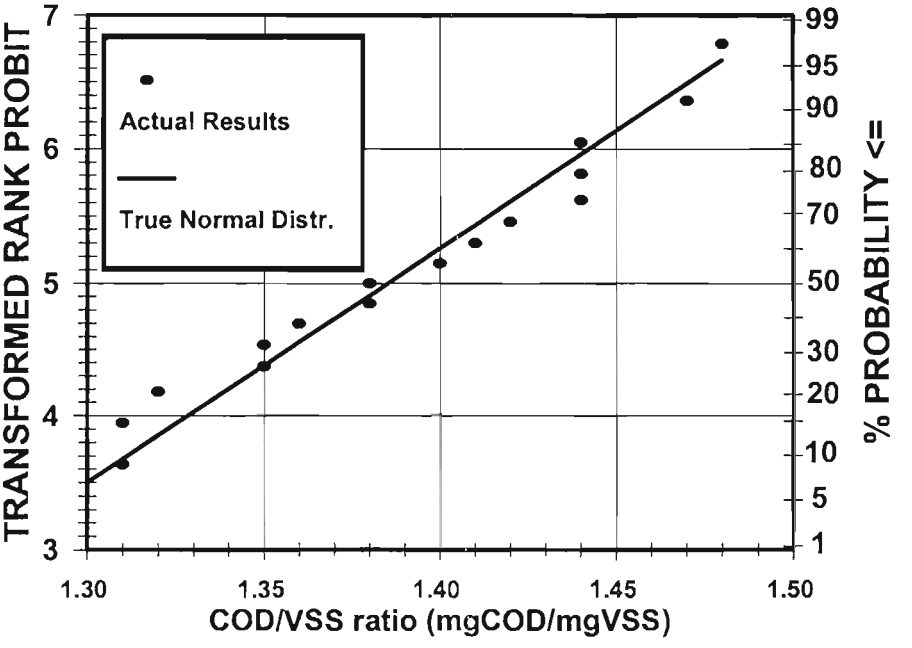


Figure 4.7: Statistical plot for the COD/VSS ratio for the parent experimental laboratory-scale activated sludge system: * outliers rejected.

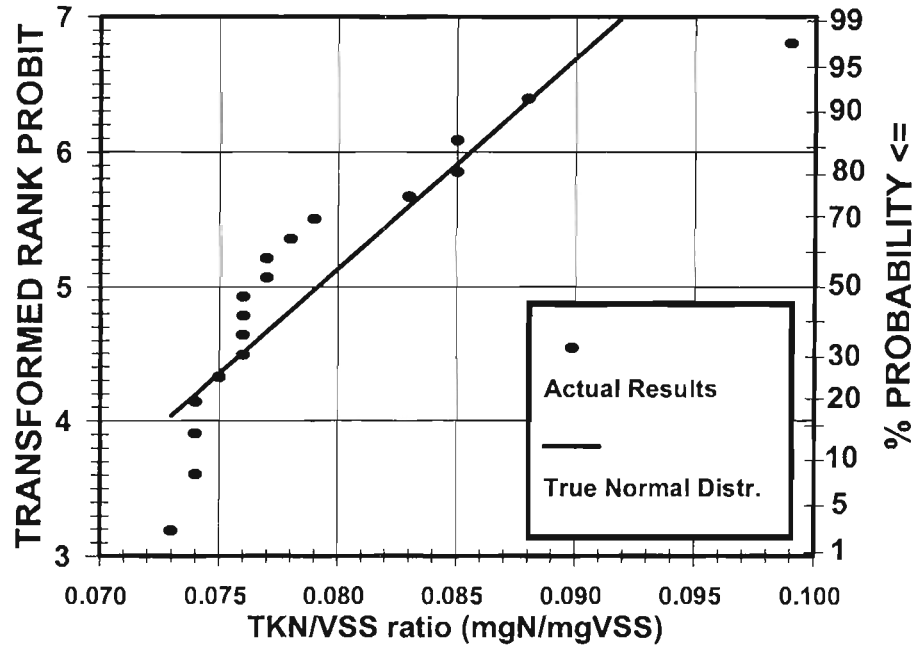


Figure 4.8: Statistical plot for the TKN/VSS ratio for the parent experimental laboratory-scale activated sludge system.

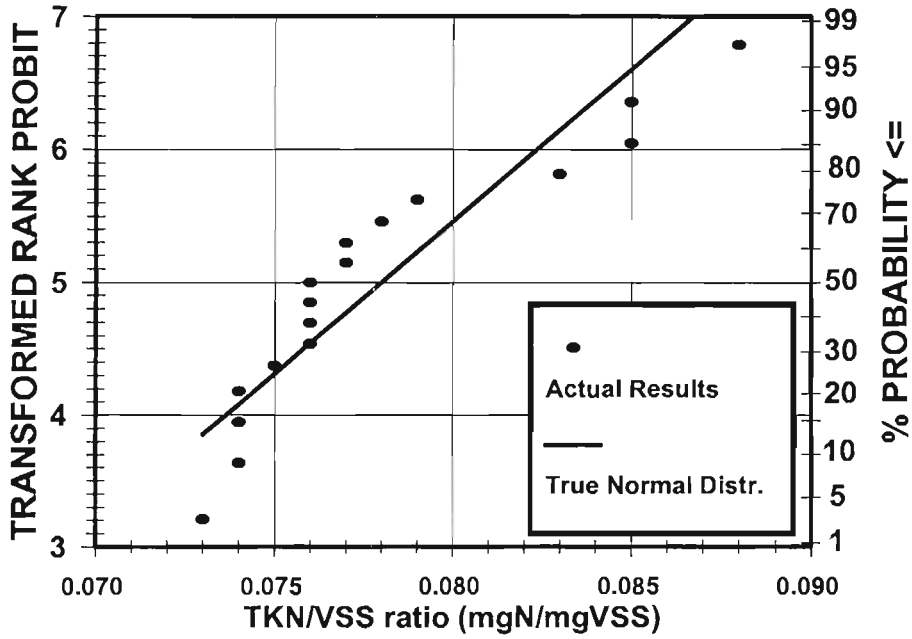


Figure 4.9: Statistical plot for the TKN/VSS ratio for the parent experimental laboratory-scale activated sludge system: * outliers rejected.

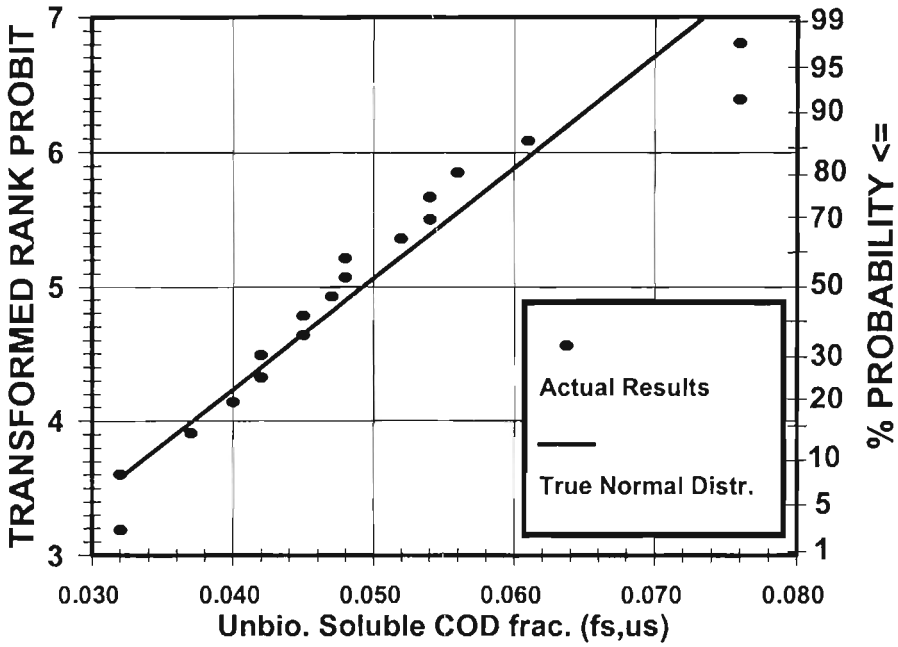


Figure 4.10: Statistical plot for unbiodegradable soluble ($f_{S,us}$) fraction for the parent experimental laboratory-scale activated sludge system fed with municipal wastewater from Mitchell's Plain Treatment Plant (Cape Town, South Africa) and toilet paper.

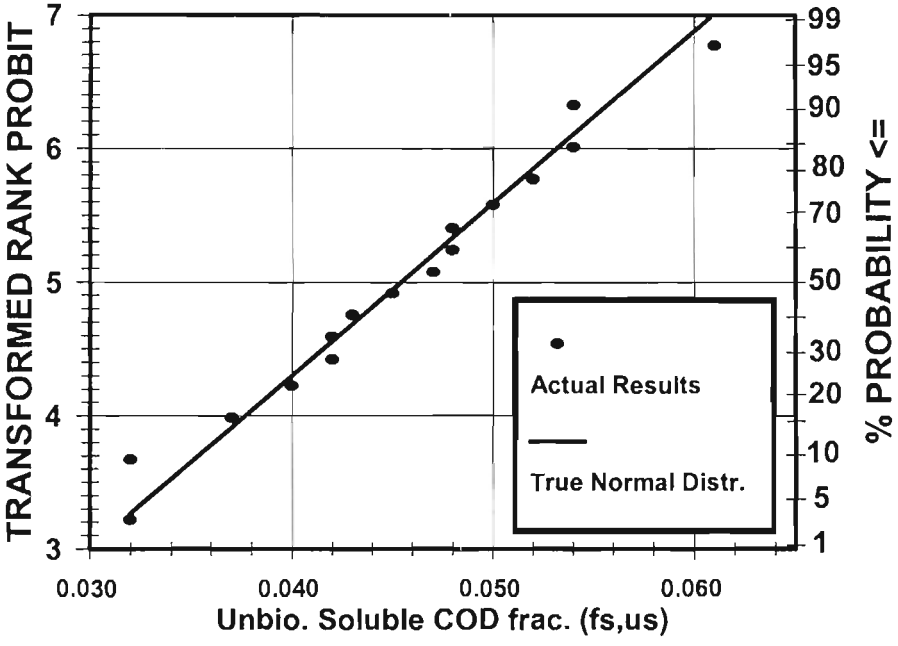


Figure 4.11: Statistical plot for unbiodegradable soluble ($f_{S,us}$) fraction for the parent experimental laboratory-scale activated sludge system fed with municipal wastewater from Mitchell's Plain Treatment Plant (Cape Town, South Africa) and toilet paper: * outliers rejected.

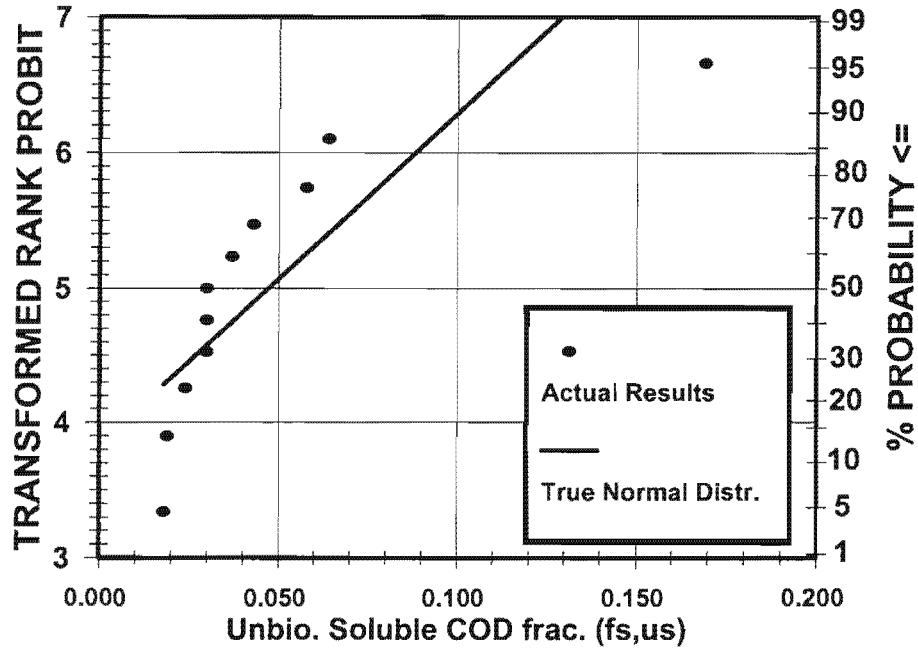


Figure 4.12: Statistical plot for unbiodegradable soluble ($f_{S,us}$) fraction of toilet paper only for the parent experimental laboratory-scale activated sludge system fed with municipal wastewater from Mitchell's Plain Treatment Plant (Cape Town, South Africa) and toilet paper.

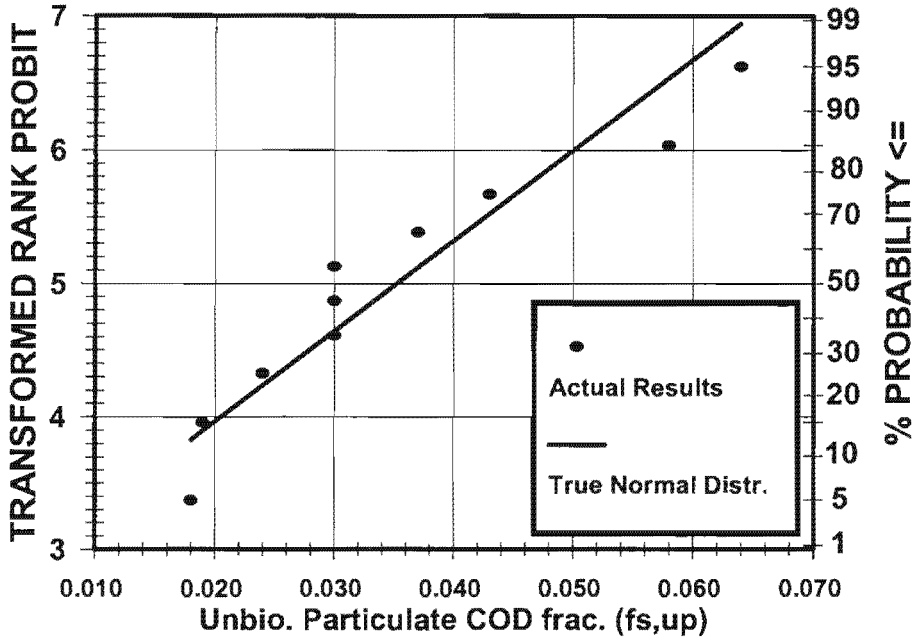


Figure 4.13: Statistical plot for unbiodegradable soluble ($f_{S,us}$) fraction of toilet paper only for the parent experimental laboratory-scale activated sludge system fed with municipal wastewater from Mitchell's Plain Treatment Plant (Cape Town, South Africa) and toilet paper: * outliers rejected.

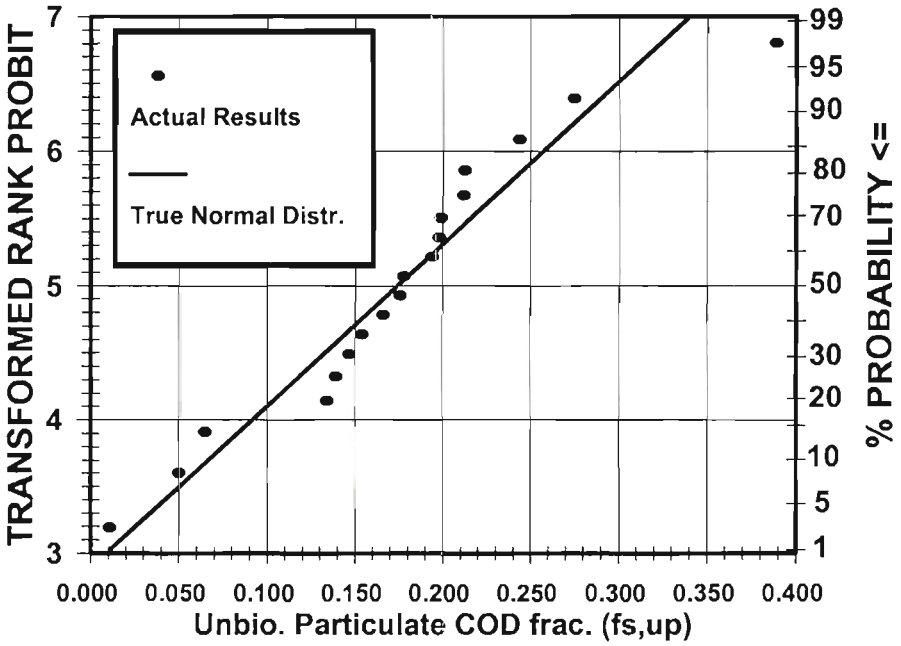


Figure 4.14: Statistical plot for unbiodegradable particulate ($f_{S,up}$) fraction for the parent experimental laboratory-scale activated sludge system fed with municipal wastewater from Mitchell's Plain Treatment Plant (Cape Town, South Africa) and toilet paper.

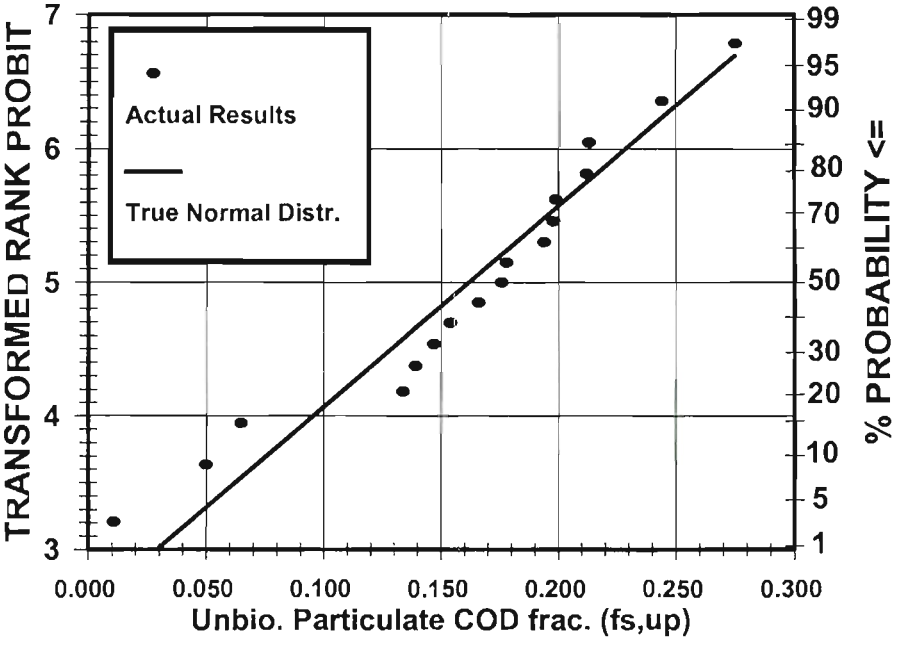


Figure 4.15: Statistical plot for unbiodegradable particulate ($f_{S,up}$) fraction for the parent experimental laboratory-scale activated sludge system fed with municipal wastewater from Mitchell's Plain Treatment Plant (Cape Town, South Africa) and toilet paper: * outliers rejected.

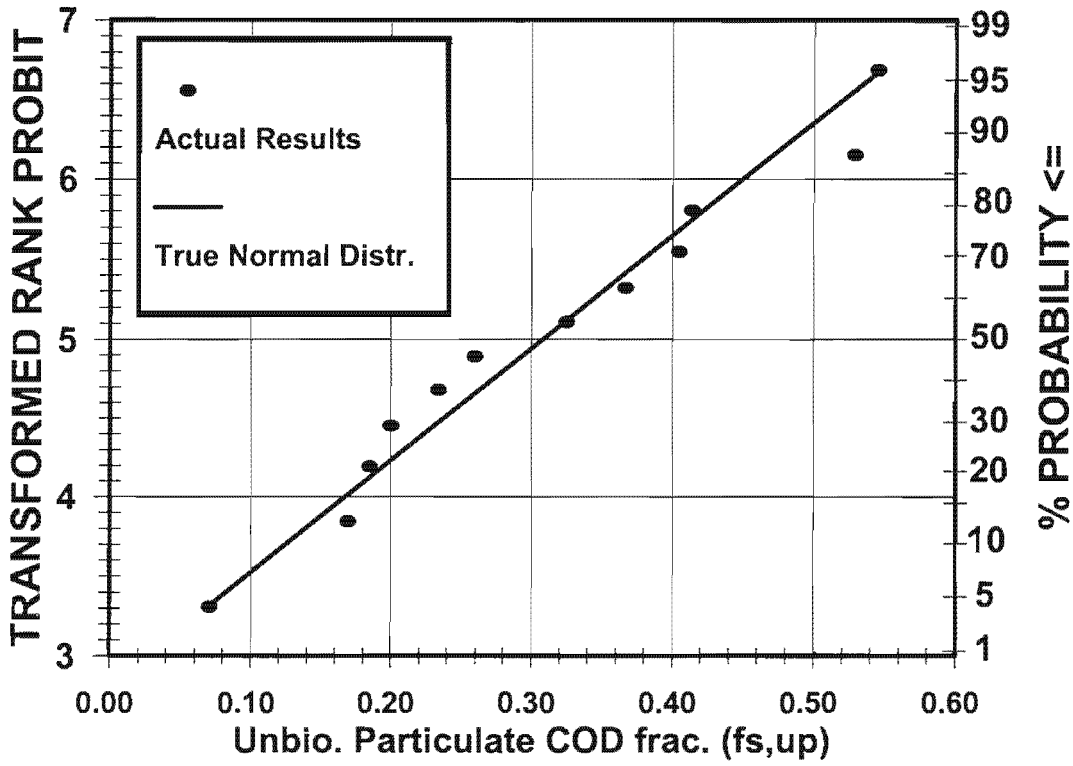


Figure 4.16: Statistical plot for unbiodegradable particulate ($fs_{,up}$) fraction of toilet paper only for the parent experimental laboratory-scale activated sludge system fed with municipal wastewater from Mitchell's Plain Treatment Plant (Cape Town, South Africa) and toilet paper.

CHAPTER 5

MODIFIED BATCH TEST PROCEDURE TO QUANTIFY OHO ACTIVE BIOMASS

5.1 INTRODUCTION

The batch test procedure developed by Kappeler and Gujer (1992) presented a means of quantifying the 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) and by Ubisi *et al.* (1997a,b) and Cronje *et al.* (2000) to quantify the OHO active biomass concentration in mixed liquor drawn from aerobic and anoxic/aerobic activated sludge systems, obtained from the Mitchell's Plain Treatment Plant, Cape Town (South Africa).

Cronje *et al.* (2000) compared the results for OHO active biomass concentration obtained from the modified batch test with theoretical values for OHO active biomass concentration from the steady state design model (WRC, 1984) for mixed liquor samples drawn from a parent anoxic/aerobic activated sludge system. From this comparison, they concluded that the results obtained showed good agreement, see Fig. 1.1. Also, there was remarkable agreement between the theoretical OHO active biomass concentration in the parent system and the mean of the measured OHO active biomass values *projected* to the parent system. However, they noted that the individually measured OHO active biomass values were prone to significant variation. They attributed this mainly to the sensitivity of the measured OHO 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 OHO active biomass values.

The good correlation that Cronje *et al.* (2000) found between the theoretical and measured values was remarkable, considering the sensitivity of the analysis. The results appeared to substantiate the modified batch test method as a reliable means of quantifying the OHO active biomass. However, the modified method does require more extensive evaluation. In this research project, ***the principle aim is to further investigate the modified batch test method of Cronje et al. (2000) as a reliable means of quantifying the concentration of OHO active biomass concentration of the mixed liquor drawn from aerobic and anoxic/aerobic activated sludge systems.***

In this Chapter the experimental procedure and the interpretation of data obtained from the modified batch tests will be described. The results of the batch tests and the speculated potential sources of error in the analysis of the batch tests data will also be described and the steps followed in solving these errors will then follow.

5.2 EXPERIMENTAL PROCEDURE

5.2.1 Experimental approach

The application of the batch test to quantify the OHO active biomass concentration hinges around the capacity of the OHO 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 *flocculated-filtered* wastewater and thus are surrounded by a high concentration of substrate in the form of readily biodegradable COD (RBCOD) and some slowly biodegradable COD (SBCOD). The wastewater is preflocculated and filtered, to remove any OHO active biomass present in the wastewater itself.

Under the conditions in the batch test, the OHO organisms utilize the substrate at a maximum rate 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), with growth/substrate utilization rates at maxima, the oxygen utilization rate (OUR) is independent of the substrate concentration and 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 to give the OUR for OHOs only. Cronje *et al.* (2000) describe the batch test procedures in detail, but this is repeated below for elucidation purposes.

5.2.2 Wastewater preparation

The preparation of the wastewater for the modified batch test incorporated flocculating and filtering the raw wastewater. Because these processes tend to be 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 3. The wastewater was diluted to approximately the same COD concentration (± 750 mgCOD/ℓ) as that fed to the parent system. A sufficient volume of diluted wastewater (± 8ℓ) was measured and placed in a separate bucket which served as wastewater source for the two separate batch tests to be performed on 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 sulphate [Aℓ(SO_4) • 15 H_2O, stock at 50g/ℓ] were added per ℓ wastewater, the mixture was stirred rapidly (~200 rpm) for 2 minutes (rapid mix phase) and then slowly (~1 rpm) for 30 minutes (flocculation and settling

phase). The flocculation and settling phase was conducted in custom-built settling cylinders of 3ℓ capacity each. The settling cylinders (110 mm dia.) were equipped with magnetic stirring arms regulated to achieve the slow rotational speed of 1 rpm. Enhanced settling was observed when the stirring was discontinued after the 30 minute period and the contents of the cylinder were allowed to settle (without stirring) for a further 30 minute period.

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 ± 8ℓ of wastewater had to be filtered at a time. In any event, with a preflocculation step, using a glass fibre filter met the requirement in that it effectively removed all active biomass from the wastewater – no measurable OUR on the *flocculated-filtered* wastewater only was observed (Cronje *et al.*, 2000).

The *flocculated-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 contamination occurred.

5.2.3 Filtered wastewater and mixed liquor batch tests

As described above, the modified batch tests were conducted using a mixture of *flocculated-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 *flocculated-filtered* wastewater from the cold room, the required volume of wastewater (i.e. 2.85ℓ in Fig. 5.1) was carefully measured, 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 *flocculated-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 3, Section 3.4) to the wastewater. The required volume of mixed liquor (0.15ℓ in Fig. 5.1) was harvested from the aerobic reactor of the parent system and added to the *flocculated-filtered* wastewater, giving a combined volume of 3ℓ for the mixture in the batch reactor. The volume of mixed liquor added was varied between 80 mℓ and 400 mℓ for all the modified batch tests conducted, with the volume of *flocculated-filtered* wastewater being concomitantly varied from 2.92ℓ to 2.6ℓ. Immediately after the mixed liquor was added to the *flocculated-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, at the end of which was an air-stone terminating 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 (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, $R^2 < 0.95$ were rejected for analysis. A typical OUR (mgO/ℓ/h) versus time (h) response is shown plotted in Fig. 5.2. During the first period of the batch test (<6h) the OUR exhibits an exponential increase due to OHO active biomass growth on both RBCOD and SBCOD added with the wastewater. After ± 6 h the OUR drops precipitously due to the depletion of the wastewater RBCOD, and the subsequent OUR reflects OHO active biomass growth on SBCOD only. Concomitantly, over the entire batch test, there is a low approximately constant OUR due to nitrification. As the first period is the important part for subsequent analysis, the batch tests were generally continued for approximately 1 hour after the precipitous drop in OUR was observed. Because the mixed liquor OHOs exhibited a low growth rate, some of the batch tests conducted on small sample volumes 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 6 and 12 hours, depending on the volume of mixed liquor added.

For the batch tests conducted on mixed liquor samples drawn when the parent laboratory-scale activated sludge system was receiving Sewage Batch No. 18 (Batch Tests No. 1-8), the surface of the water was covered by small plastic balls (roll-on deodorant balls) to limit surface exchange of oxygen. However, this technique gave difficulties: Particulate matter and organisms had a tendency to adhere to the plastic balls, which were only partially submerged in the batch test liquid; thus, some organisms were in fact not participating in the batch test. Later, it was deduced that the amount of oxygen entrained into the batch reactor was negligible and from Sewage Batch No. 19 (Batch Test No. 9) onwards, the water surface was left exposed to the air.

Also, as a result of the intermittent aeration process, particulate matter and organisms were 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 batch reactor, immediately filtered through 0.45 μm filter paper, 2 – 3 drops of $HgCl_2$ 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 were homogenized in a liquidizer, a sample drawn and the final total COD concentration measured. The OUR results were downloaded from the DO meter to a PC.

5.3 DATA INTERPRETATION

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

- COD recovery (%)
- OHO active biomass concentration, $Z_{BH(0)}$ (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 model of the processes involved in the batch test and thus will be repeated here to clarify the interpretation of the OUR-time response. The data from one batch test (Batch Test No. 8, Sewage Batch No. 18) is used as an example to illustrate the calculation procedures, see Figs. 5.2 to 5.5.

5.3.1 Batch test data

5.3.1.1 Separating the OUR into its OHO and nitrification components

In the batch test, mixed liquor is drawn from a nitrifying activated sludge system and thus nitrification can be expected and indeed was observed, see Fig. 5.3. The OUR due to nitrification must be taken into account in deriving estimates for %COD recovery and $Z_{BH(0)}$ since both parameters are determined from the OUR for OHOs 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 OHO growth ($OUR_{H(t)}$) and due to nitrification by autotrophic organisms (AO) ($OUR_{N(t)}$), i.e.

$$OUR_{M(t)} = OUR_{H(t)} + OUR_{N(t)} \qquad (mgO/\ell/h) \qquad (5.1)$$

Rearranging Eq. (5.1):

$$OUR_{H(t)} = OUR_{M(t)} - OUR_{N(t)} \qquad (mgO/\ell/h) \qquad (5.2)$$

Accordingly, to determine $OUR_{H(t)}$, an estimate for $OUR_{N(t)}$ is essential. The $OUR_{N(t)}$ can be determined from the nitrate concentration – time profile (for example Fig. 5.3). In determining $OUR_{N(t)}$ from the nitrate concentration – time profile, Ubisi *et al.* (1997a,b) noted that for the batch tests, ammonia-N is available in excess and nitrification proceeds at the maximum rate. Further, they noted that since the yield and maximum specific growth rate of the AOs are relatively low, the nitrification rate can be assumed to be constant within the time scale of the batch test. Accepting a constant nitrification rate, the

slope of a "best-fit" linear line to the nitrate (mgN/ℓ) time (h) profile is the constant nitrification rate ($\Delta NO_3^- / \Delta t$, mgN/ℓ/h), and the $OUR_{N(t)}$ is given by:

$$OUR_{N(t)} = 4.57 \cdot (\Delta NO_3^- / \Delta t) \qquad (mgO/\ell/h) \qquad (5.3)$$

In their evaluation of the batch test method, Cronje *et al.* (2000) proposed that the nitrification rate is not constant, but is better approximated by an exponential fit to the measured NO_3^- concentration – time data. From such an exponential fit, the nitrification rate can be found at each time interval from the slope of the exponential equation, i.e. from the differential of the exponential equation and the corresponding $OUR_{N(t)}$ determined via Eq. (5.3). This proposal will be evaluated in this investigation, see below. However, irrespective of the linear or exponential fit, the nitrification OUR can be found from Eq. (5.3), and hence the OHO OUR via Eq. (5.2).

In interpreting the nitrate and nitrite concentrations with time observed in their batch tests, both Ubisi *et al.* (1997a,b) and Cronje *et al.* (2000) found that the nitrite concentrations were very low, and hence could be neglected. However, in this investigation nitrite concentrations were found to be significant compared to nitrate concentrations (for example see Fig. 5.3), and hence need to be taken into account to determine OUR_N. This arises because the oxygen requirement to nitrify ammonia-N to nitrite is lower than that for nitrification of ammonia-N to nitrate:

$$NH_4^+ + \tfrac{3}{2} O_2 \rightarrow NO_2^- + H_2O + 2H^+ \qquad (5.4)$$

$$NO_2^- + \tfrac{1}{2} O_2 \rightarrow NO_3^- \qquad (5.5)$$

From the stoichiometric equations above, for every 1 mg NH_4^+ - N nitrified to 1 mg NO_2^- - N, 3.43 mg O are required, and for 1 mg NO_2^- - N nitrified to 1 mg NO_3^- - N, 1.14 mg O are required. Thus, for nitrification of NH_4^+ - N to NO_3^- - N, 4.57 mgO/mgN are required.

Thus, in this investigation the nitrate and nitrite concentrations were separated, and "best-fit" lines fitted to the individual profiles, linear or exponential to be evaluated. For the example, (Fig. 5.3), linear fits gave good correlation to the experimental data: From regression analysis of the data in Fig. 5.3,

$$NO_{2(t)} = 0.0930\, t + 0.1209 \qquad [R^2 = 0.988] \qquad (mgN/\ell) \qquad (5.6)$$

$$NO_{3(t)} = 0.1766\, t + 0.2954 \qquad [R^2 = 0.975] \qquad (mgN/\ell) \qquad (5.7)$$

Hence, the $\Delta NO_2^- / \Delta t = 0.0930$ (mgN/ℓ/h) and $\Delta NO_3^- / \Delta t = 0.1766$ (mgN/ℓ/h) for Batch Test No. 8. From the individual profile fits, the OUR for NO_2^- nitrification (OUR_{NO2}) and NO_3^- nitrification (OUR_{NO3}) could be determined:

$$OUR_{NO2} = 3.43 \cdot (\Delta NO_2^- / \Delta t) \qquad (mgO/\ell/h) \qquad (5.8)$$

$$OUR_{NO3} = 4.57 \cdot (\Delta NO_3^- / \Delta t) \quad (mgO/\ell/h) \quad (5.9)$$

and

$$OUR_N = OUR_{NO2} + OUR_{NO3} \quad (mgO/\ell/h) \quad (5.10)$$

For the example here, this gives OUR_{NO2} = 0.319 mgO/ℓ/h, OUR_{NO3} = 0.807 mgO/ℓ/h and OUR_N = 1.126 mgO/ℓ/h respectively. These two OURs were subtracted from the measured OUR_M (Fig. 5.2) at each time interval, to give the OUR_H for OHOs only (Fig. 5.4).

A more detailed explanation of the analysis method is given in Section 5.4.2 below, together with an evaluation on the suitability of linear versus exponential fits to the experimental data.

5.3.1.2 Derivation of equations for COD recovery

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

$$\% \text{ COD recovery} = \frac{COD_{t=T} + \int_{t=0}^{t=T} OUR_{H(t)} \cdot dt}{COD_{t=0}} \cdot 100 \quad (5.11)$$

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)
$OUR_{H(t)}$	=	OHO active biomass oxygen utilization rate at time t (mgO/ℓ/h)
$\int_{t=0}^{t=T} OUR_{H(t)} \cdot dt$	=	integral (area) under the OHO OUR versus time plot between start and end of batch test (mgO/ℓ)
	=	oxygen concentration consumed over the batch test by OHO active biomass

Table 5.1: 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				$K_A S_{enm} Z_{BH}(f_{MA} - S_{ads}/Z_{BH})$
Stoichiometric constants Y_{ZH} = Heterotroph yield f_E = Endogenous residue f_{MA} = Max. ratio S_{ads}/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}	Kinetic constants μ_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. (5.11), the OUR due to OHO growth only ($OUR_{H(t)}$) equals the measured total OUR ($OUR_{M(t)}$) minus the OUR due to nitrification ($OUR_{N(t)}$), see Section 5.3.1 above. Integrating the area under the measured OUR_H – time profile and substituting into Eq. (5.11), the COD recoveries for the different batch tests can be calculated. Using the data in Figs. 5.2 and 5.4 as an example, $COD_{t=0}$ = 356.4 mgCOD/ℓ ; $COD_{t=T}$ = 290.5 mgCOD/ℓ ;

$$\int_{t=0}^{t=T} OUR_{H(t)} \cdot dt = 42.7 \text{ mgO}/\ell, \text{ then:}$$

$$\% \text{ COD recovery} = \frac{290.5 + 42.7}{356.4} \cdot 100 = 93.5 \%$$

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.

5.3.1.3 Derivation of equations for OHO active biomass concentration, $Z_{BH(0)}$

The simplified UCT model is presented in Table 1 of Wentzel *et al.* (1995), and duplicated in Table 5.1. From the simplified UCT model, the rate of growth of OHO active biomass (dZ_{BH}/dt) is given by:

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

$$\frac{dZ_{BH}}{dt} = \mu_{HT} \frac{S_{bs}}{K_{SH} + S_{bs}} \cdot Z_{BH} + K_{MPT} \frac{S_{ads} / Z_{BH}}{K_{SP} + S_{ads} / Z_{BH}} \cdot Z_{BH} - b_{HT} \cdot Z_{BH} \quad (5.12)$$

where

Z_{BH}	=	OHO active biomass concentration (mgCOD/ℓ)
μ_{HT} *	=	maximum specific growth rate of OHO on RBCOD at temperature T (/d)
S_{bs}	=	RBCOD concentration (mgCOD/ℓ)
K_{SH}	=	half saturation constant for RBCOD
	=	5 mgCOD/ℓ
K_{MPT} *	=	maximum specific growth rate of OHO on SBCOD at temperature T (/d)
S_{ads}	=	adsorbed SBCOD concentration (mgCOD/ℓ)
K_{SP}	=	half saturation constant for SBCOD
	=	0.027 mgCOD/mgCOD
b_{HT} *	=	OHO specific death rate at temperature T (/d)

* *μ_{HT}, K_{MPT} and b_{HT} are temperature dependent.*

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,

$$\frac{dZ_{BH}}{dt} = (\mu_{HT} + K_{MPT} - b_{HT})\, Z_{BH} \qquad (5.13)$$

Integrating Eq. (5.13) and solving yields the OHO active biomass concentration at time t ($Z_{BH(t)}$, mgCOD/ℓ) in terms of the initial OHO active biomass concentration ($Z_{BH(0)}$, mgCOD/ℓ), time (t, in h) and the net specific growth rate ($\mu_{HT} + K_{MPT} - b_{HT}$) *viz*;

$$Z_{BH(t)} = Z_{BH(0)}\, e^{(\mu_{HT} + K_{MPT} - b_{HT})\, t / 24} \qquad (5.14)$$

The OHO active biomass OUR at time t ($OUR_{H(t)}$, mgO/ℓ) is a function of $Z_{BH(t)}$, the net specific growth rate and OHO yield coefficient, Y_{ZH} = 0.666 mgCOD/mgCOD:

$$OUR_{H(t)} = \frac{1-Y_{ZH}}{Y_{ZH}} (\mu_{HT} + K_{MPT})\, Z_{BH(t)} / 24 \qquad (5.15)$$

Substituting Eq. (5.14) for $Z_{BH(t)}$ in Eq. (5.15) and taking natural logs yields

$$\ell n\, OUR_{H(t)} = \ell n \left[\frac{1-Y_{ZH}}{Y_{ZH}} (\mu_{HT} + K_{MPT})\, Z_{BH(0)} / 24 \right] + (\mu_{HT} + K_{MPT} - b_{HT})\, t / 24 \qquad (5.16)$$

which is a straight line with,

$$\text{slope} = (\mu_{HT} + K_{MPT} - b_{HT}) / 24 \qquad (5.17)$$

$$\text{y-intercept} = \ell n(OUR_{H(t=0)}) = \ell n \left[\frac{1-Y_{ZH}}{Y_{ZH}} (\mu_{HT} + K_{MPT})\, Z_{BH(0)} / 24 \right] \qquad (5.18)$$

To determine the OHO active biomass at the start of the batch test ($Z_{BH(0)}$), the OHO active biomass OUR values for the data up to the precipitous drop in OUR were plotted as ℓn (OUR_H)versus time (for the example, the ℓn (OUR_H)from the data in Fig. 5.4 are shown plotted in Fig. 5.5), and linear regression applied to determine the y-intercept, slope and correlation coefficient. For the example, these are listed in Table 5.2. From the slopes and y-intercepts, Eqs. (5.17) and (5.18) respectively, $Z_{BH(0)}$ can be determined from (Wentzel *et al.*, 1995):

$$Z_{BH(0)} = \frac{(e^{\text{y-intercept}}) \cdot 24}{\frac{1-Y_{ZH}}{Y_{ZH}} \cdot (\text{slope} \cdot 24 + b_{HT})} \quad (\text{mgCOD}/\ell) \qquad (5.19)$$

where

$Z_{BH(0)}$ = OHO active biomass concentration at the start of the batch test (mgCOD/ℓ *batch reactor*)
Y_{ZH} = OHO active biomass yield, COD units (mgCOD/mgCOD)
= 0.666 mgCOD/mgCOD (Dold *et al.*, 1980, 1991; Wentzel *et al.*, 1995)
b_{HT} = OHO specific death rate at temperature T (/d)
= $b_{H20}\ 1.029^{(T-20)}$
b_{H20} = OHO specific death rate at 20°C
= 0.62/d (death/regeneration theory, Dold *et al.*, 1980; Wentzel *et al.*, 1995).

Accepting Eq. (5.19) and substituting into the equation the regression data listed in Table 5.2 for the example batch test, the value for $Z_{BH(0)}$ for Batch Test No. 8 is calculated and is shown in Table 5.2 as a concentration and percentage of the total wastewater COD: As reported by Mbewe *et al.* (1995), Wentzel *et al.* (1995), Ubisi *et al.* (1997a,b) and Cronje *et al.* (2000) for Mitchell's Plain wastewater, OHO active biomass was found to be present at low concentrations, 4.6% of total COD for the example here.

Table 5.2: Batch Test No. 8: COD recovery, regression data from ℓn (OUR_H) versus time plot and OHO active biomass at the start of the batch test [$Z_{BH(0)}$].

BATCH TEST : FLOCCULATED-FILTERED WASTEWATER (WW) AND MIXED LIQUOR (ML)										
Sew. Batch No.	Batch test No.	Batch test date	Volume (ℓ)		COD Recov. (%)	Regression			$Z_{BH(0)}$	
			WW	ML		Y-int.	Slope	R^2	Conc. (mgCOD/ℓ)	Fraction of Total COD (%)
18	8	05-03	2.85	0.15	93.5	0.7980	0.242	0.990	16.27	4.57

5.3.2 Theoretical OHO active biomass concentration in the parent system

To evaluate the measured OHO active biomass concentrations determined in the batch tests, these will be compared with the theoretical values for the parent system from which the mixed liquor is drawn. Ubisi *et al.* (1997a,b) and Cronje *et al.* (2000) found that the steady state theory (WRC, 1984) and the kinetic simulation models (Dold *et al.*, 1991) for anoxic/aerobic activated sludge systems gave theoretical OHO active biomass concentrations that were in close agreement. Since the steady state theory is simpler and provides a direct analytical solution, this will be used to calculate the theoretical OHO active biomass concentration in the parent system, and added to the batch test. This calculation procedure is summarized below.

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

$$f_{av} = MX_{BH}/MX_V$$
$$= MX_{BH}/(MX_{BH} + MX_E + MX_I + MX_{BA}) \quad (5.20)$$

where

MX_V = mass of volatile suspended solids = $V \cdot X_V$, VSS units (mg VSS)
MX_{BH} = mass of OHO 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 AO active biomass = $V \cdot X_{BA}$, VSS units (mgVSS)
V = system volume (ℓ)
X_{BH} = OHO 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. (5.20), for activated sludge systems receiving "normal" municipal wastewaters (influent TKN/COD ratio < 0.12 mgN/mgCOD) the AO 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 AO active biomass can be neglected when calculating the mixed liquor VSS. Accordingly, from WRC (1984), substituting in Eq. (5.20) for $MX_{BA} = 0$ and $MX_V = (MX_{BH} + MX_E + MX_I)$:

$$\frac{1}{f_{av}} = 1 + f_E^* b_{HT}^* R_S + \frac{f_{S,up}(1 + b_{HT}^* R_S)}{f_{cv} Y_H^* (1 - f_{S,us} - f_{S,up})} \qquad (5.21)$$

where

f_E^* = fraction of OHO 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^* = OHO active biomass yield, VSS units (mgVSS/mgCOD)
= 0.45 mgVSS/mgCOD (WRC, 1984)

Accepting each sewage batch as a steady state period, values for $f_{S,us}$ and $f_{S,up}$ have been determined for both the *control* (Table 3.4) and *experimental* (Table 4.4) parent systems. Also, f_{cv} values were measured and averaged for each sewage batch (Tables 3.4 and 4.4). Hence, values for f_{av} for each sewage batch could be calculated using Eq. (5.21). For the example Sewage Batch No. 18, for the *experimental* parent system $f_{S,us} = 0.047$, $f_{S,up} = 0.198$, $f_{cv} = 1.29$ and T = 26°C (Table 4.4). Substituting these values in Eq. (5.21) gives $f_{av} = 0.3027$.

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 steady state VSS concentration, measured in the parent system and averaged for each sewage batch; Tables 3.3(b) and 4.3(b)], the *theoretical* OHO active biomass concentration in the batch reactor due to the added mixed liquor [$X_{BH}(theor)_{BT}$, COD units] is given by:

$$Z_{BH}(theor)_{BT} = [X_V(PS) \cdot f_{av} \cdot f_{cv} \cdot V_{ML}] / (V_{ML}+V_{WW}) \quad (5.22)$$

where

$Z_{BH}(theor)_{BT}$ = theoretical OHO active biomass concentration in batch test reactor due to added mixed liquor, COD units (mgCOD/ℓ batch reactor)
$X_V(PS)$ = mixed liquor VSS concentration measured in parent system, from Tables 3.3(b) and 4.3(b) (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 No. 8 the *theoretical* OHO active biomass concentration in the batch reactor due to the addition of the mixed liquor sample drawn from the parent system was calculated as follows:

The average VSS concentration for the *experimental* parent system during Sewage Batch No. 18 is $X_V(PS)$ = 3103 mgVSS/ℓ [Table 4.3(b)]. For Sewage Batch No. 18, f_{av} = 0.3027 from above; f_{cv} for this sewage batch = 1.29 mgCOD/mgVSS and the volumes used in Batch Test No. 8, V_{ML} = 0.15ℓ ; V_{WW} = 2.85ℓ. Substituting these values into Eq. (5.22):

$$Z_{BH}(theor)_{BT} = \frac{(3103 \cdot 0.3027 \cdot 1.29 \cdot 0.15)}{(0.15 + 2.85)} = 61 \text{ mgCOD}/\ell$$

Note that in Eq. (5.22) the parent system mixed liquor organic suspended solids are expressed in VSS units [$X_V(PS)$], whereas the OHO 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 model 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 available from direct measurements.

5.4 BATCH TEST DATA ANALYSIS

5.4.1 Nitrate concentration-time profiles

As noted above, in the batch tests with wastewater and mixed liquor conducted by Ubisi *et al.* (1997a,b), they observed that nitrification in these batch tests caused a linear increase in the nitrate concentration with time. Cronje *et al.* (2000) evaluated this batch test method and concluded that the linearity of the nitrate concentration-time profiles proved to be unduly simplified. In agreement with the observations of Antoniou *et al.* (1990) and Sözen *et al.* (1996), Cronje *et al.* (2000) observed that the generation of nitrate in the batch reactor was better represented by an exponential increase rather than a linear increase with time. This observation was to be evaluated in this investigation.

During the course of this experimental investigation, as noted earlier it was observed that apart from the generation of nitrate with time, a detectable amount of nitrite was also generated in all the batch tests. Thus, increases in both the nitrate and nitrite concentrations had to be taken into account when analyzing the results of the batch tests. In addition, it was observed that the nitrate/nitrite concentrations could be represented by either a linear or an exponential increase. To select the best type of fit for a particular batch test, this was done by visually checking which of the linear or exponential lines best fitted the data, and confirming the best-fit line by doing a regression analysis and noting the correlation coefficient. A reasonable correlation coefficient ($R^2 > 0.90$) implies that the selected best-fit line gives a good approximation of the experimental data. For the various batch tests, both linear and exponential fits were used. Thus, selecting the type of fit is not general, but must be based on the data for a particular batch test.

5.4.2 Batch test calculation procedure

The derivation of the equations to calculate OHO active biomass in the batch test has been detailed in Section 5.3.1 above; the procedure in applying these equations is summarized here. As an example, the measured OUR_M (mgO/ℓ/h, Fig. 5.2) and observed nitrate/nitrite concentration (mgN/ℓ, Fig. 5.3) versus time responses for Batch Test No. 8 conducted on 05th March 2001 (Sewage Batch No. 18) with a mixture of *flocculated-filtered* wastewater (2.85ℓ) and mixed liquor (0.15ℓ) are used here to briefly explain the calculation procedures.

- From the measured nitrate/nitrite concentration – time profile (Fig. 5.3 for this example), the most appropriate linear or exponential expression was obtained through regression analysis. For the example here, this was *linear*:

$$[NO_3]_t = 0.1766\,t + 0.2954 \qquad (mgN/\ell) \qquad (5.23)$$

$$[NO_2]_t = 0.0930\,t + 0.1209 \qquad (mgN/\ell) \qquad (5.24)$$

  From Fig. 5.3 it is evident that *linear* expressions [Eqs. (5.23 and 5.24)] best represented the experimental nitrate/nitrite concentration data with time, which is

reflected by the good correlation coefficients, R^2 = 0.9750 for the nitrate concentration – time data and R^2 = 0.9880 for the nitrite concentration – time data. It should be noted that in other batch tests, the *exponential* fit was superior.

- At each recorded point in time (Column 1, Table 5.3) the nitrate/nitrite concentrations (Columns 2 and 3, Table 5.3) were calculated, using Eqs. (5.23) and (5.24) respectively.

- The OUR_{NO3} at each point in time (Column 4) was calculated as being 4.57 • nitrification rate, Eq. (5.9): To obtain the nitrification rate (due to NO_3) at each point in time, for the linear fit to the nitrate-time data, this is simply the slope of the fitted line which is constant. Where an exponential fit was used, the exponential fit equation was differentiated which gives the general equation for the slope (i.e. nitrification rate) at any time. In this case, at each time (Column 1) the time was inserted in the differential equation and the slope at that time (i.e. nitrification rate) determined. Similarly, the OUR_{NO2} at each point in time (Column 5) was calculated as being 3.43 • nitrification rate, Eq. (5.8): To obtain the nitrification rate (due to NO_2) at each point in time, the procedures for NO_3 above were followed, but with the nitrite-time profile.

- At each time, subtracting the calculated OUR_{NO3} and OUR_{NO2} from the OUR measured in the batch test (OUR_M) gives an estimate of the OUR due to the OHO active biomass only (OUR_H), Eq. (5.2). In Table 5.3: Column 7 = Column 6 - Column 5 - Column 4.

- To determine the OHO active biomass at the start of the batch test ($Z_{BH(0)}$), a plot of $\ell n(OUR_H)$ versus time needs to be constructed up to the precipitous drop in OUR; the $\ell n(OUR_H)$ versus time plot is shown in Fig. 5.5. Taking the ℓn of the values in Column 7 gives Column 8. A linear regression performed on the $\ell n(OUR_H)$ versus time plot gives the y-intercept and slope required to determine the OHO active biomass at the start of the batch test, via Eq. (5.19).

Table 5.3: Determination of the OUR due to OHO active biomass (OUR_H) from a constant OUR due to NO_3 and NO_2. Key parameters calculated for Batch Test No. 8, 05-03, conducted with a 0.15ℓ mixed liquor sample.

1	Approximate		4	5	6	7	8
Time (h)	2 NO_3 conc. (mgN/ℓ)	3 NO_2 conc. (mgN/ℓ)	OUR_{NO3} (mgO/ℓ/h)	OUR_{NO2} (mgO/ℓ/h)	ML + WW OUR_M (mgO/ℓ/h)	ML + WW OUR_H (mgO/ℓ/h)	ℓn OUR_H (mgO/ℓ/h)
0.08	0.31	0.13	0.81	0.32	4.20		
0.49	0.38	0.17	0.81	0.32	3.78	2.65	0.98
0.98	0.47	0.21	0.81	0.32	4.04	2.91	1.07
1.43	0.55	0.25	0.81	0.32	4.43	3.30	1.20
1.83	0.62	0.29	0.81	0.32	4.78	3.65	1.30
2.20	0.68	0.33	0.81	0.32	4.94	3.81	1.34
2.58	0.75	0.36	0.81	0.32	5.07	3.94	1.37
2.95	0.82	0.39	0.81	0.32	5.20	4.07	1.40
3.30	0.88	0.43	0.81	0.32	5.83	4.70	1.55
3.66	0.94	0.46	0.81	0.32	6.26	5.13	1.64

For the example, in Fig. 5.2 the measured OUR response, OUR_M (listed in Table 5.3, Column 6) is shown plotted with the OUR_{NO3} and OUR_{NO2} response calculated from Eqs. (5.9) and (5.8) respectively (listed in Table 5.3, Columns 4 and 5 respectively). The OUR_H response, calculated from Eq. (5.2) is listed in Table 5.3 (Column 7) and is shown plotted in Fig. 5.4.

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 (%), via Eq. (5.11).
- OHO active biomass at the start of the batch test ($Z_{BH(0)}$), via Eq. (5.19).

A detailed derivation of equations for these two parameters are given in Section 5.3.1 above, and, accordingly, are not repeated here.

COD recovery

The acceptability of the data from the batch test can be evaluated by doing a COD mass balance, as defined by Eq. (5.11).

- %COD recoveries ranging from 90 – 100 % provide support for the reliability of the measurements. For the example here, %COD recovery = 93.5%, see Section 5.3.1.2 above.

Determining the OHO active biomass concentration

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

From the y-intercepts and slopes, the OHO active biomass concentration at the start of each batch test was calculated using Eq. (5.19). This was compared to the theoretical concentration in the batch test, calculated using the procedures in Section 5.3.2 above. Additionally, the *projected* OHO active biomass concentrations in the *parent system* were calculated using Eq. (5.25) and the values are listed in Table 5.7 (Z_{BH} - Measured ML).

$$Z_{BH(ML)} = Z_{BH(0)BT} \cdot (V_{ML} + V_{WW}) / V_{ML} \quad (mgCOD/\ell) \qquad (5.25)$$

where

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

5.5 RESULTS FOR THE MLE *CONTROL* ACTIVATED SLUDGE SYSTEM

The *control* parent laboratory-scale activated sludge system and its operation have been described in detail in Chapter 3. The aspects of importance here are: The parent laboratory-scale nitrification/denitrification system was operated at 10 days sludge age and was continually monitored; the sewage was fed to the parent system in batches which lasted for approximately 2 weeks and each sewage batch constituted a steady state period. A total number of 18 batch tests were conducted using mixed liquor drawn from the *MLE control* activated sludge system. The sewage batches during which batch tests were conducted on the *control* system are given in Table 5.4.

Table 5.4: The sewage batch number and the dates it was used as feed for the parent *control* activated sludge system together with the number of batch tests with *flocculated-filtered* wastewater and mixed liquor conducted during each sewage batch.

Sewage Batch No.	Dates (2001)	Number of batch tests
18	16 Feb – 11 Mar	4
19	12 Mar – 23 Mar	7
20	24 Mar – 08 Apr	7
TOTAL		18

5.5.1 Parent system data

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 OHO active biomass concentration would exhibit a corresponding fluctuation between different sewage batches. To formulate a theoretical estimate for the OHO active biomass concentration present in the parent system during each sewage batch period, some crucial parameters were monitored on a daily basis (see Chapter 3). Detailed data on the parent system are given in Appendix B, Tables B3 and B4. For each sewage batch tested, the daily results have been averaged and the average values are listed in Table 5.5 below.

Table 5.5: Steady state results for parent *control* laboratory-scale anoxic/aerobic activated sludge system receiving sewage batches during which batch tests were performed. Averages are listed with sample standard deviations in brackets.

CONTROL 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)		
		Inf	Eff	Inf	Eff	Anoxic	Aerobic	Eff		VSS	COD	TKN
18	8	655 (56)	36 (13)	63 (3)	8.1 (0.7)	1.6 (0.4)	9.2 (1.1)	8.6 (0.9)	37.5 (1.3)	2409 (138)	3119 (282)	208 (14)
19	8	728 (35)	52 (9)	85 (4)	6.2 (1.8)	4.8 (1.9)	17.3 (1.5)	15.2 (1.2)	41.6 (0.9)	3042 (80)	4073 (243)	243 (14)
20	8	741 (52)	65 (13)	70 (4)	5.1 (0.9)	5.4 (3.5)	14.6 (5.2)	12.6 (3.0)	40.9 (2.2)	3760 (106)	3936 (148)	239 (7)

Using the average values in Table 5.5, 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 3 and the results for each sewage batch are listed in Table 5.6.

- The OHO 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. (5.21)].

It should be noted that during Sewage Batch No. 18, the laboratory air-conditioner failed causing the temperature to increase above 20°C. This increase in temperature was taken into account by using the average recorded temperature to appropriately adjust the temperature dependent constants, namely b_{HT}*, in Eq. (5.21) and in the equation for $f_{S,up}$ determination (Chapter 3).

Table 5.6: Steady state COD and N mass balances, wastewater fractions and mixed liquor parameters for parent *control* laboratory-scale anoxic/aerobic activated sludge system. Data calculated from data Table 5.5 using the steady state (SS) design model (WRC, 1984).

CONTROL PARENT ANOXIC/AEROBIC STEADY STATE SYSTEM								
WW Batch	No. of tests	Mass Balance (%)		Wastewater fractions		Mixed liquor		
		COD	N	Unbio. 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})
18	8	98	93	0.043	0.171	1.31	0.087	0.3323
19	8	99	94	0.066	0.249	1.35	0.080	0.2659
20	8	100	96	0.081	0.198	1.44	0.087	0.3407

For all sewage batches when the batch tests were conducted, the COD and N mass balances fell within the acceptable range. This lends credibility to the reliability of the parent system steady state data during these periods.

Examination of the $f_{S,up}$ and f_{av} values calculated for Sewage Batch No. 19 shows that these are significantly different from the corresponding values for Sewage Batches No. 18 and 20. A higher unbiodegradable particulate COD fraction and a corresponding lower mixed liquor active fraction were obtained for Sewage Batch No. 19; $f_{S,up} = 0.249$ and $f_{av} = 0.2659$ compared to the average $f_{S,up} = 0.185$ and $f_{av} = 0.3365$ calculated for the two other sewage batches (the high $f_{S,up}$ of the wastewater is inversely related to the low f_{av} value).

The unusually high unbiodegradable particulate COD fraction ($f_{S,up}$) and low mixed liquor active fraction (f_{av}) recorded for Sewage Batch No. 19 would suggest that the

parent system's behavioural response during this period deviated significantly from the steady state behaviour in the other two periods. In seeking an explanation for the deviation, the following were noted:

- Just prior to Sewage Batch No. 19, a strong dose of aluminium sulphate was added to the parent system and thereafter a lower dose was continued throughout the experimental investigation to control bulking (see Chapter 3, Section 3.2). This possibly caused some changes in the parent system. Although, it is known that aluminium sulphate causes an increase in Total Suspended Solids (TSS) concentration only, by increasing the Inorganic Suspended Solids (ISS), and seemingly does not have any effect on the Volatile Suspended Solids (VSS) concentration in an activated sludge system, it is clear from Table 5.5 that the VSS increased significantly from Sewage Batch No. 18 to 19.

- A similar behaviour was observed in the *experimental* parent system during Sewage Batch No. 19, see Section 5.6 below.

- For both the *control* and *experimental* systems, the COD and N mass balances for Sewage Batch No. 19 fell within the acceptable range. If the increased VSS was due to aluminium sulphate dosing, this would cause the COD mass balance to increase significantly.

Thus, the increased VSS concentration observed for Sewage Batch No. 19 appeared to be related to the characteristics of this sewage batch. This is taken into account automatically in the batch test analysis procedures. This was further substantiated by the observation that batch tests conducted during this period gave correlations of measured OHO active biomass to theoretical values that were similar to those conducted during other sewage batches, see below.

5.5.2 Batch test data

For the batch tests, measured OUR, NO_3 and NO_2 – time data are shown graphically in Appendix A. Following the procedures set out in Section 5.3.1.3 above, the OHO active biomass concentrations measured at the start of each batch test were calculated and are listed in Table A1, Appendix A. The results are summarized in Table 5.7. As noted above, during Sewage Batch No. 18, the laboratory air conditioning unit failed causing the temperature to increase above 20°C. When the batch tests were conducted during this period, the temperature was recorded, and temperature dependent constants in the equations to calculate OHO active biomass concentration at the start of the batch tests appropriately adjusted, i.e. b_{HT} in Eq. (5.19).

The R^2 values obtained by linear regression of the $\ell n(OUR_H)$ – time data in the batch tests are listed in Table A1, Appendix A. The R^2 values obtained for the batch tests were reasonable with a mean R^2 value of 0.90 and a sample standard deviation of 0.11.

The %COD recoveries for all the batch tests were calculated using Eq. (5.11) and the results are listed in Table A1, Appendix A. The %COD recovery for each batch test is summarized in Table 5.7 below.

- In general, good %COD recoveries were achieved, with only two batch tests (No. 3 and 7) yielding %COD recoveries < 90 %. It would seem the results from these batch tests should be rejected for further analysis. However, statistical analysis indicated that these poor COD mass balances may have arisen from random effects and accordingly these batch tests data were not rejected for further analysis, but will be marked: 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 97.8 % with sample standard deviation of 6.9 %. The good %COD recoveries lend credibility to the reliability of the measurements and the batch test procedure.

Table 5.7: Results for batch tests with a mixture of *flocculated-filtered* wastewater (WW) and mixed liquor (ML): Batch test numbers, dates of batch tests, volumes added, COD recoveries, *measured* OHO active biomass present the start of the batch test ($Z_{BH(0)}$) and the *projected* parent system mixed liquor (ML) active biomass. The *theoretical* parent system mixed liquor (ML) OHO active biomass and the *projected* active biomass present the start of the batch test are also given.

Sew. Batch No.	Batch Test No.	Date of Test	Volume (ℓ)		COD recov. (%)	$Z_{BH(0)}$ (mgCOD/ℓ)			
						Measured		Theoretical	
			ML	WW		Batch Test	ML	ML	Batch Test
18	1	28-02	0.25	2.75	104.3	35	415	1036	86
	3	02-03	0.30	2.70	86.7*	7	71	1036	104
	5	04-03	0.20	2.80	102.4	27	400	1036	69
	7	05-03	0.15	2.85	84.1*	23	453	1036	52
19	9	12-03	0.40	2.60	109.6	120	900	1083	144
	11	13-03	0.35	2.65	105.0	92	790	1083	126
	13	14-03	0.30	2.70	106.3	62	619	1083	108
	15	15-03	0.25	2.75	100.6	65	782	1083	90
	17	16-03	0.20	2.80	102.9	57	704	1083	72
	19	17-03	0.15	2.85	90.7	33	663	1083	54
	21	18-03	0.10	2.90	92.7	9	264	1083	36
20	23	28-03	0.10	2.90	91.7	49	1480	1341	45
	25	30-03	0.15	2.85	98.0	29	576	1341	67
	27	31-03	0.20	2.80	92.4	62	936	1341	89
	29	02-04	0.25	2.75	92.5	138	1651	1341	112
	31	03-04	0.30	2.70	101.5	165	1650	1341	134
	33	04-04	0.35	2.65	99.3	147	1259	1341	156
	35	05-04	0.40	2.60	100.4	166	1247	1341	179

* Poor COD mass balance

5.5.3 Comparison between measured and theoretical active biomass for the *MLE control* activated sludge system

In Table 5.7 the measured OHO active biomass concentration at the start of each batch test is compared with the theoretical OHO active biomass concentration at the start of the batch test due to the mixed liquor sample drawn from the parent system and added to the batch test; theoretical values predicted via the steady state design model. To illustrate the comparison, the measured versus theoretical mixed liquor OHO active biomass data for all the batch tests are shown plotted in Fig. 5.7. In Fig. 5.7 the comparative data for the batch test results that should be rejected on the basis of poor %COD recovery (Batch Tests No. 3 and 7) are also shown, but are appropriately marked.

The comparisons for batch tests conducted during Sewage Batch No. 18 show poor agreement between the measured and theoretical results. As mentioned in Chapter 3 Section 3.9.4, when the parent laboratory-scale system was receiving Sewage Batch No. 18, the laboratory's air-conditioning system failed and resulted in higher ambient temperatures (22°C to 30°C) than the controlled 20°C. Although it was attempted to take this temperature effect into account, this was done by assuming a constant temperature for the entire sewage batch and using this temperature to adjust the temperature dependent constants in Eq. (5.21) to calculate the theoretical OHO active biomass concentration. In practice, the temperature varied daily so that mixed liquor drawn from the parent system for a particular batch test may have been at a temperature that deviated from the sewage batch average, and hence the theoretical OHO active biomass concentration would be different from that calculated with the sewage batch average temperature. However, the effect is not large, as the correlation between theoretical and measured OHO active biomass concentrations for Sewage Batch No. 18 is similar to that for Sewage Batch No. 19 when the constant temperature was re-established in the laboratory.

Comparing the measured and the theoretical OHO active biomass concentrations for Sewage Batch No. 19, it would appear that there is close correspondance between theoretical and measured OHO active biomass concentrations; the "serial dilutions" of mixed liquor give an almost linear decrease in OHO active biomass concentration. However, the values plot virtually parallel to the 45° line (i.e. 1:1 correspondance). This implies that there is a constant (i.e. independent of volume of mixed liquor added) difference between the measured and theoretical values – when the measured OHO active biomass concentration is zero, the theoretical OHO active biomass concentration in the batch test is approximately 25 mgCOD/ℓ. No explanation for this deviation was apparent.

For Sewage Batch No. 20, the data is mixed with some batch tests falling close to the 1:1 correspondance line, and some data similar to that for Sewage Batch No. 19 above.

Although some correlation does exist between the theoretical and measured OHO active biomass concentrations (Fig. 5.7) for the range of mixed liquor volumes used in the batch tests, individual data points tend to exhibit some variation from the appropriate correlation line. This variation can be attributed to the sensitivity of the measured OHO active biomass concentration, calculated via Eq. (5.19), to the slope of the ℓn (OUR_H) –

time plot (Fig. 5.5). Even the smallest change in the slope (magnitude ~ 0.05) can result in marked variations in the OHO active biomass concentration values. This would suggest that a number of batch tests need to be conducted to establish a reasonable estimate for OHO active biomass concentration.

To further examine the batch test results, a statistical plot of the OHO active biomass concentration measured in the batch tests, but *projected* to the concentration in the parent system was constructed; see Fig. 5.8. The projected values were calculated from the measured values obtained in all batch tests, using Eq. (5.25). The projected values are listed in Table 5.7. The statistical plot (Fig. 5.8) represents the results obtained in all batch tests, including the rejected results from Batch Tests No. 3 and 7.

- From the statistical plot, the *projected* values appear to be normally distributed with a *mean* projected Z_{BH} of 826 mgCOD/ℓ and a sample standard deviation of 453 mgCOD/ℓ.

- The large sample standard deviation value of 453 mgCOD/ℓ underlines the marked variation associated with the sensitivity of the batch test analysis (described above).

- The weighted average (taking into account the number of batch tests conducted in each of the three sewage batches) for the theoretical OHO active biomass concentration in the parent system was 1173 mgCOD/ℓ.

- Comparing the measured and theoretical values, it is apparent that these differ significantly, but the standard deviation for the measured values cover the theoretical value.

From the comparisons above, clearly, the batch test method requires refinement to make it more repeatable and precise.

5.6 RESULTS FOR THE MLE *EXPERIMENTAL* ACTIVATED SLUDGE SYSTEM

The *experimental* parent laboratory-scale system and its operation have been described in detail in Chapter 4. The aspects of importance here are: The *experimental* and the *control* parent laboratory-scale nitrification/denitrification activated sludge system were identical in operation, except that the *experimental* system had a reduced OHO active biomass fraction of the mixed liquor: To achieve the desired OHO active biomass fraction of the mixed liquor for the *experimental* system, a known concentration of macerated toilet paper solution was dosed to this system (see Chapter 4). A total number of 18 batch tests were conducted using mixed liquor drawn from the *MLE experimental* activated sludge system and were done in parallel to the batch tests on the *control* system. The sewage batches during which batch tests were conducted on the *experimental* system are given in Table 5.8.

Table 5.8: The sewage batch number and the dates it was used as feed for the parent *experimental* activated sludge system together with the number of batch tests with *flocculated-filtered* wastewater and mixed liquor conducted during each sewage batch.

Sewage Batch No.	Dates (2001)	Number of batch tests
18	16 Feb – 11 Mar	4
19	12 Mar – 23 Mar	7
20	24 Mar – 08 Apr	7
TOTAL		18

5.6.1 Parent system data

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 OHO active biomass concentration would exhibit a corresponding fluctuation between different sewage batches. To formulate a theoretical estimate for the OHO active biomass concentration present in the parent system during each sewage batch period, some crucial parameters were monitored on a daily basis (see Chapter 4). Detailed data on the parent *experimental* 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 5.9 below.

Table 5.9: Steady state results for *experimental* parent laboratory-scale anoxic/aerobic activated sludge system receiving sewage batches during which batch tests were performed. Averages are listed with sample standard deviations in brackets.

EXPERIMENTAL 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)		
		Inf	Eff	Inf	Eff	Anoxic	Aerobic	Eff		VSS	COD	TKN
18	8	799 (58)	66 (24)	63 (3)	9.2 (1.7)	0.7 (0.1)	7.7 (1.6)	8.0 (0.7)	41.8 (2.2)	3103 (178)	4011 (235)	227 (12)
19	8	807 (32)	66 (12)	83 (5)	7.8 (0.8)	2.3 (1.1)	15.8 (1.5)	14.8 (1.5)	49.0 (4.1)	3482 (72)	4720 (251)	266 (14)
20	8	904 (22)	81 (18)	71 (2)	6.6 (1.3)	3.2 (1.0)	11.3 (2.4)	15.5 (2.3)	42.6 (1.0)	3359 (92)	4778 (271)	247 (12)

Using the average values in Table 5.9, 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 3 and the results for each sewage batch are listed in Table 5.10.

- The OHO 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. (5.21)].

Again, it should be noted that during Sewage Batch No. 18, the laboratory air-conditioner failed causing the temperature to increase above 20°C. This increase in temperature was taken into account by using the average recorded temperature to appropriately adjust the temperature dependent constants, namely b_{HT}*, in Eq. (5.21) and in the equation for $f_{S,up}$ determination (Chapter 3).

Table 5.10: Steady state COD and N mass balances, wastewater fractions and mixed liquor parameters for parent *experimental* laboratory-scale anoxic/aerobic activated sludge system. Data calculated from data Table 5.9 using the steady state (SS) design model (WRC, 1984).

EXPERIMENTAL PARENT ANOXIC/AEROBIC STEADY STATE SYSTEM											
WW Batch	No. of tests	Mass Balances (%)		WW + toilet paper fractions		Toilet paper fractions only		Mixed liquor			
		COD	N	$f_{S,us}$	$f_{S,up}$	$f_{S,us}$	$f_{S,up}$	COD/VSS ratio (mgN/mg VSS) (f_{cv})	TKN/VSS ratio (mgN/mg VSS) (f_n)	Active Frac. (f_{av}) of WW + TP	Active Frac (f_{av}) of toilet paper only
18	8	99	94	0.047	0.198	0.064	0.325	1.29	0.073	0.3027	0.1964
19	8	107	107	0.076	0.275	0.169	0.529	1.36	0.076	0.2443	0.0823
20	8	90	110	0.076	0.194	0.058	0.185	1.42	0.074	0.3453	0.3603

For all sewage batches when the batch tests were conducted, the COD and N mass balances fell within the acceptable range. This lends credibility to the reliability of the parent system steady state data during these periods.

As for the *control* parent system, during Sewage Batch No. 18 the temperature in the laboratory increased above 20°C; this was taken into account as detailed for the *control* system above.

From Table 5.10 above, the $f_{S,up}$ and f_{av} values for wastewater plus toilet paper calculated for Sewage Batch No. 19 differ from the $f_{S,up}$ and f_{av} values for Sewage Batches No. 18 and 20. A higher unbiodegradable particulate COD fraction and a corresponding lower mixed liquor active fraction of wastewater plus toilet paper was obtained for sewage batch 19; $f_{S,up}$ = 0.275 and f_{av} = 0.2443 compared to the average $f_{S,up}$ = 0.196 and f_{av} = 0.3240 calculated for the two other sewage batches (the high $f_{S,up}$ of the wastewater is inversely related to the low f_{av} value). A similarly high $f_{S,up}$ and low f_{av} were noted for the

control parent system for the same wastewater batch. From an examination of the data it was concluded that this behaviour was related to the wastewater characteristics for this sewage batch, which are taken into account in the calculation procedure.

5.6.2 Batch test data

For the batch tests, measured OUR, NO_3 and NO_2 – time data are shown graphically in Appendix A. Following the procedures set out in Section 5.3.1.3 above, the OHO active biomass concentrations measured at the start of each batch test were calculated and are listed in Table A2, Appendix A. The OHO active biomass values measured at the start of each batch test are listed in Table A2, Appendix A. As for the *control* system, the increase in temperature during Sewage Batch No. 18 was taken into account in the calculation procedure. The results are summarized in Table 5.11.

The R^2 values obtained by linear regression of the OUR_H – time data in all batch tests are listed in Table A2, Appendix A. The R^2 values obtained for the batch tests were reasonable with a mean R^2 value of 0.90 and a sample standard deviation of 0.06.

The %COD recoveries for all the batch tests were calculated using Eq. (5.11) and the results are listed in Table A2, Appendix A. The %COD recovery for each batch test is summarized in Table 5.11 below.

- In general, good %COD recoveries were achieved with only one batch test (No. 32) yielding %COD recovery < 90 %. The %COD recovery for Batch Test No. 32 was marginally < 90 % (89.0 %); however, statistical analysis indicated that this COD mass balance may have arisen from random effects and accordingly this batch test data was not rejected for further analysis. A statistical plot of the %COD recovery for all the modified batch tests was constructed, see Fig. 5.9. From Fig. 5.9 the mean %COD recovery was 95.9 % with sample standard deviation of 5.2 %. The good %COD recoveries lend credibility to the reliability of the measurements and the batch test procedure.

Table 5.11: Results for batch tests with a mixture of *flocculated-filtered* wastewater (WW) and mixed liquor (ML): Batch test numbers, dates of batch tests, volumes added, COD recoveries, *measured* OHO active biomass present the start of the batch test ($Z_{BH(0)}$) and the *projected* parent system mixed liquor (ML) active biomass. The *theoretical* parent system mixed liquor (ML) OHO active biomass and the *projected* active biomass present the start of the batch test are also given.

Sew. Batch No.	Batch Test No.	Date of Test	Volume (ℓ)		COD recov. (%)	$Z_{BH(0)}$ (mgCOD/ℓ)			
						Measured		Theoretical	
			ML	WW		Batch Test	ML	ML	Batch Test
18	2	28-02	0.25	2.75	105.4	27	322	1214	101
	4	02-03	0.30	2.70	90.7	44	444	1214	121
	6	04-03	0.20	2.80	106.0	36	545	1214	81
	8	05-03	0.15	2.85	93.5	16	325	1214	61
19	10	12-03	0.40	2.60	98.4	158	1183	1153	154
	12	13-03	0.35	2.65	106.2	100	856	1153	134
	14	14-03	0.30	2.70	91.0	77	769	1153	115
	16	15-03	0.25	2.75	91.1	64	770	1153	96
	18	16-03	0.20	2.80	97.9	36	541	1153	77
	20	17-03	0.15	2.85	99.3	27	532	1153	58
	22	18-03	0.10	2.90	95.9	15	463	1153	38
20	24	28-03	0.10	2.90	92.2	16	480	1650	55
	26	30-03	0.15	2.85	93.3	38	765	1650	82
	28	31-03	0.20	2.80	94.3	99	1480	1650	110
	30	02-04	0.25	2.75	93.5	81	976	1650	137
	32	03-04	0.30	2.70	89.0*	136	1360	1650	165
	34	04-04	0.35	2.65	96.8	53	455	1650	192
	36	05-04	0.40	2.60	92.0	326	2448	1650	220

* Poor COD mass balance

5.6.3 Comparison between measured and theoretical active biomass for the *MLE experimental* activated sludge system

In Table 5.11 the measured OHO active biomass concentration at the start of each batch test is compared with the theoretical OHO active biomass concentration at the start of the batch test due to the mixed liquor sample drawn from the parent system and added to the batch test; theoretical values predicted via the steady state design model. To illustrate the comparison, the measured versus theoretical mixed liquor OHO active biomass data for all the batch tests are shown plotted in Fig. 5.10.

As noted above for the *control* system, the comparisons for batch tests conducted during Sewage Batch No. 18 show poor agreement between the measured and theoretical results. Again, as the *control* system, more than likely this was related to the temperature effect, see Section 5.6.1 above.

Comparing the measured and the theoretical OHO active biomass concentrations for Sewage Batches No. 19 and 20, the correlations show remarkable similarity to those obtained for the *control* system with Sewage Batch No. 19 – there is a close correlation but the values plot parallel to the 45° line. Again, this implies that there is a constant difference between measured and theoretical OHO active biomass concentrations; as for the *control* system, this difference is approximately 25 mgCOD/ℓ.

Although a correlation does exist between the theoretical and measured OHO active biomass concentrations (Fig. 5.7) for the range of mixed liquor volumes used in the batch tests, individual data points tend to exhibit some variation from the appropriate correlation line. This variation can be attributed to the sensitivity of the measured OHO active biomass concentration (as explained in Section 5.5.3 above).

To further examine the batch test results, a statistical plot of the OHO active biomass concentration measured in the batch tests, but *projected* to the concentration in the parent system was constructed; see Fig. 5.11. The *projected* values were calculated from the measured values obtained in all batch tests, using Eq. (5.25). The projected values are listed in Table 5.11. The statistical plot (Fig. 5.11) represents the results obtained in all batch tests.

- From the statistical plot the *projected* values appear to be normally distributed with a *mean* projected Z_{BH} of 817 mgCOD/ℓ and a sample standard deviation of 516 mgCOD/ℓ.

- One of the data points was rejected as an outlier (Z_{BH} = 2448 mgCOD/ℓ) and the remaining values were replotted in Fig. 5.12. From Fig. 5.12, the *mean* projected Z_{BH} is 722 mgCOD/ℓ and the sample standard deviation is 341 mgCOD/ℓ.

- The large sample standard deviation value of 341 mgCOD/ℓ underlines the marked variation associated with the sensitivity of the batch test analysis.

- The weighted average (taking into account the number of batch tests conducted in each of the three sewage batches) for the theoretical OHO active biomass concentration in the parent system was 1360 mgCOD/ℓ.

- Comparing the measured and theoretical values, it is apparent that these differ significantly.

5.7 COMPARISON BETWEEN OHO ACTIVE BIOMASS BETWEEN THE *CONTROL* AND *EXPERIMENTAL* SYSTEMS

As described in detail in Chapters 3 and 4, two primary objectives for this investigation were identified, *viz*:

(1) Evaluate the reliability of the modified batch test method, by comparing the measured OHO active biomass concentrations with the theoretical values predicted by the steady state design model.

(2) Evaluate the ability of the modified batch test procedure to detect a decreased OHO active biomass fraction of the mixed liquor. To reduce the OHO active biomass fraction of the mixed liquor, a known concentration of macerated toilet paper solution was dosed to the *experimental* system. At the outset of the investigation, it was envisaged that the toilet paper was largely unbiodegradable in the activated sludge system. Accordingly, toilet paper should contribute significantly to the inert sludge mass in the activated sludge system, thereby significantly increasing the MLOSS concentration in the system, and reducing the OHO active biomass fraction of the MLOSS. One of the specific tasks highlighted to address objective (2) was to determine the biodegradability of the toilet paper, and hence its effect on the components making up the MLOSS, to derive a theoretical value of OHO active biomass.

In Chapter 4, it was concluded that the unbiodegradable particulate fraction of toilet paper was about 31% ($f_{S,up}$ = 0.309). Accordingly, the initial thought that toilet paper would contribute a high unbiodegradable particulate fraction ($f_{S,up}$) to the *experimental* activated sludge system was not true: This implies that toilet paper did not cause a large increase in inert material in the *experimental* activated sludge system, thus the OHO active biomass fraction of the mixed liquor would not be expected to decrease significantly.

The measured versus theoretical OHO active biomass (Z_{BH}) values for Sewage Batches No. 18, 19 and 20 for both the *control* and the *experimental* systems are plotted in Fig. 5.13. Excluding the outliers, it is apparent that the data for the *control* and *experimental* systems are remarkably similar: Both data sets plot largely on a line parallel to the 1:1 correspondance (45°) line – as noted above, this implies that there is a constant (i.e. independent of volume of mixed liquor added) difference between measured and

theoretical values, of about 25 mgCOD/ℓ. No explanation for this difference could be found, although a number of aspects were examined, e.g. Section 5.8 below.

That the two data sets are similar would indicate that the batch test has correctly detected the change in OHO active biomass fraction due to the toilet paper added to the *experimental* system: The effect of the toilet paper is taken into account automatically in calculating the theoretical OHO active biomass concentration.

Thus, the original objectives of this investigation were achieved. However, since the toilet paper proved largely biodegradable, its effect was not as marked as was hoped. Hence, it was decided to increase the dosage of toilet paper to the *experimental* activated sludge system (to further increase the contribution of inert sludge mass in the system, thereby achieving a larger increase in the MLOSS concentration in the system, and a more significant reduction of the OHO active biomass fraction of the MLOSS). Unfortunately, in practice it proved not possible to operate the laboratory-scale *experimental* activated sludge system with the higher toilet paper dose; the toilet paper caused frequent blockages of pipes between reactors which caused reactor overflows (see Chapter 4, Section 4.13).

5.8 EFFECT OF ALUMINIUM SULPHATE

The preparation of the wastewater for the modified batch tests incorporated flocculating and filtering the raw wastewater to remove all the particulate material. Aluminium sulphate (a coagulant) was chosen for the flocculation step, because as soon as it was added to the raw wastewater, the pH dropped to ≈ 6.5, which is close to the optimum pH for aluminium sulphate flocculation. During the course of the experimental investigation, it was thought that the use of aluminium sulphate as a flocculant possibly removed a large fraction of the available phosphorus required for the growth of the OHO active biomass. If true, this would have a direct impact on the OHO active biomass concentration measured in the batch tests since the growth of OHOs would be restricted by non-availability of phosphorus. It was postulated that this may be the reason for the deviation from the 1:1 correlation line observed above.

To evaluate this possibility, the soluble ortho-P concentration of both the *raw* and the *flocculated-filtered* wastewaters were measured on a number of occasions. The soluble ortho-P concentration averaged 12 mgP/ℓ in the *raw* wastewater and 1.6 mgP/ℓ in the *flocculated-filtered* wastewater. Thus, it appeared that, phosphorus could be the limiting factor in the growth of OHO active biomass, which may have caused the deviation between measured and theoretical OHO active biomass concentrations noted above. Accordingly, it was deemed necessary to further investigate this aspect.

Modified batch tests using mixed liquor drawn from the *control* activated sludge system only were run in parallel for Sewage Batches No. 21, 22 and 26; to the one batch test *flocculated-filtered* wastewater plus mixed liquor were added and to the other, *flocculated-filtered* wastewater plus mixed liquor plus 5 mℓ of stock potassium hydrogen

phosphate (K_2HPO_4, stock at 33.68 g/ℓ) were added per ℓ of wastewater (10 mgP/ℓ *batch reactor*). It must be emphasized that 6 batch tests were conducted during Sewage Batches No. 21 and 22 using mixed liquor drawn from the *MLE control* activated sludge system and another 6 batch tests were conducted during Sewage Batch No. 26 using mixed liquor drawn from the *fully aerobic control* activated sludge system (see Section 5.9). The sewage batches during which batch tests were conducted on the *control* system are given in Table 5.12.

Table 5.12: The sewage batch number and the dates it was used as feed for the parent *control* activated sludge system together with the number of batch tests with *flocculated-filtered* wastewater and mixed liquor conducted during each sewage batch.

Sewage Batch No.	Dates (2001)	Number of batch tests
21	09 Apr – 22 Apr	4
22	23 Apr – 08 May	2
26	14 Jun – 20 Jun	6
TOTAL		12

5.8.1 Parent system data

For each sewage batch tested, the parent system daily results have been averaged and the average values are listed in Table 5.13 below.

Table 5.13: Steady state results for parent laboratory-scale *control* activated sludge system receiving sewage batches during which batch tests were performed. Averages are listed with sample standard deviations in brackets.

CONTROL 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)		
		Inf	Eff	Inf	Eff	Anoxic	Aerobic	Eff		VSS	COD	TKN
21	9	774 (28)	40 (9)	70 (3)	5.6 (1.3)	5.7 (1.4)	14.7 (1.4)	11.5 (1.8)	36.4 (1.3)	2890 (164)	3908 (86)	241 (11)
22	10	749 (35)	46 (17)	73 (2)	8.3 (0.6)	3.3 (1.5)	11.5 (2.1)	9.1 (1.3)	37.1 (2.4)	2736 (143)	3862 (205)	224 (16)
26	5	787 (26)	31 (11)	72 (5)	4.5 (0.5)		50.0 (1.0)	52.6 (0.3)	45.9 (1.5)	3526 (72)	5112 (156)	250 (6)

Using the average values in Table 5.13, 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; the COD and TKN to VSS

ratios for the mixed liquor (f_{cv} and f_N respectively) and the OHO active biomass fractions of the mixed liquor organic suspended solids (f_{av}). The values are given in Table 5.14 below.

Table 5.14: Steady state COD and N mass balances, wastewater fractions and mixed liquor parameters for parent laboratory-scale anoxic/aerobic *control* activated sludge system. Data calculated from data Table 5.13 using the steady state (SS) design model (WRC, 1984).

CONTROL PARENT ANOXIC/AEROBIC STEADY STATE SYSTEM								
WW Batch	No. of tests	Mass Balance (%)		Wastewater fractions		Mixed liquor		
		COD	N	Unbio. 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})
21	9	86	92	0.040	0.170	1.38	0.085	0.3725
22	10	93	86	0.038	0.168	1.45	0.084	0.3826
26	5	101	96	0.026	0.159	1.45	0.071	0.4009

From Table 5.14 above:

- The $f_{S,up}$ and f_{av} values are reasonably consistent for Sewage Batches No. 21, 22 and 26.

- The N mass balance for Sewage Batch No. 22 was < 90 %, hence the batch test results conducted during this sewage batch should be rejected for further analysis, but they will be included where appropriate, and will be analysed.

5.8.2 Batch test data

The OHO active biomass values measured at the start of each batch test are summarized in Table 5.15 and are listed in Table A3, Appendix A. The R^2 values obtained for the batch tests were reasonable with a mean R^2 value of 0.90 and a sample standard deviation of 0.14; these are listed in Table A3, Appendix A.

The %COD recovery for each batch test is summarized in Table 5.15 below.

- In general, good %COD recoveries were achieved with only three batch tests (No. 61, 63 and 65) yielding %COD recoveries < 90 %. Although the results from these batch tests should be rejected for further analysis, statistical analysis indicated that these COD mass balance may have arisen from random effects and accordingly these batch tests data were not rejected for further analysis. A statistical plot of the %COD recovery for all the modified batch tests was constructed, see Fig. 5.14. From Fig. 5.14, the mean %COD recovery was 93.9 % with sample standard deviation of 5.5 %.

The good %COD recoveries lend credibility to the reliability of the measurements and the batch test procedure.

Table 5.15: Results for batch tests with a mixture of *flocculated-filtered* wastewater (WW) and mixed liquor (ML) {and phosphorus, P}: Batch test numbers, dates of batch tests, volumes added, COD recoveries, *measured* OHO active biomass present the start of the batch test ($Z_{BH(0)}$) and the *projected* parent system mixed liquor (ML) active biomass. The *theoretical* parent system mixed liquor (ML) OHO active biomass and the *projected* active biomass present the start of the batch test are also given.

Sew. Batch No.	Batch Test No.	Date of Test	Volume (ℓ)		COD recov. (%)	$Z_{BH(0)}$ (mgCOD/ℓ)			
						Measured		Theoretical	
			ML	WW		Batch Test	ML	ML	Batch Test
21	37	12-04	0.35	2.65	92.7	12	102	1456	170
	38 (P)	12-04	0.35	2.65	90.1	37	319	1456	170
	39	19-04	0.35	2.65	101.5	137	1176	1456	170
	40 (P)	19-04	0.35	2.65	102.8	169	1446	1456	170
22	41	23-04	0.35	2.65	95.2	142	1218	1478	172
	42 (P)	23-04	0.35	2.65	95.4	225	1929	1478	172
26 Fully Aerobic System	61	15-06	0.08	2.92	87.5*	18	659	2049	55
	62 (P)	15-06	0.08	2.92	94.7	17	630	2049	55
	63	16-06	0.16	2.84	86.0*	29	552	2049	109
	64 (P)	16-06	0.16	2.84	97.8	29	552	2049	109
	65	17-06	0.24	2.76	85.1*	61	759	2049	164
	66 (P)	17-06	0.24	2.76	97.4	40	497	2049	164

* Poor COD mass balance
(P) Solution of phosphate added

5.8.3 Comparison of OHO active biomass between batch tests with and without phosphate addition

In Table 5.15 the measured OHO active biomass concentration at the start of each batch test with and without P addition is compared with the theoretical OHO active biomass concentration at the start of the batch test due to the mixed liquor sample drawn from the parent system and added to the batch test; theoretical values predicted via the steady state design model. To illustrate the comparison, the measured versus theoretical mixed liquor OHO active biomass data for all the batch tests are shown plotted in Fig. 5.15. In Fig. 5.15 the comparative data for the batch test results rejected on the basis of poor % N recovery on the parent system (Batch Tests No. 41 and 42, Sewage Batch No. 22) are also shown.

For Sewage Batch No. 21, the addition of P caused the measured OHO active biomass concentration to increase. However, the comparisons for Batch Tests No. 37 and 38 conducted during Sewage Batch No. 21 show poor agreement between the measured and theoretical results. Careful analysis of these batch tests data revealed that during these 2 batch tests, nitrification only started after 3 to 4 hours after the start of the batch tests. Consequently, it was difficult to determine the OUR due to nitrification precisely; this uncertainty influenced the determination of the OUR due to OHO growth; the uncertainty was carried over to the $\ell n\ (OUR_H)$ – time plot, and hence inaccurate values were obtained for the slope and the y-intercept, which gave a very poor estimation of the OHO active biomass concentration at the start of the batch test. The possible reason for the slow nitrification was because the pH of the batch test was low ($\approx$ 6.94). In these tests insufficient buffer in the form of sodium hydrogen carbonate was added to raise the pH to the optimum range (pH $\approx$ 7.2 – 7.8). Hence, it took time for the organisms to begin to nitrify. Accordingly, caution should be exercised in including these batch test data.

For Sewage Batch No. 22, again addition of P caused a significant increase in the measured OHO active biomass concentration. However, for Sewage Batch No. 26, either no significant change was observed, or a slight decrease in OHO active biomass concentration with P addition. Comparing the measured and the theoretical OHO active biomass concentrations for Sewage Batch No. 26, it is clear that the measured values are significantly lower than the theoretical OHO values. This aspect will be dealt with in the next section, Section 5.9.

From the results above, it appears that the effect of adding P to the batch test is inconsistent, and not entirely conclusive. For some sewage batches, the effect is negligible, while for others adding P caused an increase in the OHO active biomass concentration. Thus, it appears that the effect of adding P may be dependent on the particular sewage batch used in the batch test, possibly depending on the P concentration available after flocculation and filtration. With the clarity of hindsight, P should have been supplemented to all subsequent batch tests when this became apparent, but at the time from the results on Sewage Batch No. 26, it was thought that the effect of P addition was negligible, so this was not done. Clearly, this aspect deserves further attention. However, from the results of Sewage Batch No. 26 it is evident that P limitation was not the cause for the significant deviation in measured values from theoretical values observed for the fully aerobic parent system, see below.

5.9 BATCH TESTS DONE ON THE *FULLY AEROBIC* SYSTEM

Throughout the experimental investigation, bulking in the parent systems was a continual problem. Bulking was especially prominent in the *control* activated sludge system. At the very early stages of the investigation, bulking was so severe that the system had to be reseeded with mixed liquor from the Mitchell's Plain Wastewater Treatment Plant. Thereafter, whenever bulking manifested itself, a short-term remedy to mitigate its effects was to dose aluminium sulphate to the aerobic reactor of the MLE activated sludge system. However, during the final stages of the experimental investigation, to try

to permanently cure bulking, it was decided to modify the *MLE* activated sludge system to a *fully aerobic* system (single aerobic reactor and secondary settling tank, i.e. the anoxic reactor was removed). However, sludge settleability did not improve significantly and the aluminium sulphate dose was continued till the end of the experimental investigation.

5.9.1 Parent system data

The *control fully aerobic* parent laboratory-scale system and its operation have been described in detail in Chapter 3. A total number of 24 batch tests (including 6 batch test where the effect of phosphate addition was monitored) were conducted using mixed liquor drawn from the *fully aerobic* activated sludge system. The sewage batches during which batch tests were conducted on the *fully aerobic* system are given in Table 5.16.

Table 5.16: The sewage batch number and the dates it was used as feed for the *control fully aerobic* system together with the number of batch tests with *flocculated-filtered* filtered wastewater and mixed liquor conducted during each sewage batch.

Sewage Batch No.	Dates (2001)	Number of batch tests
23A	09 May – 11 May	2
23B	12 May – 20 May	4
24	21 May – 03 Jun	6
25	04 Jun – 13 Jun	6
26	14 Jun – 20 Jun	6
TOTAL		24

Sewage Batch No. 23 is divided into 23A and 23B, because the system configuration was changed from *MLE* to *fully aerobic* in the middle of Sewage Batch No. 23. 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 OHO active biomass concentration would exhibit a corresponding fluctuation between different sewage batches. To formulate a theoretical estimate for the OHO active biomass concentration present in the parent system during each sewage batch period, some crucial parameters were monitored on a daily basis (see Chapter 3). Detailed data on the parent system are given in Appendix B, Tables B3 and B4. For each sewage batch tested, the daily results have been averaged and the average values are listed in Table 5.17 below.

Table 5.17: Steady state results for parent laboratory-scale *control fully aerobic* activated sludge system receiving sewage batches during which batch tests were performed. Averages are listed with sample standard deviations in brackets.

CONTROL 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)		
		Inf	Eff	Inf	Eff	Anoxic	Aerobic	Eff		VSS	COD	TKN
23A	3	795 (19)	57 (6)	86 (5)	5.5 (1.4)	24.8 (0.3)	36.4 (0.3)	35.6 (1.1)	41.4 (0.7)	3111 (88)	4110 (105)	252 (11)
23B	5	785 (26)	66 (24)	80 (3)	6.1 (1.4)		60.4 (3.1)	58.8 (4.7)	47.9 (1.1)	3220 (69)	4429 (99)	257 (11)
24	6	798 (14)	52 (19)	57 (6)	7.1 (1.6)		28.8 (3.9)	30.0 (6.0)	43.0 (1.9)	3625 (160)	5083 (114)	258 (21)
25	5	815 (22)	54 (11)	104 (2)	4.3 (0.4)		69.3 (3.0)	73.1 (1.6)	43.7 (0.8)	3726 (128)	5313 (57)	277 (7)
26	5	787 (26)	31 (11)	72 (5)	4.5 (0.5)		50.0 (1.0)	52.6 (0.3)	45.9 (1.5)	3526 (72)	5112 (156)	250 (6)

Using the average values in Table 5.17, 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; the COD and TKN to VSS ratios for the mixed liquor (f_{cv} and f_N respectively) and the OHO active biomass fractions of the mixed liquor organic suspended solids (f_{av}). The values are given in Table 5.18 below.

Table 5.18: Steady state COD and N mass balances, wastewater fractions and mixed liquor parameters for parent laboratory-scale *control fully aerobic* activated sludge system. Data calculated from data Table 5.17 using the steady state (SS) design model (WRC, 1984).

CONTROL PARENT ANOXIC/AEROBIC STEADY STATE SYSTEM								
WW Batch	No. of tests	Mass Balance (%)		Wastewater fractions		Mixed liquor		
		COD	N	Unbio. 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})
23A	3	86	98	0.044	0.210	1.42	0.081	0.3354
23B	5	96	101	0.041	0.111	1.38	0.080	0.4551
24	6	105	91	0.026	0.172	1.40	0.071	0.3583
25	5	100	90	0.022	0.165	1.43	0.074	0.3914
26	5	96	101	0.026	0.159	1.45	0.071	0.4009

From Table 5.18 above:

- The $f_{S,up}$ and f_{av} values are reasonably close for Sewage Batch No. 23 through to 26, although a slightly higher value for $f_{S,up}$ was obtained for Sewage Batch No. 23A. This was attributed to the fact that only three daily tests were conducted on the anoxic/aerobic parent system after Sewage Batch No. 23 was introduced, which could have resulted in significant uncertainties in measurements. This is reflected in the COD mass balance of 86% obtained for this period.

- The fact that the COD and N mass balances were good during all other sewage batches lends credibility to the measurements done on the parent system.

5.9.2 Batch test data

The OHO active biomass values measured at the start of each batch test are summarized in Table 5.19 and are listed in Table A4, Appendix A. The R^2 values obtained for the batch tests were generally good with a mean R^2 value of 0.90 and a sample standard deviation of 0.11; these are listed in Table A4, Appendix A.

The %COD recoveries for all the batch tests were calculated using Eq. (5.11) and the results are listed in Table A4, Appendix A. The %COD recovery for each batch test is summarized in Table 5.19 below.

- In general, good %COD recoveries were achieved with only three batch tests (No. 61, 63 and 65 – all from Sewage Batch No. 26) yielding %COD recoveries < 90 %. Although the results from these batch tests should be rejected for further analysis, statistical analysis indicated that these COD mass balance may have arisen from random effects and accordingly these batch tests data were not rejected for further analysis. A statistical plot of the %COD recovery for all the modified batch tests was constructed, see Fig. 5.16. From Fig. 5.16 the mean %COD recovery was 93.9 % with sample standard deviation of 3.8 %. The good %COD recoveries lend credibility to the reliability of the measurements and the batch test procedure.

Table 5.19: Results for batch tests with a mixture of *flocculated-filtered* wastewater (WW) and mixed liquor (ML) for *control fully aerobic* activated sludge system: Batch test numbers, dates of batch tests, volumes added, COD recoveries, *measured* OHO active biomass present the start of the batch test ($Z_{BH(0)}$) and the *projected* parent system mixed liquor (ML) active biomass. The *theoretical* parent system mixed liquor (ML) OHO active biomass and the *projected* active biomass present the start of the batch test are also given.

Sew. Batch No.	Batch Test No.	Date of Test	Volume (ℓ)		COD recov. (%)	$Z_{BH(0)}$ (mgCOD/ℓ)			
						Measured		Theoretical	
			ML	WW		Batch Test	ML	ML	Batch Test
23A	43	14-05	0.08	2.92	95.5	44	1667	1479	39
	44	14-05	0.32	2.68	95.6	234	2192	1479	158
23B	45	15-05	0.16	2.84	90.9	61	1137	2016	107
	46	15-05	0.24	2.76	94.1	88	1105	2016	161
	47	16-05	0.12	2.88	93.0	20	491	2016	81
	48	16-05	0.28	2.72	94.7	58	626	2016	188
24	49	21-05	0.08	2.92	97.4	17	537	1821	49
	50	21-05	0.12	2.88	95.9	26	496	1821	73
	51	22-05	0.16	2.84	92.4	35	417	1821	97
	52	22-05	0.20	2.80	92.9	43	453	1821	121
	53	23-05	0.24	2.76	96.6	52	343	1821	146
	54	23-05	0.28	2.72	101.1	60	473	1821	170
25	55	06-06	0.08	2.92	90.1	16	613	2080	55
	56	06-06	0.16	2.84	94.9	26	485	2080	111
	57	07-06	0.24	2.76	95.0	52	650	2080	166
	58	07-06	0.28	2.72	94.7	74	792	2080	194
	59	09-06	0.12	2.88	90.9	15	385	2080	83
	60	09-06	0.20	2.80	98.4	27	398	2080	139
26	61	15-06	0.08	2.92	87.5*	18	659	2049	55
	62	15-06	0.08	2.92	94.7	17	630	2049	55
	63	16-06	0.16	2.84	86.0*	29	552	2049	109
	64	16-06	0.16	2.84	97.8	29	552	2049	109
	65	17-06	0.24	2.76	85.1*	61	759	2049	164
	66	17-06	0.24	2.76	97.4	40	497	2049	164

* Poor COD mass balance

\- - - Change from *MLE* to *fully aerobic* system

5.9.3 Comparison between measured and theoretical active biomass for *fully aerobic* activated sludge system

In Table 5.19 the measured OHO active biomass concentration at the start of each batch test is compared with the theoretical OHO active biomass concentration at the start of the batch test due to the mixed liquor sample drawn from the *fully aerobic* system and added to the batch test; theoretical values predicted via the steady state design model. To illustrate the comparison, the measured versus theoretical mixed liquor OHO active biomass data for all the batch tests are shown plotted in Fig. 5.17.

The batch tests done using mixed liquor drawn from the *MLE* activated sludge system during Sewage Batch No. 23A show a reasonable agreement between the measured and the theoretical OHO active biomass concentrations.

However, the batch tests performed using mixed liquor drawn from the *fully aerobic* activated sludge system during all other sewage batches show a very poor agreement between the measured and the theoretical OHO active biomass concentrations: There is a close correlation between the theoretical and measured values, but the theoretical values are approximately 3 to 4 times those measured. The fact that the COD and N mass balances both on the parent system and for the batch tests were good during all these sewage batches lends credibility to the measurements.

Comparing the data obtained with the mixed liquor drawn from the *fully aerobic* system (Fig. 5.17) with that from the *MLE anoxic/aerobic* systems (*control* and *experimental* – Figs. 5.7 and 5.10 respectively), the trends are completely different: For the *anoxic/aerobic* system mixed liquor, there is a close correlation between measured and theoretical values, but with a constant difference between the actual values (i.e. the values fall on a line parallel to the 1:1 correlation line); for the aerobic system mixed liquor, the measured values are about $\frac{1}{3}$ to $\frac{1}{4}$ the theoretical values [i.e. the values fall on a line that passes through the (0,0) origin, but the line has a reduced slope]. In seeking an explanation for this difference in response, the data collected during Sewage Batch No. 23 is of interest: For the batch test conducted during Sewage Batch No. 23A, the system was operated as an *MLE* and the batch test data falls close to or higher than the 1:1 correlation line. The system was then changed to *fully aerobic*, and shortly after batch tests marked Sewage Batch No. 23B – Set 1 in Fig. 5.17 were conducted, followed by Sewage Batch No. 23B – Set 2. With each successive set of batch tests, the measured OHO active biomass concentration decreased, to reach the trend line for the *fully aerobic* system apparent for the batch tests that followed. This would suggest that changing from the *anoxic/aerobic* to *aerobic* configuration caused a significant change in the behaviour of the mixed liquor. Such a change in population dynamics is to be expected. However, why the population does not re-establish to the theoretical values after 3 sludge ages of operation is not clear: It would be expected that with time the data should return to 1:1 correlation line; this clearly does not happen.

5.10 DILUTION EFFECT

During the course of the experimental investigation, whilst examining in detail the batch tests nitrification data, it was observed that, whenever sample dilution was increased to bring the nitrate concentrations into the range that can be measured, for example, increasing dilution of samples from two times dilution to five times dilution, there was always a step increase in the nitrate/nitrite concentration. A typical example is shown in Fig. 5.18. As explained in Section 5.4 above, the best exponential or linear fit to the experimental data was of crucial importance in the analysis of the results, as this impacted on the OUR due to nitrification, and consequently on OUR due to OHO growth, which in turn influenced, to a large extent, the slope and the y-intercept value required to calculate the OHO active biomass concentration at the start of a batch test. Hence, it was decided to further investigate this aspect.

5.10.1 Investigation of wastewater interference in nitrate/nitrite determination

Samples taken during the course of a batch test were analysed for nitrate/nitrite concentrations using the *Technicon Auto Analyzer* automated method; the testing procedure is given in *Technicon Auto Analyzer methodology industrial methods 33, 68 and 35, 67w*. From the observations above, it was inferred that there was interference with this method, probably caused by the batch test solution; as the dilution increased, the interference would diminish. Accordingly, interference by *flocculated-filtered* wastewater in the measurement method was investigated.

The same *flocculated-filtered* wastewater that was used in the batch tests served to determine whether the wastewater matrix produced any interference in the nitrate/nitrite determination on the *Auto-Analyzer*. Based on the dilutions used in determining the nitrate/nitrite concentrations of the various batch test samples, the *flocculated-filtered* wastewater was diluted in a similar way. Undiluted, twice-diluted and five times diluted samples of *flocculated-filtered* wastewater were used to analyze the speculated interference in nitrate/nitrite determination on the *Auto Analyzer*. The underlying principle was to set up standard curves for nitrate/nitrite in, distilled water, undiluted, two and five times diluted *flocculated-filtered* wastewater. The procedure that was followed is given below:

(I) Distilled water

Stock solutions containing 1000 mgN/ℓ (as NO_3^- and NO_2^-) were accurately made up in distilled water. These solutions were called stock solutions (A) and (B). Then, "top standard" solutions of 2 mgN/ℓ (as NO_3^- and NO_2^-) were made up by accurately diluting 1 mℓ stock (A) and (B) respectively to 500 mℓ *distilled water*. Sub-dilutions were made as follows, see Table 5.20.

Table 5.20: Table showing the nitrate/nitrite concentration used in determining the wastewater interference on *Auto-Analyzer*, the relative volumes of "top-standard" stock solution and distilled water used.

Concentration of NO_3^-/NO_2^- (mgN/ℓ)	Volume of "top standard" solution in mℓ (at 2 mgN/ℓ)	Volume of distilled water (mℓ)
0.0	0.0	10.0
0.4	2.0	8.0
0.8	4.0	6.0
1.2	6.0	4.0
1.6	8.0	2.0
2.0	10.0	0.0

(II) Undiluted wastewater

The same stock solutions of nitrate/nitrite, as above, namely (A) and (B) were used. However, in this case, the "top standard" solutions of 2 mgN/ℓ (as NO_3^- and NO_2^-) were made up in *undiluted flocculated-filtered wastewater* by accurately diluting 1 mℓ stock (A) and (B) respectively to 500 mℓ *undiluted wastewater*. The same sub-dilutions, as given in Table 5.20 above were followed.

- For two and five times diluted wastewater, the same procedure as above was repeated, except that the wastewater was diluted two times and five times respectively with distilled water.

The results for nitrate and nitrite concentrations in the various dilutions determination are shown in Figs. 5.20 and 5.21 respectively.

- From Fig. 5.20, the *flocculated-filtered* wastewater matrix clearly interfered with the nitrate measurement.

- It is also clear from Fig. 5.20 that the more the samples were diluted, the lesser the interference on nitrate determination, with the undiluted samples having the largest impact of all. As an example, a sample of height 100 mm diluted in *distilled water* would apparently have a nitrate concentration of about 1 mgN/ℓ, whereas in *undiluted flocculated-filtered wastewater*, the nitrate concentration would be as high as 2 mgN/ℓ. This is a significant issue, when considering the sensitivity of the batch test analysis. Accordingly, all the batch test nitrate data were corrected using appropriate nitrate standard curve for the specific dilution. This eliminated the step increase in nitrate concentration when dilutions were changed, see Fig. 5.19.

- From Fig. 5.21, it can be observed that the wastewater matrix had a very small impact on nitrite determination. Nonetheless, to ensure accuracy of the batch test analysis, the slopes generated by the nitrite standard curve were used in correcting the nitrite data for all batch tests.

5.11 CLOSURE

This Chapter describes the modified batch test procedure, the interpretation of the recorded data and the evaluation of the batch test results for the *MLE control, experimental* and *fully aerobic* activated sludge systems. The following aspects and conclusions are of significance:

- In interpreting the nitrate and nitrite concentrations with time observed in their batch tests, both Ubisi *et al.* (1997a,b) and Cronje *et al.* (2000) found that the nitrite concentrations were very low, and hence could be neglected. However, in this investigation nitrite concentrations were found to be significant compared to nitrate concentrations, and hence need to be taken into account to determine OUR_N. This arises because the oxygen requirement to nitrify ammonia-N to nitrite is lower than that for nitrification of ammonia-N to nitrate.

- In the batch tests with wastewater and mixed liquor conducted by Ubisi *et al.* (1997a,b), they observed that nitrification in these batch tests caused a linear increase in the nitrate concentration with time. Cronje *et al.* (2000) observed that the generation of nitrate in the batch reactor was better represented by an exponential increase rather than a linear increase with time. In this experimental investigation, it was observed that the nitrate/nitrite concentrations could be represented by either a linear or an exponential increase. To select the best type of fit for a particular batch test, this was done by visually checking which of the linear or exponential lines best fitted the data, and confirming the best-fit line by doing a regression analysis and noting the correlation coefficient. A reasonable correlation coefficient (R^2 >0.90) implies that the selected best-fit line gives a good approximation of the experimental data. For the various batch tests, both linear and exponential fits were used. Thus, selecting the type of fit is not general, but must be based on the data for a particular batch test.

- The modified batch tests done using mixed liquor drawn from the *MLE control* activated sludge system yielded good %COD recoveries, with only 2 out of 18 batch tests (No. 3 and 7) yielding %COD recoveries < 90 %. Statistical analysis indicated that these poor COD mass balances may have arisen from random effects and accordingly these batch tests data were not rejected. The mean %COD recovery was 97.8 % with sample standard deviation of 6.9 %. The good %COD recoveries lend credibility to the reliability of the measurements and the batch test procedure.

- The modified batch tests done using mixed liquor drawn from the *MLE experimental* activated sludge system yielded good %COD recoveries, with only 1 out of 18 batch tests (No. 32) yielding %COD recoveries < 90 %. Statistical analysis indicated that this poor COD mass balance may have arisen from random effects and accordingly this batch tests data was not rejected. The mean %COD recovery was 95.9 % with sample standard deviation of 5.2 %. The good %COD recoveries lend credibility to the reliability of the measurements and the batch test procedure.

- From the plot of the measured versus theoretical OHO active biomass values (excluding outliers) for Sewage Batches No. 18, 19 and 20 for the *control* and the *experimental* systems, it is apparent that the data for both systems are remarkably similar: Both data sets plot largely on a line parallel to the 1:1 correspondance (45°) line. This implies that there is a constant (i.e. independent of volume of mixed liquor added) difference between measured and theoretical values, of about 25 mgCOD/ℓ. No explanation for this difference could be found.

- The two similar data sets would indicate that the batch test has correctly detected the change in OHO active biomass fraction due to the toilet paper added to the *experimental* system.

- When it was decided to increase the dosage of toilet paper to the *experimental* activated sludge system (to further increase the contribution of inert sludge mass in the system, thereby achieving a larger increase in the MLOSS concentration in the system, and a more significant reduction of the OHO active biomass fraction of the MLOSS), it proved not possible to operate the laboratory-scale *experimental* activated sludge system; the toilet paper caused frequent blockages of pipes between reactors which caused reactor overflows. In future investigations, an alternative method to changing the OHO active fraction should be implemented, by for example, changing the sludge age.

- For the modified batch tests done using mixed liquor drawn from both the *control MLE* and the *control fully aerobic* activated sludge system, investigating the effect of aluminium sulphate on the *flocculated-filtered* wastewater, reasonable %COD recoveries were achieved with 3 out of 12 batch tests (No. 61, 63 and 65) yielding %COD recoveries < 90 %. Statistical analysis indicated that these poor COD mass balances may have arisen from random effects and accordingly these batch tests data were not rejected. The mean %COD recovery was 93.9 % with sample standard deviation of 5.5 %. The good %COD recoveries lend credibility to the reliability of the measurements and the batch test procedure.

- From the results obtained, the effect of adding P to the batch test is inconsistent, and not entirely conclusive. For some sewage batches, the effect is negligible, while for others adding P caused an increase in the OHO active biomass concentration. Thus, it appears that the effect of adding P may be dependent on the particular sewage batch used in the batch test, possibly depending on the P concentration available after flocculation and filtration. This aspect requires further investigation.

- The modified batch tests done using mixed liquor drawn from the *fully aerobic control* activated sludge system yielded good %COD recoveries, with only 3 out of 24 batch tests (No. 61, 63 and 65) yielding %COD recoveries < 90 %. Statistical analysis indicated that these poor COD mass balances may have arisen from random effects and accordingly these batch tests data were not rejected. The mean %COD recovery was 93.9 % with sample standard deviation of 3.8 %. The good %COD

recoveries lend credibility to the reliability of the measurements and the batch test procedure.

- The batch tests performed using mixed liquor drawn from the *fully aerobic* activated sludge system during all sewage batches show a very poor agreement between the measured and the theoretical OHO active biomass concentrations: There is a close correlation between the theoretical and measured values, but the theoretical values are approximately 3 to 4 times those measured. The fact that the COD and N mass balances both on the parent system and for the batch tests were good during all these sewage batches lends credibility to the measurements.

- Changing the parent system from the *anoxic/aerobic* to *fully aerobic* configuration caused a significant change in the behaviour of the mixed liquor. Such a change in population dynamics is to be expected. However, why the population does not re-establish to the theoretical values after 3 sludge ages of operation is not clear.

- Samples taken during the course of a batch test were analysed for nitrate/nitrite concentrations. Whenever sample dilution was increased to bring the nitrate concentrations into the range that can be measured, there was always a step increase in the nitrate/nitrite concentration. This aspect was investigated. From the results obtained, the *flocculated-filtered* wastewater matrix clearly interfered with the nitrate measurement. The more the samples were diluted, the lesser the interference on nitrate determination, with the undiluted samples having the largest impact of all. The wastewater matrix had a very small impact on nitrite determination. All batch test nitrate/nitrite data were corrected using appropriate nitrate/nitrite standard curve respectively for the specific dilution.

In closure, (i) the remarkable similarity in the correlation between theoretical and measured OHO active biomass concentrations for mixed liquor drawn from the *control* and *experimental MLE* systems, (ii) the linearity of results with "serial" dilutions, and (iii) the consistent progressive change in behaviour detected by the batch test in changing from the *MLE* to *fully aerobic* configurations all indicate that the batch test method is a valuable tool for examining activated sludge system behaviour. However, the lack of a 1:1 correlation between theoretical and measured values requires further investigation. In this regard, the possibility of P limitation due to aluminium sulphate flocculation of the wastewater should be examined more closely.

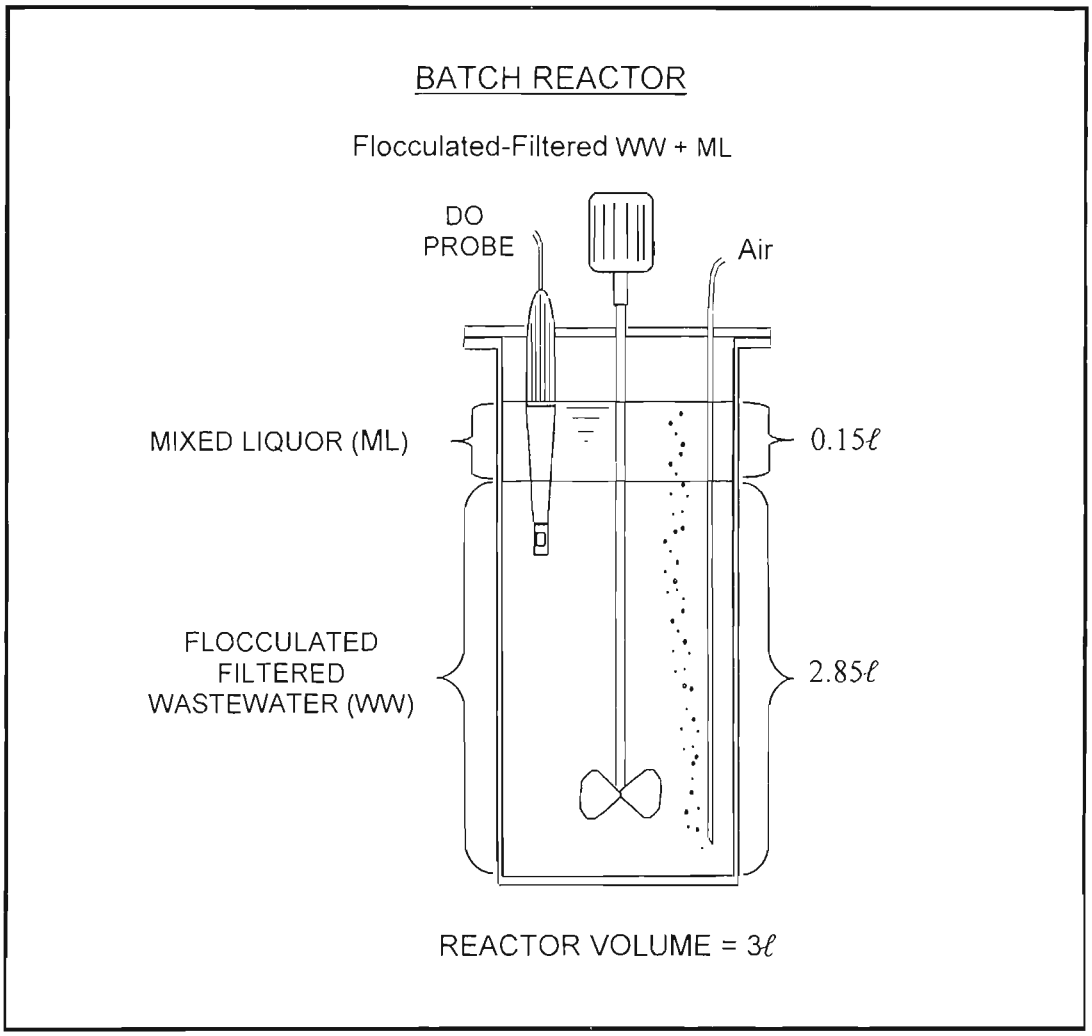


Figure 5.1: Single batch reactor arrangement used to conduct batch tests with mixed liquor added to *flocculated -filtered* wastewater.

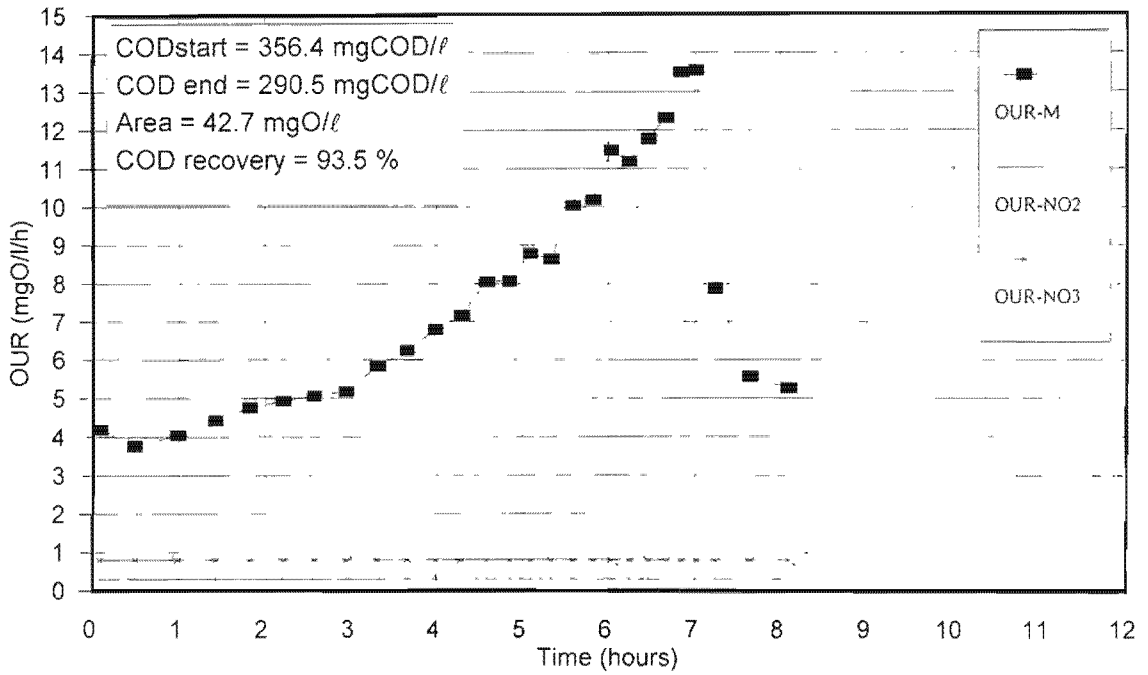


Figure 5.2: Oxygen utilization rate (OUR) response with time for a modified batch test on a mixture of flocculated-filtered wastewater (2.85ℓ) and mixed liquor (0.15ℓ) drawn from the aerobic reactor of the *experimental* system (Chapter 4). Batch Test No. 8, 05-03, Sewage Batch No. 18.

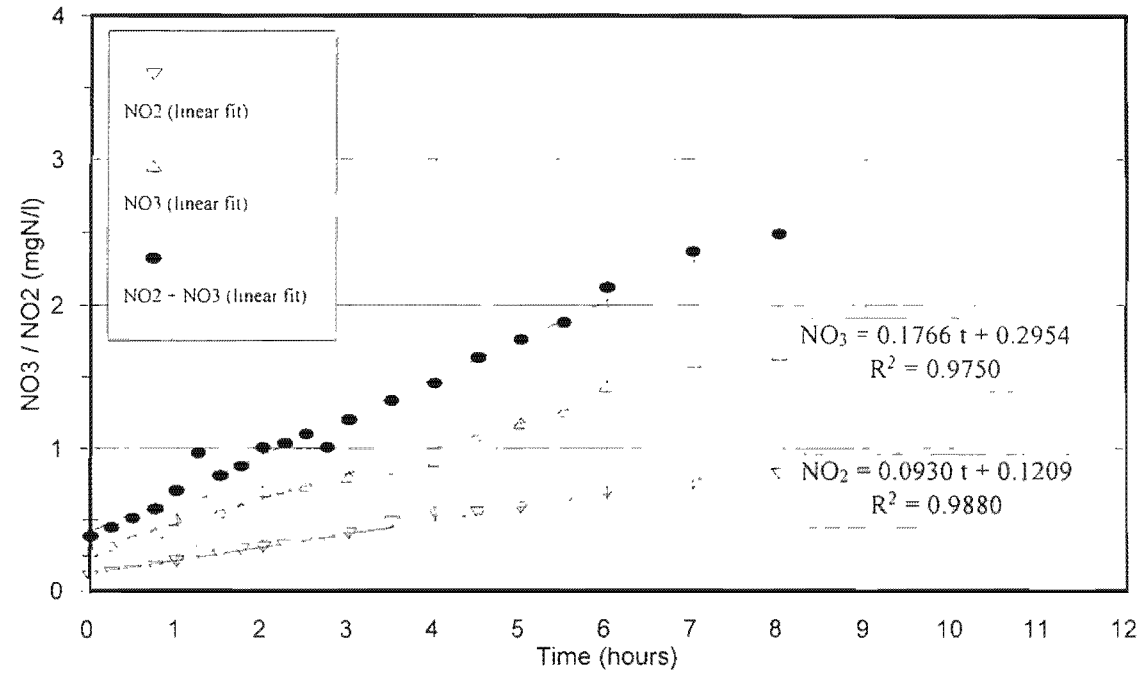


Figure 5.3: Nitrate and nitrite variation with time for the modified batch test in Fig. 5.2. Also shown are the linear expressions and correlation coefficients (R^2), obtained through linear regression. The nitrification OUR is shown in Fig. 5.2.

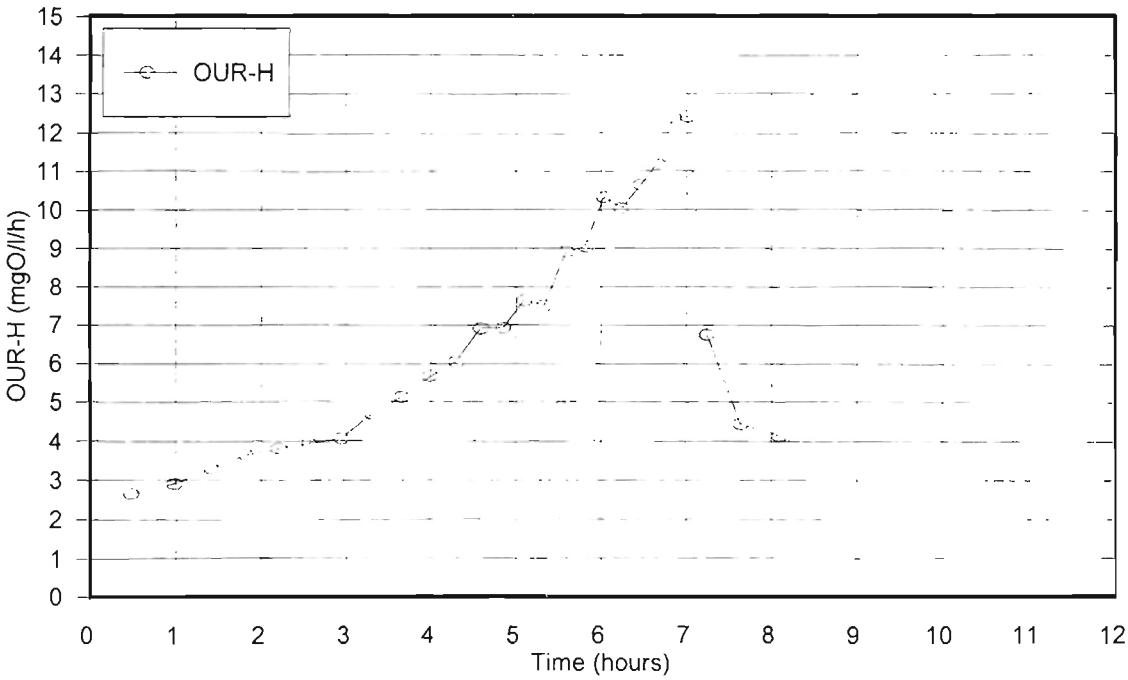


Figure 5.4: Oxygen utilization rate (OUR) due to OHO active biomass (OUR_H) versus time for modified Batch Test No. 8, 05-03, Sewage Batch No. 18. The OUR due to nitrification (OUR_N) was subtracted from the measured OUR data in Fig. 5.2.

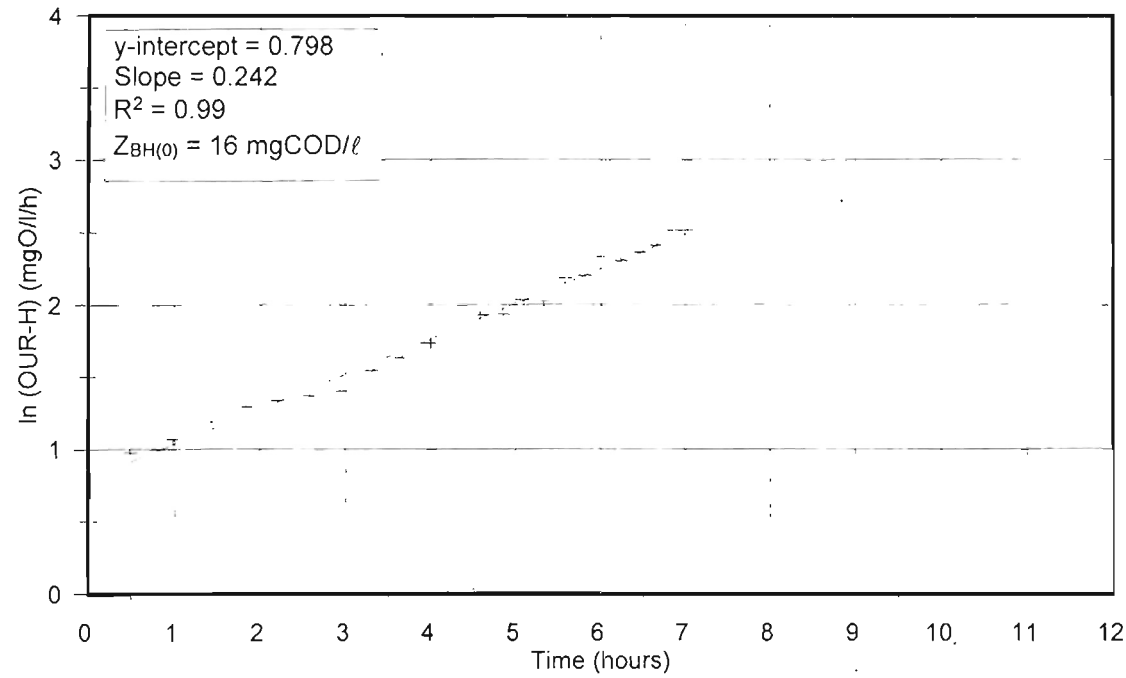


Figure 5.5: ℓn oxygen utilization rate (OUR) due to OHO active biomass (ℓn OUR_H) versus time for the OUR data in Fig. 5.4 up to the precipitous drop in OUR. Batch Test No. 8, 05-03, Sewage Batch No. 18.

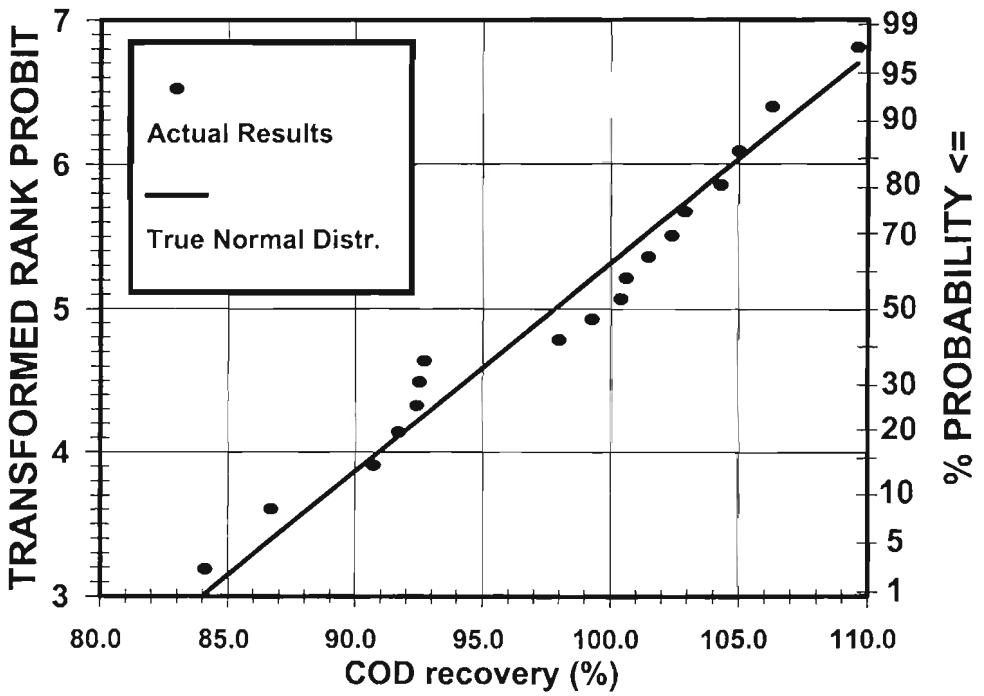


Figure 5.6: Statistical plot of %COD recovery for all the modified batch tests conducted with flocculated-filtered wastewater and mixed liquor performed on the MLE control activated sludge system.

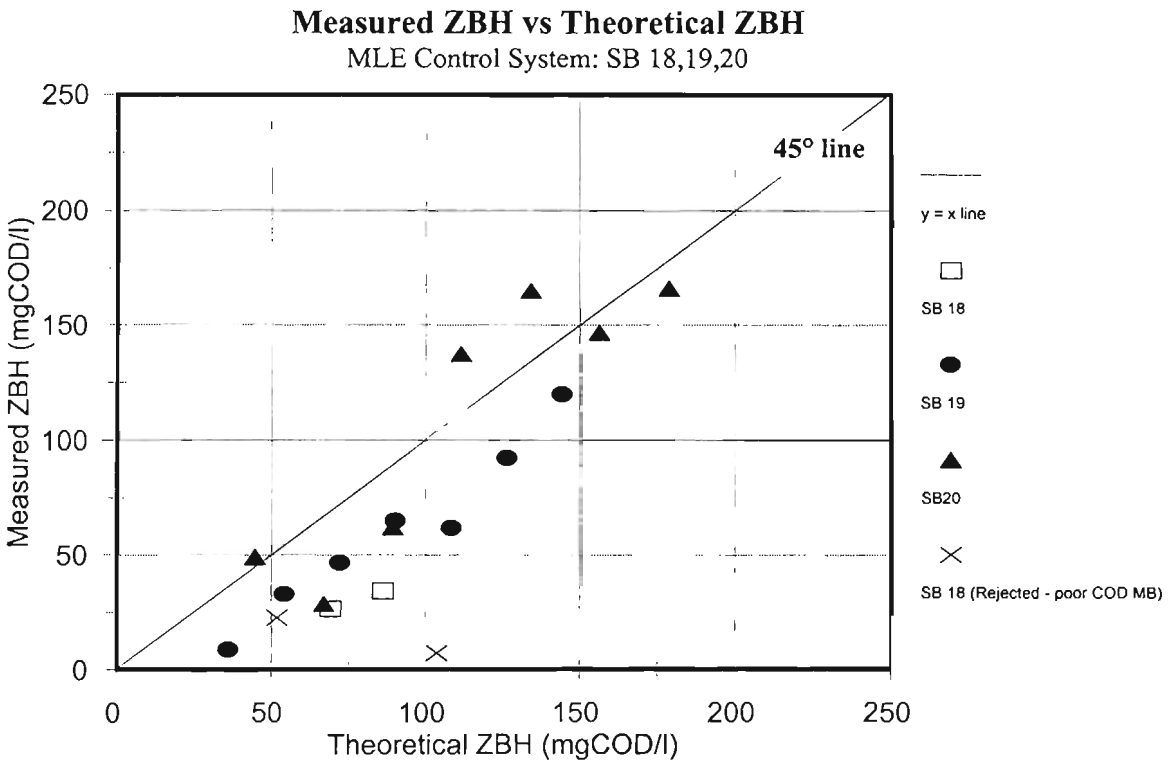


Figure 5.7: Modified batch tests results; graph of measured versus theoretical OHO active biomass concentration at the start of the batch test [$Z_{BH(0)}$] for the various wastewater batches for the MLE control activated sludge system operated at 10 days sludge age.

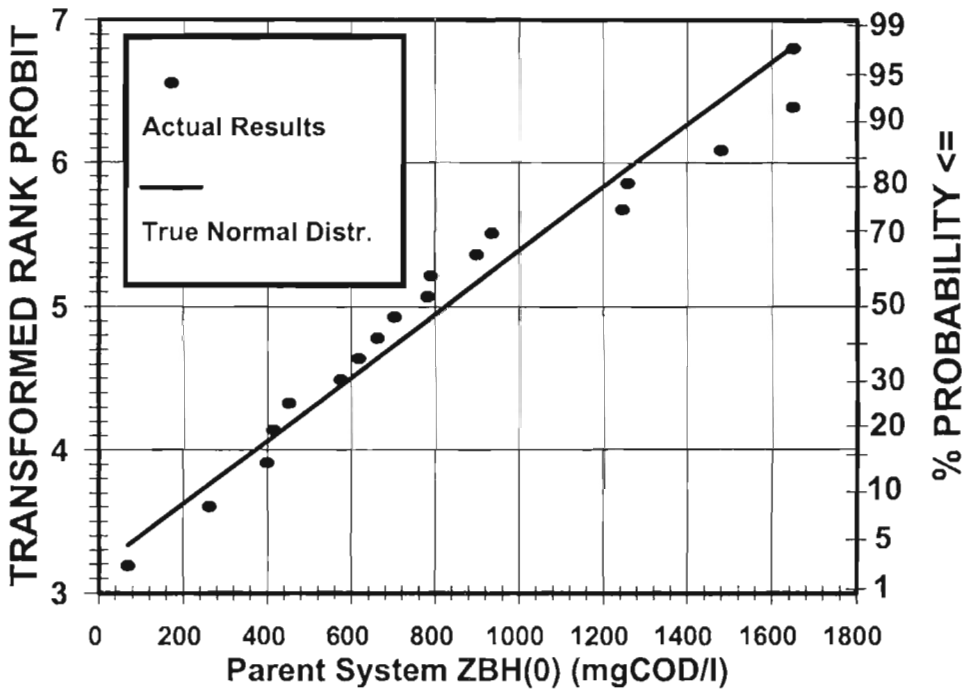


Figure 5.8: Statistical plot of the measured OHO active biomass concentration at the start of the batch test $Z_{BH(0)}$ for the various wastewater batches projected to the concentration in the MLE control activated sludge system operated at 10 days sludge age.

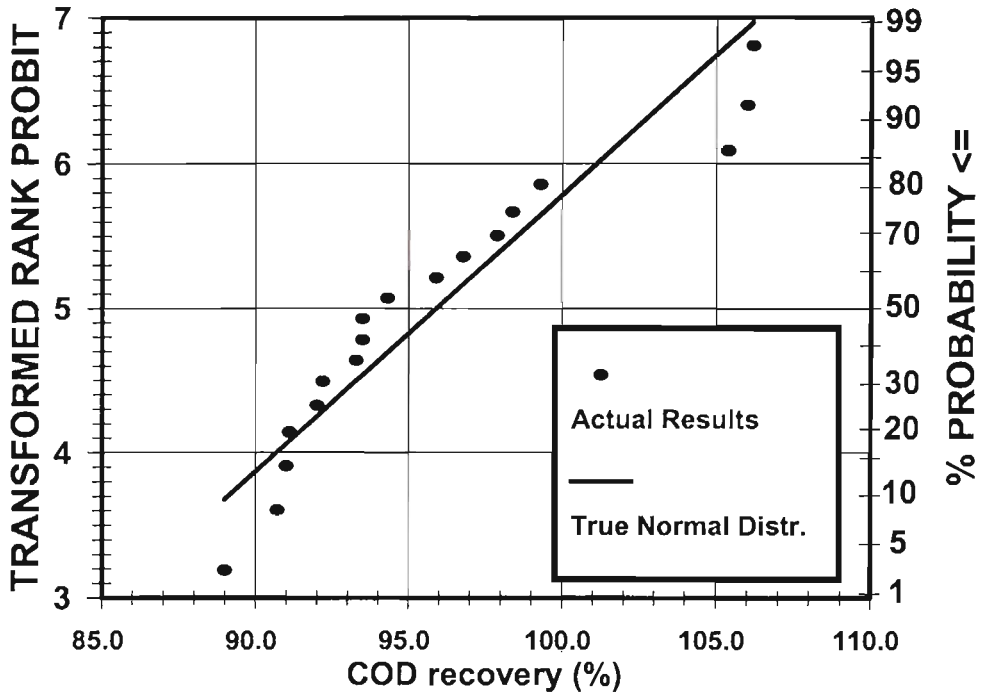


Figure 5.9: Statistical plot of %COD recovery for all the modified batch tests conducted with flocculated-filtered wastewater and mixed liquor performed on the MLE experimental activated sludge system.

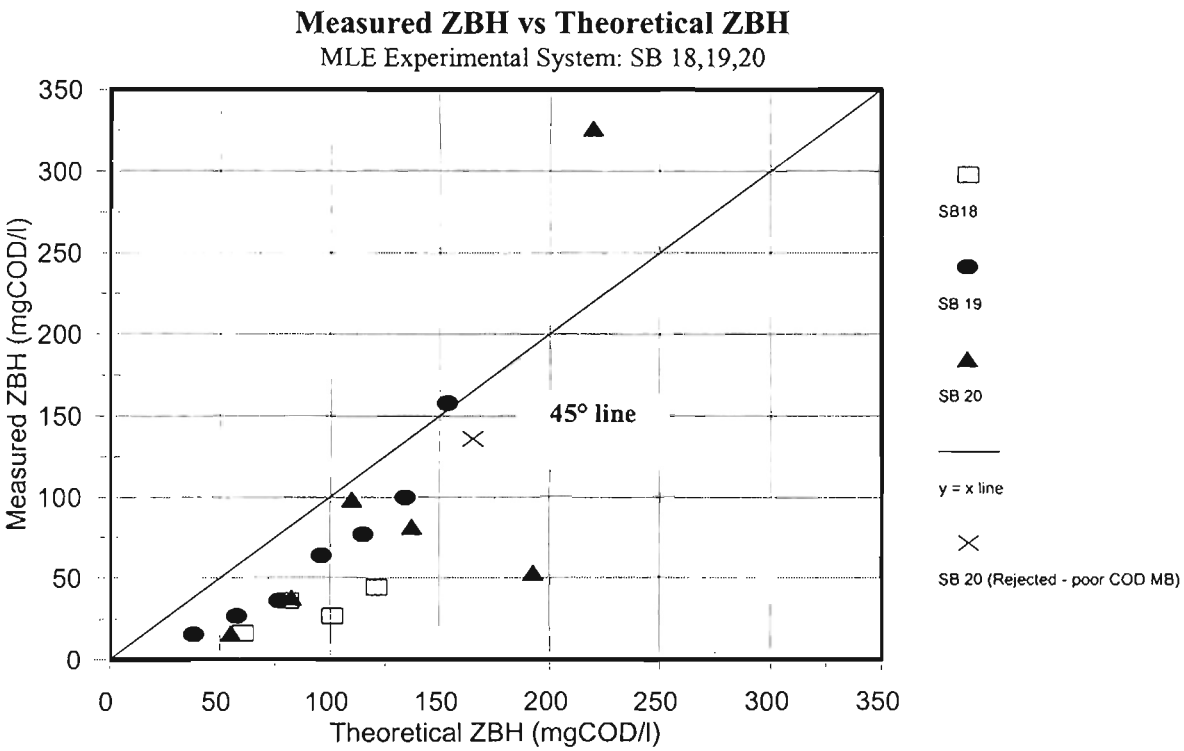


Figure 5.10: Modified batch tests results; graph of measured versus theoretical OHO active biomass concentration at the start of the batch test [$Z_{BH(0)}$] for the various wastewater batches for the MLE experimental activated sludge system operated at 10 days sludge age.

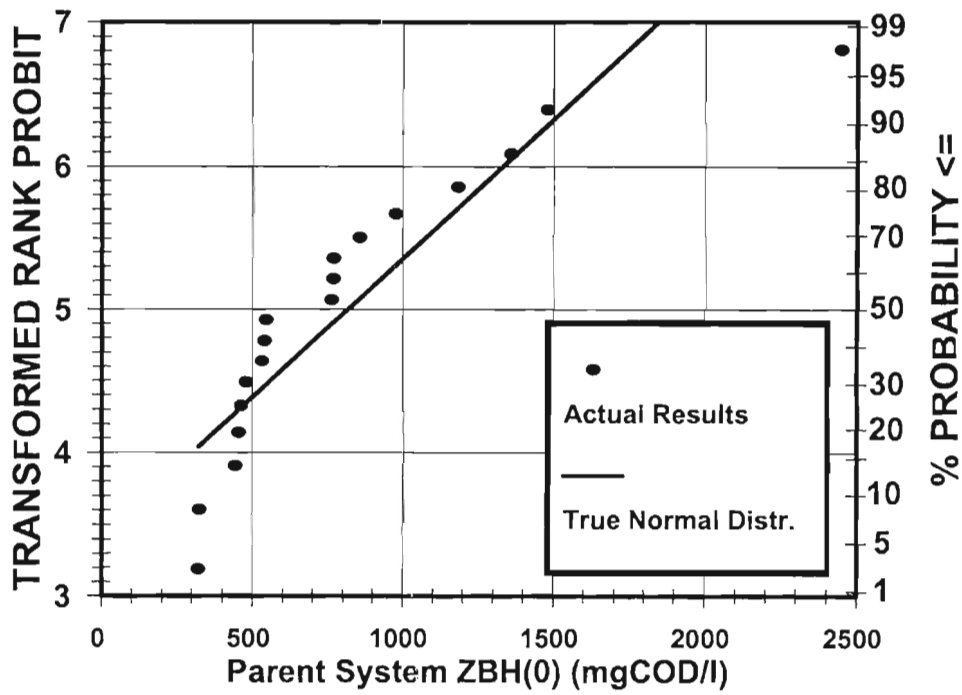


Figure 5.11: Statistical plot of the measured OHO active biomass concentration at the start of the batch test ZBH(0) for the various wastewater batches projected to the concentration in the MLE experimental activated sludge system operated at 10 days sludge age.

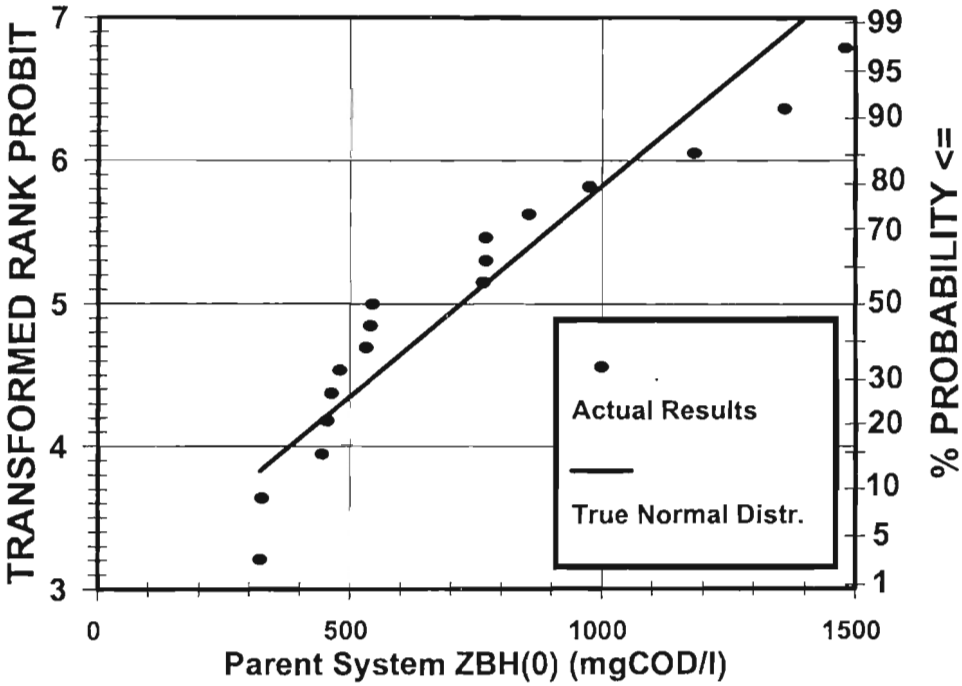


Figure 5.12: Statistical plot of the measured OHO active biomass concentration at the start of the batch test ZBH(0) for the various wastewater batches projected to the concentration in the MLE experimental activated sludge system operated at 10 days sludge age:
* outliers rejected.

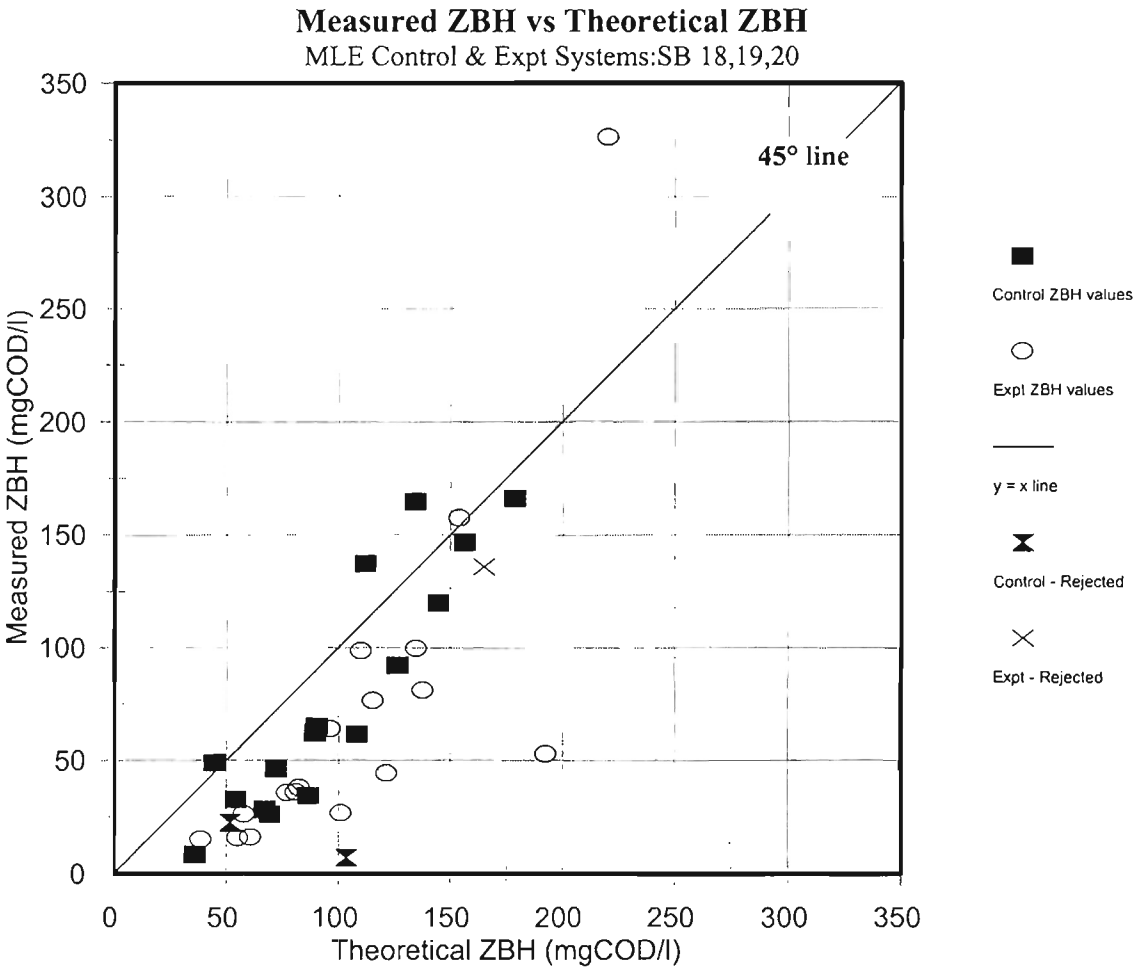


Figure 5.13: Modified batch tests results; graph of measured versus theoretical OHO active biomass concentration at the start of the batch test $Z_{BH(0)}$ for the various wastewater batches comparing the MLE control and experimental activated sludge systems operated at 10 days sludge age.

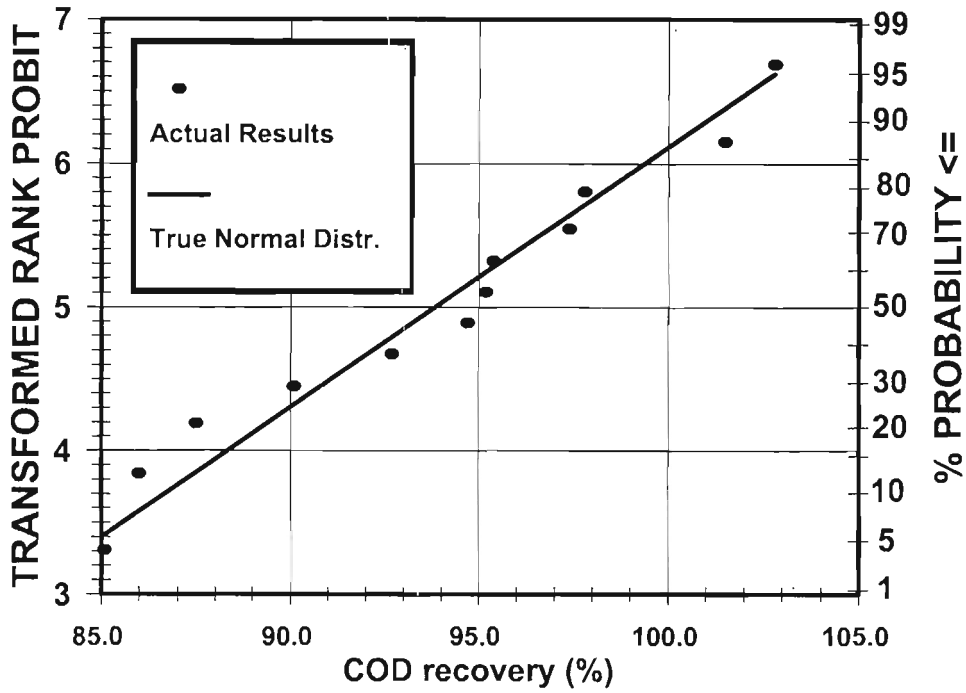


Figure 5.14: Statistical plot of %COD recovery for all the modified batch tests conducted with flocculated-filtered wastewater and mixed liquor for Sewage Batches No. 21, 22 and 26 performed on the MLE and fully aerobic control activated sludge system operated at 10 days sludge age.

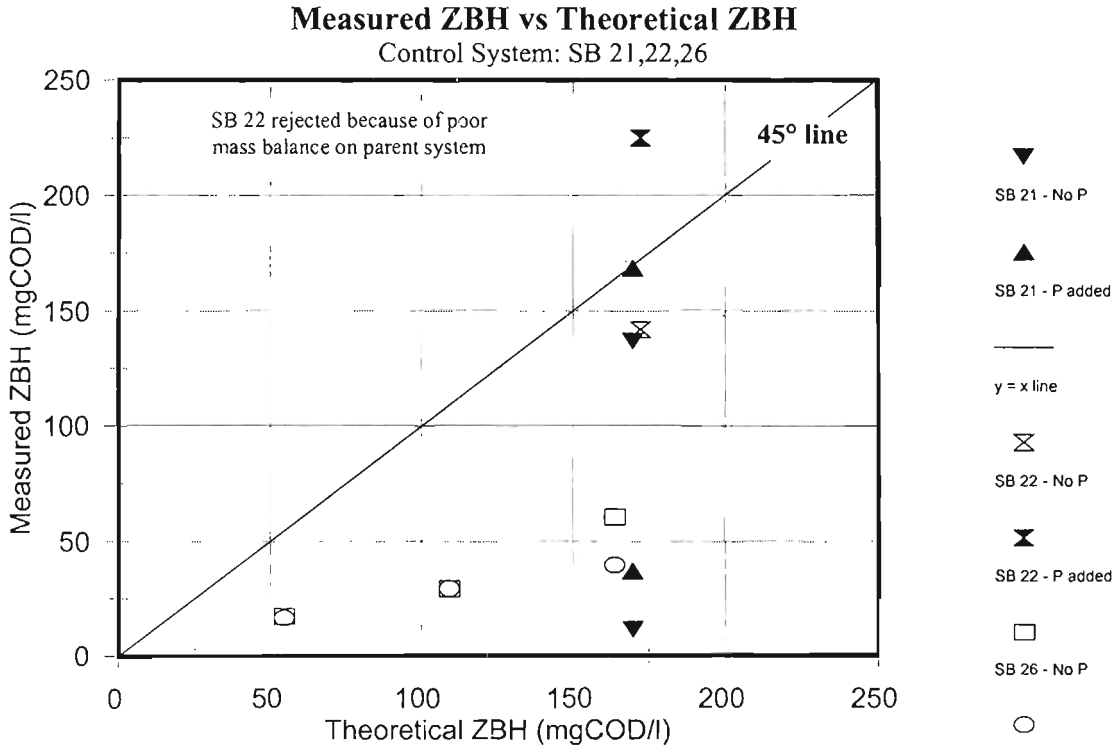


Figure 5.15: Modified batch tests results; graph of measured versus theoretical OHO active biomass concentration at the start of the batch test $Z_{BH(0)}$ for Sewage Batches 21, 22 and 26 for the MLE and fully aerobic control activated sludge systems operated at 10 days sludge age.

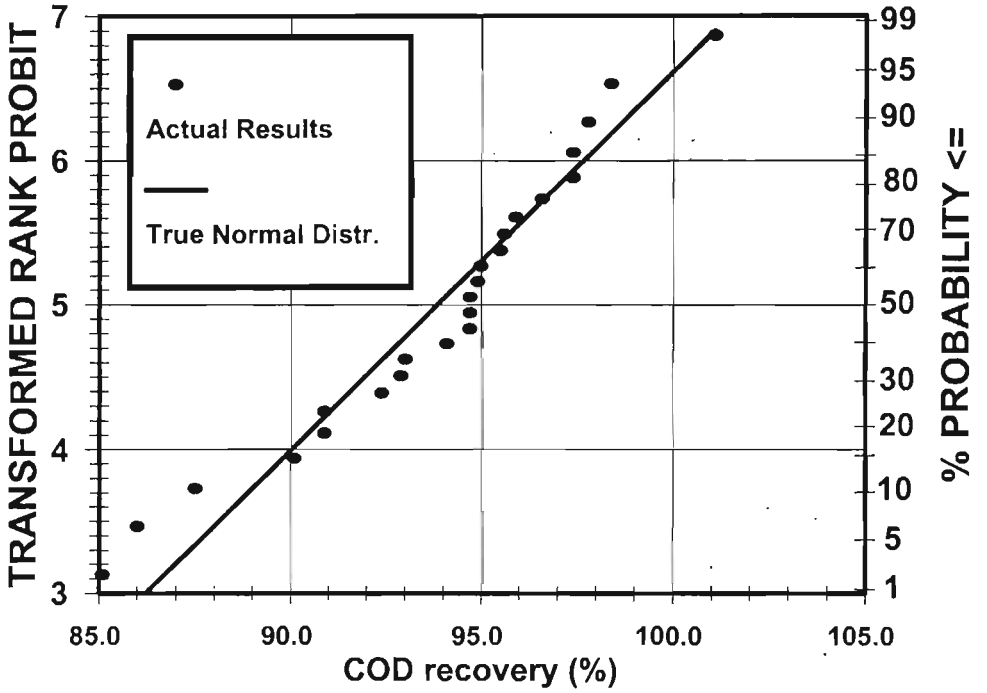


Figure 5.16: Statistical plot of %COD recovery for all the modified batch tests conducted with flocculated-filtered wastewater and mixed liquor performed on the fully aerobic activated sludge system.

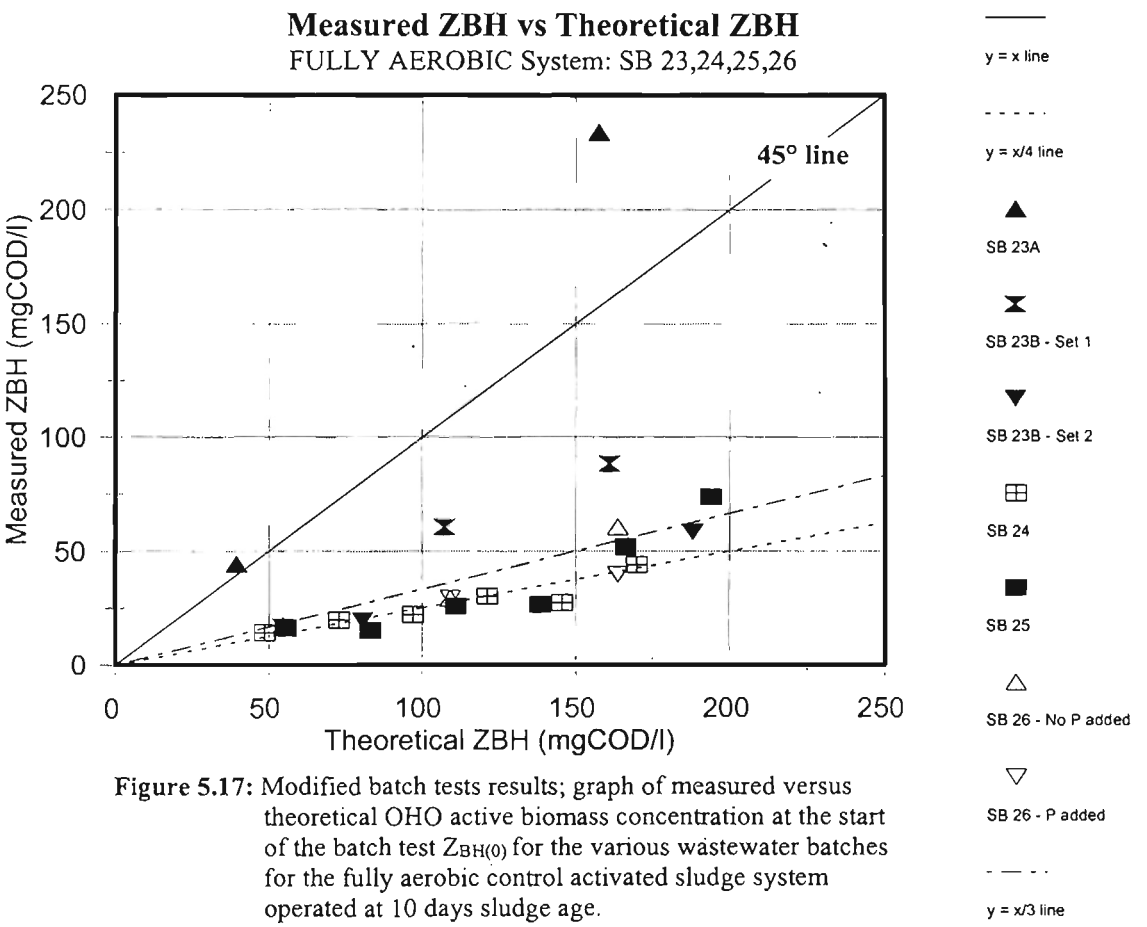


Figure 5.17: Modified batch tests results; graph of measured versus theoretical OHO active biomass concentration at the start of the batch test $Z_{BH(0)}$ for the various wastewater batches for the fully aerobic control activated sludge system operated at 10 days sludge age.

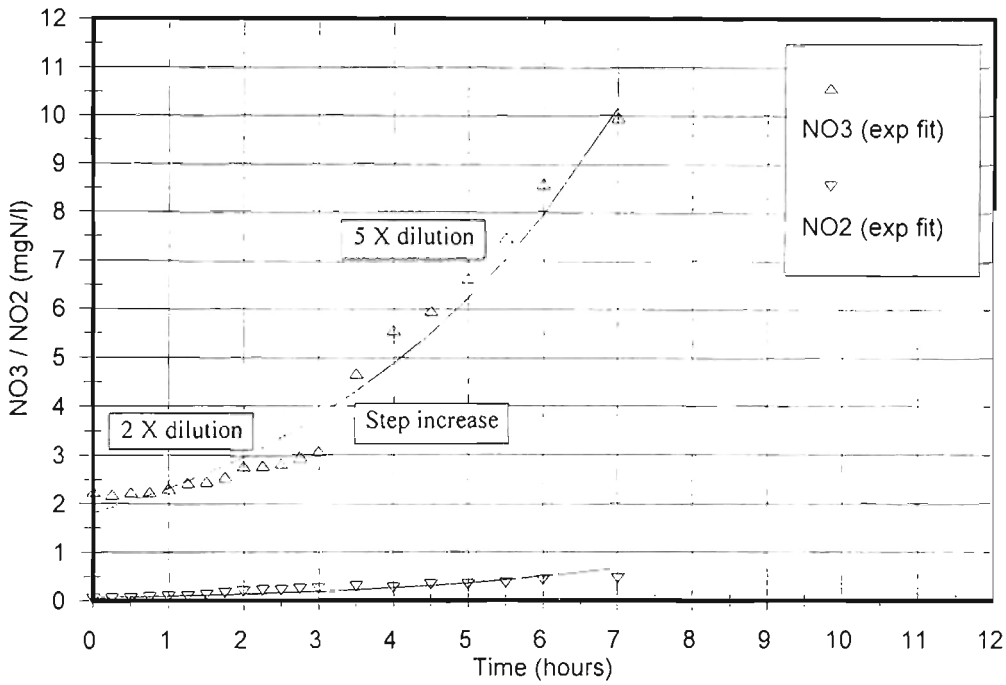


Figure 5.18: Nitrate and nitrite variation with time for the modified batch test with flocculated-filtered wastewater (2.65ℓ) and mixed liquor (0.35ℓ) drawn from the aerobic reactor of the control activated sludge system, showing the step increase in nitrate concentration with time. Batch Test No. 33, 04-04, Sewage Batch No. 20.

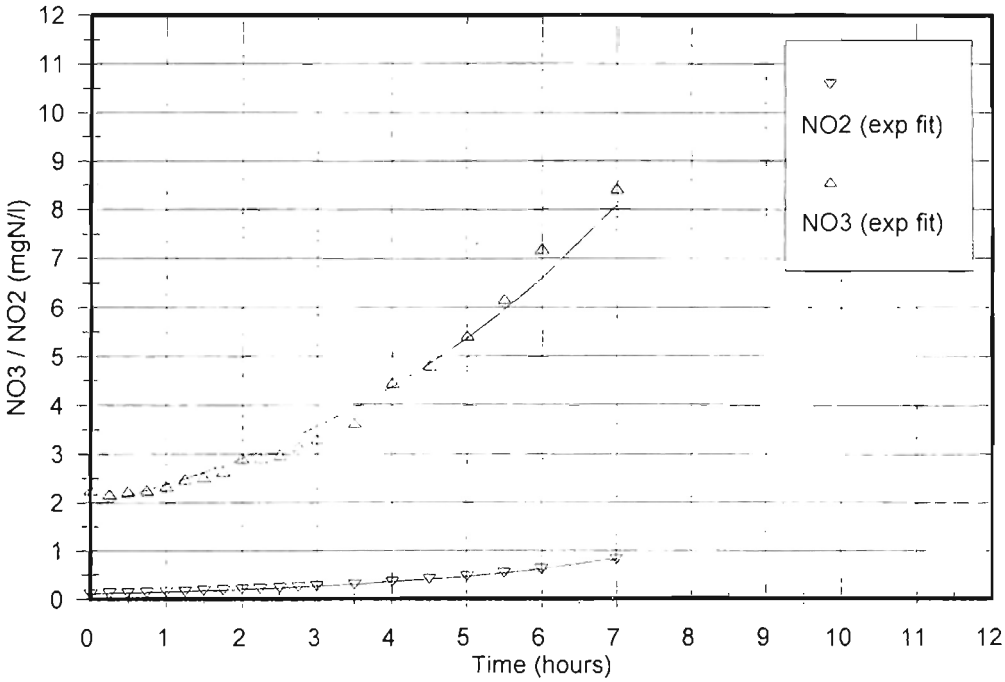


Figure 5.19: Nitrate and nitrite variation with time for the modified batch test with flocculated-filtered wastewater (2.65ℓ) and mixed liquor (0.35ℓ) drawn from the aerobic reactor of the control activated sludge system, after correcting the step increase in nitrate and nitrite concentration using the nitrate and nitrite standard curves respectively (Fig. 5.20 and 5.21). Batch Test No. 33, 04-04, Sewage Batch No. 20.

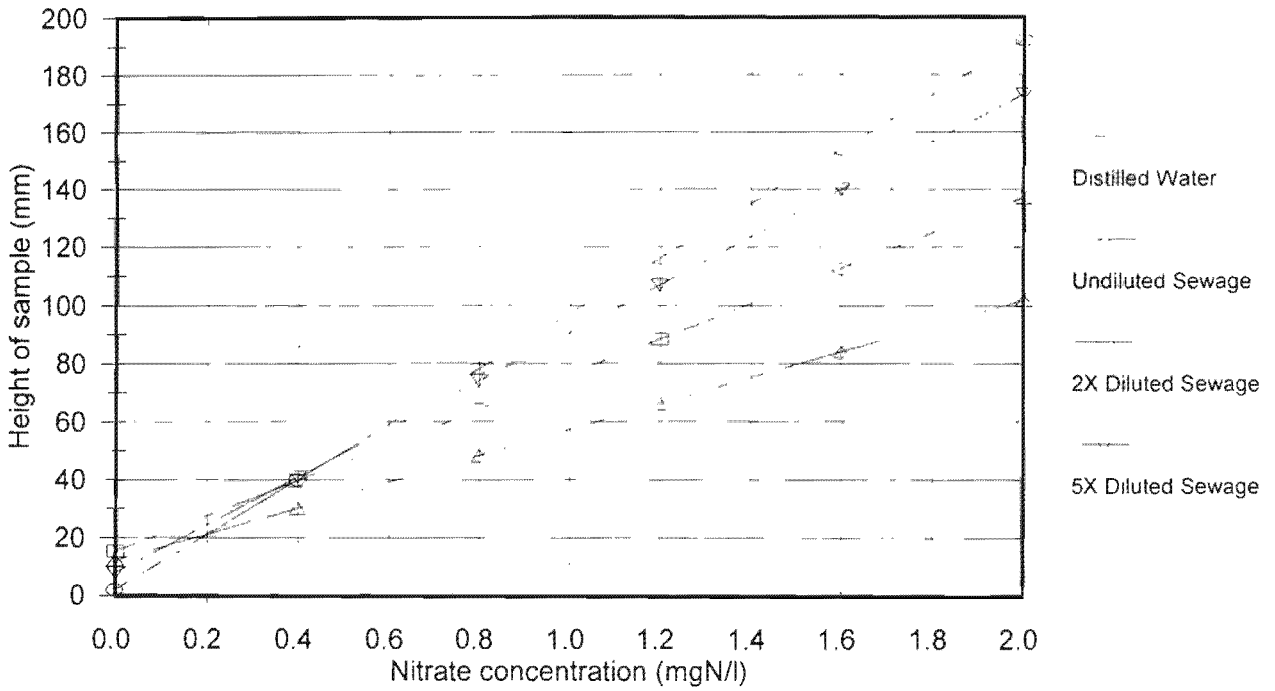


Figure 5.20: Nitrate standard curve developed for various dilutions of flocculated-filtered wastewater.

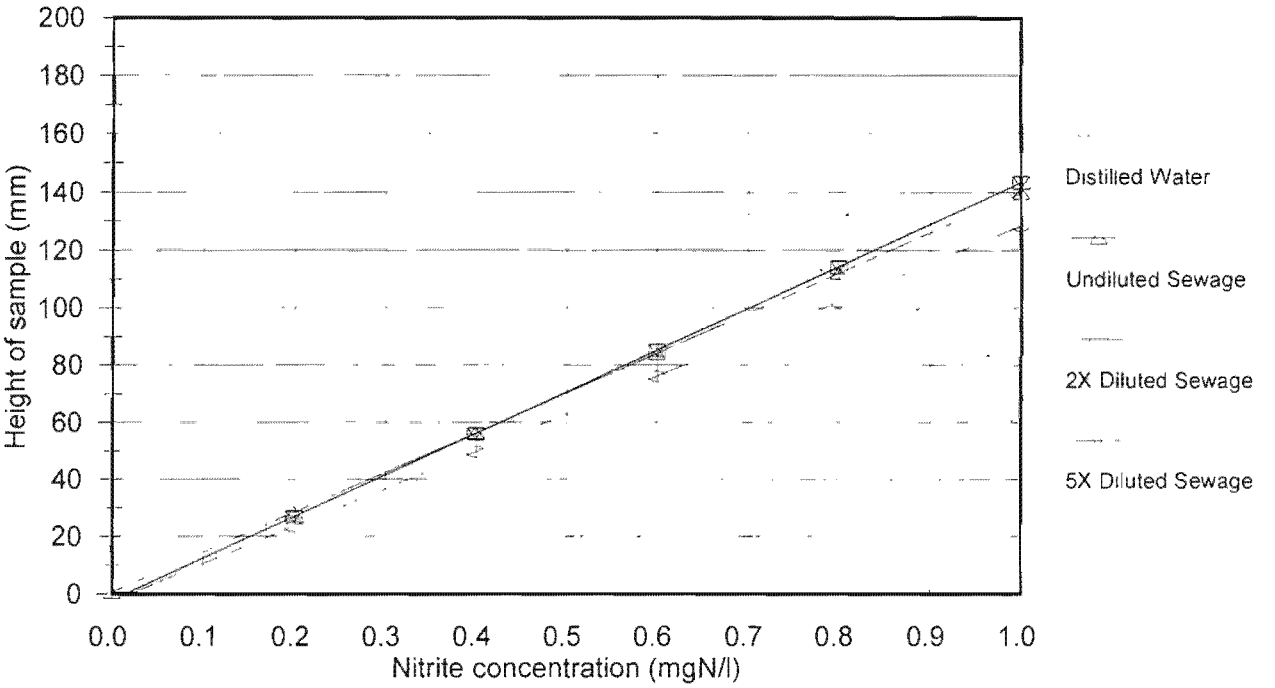


Figure 5.21: Nitrite standard curve developed for various dilutions of flocculated-filtered wastewater.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 OBJECTIVES

Fundamental to the steady state design and kinetic simulation models for activated sludge systems is the parameter OHO 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 OHO 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 weakness in the models. Although recently the OHO 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 in the models themselves, it is essential that the OHO active biomass parameter is validated by experimental measurement.

Recently, a modified batch test method developed by Cronje *et al.* (2000) has shown considerable promise as an *independent* means to quantify the hypothesized concentration of OHO active biomass present in an activated sludge system. Cronje *et al.* (2000) compared the results from the modified batch test with theoretical values for OHO active biomass concentrations from the steady state design model (WRC, 1984). From this comparison they concluded that the results obtained showed good agreement. Also, there was remarkable agreement between the theoretical OHO active biomass concentration in the parent system and the mean of the measured OHO active biomass values *projected* to the parent system. ***The principle aim of this research project was to further investigate the modified batch test method of Cronje et al. (2000) as a reliable means of quantifying the concentration of OHO active biomass.*** To achieve this aim, two primary objectives for this research project were identified.

6.1.1 Verification of consistent modified batch test results

Cronje *et al.* (2000) developed a modified batch test method to quantify the concentration of OHO active biomass present in an activated sludge system. They 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 a sludge age of 10 days, and compared the measured OHO active biomass values to those calculated theoretically with the models.

Cronje *et al.* (2000) found that a good correlation existed between the theoretical and measured OHO active biomass values. The results appeared to substantiate the modified batch test method as a reliable means of quantifying the OHO active biomass. However, the modified batch test method does require more extensive evaluation.

Thus, ***the first objective of this research project was to repeat the modified batch test procedure,*** using an identical parent laboratory-scale anoxic/aerobic activated sludge system, termed the *control* system. This would establish whether the results of Cronje *et al.* (2000) are reproducible, or whether their good correlation was fortuitous.

6.1.2 Evaluation of the modified batch test method to detect a change in OHO active biomass

In addressing the main aim above, it was decided to run and operate a parallel parent laboratory-scale anoxic/aerobic activated sludge system having a different OHO active biomass fraction of the mixed liquor. This system was termed the *experimental* system. To change the OHO active biomass fraction of the mixed liquor, a known concentration of macerated toilet paper solution was dosed to this system. Toilet paper is mainly constituted of wood pulp, which is composed of 75% cellulose and 25% lignin. These two organic components are believed to be largely unbiodegradable in the activated sludge system. Accordingly, toilet paper should contribute significantly to the inert sludge mass in the laboratory-scale anoxic/aerobic activated sludge system, thereby significantly increasing the MLOSS concentration in the system, and reducing the OHO active biomass fraction of the MLOSS. ***This would provide the opportunity to evaluate the ability of the modified batch test procedure to detect the decreased active biomass fraction.***

6.2 RESEARCH APPROACH

The research approach adopted was to operate and monitor two well-defined and controlled continuously fed parent activated sludge systems. The *control* activated sludge system provided the mixed liquor samples for measuring the OHO active biomass to address the first objective. To address the second objective, the *experimental* activated sludge system provided the mixed liquor samples for measuring the OHO active biomass.

6.3 VERIFICATION OF THE CONSISTENT BATCH TEST RESULTS

6.3.1 Control parent system

To further investigate the modified batch test method of Cronje *et al.* (2000), a well-defined and controlled parent laboratory-scale nitrification / denitrification activated sludge system identical to that of Cronje *et al.* (2000) was operated. The system was monitored closely and provided the mixed liquor samples required for measuring OHO active biomass. From the results obtained for the *control* system:

- N mass balances were consistent and were generally in the range 90 to 110%. 8 out of 26 sewage batches gave mass balances falling outside this range. Batch tests were conducted, only during one of these sewage batches. The batch test data collected on this sewage batch was included where appropriate and analysed, but it was noted that the data should be interpreted with caution.

- Generally COD mass balances were poor, with 15 out of 26 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 = 1.42 mgCOD/mgVSS (sample standard deviation = 0.05) and TKN/VSS = 0.086 mgN/mgVSS (sample standard deviation = 0.008). These values are close to 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 OHO active biomass. Accordingly, the lower limit for the COD mass balance was set at 80%. On this basis, only 2 sewage batches 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 0.050 (sample standard deviation = 0.014). This value is lower than the $f_{S,us}$ values obtained by both Ubisi *et al.* (1997a,b), $f_{S,us}$ = 0.095 and Cronje *et al.* (2000), $f_{S,us}$ = 0.085 for the same Mitchell's Plain raw wastewater. Of interest is the fact that both Ubisi *et al.* (1997a,b) and Cronje *et al.* (2000) were feeding a COD concentration of 500 ± 50 mgCOD/ℓ to their parent systems. In this experimental investigation, the same feed concentration of 500 ± 50 mgCOD/ℓ was fed to the parent system for the first 107 days, whereafter, the feed concentration was increased to 750 ± 75 mgCOD/ℓ and this increased COD concentration was fed to the parent system till closure (day 417). Thus, despite that the $f_{S,us}$ value would be expected to be the same, given that the influent wastewater being treated was the same, the high influent COD concentration gave a lower $f_{S,us}$. The lower $f_{S,us}$ value is however, in the range of accepted values of 0.04 – 0.10 mgCOD/mgCOD for municipal raw wastewaters in South Africa (WRC, 1984).

- The wastewater mean unbiodegradable particulate COD fraction ($f_{S,up}$) was determined to be 0.161 (sample standard deviation = 0.037). This value compares favourably with that observed by Ubisi *et al.* (1997a,b), $f_{S,up}$ = 0.120, Cronje *et al.* (2000), $f_{S,up}$ = 0.103 for the same Mitchell's Plain raw wastewater, and conforms to the accepted range of 0.07 – 0.20 mgCOD/mgCOD for municipal raw wastewaters in South Africa (WRC, 1984). This indicates that the value obtained for $f_{S,up}$ is reasonable.

6.3.1.1 Batch tests

The modified batch tests were conducted in accordance with the procedures detailed by Cronje *et al.* (2000). A total of 18 batch tests were conducted. From an analysis of the modified batch test results, the following were concluded:

- The modified batch tests done using mixed liquor drawn from the *MLE control* activated sludge system yielded good %COD recoveries, with only 2 out of 18 batch tests (No. 3 and 7) yielding %COD recoveries < 90 %. Statistical analysis indicated that these poor COD mass balances may have arisen from random effects and accordingly these batch tests data were not rejected. The mean %COD recovery was 97.8 % with sample standard deviation of 6.9 %. The good %COD recoveries lend credibility to the reliability of the measurements and the batch test procedure.

- Comparing the measured and the theoretical OHO active biomass concentrations, it would appear that there is reasonably close correspondance between theoretical and measured OHO active biomass concentrations; the "serial dilutions" of mixed liquor gave an almost linear decrease in OHO active biomass concentration. However, the values plot virtually parallel to the 45° line (i.e. 1:1 correspondance). This implies that there is a constant (i.e. independent of volume of mixed liquor added) difference between the measured and theoretical values – when the measured OHO active biomass concentration is zero, the theoretical OHO active biomass concentration in the batch test is approximately 25 mgCOD/ℓ. No explanation for this deviation was apparent.

- Although some correlation does exist between the theoretical and measured OHO active biomass concentrations for the range of mixed liquor volumes used in the batch tests, individual data points tend to exhibit some variation from the appropriate correlation line. This variation can be attributed to the sensitivity of the measured OHO active biomass concentration, to the slope of the ℓn (OUR_H) – time plot. Even the smallest change in the slope (magnitude ~ 0.05) can result in marked variations in the OHO active biomass concentration values. This would suggest that a number of batch tests need to be conducted to establish a reasonable estimate for OHO active biomass concentration.

6.3.2 Experimental parent system

To address the second objective of this research project, a parallel parent laboratory-scale anoxic/aerobic activated sludge system, termed the *experimental* system, having a different OHO active biomass fraction of the mixed liquor was run and operated identically to the *control* parent system, but the *experimental* system additionally received a known mass of toilet paper. It was envisaged that the toilet paper would be largely unbiodegradable, and hence the *experimental* system would have a mixed liquor OHO active biomass fraction that would deviate significantly from the parallel *control* system. The ability of the batch test procedure of Cronje *et al.* (2000) to correctly detect this difference in OHO active biomass was also evaluated. From a comparison of the

steady state results of the *control* and *experimental* parent systems: (i) the biodegradability of the toilet paper, and (ii) its effect on the components making up the MLOSS, to derive a theoretical value of OHO active biomass were determined. From the results obtained for the *experimental* system:

- N mass balances were consistent and were generally in the range 90 to 110%. Only 6 out of 19 sewage batches gave mass balances falling outside this range, 3 only marginally outside the range. No batch tests were conducted during these sewage batches.

- Generally COD mass balances were reasonable, with 7 out of 19 sewage batches giving mass balances < 90%. Of these, 3 sewage batches had COD mass balances only marginally less than 90%. No batch tests were conducted during any of these sewage batches.

- The mixed liquor organic solids were determined by three independent tests – VSS, COD and TKN. Mean ratios for these measurements gave COD/VSS = 1.39 mgCOD/mgVSS (sample standard deviation = 0.06) and TKN/VSS = 0.078 mgN/mgVSS (sample standard deviation = 0.004). These values are lower than the values measured for the *control* parent system (1.42 mgCOD/mgVSS and 0.086 mgN/mgVSS respectively) and the accepted standard values of 1.48 mgCOD/mgVSS and 0.10 mgN/mgVSS respectively (WRC, 1984). More than likely, the lower values were caused by the toilet paper dose.

6.3.2.1 Biodegradability of toilet paper

From the literature review on the composition and biodegradation of toilet paper, it was evident that the main organic materials present in toilet paper are cellulose and lignin, and that these are very difficult to biodegrade. Accordingly, this aspect was further investigated.

From the steady state data on the *experimental* parent system, for each sewage batch, the unbiodegradable soluble and particulate fractions of the influent COD + toilet paper solution ($f_{S,us\ -\ WW+TP}$ and $f_{S,up\ -\ WW+TP}$ respectively) were determined. The $f_{S,us}$ and $f_{S,up}$ for the wastewater could be determined from the *control* system. By difference, the $f_{S,us}$ and $f_{S,up}$ for the toilet paper was calculated, taking due account of dilution effects.

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

- The $f_{S,us}$ values for {Wastewater + Toilet Paper} for the *experimental* system gave a mean $f_{S,us}$ of 0.045 and sample standard deviation of 0.008. The $f_{S,us}$ for {Toilet Paper only} was 0.035 with a sample standard deviation of 0.015.

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

- The $f_{S,up}$ values for {Wastewater + Toilet Paper} for the *experimental* system gave a mean $f_{S,up}$ of 0.162 and sample standard deviation of 0.066. The $f_{S,up}$ for {Toilet Paper only} was 0.309 with a sample standard deviation of 0.141.

Hence, it can be concluded that the total unbiodegradable fraction of the toilet paper only was about 34%. In fact, this does not differ that much from the total unbiodegradable fraction of sewage (~ 21%), determined in this investigation. This indicated that the initial proposal that the toilet paper would contribute a high unbiodegradable particulate fraction ($f_{S,up}$) to the *experimental* activated sludge system was not true. This was attributed to the fact that the active organisms were degrading the toilet paper, in the process consuming oxygen. Hence, in terms of the second objective of this research project, the original amount of toilet paper solution added did not cause a significant change in the active fraction of the VSS of the *experimental* system compared to the *control* system: This implies that the toilet paper did not cause a sufficient increase in inert material to the *experimental* system, thus the concentration of OHO active biomass fraction of the mixed liquor would not be expected to decrease significantly (this assumption was confirmed by the batch test results). Increasing the dosage of toilet paper proved inconclusive as the *experimental* system became difficult to operate, with frequent blockages of pipes connecting reactors so that reactor overflows occurred very often, making it difficult to maintain steady state. Since it became evident that the toilet paper was more biodegradable than initially surmised, and would not achieve the desired effect of substantially decreasing the OHO active biomass fraction of the mixed liquor, this investigation was terminated.

6.3.2.2 Batch tests

To further evaluate the reliability of the modified batch test procedure and its application to anoxic/aerobic activated sludge systems, subjected to decreased OHO active biomass fractions, a total number of 18 modified batch tests were conducted using mixed liquor drawn from the *MLE experimental* activated sludge system and were done in parallel to the batch tests on the *control* system.

From an analysis of the batch test results, the following were concluded:

- In general, good %COD recoveries were achieved with only one batch test (No. 32) yielding %COD recovery < 90 %. The %COD recovery for Batch Test No. 32 was marginally < 90 % (89.0 %); statistical analysis indicated that this COD mass balance arose from random effects and accordingly this batch test data was not rejected. The mean %COD recovery was 95.9 % with sample standard deviation of 5.2 %. The good %COD recoveries lend credibility to the reliability of the measurements and the batch test procedure.

- Comparing the measured and the theoretical OHO active biomass concentrations, the correlations show remarkable similarity to those obtained for the *control* system –

there is a close correlation but the values plot parallel to the 45° line. Again, this implies that there is a constant difference between measured and theoretical OHO active biomass concentrations; as for the *control* system, this difference is approximately 25 mgCOD/ℓ.

- Although some correlation does exist between the theoretical and measured OHO active biomass concentrations for the range of mixed liquor volumes used in the batch tests, individual data points tend to exhibit some variation from the appropriate correlation line. This variation can be attributed to the sensitivity of the measured OHO active biomass concentration (as explained above).

6.3.3 Comparison between OHO active biomass between the *control* and *experimental* systems

From a comparison of the results, it was apparent that the data for the *control* and *experimental* systems are remarkably similar: Both data sets plot on a line parallel to the 1:1 correspondance (45°) line – as noted above, this implies that there is a constant (i.e. independent of volume of mixed liquor added) difference between measured and theoretical values, of about 25 mgCOD/ℓ. No explanation for this difference could be found. That the two data sets are similar would indicate that the batch test has correctly detected the change in OHO active biomass fraction due to the toilet paper added to the *experimental* system: The effect of the toilet paper is taken into account automatically in calculating the theoretical OHO active biomass concentration.

Thus, the original objectives of this investigation were achieved. However, since the toilet paper proved largely biodegradable, its effect was not as marked as was hoped. Difficulties in operating and maintaining steady state in the *experimental* activated sludge system arose when the toilet paper dose was increased; the higher toilet paper dose caused frequent blockages of pipes between reactors which caused reactor overflows.

6.3.4 Effect of aluminium sulphate on batch test results

In the flocculation of the wastewater, it was thought that perhaps P was also flocculated and may be limiting in the batch test, and hence would influence the results. To evaluate this possibility, modified batch tests using mixed liquor drawn from the *control* activated sludge system only were run in parallel for Sewage Batches No. 21, 22 and 26; to the one batch test *flocculated-filtered* wastewater plus mixed liquor were added and to the other, *flocculated-filtered* wastewater plus mixed liquor plus 5 mℓ of stock potassium hydrogen phosphate (K_2HPO_4, stock at 33.68 g/ℓ) were added per ℓ of wastewater (10 mgP/ℓ *batch reactor*). It must be emphasized that 6 batch tests were conducted during Sewage Batches No. 21 and 22 using mixed liquor drawn from the *MLE control* activated sludge system and another 6 batch tests were conducted during Sewage Batch No. 26 using mixed liquor drawn from the *fully aerobic control* activated sludge system.

- The N mass balance for the *parent* system for Sewage Batch No. 22 was < 90 %, hence the batch test results conducted during this sewage batch should be rejected for further analysis, but were included, and analysed.

- In general, good %COD recoveries were achieved with only three batch tests (No. 61, 63 and 65) yielding %COD recoveries < 90 %. Statistical analysis indicated that these COD mass balance may have arisen from random effects and accordingly these batch tests data were not rejected. The mean %COD recovery was 93.9 % with sample standard deviation of 5.5 %. The good %COD recoveries lend credibility to the reliability of the measurements and the batch test procedure.

For Sewage Batch No. 21, the addition of P caused the measured OHO active biomass concentration to increase. For Sewage Batch No. 22, again addition of P caused a significant increase in the measured OHO active biomass concentration. However, for Sewage Batch No. 26, either no significant change was observed, or a slight decrease in OHO active biomass concentration with P addition.

From the results above, it was noted that the effect of adding P to the batch test was inconsistent, and not entirely conclusive. For some sewage batches, the effect was negligible, while for others adding P caused an increase or decrease in the OHO active biomass concentration. Thus, it appears that the effect of adding P may be dependent on the particular sewage batch used in the batch test, possibly depending on the P concentration available after flocculation and filtration. With the clarity of hindsight, P should have been supplemented to all subsequent batch tests when this became apparent, but at the time from the results on Sewage Batch No. 26, it was thought that the effect of P addition was negligible, so this was not done. Clearly, this aspect deserves further attention. However, for the results for Sewage Batch No. 26, it is evident that P limitation was not the cause for the significant deviation between measured and theoretical values observed for the *fully aerobic* parent system.

6.3.5 Batch tests done on the *fully aerobic* system

Throughout the experimental investigation, bulking in the parent systems was a continual problem. Whenever bulking manifested itself, a short-term remedy to mitigate its effects was to dose aluminium sulphate to the aerobic reactor of the *MLE* activated sludge system. However, during the final stages of the experimental investigation, to try to permanently cure bulking, it was decided to modify the *MLE* system to a *fully aerobic* activated sludge system.

A total number of 24 modified batch tests were conducted using mixed liquor drawn from the *fully aerobic control* activated sludge system. The sewage batches during which batch tests were conducted on the *fully aerobic* system were Sewage Batches No. 23A, 23B, 24, 25 and 26. Sewage Batch No. 23 is divided into 23A and 23B, because the system configuration was changed from *MLE* to *fully aerobic* in the middle of Sewage Batch No. 23.

- The fact that the COD and N mass balances for the parent system were good during these sewage batches lends credibility to the measurements done on the parent system.

- In general, good %COD recoveries were achieved with only three batch tests (No. 61, 63 and 65 – all from Sewage Batch No. 26) yielding %COD recoveries < 90 %. Statistical analysis indicated that these COD mass balance may have arisen from random effects and accordingly these batch tests data were not rejected. The mean %COD recovery was 93.9 % with sample standard deviation of 3.8 %. The good %COD recoveries lend credibility to the reliability of the measurements and the batch test procedure.

- The batch tests done using mixed liquor drawn from the *MLE* activated sludge system during Sewage Batch No. 23A show a reasonable agreement between the measured and the theoretical OHO active biomass concentrations.

- However, the batch tests performed using mixed liquor drawn from the *fully aerobic* activated sludge system during all other sewage batches show a very poor agreement between the measured and the theoretical OHO active biomass concentrations: There is a close correlation between the theoretical and measured values, but the theoretical values are approximately 3 to 4 times those measured. The fact that the COD and N mass balances both on the parent system and for the batch tests were good during all these sewage batches lends credibility to the measurements.

- Comparing the data obtained with the mixed liquor drawn from the *fully aerobic* system with that from the *MLE anoxic/aerobic* system, the trends are completely different: For the *anoxic/aerobic* system mixed liquor, there is a close correlation between measured and theoretical values, but with a constant difference between the actual values (i.e. the values fall on a line parallel to the 1:1 correlation line); for the *fully aerobic* system mixed liquor, the measured values are about $\frac{1}{3}$ to $\frac{1}{4}$ the theoretical values [i.e. the values fall on a line that passes through the (0,0) origin, but which has a reduced slope].

- In seeking an explanation for this difference in response, the data collected during Sewage Batch No. 23 is of interest: For the batch test conducted during Sewage Batch No. 23A, the system was operated as an *MLE* and the batch test data falls close to or higher than the 1:1 correlation line. The system was then changed to *fully aerobic*, and shortly thereafter batch tests were conducted. With each successive set of batch tests, the measured OHO active biomass concentration decreased, to reach the trend line for the *fully aerobic* system apparent for the batch tests that followed. This would suggest that changing from the *anoxic/aerobic* to *aerobic* configuration caused a significant change in the behaviour of the mixed liquor. Such a change in population dynamics is to be expected. However, why the population did not re-establish to the theoretical values after 3 sludge ages of operation is not clear: It would be expected that with time the data should return to 1:1 correlation line – this clearly did not happen.

6.3.6 Batch test summary

In summary, (i) the remarkable similarity in the correlation between theoretical and measured OHO active biomass concentrations for mixed liquor drawn from the *control* and *experimental MLE* systems, (ii) the linearity of results with "serial" dilutions, and (iii) the consistent progressive change in behaviour detected by the batch test in changing from the *MLE* to *fully aerobic* configurations all indicate that the batch test method is a valuable tool for examining activated sludge system behaviour. However, the lack of a 1:1 correlation between theoretical and measured values requires further investigation. In this regard, the possibility of P limitation due to aluminium sulphate flocculation of the wastewater should be examined more closely.

6.4 RECOMMENDATIONS

From this investigation the following recommendations can be made:

- Dosing toilet paper to significantly change the OHO active biomass fraction of the mixed liquor in the activated sludge system was not successful. On the one hand, toilet paper is more biodegradable than expected, and thus did not exert the anticipated influence on OHO active biomass fraction of the mixed liquor; on the other hand, with increased doses of toilet paper it became very difficult to operate and maintain steady state conditions in the activated sludge system. As an alternative, to significantly change the OHO active biomass fraction of the mixed liquor, the modified batch test method needs to be tested on mixed liquor drawn from parent systems at different sludge ages, say at 10 and 20 days.

- The effect of adding P to the batch test was inconsistent, and not entirely conclusive. For some sewage batches, the effect was negligible, while for others adding P caused an increase or decrease in the OHO active biomass concentration. Thus, it appears that the effect of adding P may be dependent on the particular sewage batch used in the batch test, possibly depending on the P concentration available after flocculation and filtration. Clearly, this aspect deserves further attention and investigation.

- The results from this research project demonstrate the behavioural differences between sludges originating from different parent systems and the influence of the behavioural differences on the batch test results. Changing from the *anoxic/aerobic* to *aerobic* configuration caused a significant change in the behaviour of the mixed liquor. Such a change in population dynamics is to be expected. However, why the population did not re-establish to the theoretical values after 3 sludge ages of operation is not clear. This warrants further investigation.

- Quantifying OHO 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 OHO 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. Exploration of ways to integrate the engineering and technology paradigm with the microbiological and biochemistry paradigm should receive attention.

6.5 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. Measurement of this parameter within the engineering paradigm by means of the batch test procedure described here and within the microbiological and biochemical paradigms by means of the newly developed analytical techniques can initiate links and overlap between the two paradigm sets.

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APPENDIX A

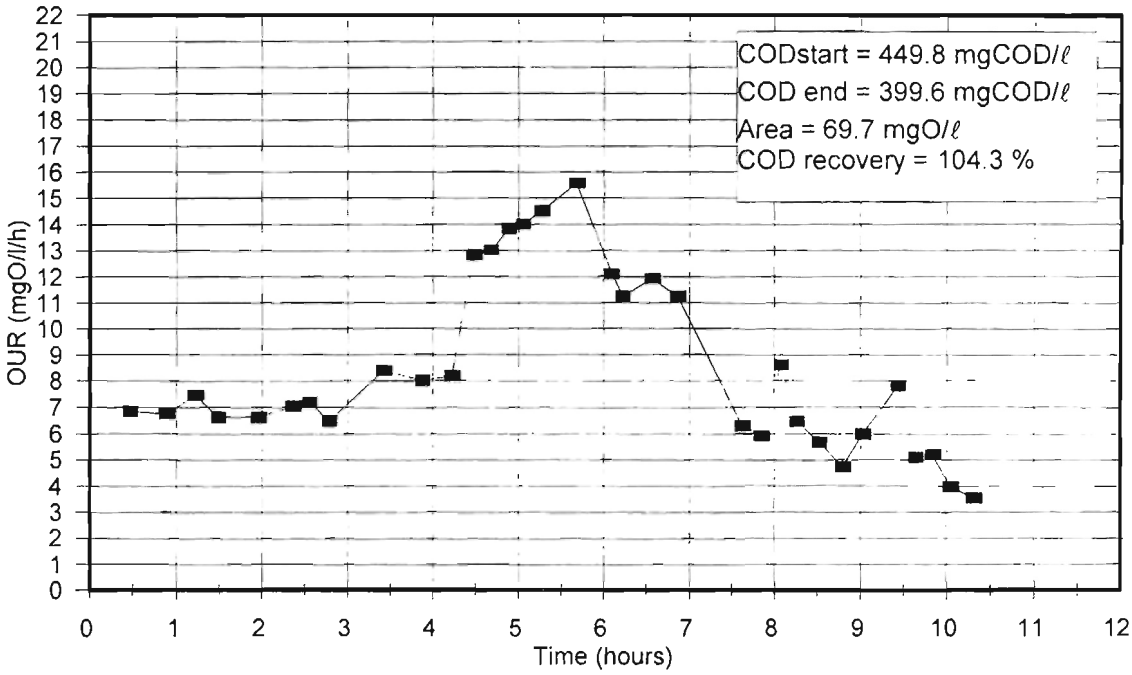
OUR vs TIME PLOTS FOR MODIFIED BATCH TESTS CONDUCTED WITH FLOCCULATED-FILTERED WASTEWATER PLUS MIXED LIQUOR

TABLE OF CONTENTS

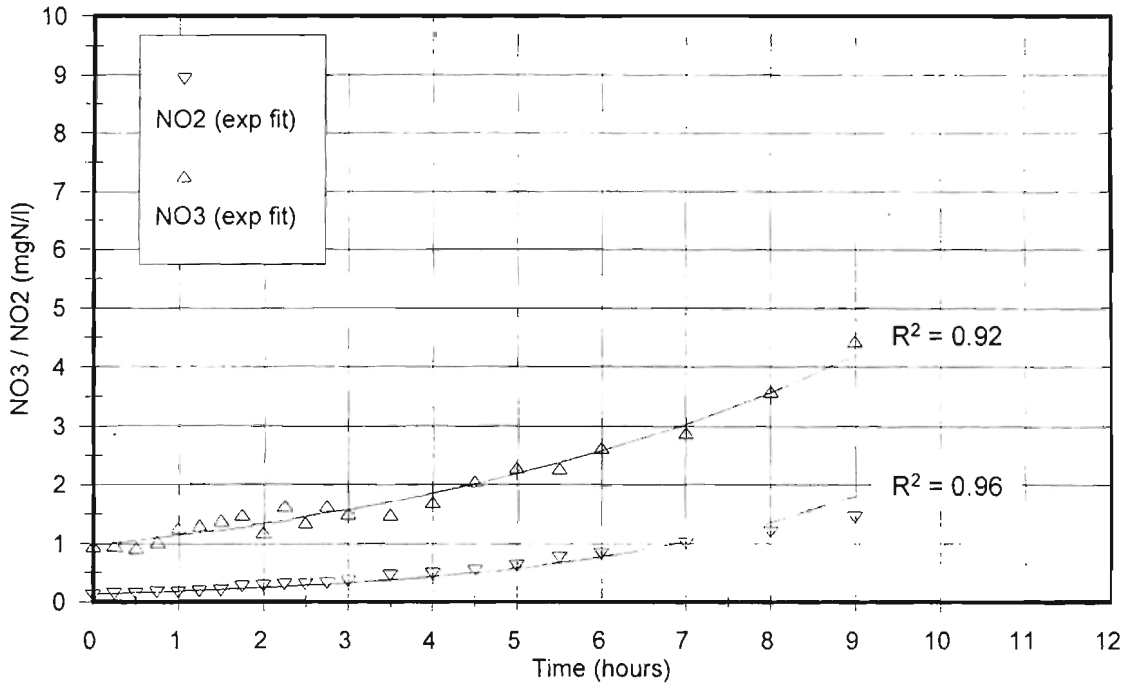
Table A1: Summarized data for modified batch tests with filtered wastewater and mixed liquor drawn from the parent MLE control activated sludge system; mixed liquor volume, COD recovery, linear regression data and measured OHO active biomass.

Sew. Batch No.	Batch Test No.	Date of Test	ML volume (ℓ)	COD (mgCOD/ℓ) Start	COD (mgCOD/ℓ) End	Area (mgO/ℓ)	% COD Recovery	Linear regresion data y-int.	Linear regresion data slope	Linear regresion data R^2	$Z_{BH(0)}$ (ML) (mgCOD/ℓ)
18	1	28-02	0.25	449.8	399.6	69.7	104.3	1.374	0.193	0.73	35
	3	02-03	0.30	496.0	371.5	58.3	86.7*	0.352	0.367	0.94	7*
	5	04-03	0.20	391.6	335.3	65.6	102.4	1.312	0.248	0.97	27
	7	05-03	0.15	331.7	232.8	46.0	84.1*	1.163	0.252	0.99	23*
19	9	12-03	0.40	550.0	558.3	44.4	109.6	2.182	0.122	0.96	120
	11	13-03	0.35	543.8	521.2	49.9	105.0	1.824	0.106	0.96	92
	13	14-03	0.30	550.0	535.6	49.3	106.3	1.740	0.155	0.95	62
	15	15-03	0.25	445.0	403.8	43.8	100.6	1.523	0.111	0.93	65
	17	16-03	0.20	416.1	379.0	49.0	102.9	1.387	0.141	0.92	47
	19	17-03	0.15	344.0	294.0	38.2	90.7	0.969	0.130	0.95	33
	21	18-03	0.10	315.2	249.3	43.0	92.7	0.163	0.239	0.97	9
20	23	28-03	0.10	342.1	277.3	36.5	91.7	0.778	0.062	0.88	49
	25	30-03	0.15	416.9	368.4	40.1	98.0	1.023	0.167	0.98	29
	27	31-03	0.20	437.2	362.3	41.6	92.4	1.395	0.103	0.99	62
	29	02-04	0.25	526.2	455.4	31.2	92.5	1.747	0.057	0.60	138
	31	03-04	0.30	537.1	491.0	54.2	101.5	1.859	0.052	0.70	165
	33	04-04	0.35	545.1	499.0	42.5	99.3	1.988	0.073	0.86	147
	35	05-04	0.40	621.2	585.2	38.8	100.4	2.196	0.082	0.86	166
				MEAN			97.8			0.90	
				Std. Deviation			6.9			0.11	

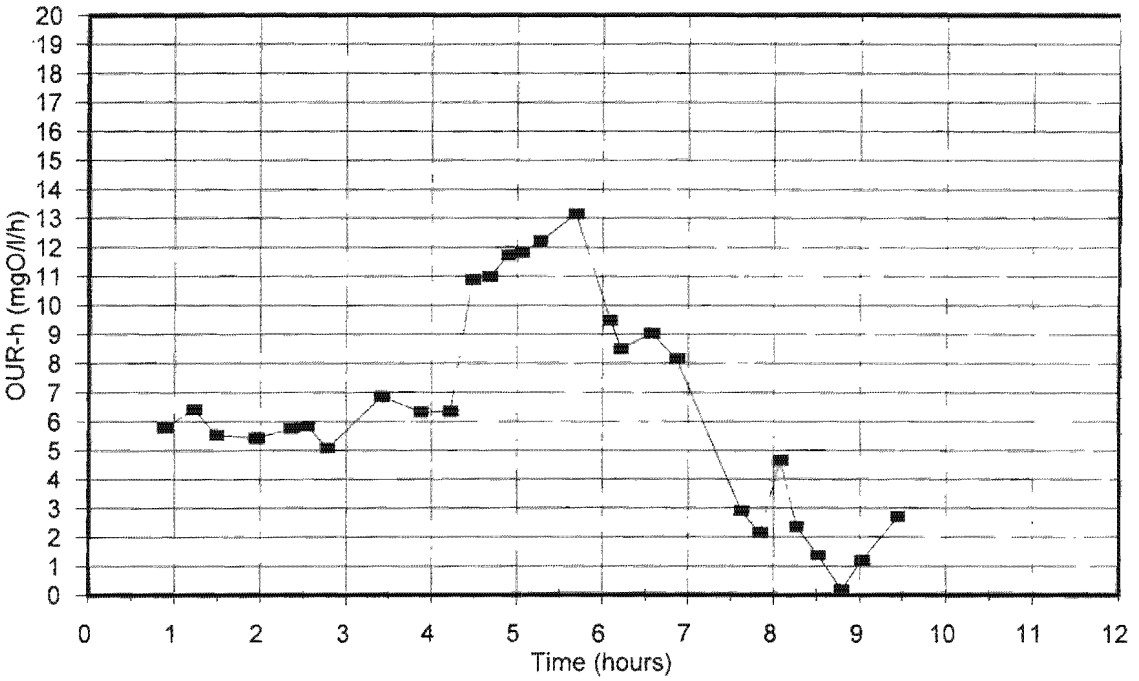
* Batch test rejected on account of poor %COD recovery



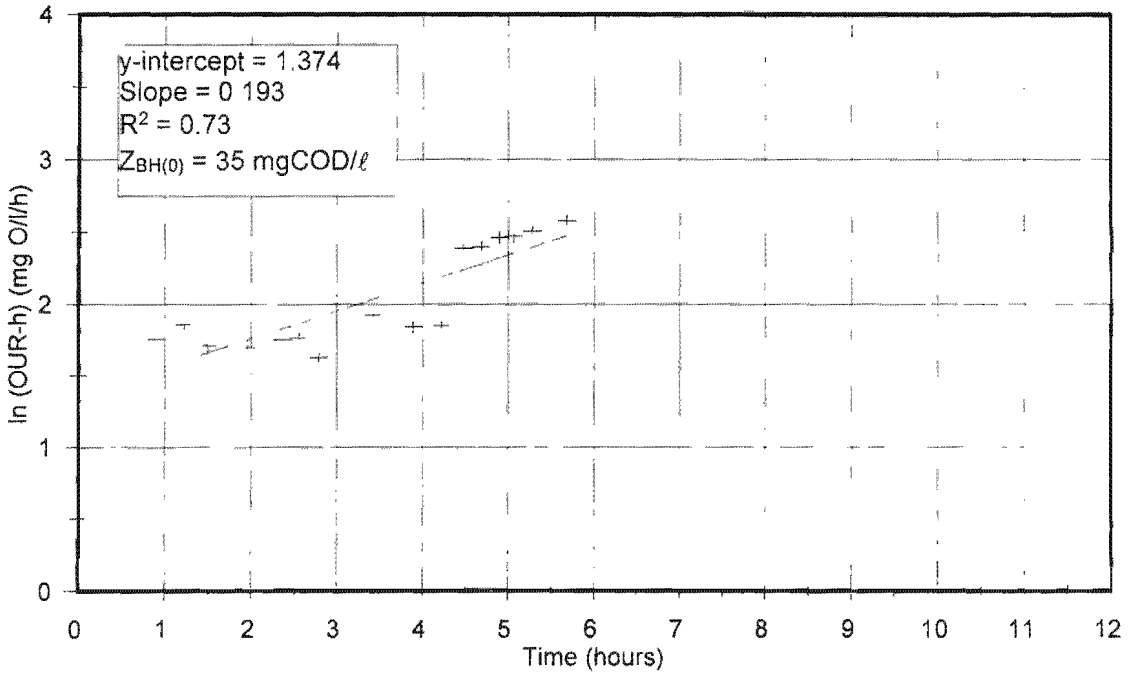
OUR graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ)
Batch Test No. 1, 28-02, Sewage Batch No. 18



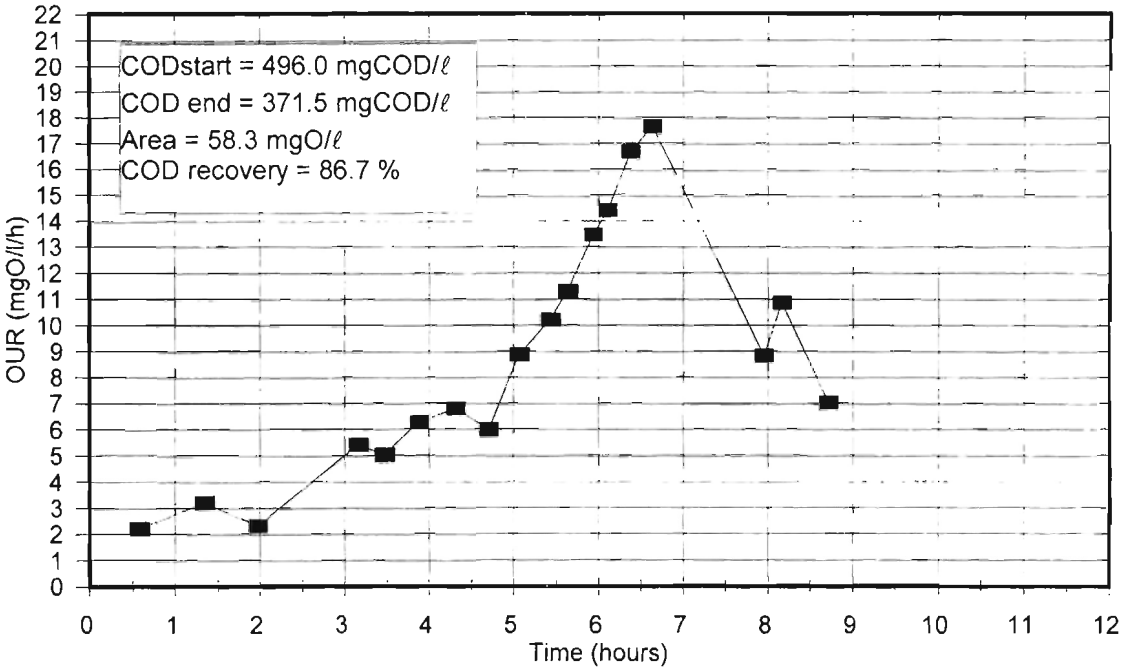
NO_3/NO_2 graph for for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ)
Batch Test No. 1, 28-02, Sewage Batch No. 18



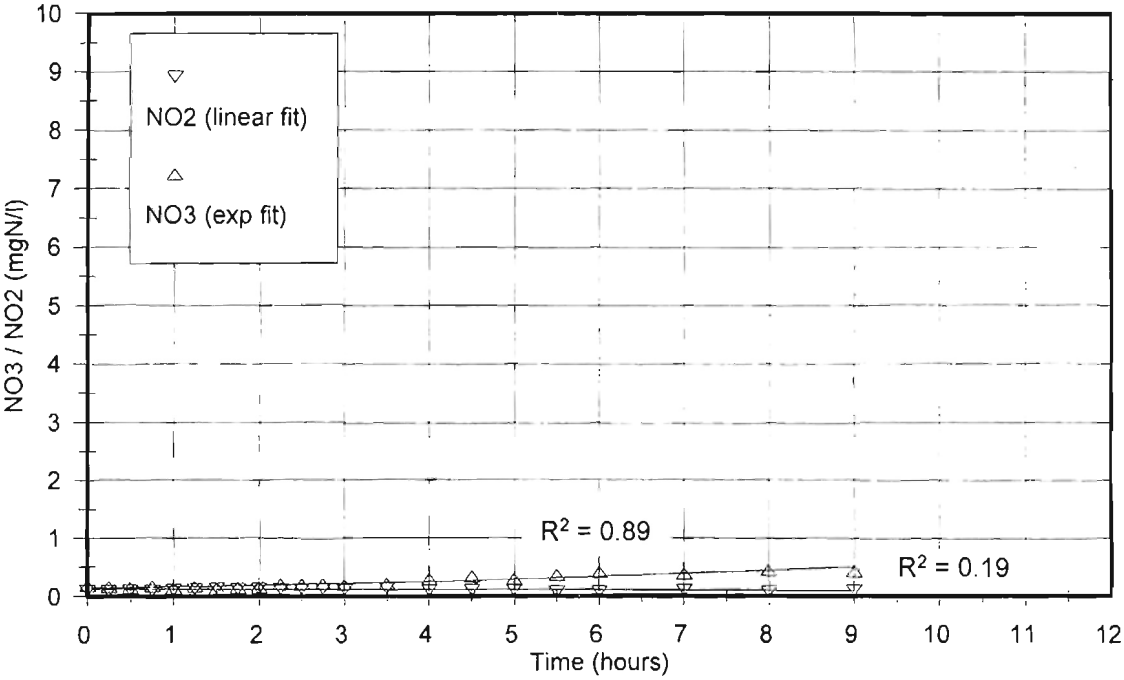
OUR-h graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ)
Batch Test No. 1, 28-02, Sewage Batch No. 18



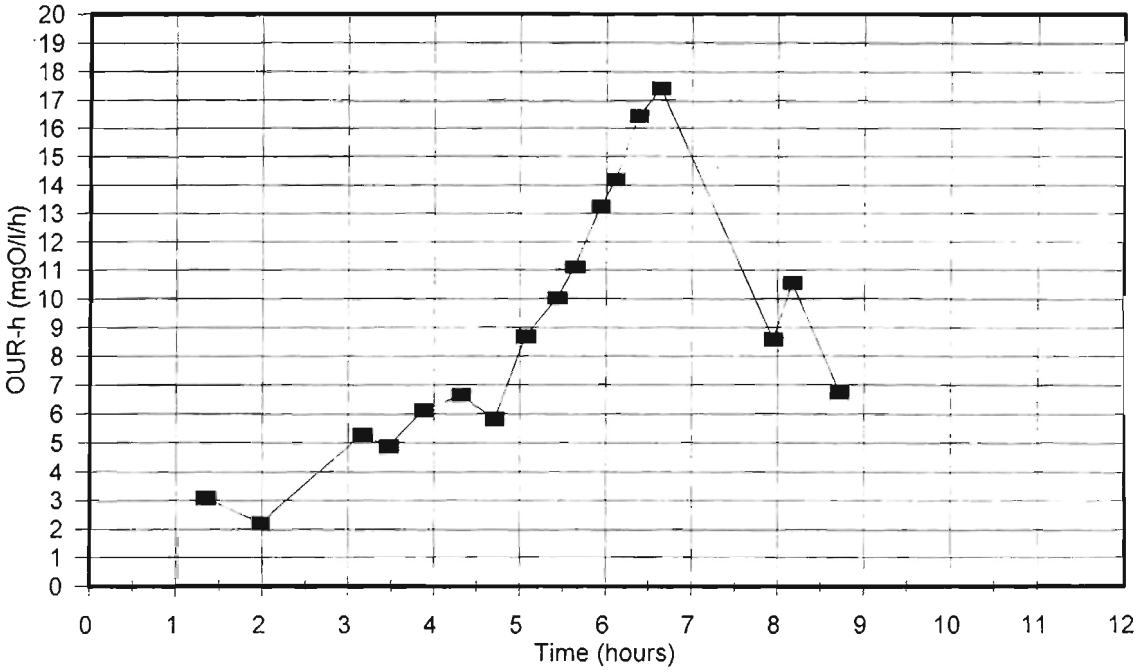
ℓn(OUR-h) graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ)
Batch Test No. 1, 28-02, Sewage Batch No. 18



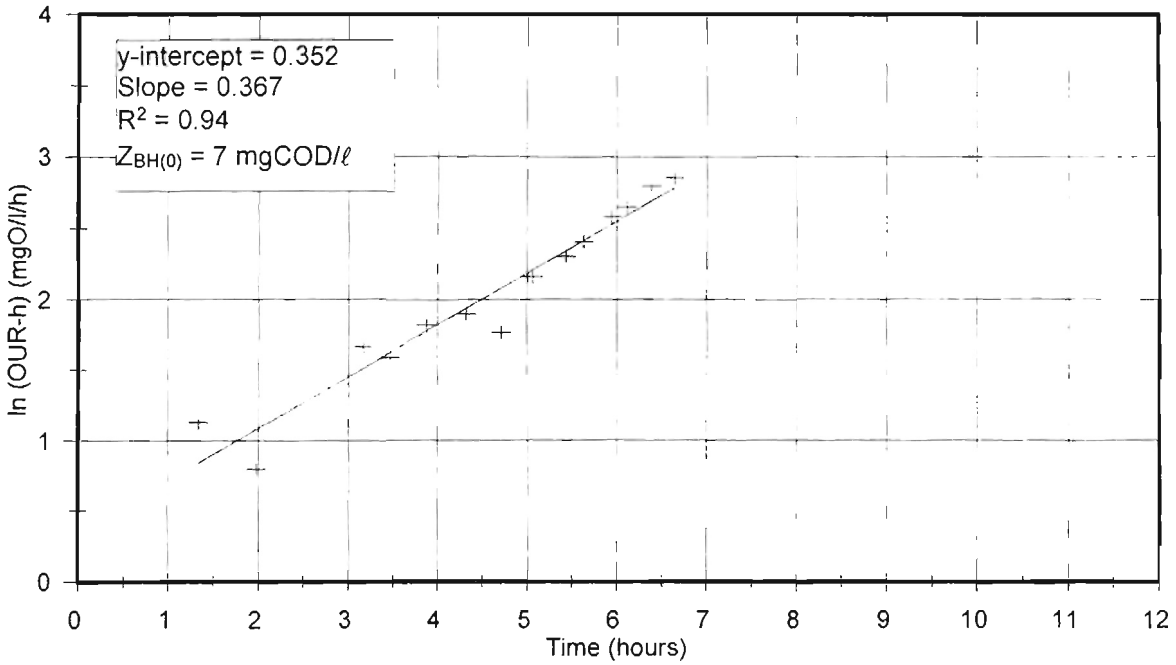
OUR graph for filtered wastewater (2.70ℓ) plus mixed liquor (0.30ℓ)
Batch Test No. 3, 02-03, Sewage Batch No. 18



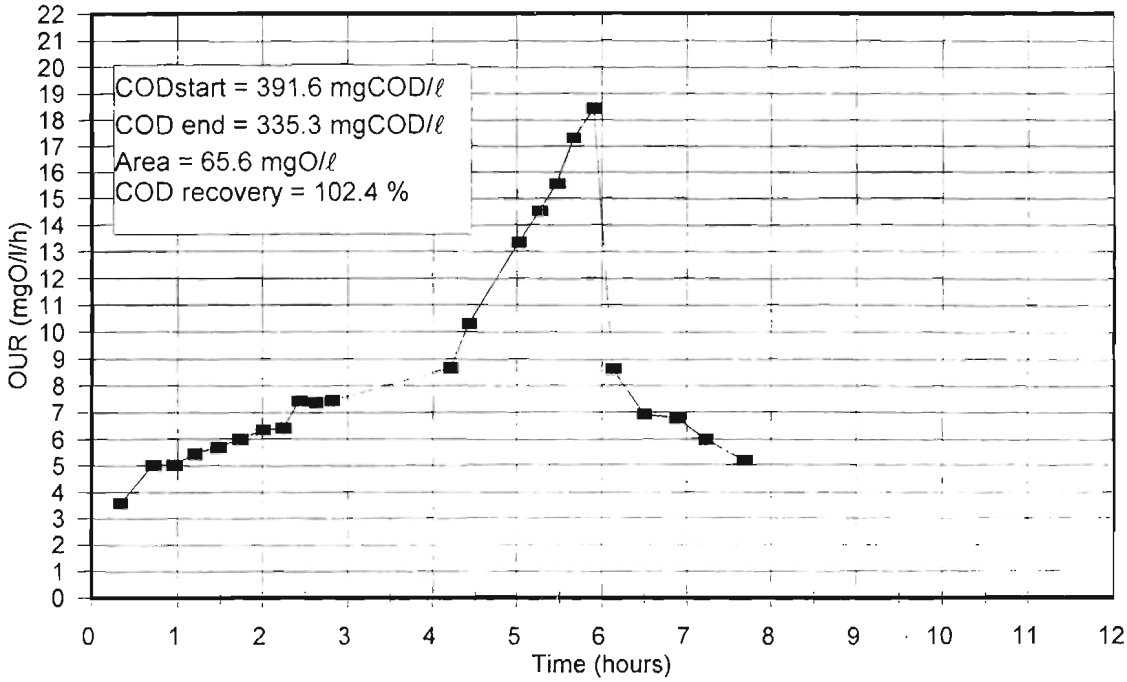
NO_3/NO_2 graph for for filtered wastewater (2.70ℓ) plus mixed liquor (0.30ℓ)
Batch Test No. 3, 02-03, Sewage Batch No. 18



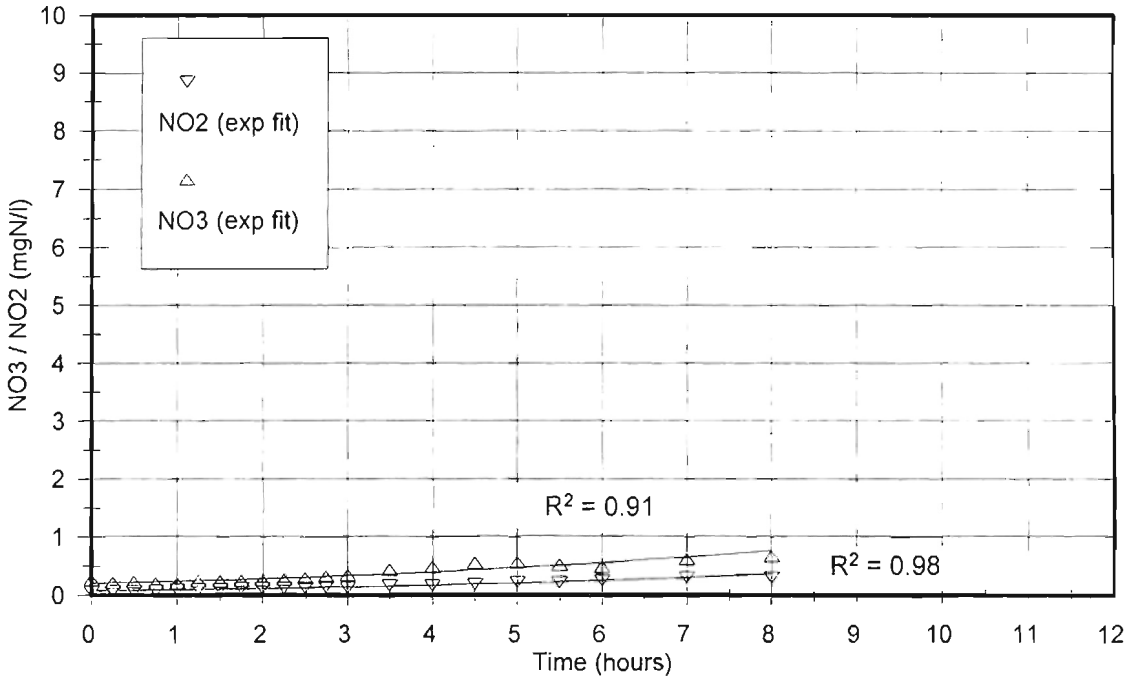
OUR-h graph for filtered wastewater (2.70ℓ) plus mixed liquor (0.30ℓ)
Batch Test No. 3, 02-03, Sewage Batch No. 18



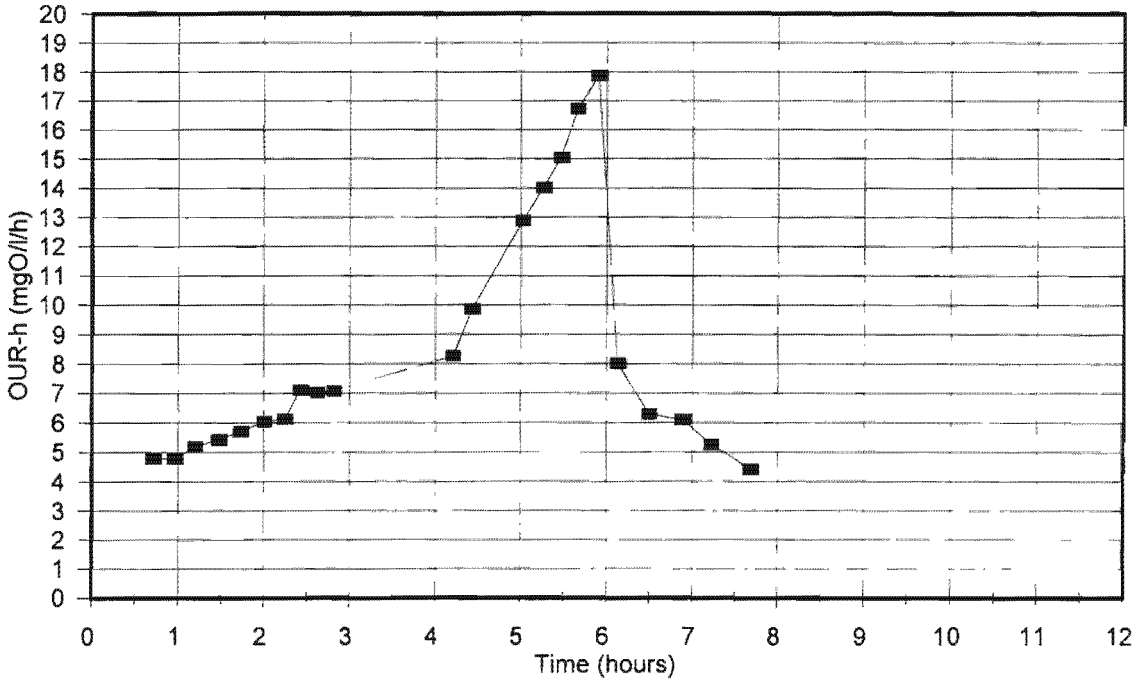
ℓn(OUR-h) graph for filtered wastewater (2.70ℓ) plus mixed liquor (0.30ℓ)
Batch Test No. 3, 02-03, Sewage Batch No. 18



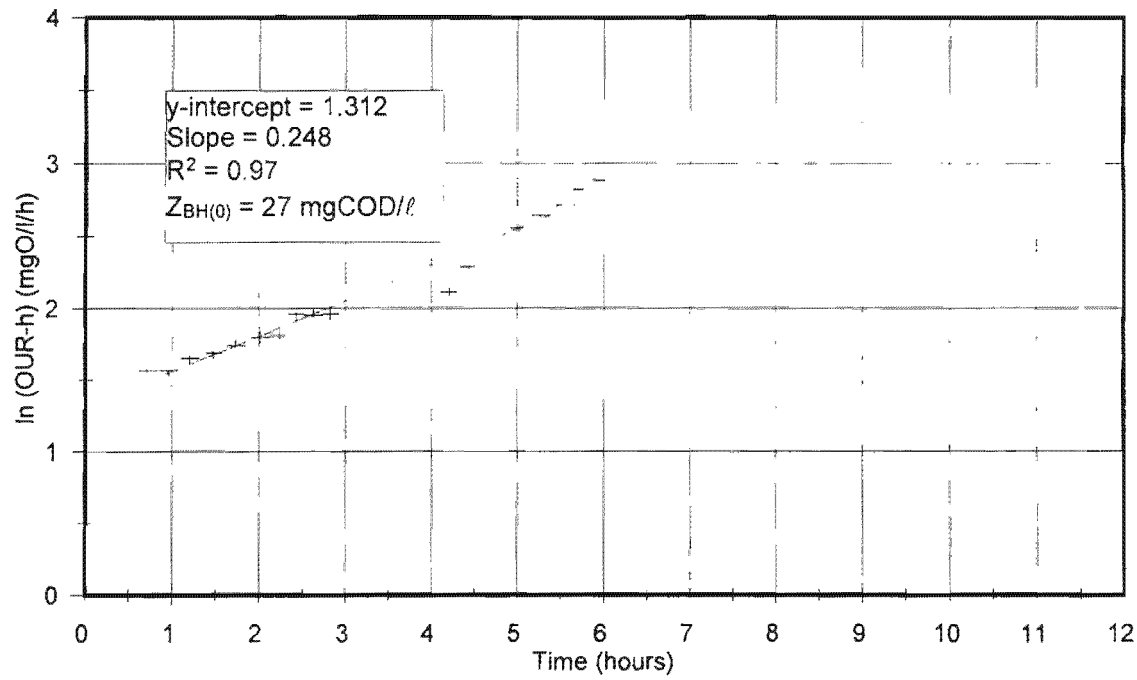
OUR graph for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 5, 04-03, Sewage Batch No. 18



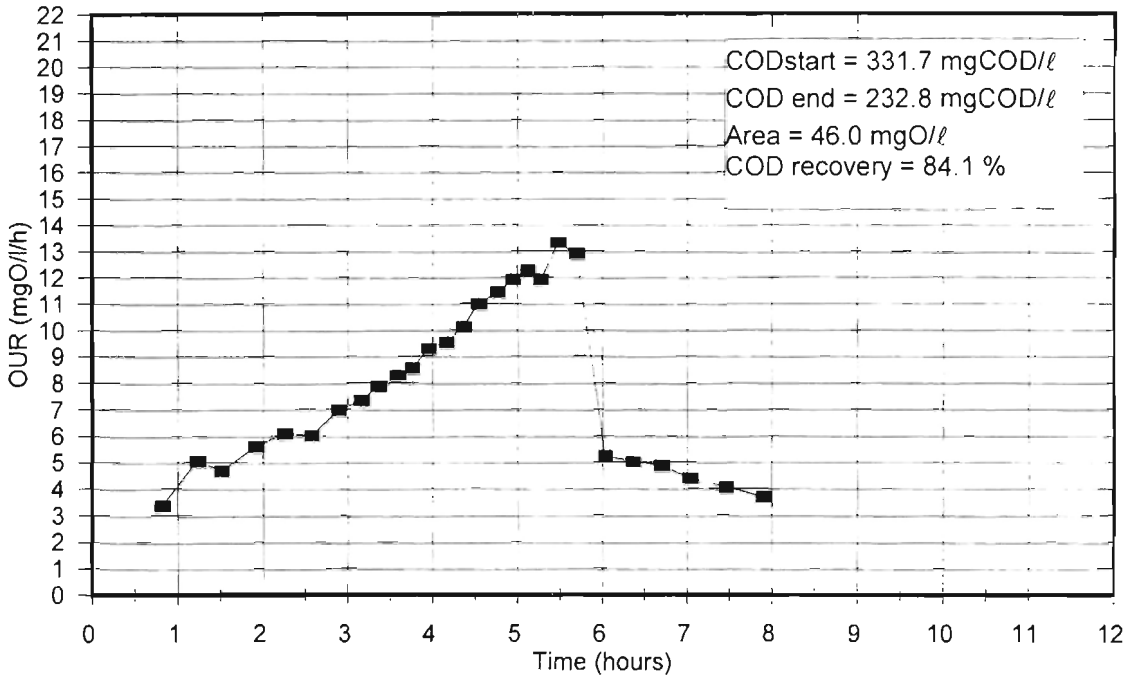
NO_3/NO_2 graph for for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 5, 04-03, Sewage Batch No. 18



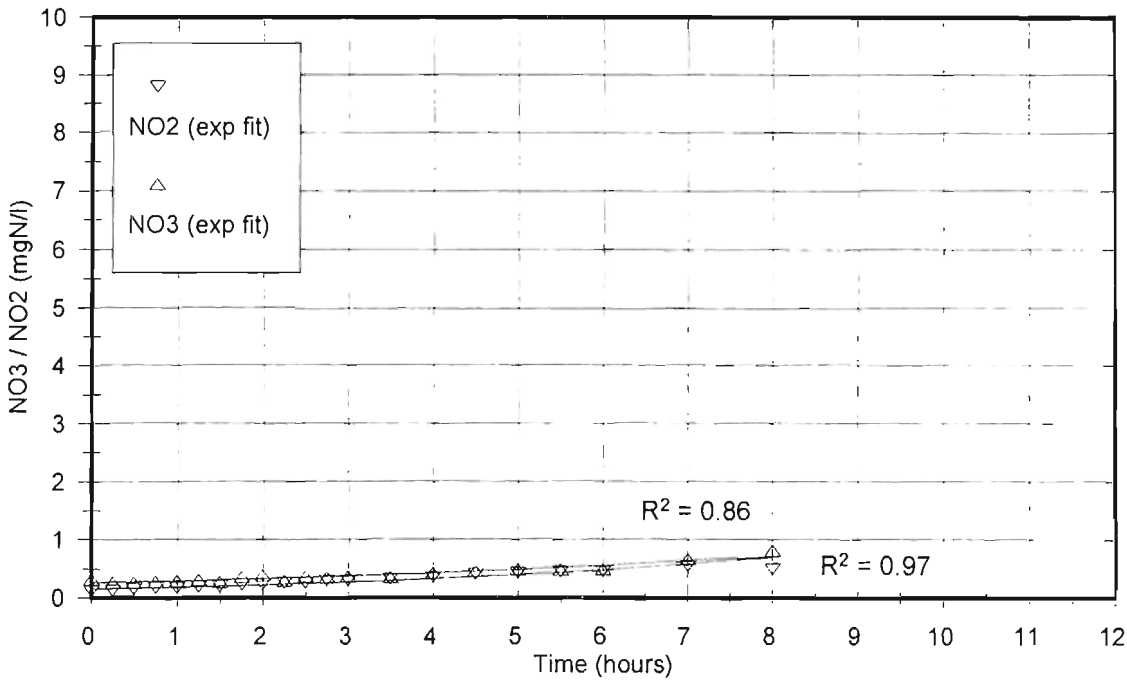
OUR-h graph for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 5, 04-03, Sewage Batch No. 18



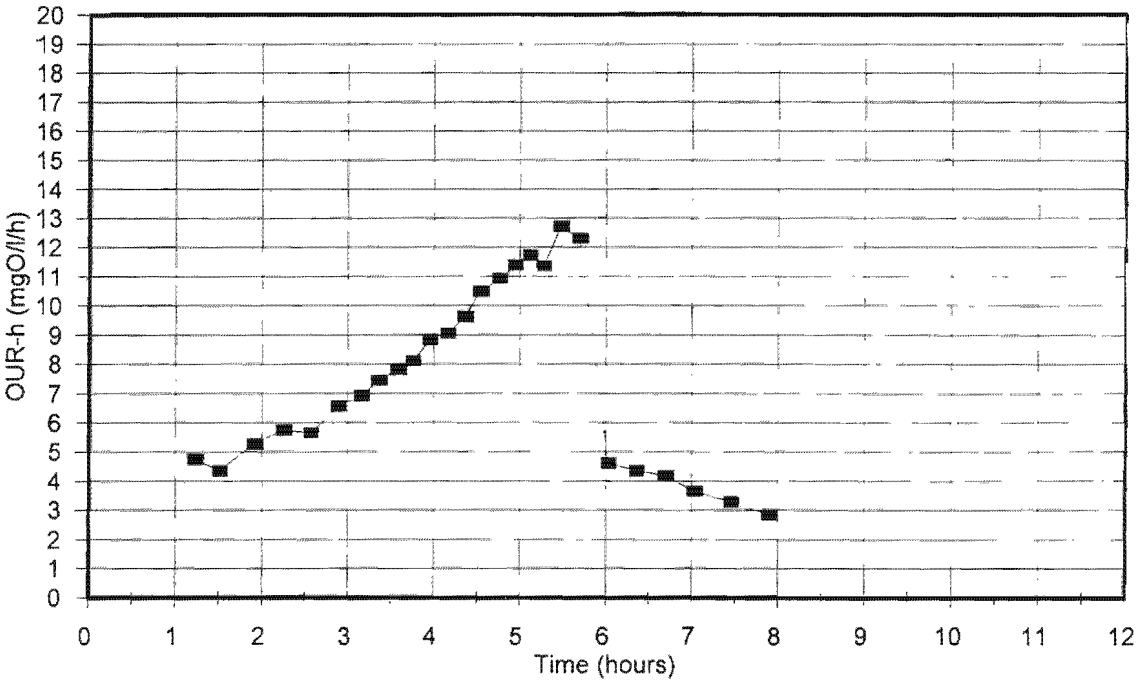
ℓn(OUR-h) graph for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 5, 04-03, Sewage Batch No. 18



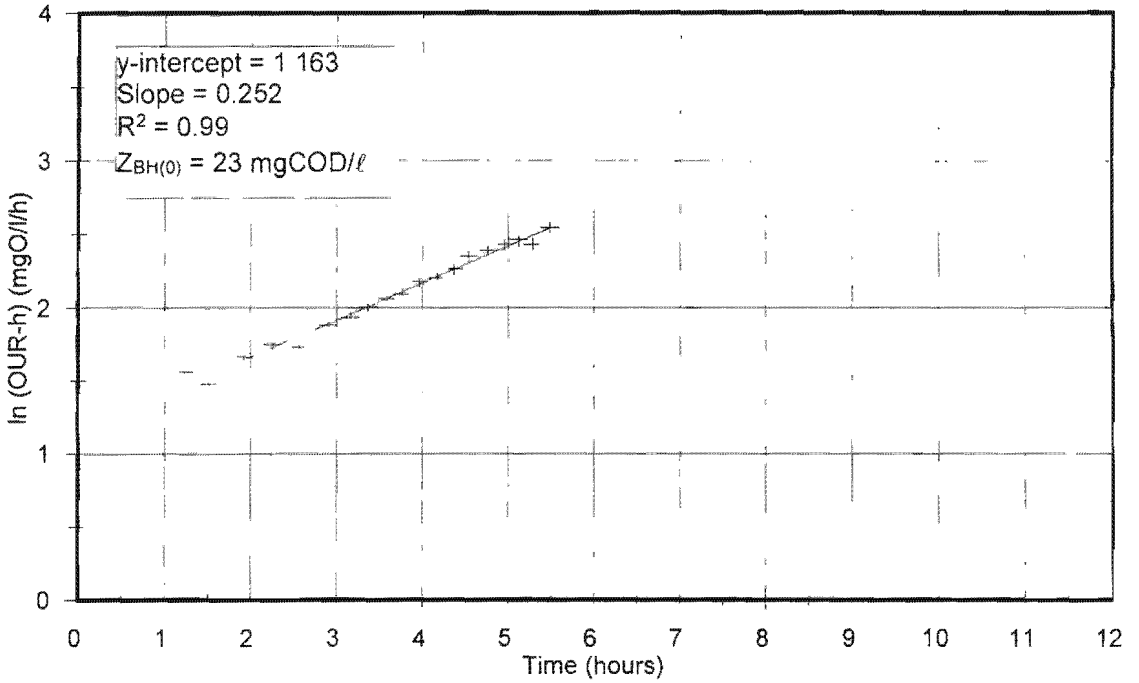
OUR graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ)
Batch Test No. 7, 05-03, Sewage Batch No. 18



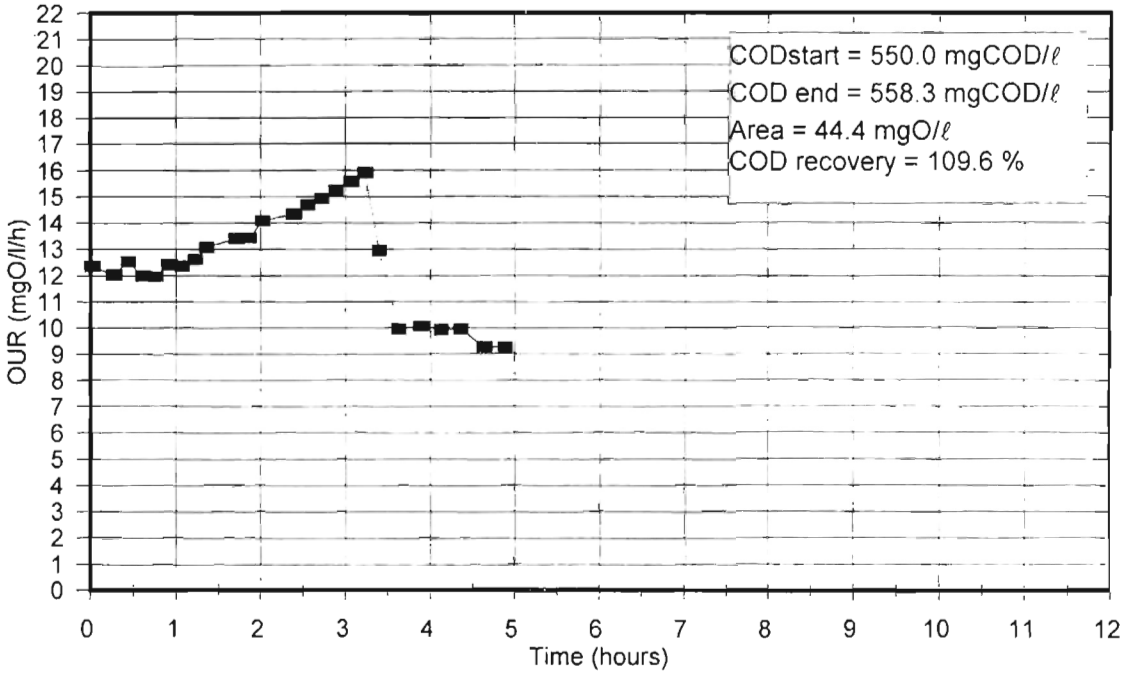
NO_3/NO_2 graph for for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ)
Batch Test No. 7, 05-03, Sewage Batch No. 18



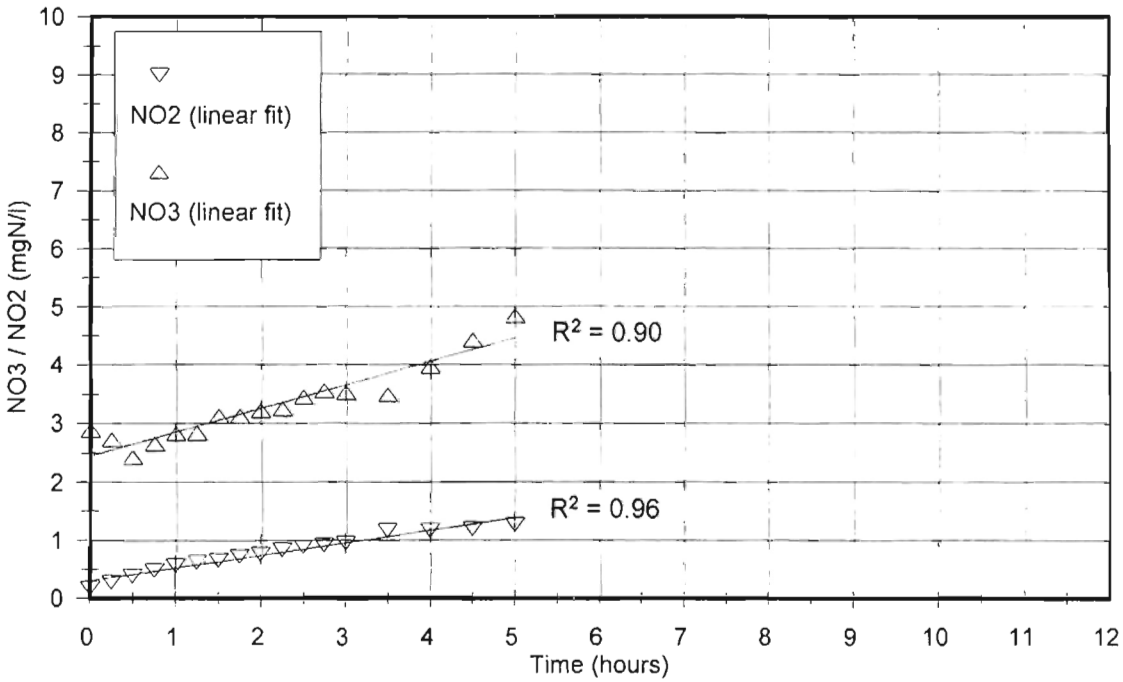
OUR-h graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ)
Batch Test No. 7, 05-03, Sewage Batch No. 18



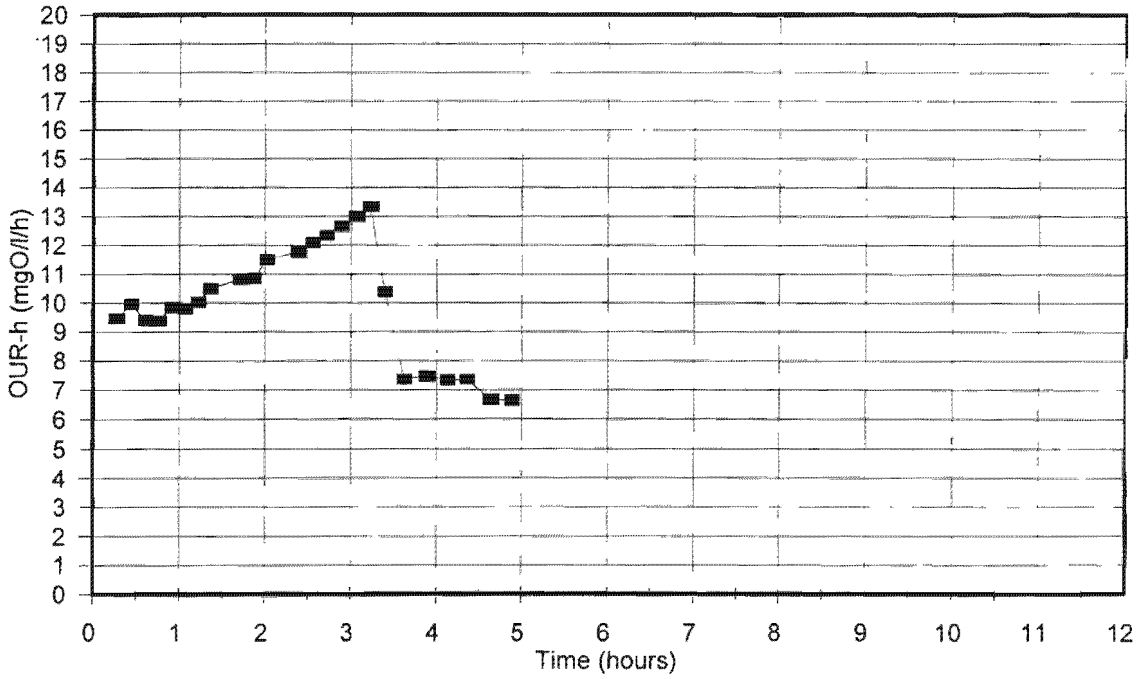
ℓn(OUR-h) graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ)
Batch Test No. 7, 05-03, Sewage Batch No. 18



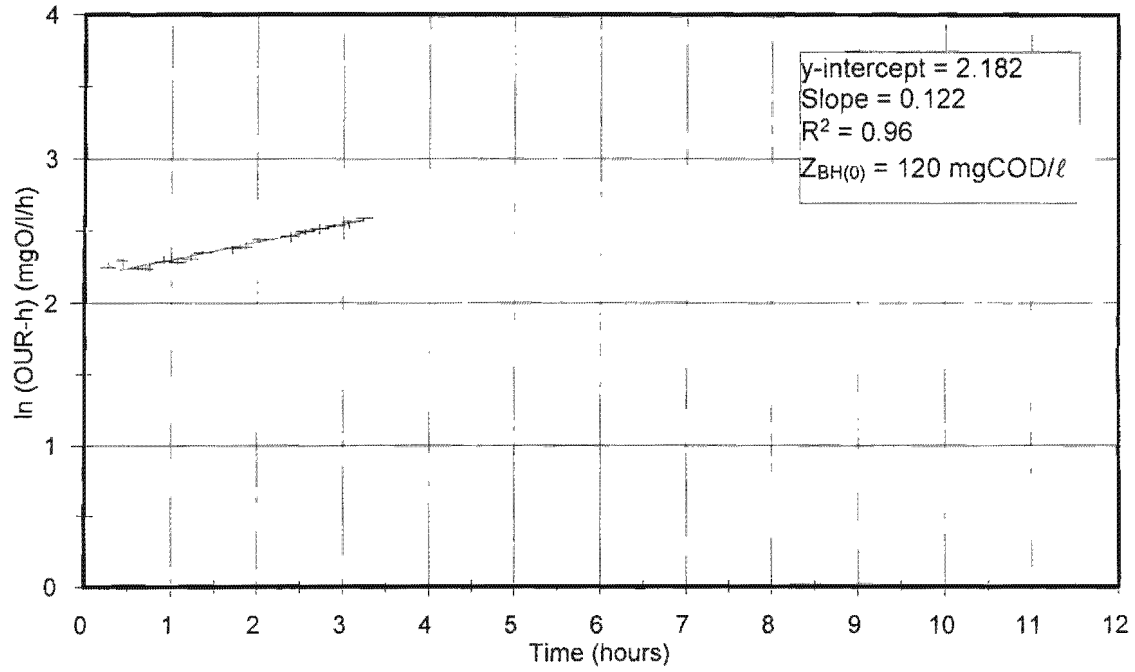
OUR graph for filtered wastewater (2.60ℓ) plus mixed liquor (0.40ℓ)
Batch Test No. 9, 12-03, Sewage Batch No. 19



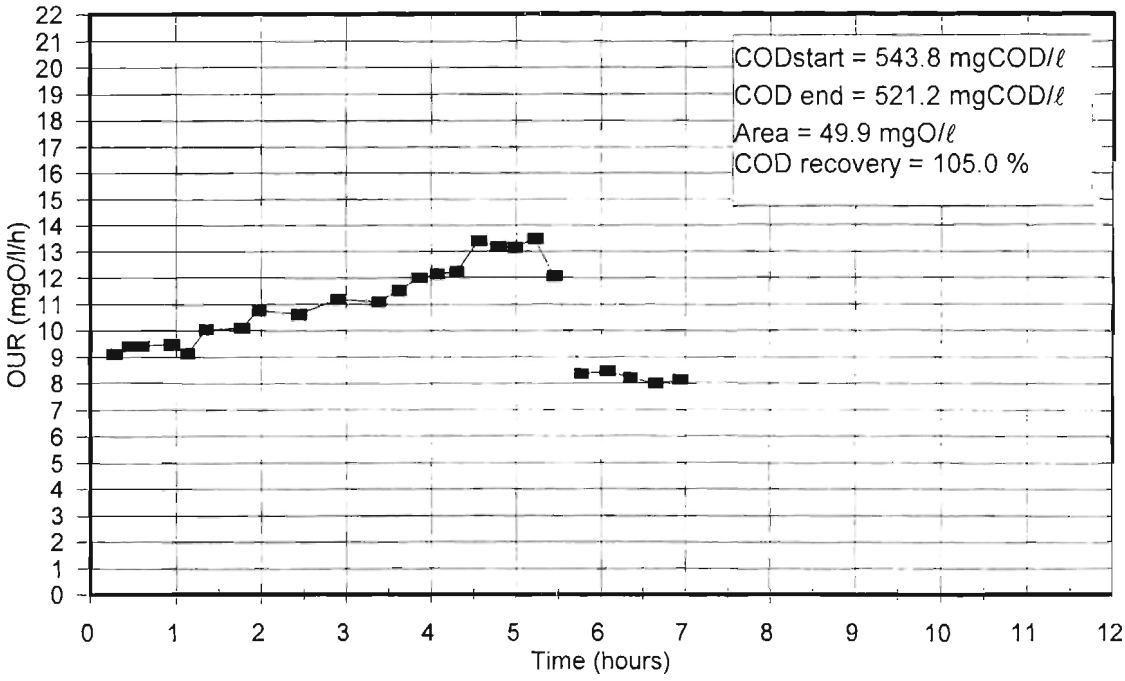
NO_3/NO_2 graph for for filtered wastewater (2.60ℓ) plus mixed liquor (0.40ℓ)
Batch Test No. 9, 12-03, Sewage Batch No. 19



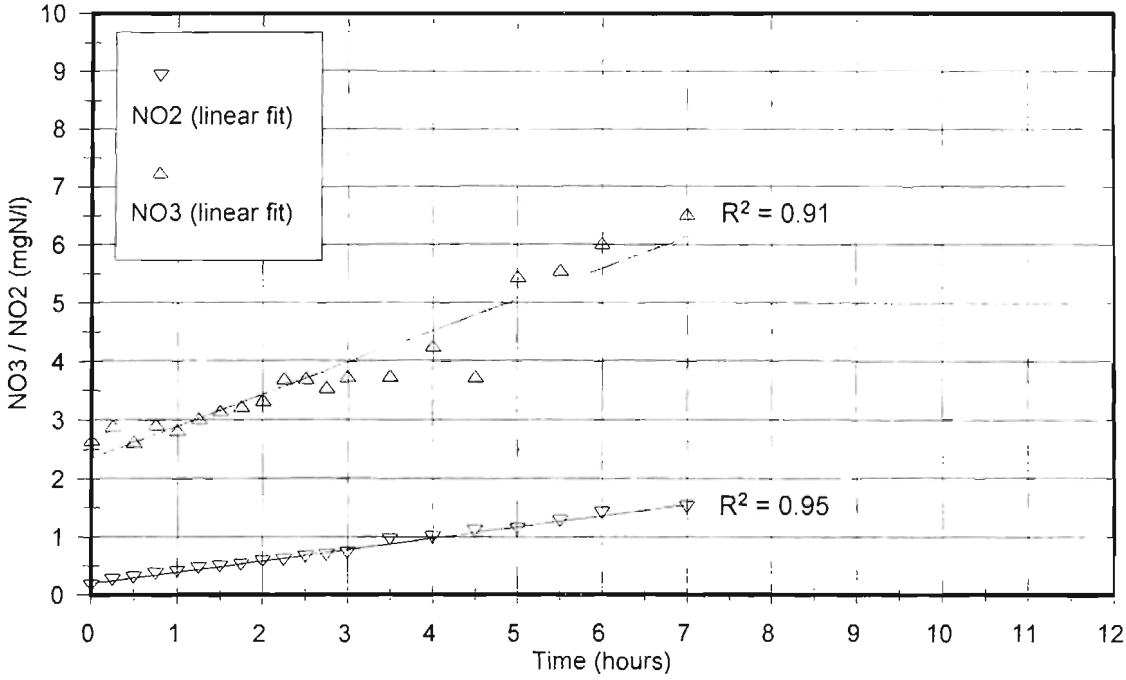
OUR-h graph for filtered wastewater (2.60ℓ) plus mixed liquor (0.40ℓ)
Batch Test No. 9, 12-03, Sewage Batch No. 19



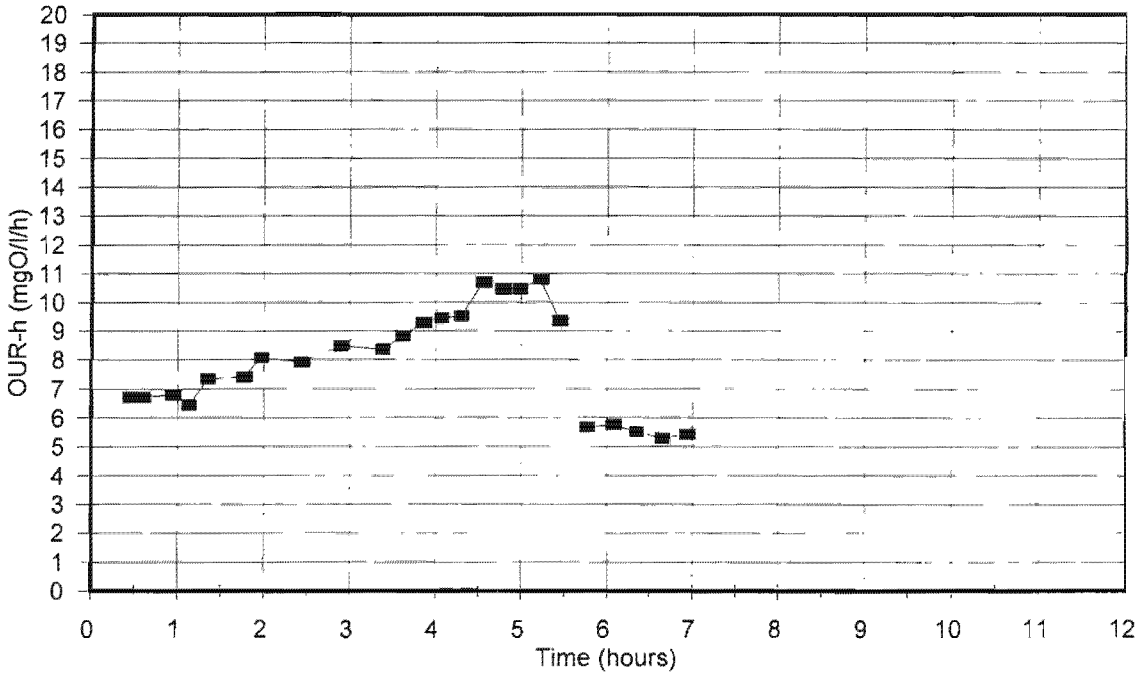
ℓn(OUR-h) graph for filtered wastewater (2.60ℓ) plus mixed liquor (0.40ℓ)
Batch Test No. 9, 12-03, Sewage Batch No. 19



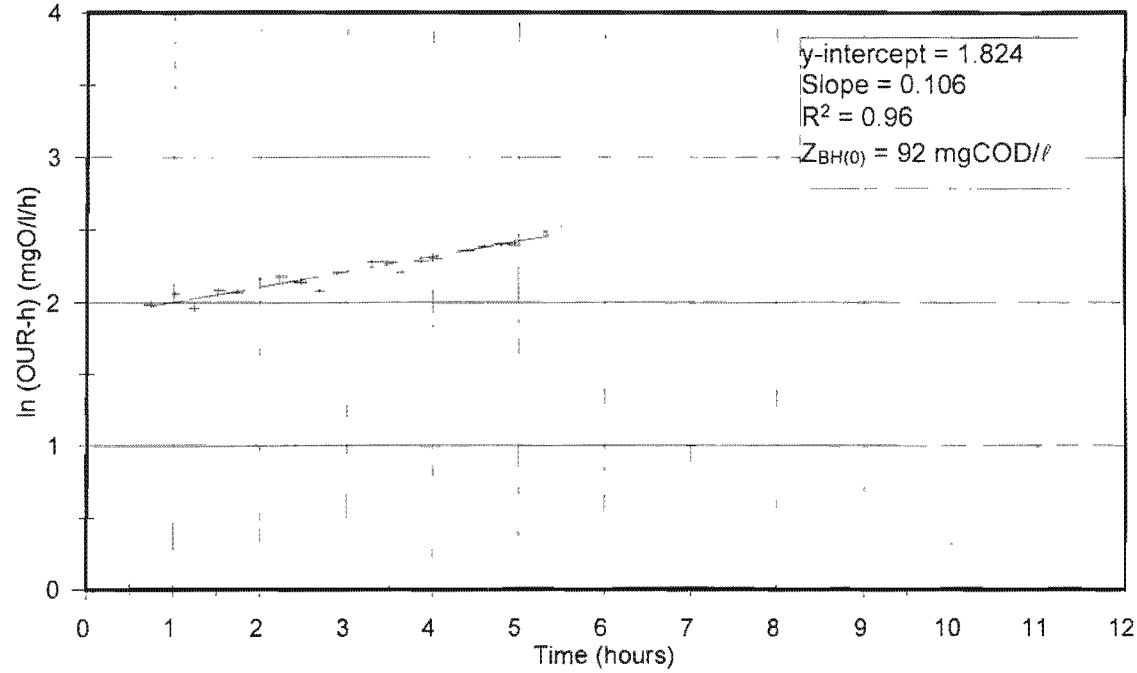
OUR graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 11, 13-03, Sewage Batch No. 19



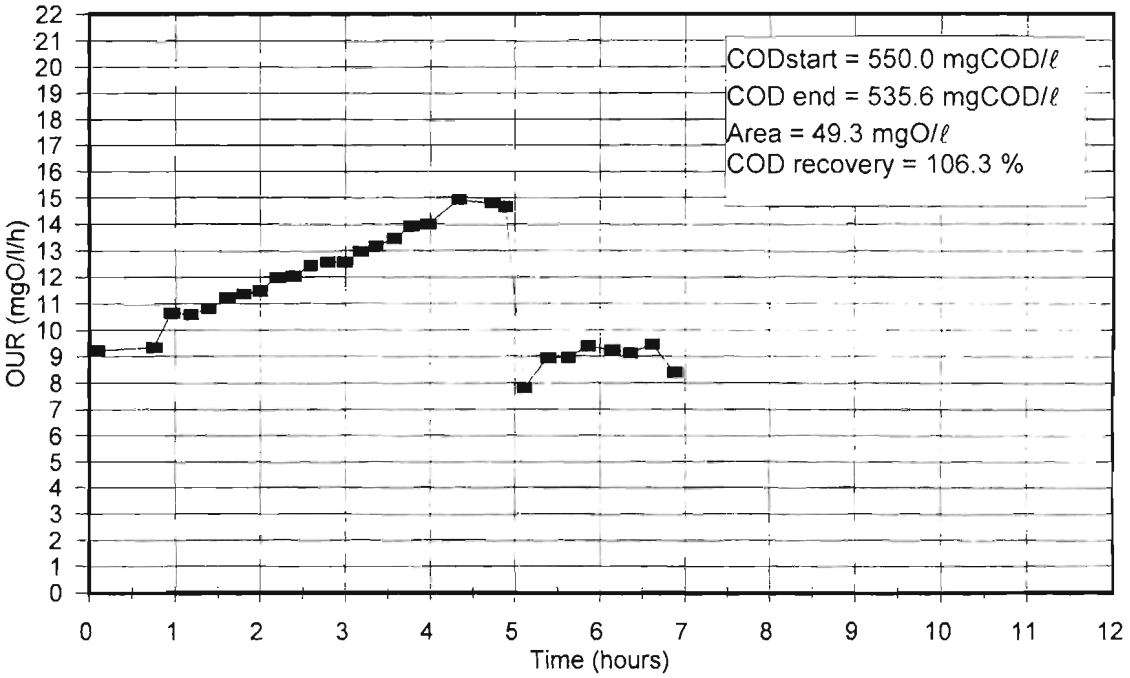
NO_3/NO_2 graph for for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 11, 13-03, Sewage Batch No. 19



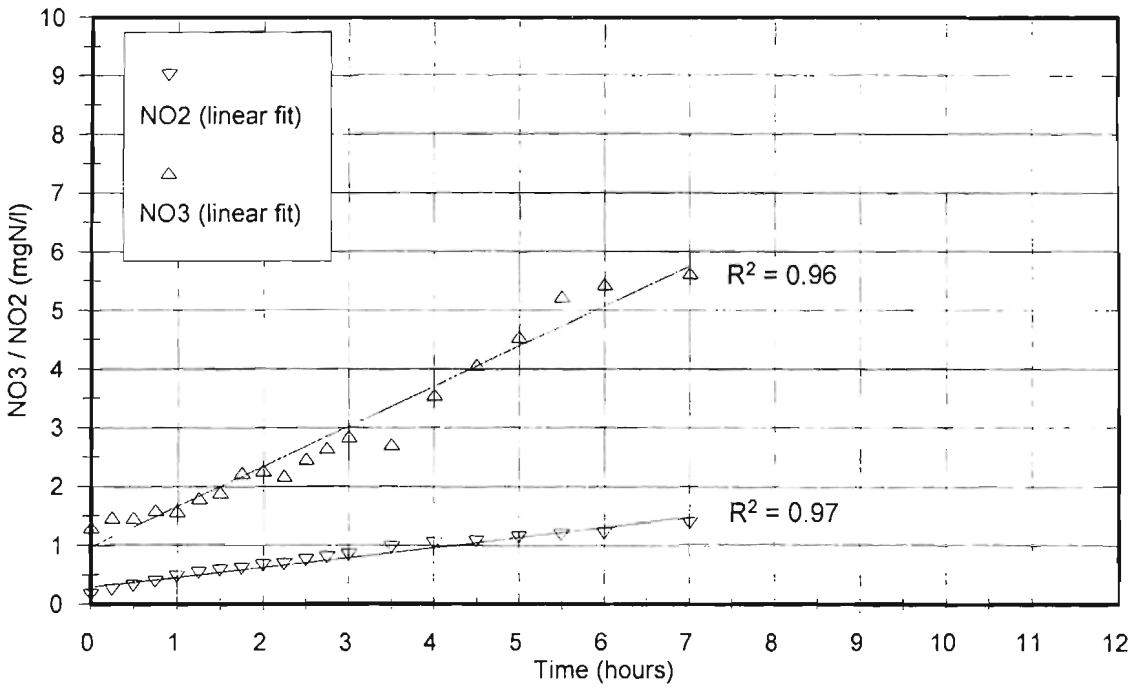
OUR-h graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 11, 13-03, Sewage Batch No. 19



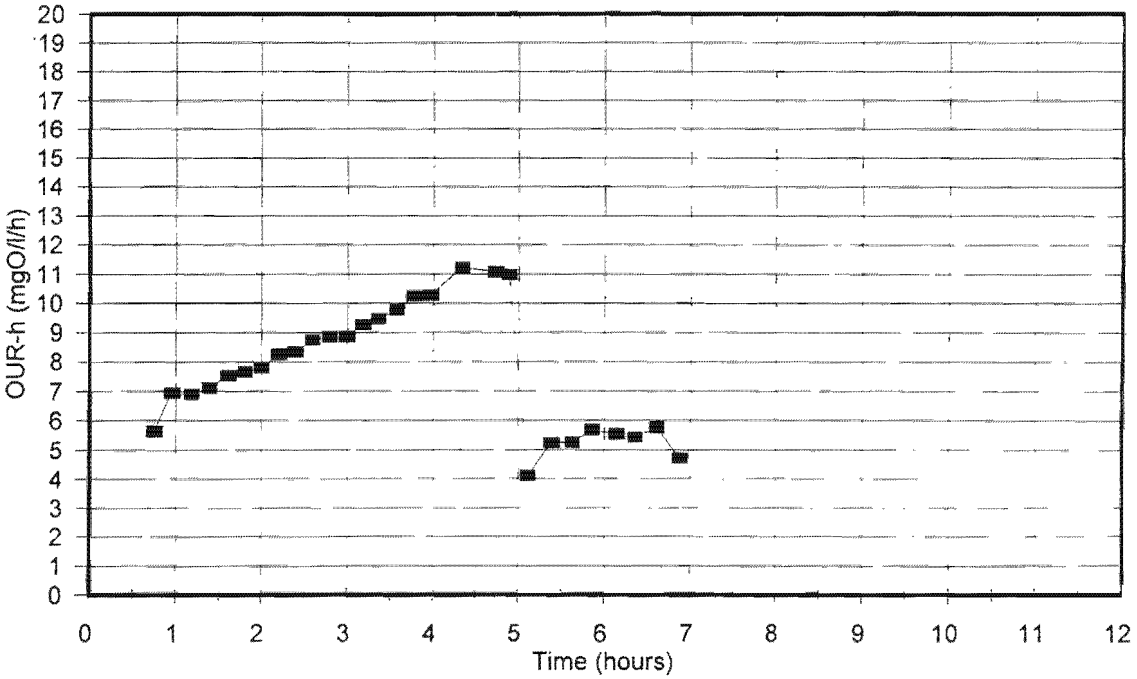
ℓn(OUR-h) graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 11, 13-03, Sewage Batch No. 19



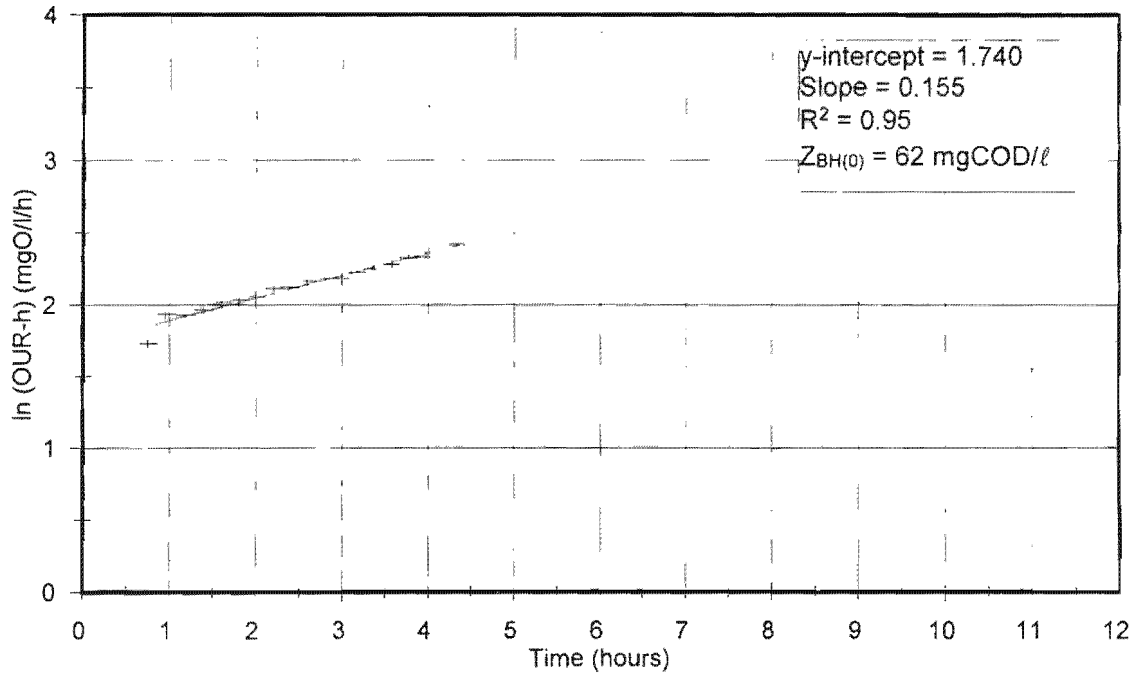
OUR graph for filtered wastewater (2.70ℓ) plus mixed liquor (0.30ℓ)
Batch Test No. 13, 14-03, Sewage Batch No. 19



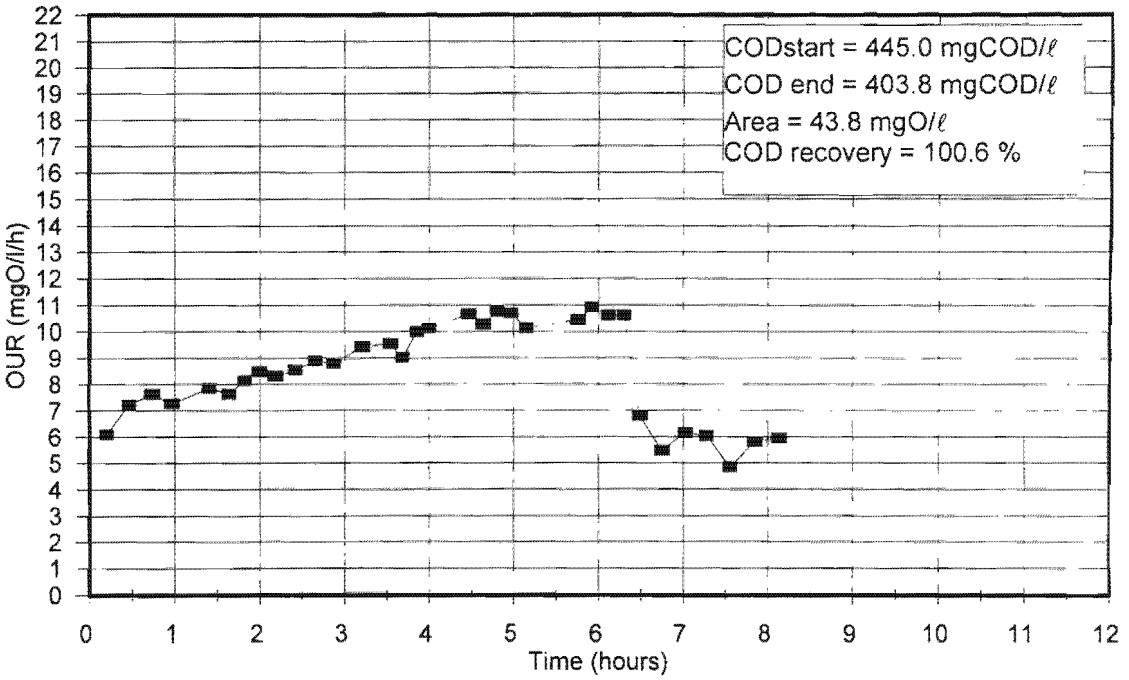
NO_3/NO_2 graph for for filtered wastewater (2.70ℓ) plus mixed liquor (0.30ℓ)
Batch Test No. 13, 14-03, Sewage Batch No. 19



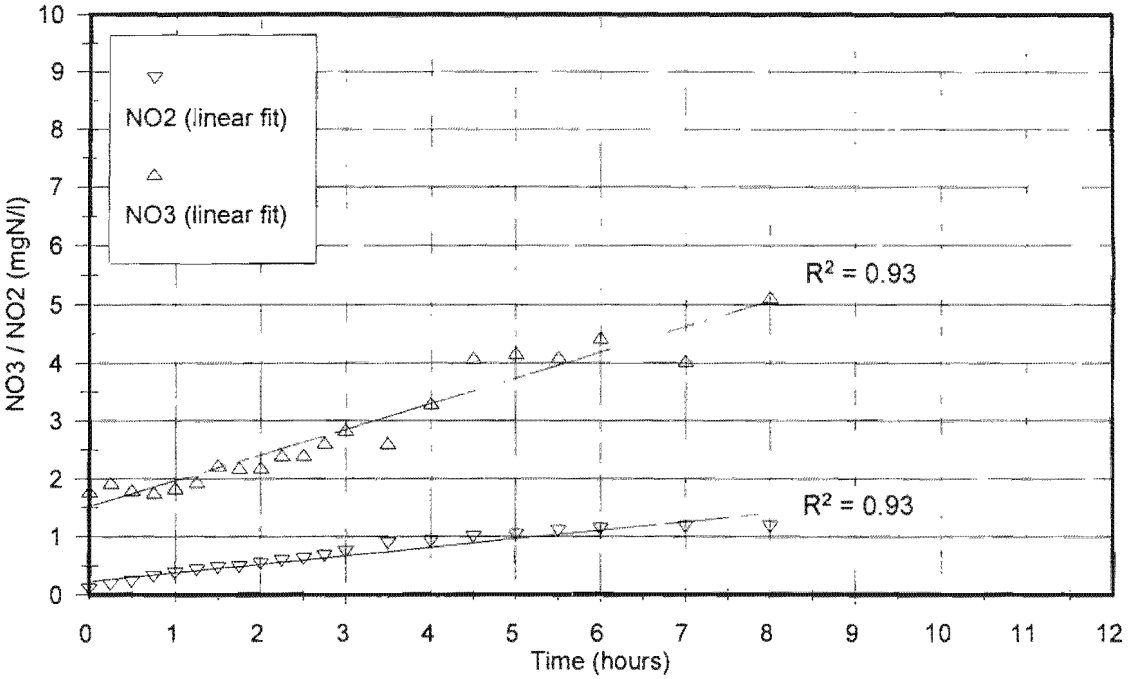
OUR-h graph for filtered wastewater (2.70ℓ) plus mixed liquor (0.30ℓ)
Batch Test No. 13, 14-03, Sewage Batch No. 19



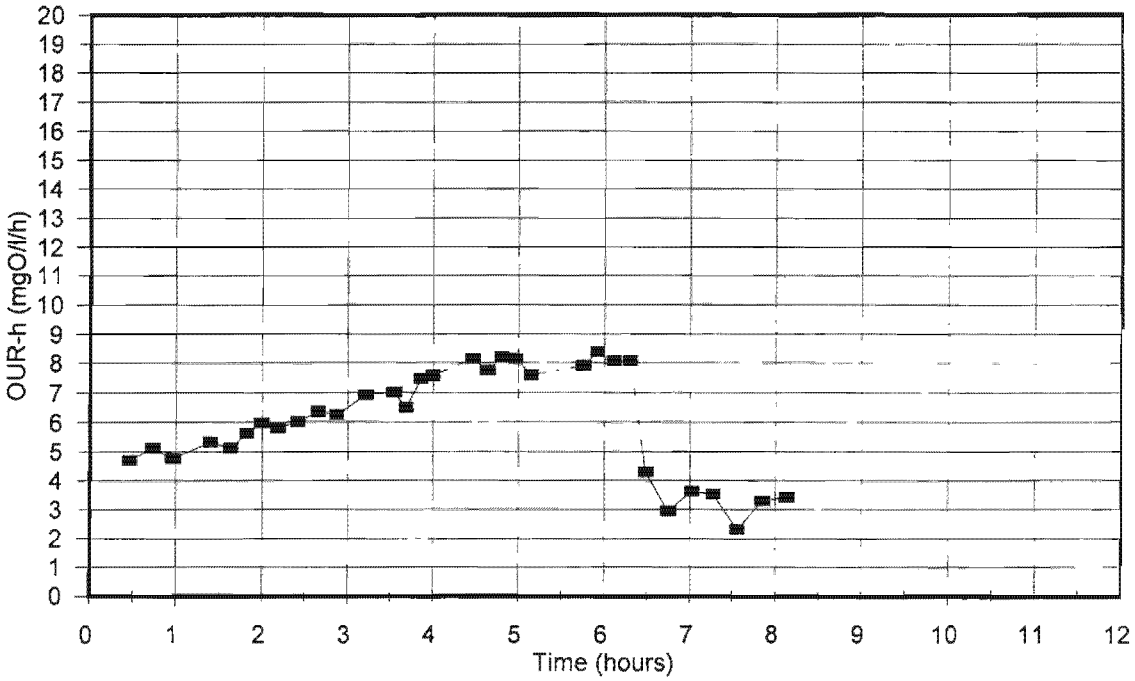
ℓn(OUR-h) graph for filtered wastewater (2.70ℓ) plus mixed liquor (0.30ℓ)
Batch Test No. 13, 14-03, Sewage Batch No. 19



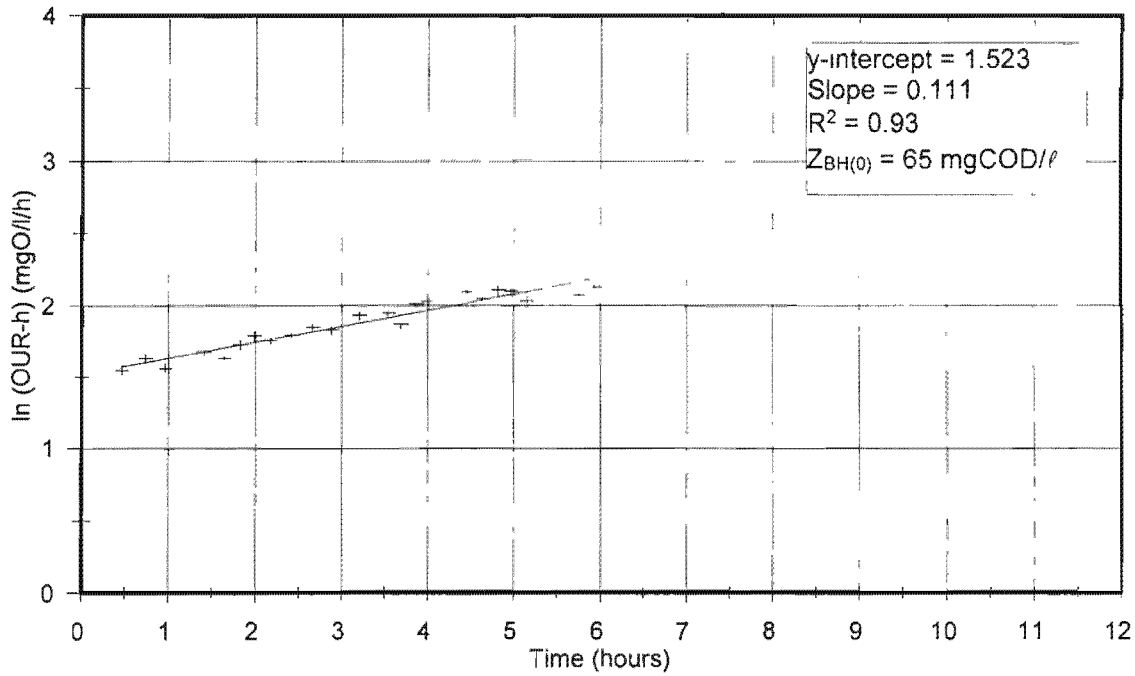
OUR graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ)
Batch Test No. 15, 15-03, Sewage Batch No. 19



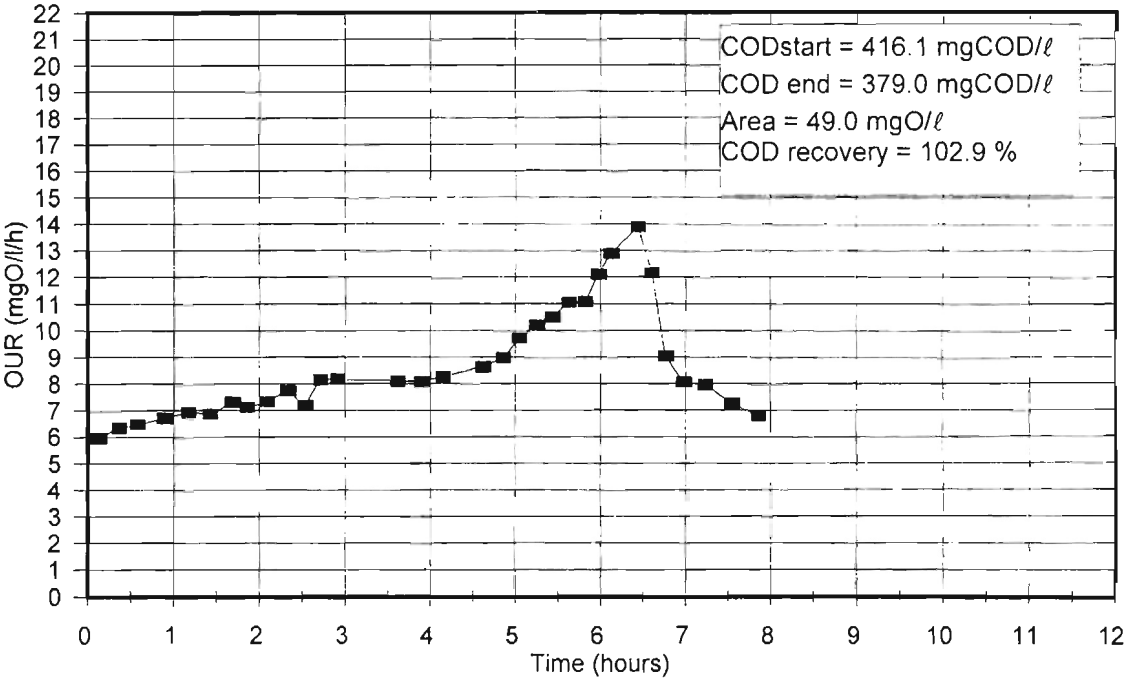
NO_3/NO_2 graph for for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ)
Batch Test No. 15, 15-03, Sewage Batch No. 19



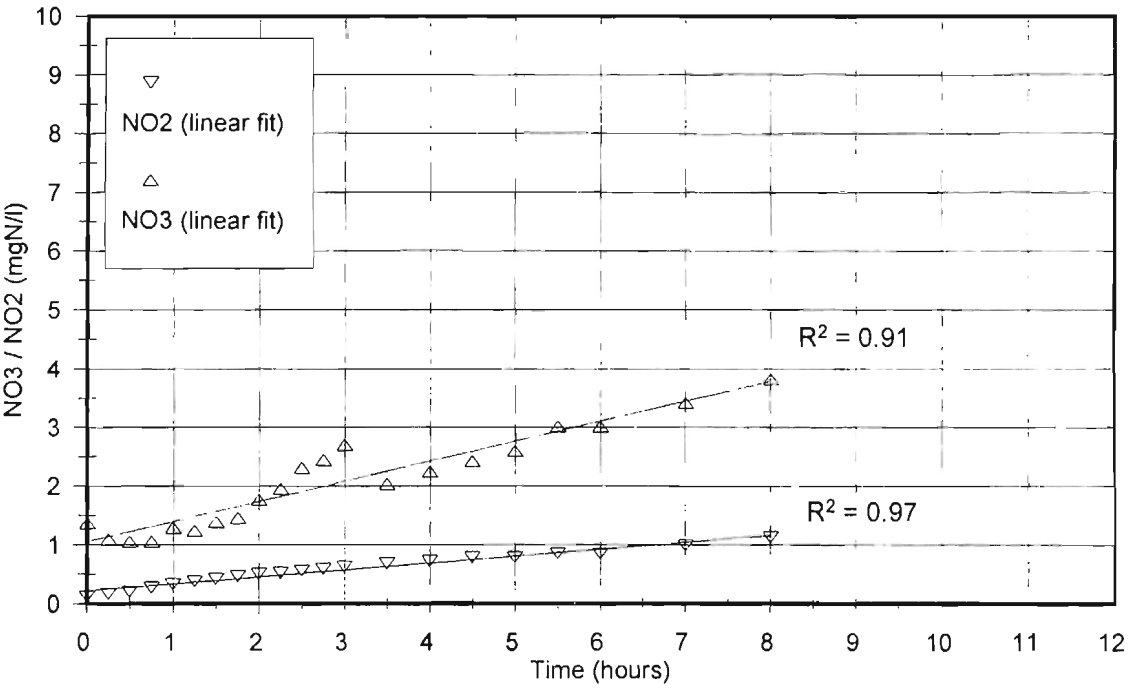
OUR-h graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ)
Batch Test No. 15, 15-03, Sewage Batch No. 19



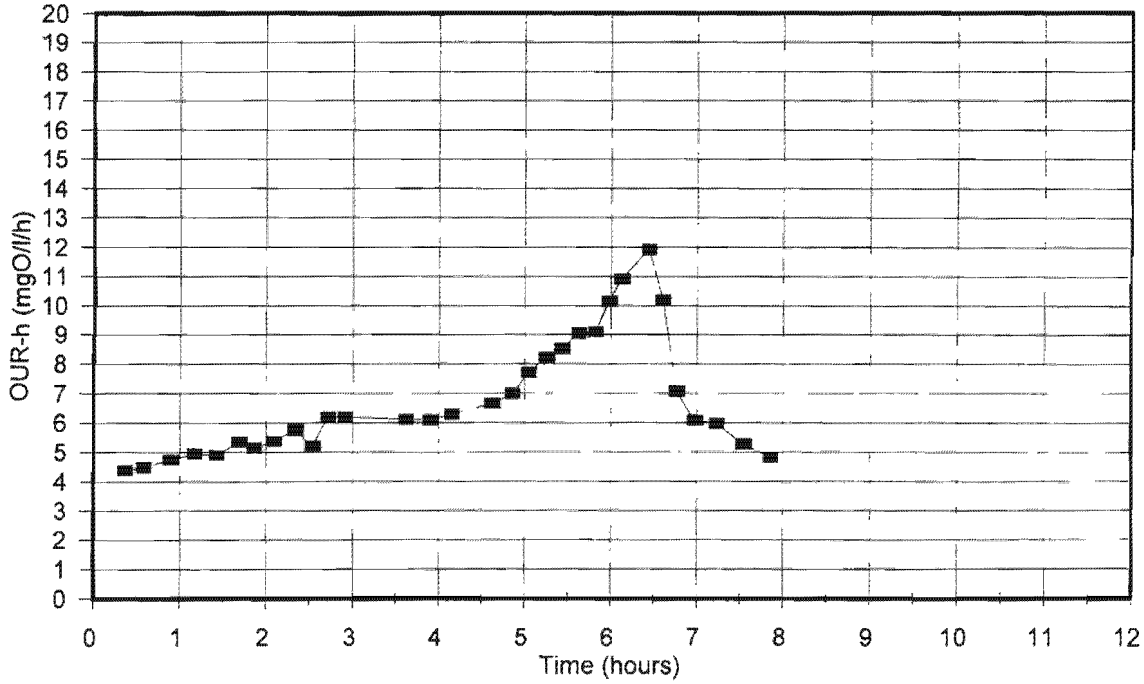
ℓn(OUR-h) graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ)
Batch Test No. 15, 15-03, Sewage Batch No. 19



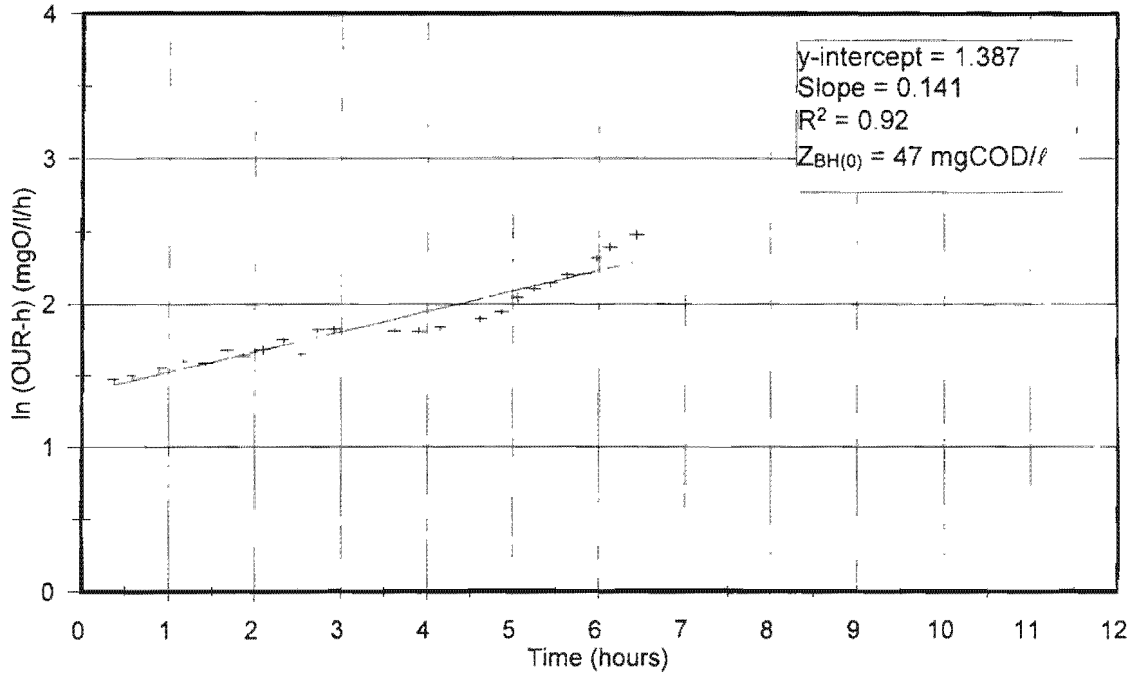
OUR graph for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 17, 16-03, Sewage Batch No. 19



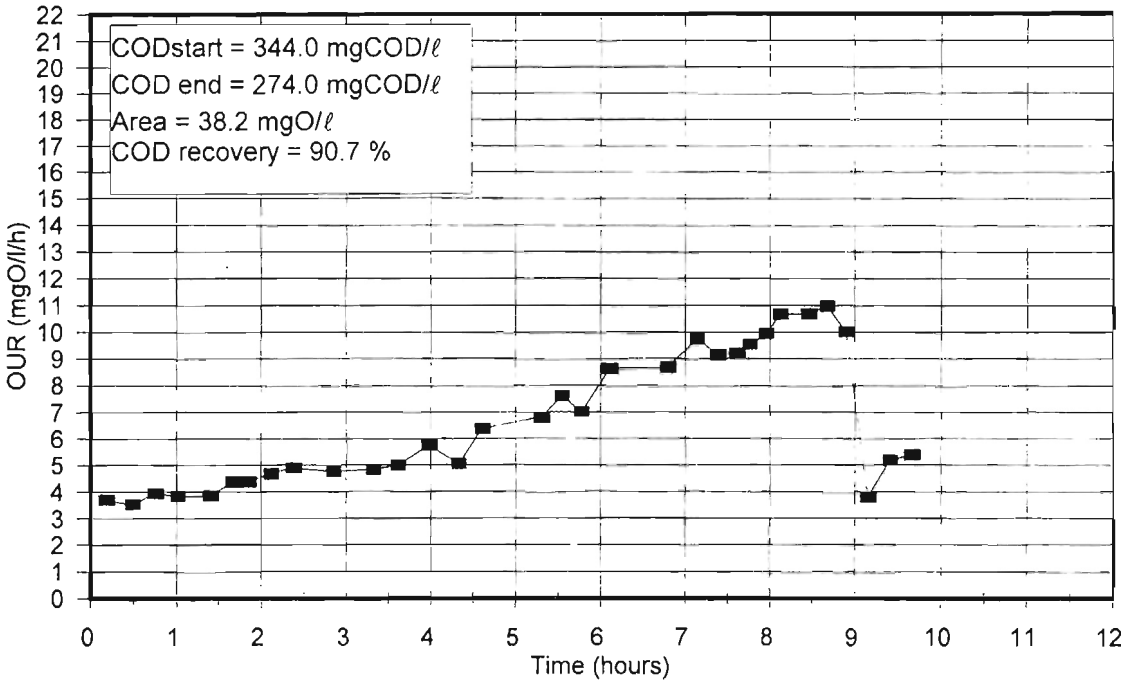
NO_3/NO_2 graph for for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 17, 16-03, Sewage Batch No. 19



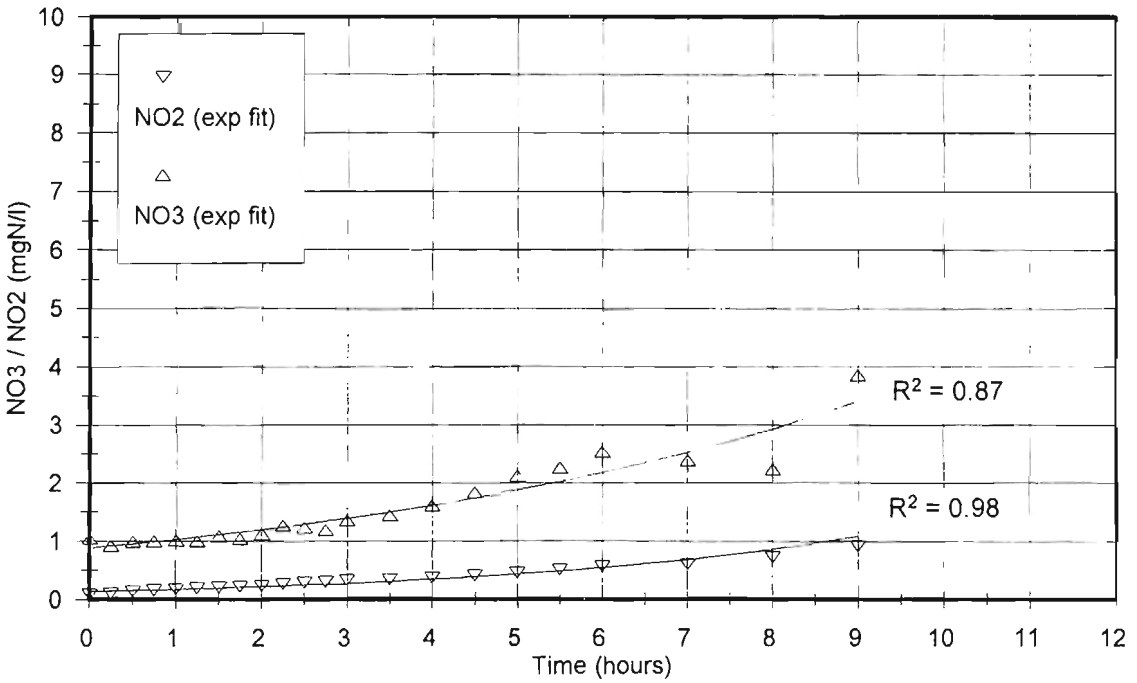
OUR-h graph for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 17, 16-03, Sewage Batch No. 19



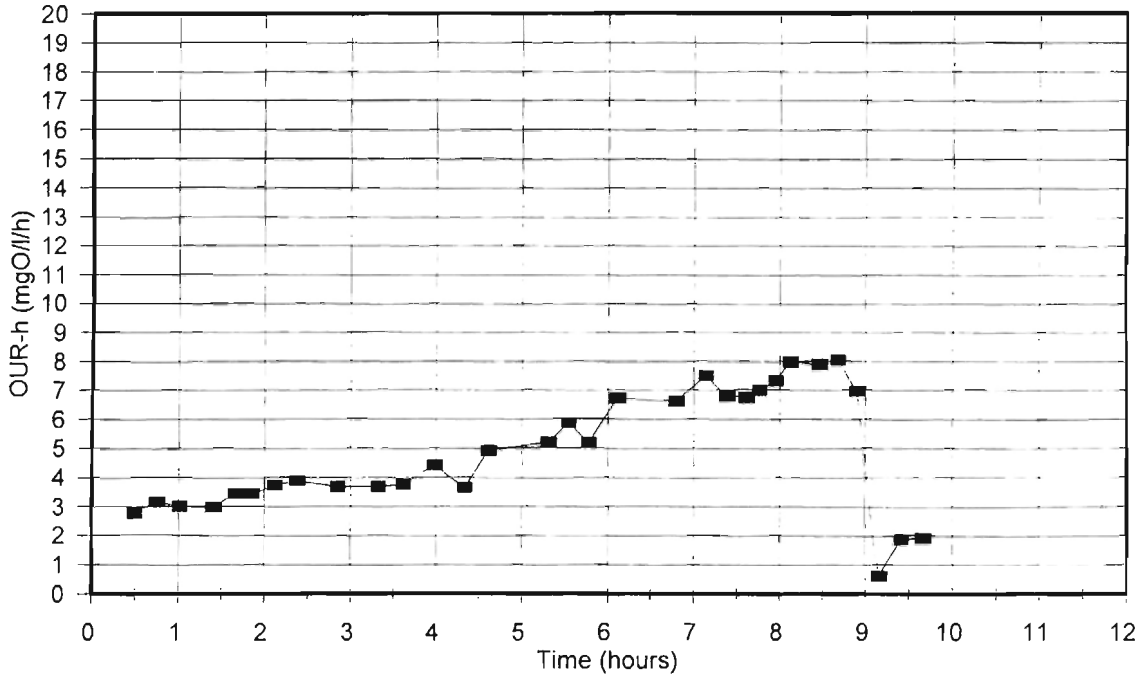
ℓn(OUR-h) graph for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 17, 16-03, Sewage Batch No. 19



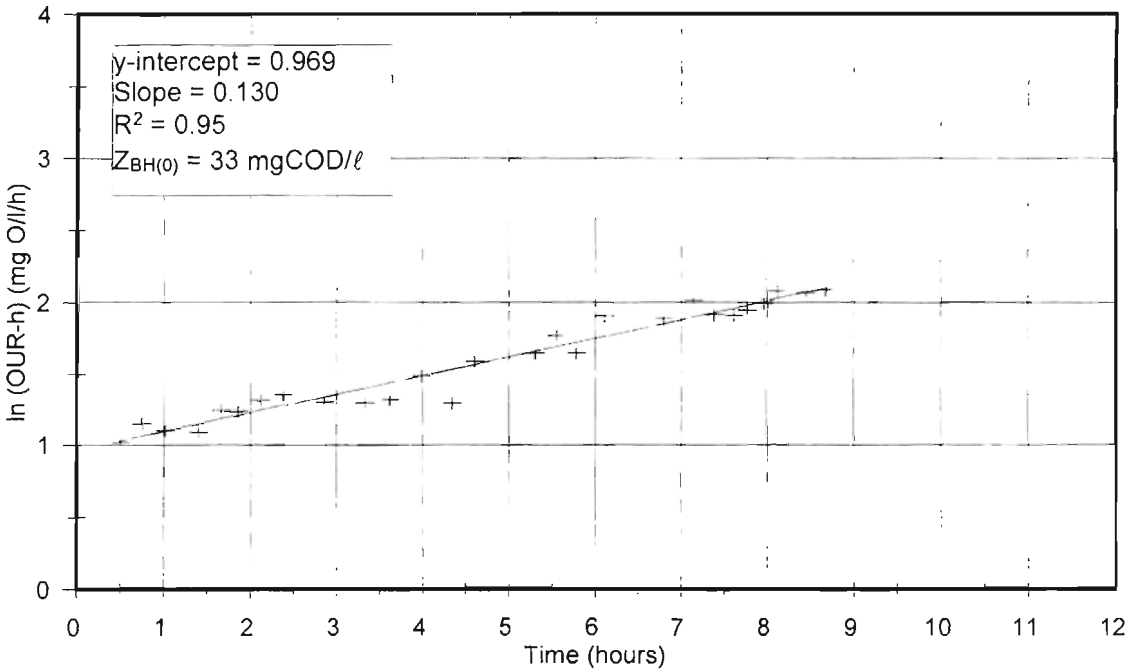
OUR graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ)
Batch Test No. 19, 17-03, Sewage Batch No. 19



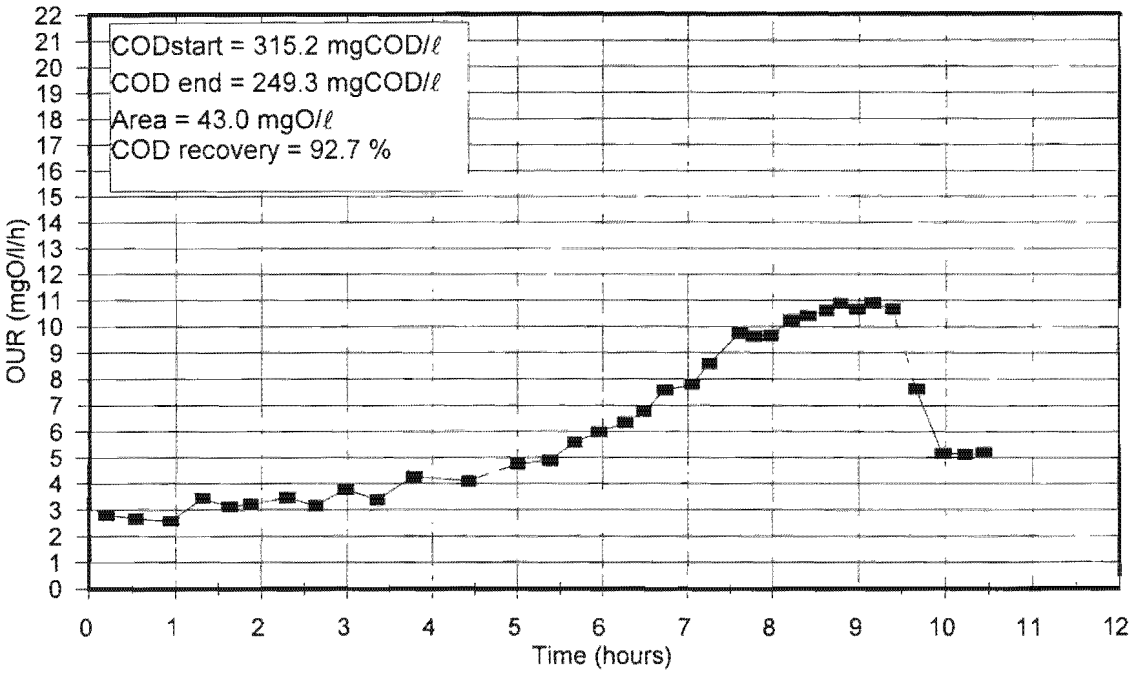
NO_3/NO_2 graph for for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ)
Batch Test No. 19, 17-03, Sewage Batch No. 19



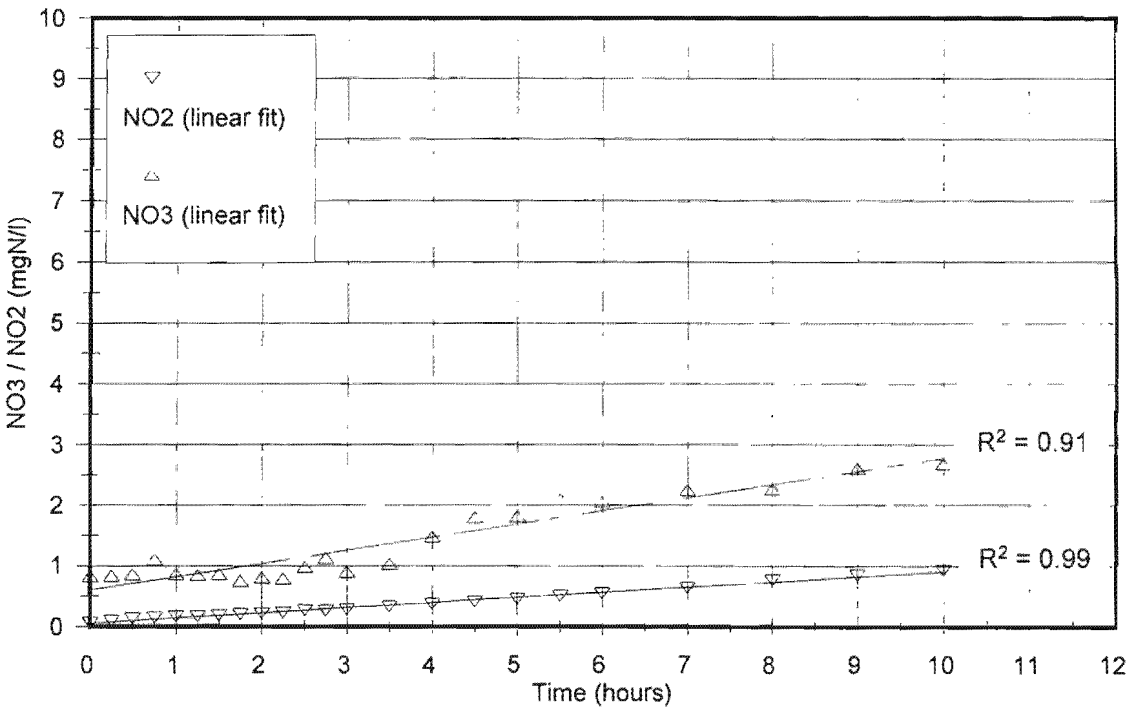
OUR-h graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ)
Batch Test No. 19, 17-03, Sewage Batch No. 19



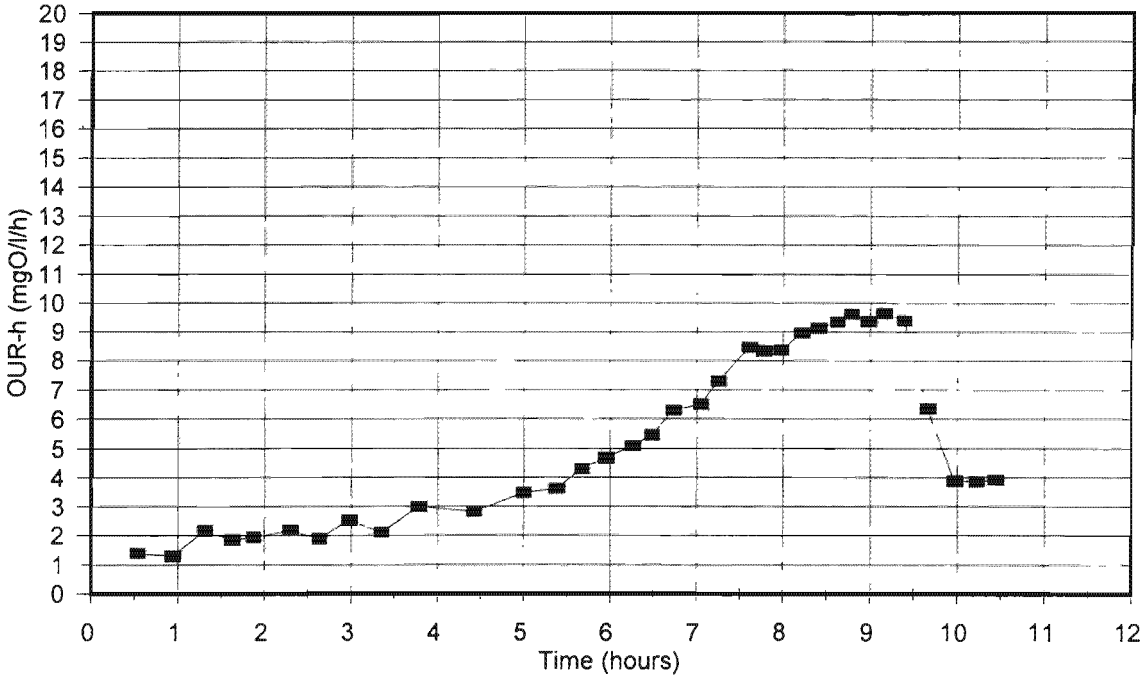
ℓn(OUR-h) graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ)
Batch Test No. 19, 17-03, Sewage Batch No. 19



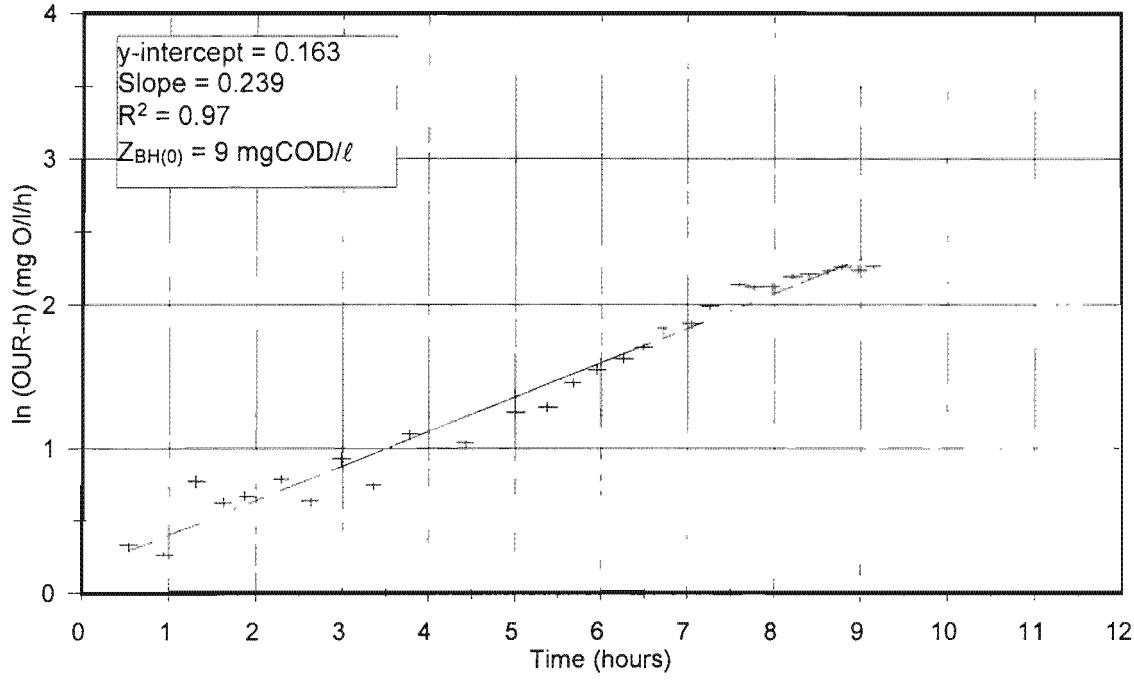
OUR graph for filtered wastewater (2.90ℓ) plus mixed liquor (0.10ℓ)
Batch Test No. 21, 18-03, Sewage Batch No. 19



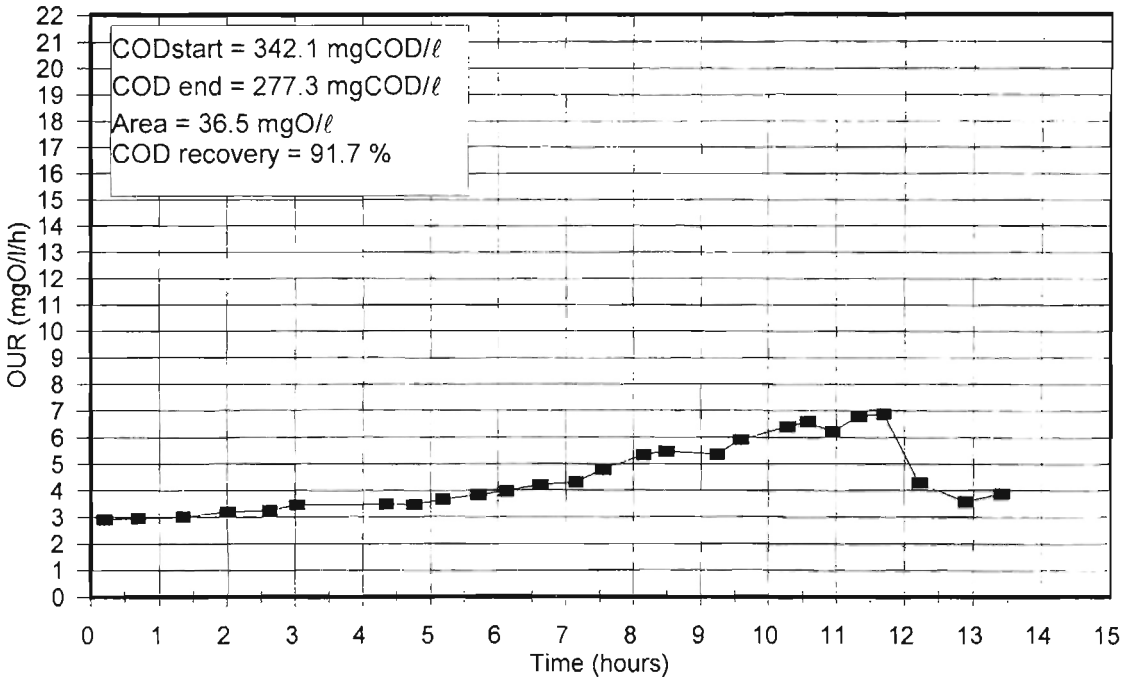
NO_3/NO_2 graph for for filtered wastewater (2.90ℓ) plus mixed liquor (0.10ℓ)
Batch Test No. 21, 18-03, Sewage Batch No. 19



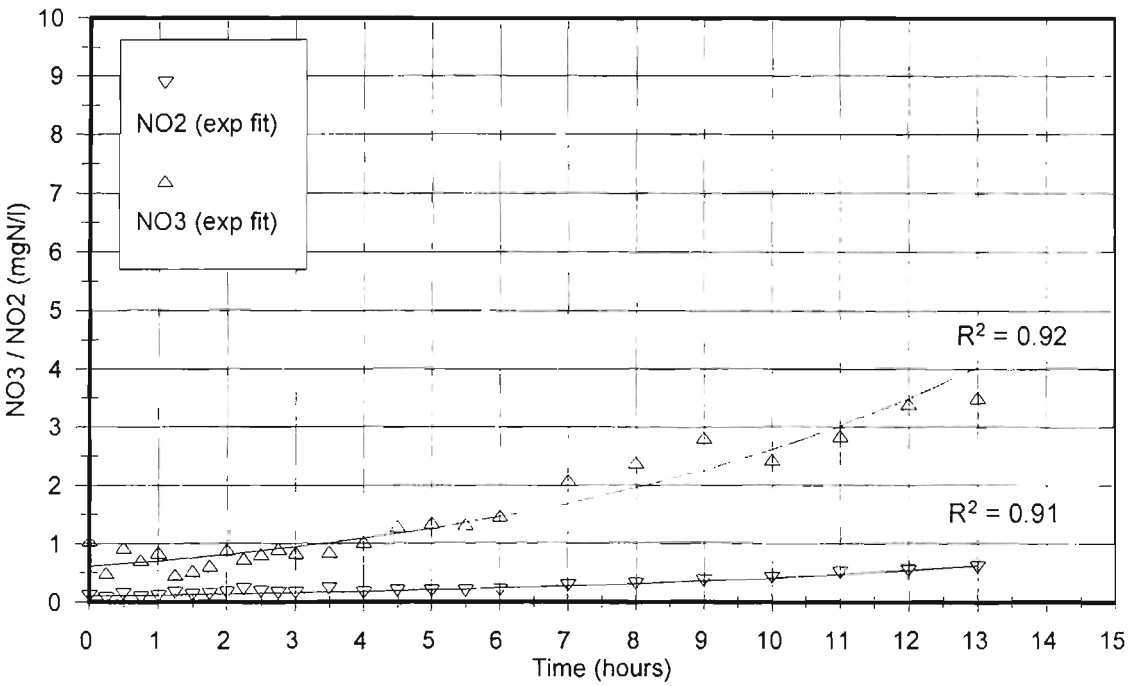
OUR-h graph for filtered wastewater (2.90ℓ) plus mixed liquor (0.10ℓ)
Batch Test No. 21, 18-03, Sewage Batch No. 19



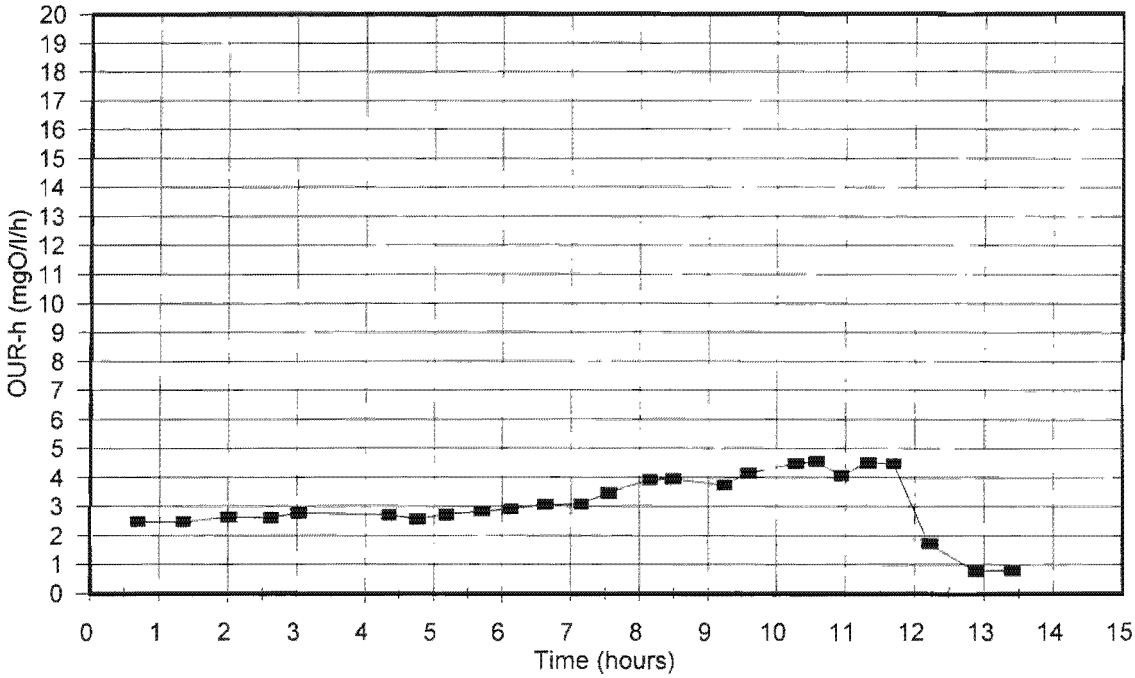
ℓn(OUR-h) graph for filtered wastewater (2.90ℓ) plus mixed liquor (0.10ℓ)
Batch Test No. 21, 18-03, Sewage Batch No. 19



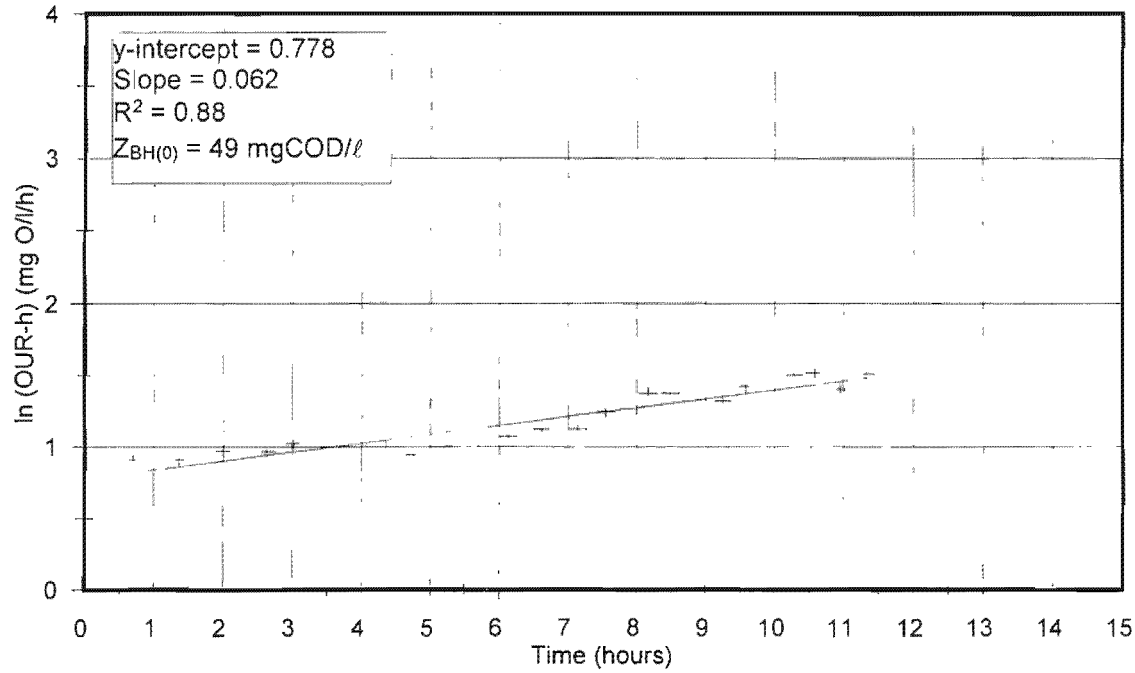
OUR graph for filtered wastewater (2.90ℓ) plus mixed liquor (0.10ℓ)
Batch Test No. 23, 28-03, Sewage Batch No. 20



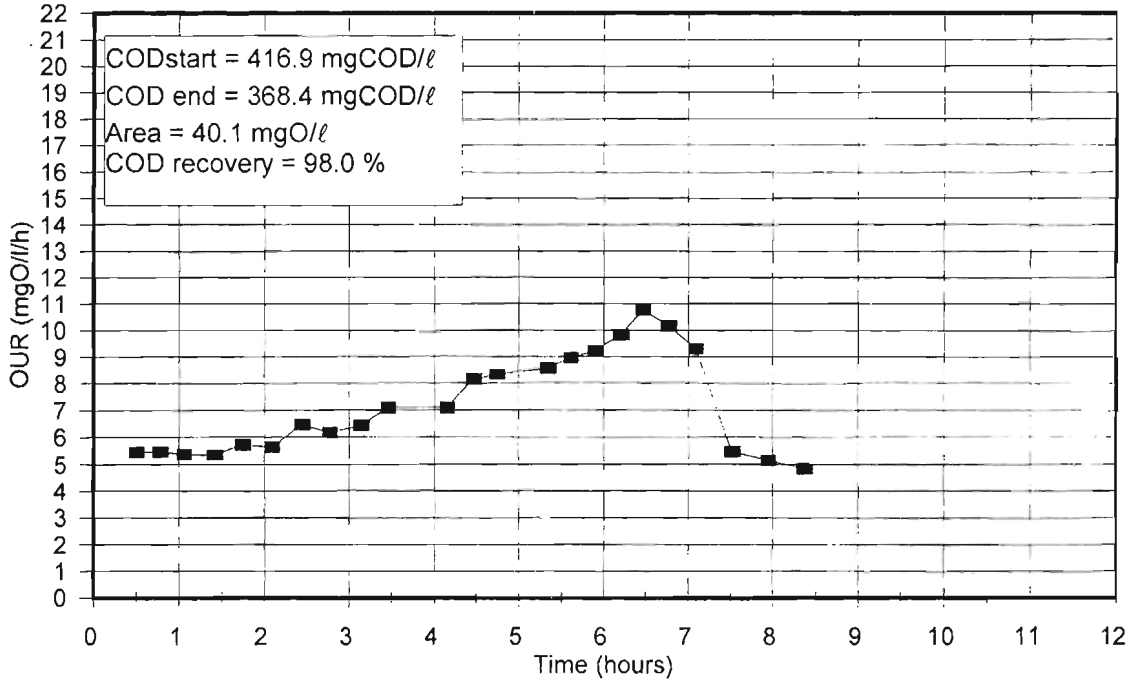
NO_3/NO_2 graph for for filtered wastewater (2.90ℓ) plus mixed liquor (0.10ℓ)
Batch Test No. 23, 28-03, Sewage Batch No. 20



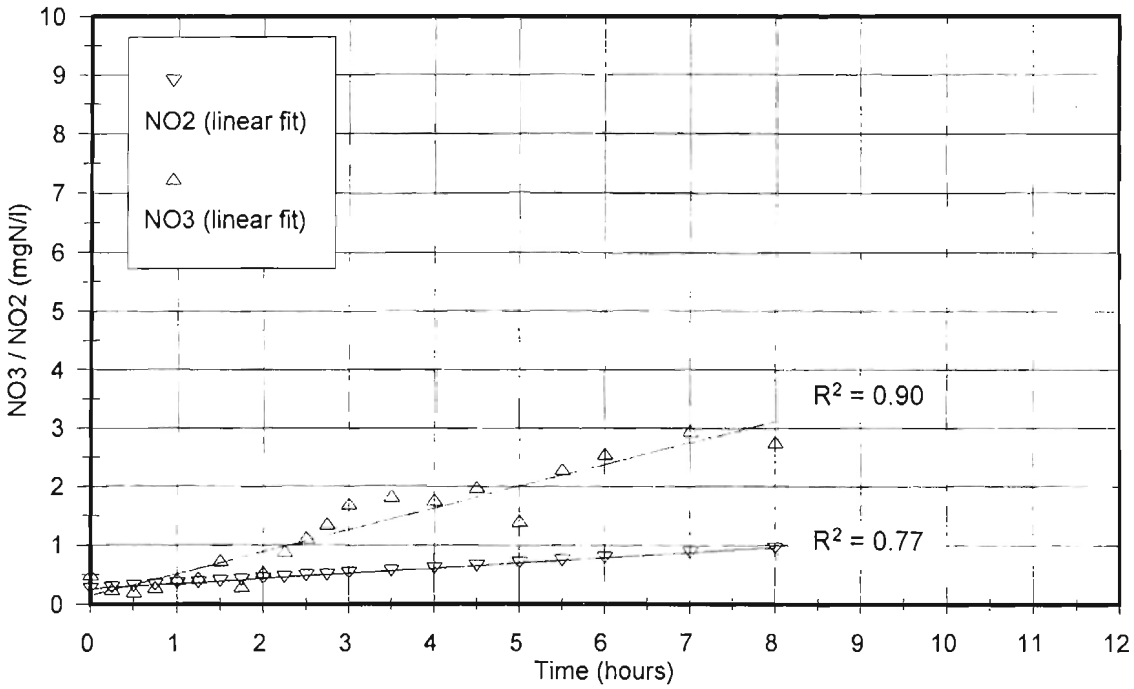
OUR-h graph for filtered wastewater (2.90ℓ) plus mixed liquor (0.10ℓ)
Batch Test No. 23, 28-03, Sewage Batch No. 20



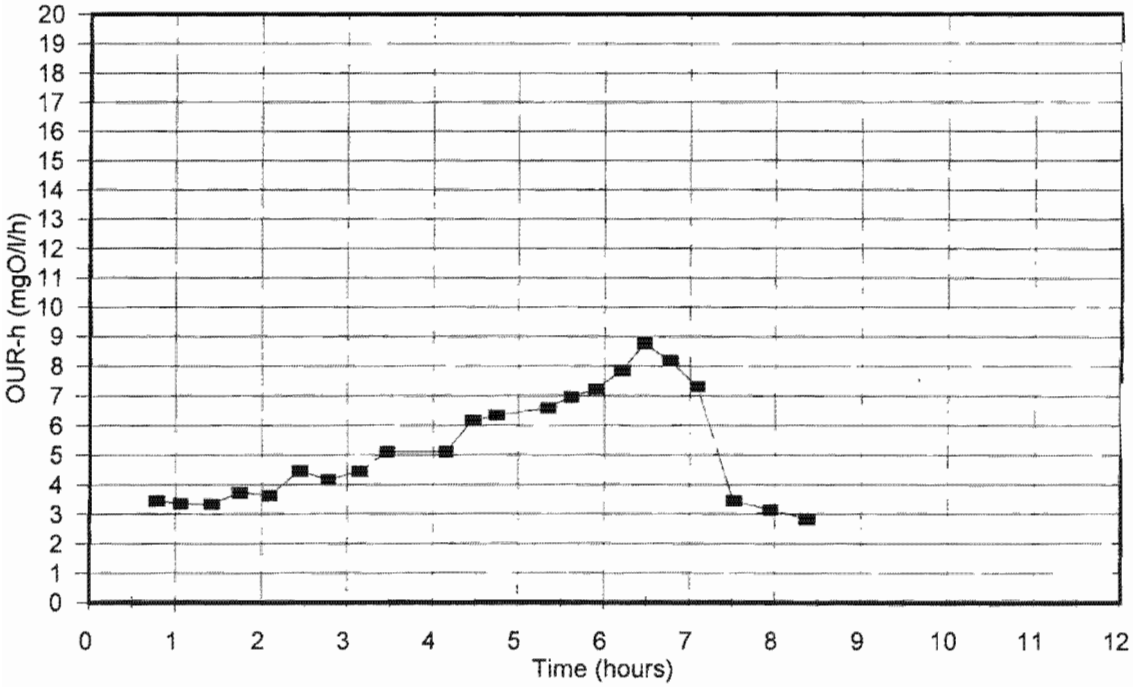
ℓn(OUR-h) graph for filtered wastewater (2.90ℓ) plus mixed liquor (0.10ℓ)
Batch Test No. 23, 28-03, Sewage Batch No. 20



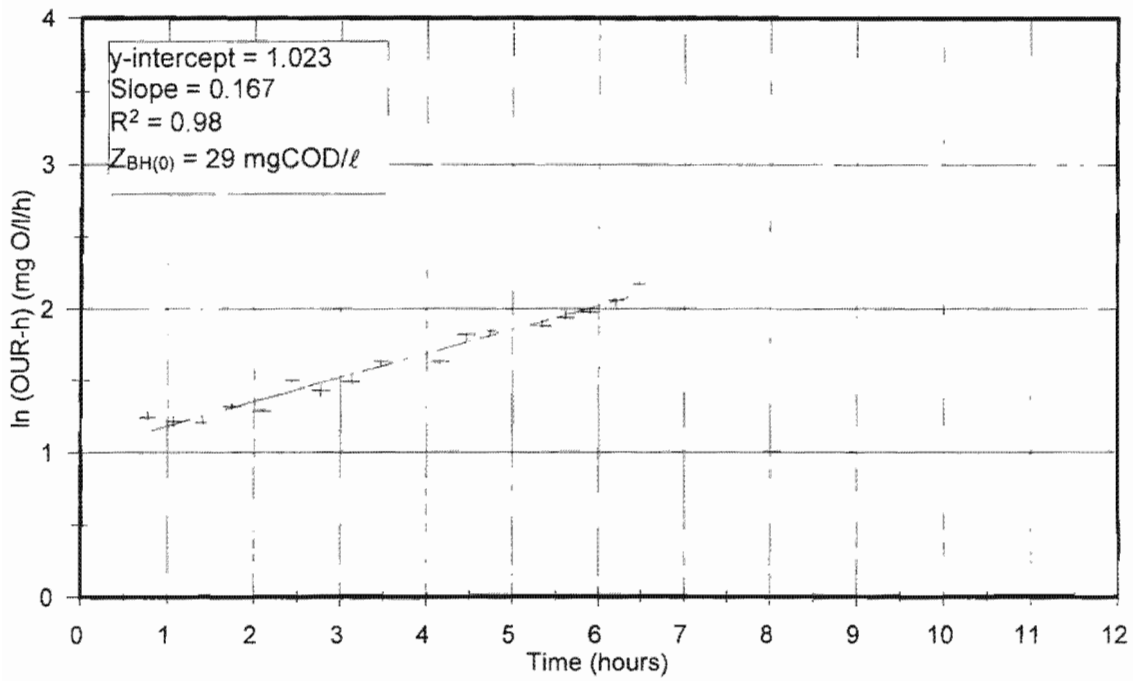
OUR graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ)
Batch Test No. 25, 30-03, Sewage Batch No. 20



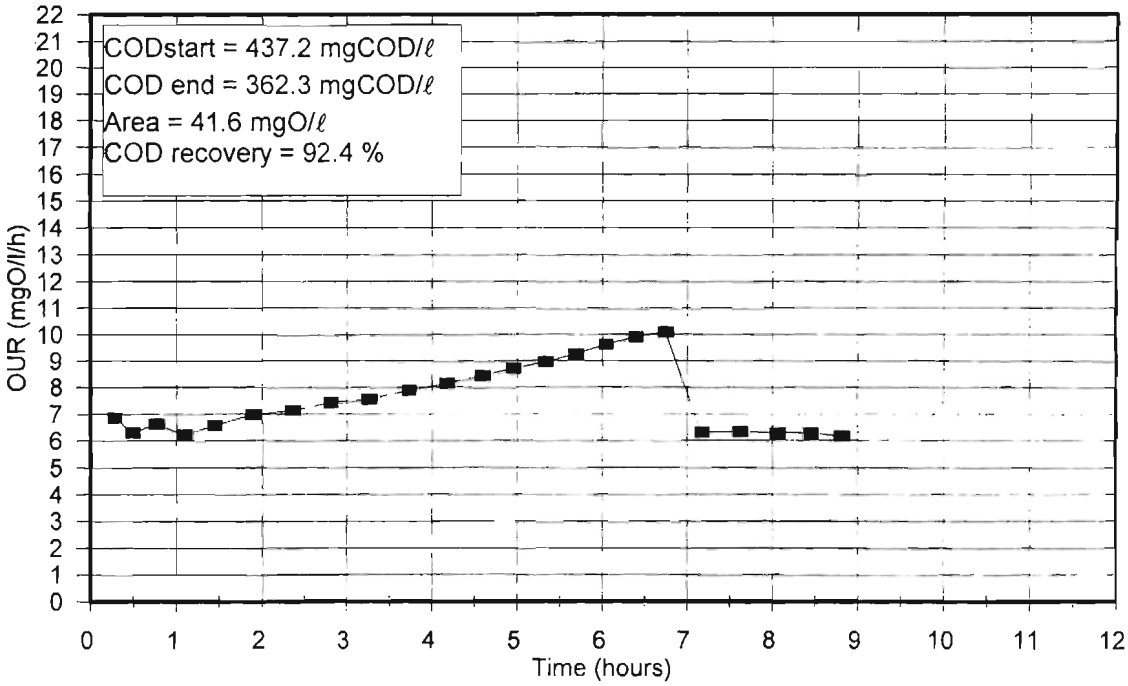
NO_3/NO_2 graph for for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ)
Batch Test No. 25, 30-03, Sewage Batch No. 20



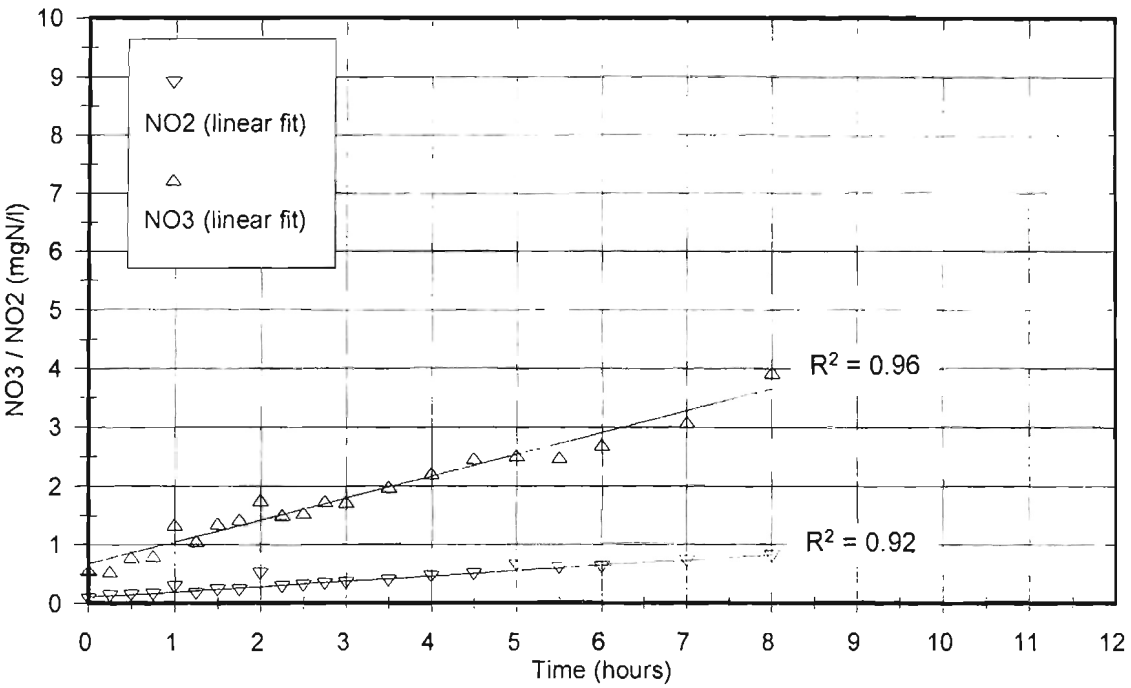
OUR-h graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ)
Batch Test No. 25, 30-03, Sewage Batch No. 20



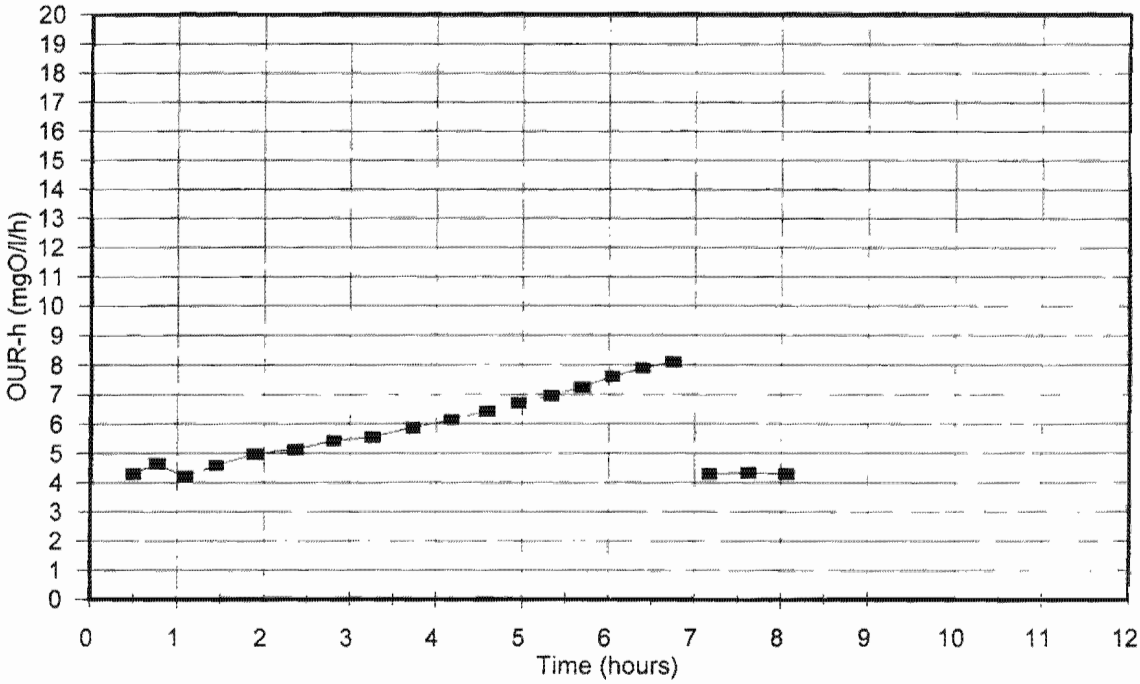
ℓn(OUR-h) graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ)
Batch Test No. 25, 30-03, Sewage Batch No. 20



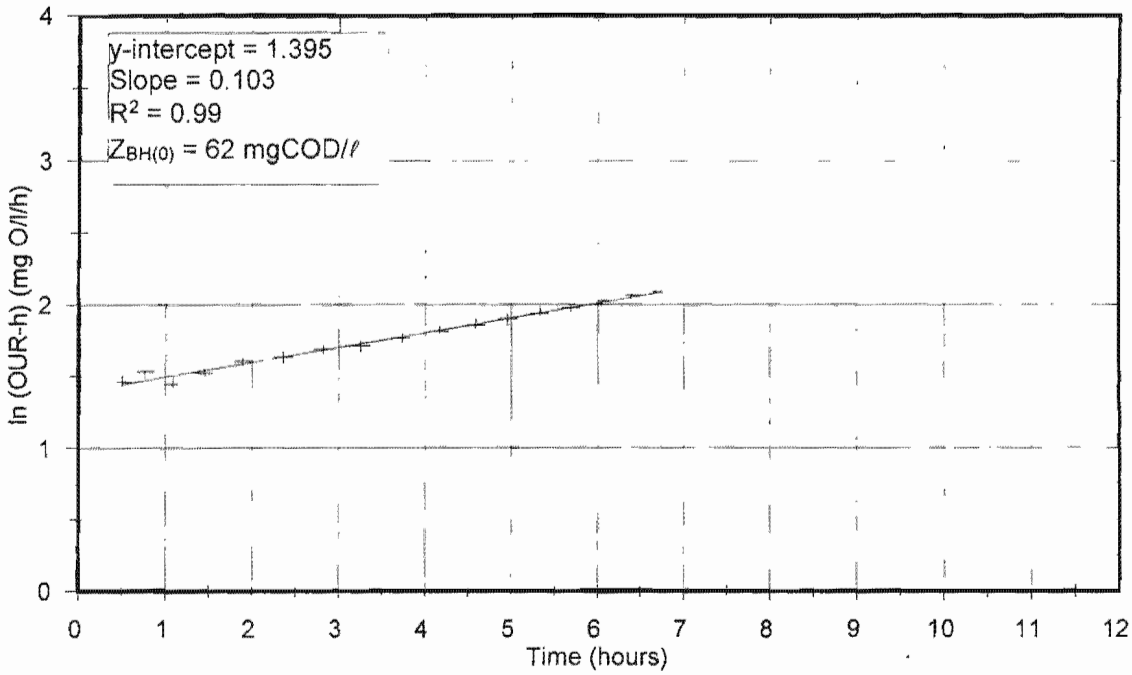
OUR graph for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)



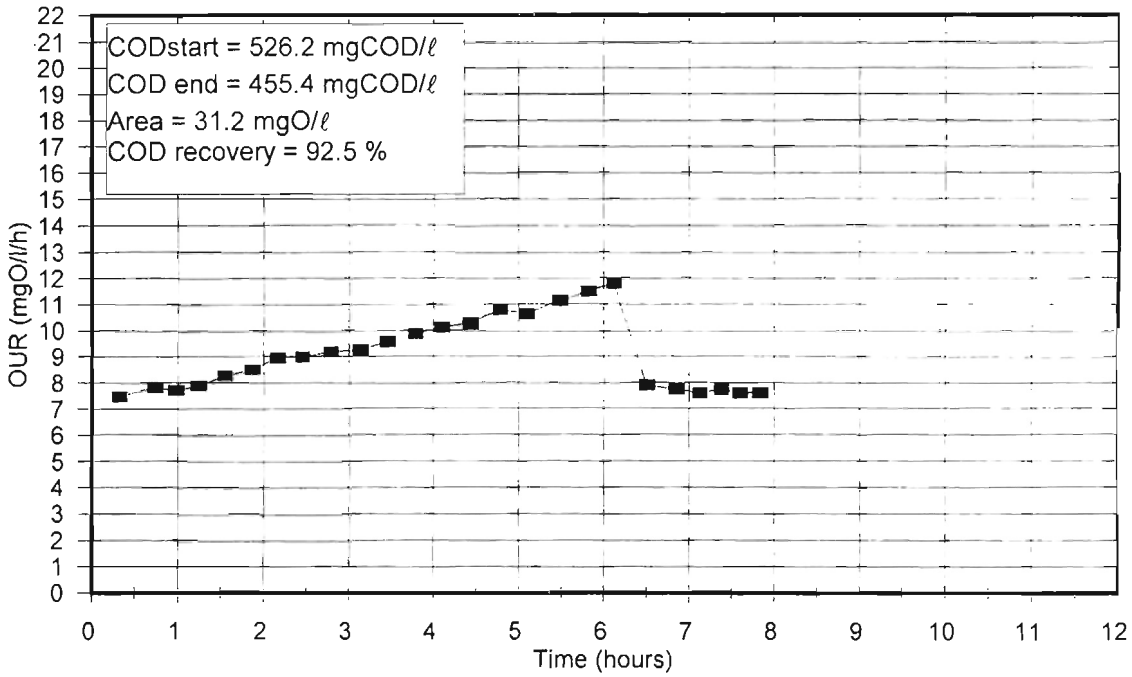
NO_3/NO_2 graph for for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 27, 31-03, Sewage Batch No. 20



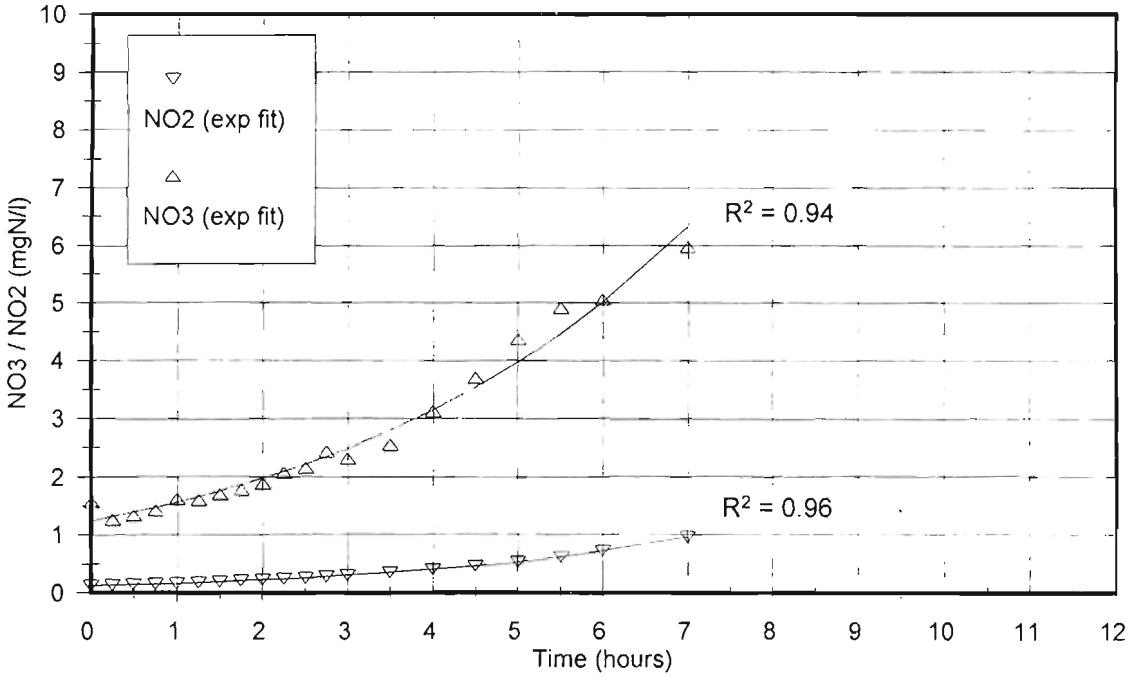
OUR-h graph for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 27, 31-03, Sewage Batch No. 20



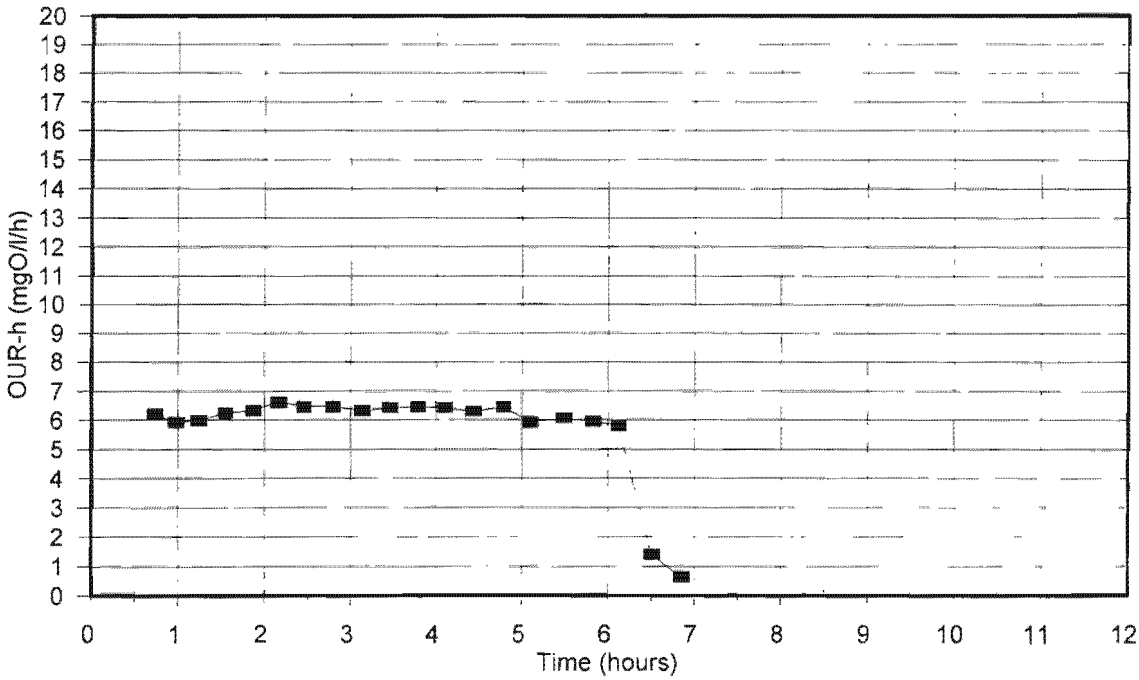
ℓn(OUR-h) graph for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 27, 31-03, Sewage Batch No. 20



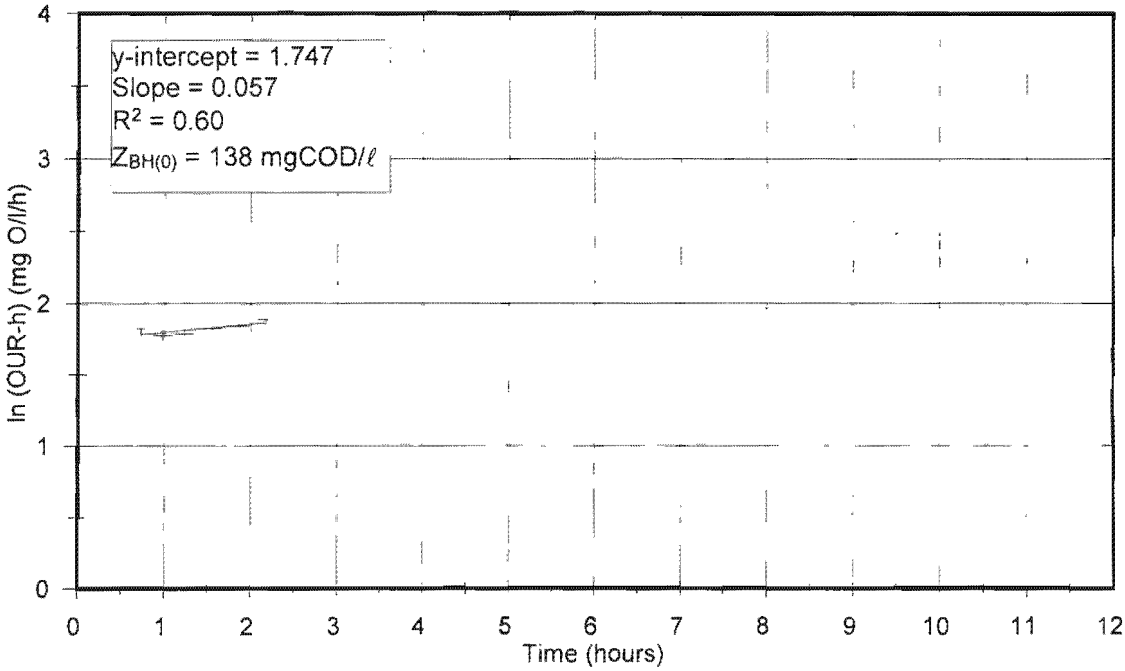
OUR graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ)
Batch Test No. 29, 02-04, Sewage Batch No. 20



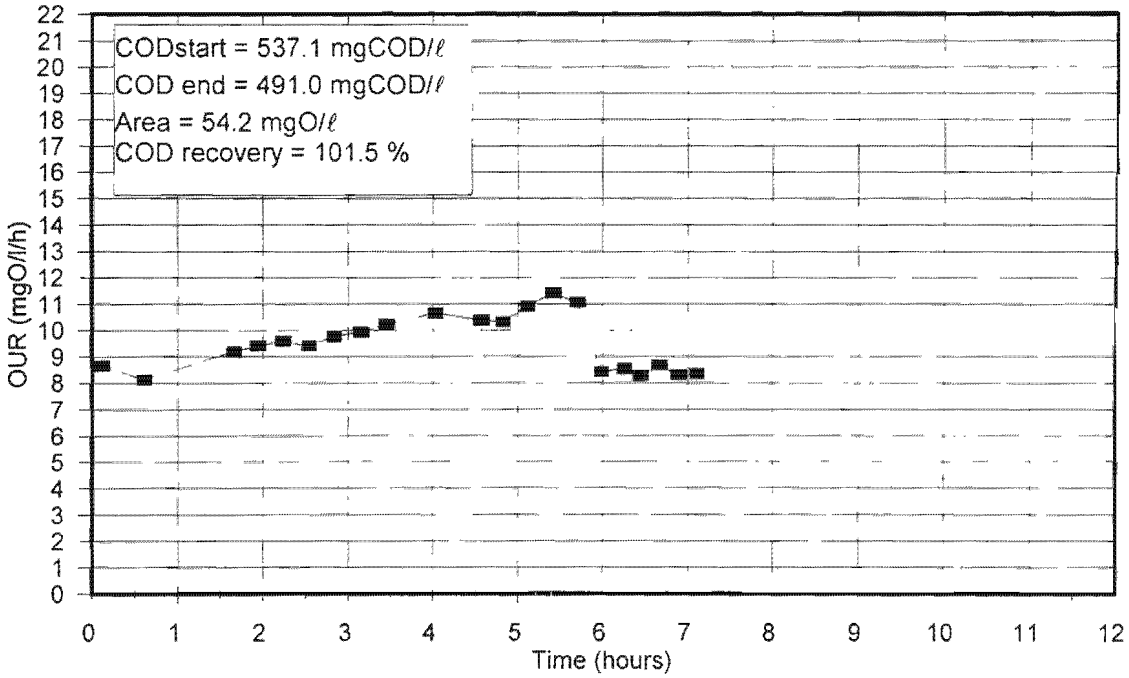
NO_3/NO_2 graph for for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ)
Batch Test No. 29, 02-04, Sewage Batch No. 20



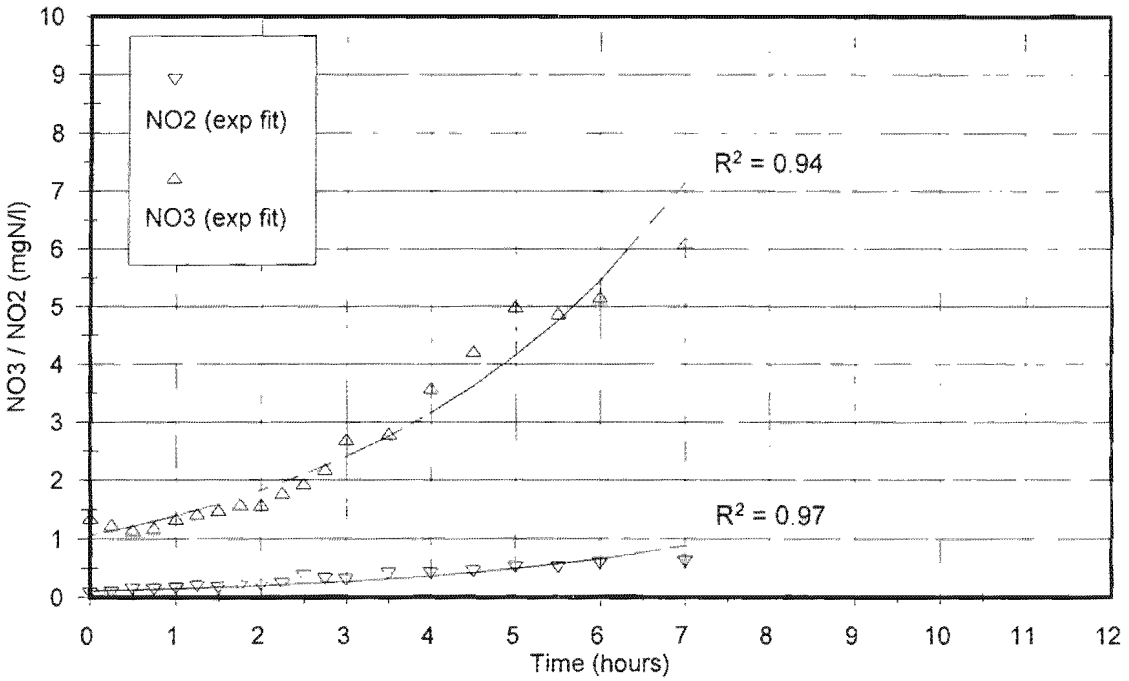
OUR-h graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ)
Batch Test No. 29, 02-04, Sewage Batch No. 20



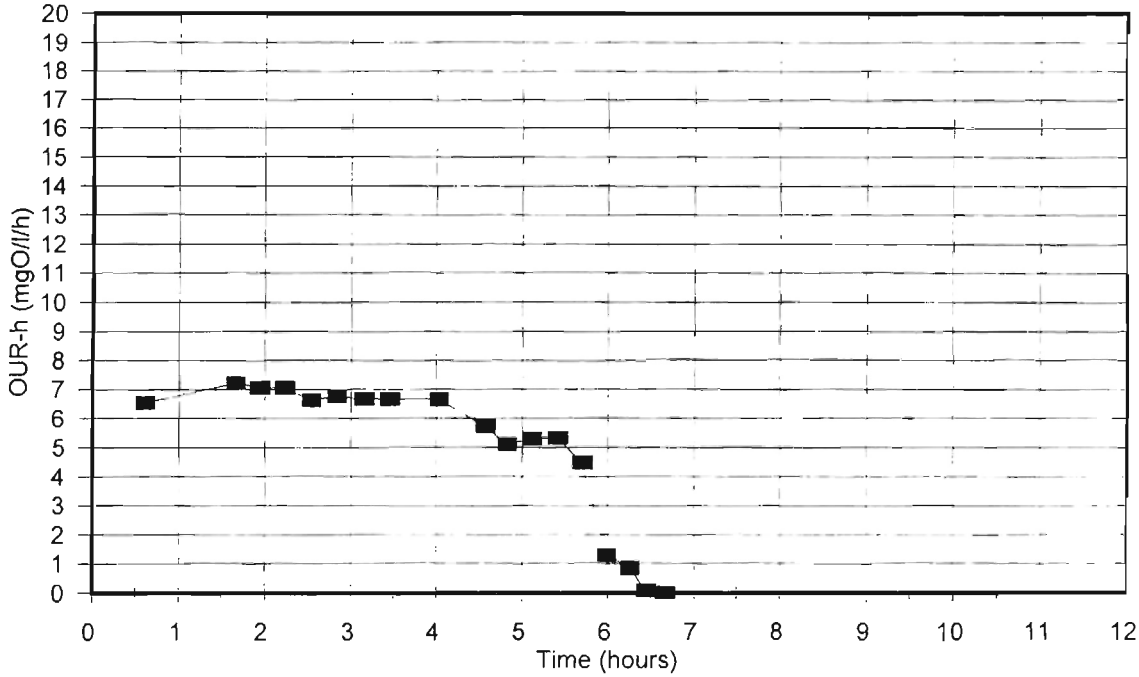
ℓn(OUR-h) graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ)
Batch Test No. 29, 02-04, Sewage Batch No. 20



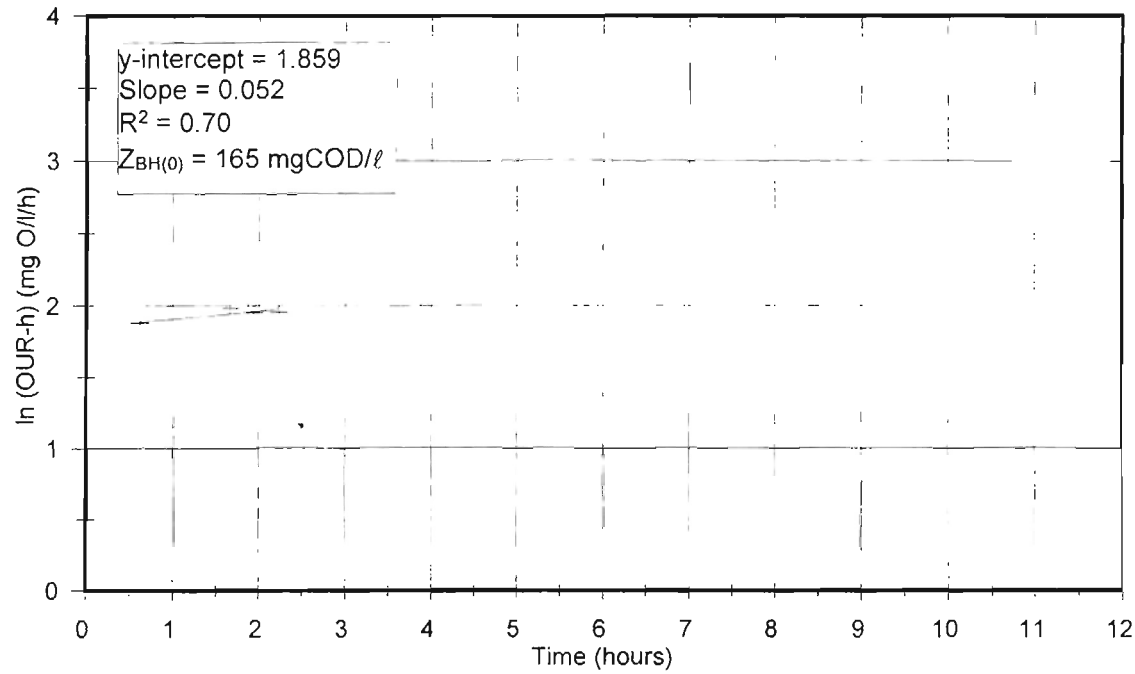
OUR graph for filtered wastewater (2.70ℓ) plus mixed liquor (0.30ℓ)
Batch Test No. 31, 03-04, Sewage Batch No. 20



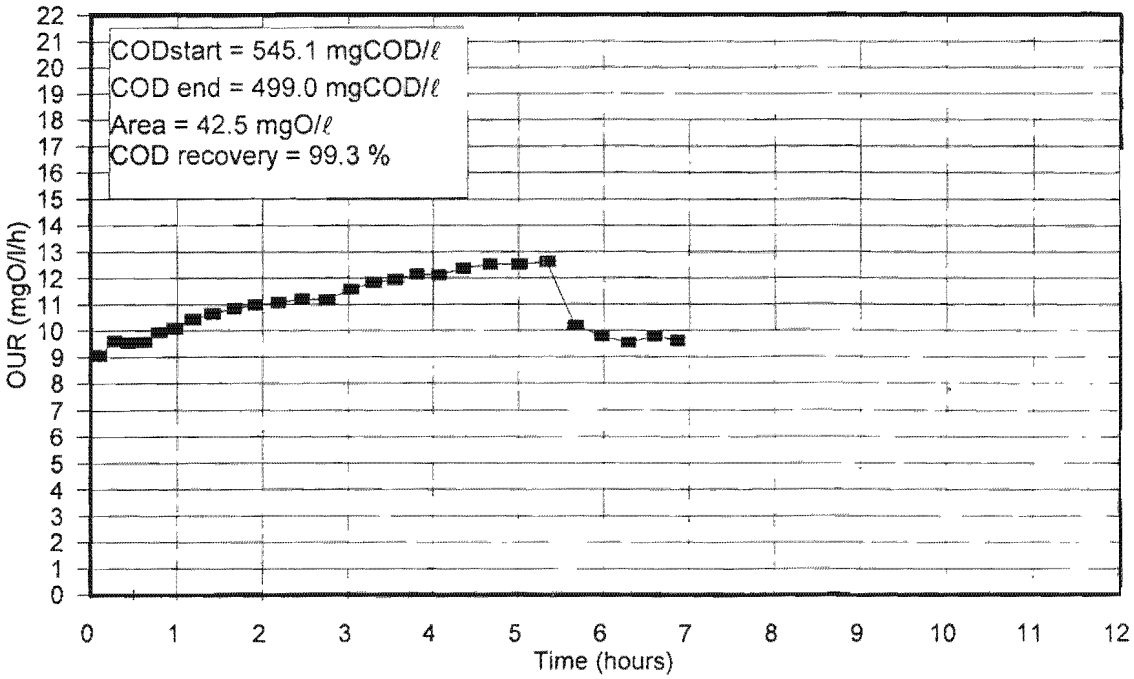
NO_3/NO_2 graph for for filtered wastewater (2.70ℓ) plus mixed liquor (0 30ℓ)
Batch Test No. 31, 03-04, Sewage Batch No. 20



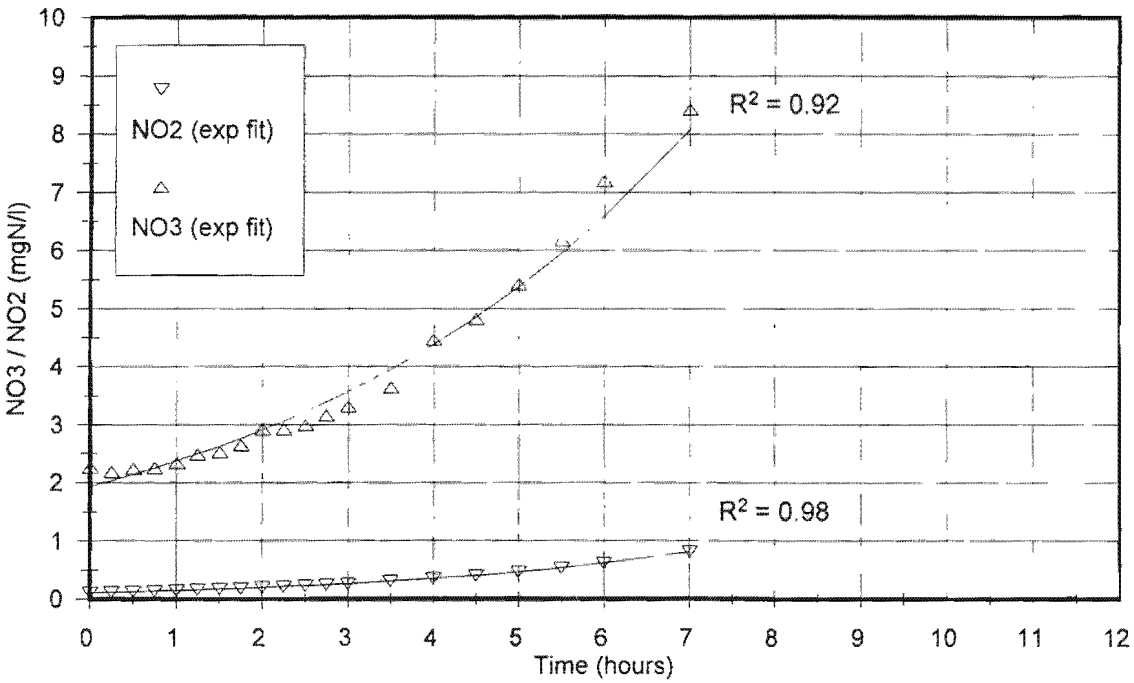
OUR-h graph for filtered wastewater (2.70ℓ) plus mixed liquor (0.30ℓ)
Batch Test No. 31, 03-04, Sewage Batch No. 20



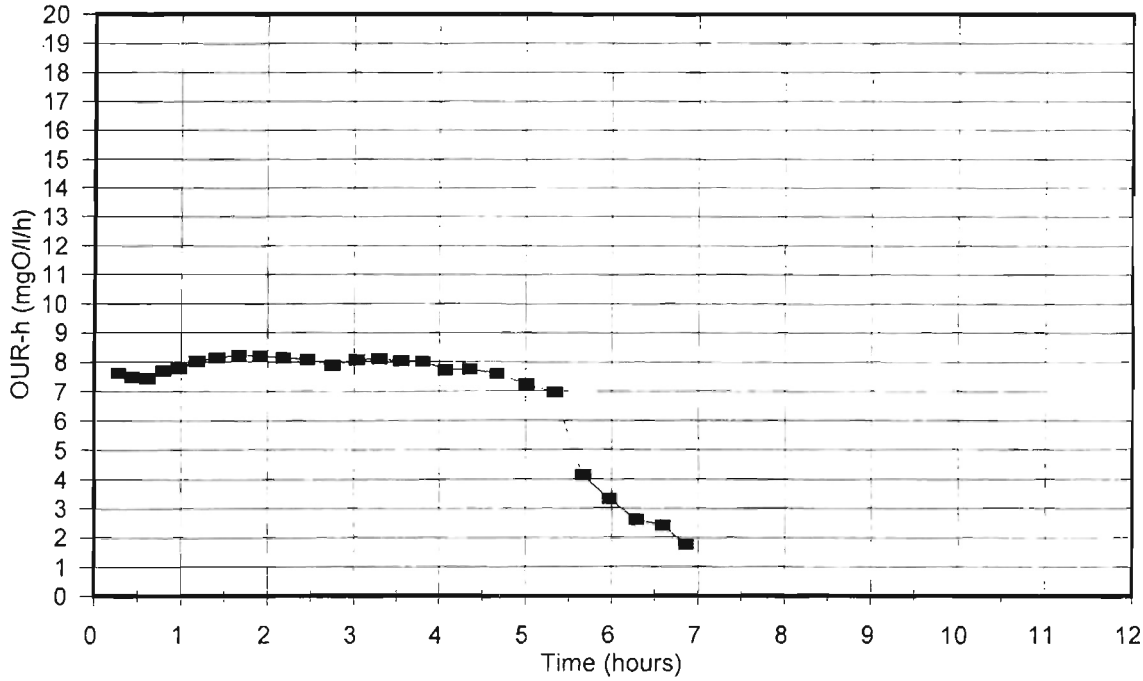
ℓn(OUR-h) graph for filtered wastewater (2.70ℓ) plus mixed liquor (0.30ℓ)
Batch Test No. 31, 03-04, Sewage Batch No. 20



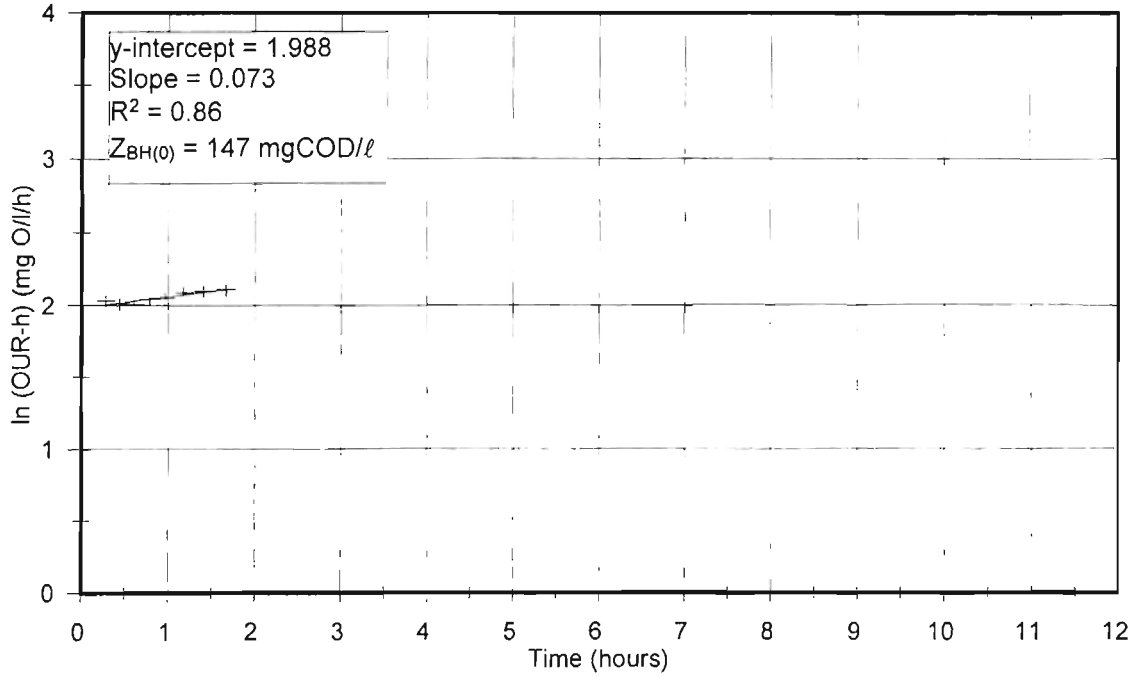
OUR graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 33, 04-04, Sewage Batch No. 20



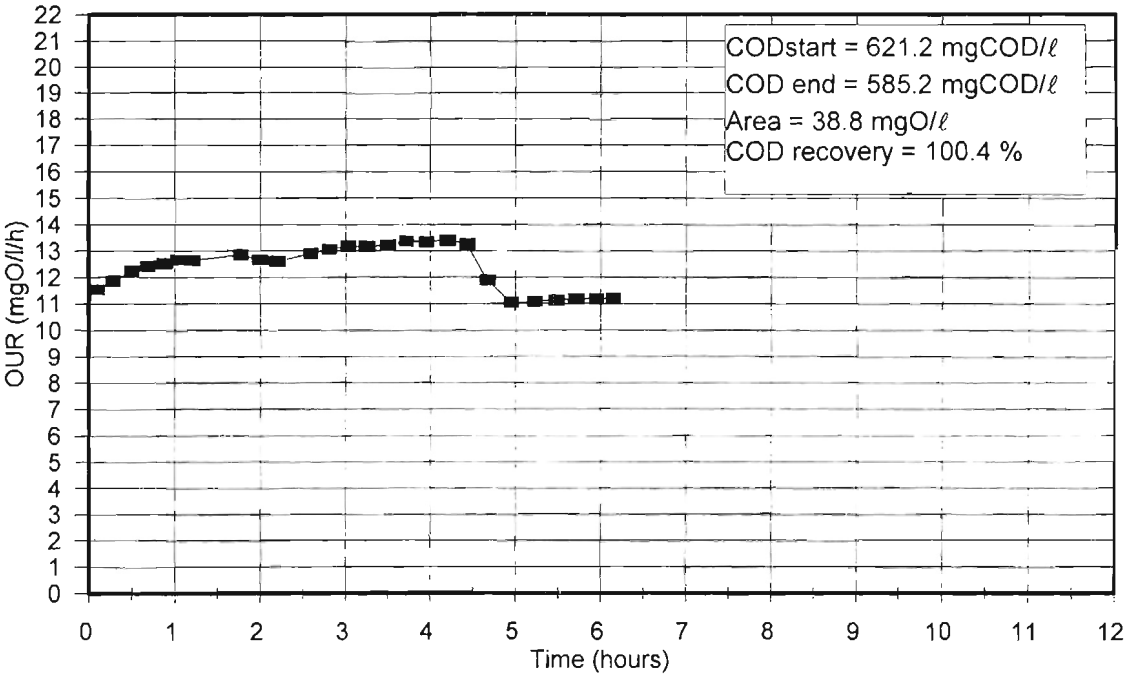
NO_3/NO_2 graph for for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 33, 04-04, Sewage Batch No. 20



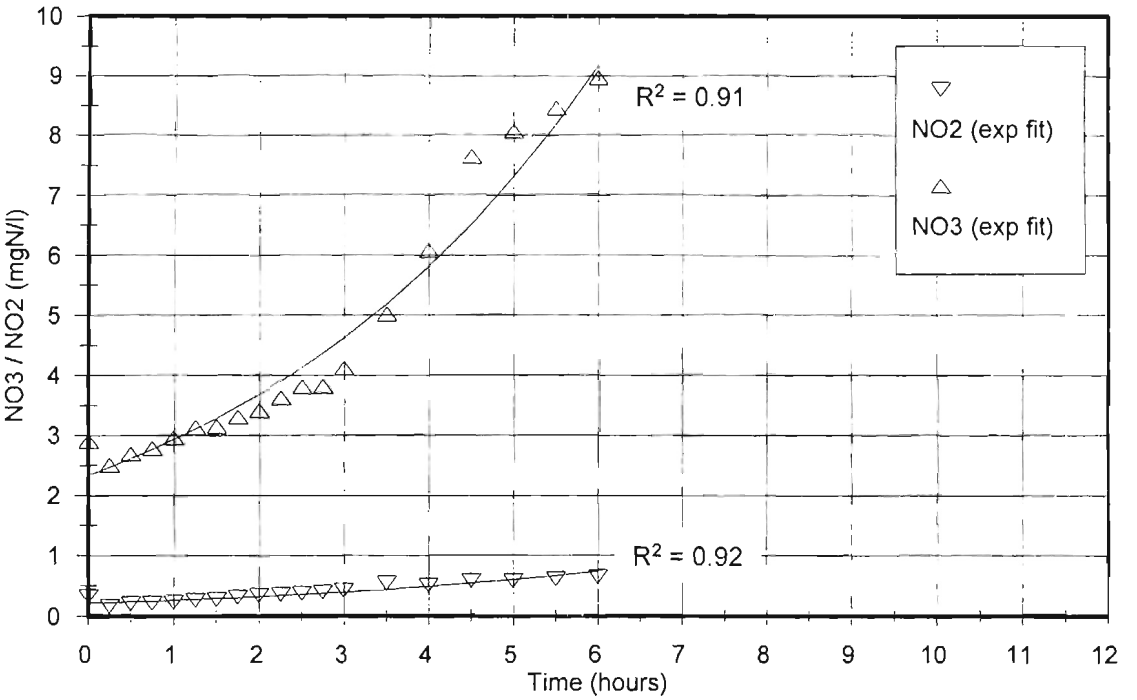
OUR-h graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 33, 04-04, Sewage Batch No. 20



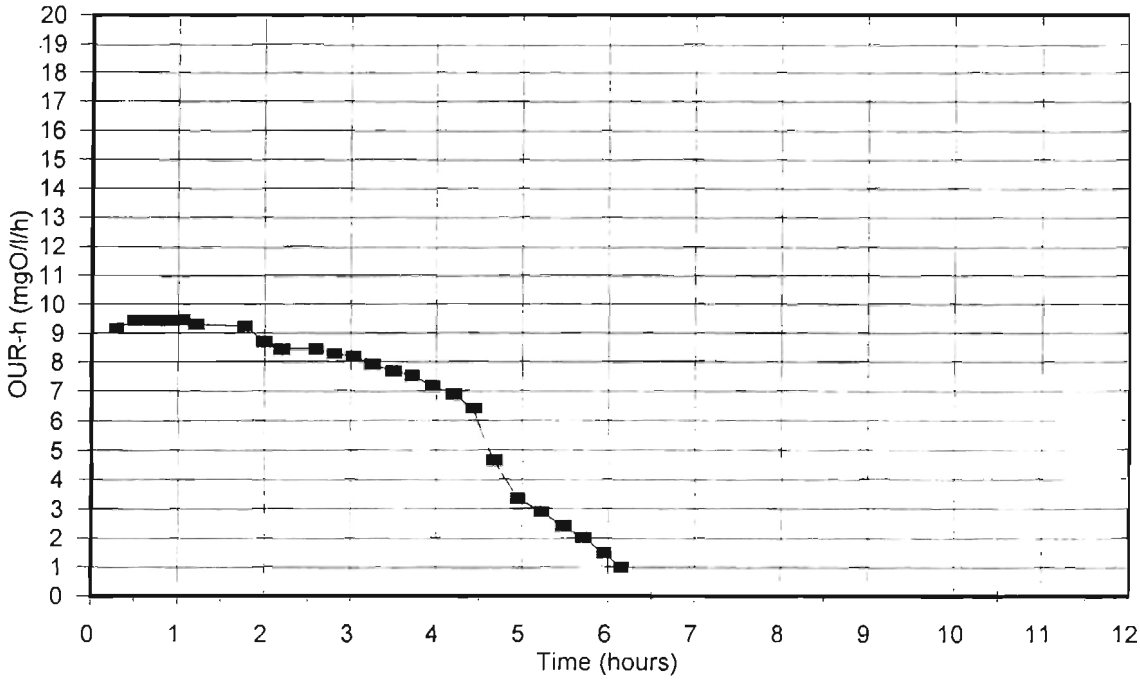
ℓn(OUR-h) graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 33, 04-04, Sewage Batch No. 20



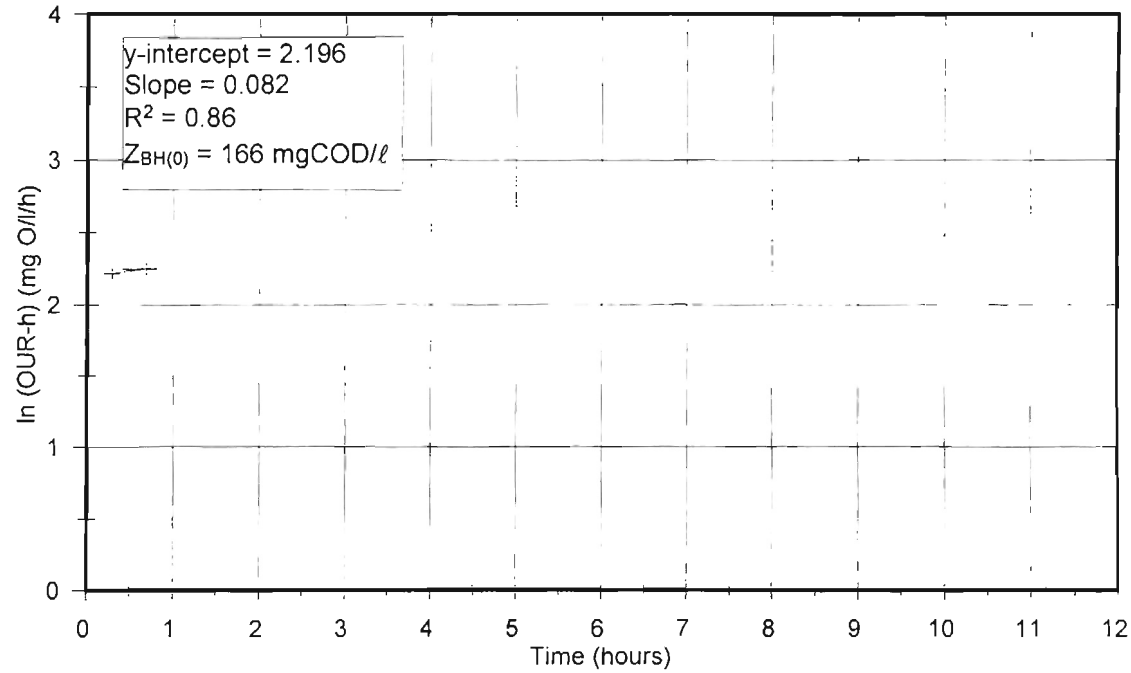
OUR graph for filtered wastewater (2.60ℓ) plus mixed liquor (0.40ℓ)
Batch Test No. 35, 05-04, Sewage Batch No. 20



NO_3/NO_2 graph for for filtered wastewater (2.60ℓ) plus mixed liquor (0.40ℓ)
Batch Test No. 35, 05-04, Sewage Batch No. 20



OUR-h graph for filtered wastewater (2.60ℓ) plus mixed liquor (0.40ℓ)
Batch Test No. 35, 05-04, Sewage Batch No. 20

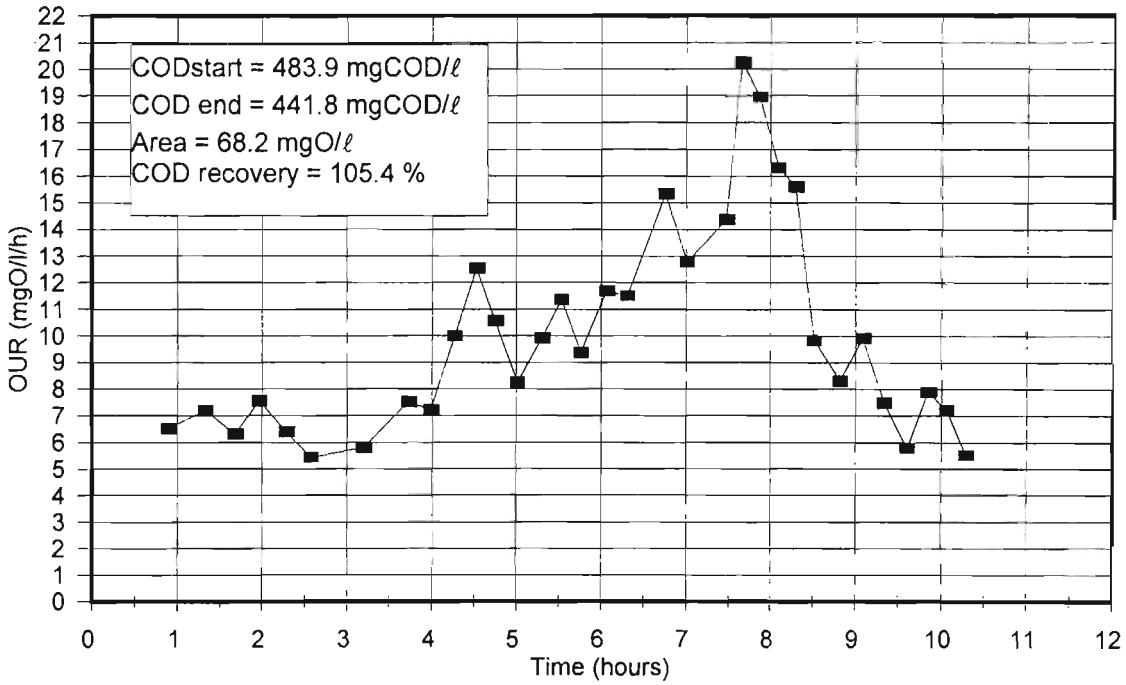


ℓn(OUR-h) graph for filtered wastewater (2.60ℓ) plus mixed liquor (0.40ℓ)
Batch Test No. 35, 05-04, Sewage Batch No. 20

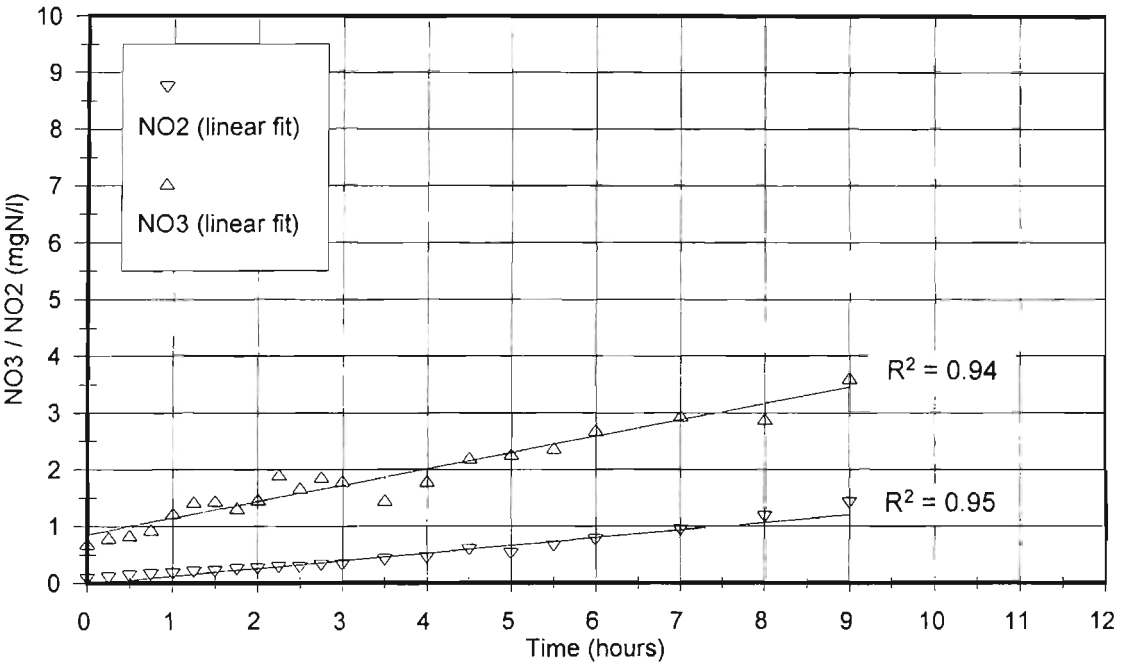
Table A2: Summarized data for modified batch tests with filtered wastewater and mixed liquor drawn from the parent MLE experimental activated sludge system; mixed liquor volume, COD recovery, linear regression data and measured OHO active biomass.

Sew. Batch No.	Batch Test No.	Date of Test	ML volume (ℓ)	COD (mgCOD/ℓ) Start	COD (mgCOD/ℓ) End	Area (mgO/ℓ)	% COD Recovery	Linear regresion data y-int.	Linear regresion data slope	Linear regresion data R^2	$Z_{BH(0)}$ (ML) (mgCOD/ℓ)
18	2	28-02	0.25	483.9	441.8	68.2	105.4	1.127	0.195	0.77	27
	4	02-03	0.30	542.2	437.7	53.9	90.7	1.327	0.139	0.85	44
	6	04-03	0.20	427.7	395.6	58.0	106.0	1.358	0.184	0.96	36
	8	05-03	0.15	356.4	290.5	42.7	93.5	0.798	0.252	0.99	16
19	10	12-03	0.40	700.4	638.6	50.6	98.4	2.272	0.097	0.87	158
	12	13-03	0.35	587.1	560.3	63.2	106.2	1.899	0.105	0.93	100
	14	14-03	0.30	585.0	477.9	54.2	91.0	1.765	0.123	0.92	77
	16	15-03	0.25	525.3	426.4	52.1	91.1	1.577	0.122	0.97	64
	18	16-03	0.20	449.1	389.3	50.5	97.9	1.282	0.170	0.96	36
	20	17-03	0.15	370.8	321.4	46.9	99.3	0.938	0.163	0.89	27
	22	18-03	0.10	313.1	249.3	50.9	95.9	0.524	0.191	0.94	15
20	24	28-03	0.10	354.2	283.4	43.2	92.2	0.198	0.126	0.87	16
	26	30-03	0.15	437.2	368.4	39.5	93.3	1.166	0.141	0.96	38
	28	31-03	0.20	483.7	410.9	45.3	94.3	1.556	0.070	0.81	99
	30	02-04	0.25	560.6	483.7	40.4	93.5	1.545	0.089	0.87	81
	32	03-04	0.30	639.3	531.1	37.7	89.0*	1.729	0.057	0.92	136*
	34	04-04	0.35	623.2	555.1	48.3	96.8	1.914	0.229	0.94	53
	36	05-04	0.40	733.5	621.2	53.2	92.0	2.147	0.026	0.80	326
				MEAN			95.9			0.90	
				Std. Deviation			5.2			0.06	

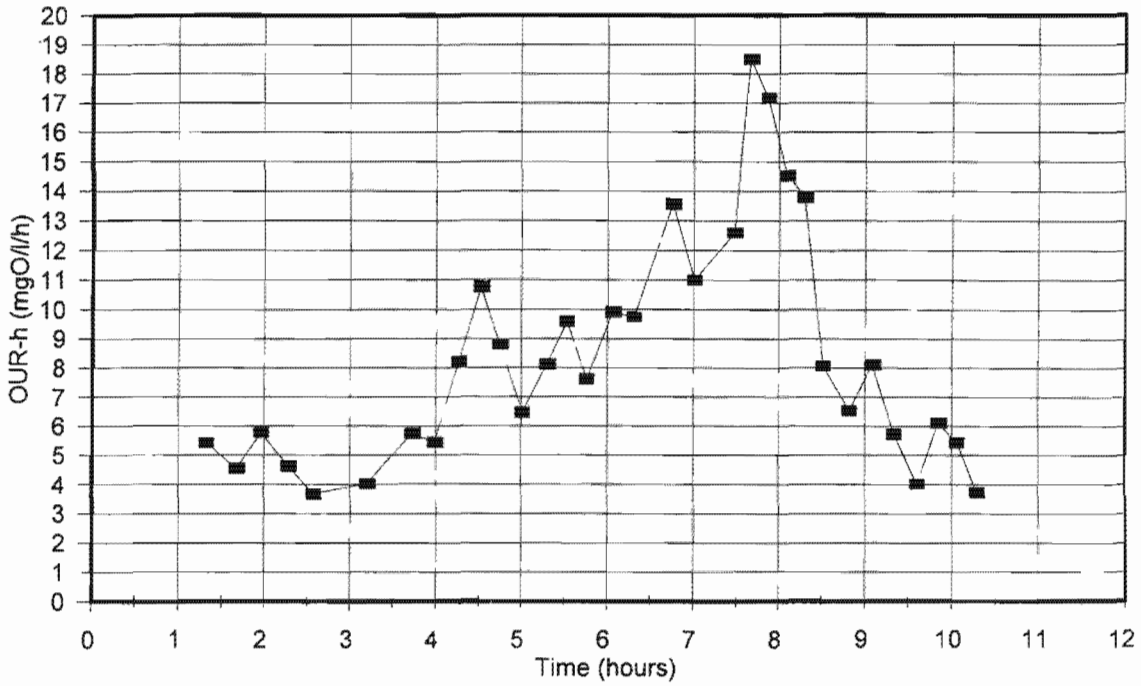
* Batch test rejected on account of poor %COD recovery



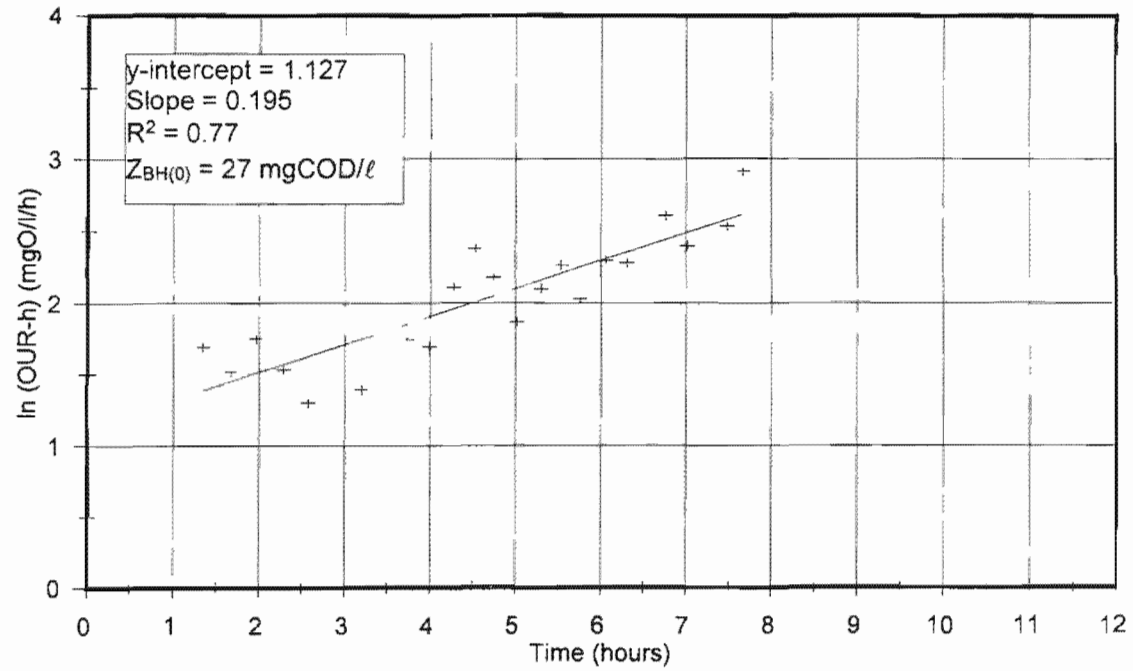
OUR graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ)
Batch Test No. 2, 28-02, Sewage Batch No. 18



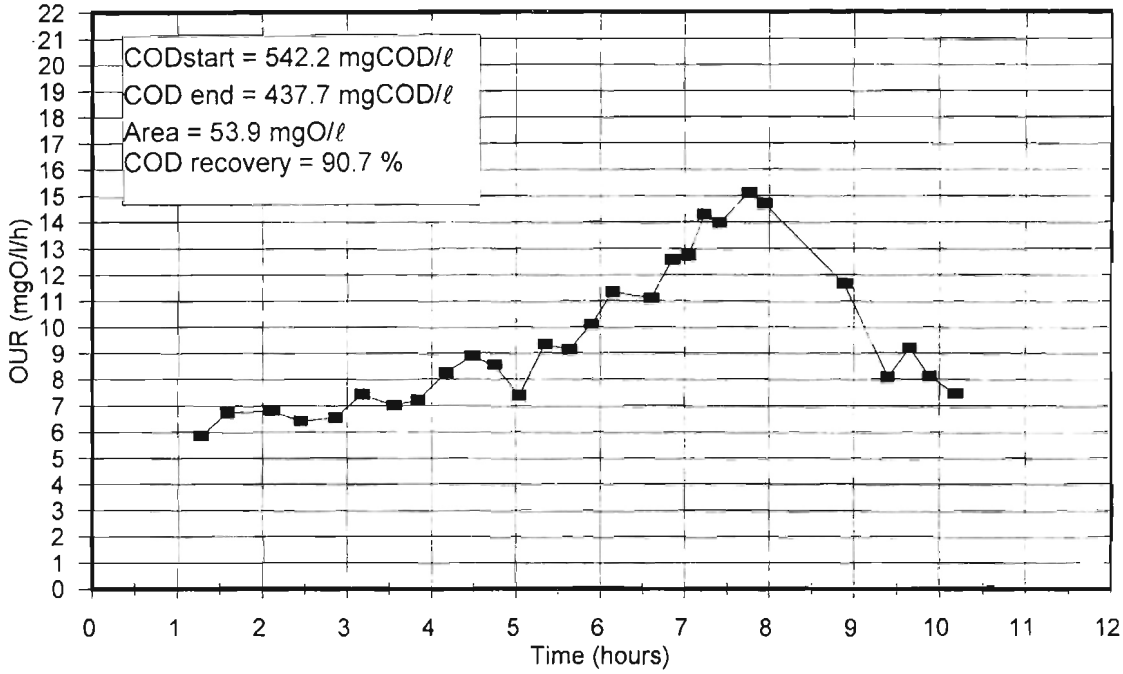
NO_3/NO_2 graph for for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ)
Batch Test No. 2, 28-02, Sewage Batch No. 18



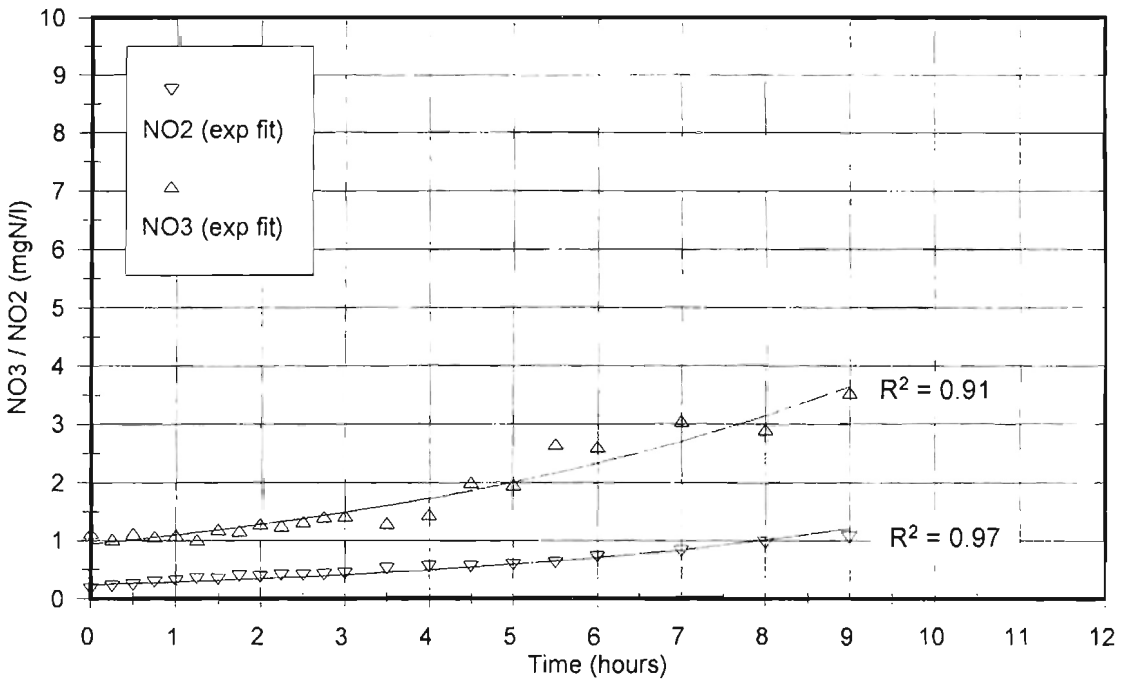
OUR-h graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ)
Batch Test No. 2, 28-02, Sewage Batch No. 18



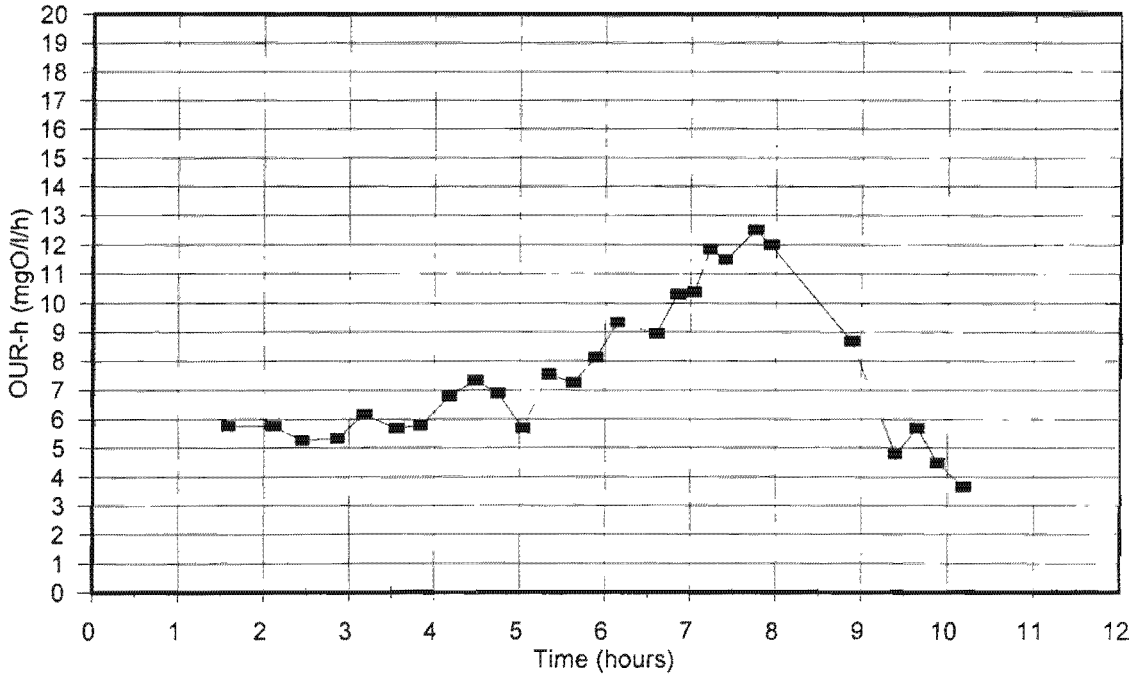
ℓn(OUR-h) graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ)
Batch Test No. 2, 28-02, Sewage Batch No. 18



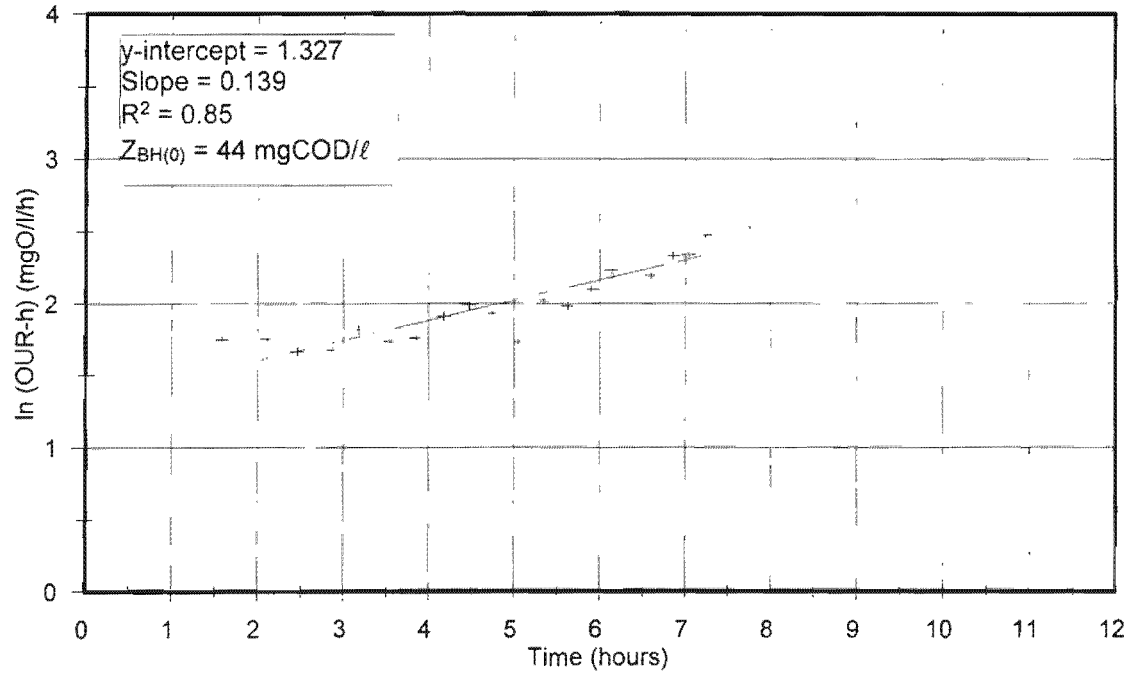
OUR graph for filtered wastewater (2.70ℓ) plus mixed liquor (0.30ℓ)
Batch Test No. 4, 02-03, Sewage Batch No. 18



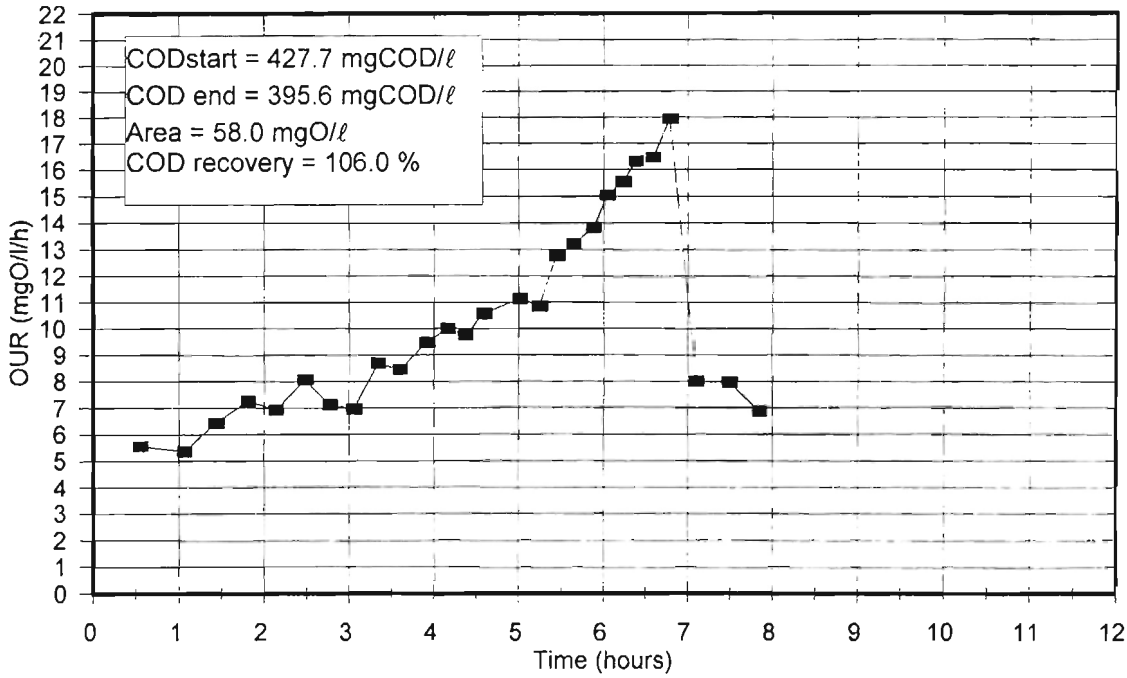
NO_3/NO_2 graph for for filtered wastewater (2.70ℓ) plus mixed liquor (0.30ℓ)
Batch Test No. 4, 02-03, Sewage Batch No. 18



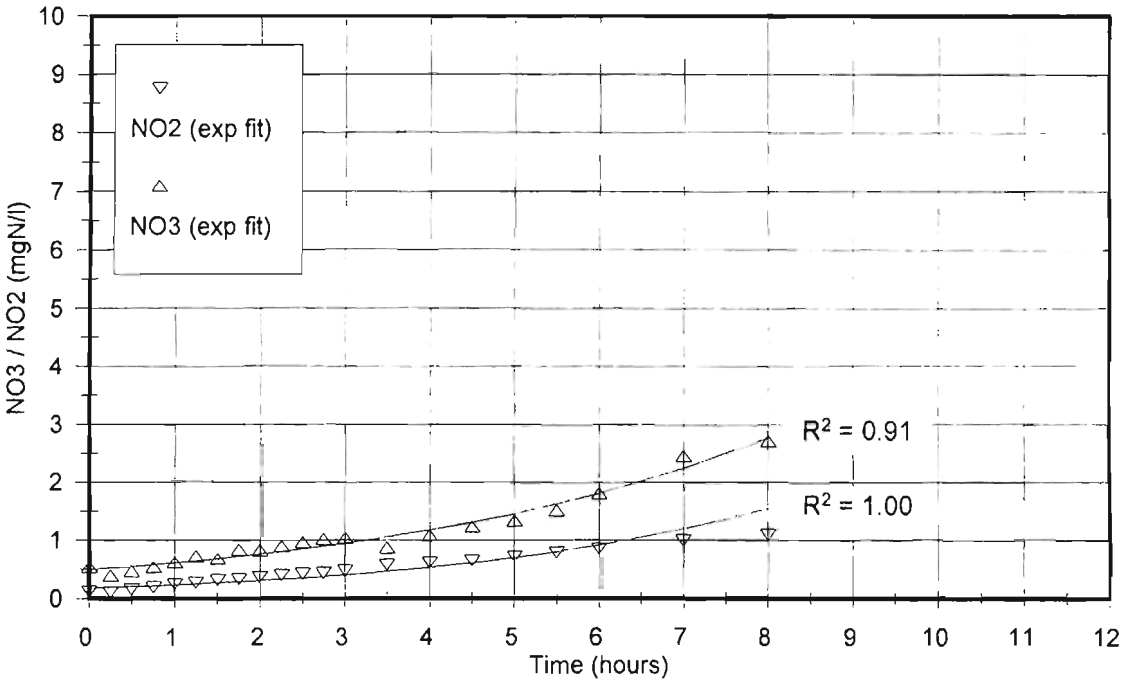
OUR-h graph for filtered wastewater (2.70ℓ) plus mixed liquor (0.30ℓ)
Batch Test No. 4, 02-03, Sewage Batch No. 18



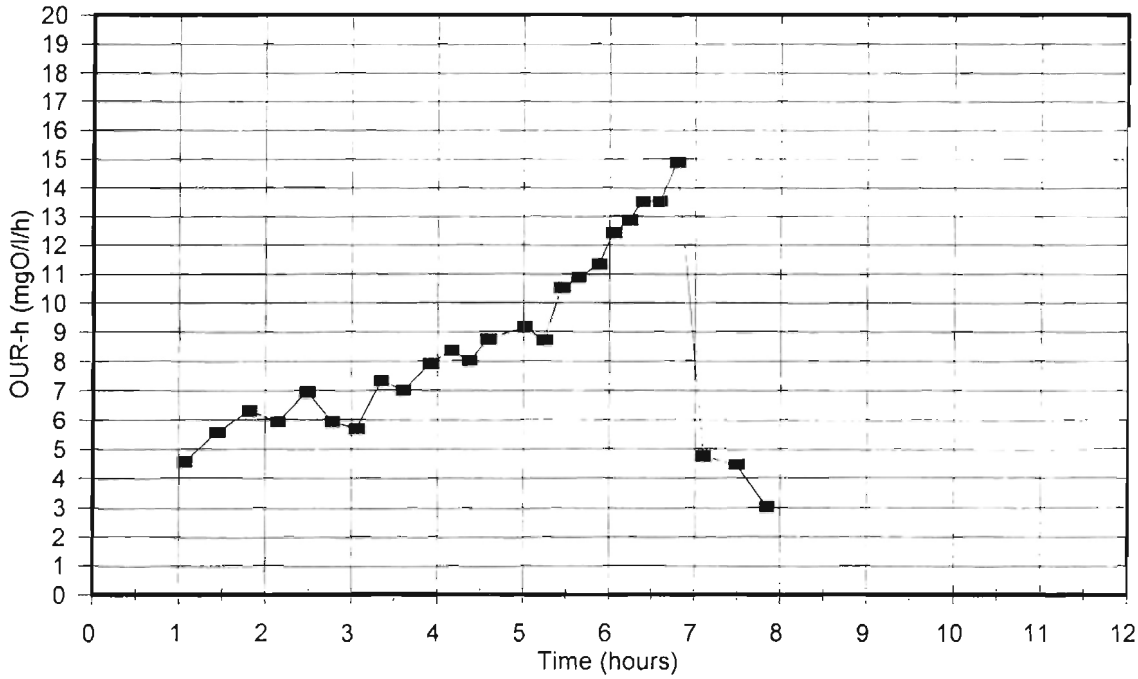
ℓn(OUR-h) graph for filtered wastewater (2.70ℓ) plus mixed liquor (0.30ℓ)
Batch Test No. 4, 02-03, Sewage Batch No. 18



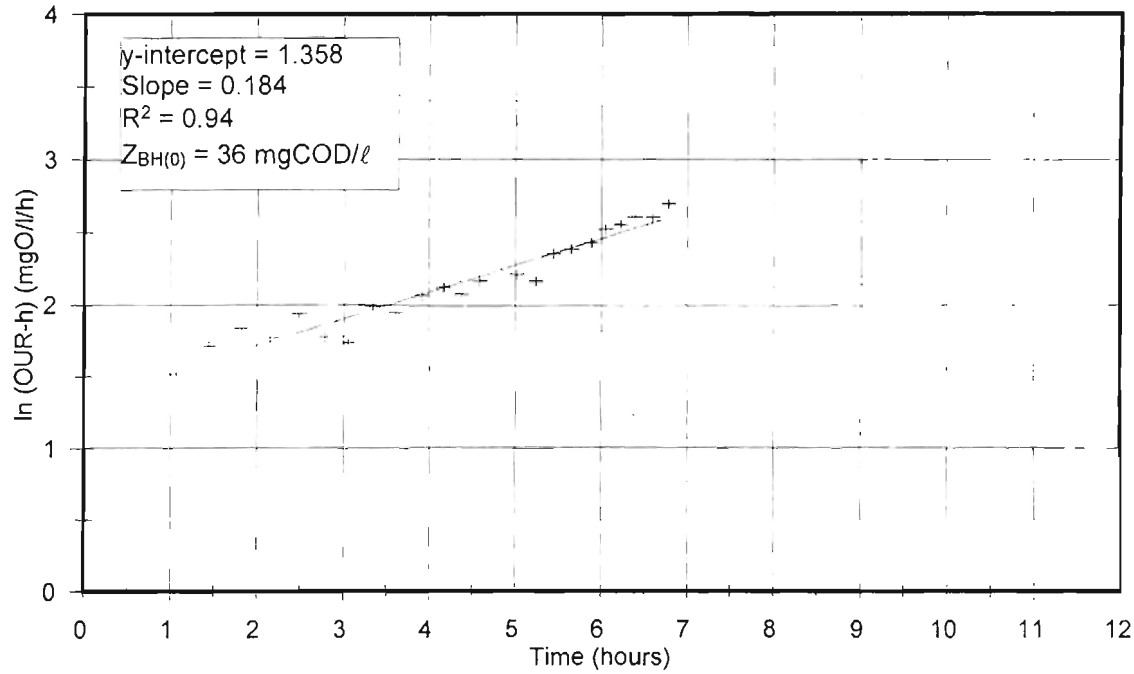
OUR graph for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 6, 04-03, Sewage Batch No. 18



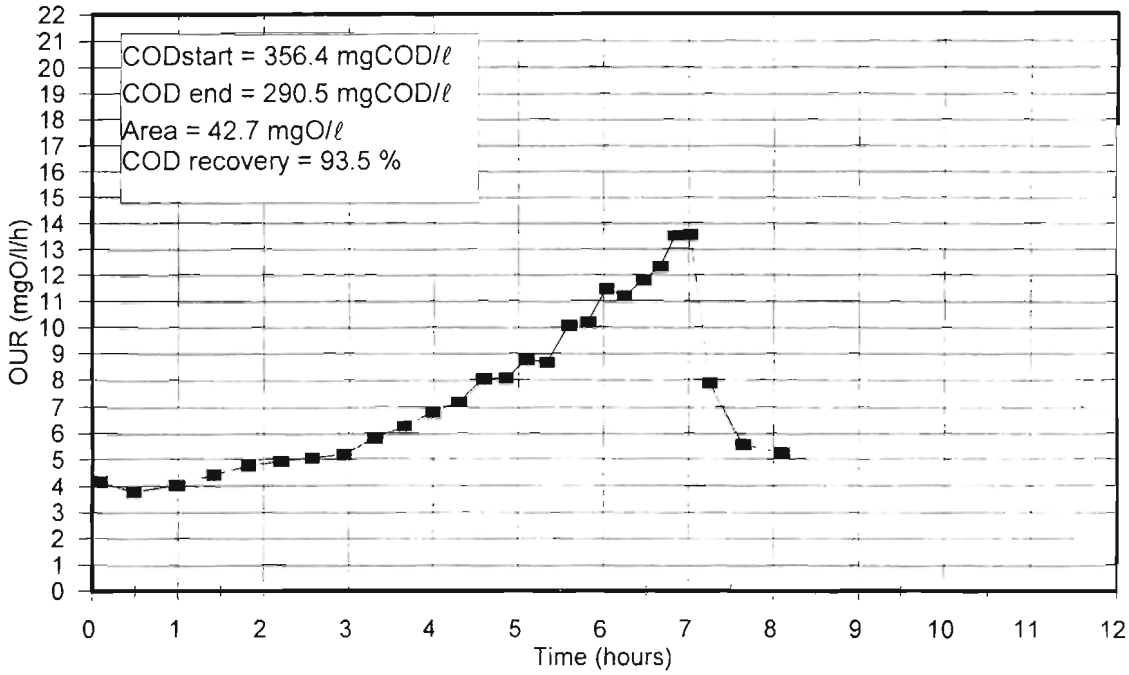
NO_3/NO_2 graph for for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 6, 04-03, Sewage Batch No. 18



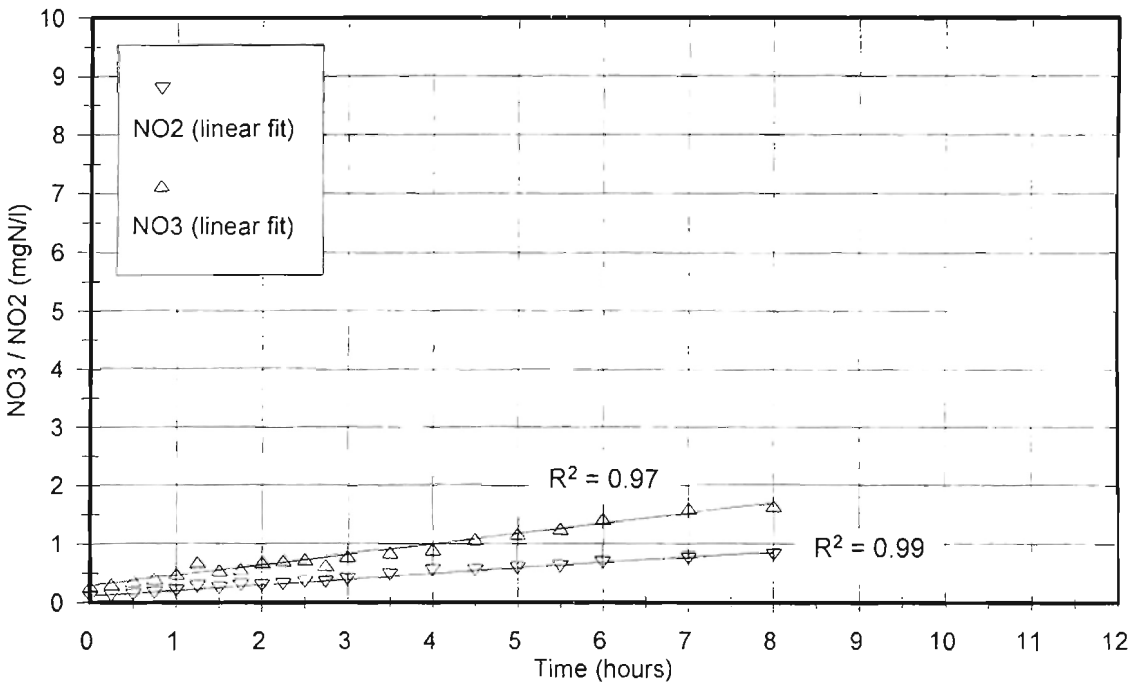
OUR-h graph for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 6, 04-03, Sewage Batch No. 18



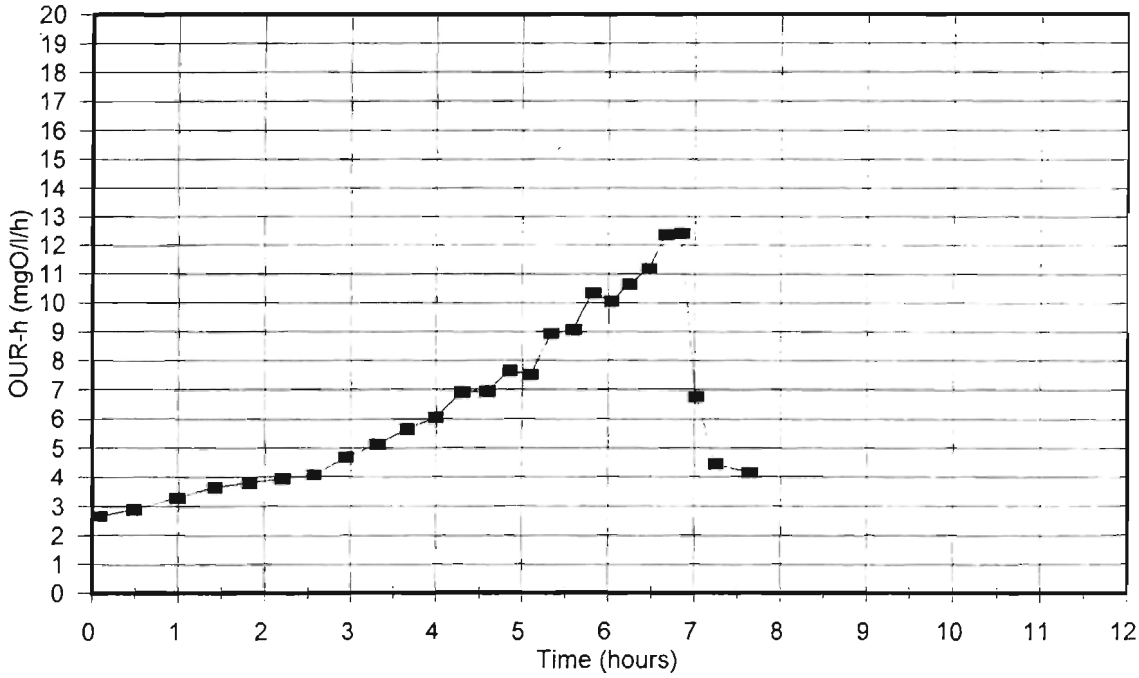
ℓn(OUR-h) graph for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 6, 04-03, Sewage Batch No. 18



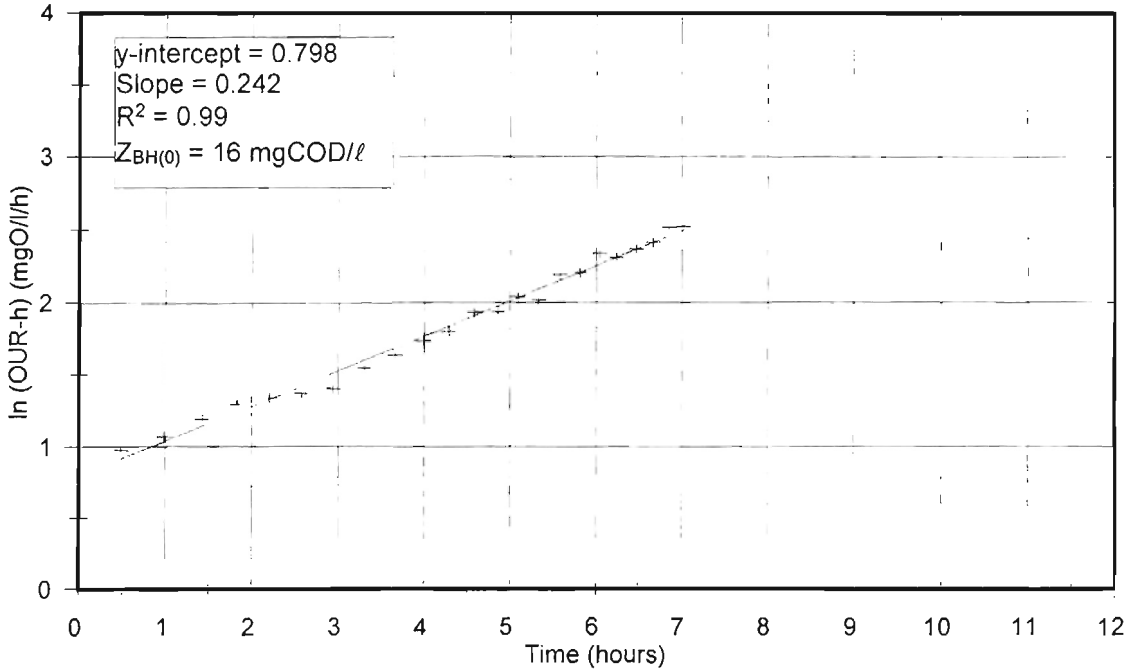
OUR graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ)
Batch Test No. 8, 05-03, Sewage Batch No. 18



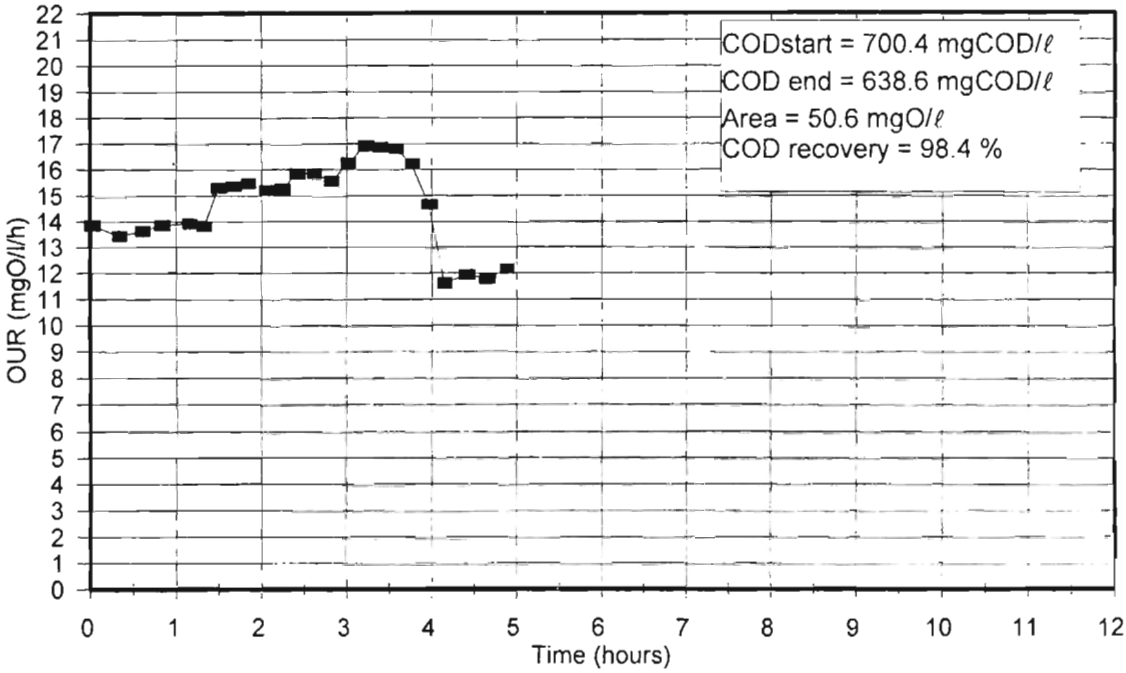
NO_3/NO_2 graph for for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ)
Batch Test No. 8, 05-03, Sewage Batch No. 18



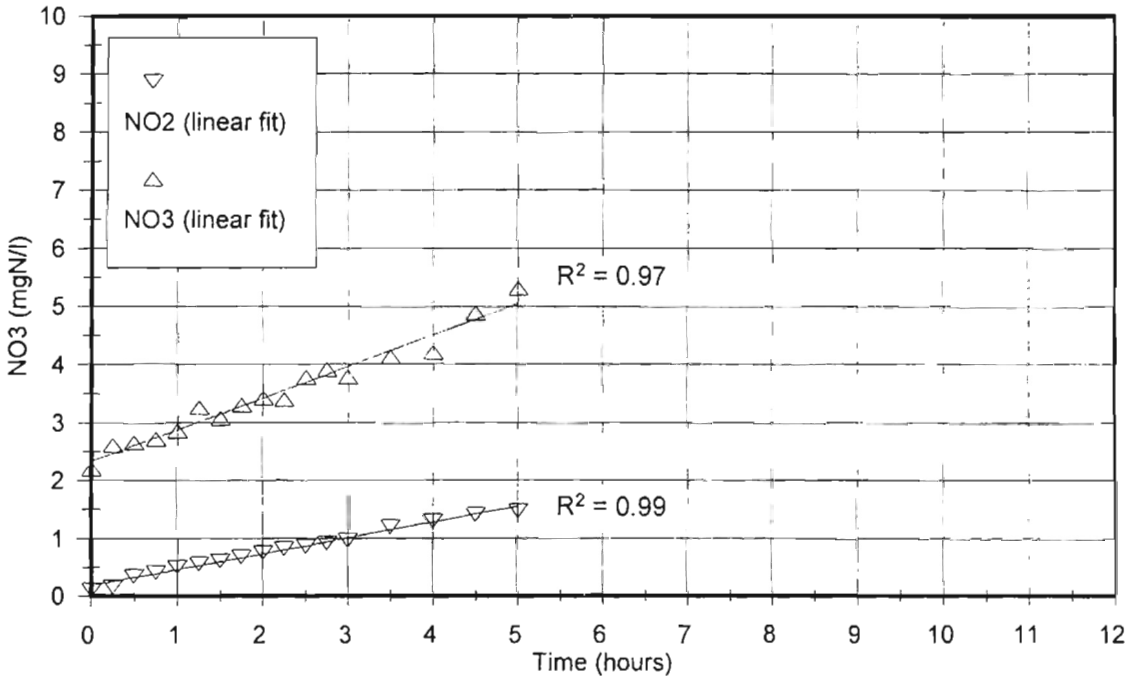
OUR-h graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ)
Batch Test No. 8, 05-03, Sewage Batch No. 18



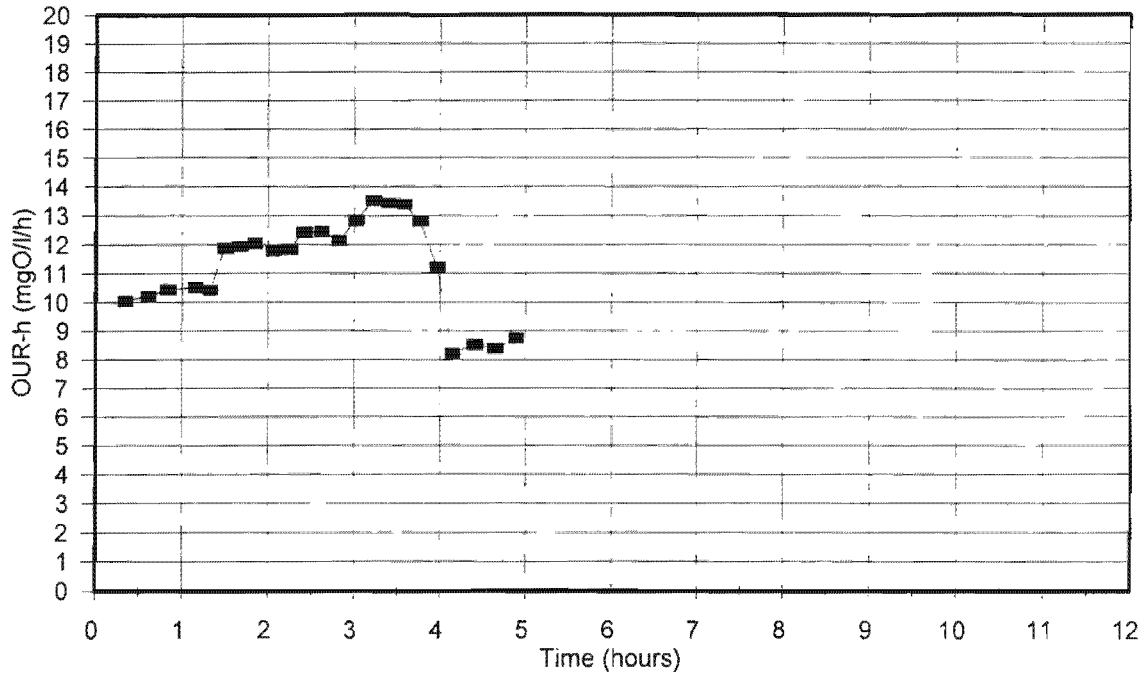
ℓn(OUR-h) graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ)
Batch Test No. 8, 05-03, Sewage Batch No. 18



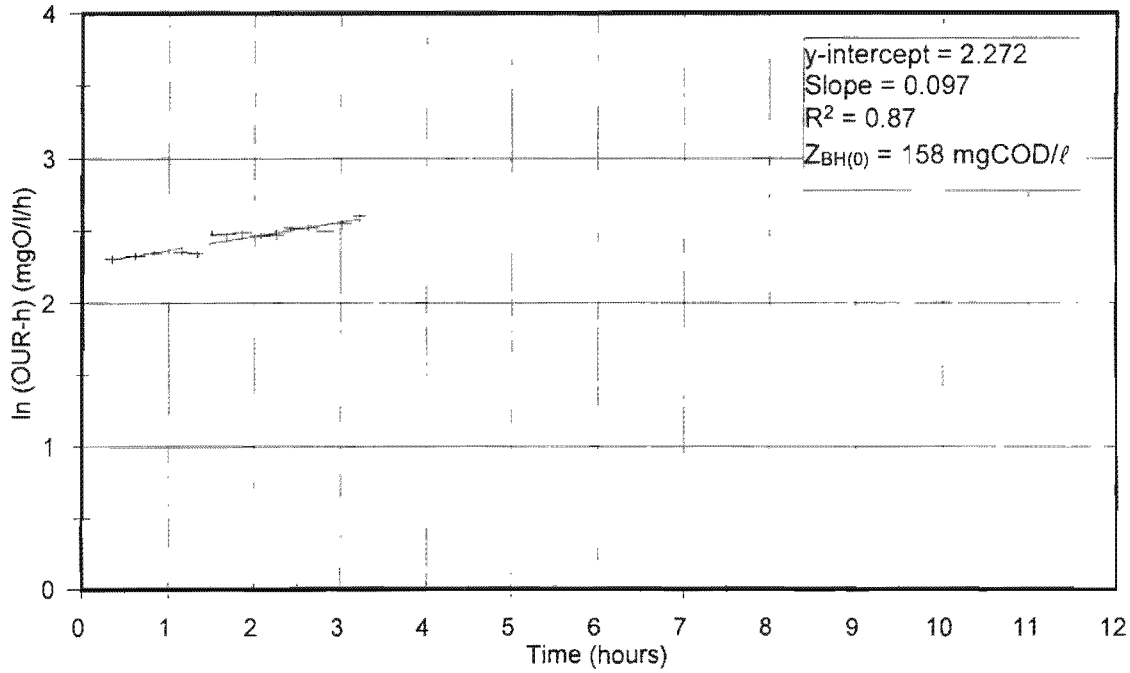
OUR graph for filtered wastewater (2.60ℓ) plus mixed liquor (0.40ℓ)
Batch Test No. 10, 12-03, Sewage Batch No. 19



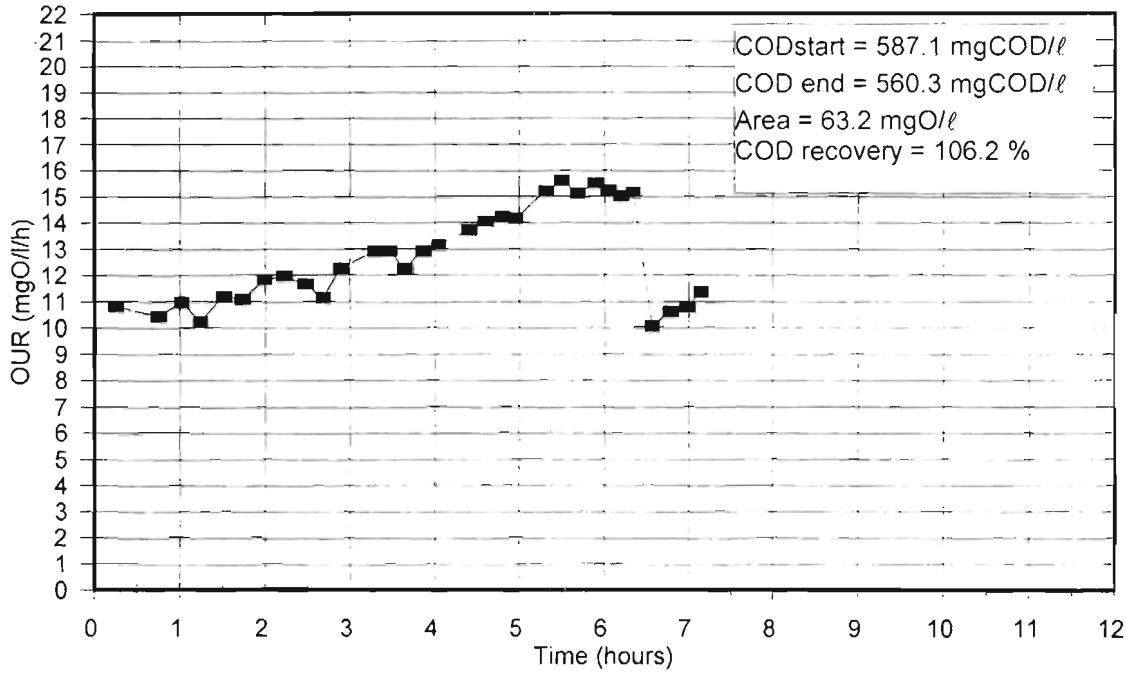
NO_3/NO_2 graph for for filtered wastewater (2.60ℓ) plus mixed liquor (0.40ℓ)
Batch Test No. 10, 12-03, Sewage Batch No. 19



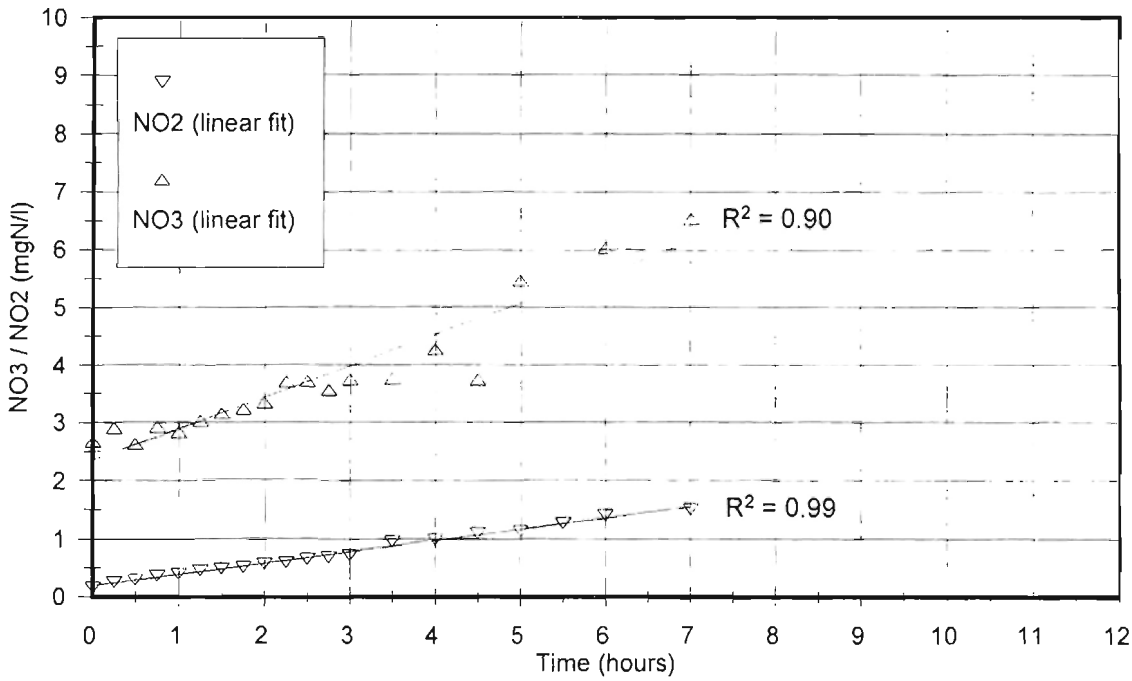
OUR-h graph for filtered wastewater (2.60ℓ) plus mixed liquor (0.40ℓ)
Batch Test No. 10, 12-03, Sewage Batch No. 19



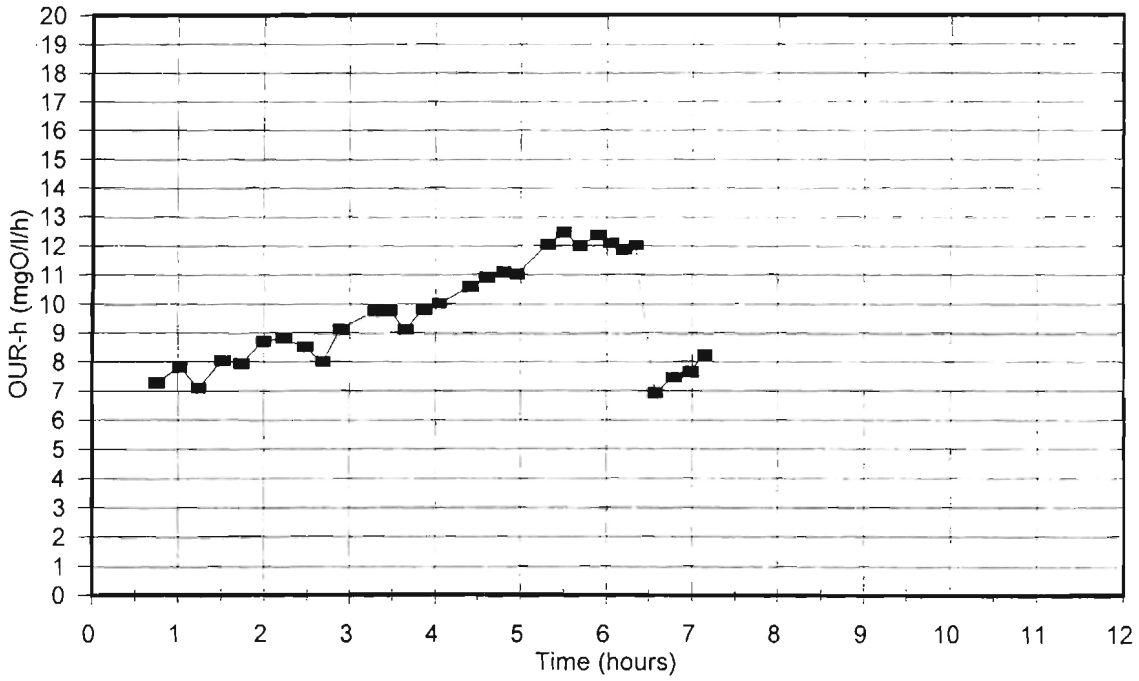
ℓn(OUR-h) graph for filtered wastewater (2.60ℓ) plus mixed liquor (0.40ℓ)
Batch Test No. 10, 12-03, Sewage Batch No. 19



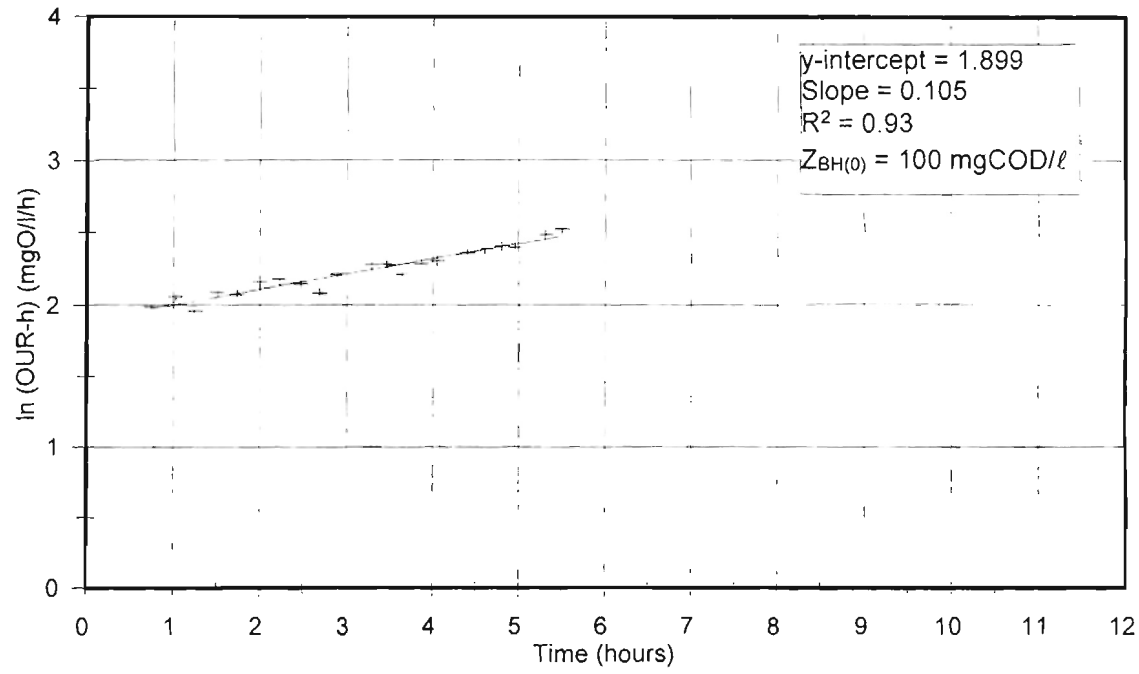
OUR graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 12, 13-03, Sewage Batch No. 19



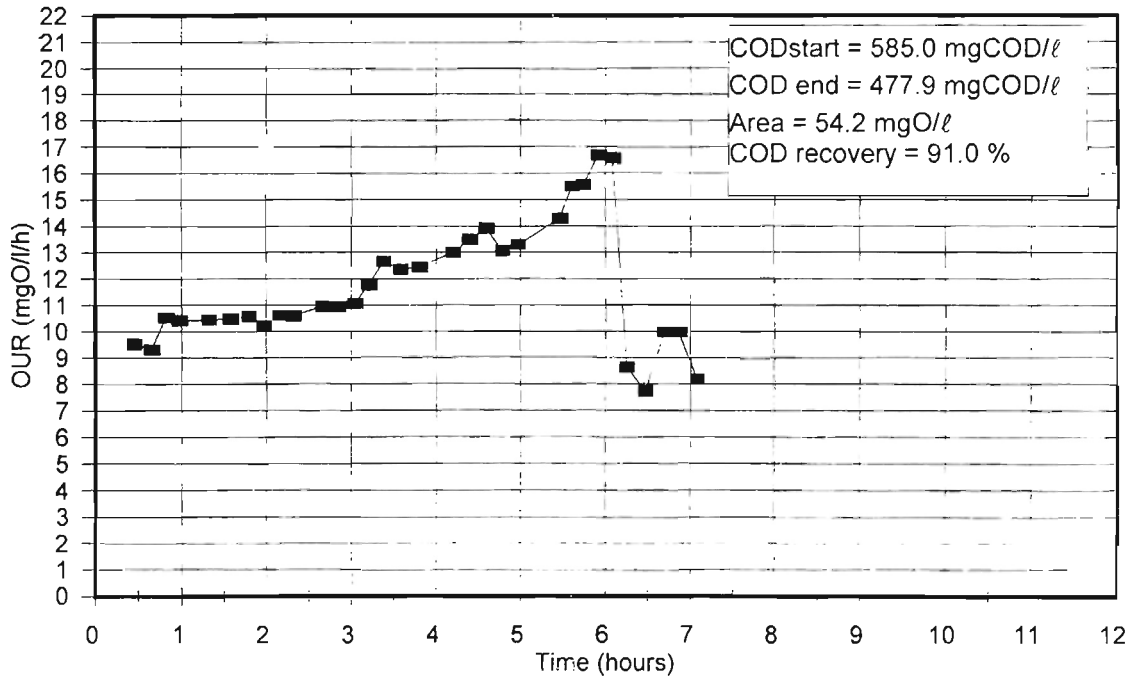
NO_3/NO_2 graph for for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 12, 13-03, Sewage Batch No. 19



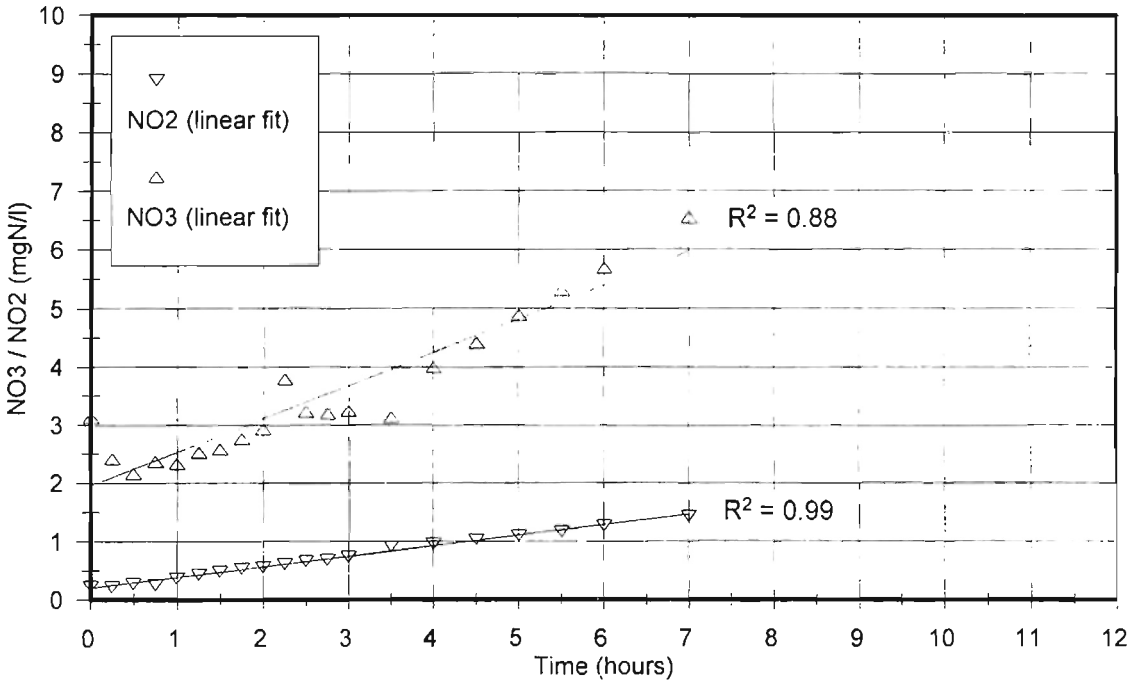
OUR-h graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 12, 13-03, Sewage Batch No. 19



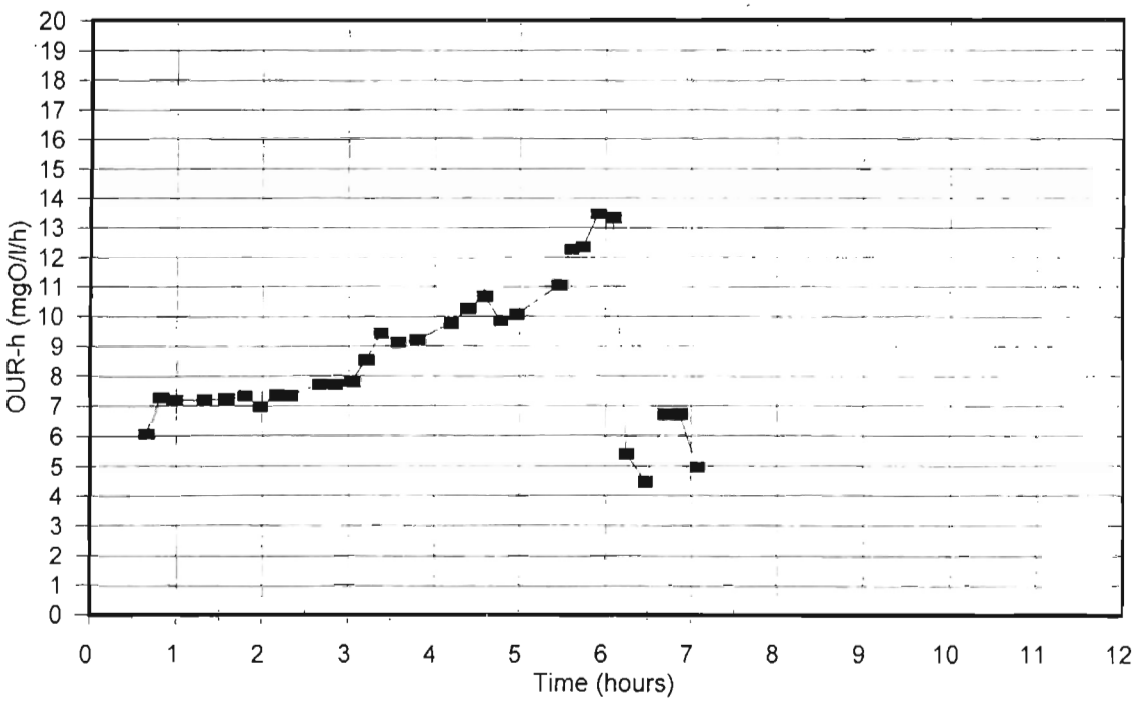
ℓn(OUR-h) graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 12, 13-03, Sewage Batch No. 19



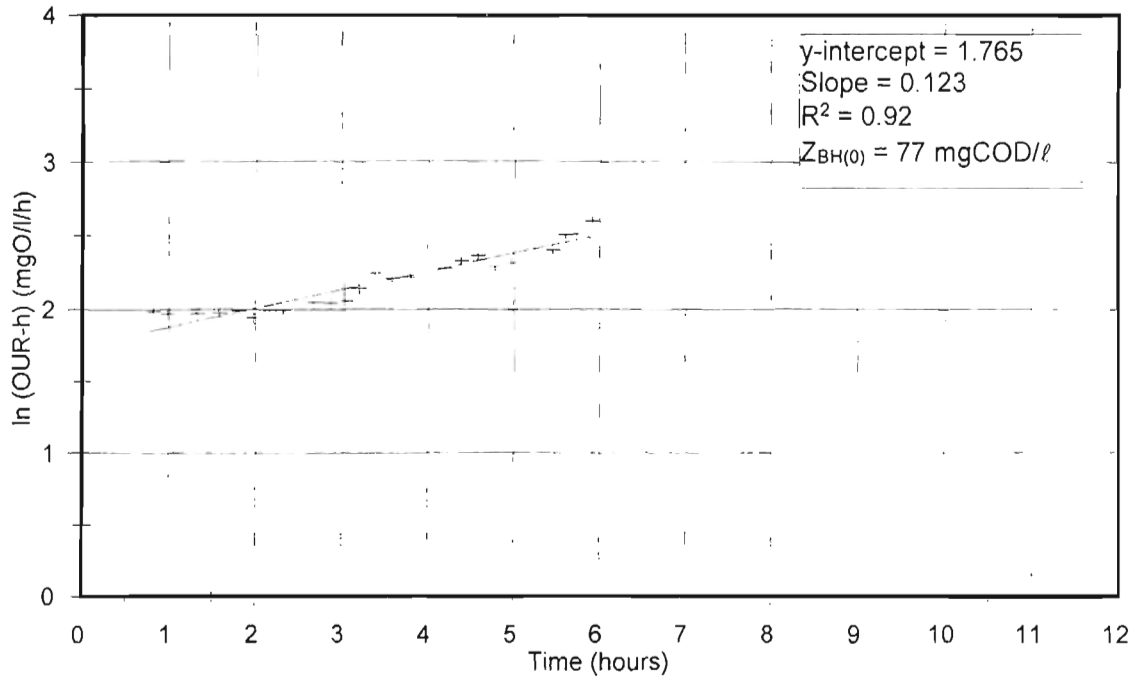
OUR graph for filtered wastewater (2.70ℓ) plus mixed liquor (0.30ℓ)
Batch Test No. 14, 14-03, Sewage Batch No. 19



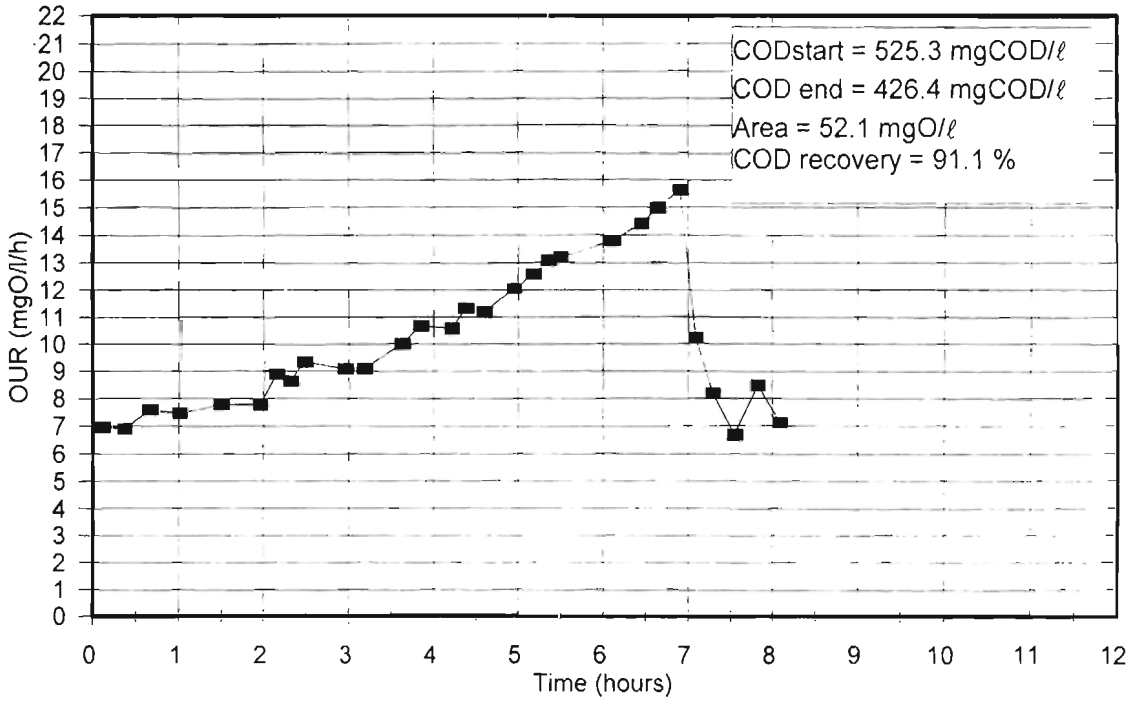
NO_3/NO_2 graph for for filtered wastewater (2.70ℓ) plus mixed liquor (0.30ℓ)
Batch Test No. 14, 14-03, Sewage Batch No. 19



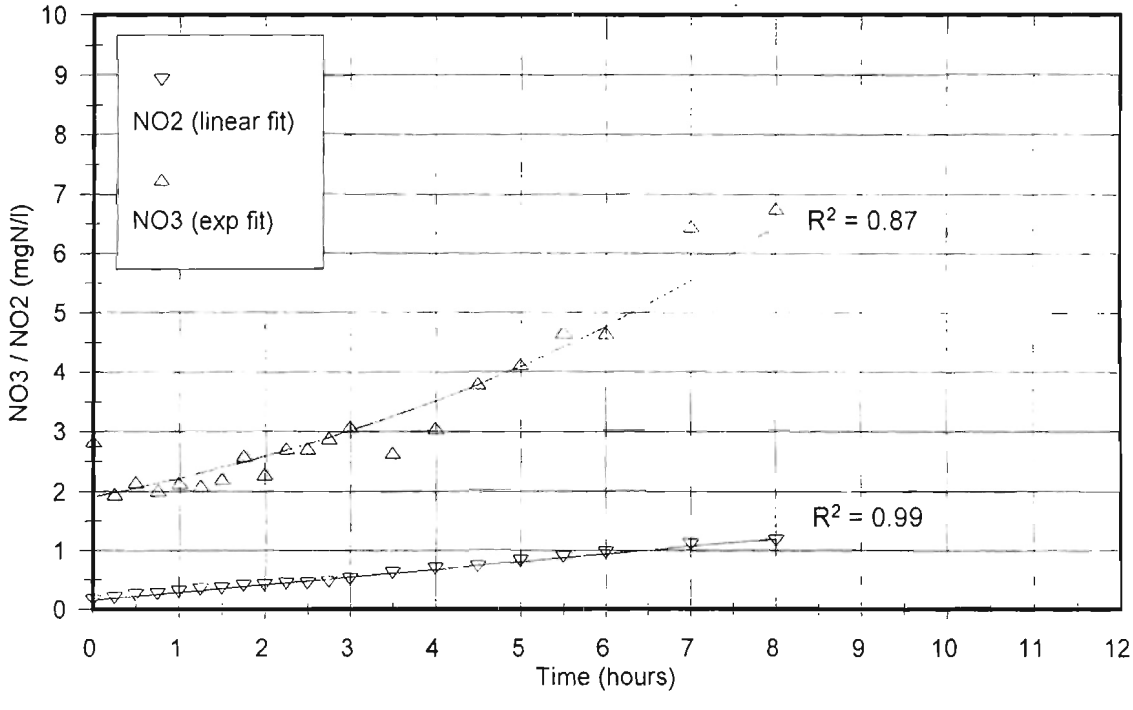
OUR-h graph for filtered wastewater (2.70ℓ) plus mixed liquor (0.30ℓ)
Batch Test No. 14, 14-03, Sewage Batch No. 19



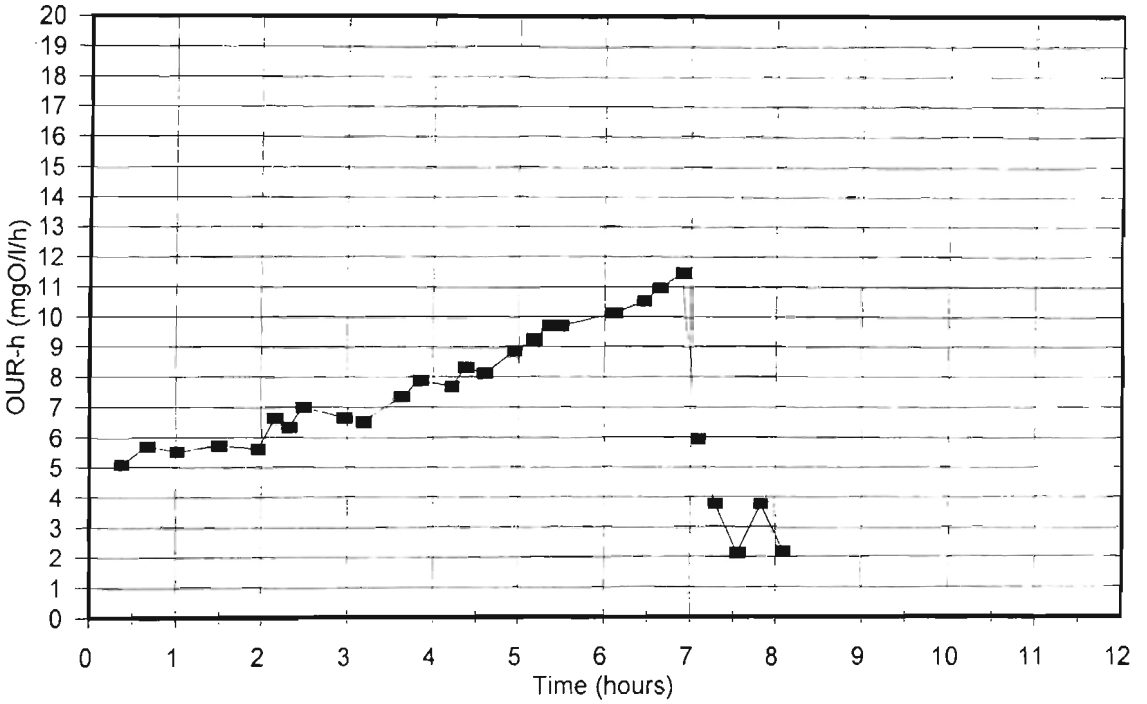
ℓn(OUR-h) graph for filtered wastewater (2.70ℓ) plus mixed liquor (0.30ℓ)
Batch Test No. 14, 14-03, Sewage Batch No. 19



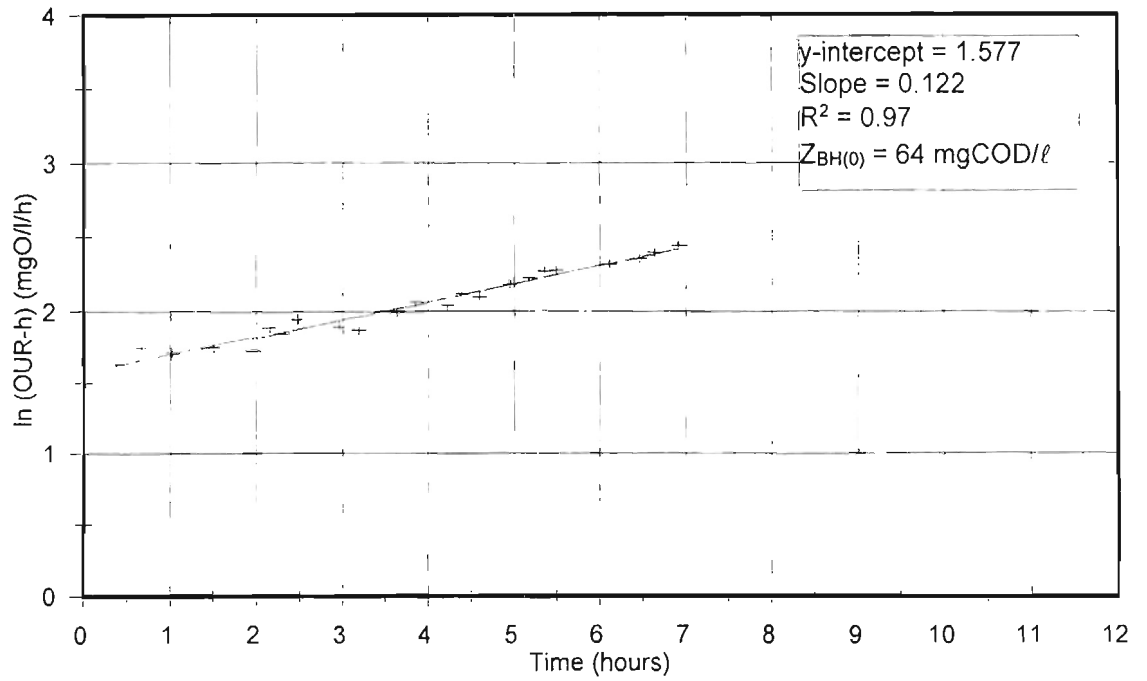
OUR graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ)
Batch Test No. 16, 15-03, Sewage Batch No. 19



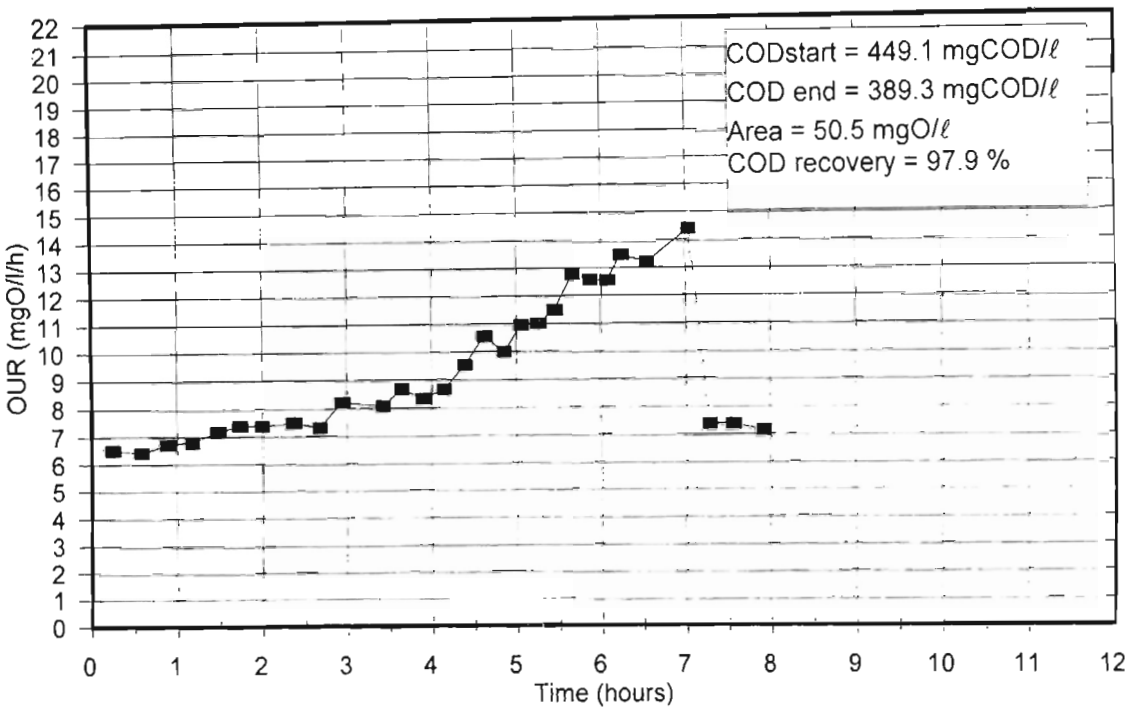
NO_3/NO_2 graph for for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ)
Batch Test No. 16, 15-03, Sewage Batch No. 19



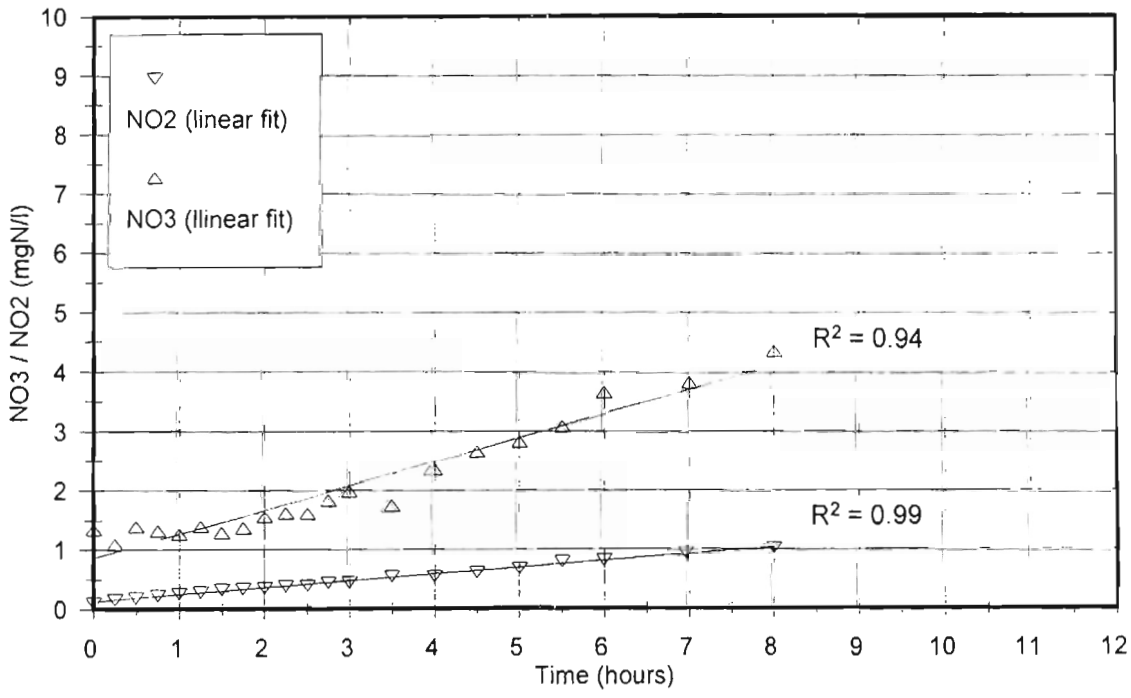
OUR-h graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ)
Batch Test No. 16, 15-03, Sewage Batch No. 19



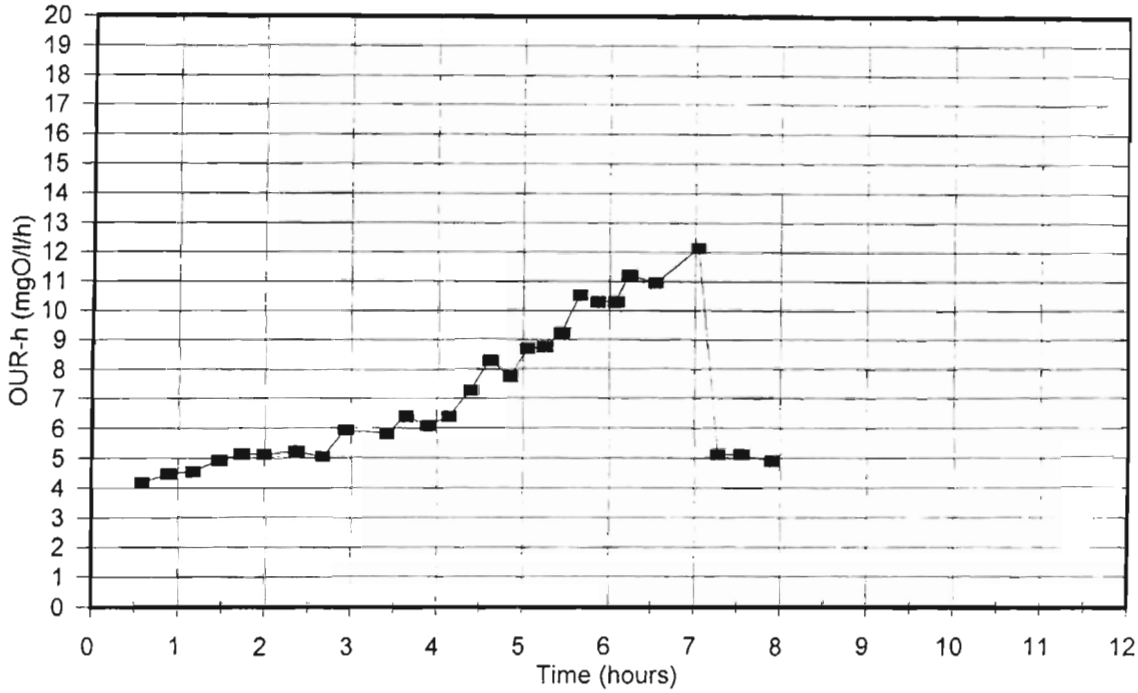
ℓn(OUR-h) graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ)
Batch Test No. 16, 15-03, Sewage Batch No. 19



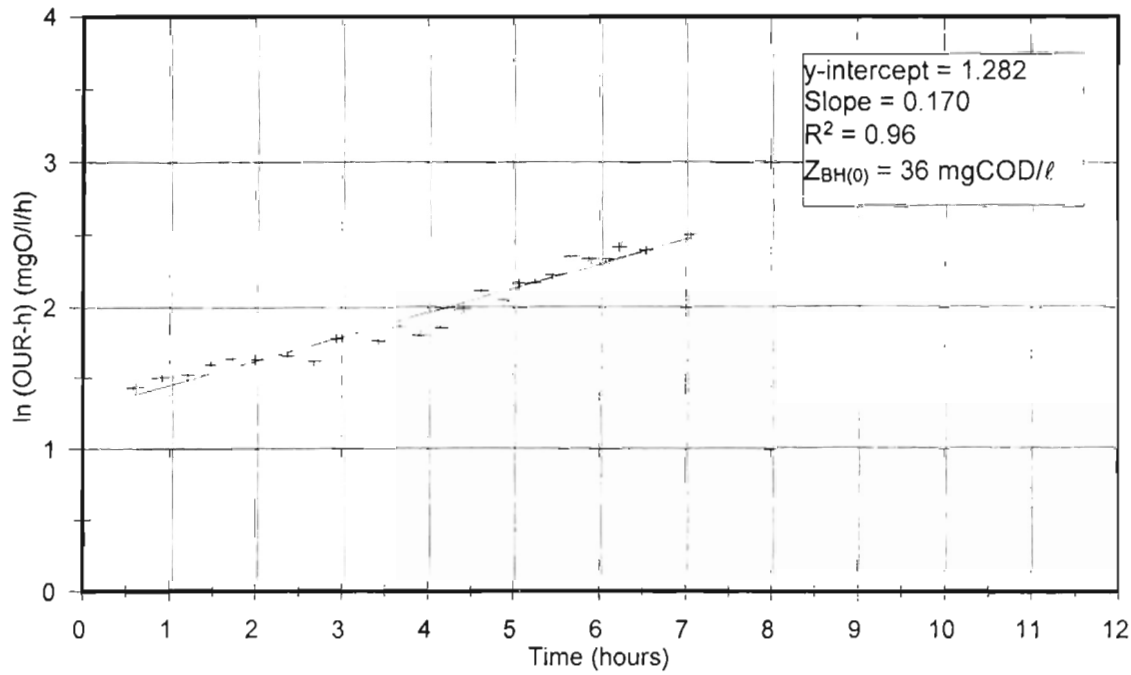
OUR graph for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 18, 16-03, Sewage Batch No. 19



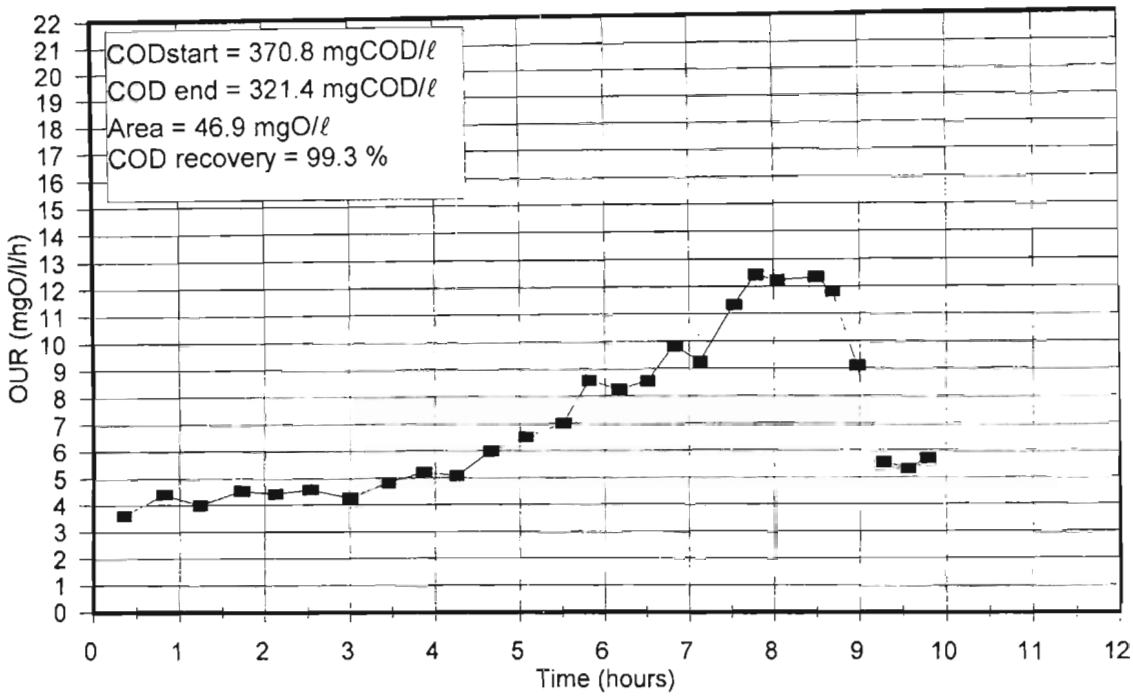
NO_3/NO_2 graph for for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 18, 16-03, Sewage Batch No. 19



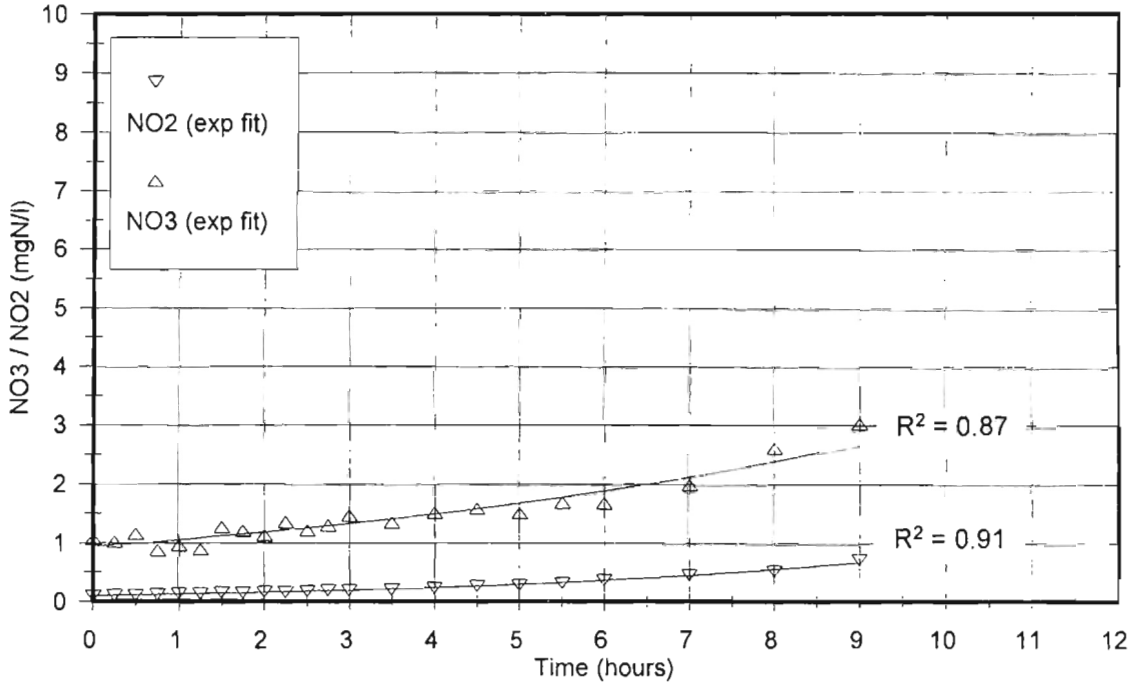
OUR-h graph for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 18, 16-03, Sewage Batch No. 19



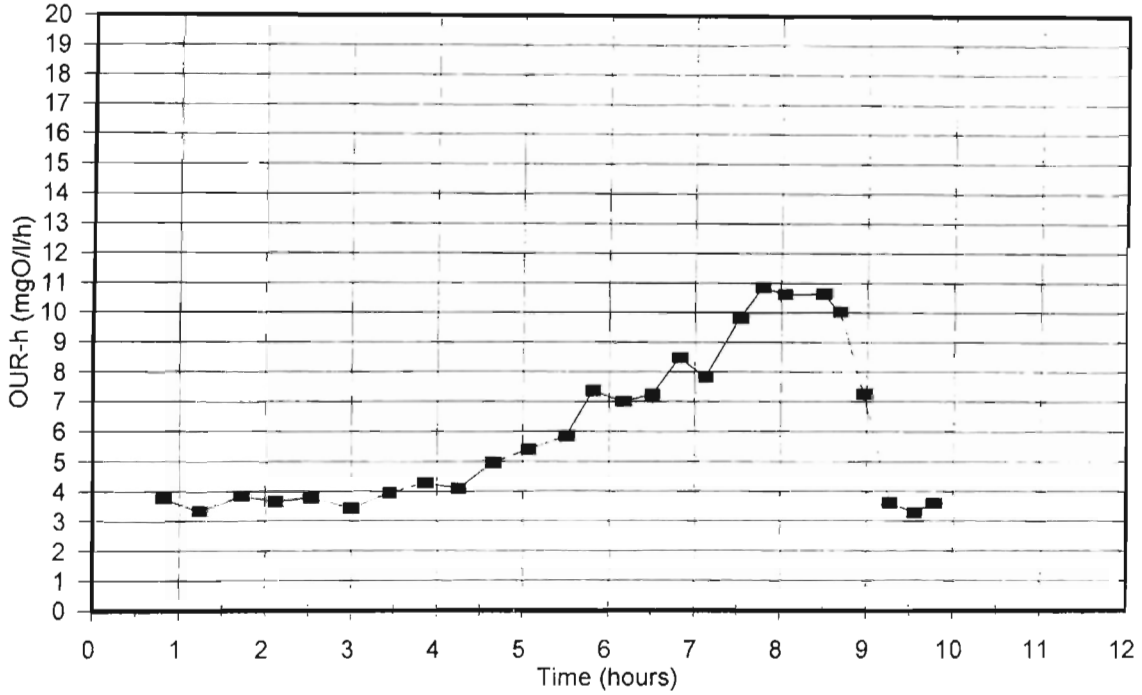
ℓn(OUR-h) graph for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 18, 16-03, Sewage Batch No. 19



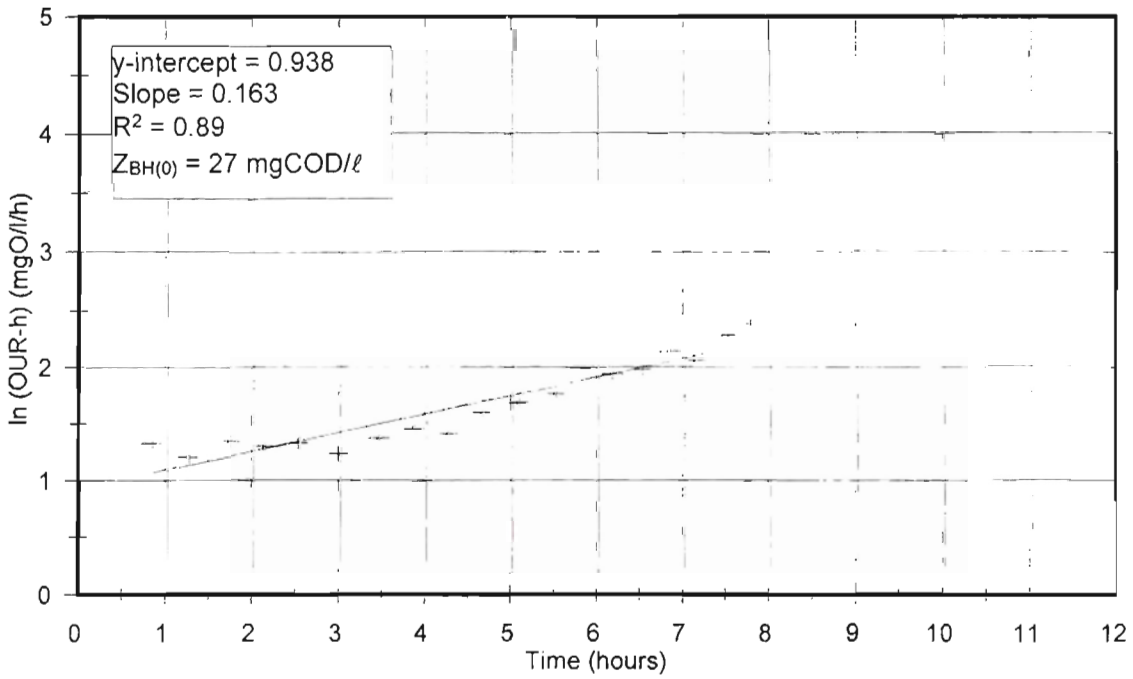
OUR graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ)
Batch Test No. 20, 17-03, Sewage Batch No. 19



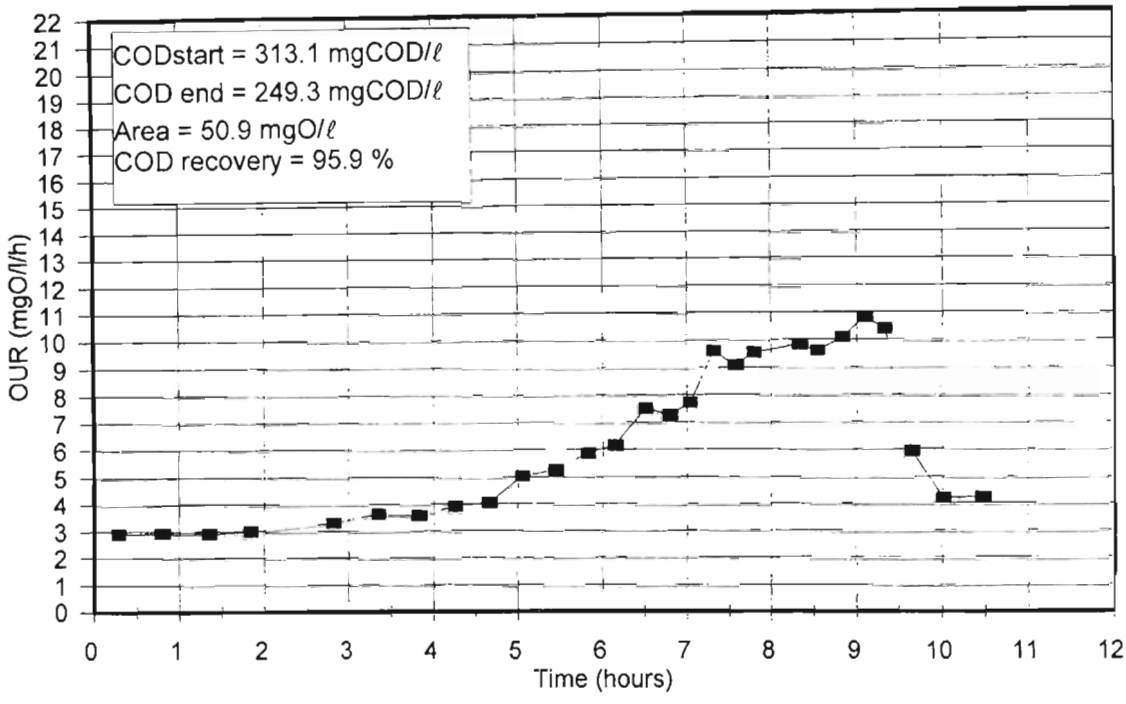
NO_3/NO_2 graph for for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ)
Batch Test No. 20, 17-03, Sewage Batch No. 19



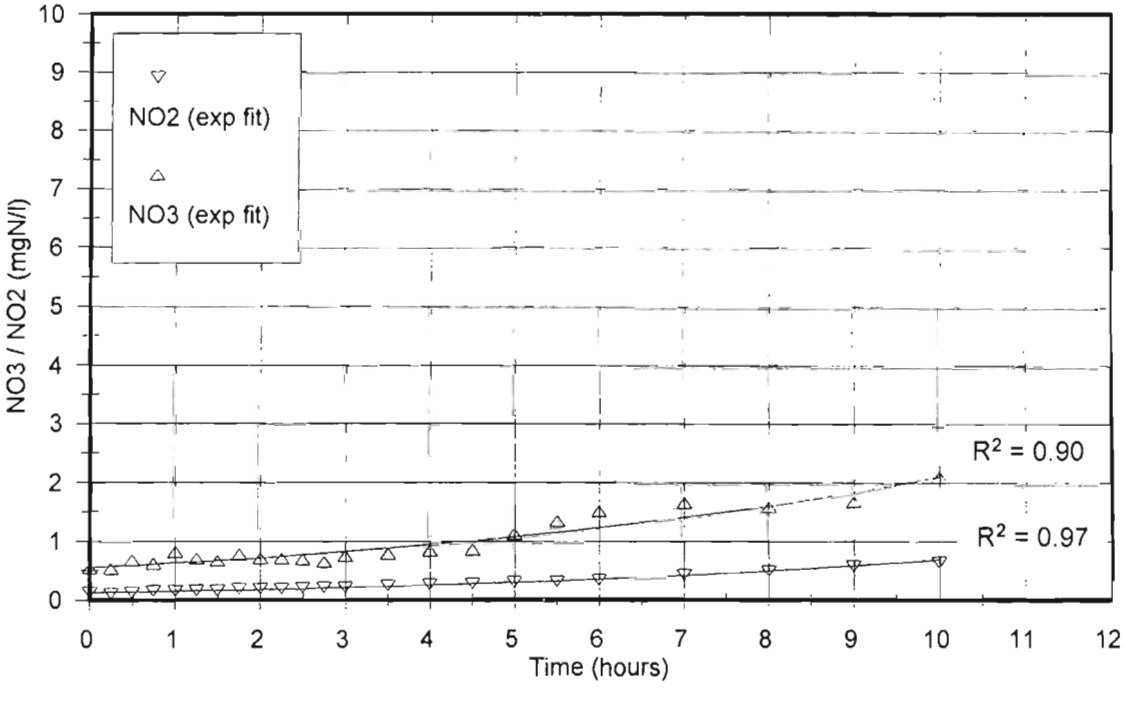
OUR-h graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ)
Batch Test No. 20, 17-03, Sewage Batch No. 19



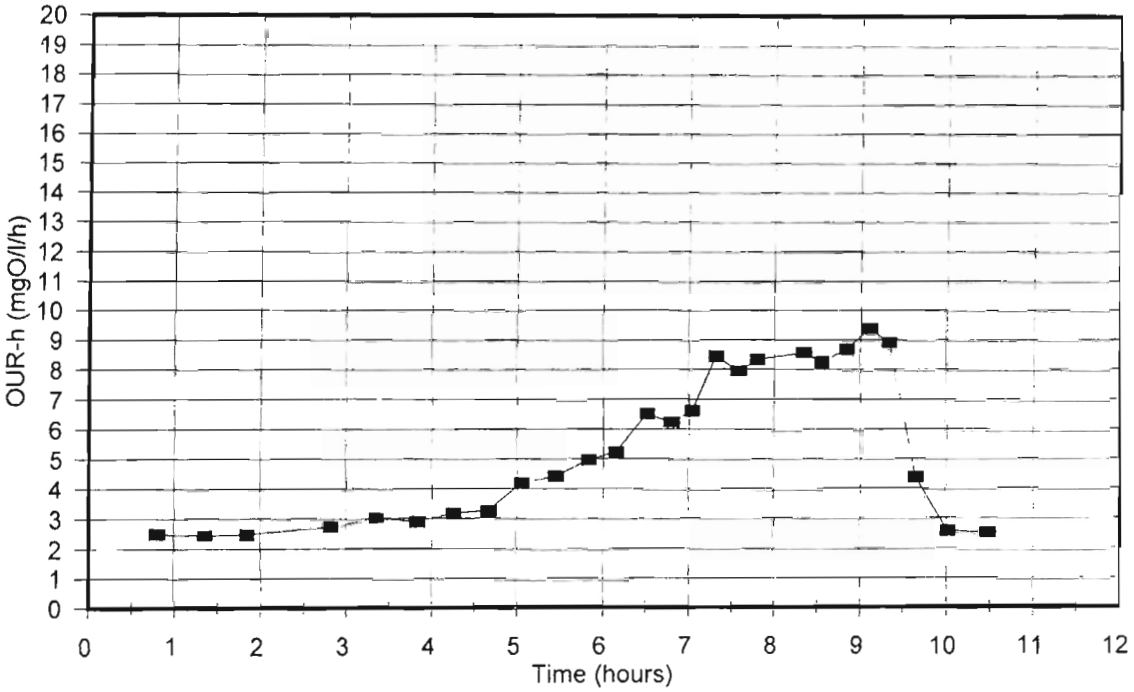
ℓn(OUR-h) graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ)
Batch Test No. 20, 17-03, Sewage Batch No. 19



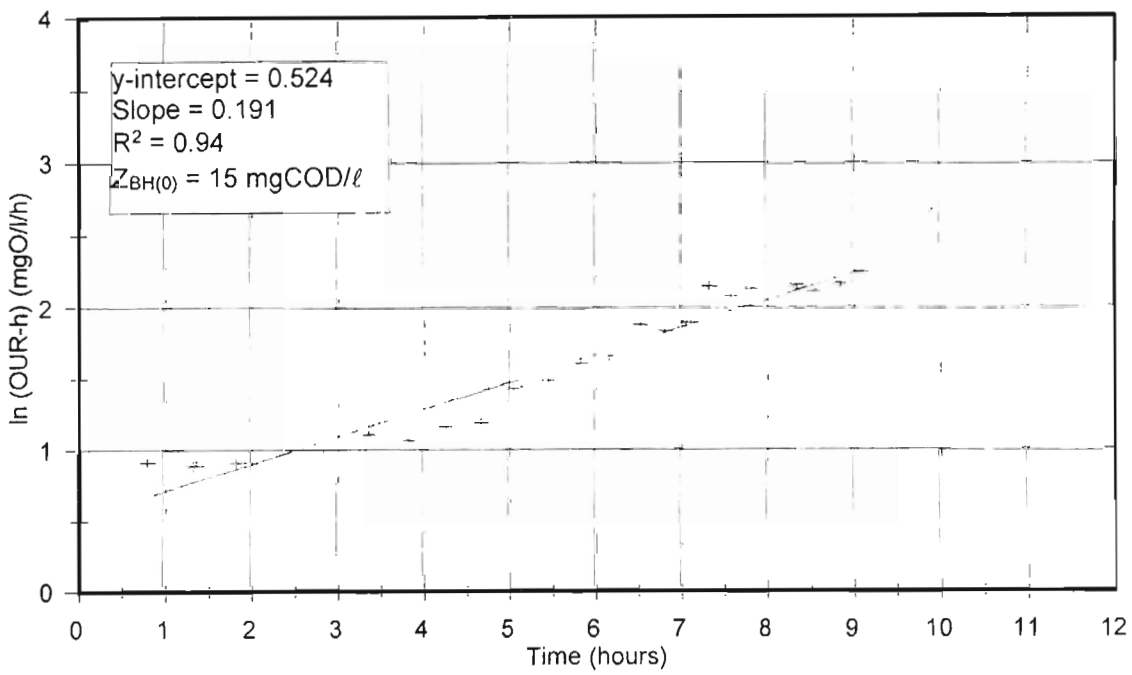
OUR graph for filtered wastewater (2.90ℓ) plus mixed liquor (0.10ℓ)
Batch Test No. 22, 18-03, Sewage Batch No. 19



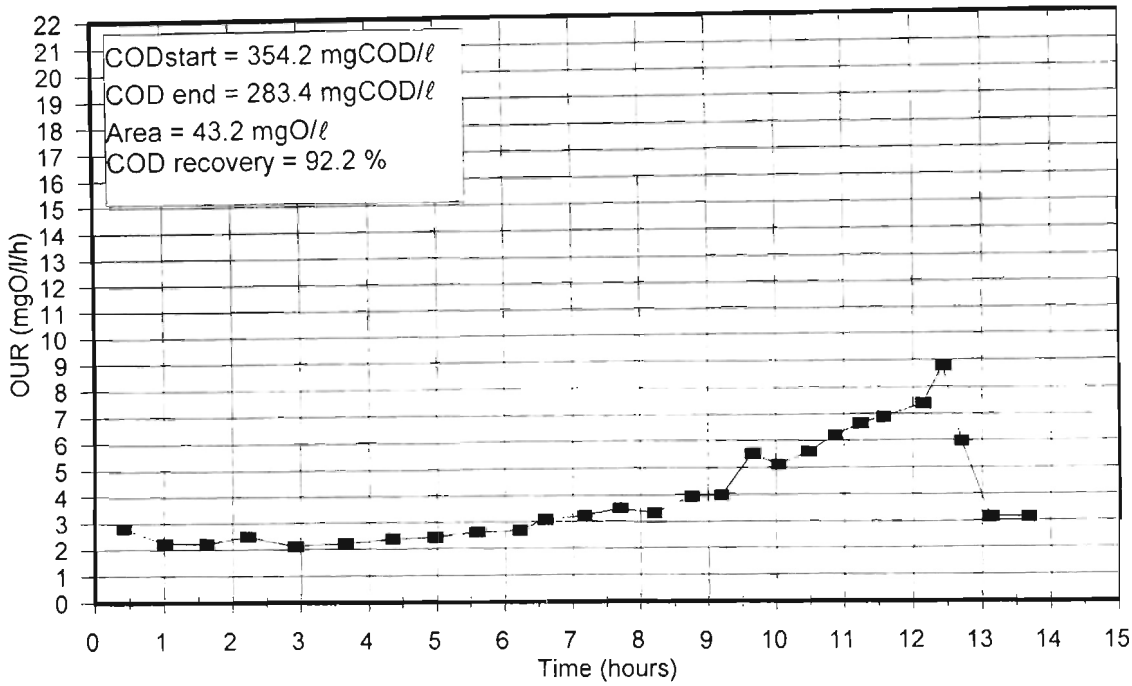
NO_3/NO_2 graph for for filtered wastewater (2.90ℓ) plus mixed liquor (0.10ℓ)
Batch Test No. 22, 18-03, Sewage Batch No. 19



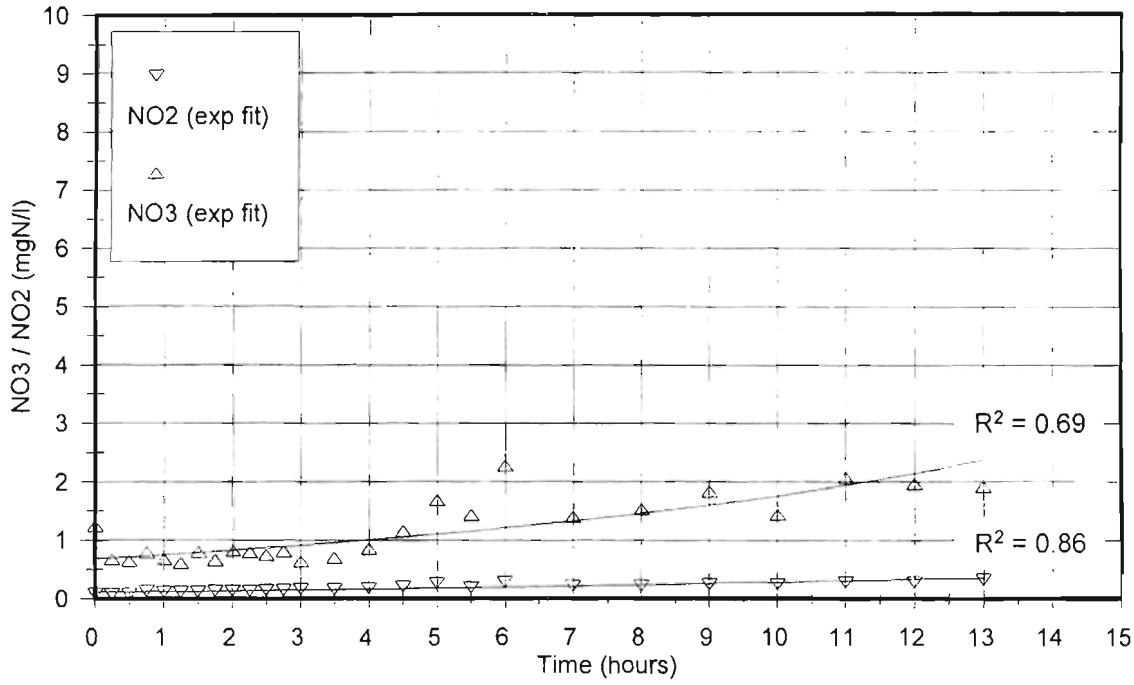
OUR-h graph for filtered wastewater (2.90ℓ) plus mixed liquor (0.10ℓ)
Batch Test No. 22, 18-03, Sewage Batch No. 19



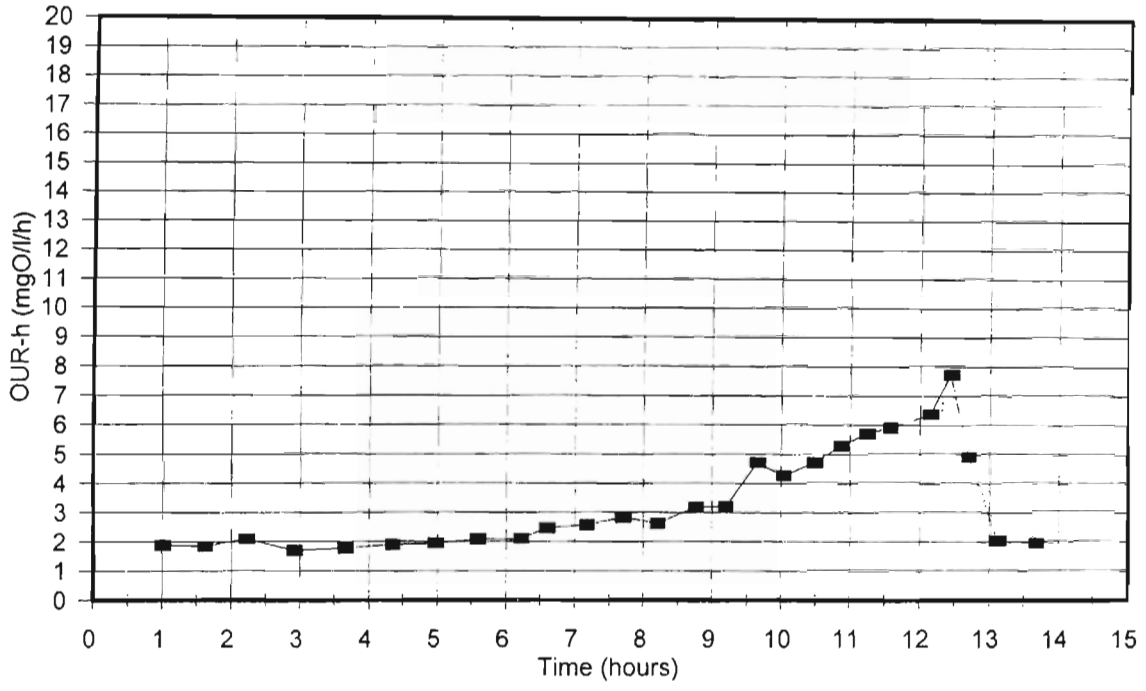
ℓn(OUR-h) graph for filtered wastewater (2.90ℓ) plus mixed liquor (0.10ℓ)
Batch Test No. 22, 18-03, Sewage Batch No. 19



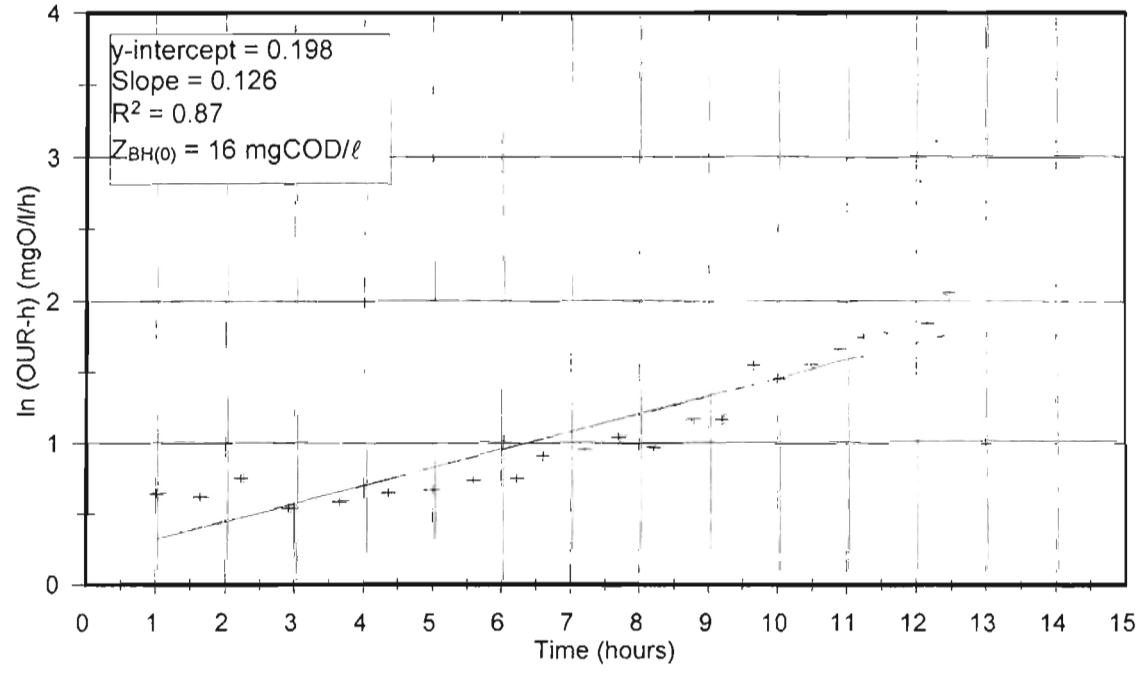
OUR graph for filtered wastewater (2.90ℓ) plus mixed liquor (0.10ℓ)
Batch Test No. 24, 28-03, Sewage Batch No. 20



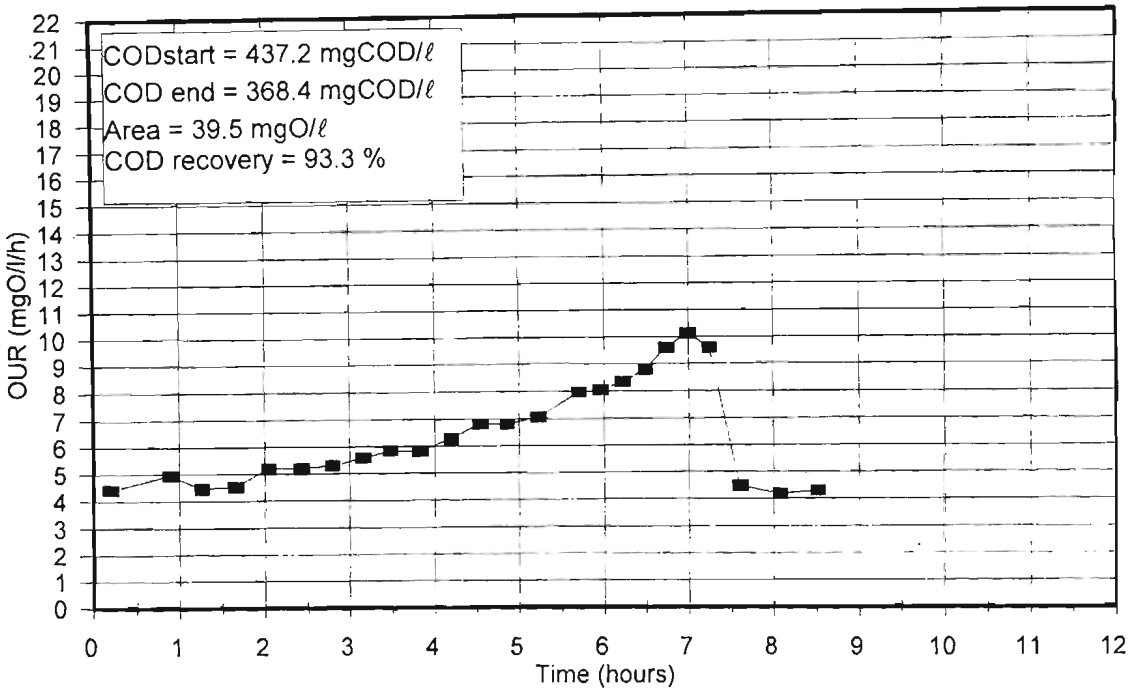
NO_3/NO_2 graph for for filtered wastewater (2.90ℓ) plus mixed liquor (0.10ℓ)
Batch Test No. 24, 28-03, Sewage Batch No. 20



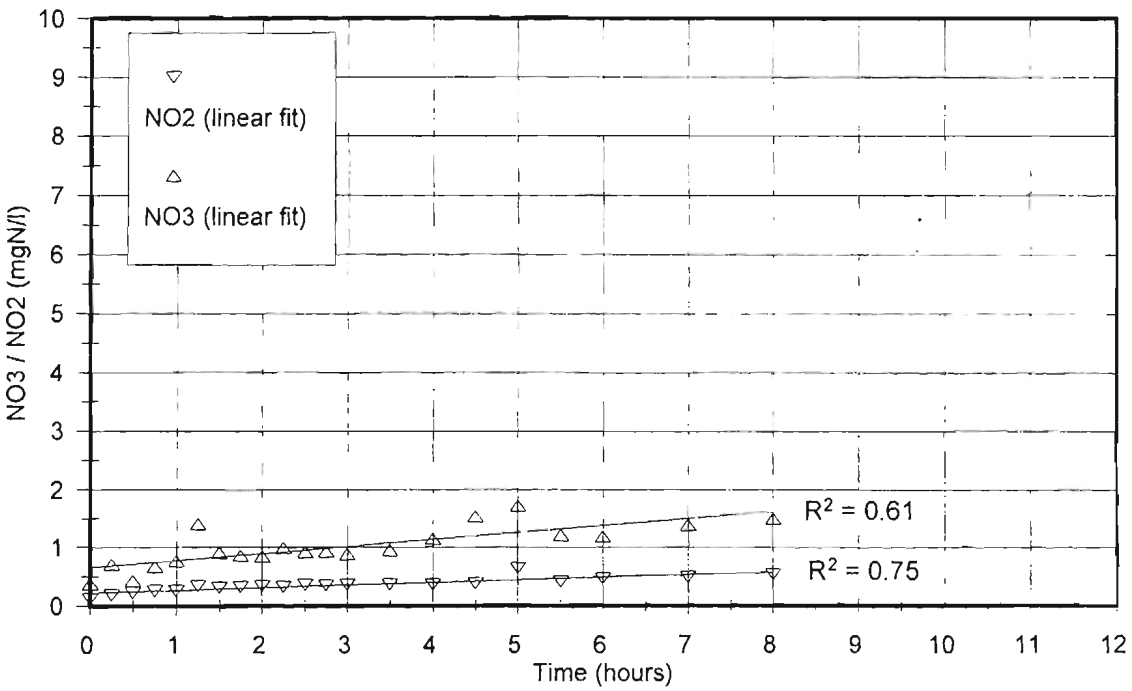
OUR-h graph for filtered wastewater (2.90ℓ) plus mixed liquor (0.10ℓ)
Batch Test No. 24, 28-03, Sewage Batch No. 20



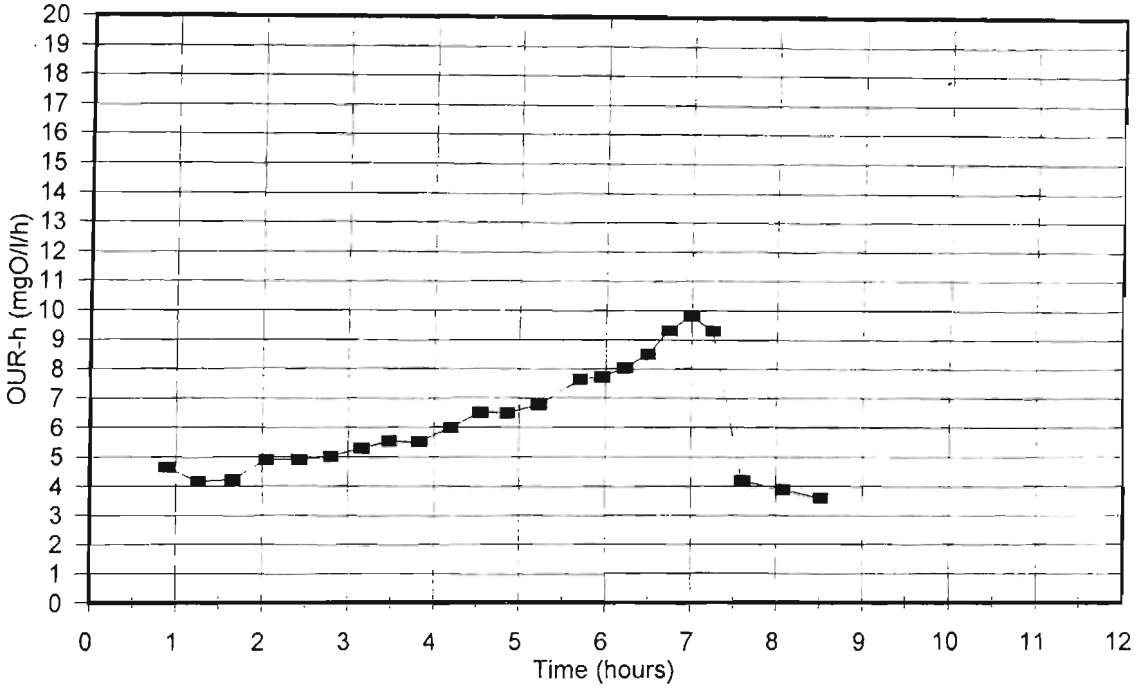
ℓn(OUR-h) graph for filtered wastewater (2.90ℓ) plus mixed liquor (0.10ℓ)
Batch Test No. 24, 28-03, Sewage Batch No. 20



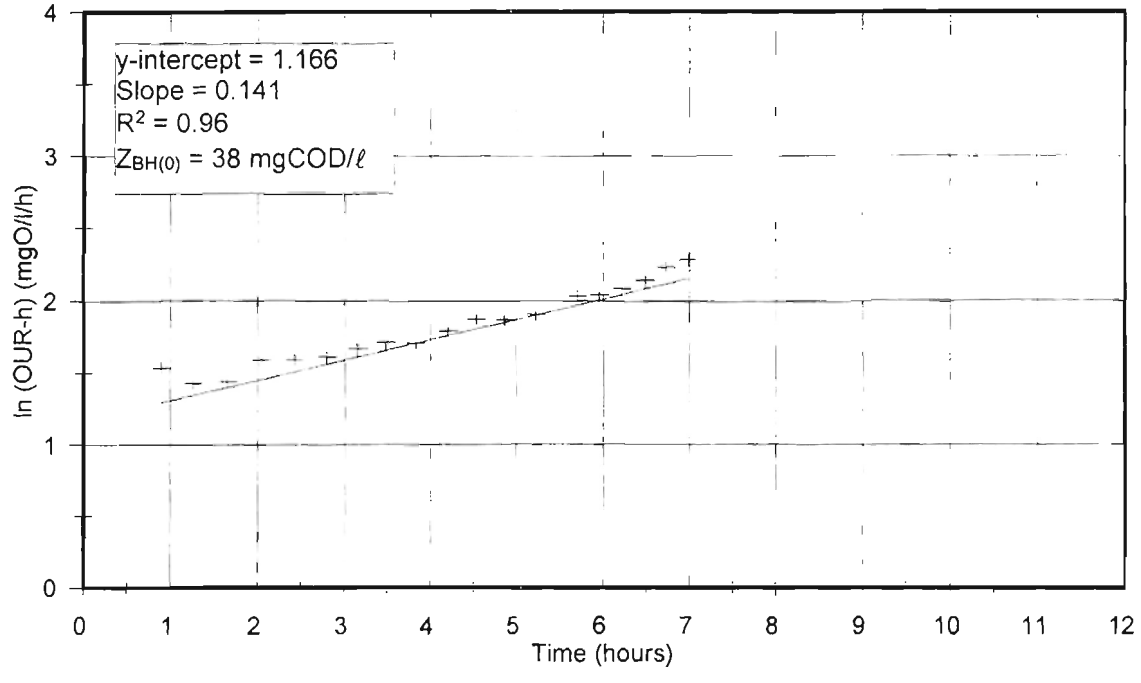
OUR graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ)
Batch Test No. 26, 30-03, Sewage Batch No. 20



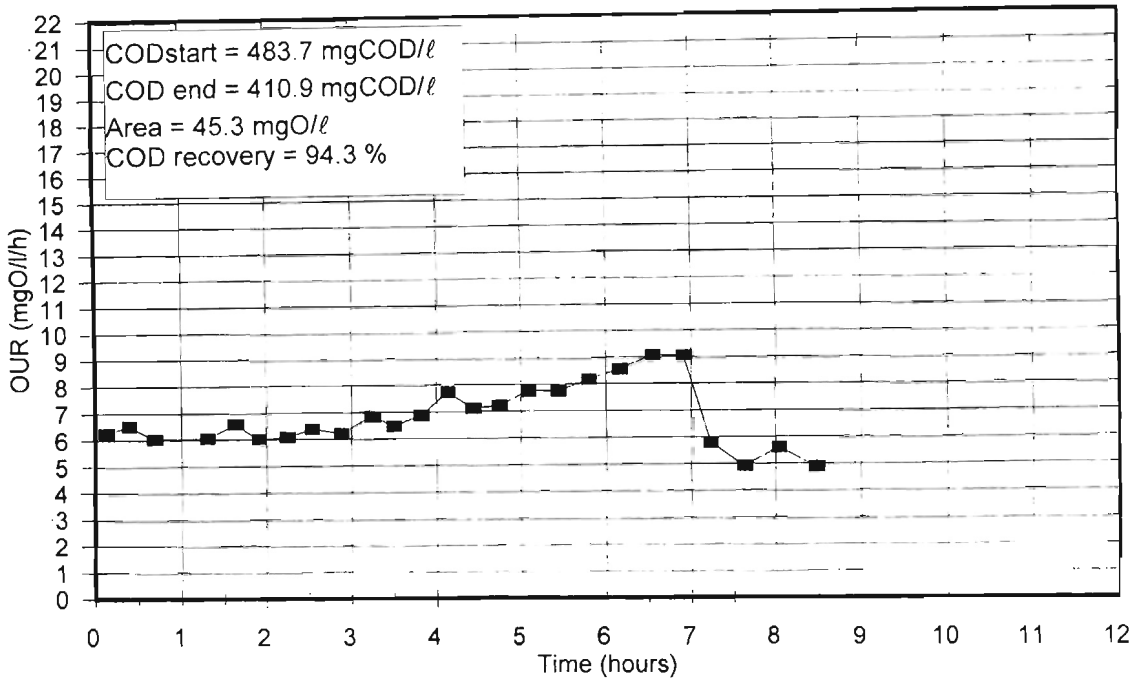
NO_3/NO_2 graph for for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ)
Batch Test No. 26, 30-03, Sewage Batch No. 20



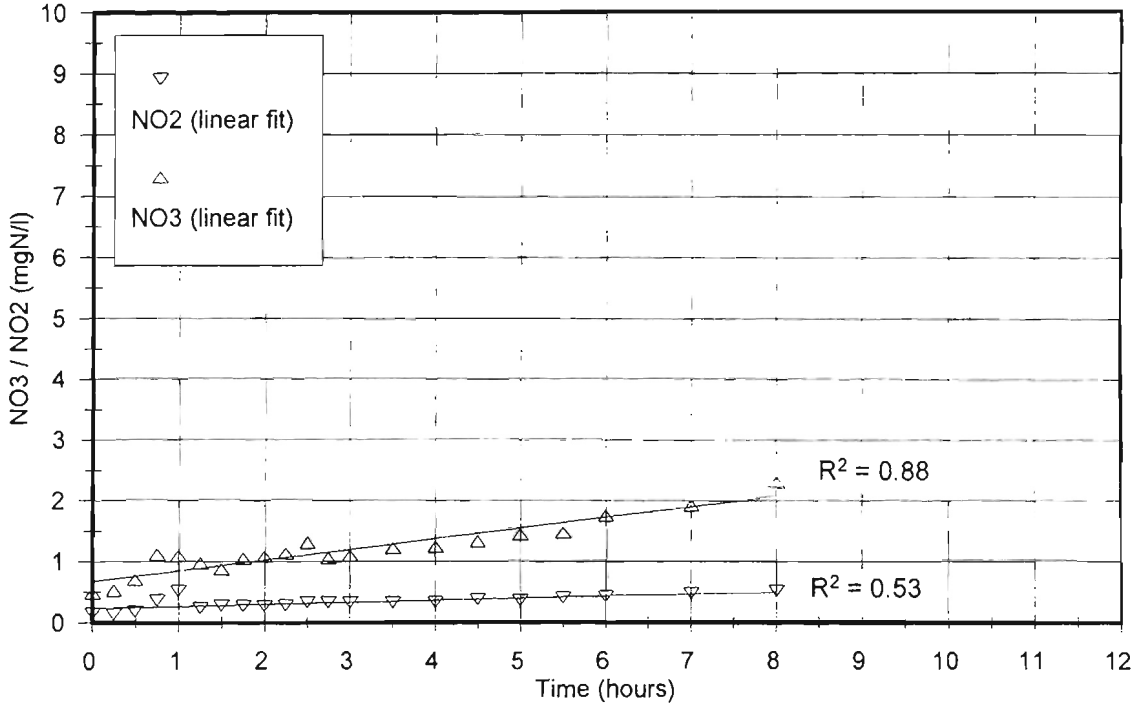
OUR-h graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ)
Batch Test No. 26, 30-03, Sewage Batch No. 20



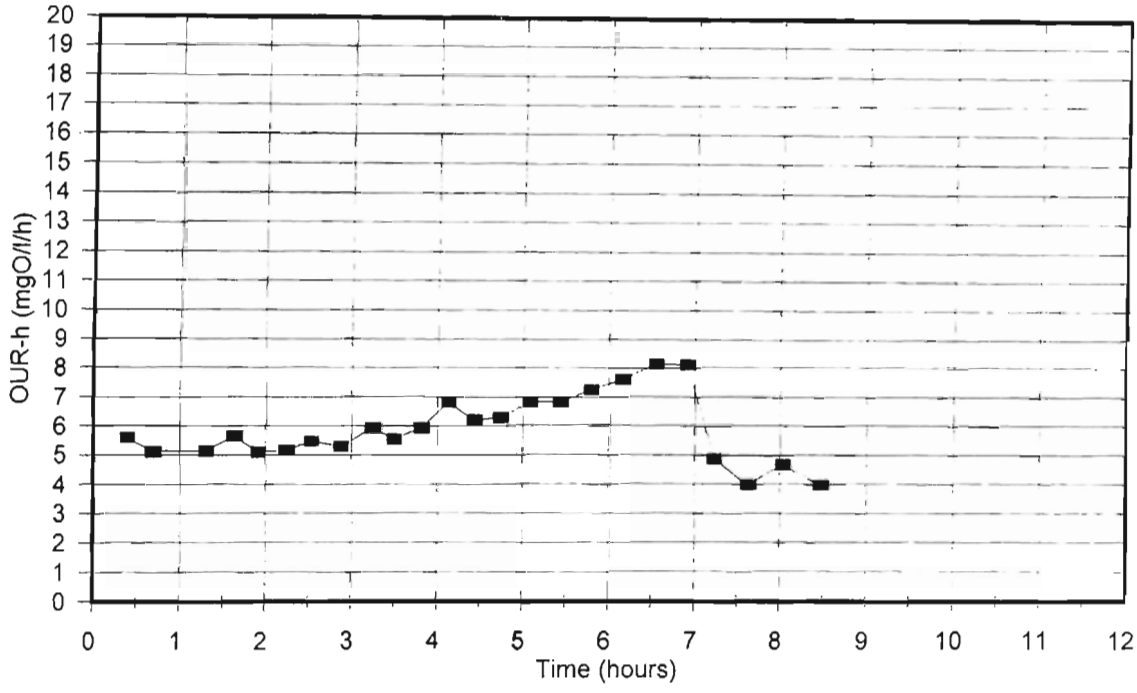
ℓn(OUR-h) graph for filtered wastewater (2.85ℓ) plus mixed liquor (0.15ℓ)
Batch Test No. 26, 30-03, Sewage Batch No. 20



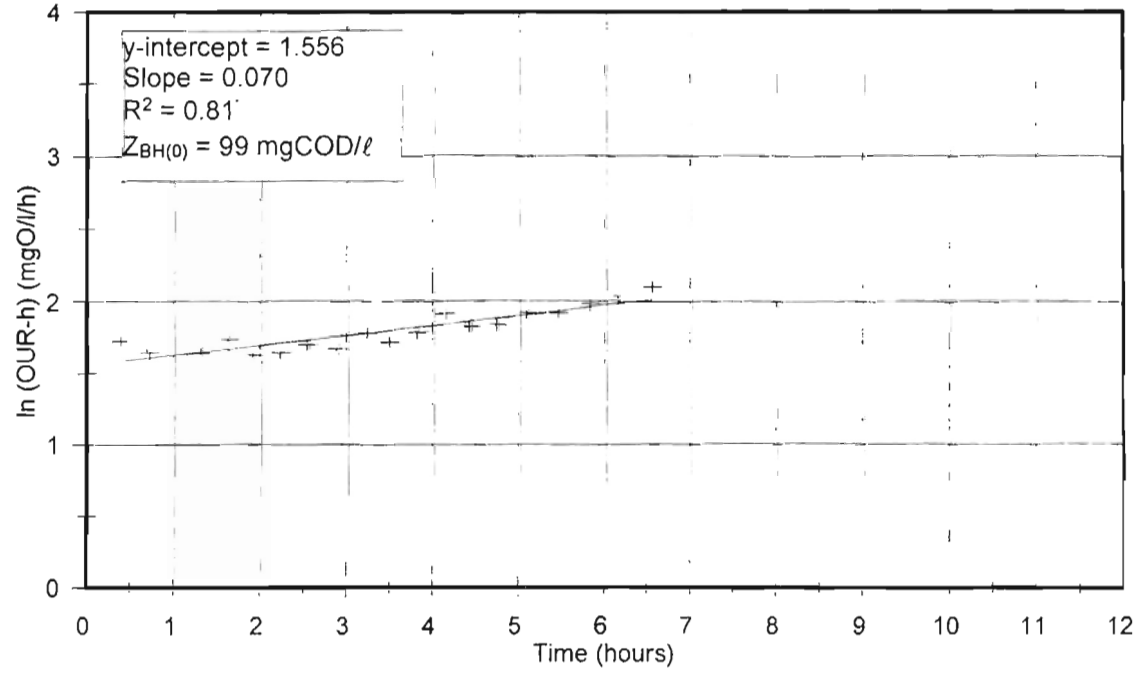
OUR graph for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 28, 31-03, Sewage Batch No. 20



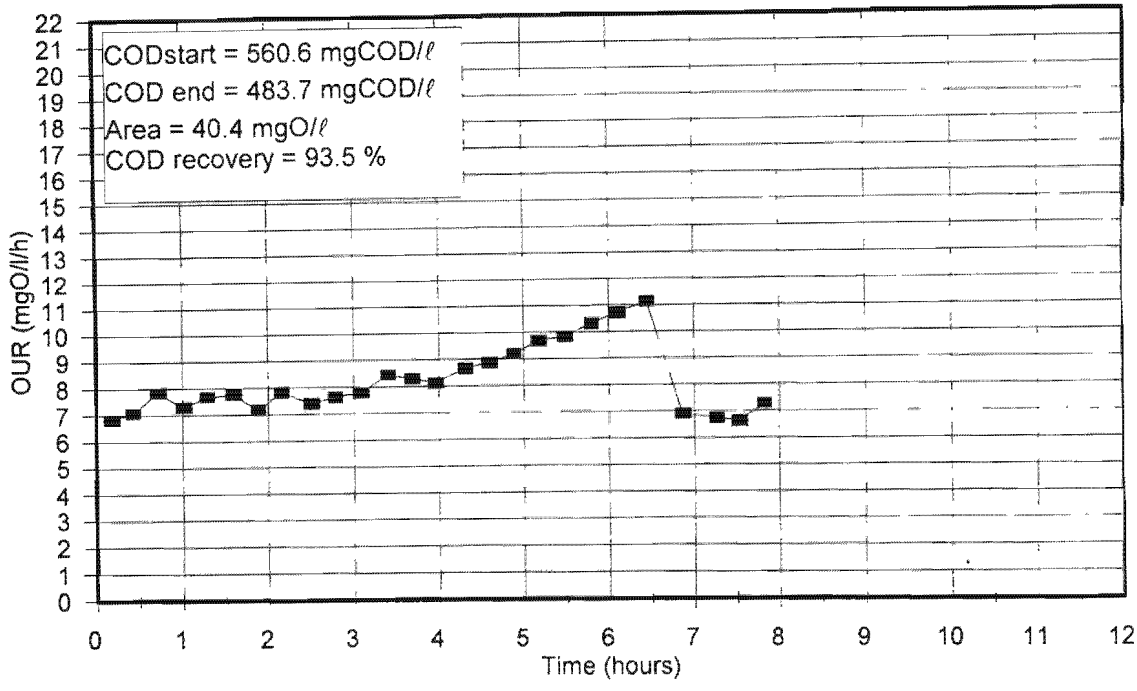
NO_3/NO_2 graph for for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 28, 31-03, Sewage Batch No. 20



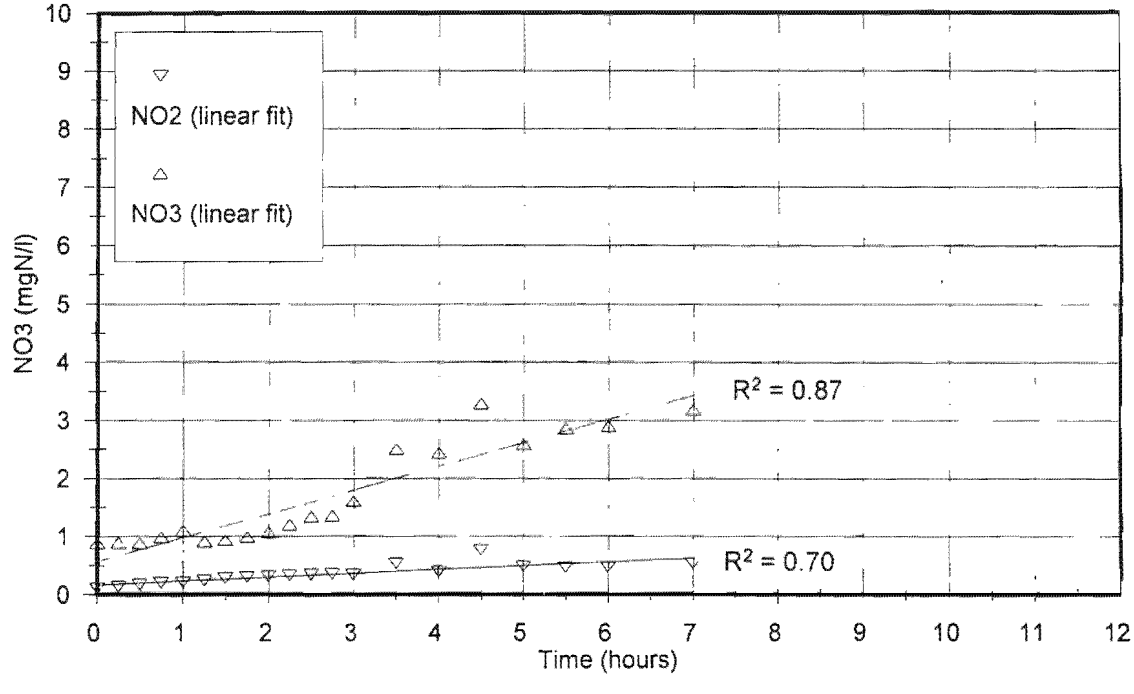
OUR-h graph for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 28, 31-03, Sewage Batch No. 20



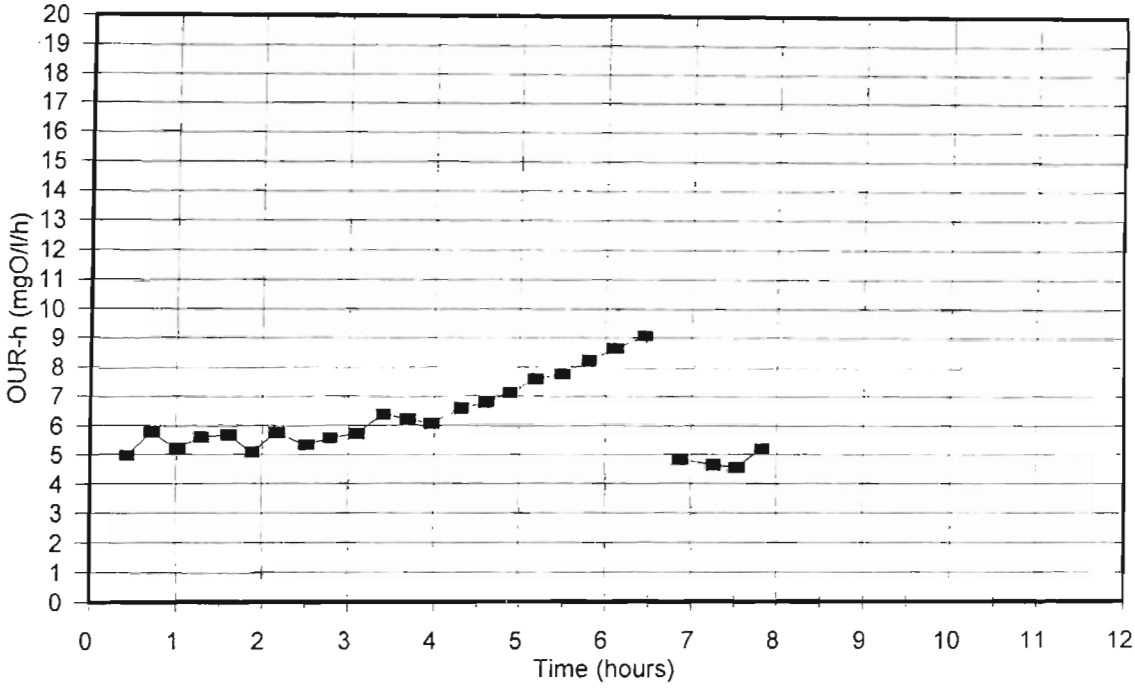
ℓn(OUR-h) graph for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 28, 31-03, Sewage Batch No. 20



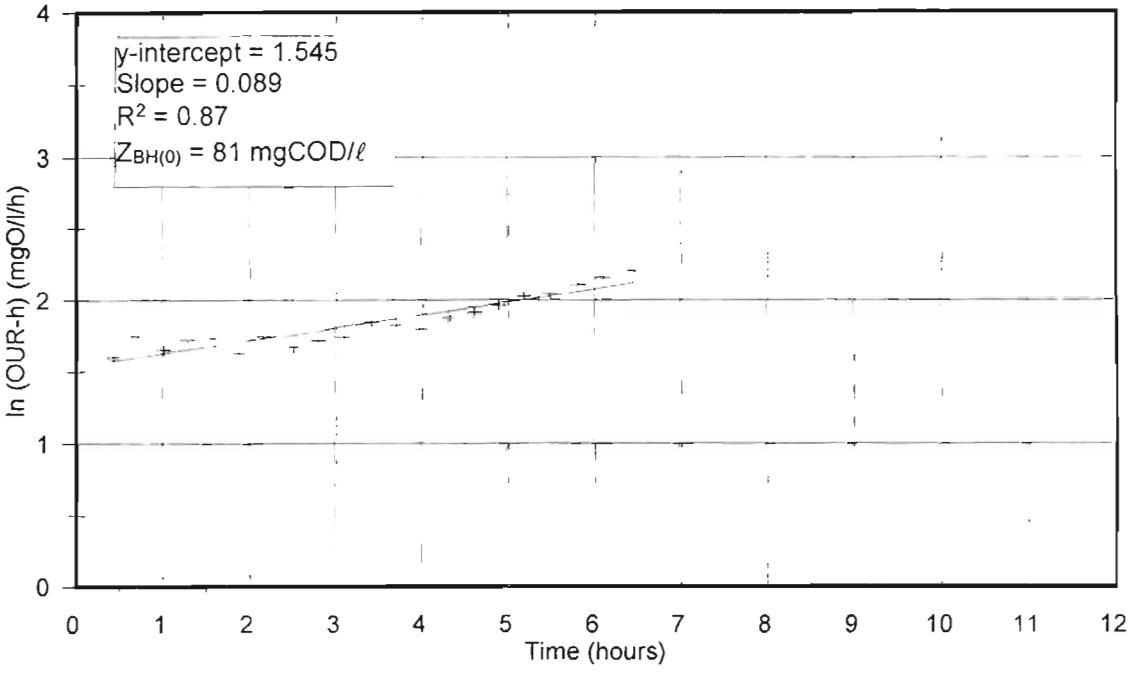
OUR graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ)
Batch Test No. 30, 02-04, Sewage Batch No. 20



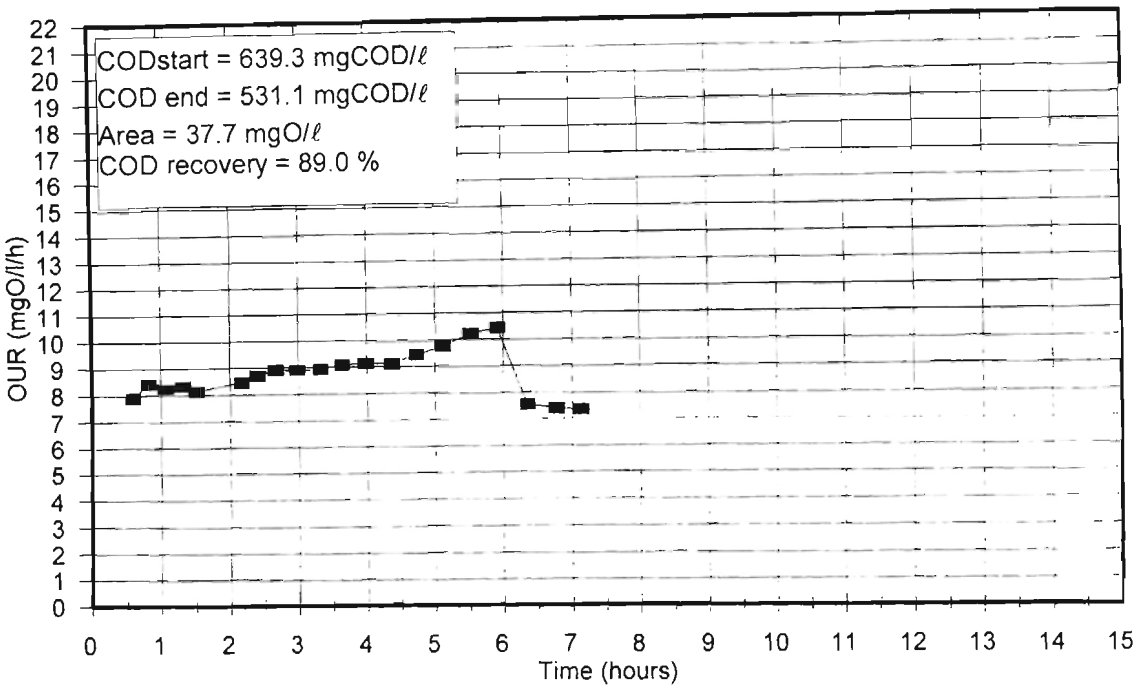
NO_3/NO_2 graph for for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ)
Batch Test No. 30, 02-04, Sewage Batch No. 20



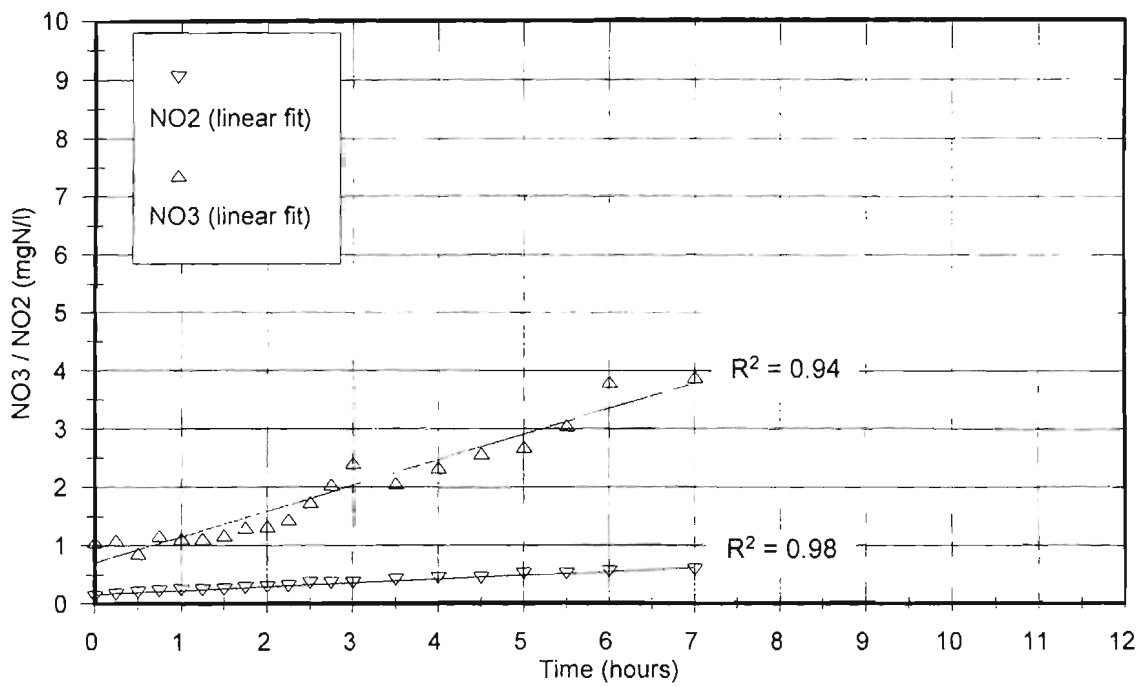
OUR-h graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ)
Batch Test No. 30, 02-04, Sewage Batch No. 20



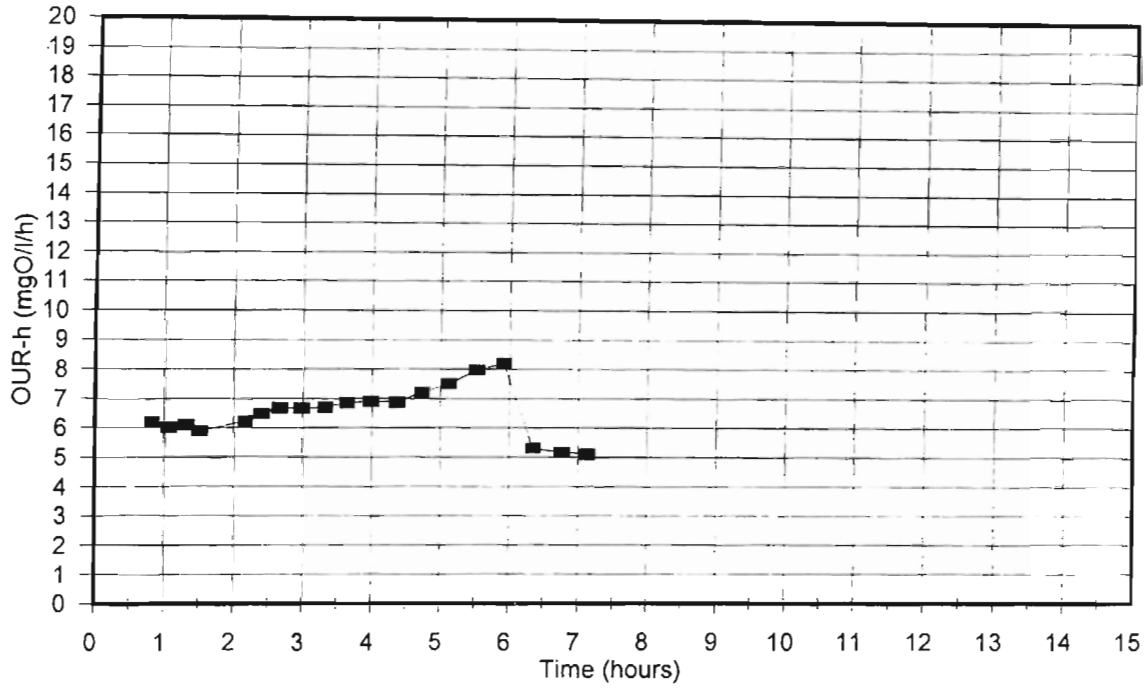
ℓn(OUR-h) graph for filtered wastewater (2.75ℓ) plus mixed liquor (0.25ℓ)
Batch Test No. 30, 02-04, Sewage Batch No. 20



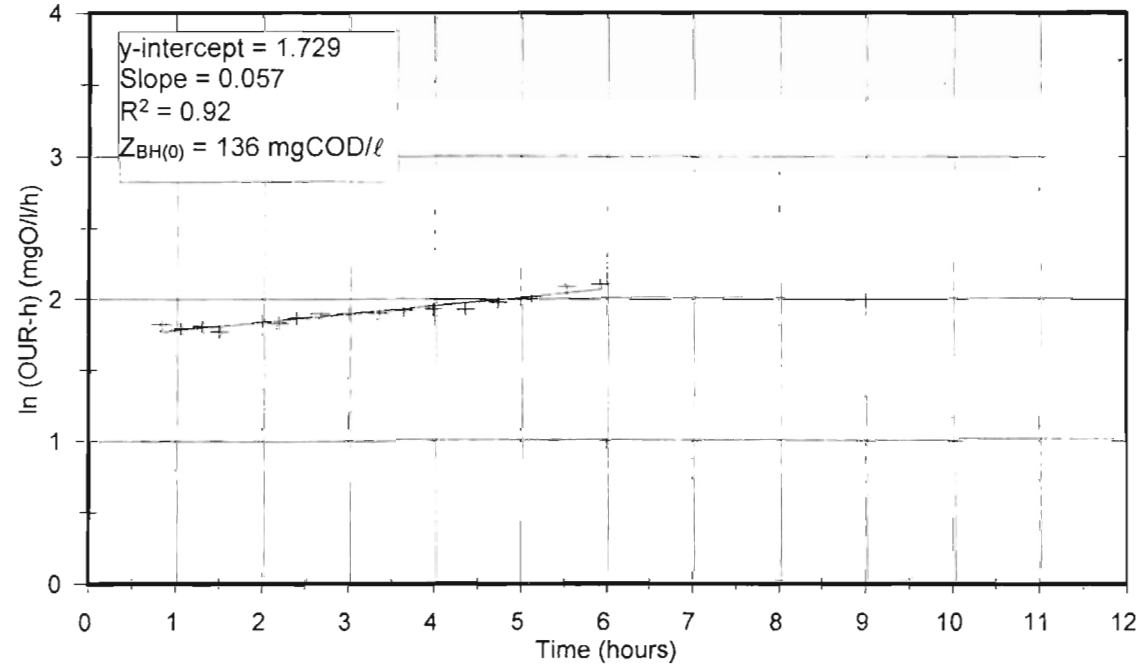
OUR graph for filtered wastewater (2.70ℓ) plus mixed liquor (0.30ℓ)
Batch Test No. 32, 03-04, Sewage Batch No. 20



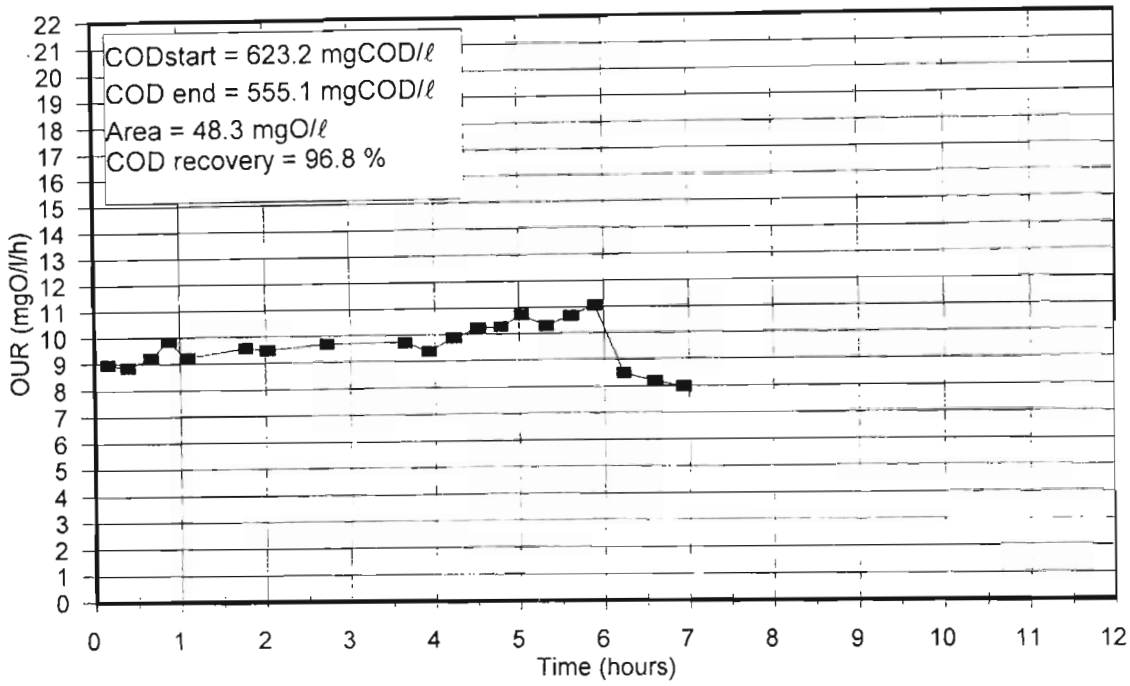
NO_3/NO_2 graph for for filtered wastewater (2.70ℓ) plus mixed liquor (0.30ℓ)
Batch Test No. 32, 03-04, Sewage Batch No. 20



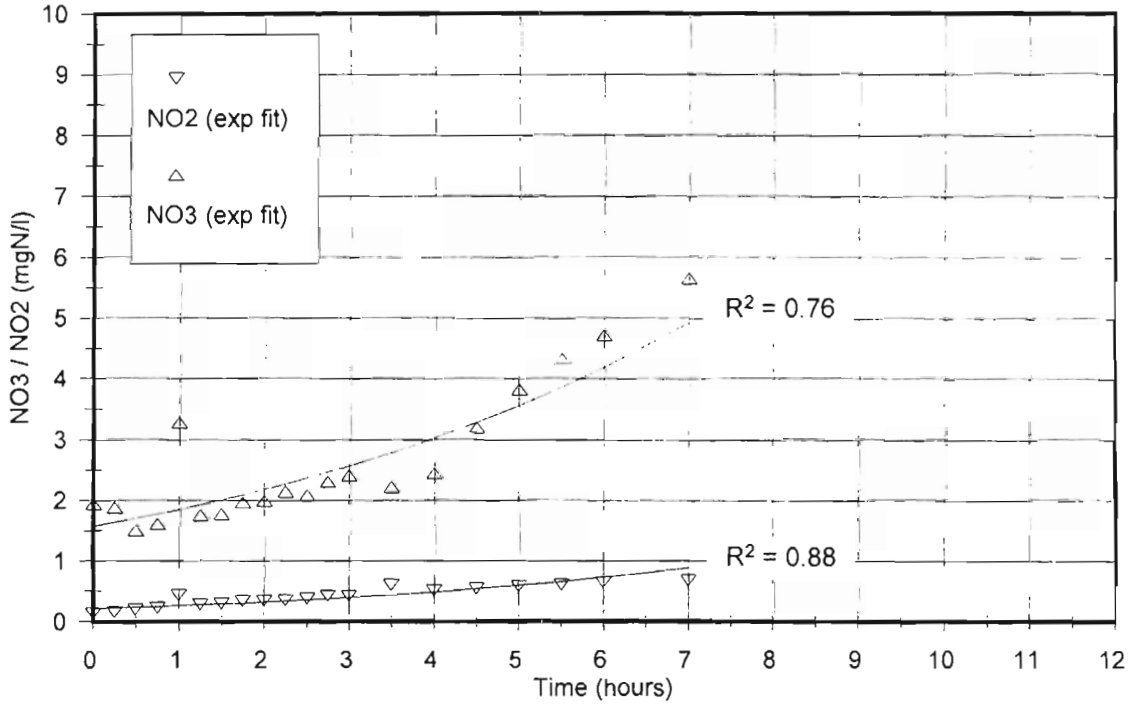
OUR-h graph for filtered wastewater (2.70ℓ) plus mixed liquor (0.30ℓ)
Batch Test No. 32, 03-04, Sewage Batch No. 20



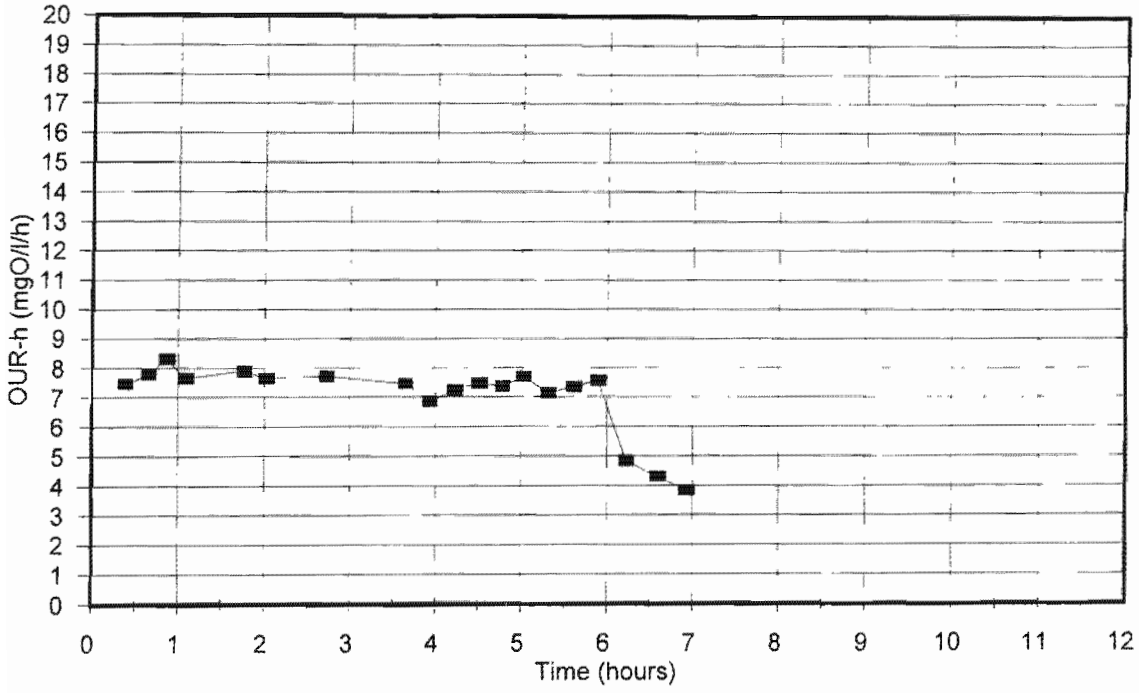
ℓn(OUR-h) graph for filtered wastewater (2.70ℓ) plus mixed liquor (0.30ℓ)
Batch Test No. 32, 03-04, Sewage Batch No. 20



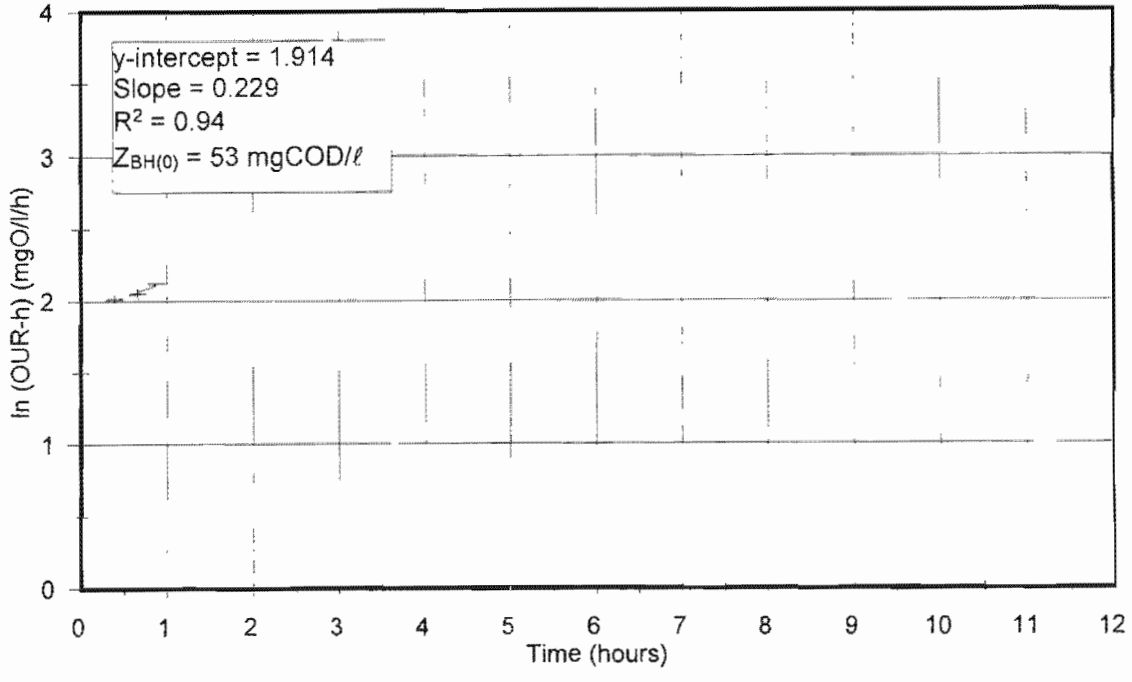
OUR graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 34, 04-04, Sewage Batch No. 20



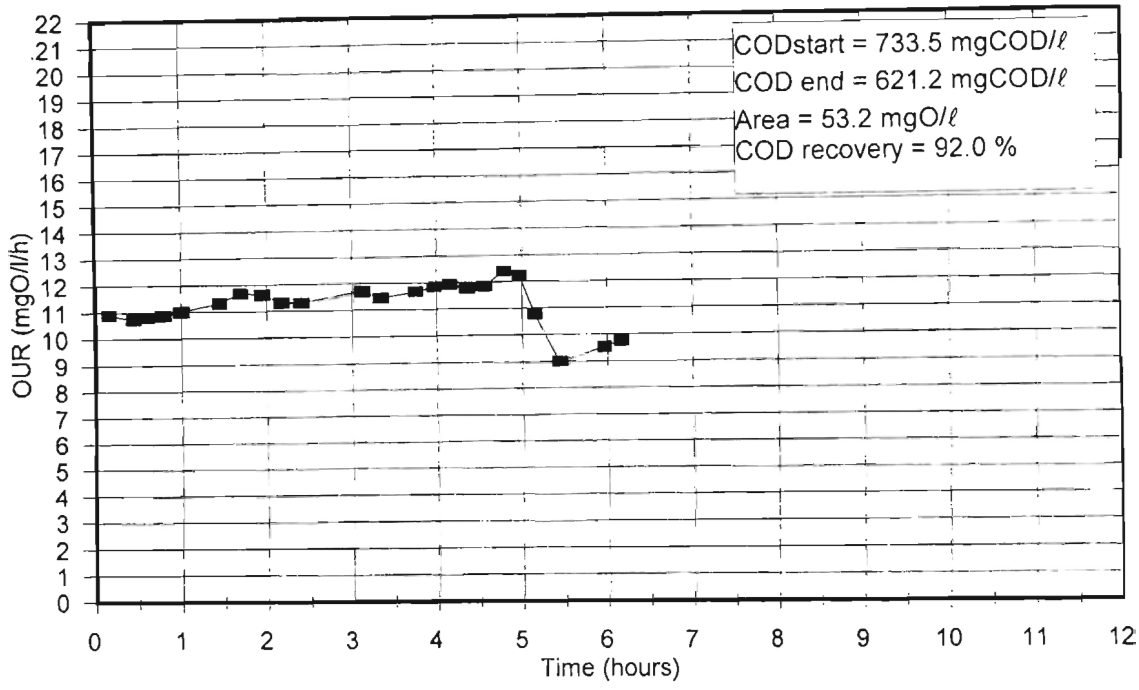
NO_3/NO_2 graph for for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 34, 04-04, Sewage Batch No. 20



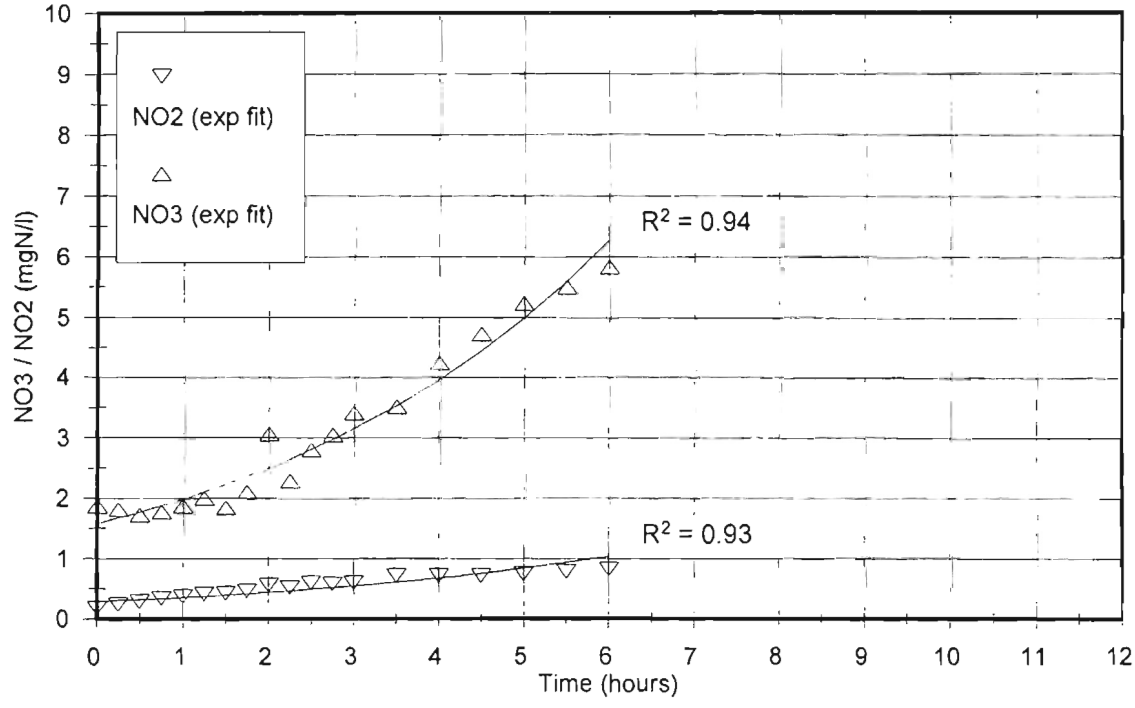
OUR-h graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 34, 04-04, Sewage Batch No. 20



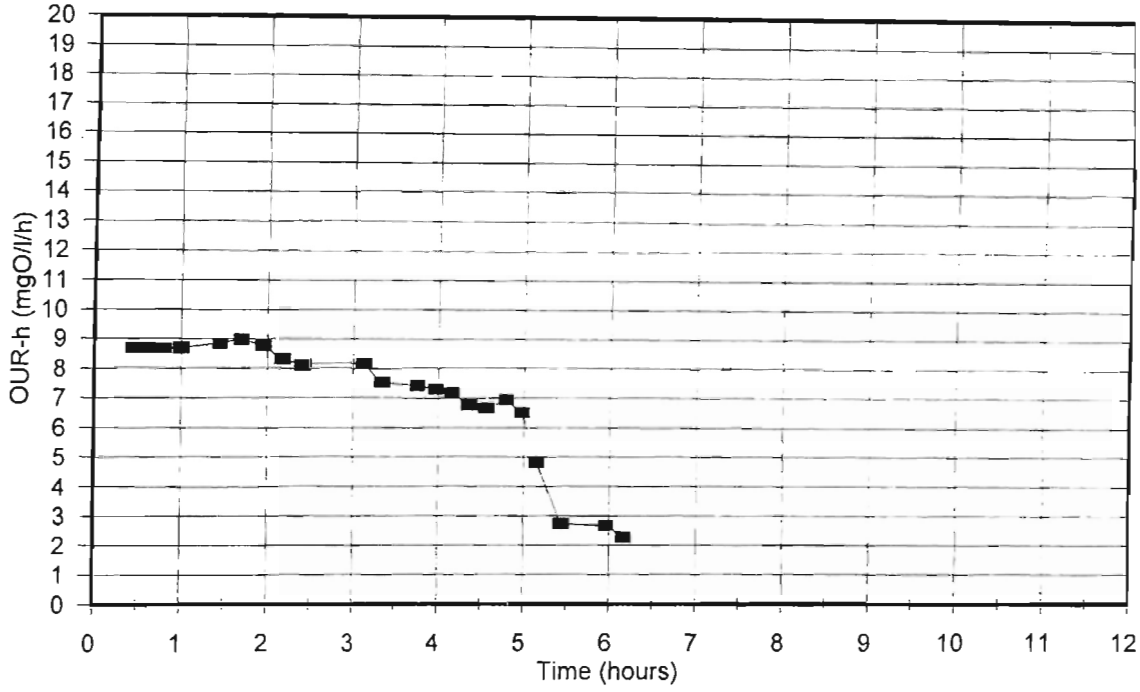
ℓn(OUR-h) graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 34, 04-04, Sewage Batch No. 20



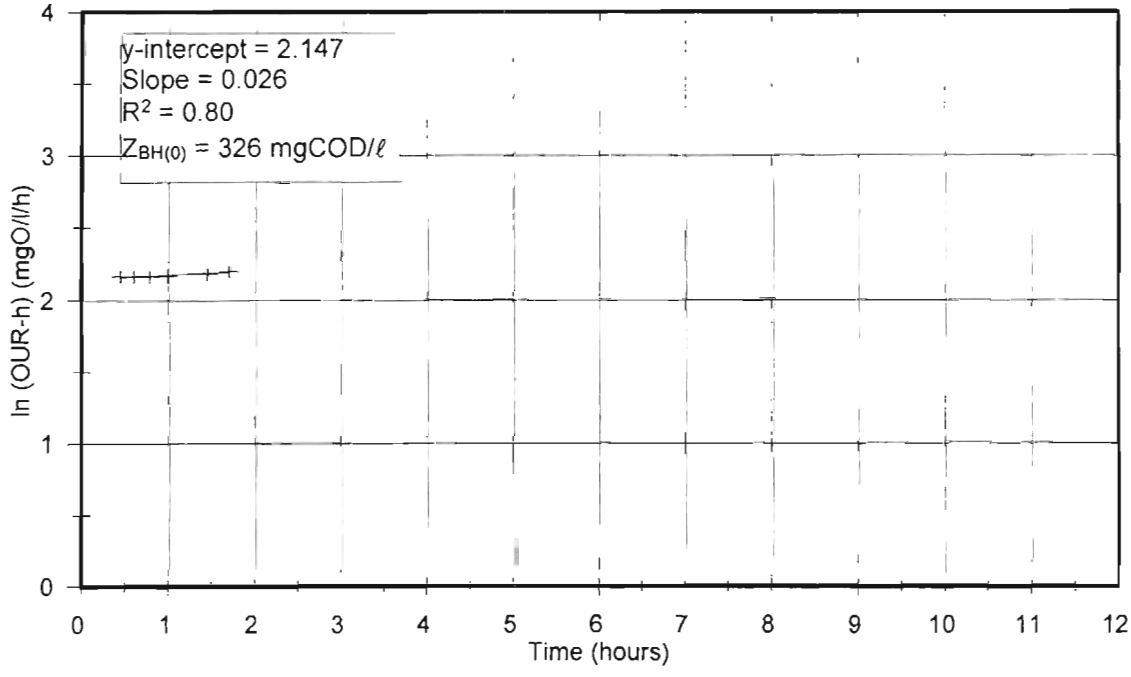
OUR graph for filtered wastewater (2.60ℓ) plus mixed liquor (0.40ℓ)
Batch Test No. 36, 05-04, Sewage Batch No. 20



NO_3/NO_2 graph for for filtered wastewater (2.60ℓ) plus mixed liquor (0.40ℓ)
Batch Test No. 36, 05-04, Sewage Batch No. 20



OUR-h graph for filtered wastewater (2.60ℓ) plus mixed liquor (0.40ℓ)
Batch Test No. 36, 05-04, Sewage Batch No. 20

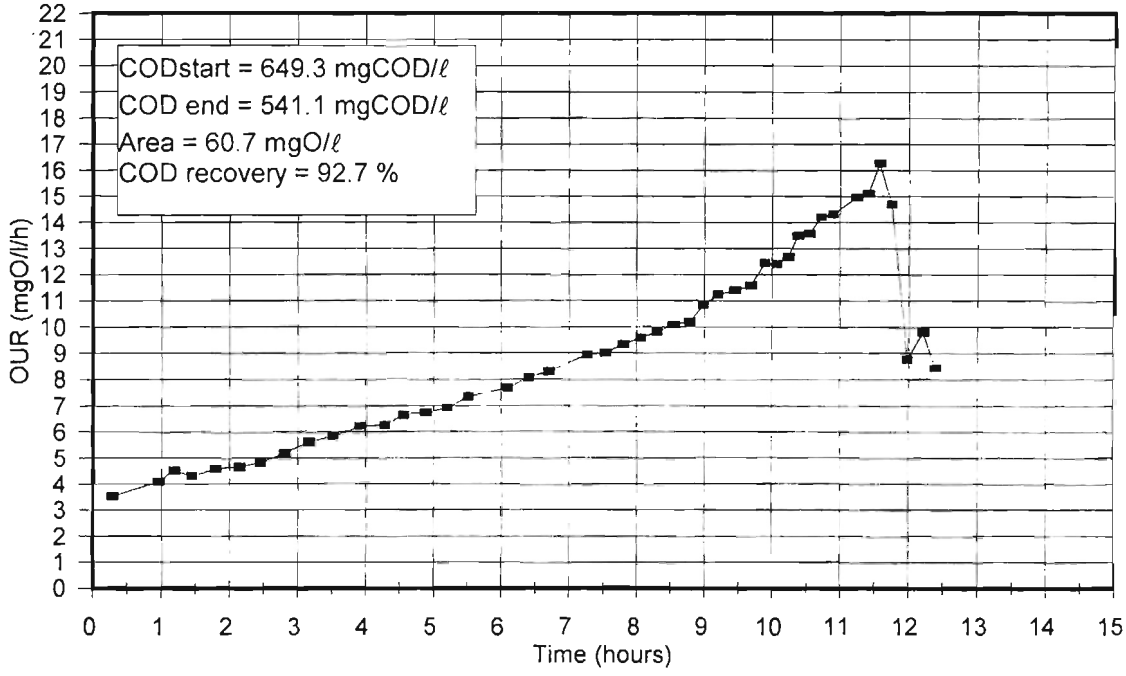


ℓn(OUR-h) graph for filtered wastewater (2.60ℓ) plus mixed liquor (0.40ℓ)
Batch Test No. 36, 05-04, Sewage Batch No. 20

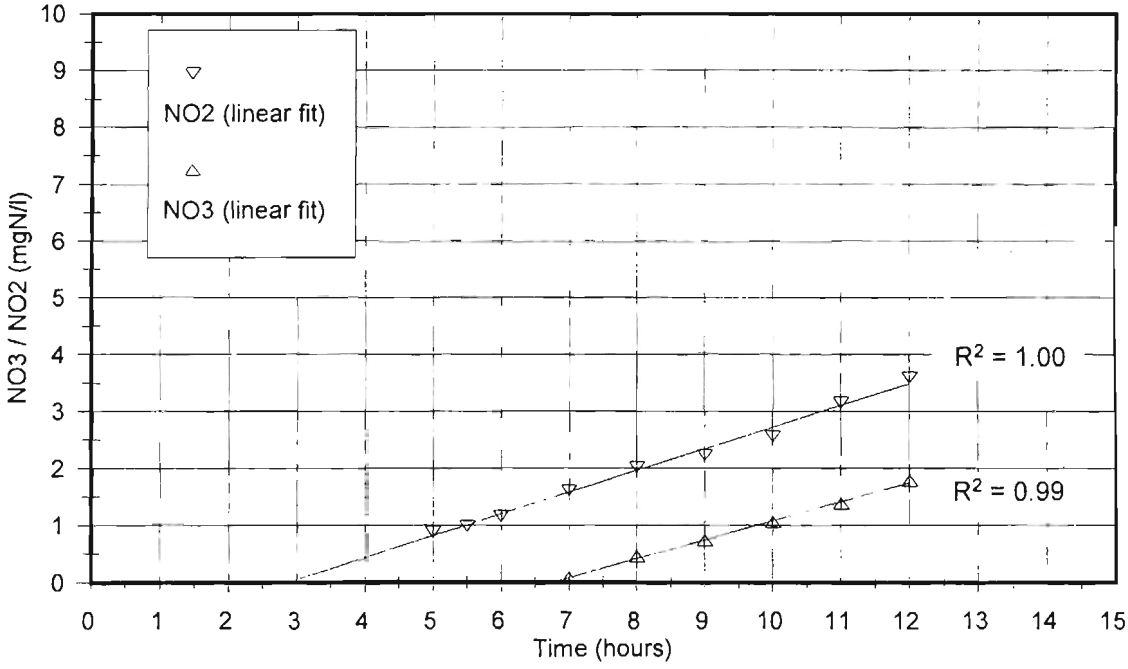
Table A3: Summarized data for modified batch tests with filtered wastewater and mixed liquor and separately with filtered wastewater, mixed liquor and phosphate drawn from the parent MLE experimental activated sludge system; mixed liquor volume, COD recovery, linear regression data and measured OHO active biomass.

Sew. Batch No.	Batch Test No.	Date of Test	ML volume (ℓ)	COD (mgCOD/ℓ) Start	COD (mgCOD/ℓ) End	Area (mgO/ℓ)	% COD Recovery	Linear regresion data y-int.	Linear regresion data slope	Linear regresion data R^2	$Z_{BH(0)}$ (ML) (mgCOD/ℓ)
21	37	12-04	0.35	649.3	541.1	60.7	92.7	0.295	0.200	0.99	12
	38	12-04	0.35	677.4	539.1	71.0	90.1	1.147	0.143	0.99	37
	39	19-04	0.35	551.1	507.0	56.2	101.5	1.832	0.065	0.86	137
	40	19-04	0.35	537.1	501.0	51.1	102.8	1.933	0.056	0.61	169
22	41	23-04	0.35	614.1	533.3	51.4	95.2	1.953	0.073	0.62	142
	42	23-04	0.35	618.1	529.2	60.7	95.4	2.015	0.041	0.48	225
26	61	15-06	0.08	267.2	185.6	48.1	87.5*	0.299	0.127	1.00	18
	62	15-06	0.08	275.4	216.2	44.5	94.7	0.235	0.124	0.96	17
	63	16-06	0.16	410.0	310.1	525.4	86.0*	0.756	0.118	0.97	29
	64	16-06	0.16	422.3	365.2	47.9	97.8	0.807	0.126	0.93	29
	65	17-06	0.24	626.3	414.1	118.6	85.1*	1.496	0.121	0.98	61
	66	17-06	0.24	579.4	467.2	97.3	97.4	1.226	0.145	0.95	40
				MEAN			93.9			0.86	
				Std. Deviation			5.5			0.17	

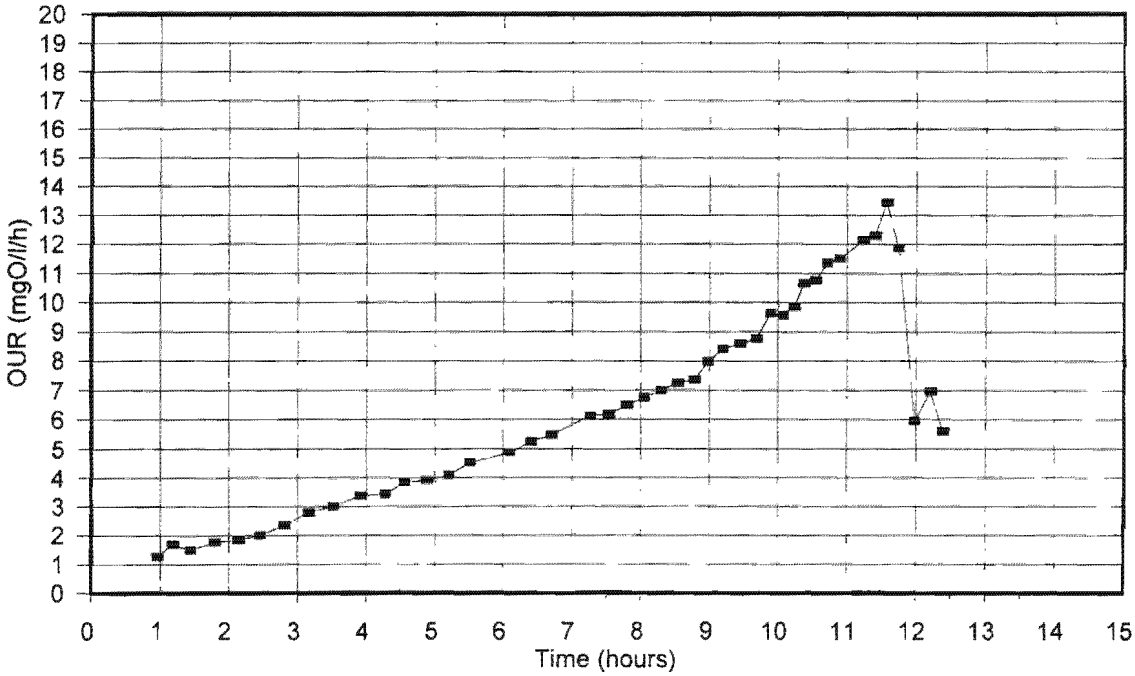
* Batch test rejected on account of poor %COD recovery



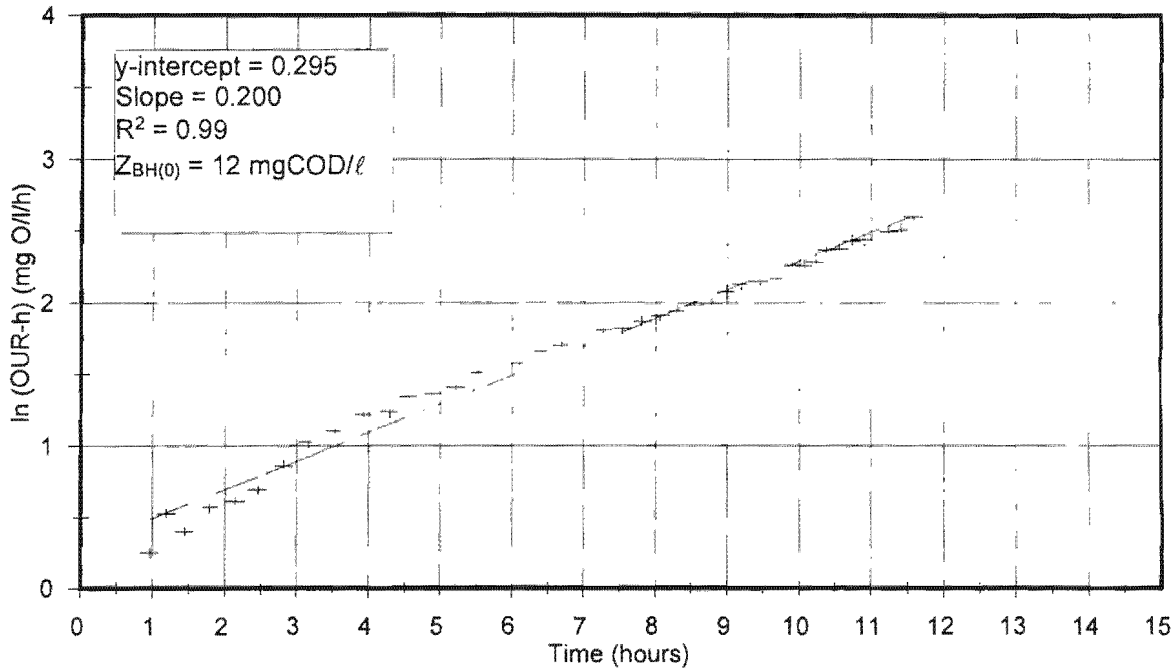
OUR graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 37, 12-04, Sewage Batch No. 21



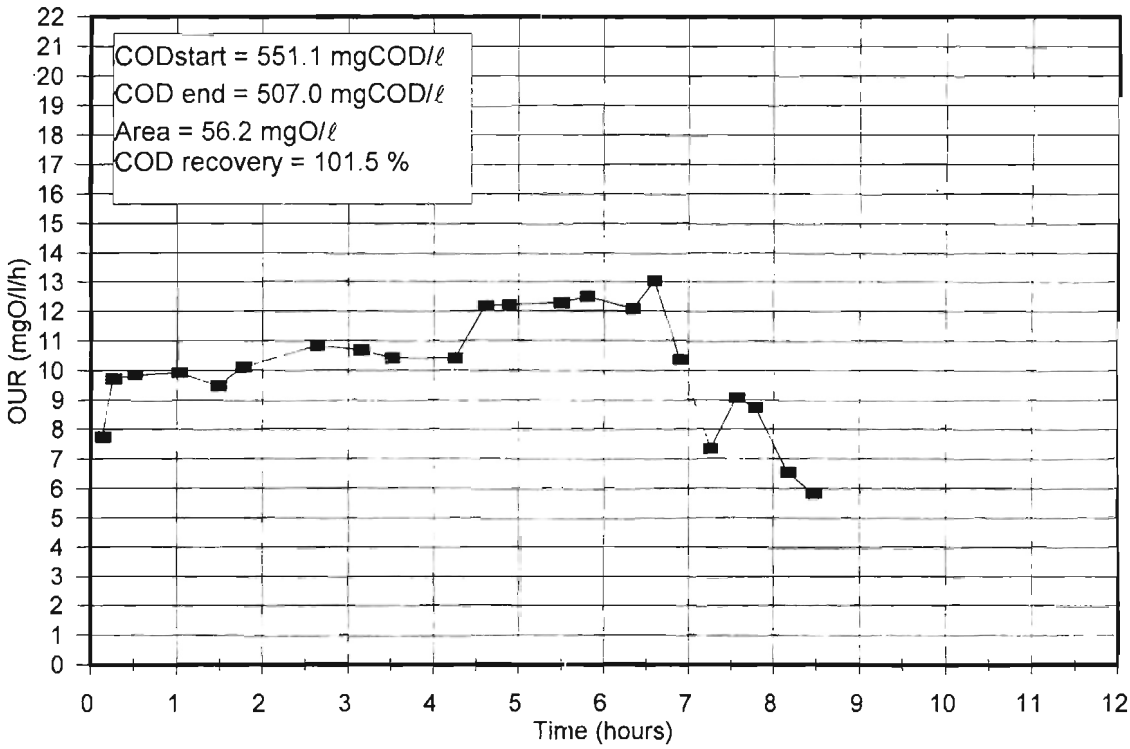
NO_3/NO_2 graph for for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 37, 12-04, Sewage Batch No. 21



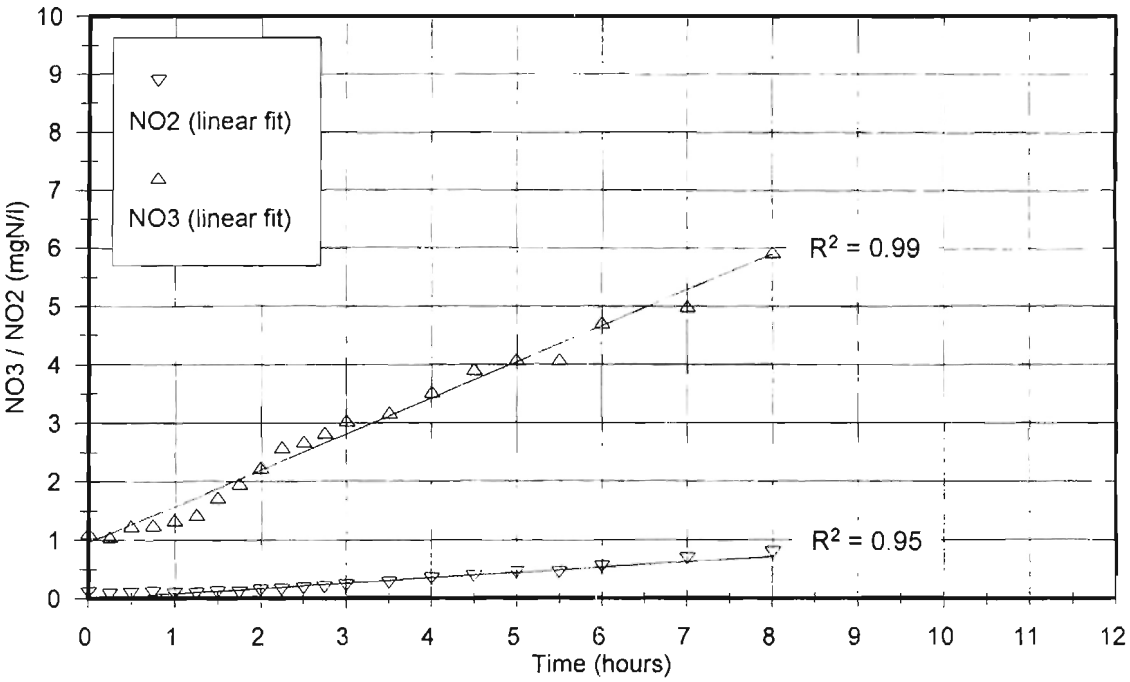
OUR-h graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 37, 12-04, Sewage Batch No. 21



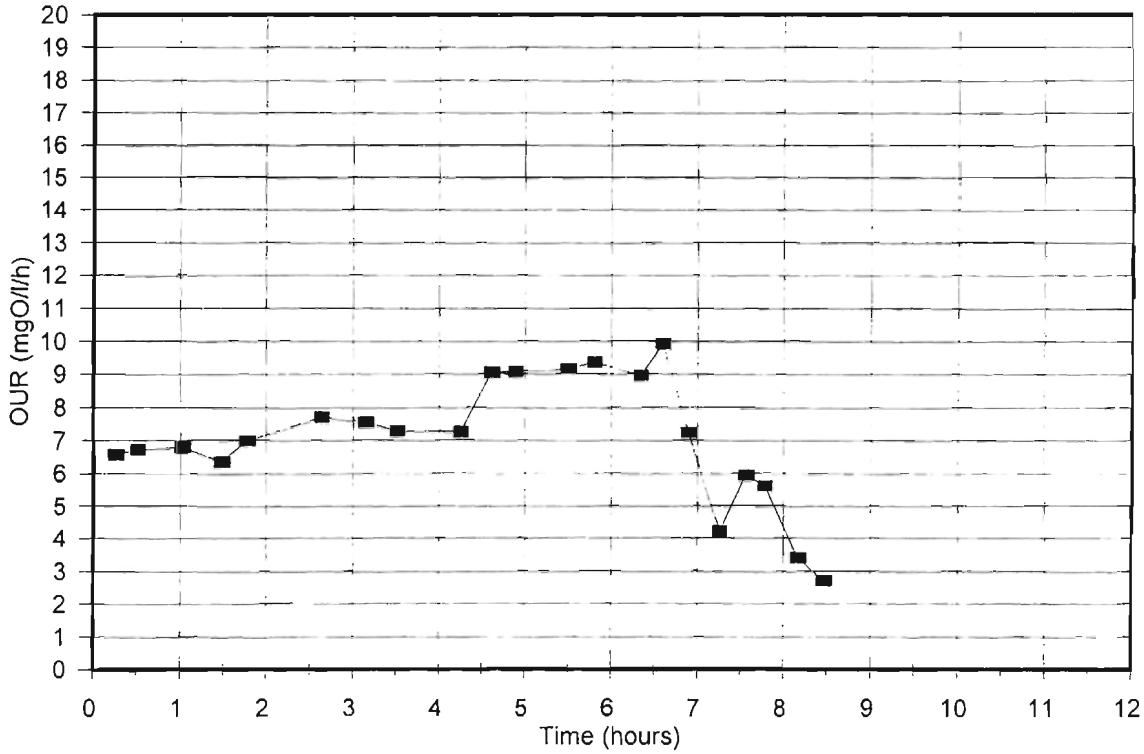
ℓn(OUR-h) graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 37, 12-04, Sewage Batch No. 21



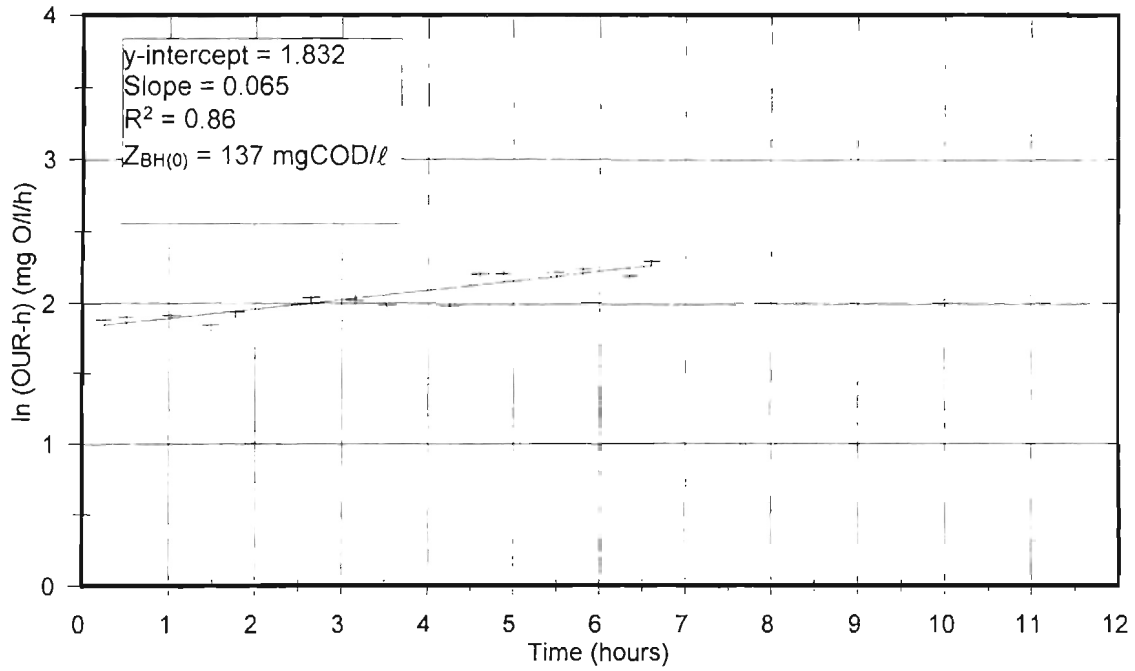
OUR graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 39, 19-04, Sewage Batch No. 21



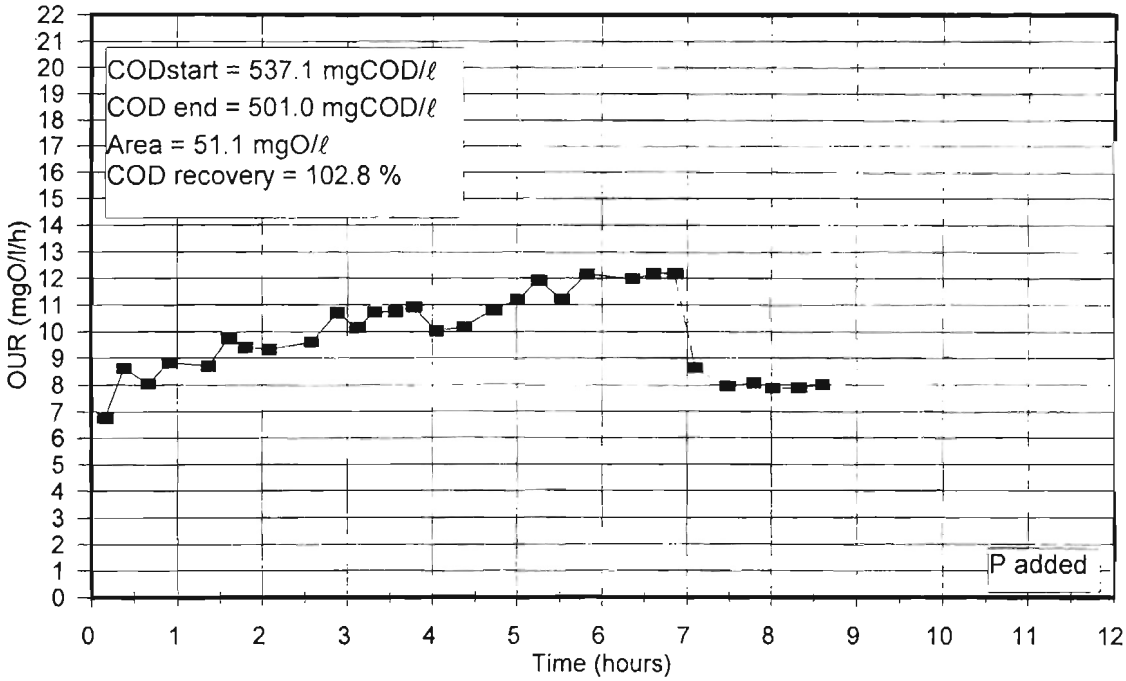
NO_3/NO_2 graph for for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 39, 19-04, Sewage Batch No. 21



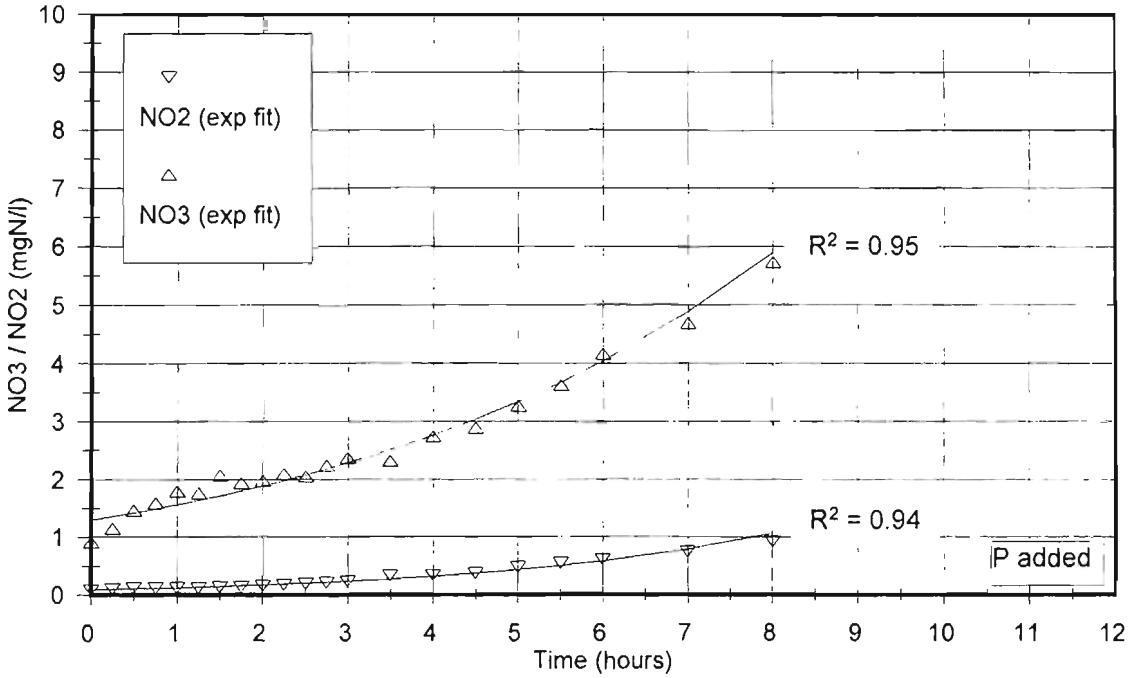
OUR-h graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 39, 19-04, Sewage Batch No. 21



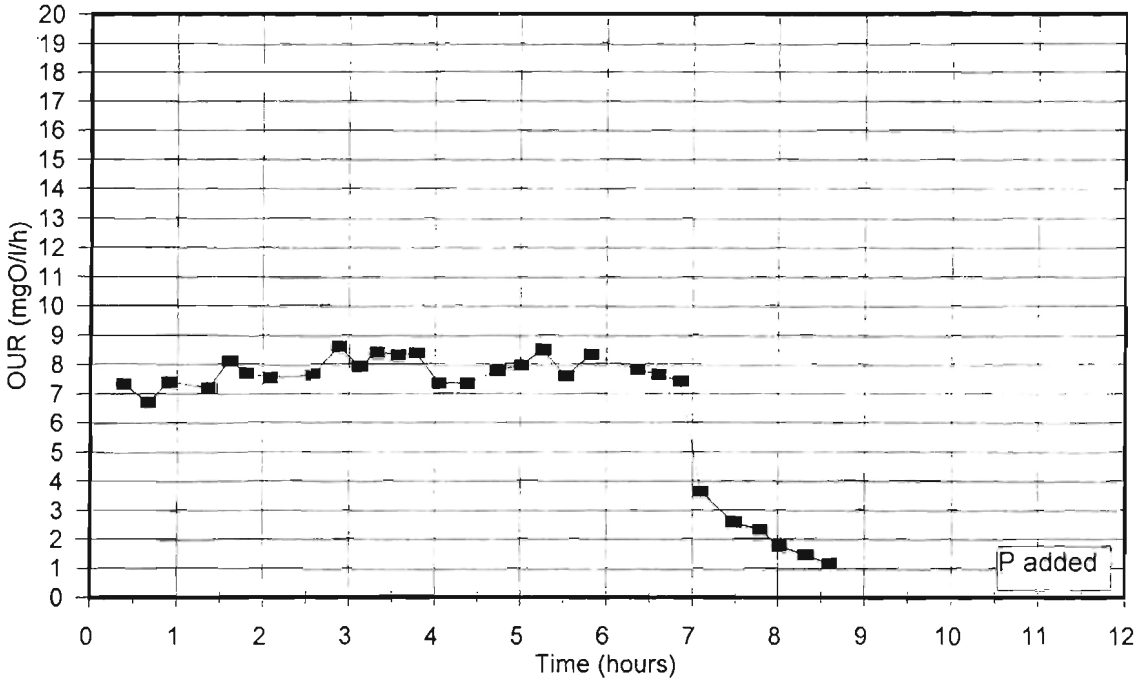
ℓn(OUR-h) graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 39, 19-04, Sewage Batch No. 21



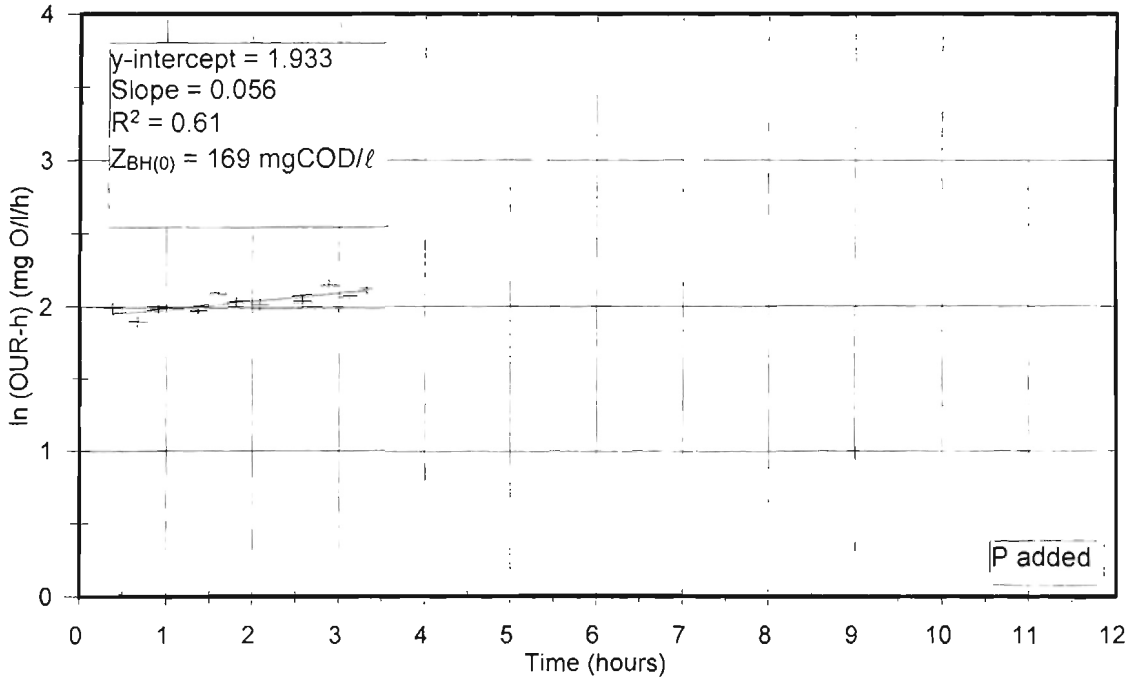
OUR graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 40, 19-04, Sewage Batch No. 21



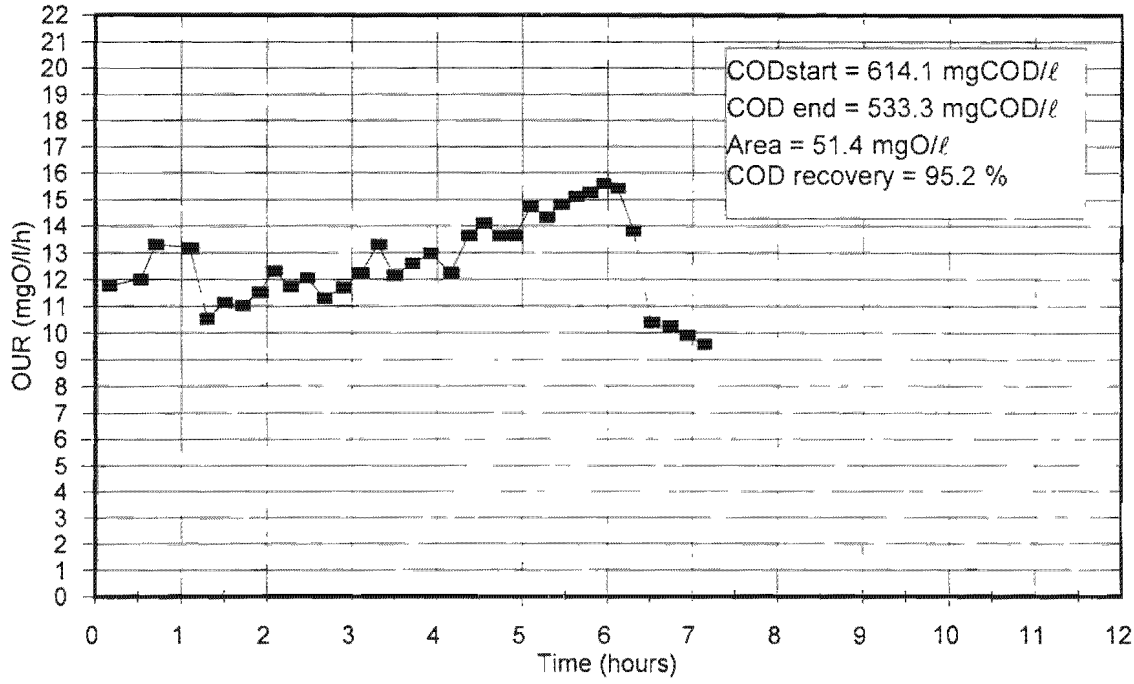
NO_3/NO_2 graph for for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 40, 19-04, Sewage Batch No. 21



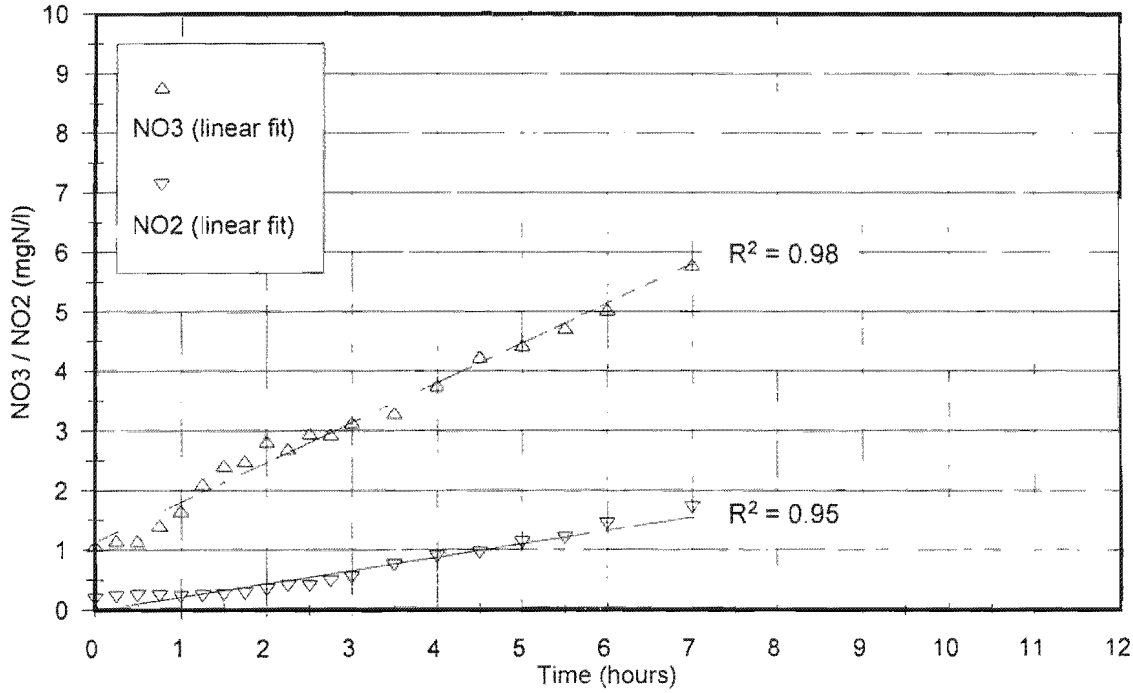
OUR-h graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 40, 19-04, Sewage Batch No. 21



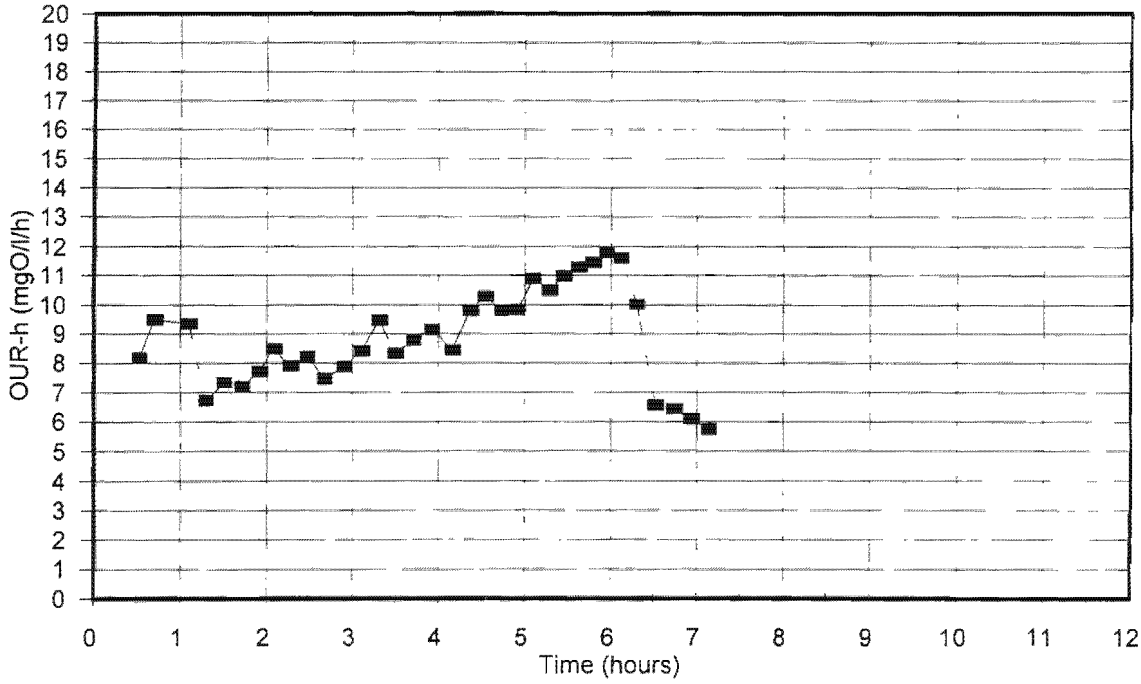
ℓn(OUR-h) graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 40, 19-04, Sewage Batch No. 21



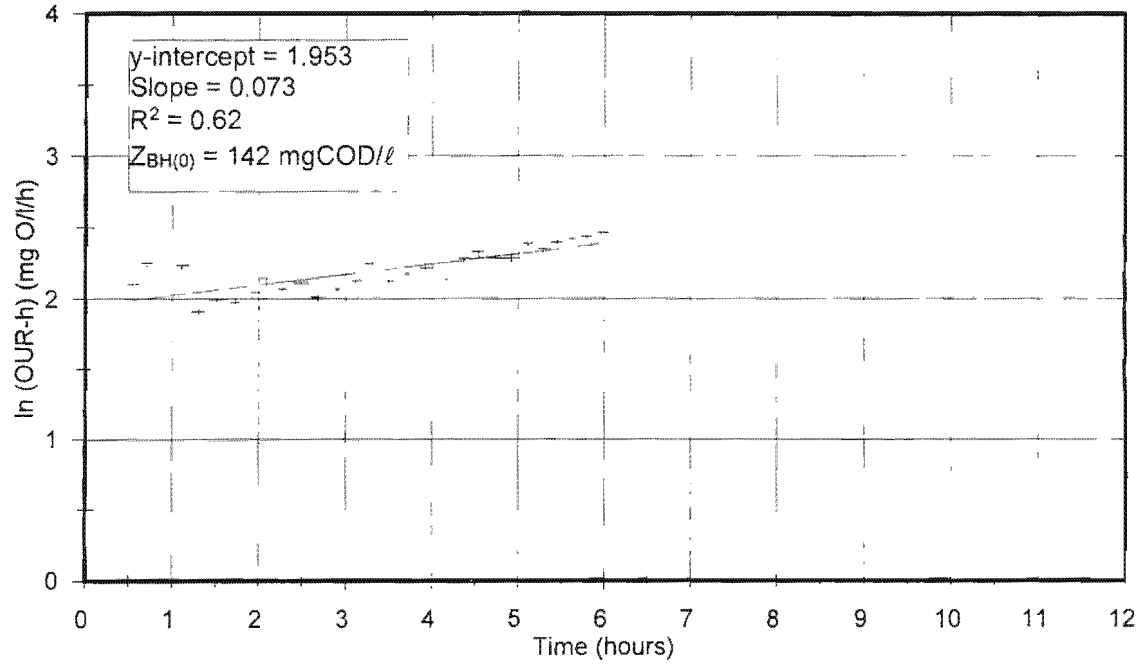
OUR graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 41, 23-04, Sewage Batch No. 22



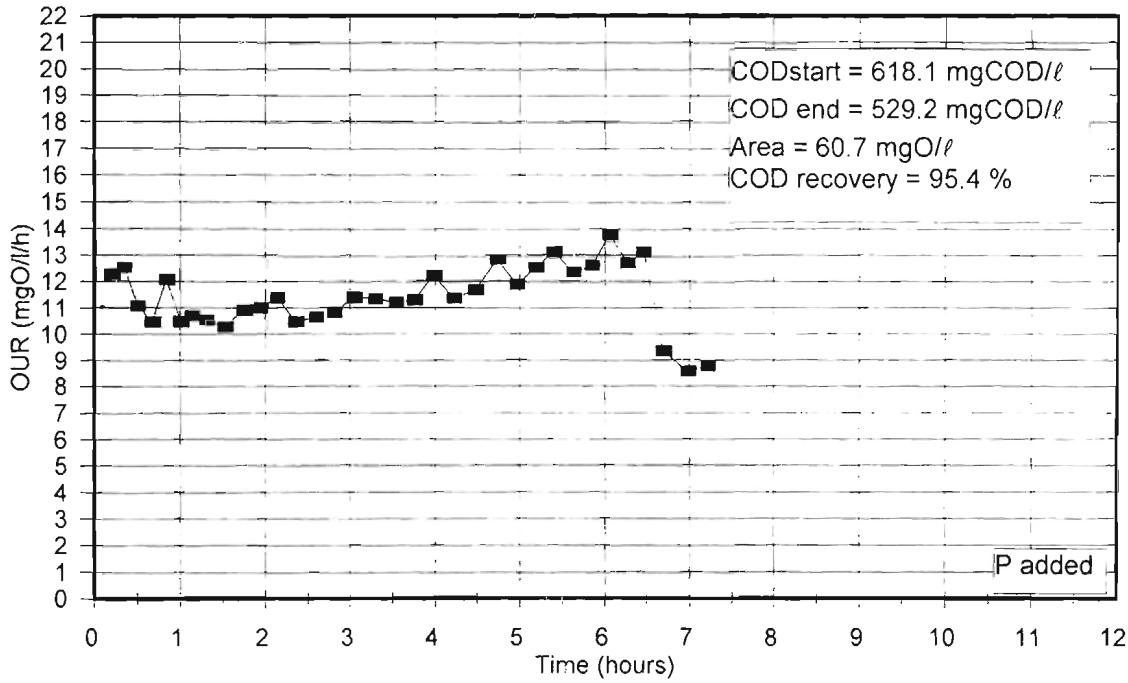
NO_3/NO_2 graph for for filtered wastewater (2.65ℓ) plus mixed liquor (0 35ℓ)
Batch Test No. 41, 23-04, Sewage Batch No. 22



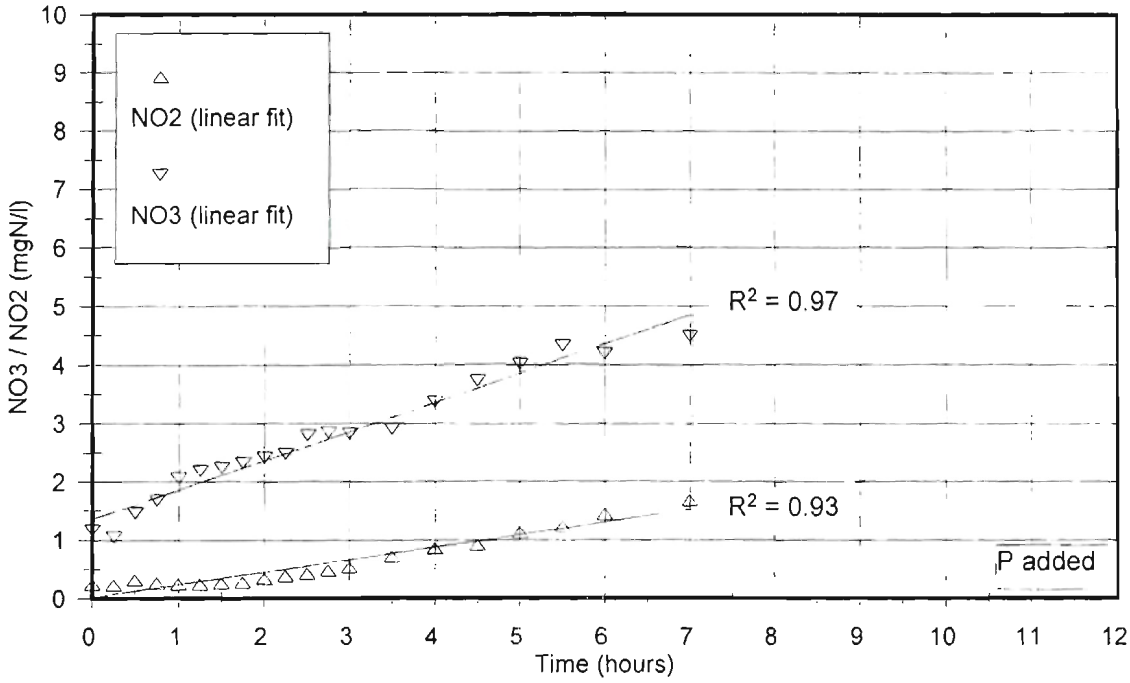
OUR-h graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 41, 23-04, Sewage Batch No. 22



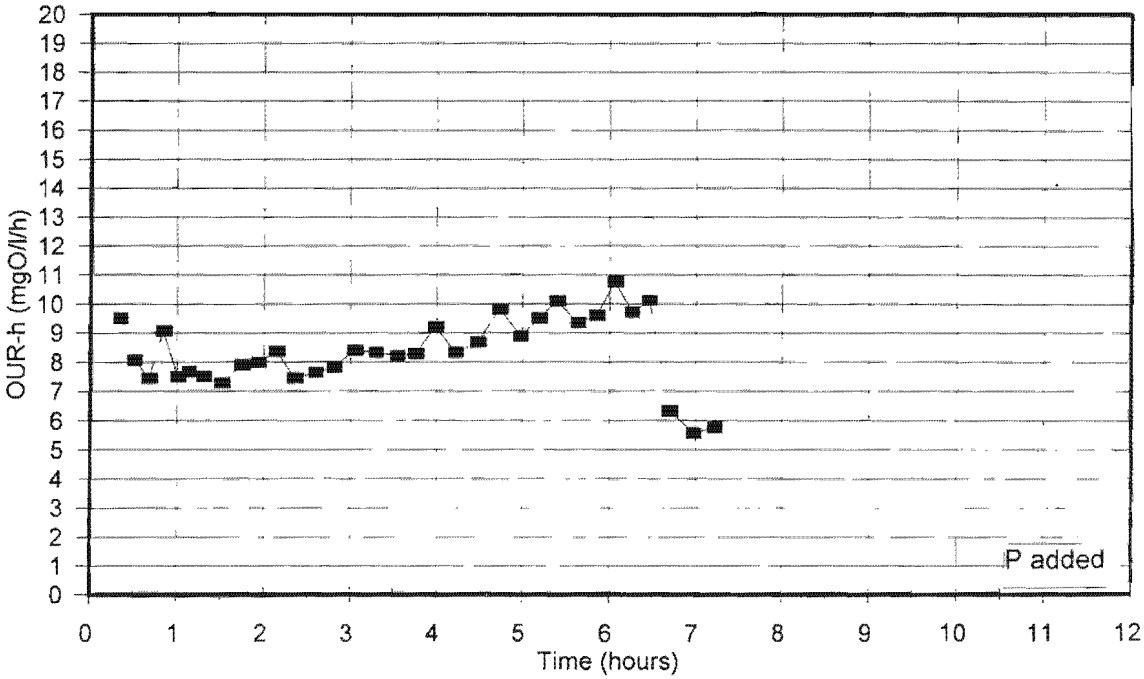
ℓn(OUR-h) graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 41, 23-04, Sewage Batch No. 22



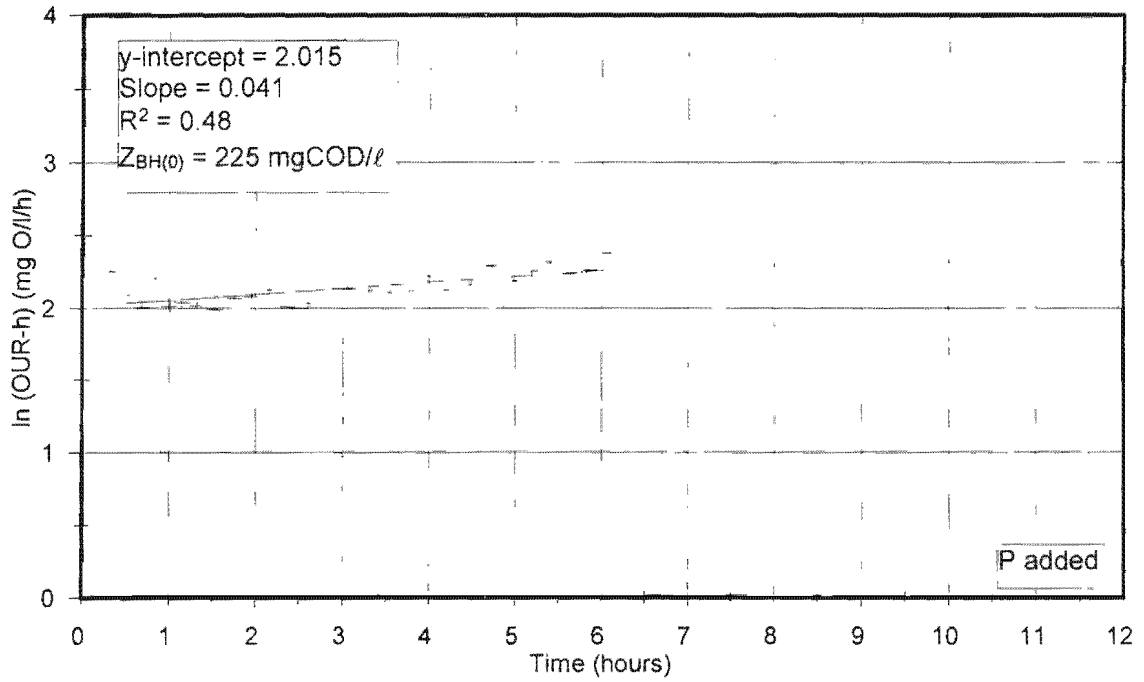
OUR graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 42, 23-04, Sewage Batch No. 22



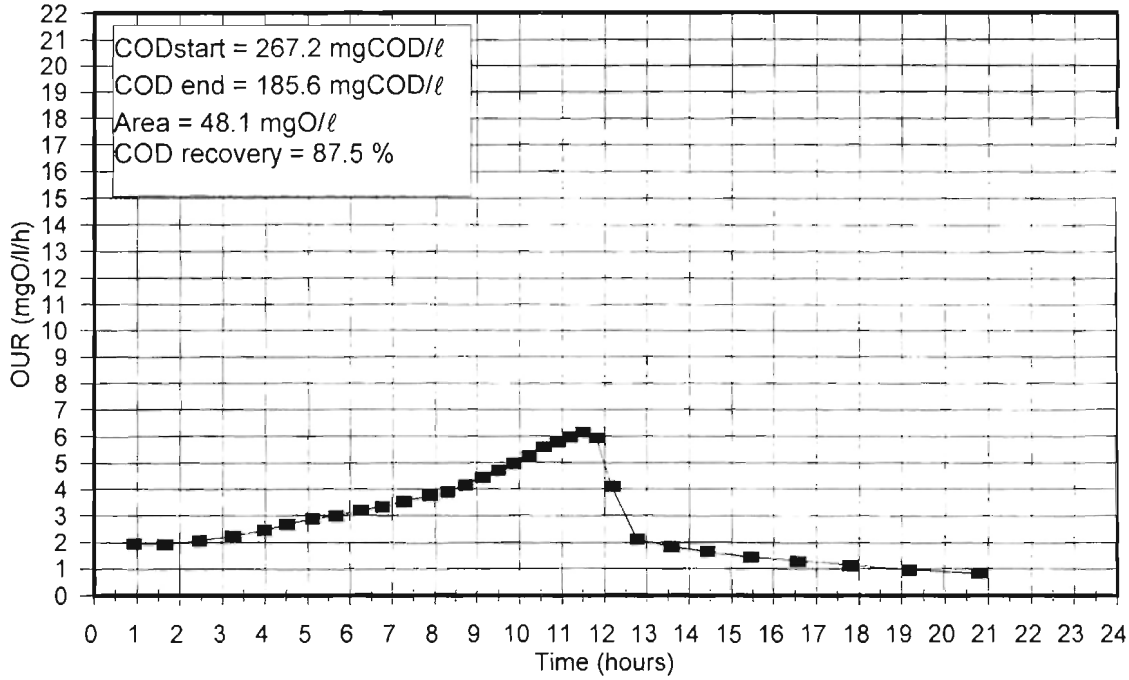
NO_3/NO_2 graph for for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 42, 23-04, Sewage Batch No. 22



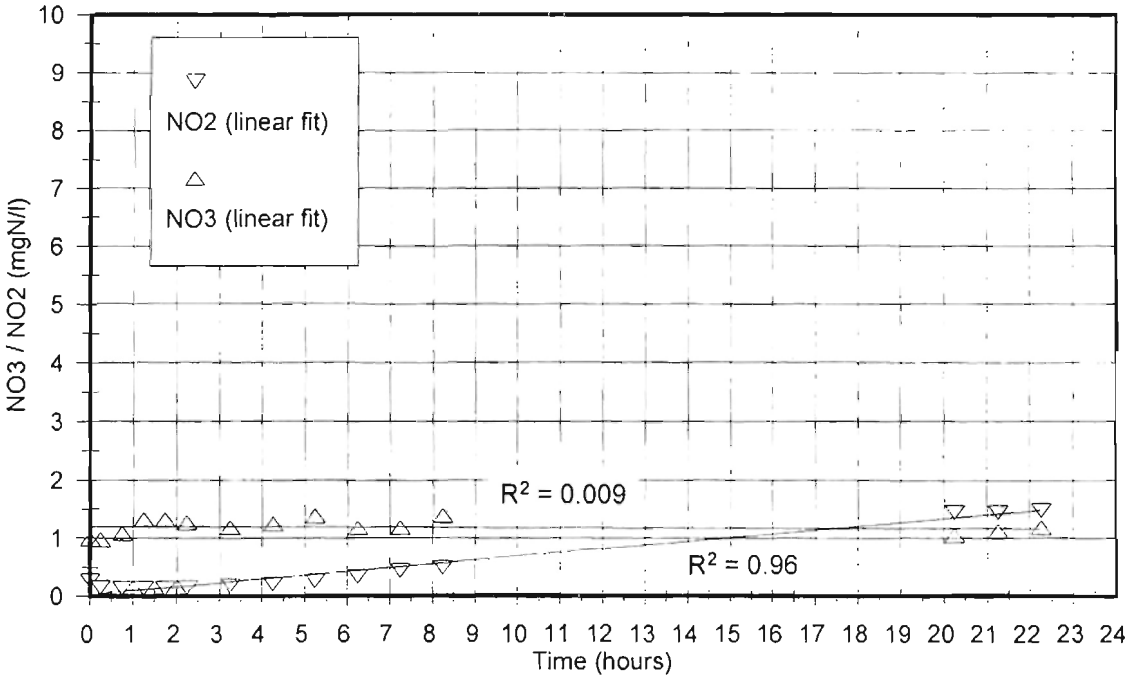
OUR-h graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 42, 23-04, Sewage Batch No 22



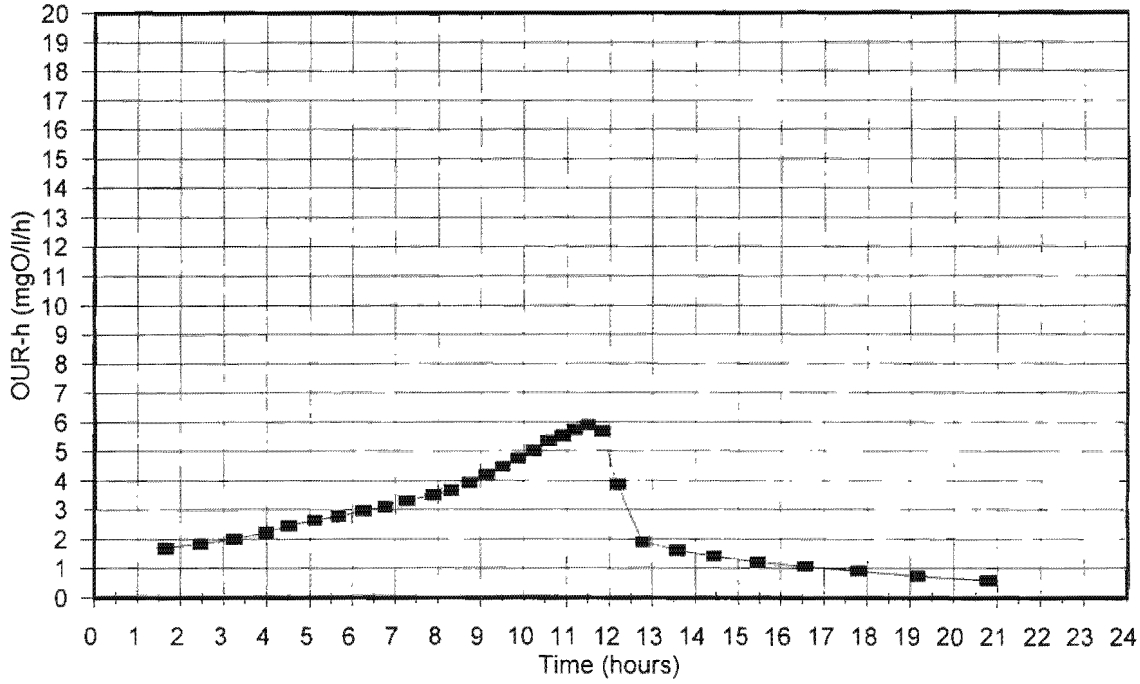
ℓn(OUR-h) graph for filtered wastewater (2.65ℓ) plus mixed liquor (0.35ℓ)
Batch Test No. 42, 23-04, Sewage Batch No. 22



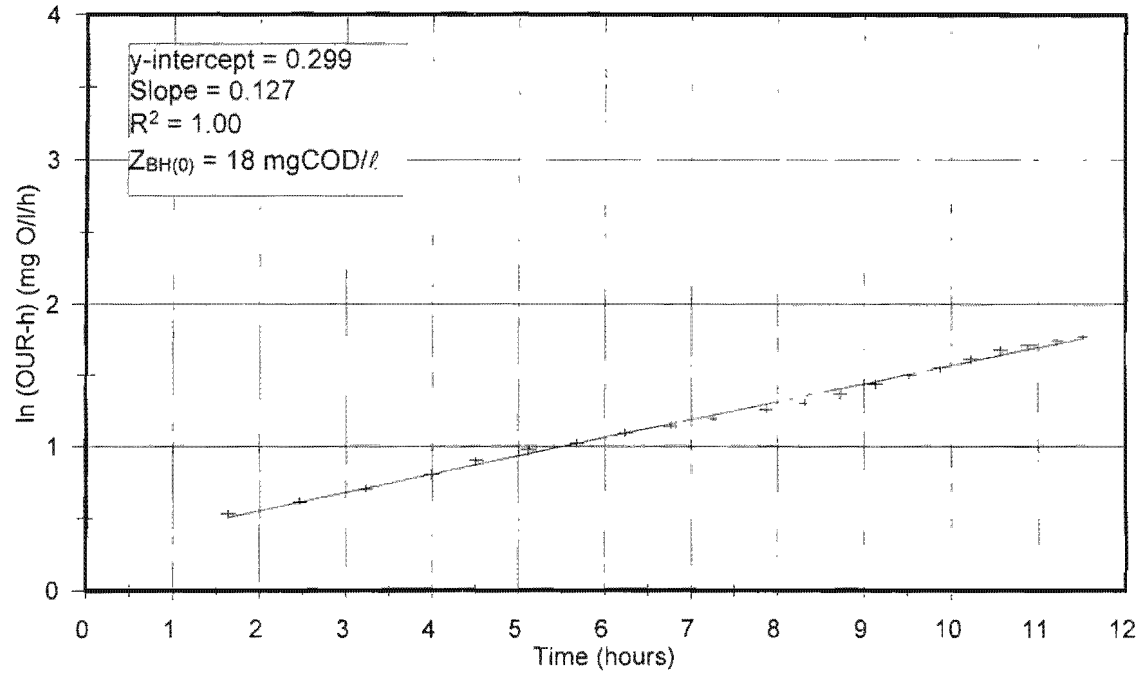
OUR graph for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 61, 15-06, Sewage Batch No. 26



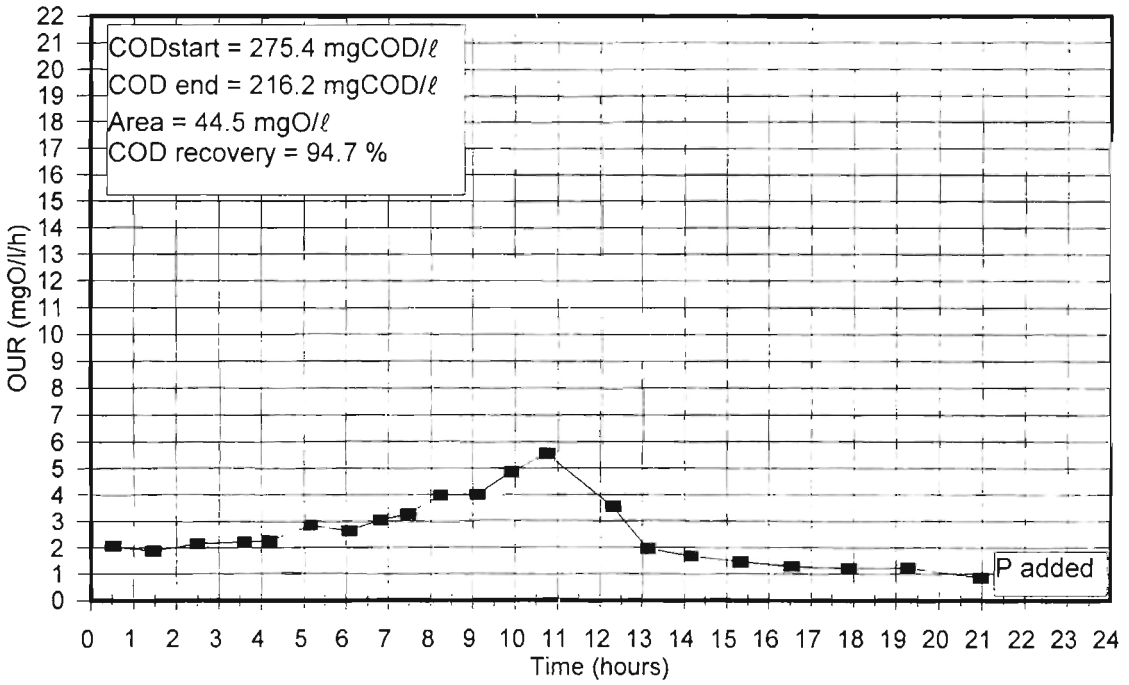
NO_3/NO_2 graph for for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 61, 15-06, Sewage Batch No. 26



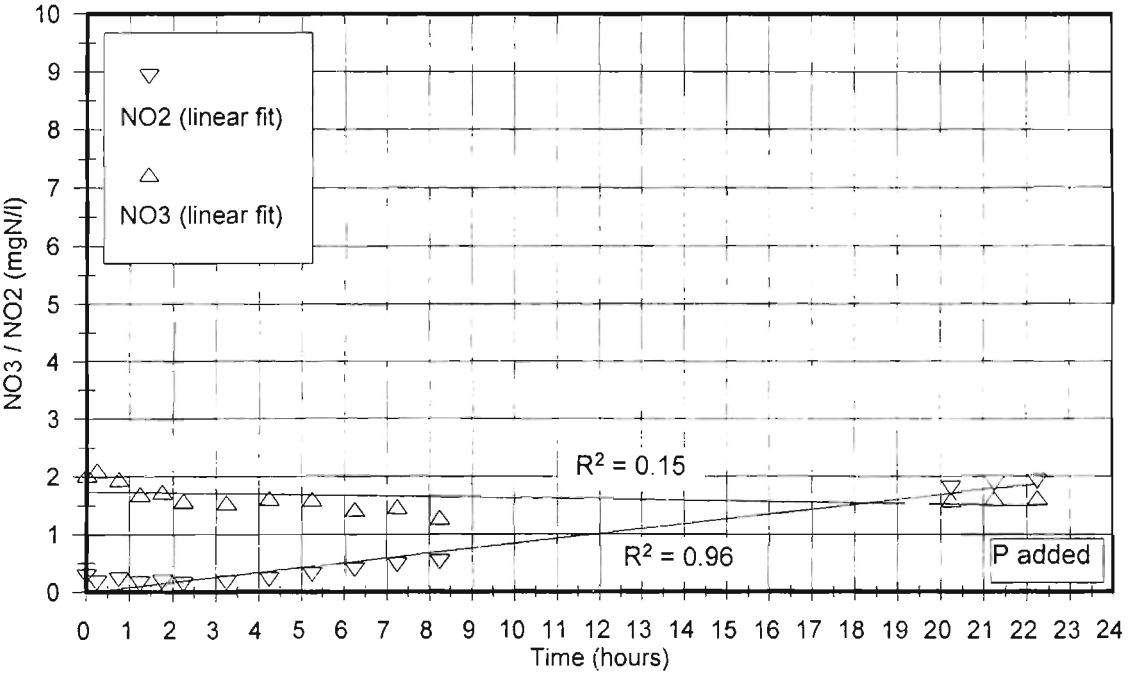
OUR-h graph for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 61, 15-06, Sewage Batch No. 26



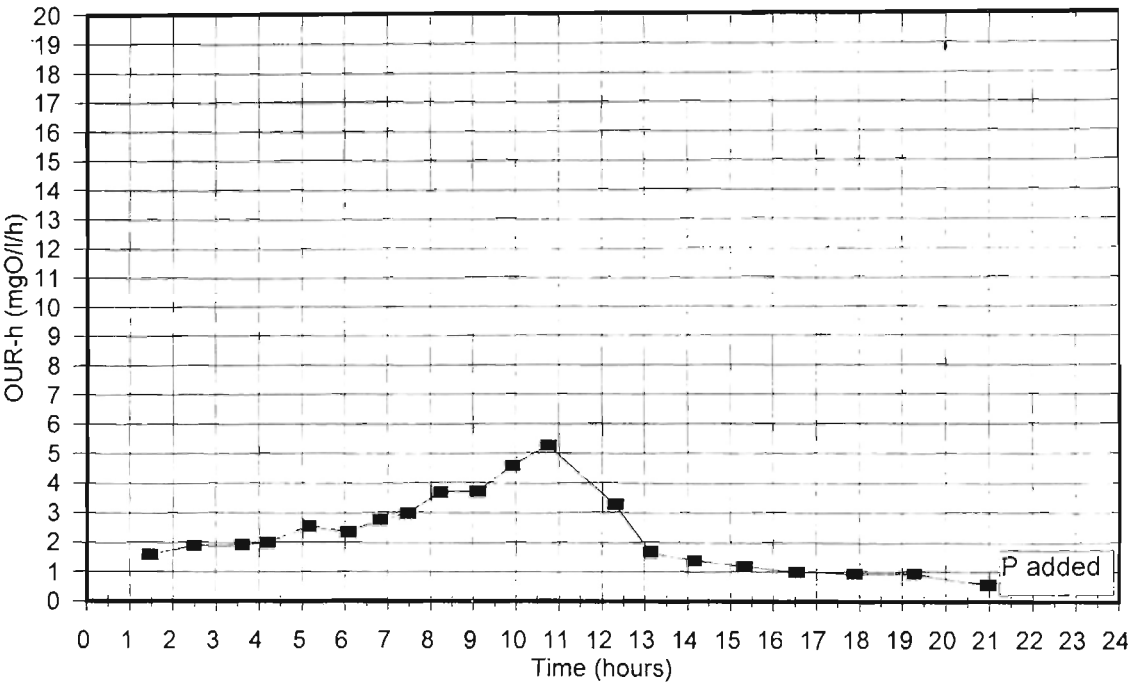
ℓn(OUR-h) graph for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 61, 15-06, Sewage Batch No. 26



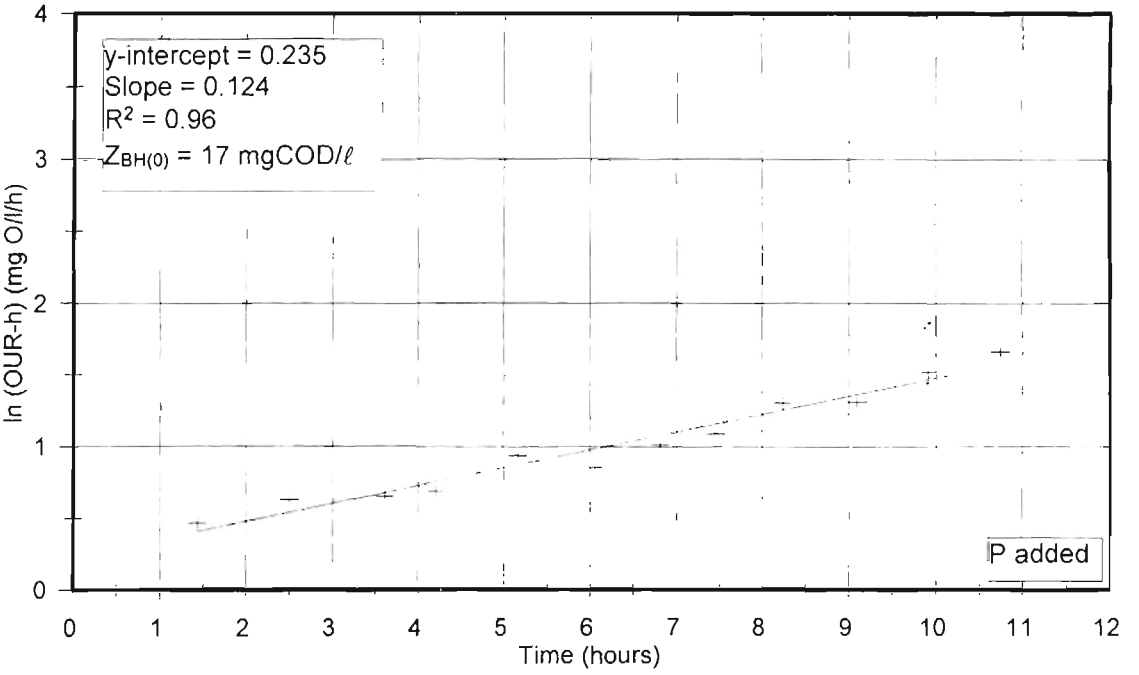
OUR graph for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 62, 15-06, Sewage Batch No. 26



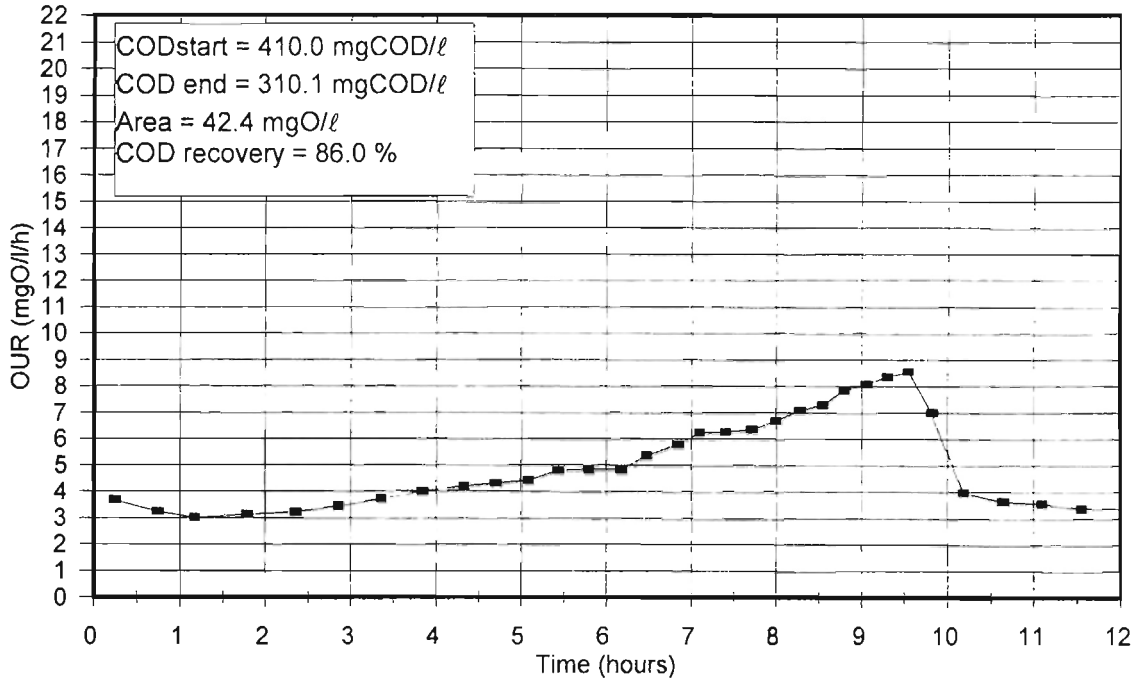
NO_3/NO_2 graph for for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 62, 15-06, Sewage Batch No. 26



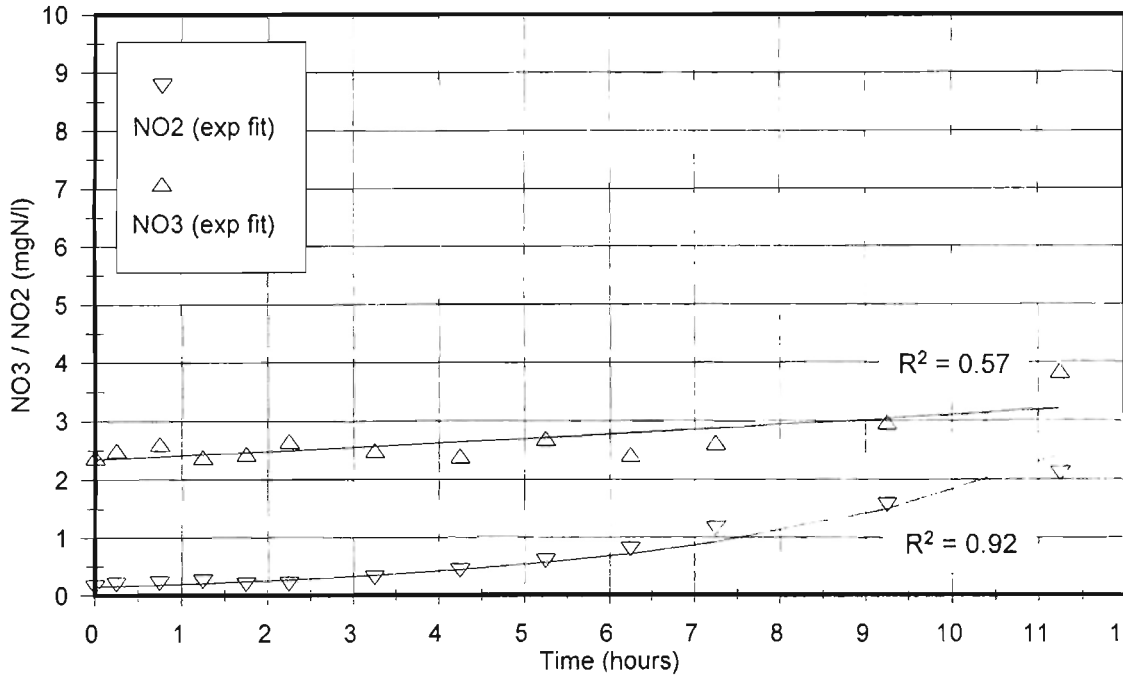
OUR-h graph for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 62, 15-06, Sewage Batch No. 26



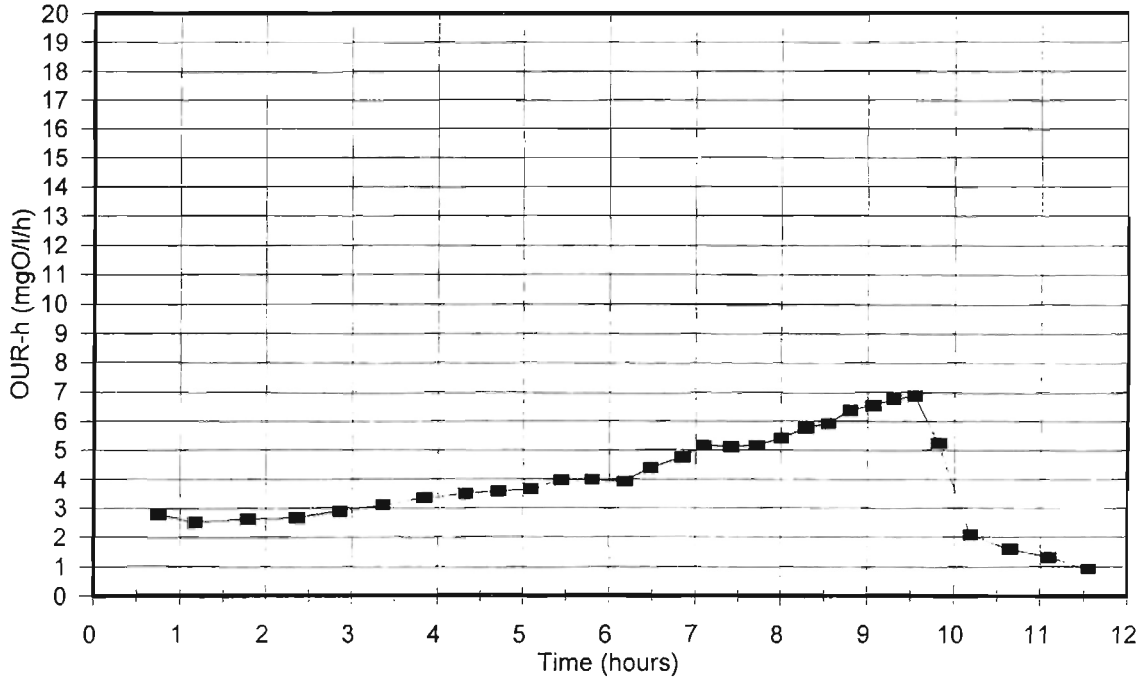
ℓn(OUR-h) graph for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 62, 15-06, Sewage Batch No. 26



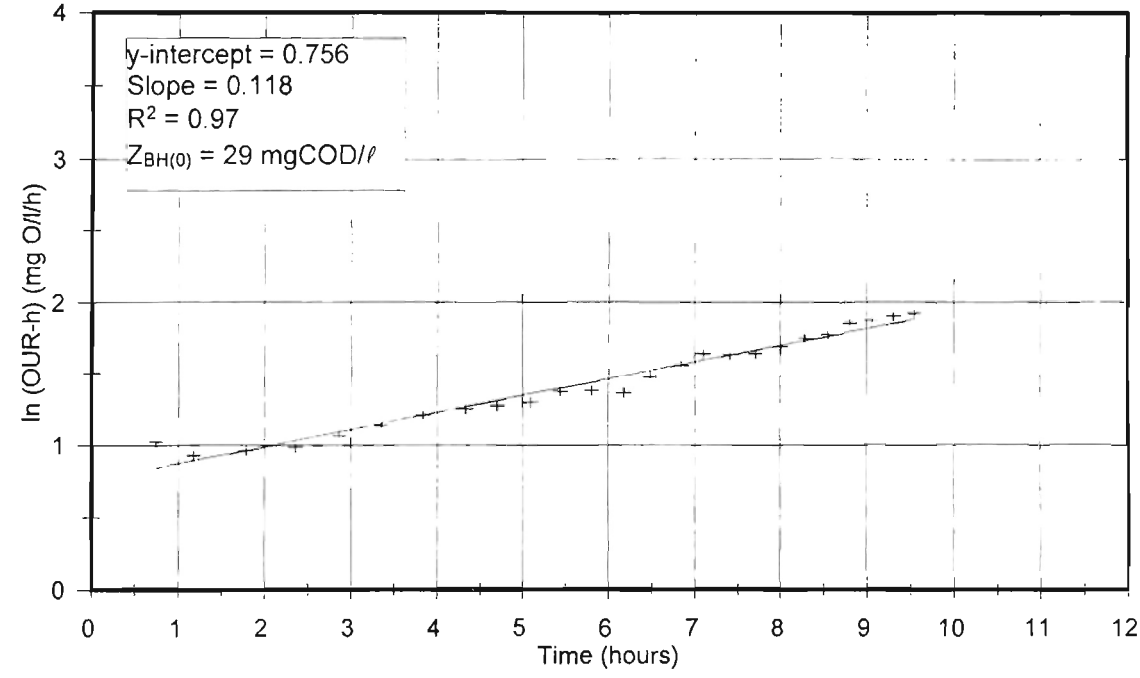
OUR graph for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 63, 16-06, Sewage Batch No. 26



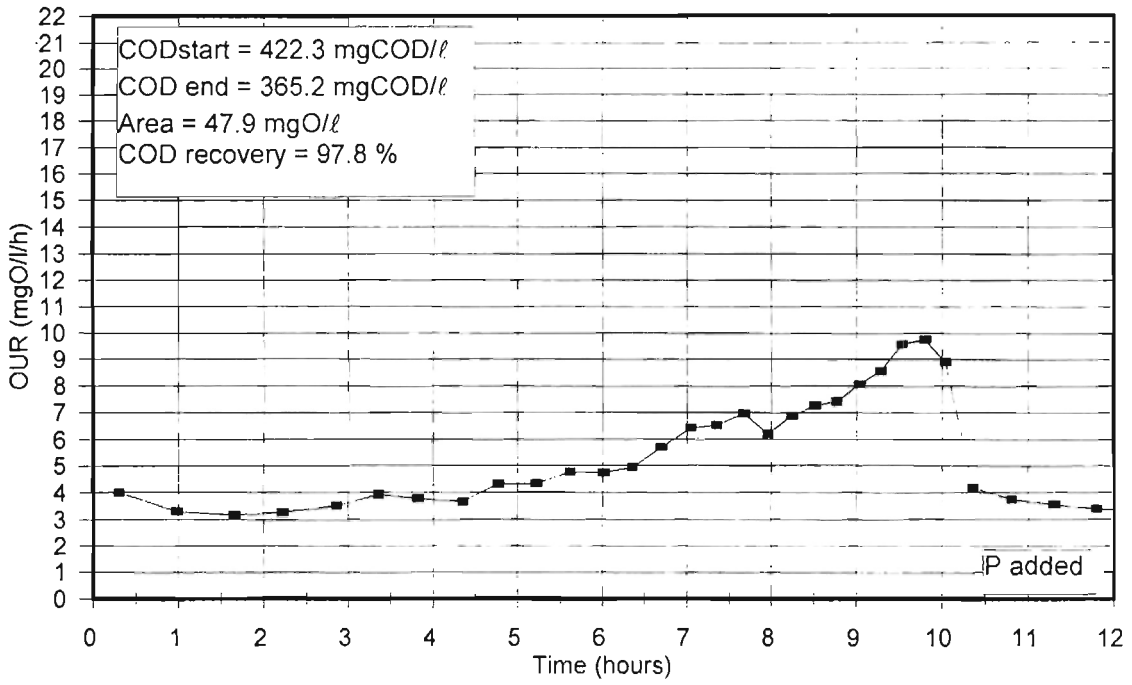
NO_3/NO_2 graph for for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 63, 16-06, Sewage Batch No. 26



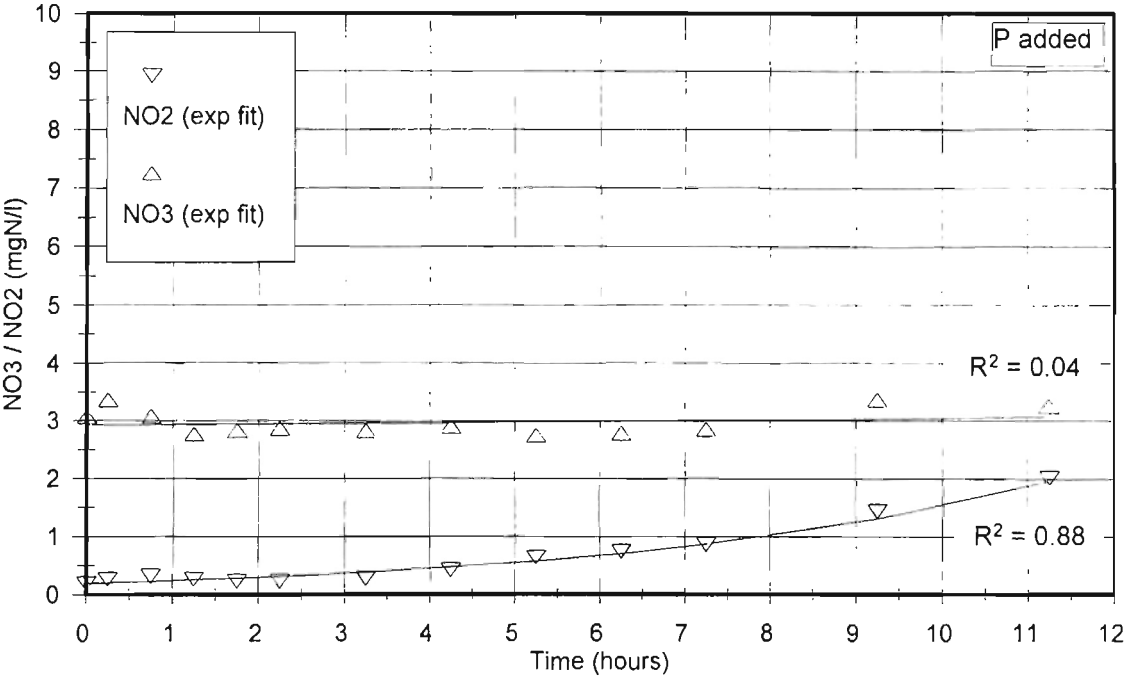
OUR-h graph for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 63, 16-06, Sewage Batch No. 26



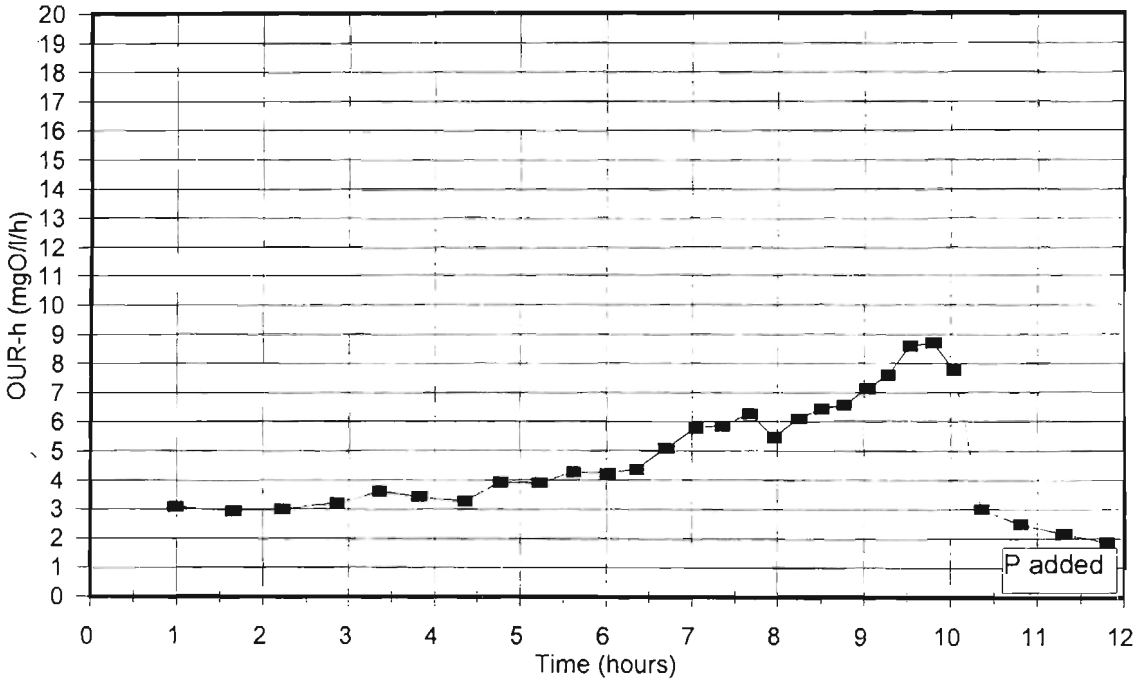
ℓn(OUR-h) graph for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 63, 16-06, Sewage Batch No. 26



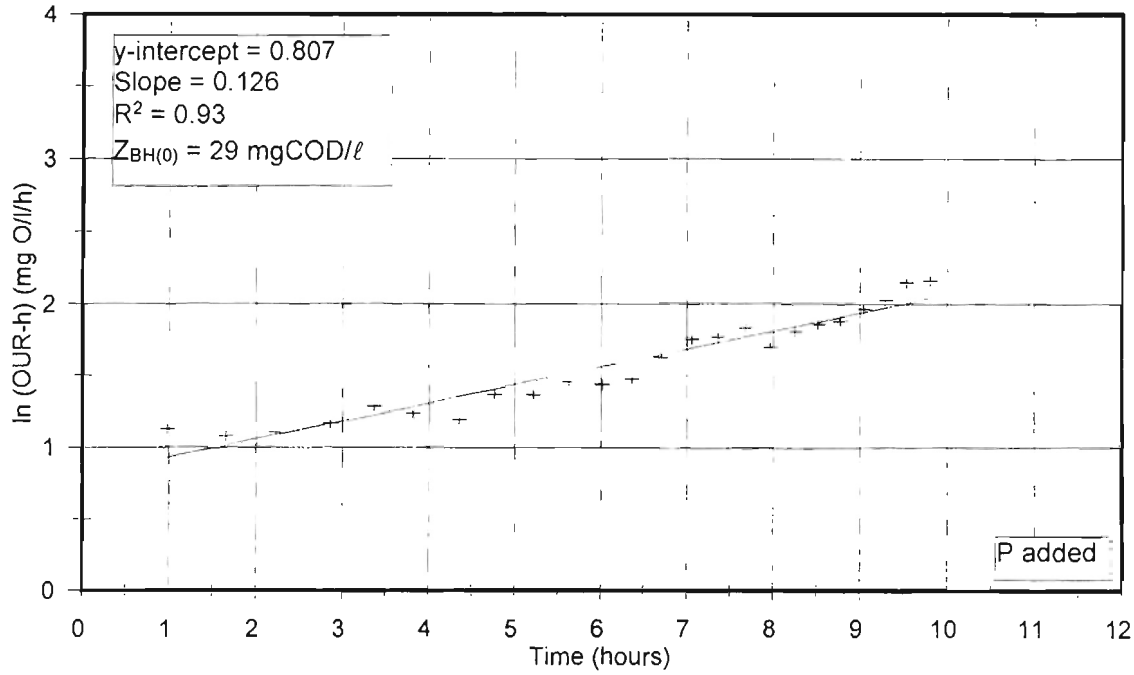
OUR graph for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 64, 16-06, Sewage Batch No. 26



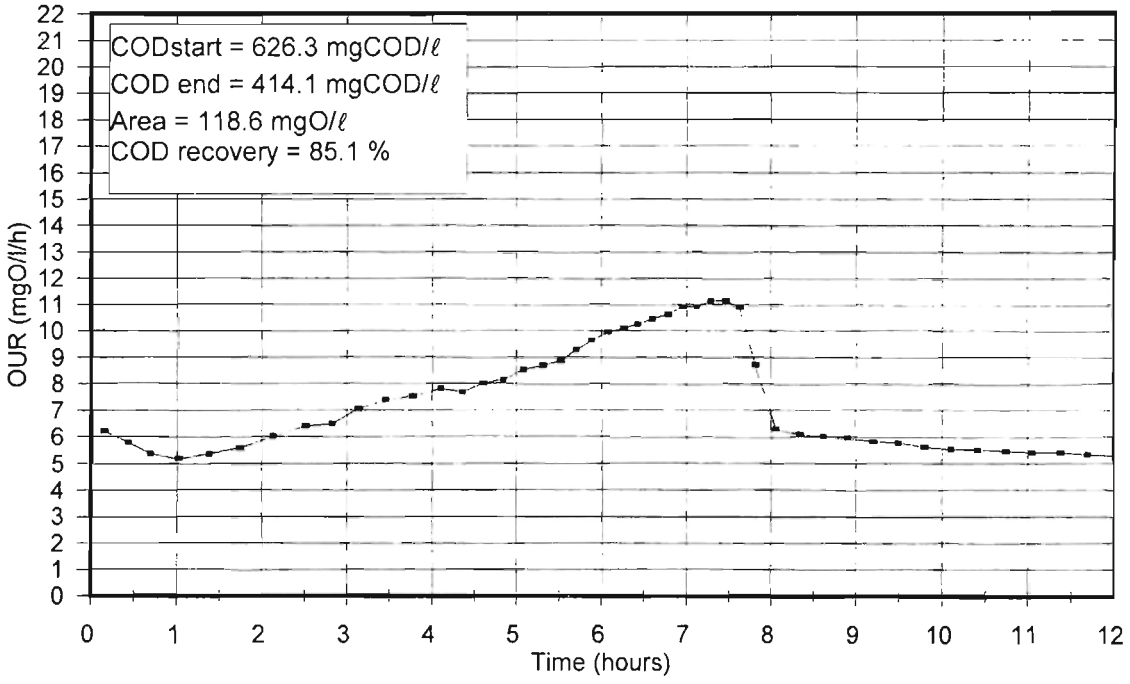
NO_3/NO_2 graph for for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 64, 16-06, Sewage Batch No. 26



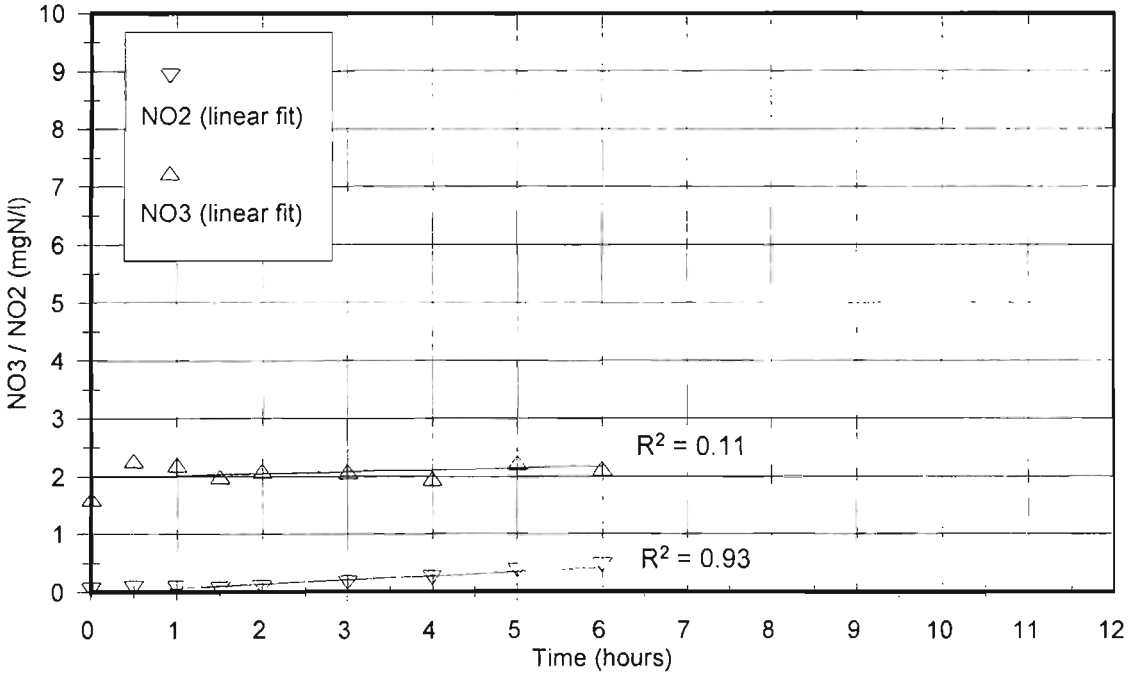
OUR-h graph for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 64, 16-06, Sewage Batch No. 26



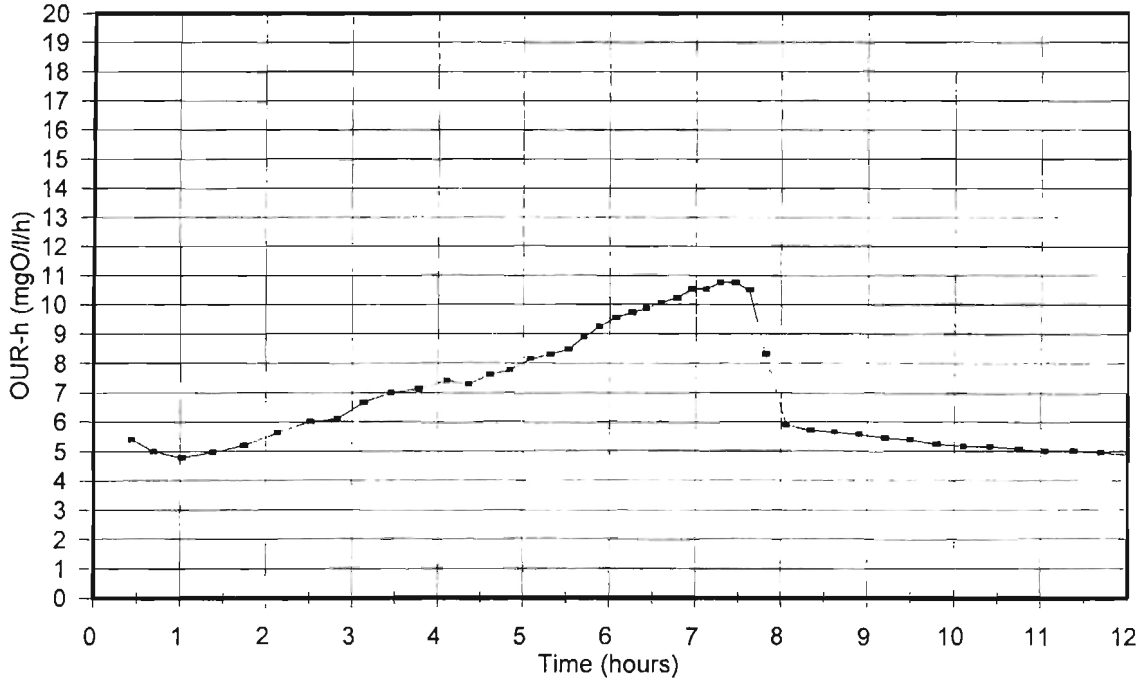
ℓn(OUR-h) graph for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 64, 16-06, Sewage Batch No. 26



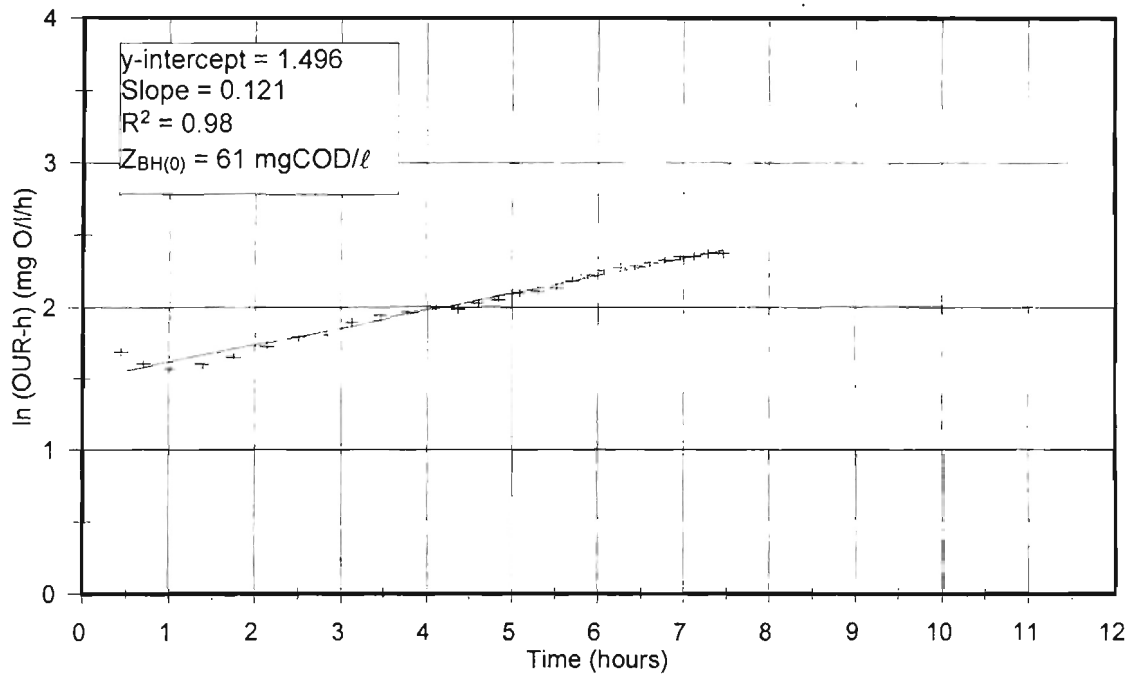
OUR graph for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 65, 17-06, Sewage Batch No. 26



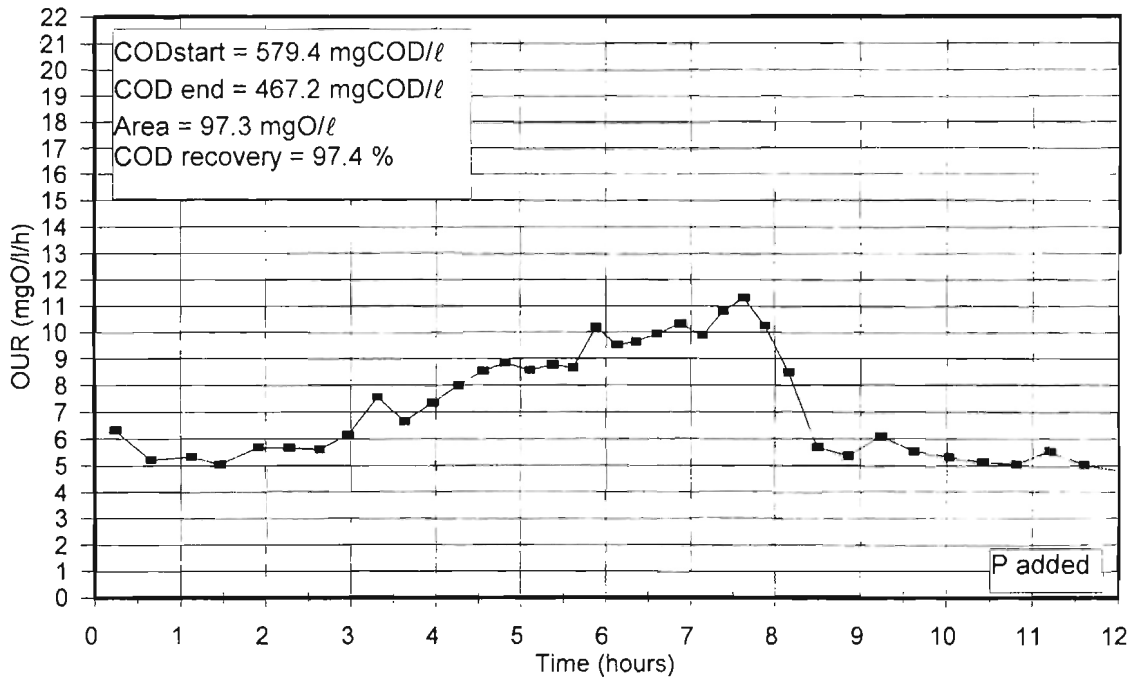
NO_3/NO_2 graph for for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 65, 17-06, Sewage Batch No. 26



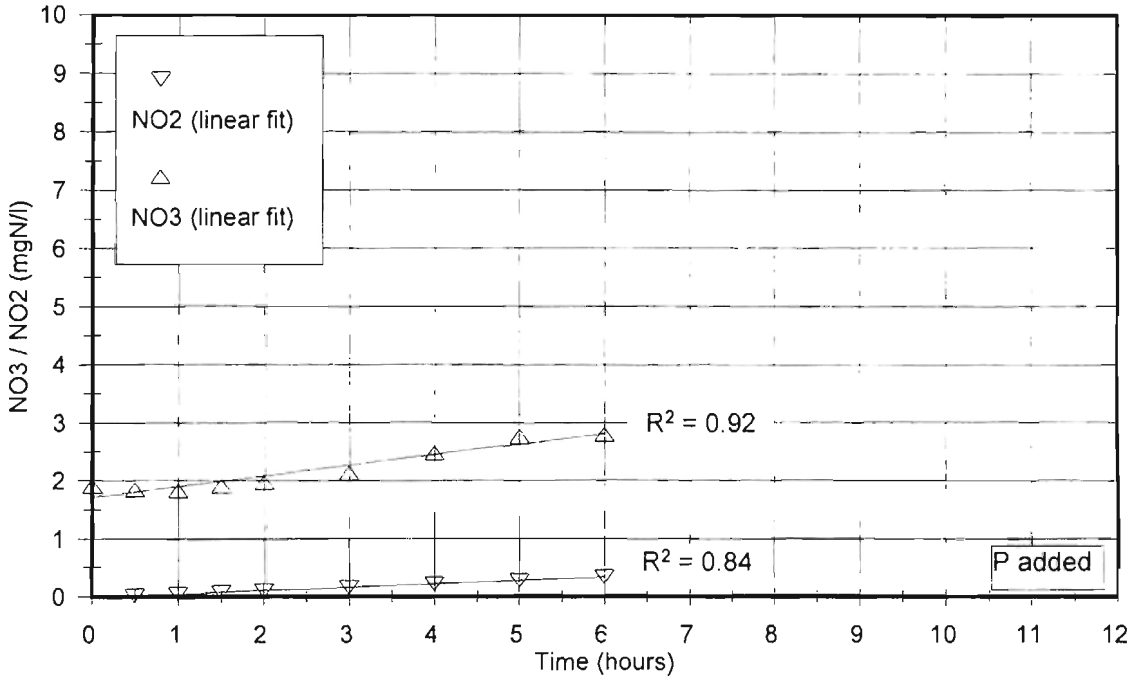
OUR-h graph for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 65, 17-06, Sewage Batch No. 26



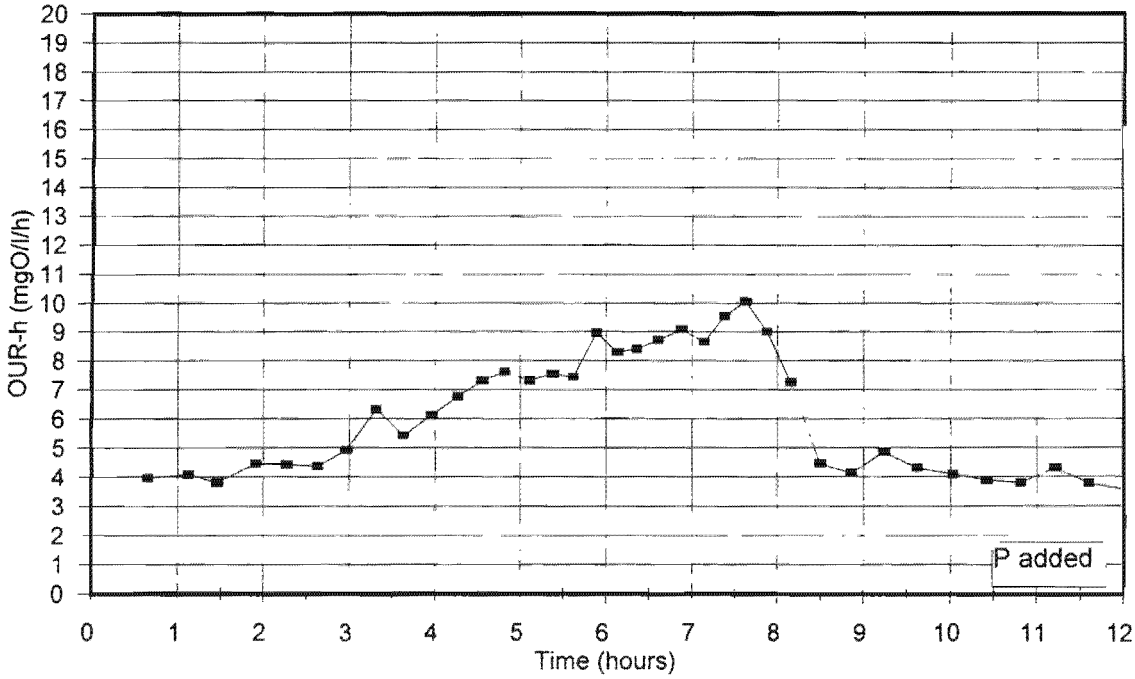
ℓn(OUR-h) graph for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 65, 17-06, Sewage Batch No. 26



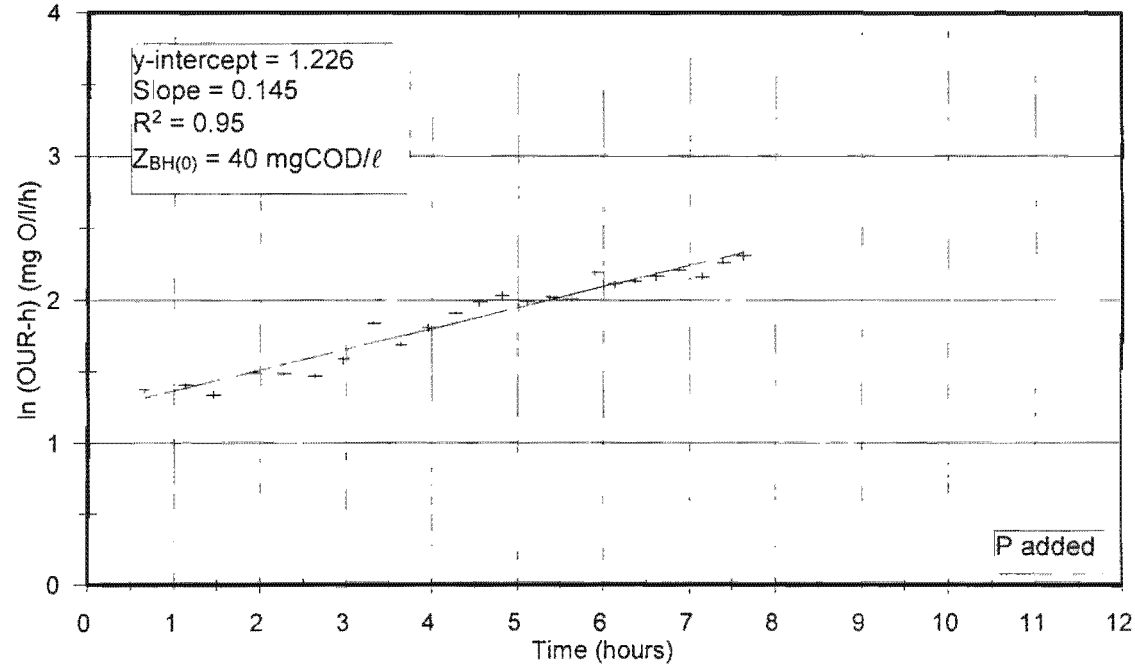
NO_3/NO_2 graph for for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 66, 17-06, Sewage Batch No. 26



NO_3/NO_2 graph for for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 66, 17-06, Sewage Batch No. 26



OUR-h graph for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 66, 17-06, Sewage Batch No. 26

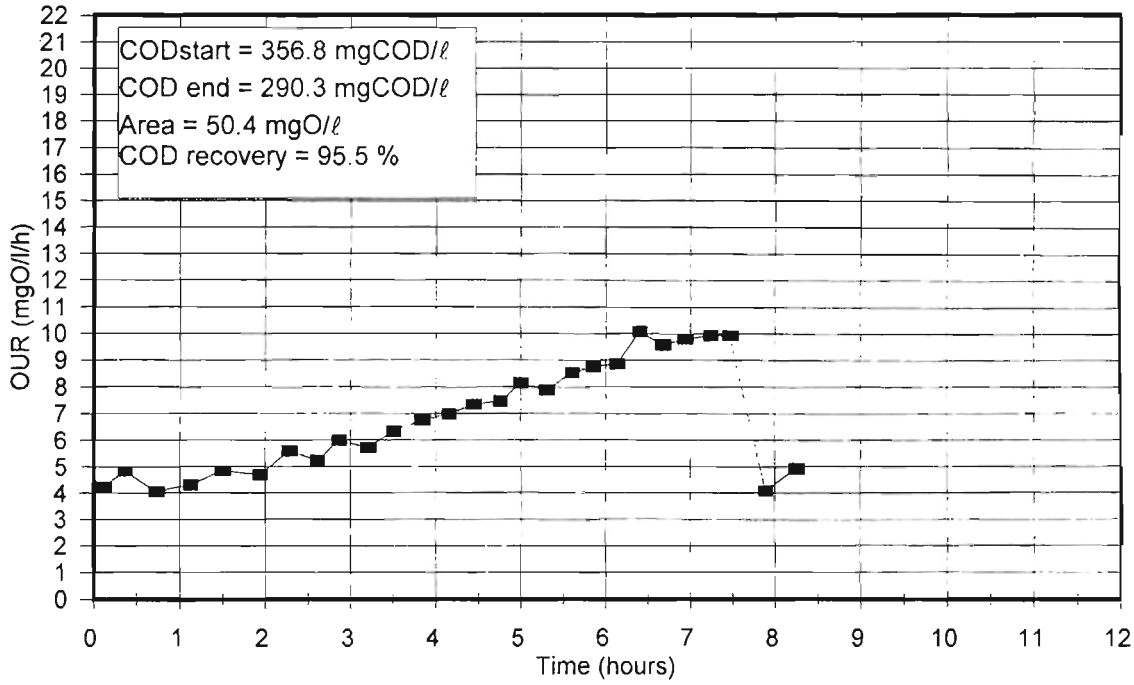


ℓn(OUR-h) graph for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 66, 17-06, Sewage Batch No. 26

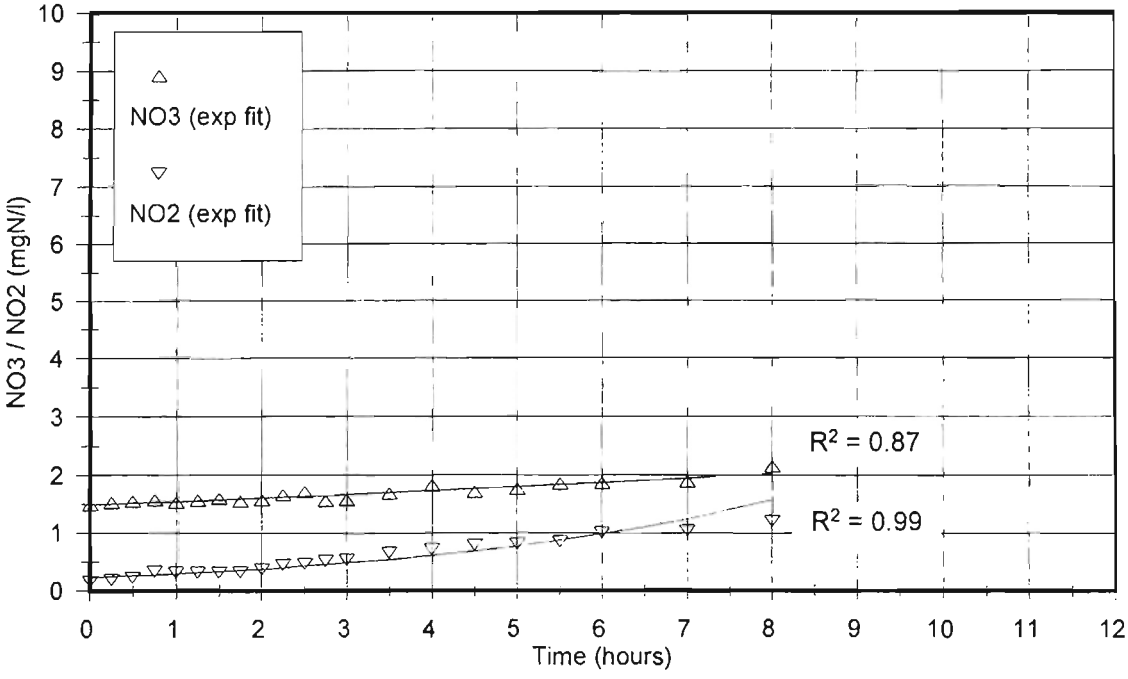
Table A4: Summarized data for modified batch tests with filtered wastewater and mixed liquor drawn from the parent Fully Aerobic control activated sludge system; mixed liquor volume, COD recovery, linear regression data and measured OHO active biomass.

Sew. Batch No.	Batch Test No.	Date of Test	ML volume (ℓ)	COD (mgCOD/ℓ) Start	COD (mgCOD/ℓ) End	Area (mgO/ℓ)	% COD Recovery	Linear regresion data y-int.	Linear regresion data slope	Linear regresion data R^2	$Z_{BH(0)}$ (ML) (mgCOD/ℓ)
23	43	11-05	0.08	356.8	290.3	50.4	95.5	1.258	0.131	0.95	44
	44	11-05	0.32	669.3	590.7	49.0	95.6	2.048	0.040	0.33	234
	45	15-05	0.16	393.1	312.5	44.9	90.9	1.151	0.078	0.92	61
	46	15-05	0.24	528.2	447.6	49.4	94.1	1.463	0.072	0.91	88
	47	16-05	0.12	362.9	292.3	45.0	93.0	0.832	0.205	0.98	20
	48	16-05	0.28	578.6	506.0	41.7	94.7	1.393	0.109	0.97	58
24	49	21-05	0.08	266.1	209.7	49.6	97.4	0.107	0.129	0.95	14
	50	21-05	0.12	312.5	243.9	55.7	95.9	0.351	0.117	0.96	20
	51	22-05	0.16	356.8	286.3	43.5	92.4	0.504	0.123	0.98	22
	52	22-05	0.20	452.9	373.3	47.4	92.9	0.724	0.110	0.95	30
	53	23-05	0.24	559.0	491.6	48.1	96.6	0.958	0.163	0.98	27
	54	23-05	0.28	607.9	563.0	51.4	101.1	1.229	0.128	0.97	44
25	55	06-06	0.08	375.2	294.1	43.9	90.1	0.898	0.274	1.00	16
	56	06-06	0.16	279.9	212.9	52.6	94.9	1.291	0.255	0.98	26
	57	07-06	0.24	649.0	551.6	64.7	95.0	1.500	0.146	1.00	52
	58	07-06	0.28	719.9	612.5	69.0	94.7	1.709	0.123	0.96	24
	59	09-06	0.12	461.0	367.2	51.9	90.9	0.510	0.190	0.96	15
	60	09-06	0.20	593.6	518.2	66.2	98.4	0.901	0.159	0.98	27
26	61	15-06	0.08	267.2	185.6	48.1	87.5*	0.299	0.127	1.00	18
	62	15-06	0.08	275.4	216.2	44.5	94.7	0.235	0.124	0.96	17
	63	16-06	0.16	410.0	310.1	525.4	86.0*	0.756	0.118	0.97	29
	64	16-06	0.16	422.3	365.2	47.9	97.8	0.807	0.126	0.93	29
	65	17-06	0.24	626.3	414.1	118.6	85.1*	1.496	0.121	0.98	61
	66	17-06	0.24	579.4	467.2	97.3	97.4	1.226	0.145	0.95	40
				MEAN			93.9			0.94	
				Std. Deviation			3.8			0.13	

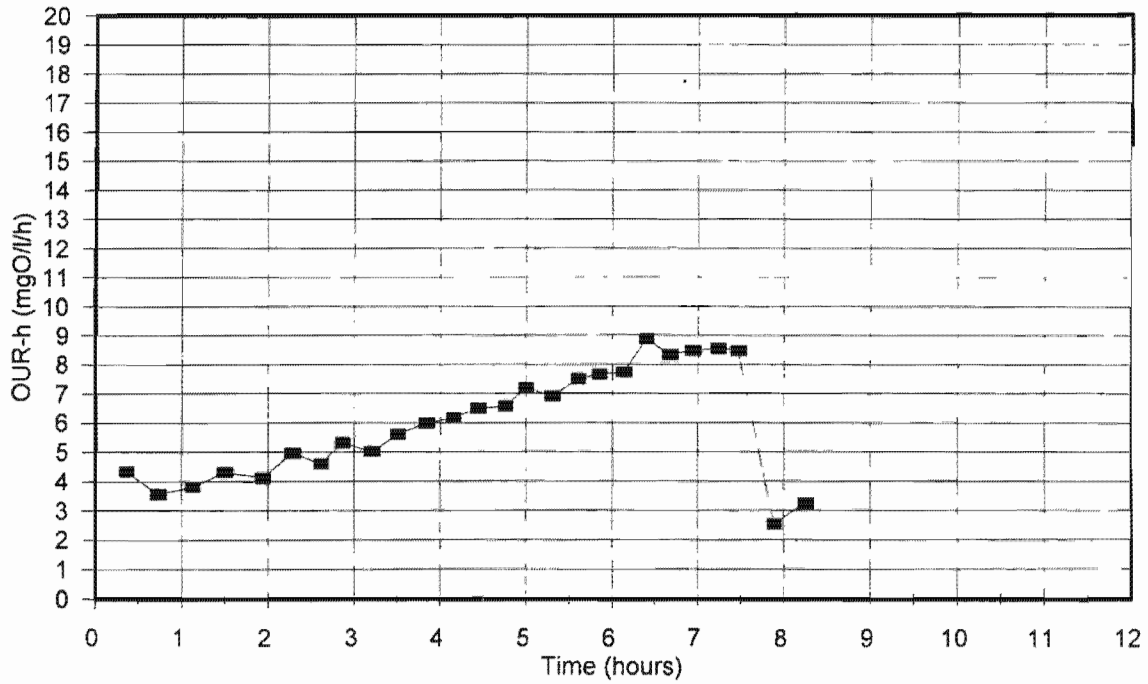
* Batch test rejected on account of poor %COD recovery



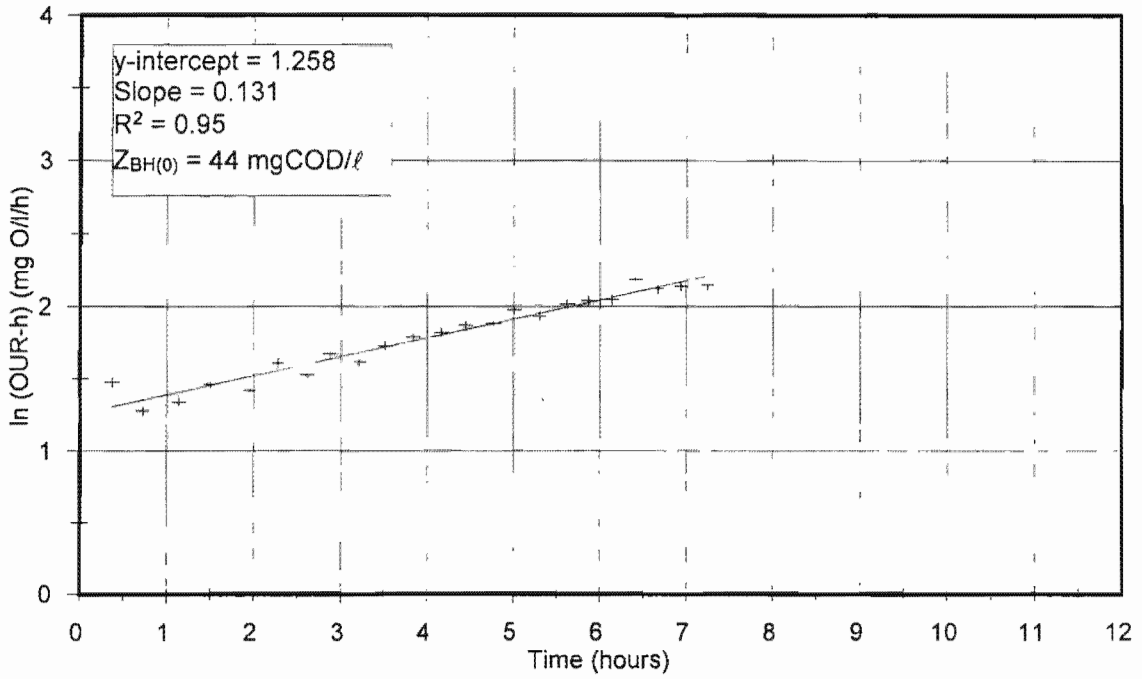
OUR graph for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 43, 11-05, Sewage Batch No. 23A



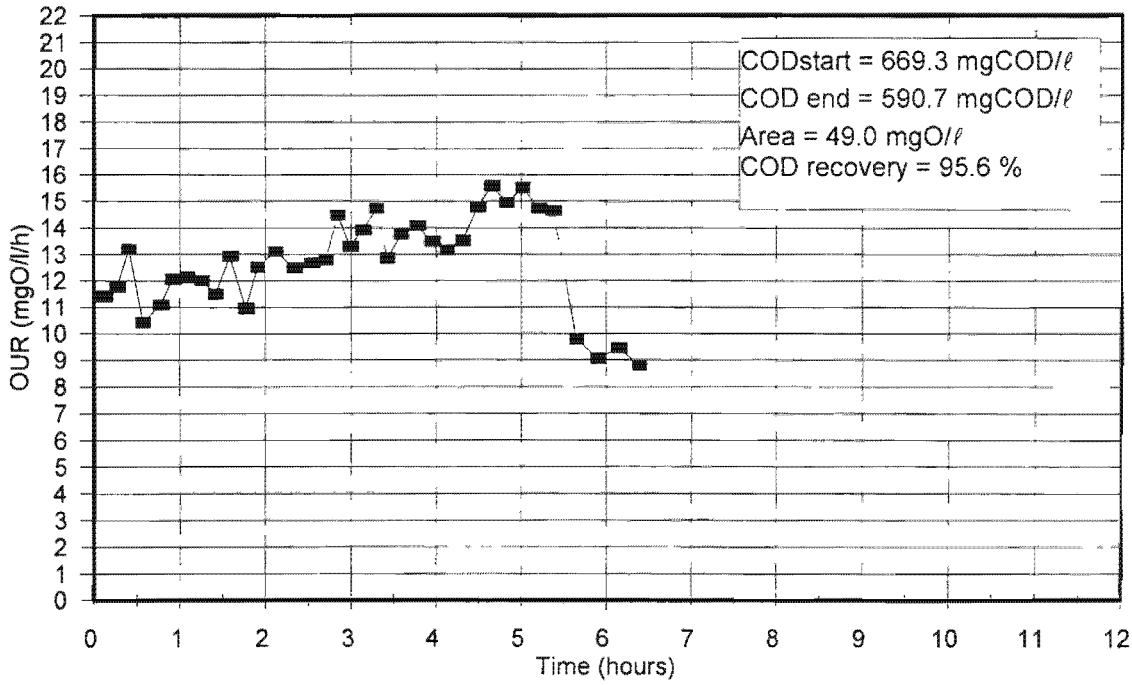
NO_3/NO_2 graph for for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 43, 11-05, Sewage Batch No. 23A



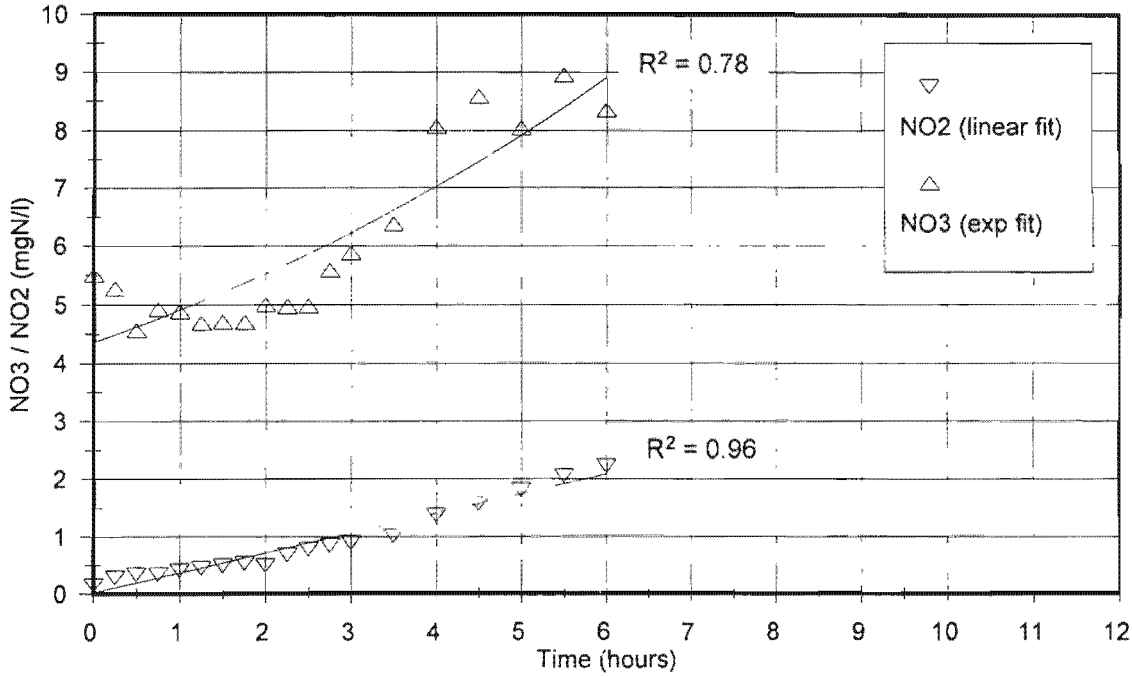
OUR-h graph for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 43, 11-05, Sewage Batch No. 23A



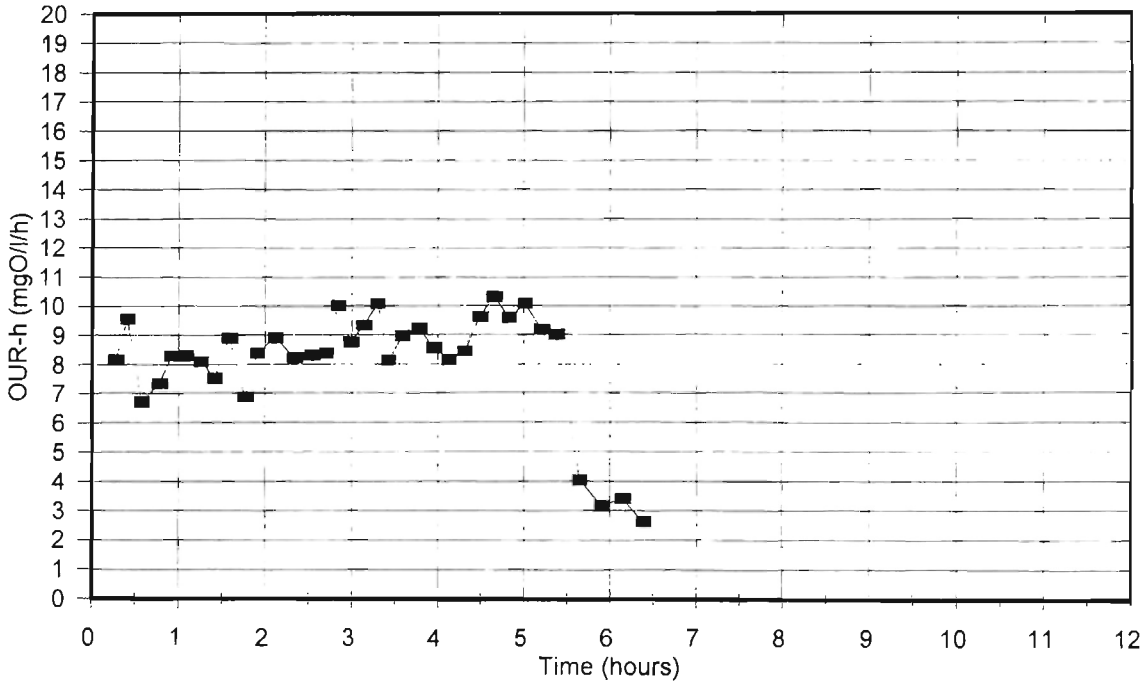
ℓn(OUR-h) graph for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 43, 11-05, Sewage Batch No. 23A



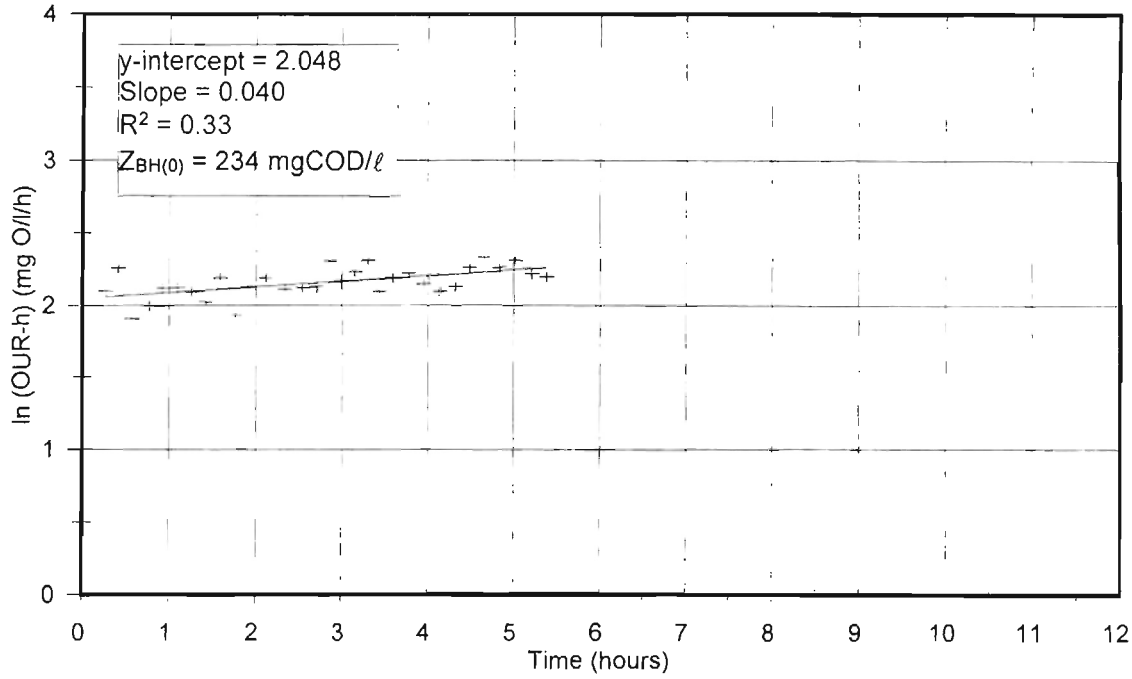
OUR graph for filtered wastewater (2.68ℓ) plus mixed liquor (0.32ℓ)
Batch Test No. 44, 11-05, Sewage Batch No. 23A



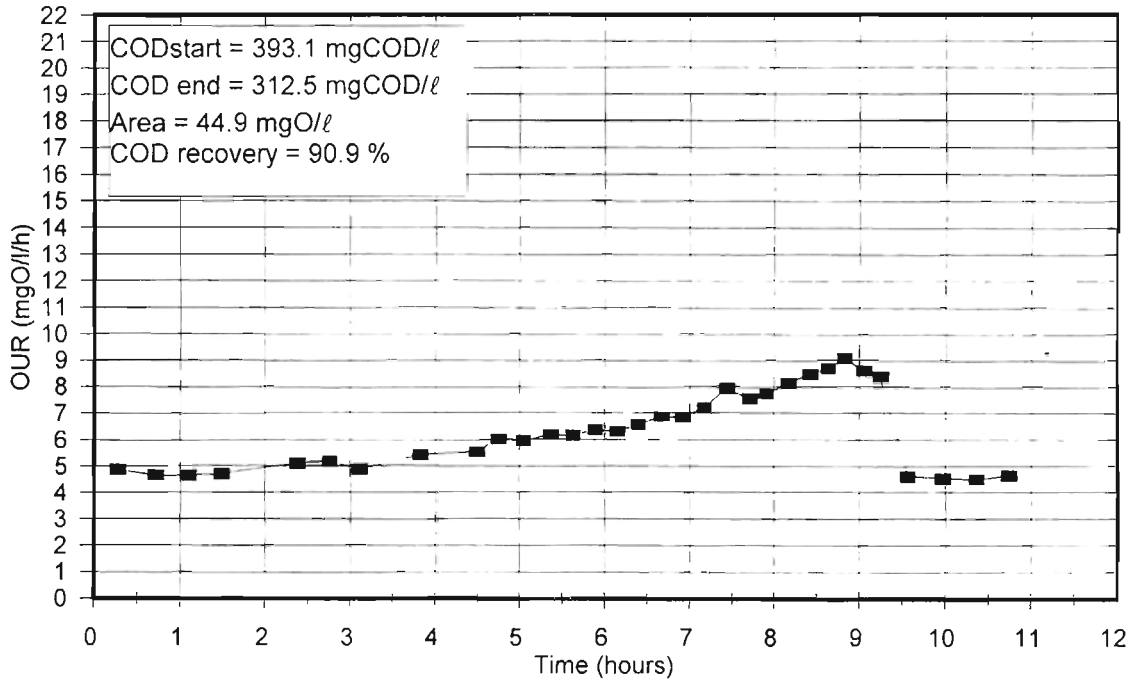
NO_3/NO_2 graph for for filtered wastewater (2.68ℓ) plus mixed liquor (0.32ℓ)
Batch Test No. 44, 11-05, Sewage Batch No. 23A



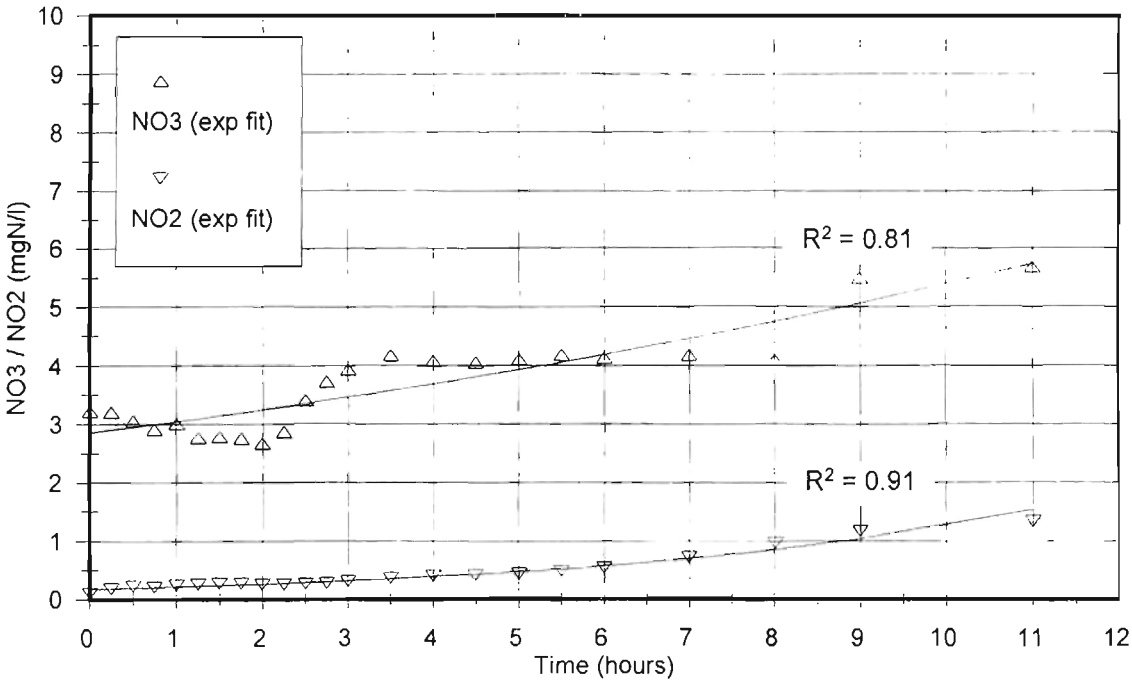
OUR-h graph for filtered wastewater (2.68ℓ) plus mixed liquor (0.32ℓ)
Batch Test No. 44, 11-05, Sewage Batch No. 23A



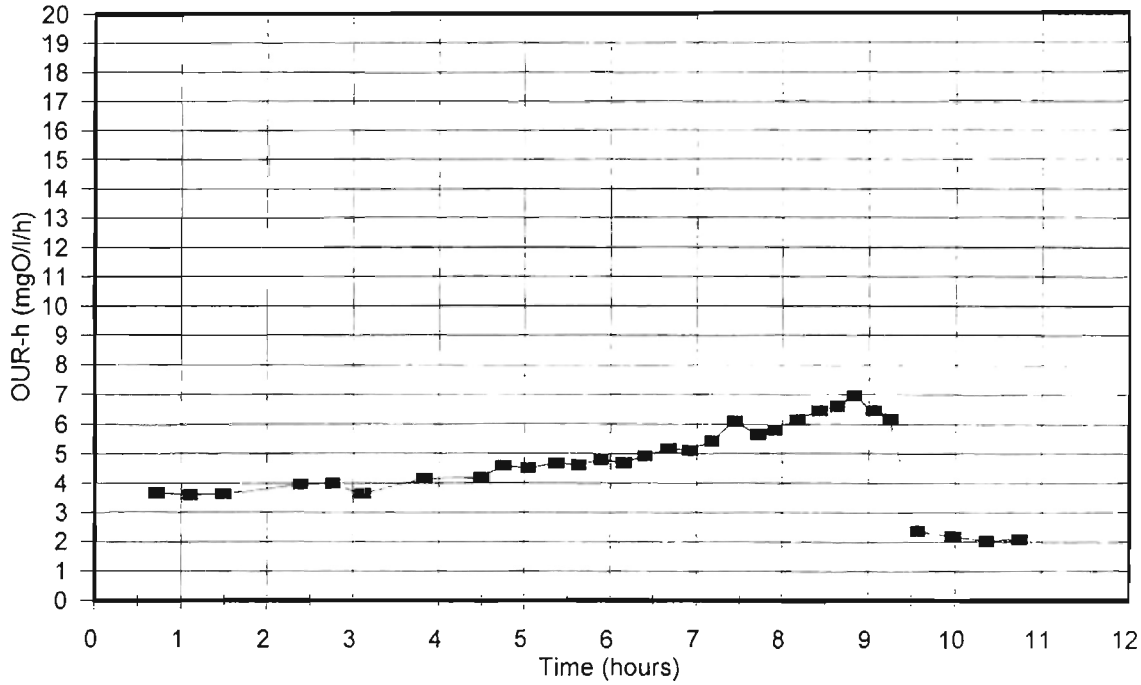
ℓn(OUR-h) graph for filtered wastewater (2.68ℓ) plus mixed liquor (0.32ℓ)
Batch Test No. 44, 11-05, Sewage Batch No. 23A



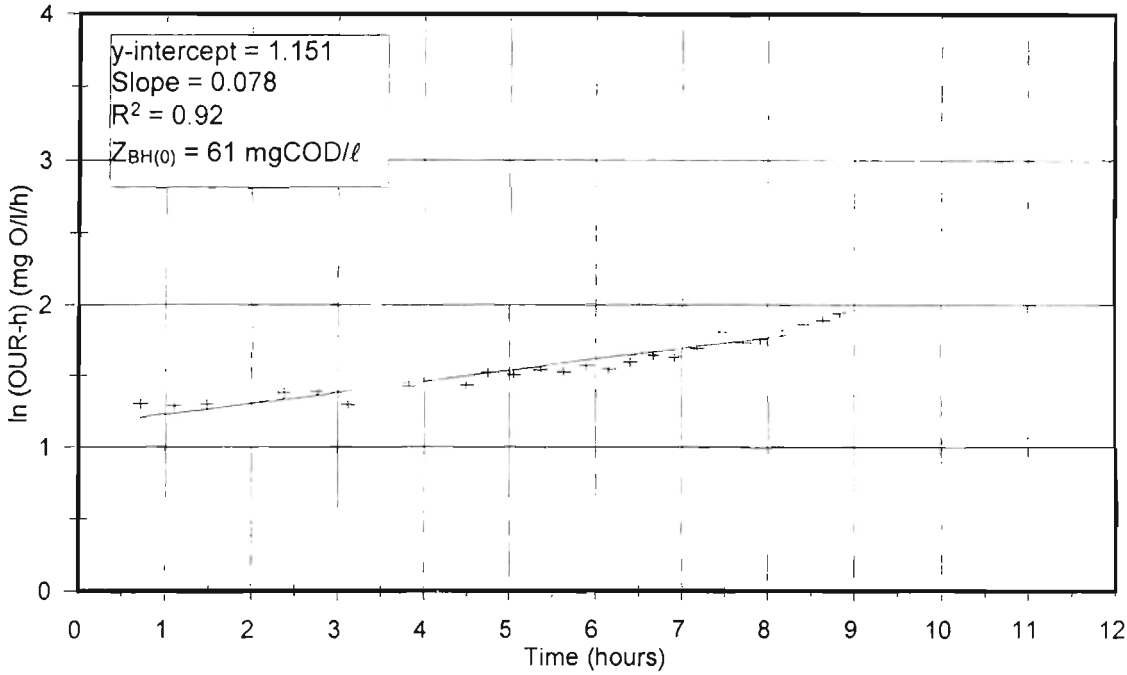
OUR graph for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 45, 15-05, Sewage Batch No. 23B



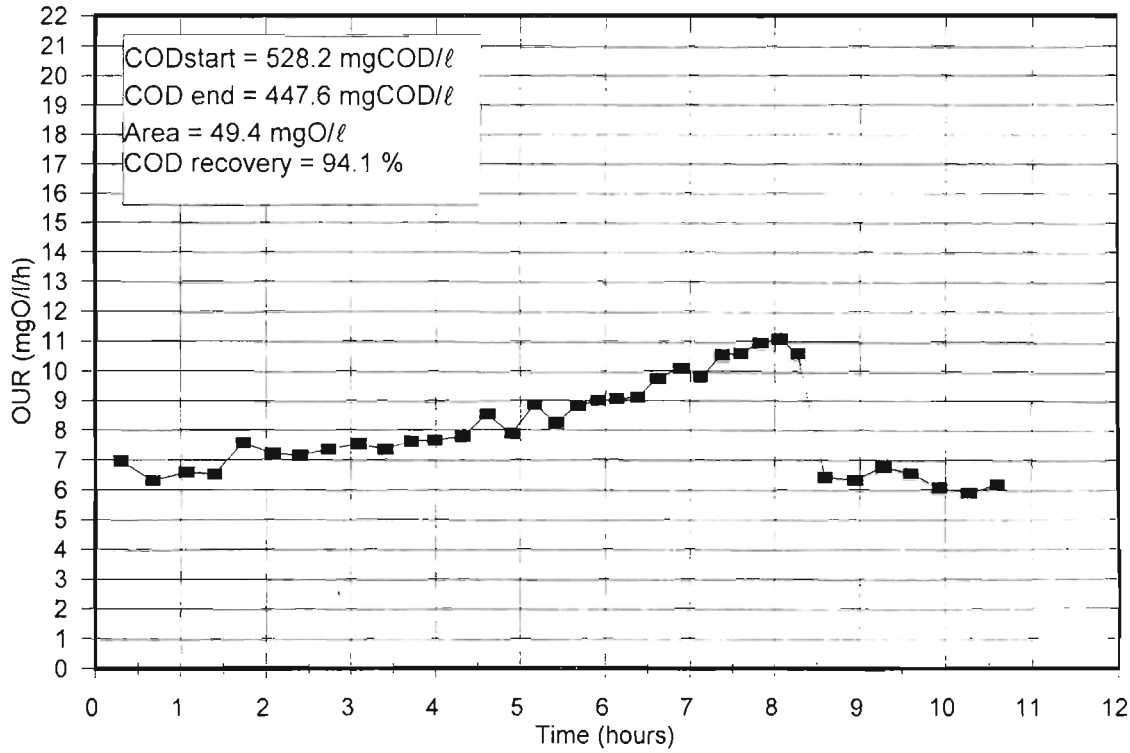
NO_3/NO_2 graph for for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 45, 15-05, Sewage Batch No. 23B



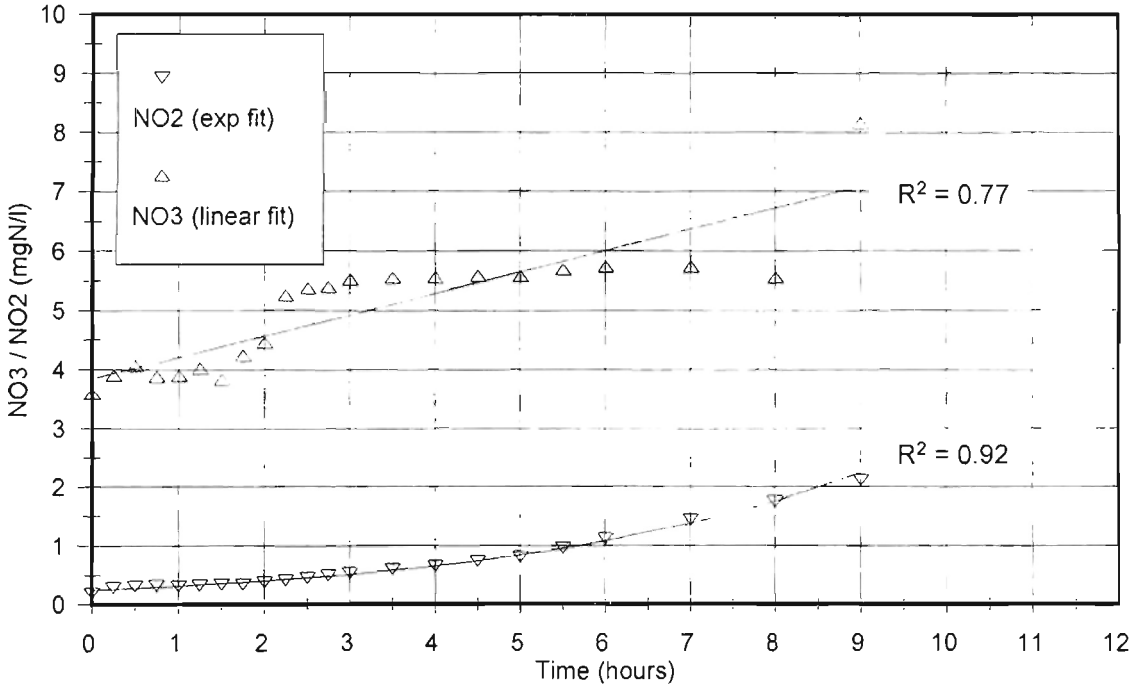
OUR-h graph for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 45, 15-05, Sewage Batch No. 23B



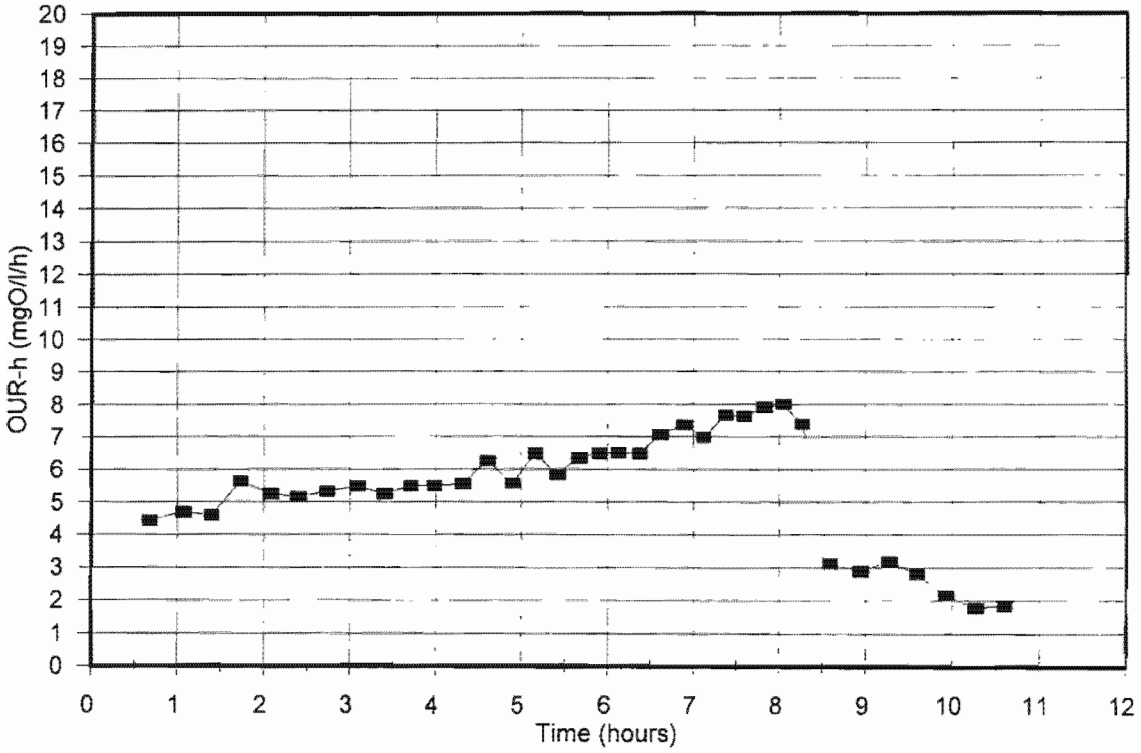
ℓn(OUR-h) graph for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 45, 15-05, Sewage Batch No. 23B



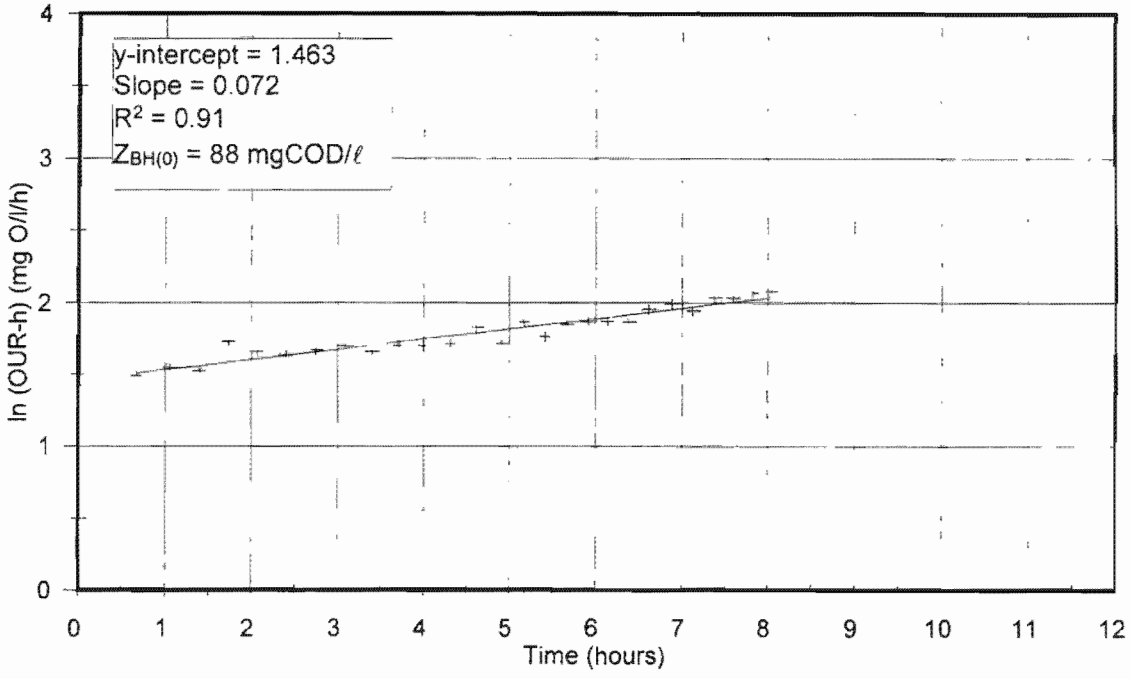
OUR graph for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 46, 15-05, Sewage Batch No. 23B



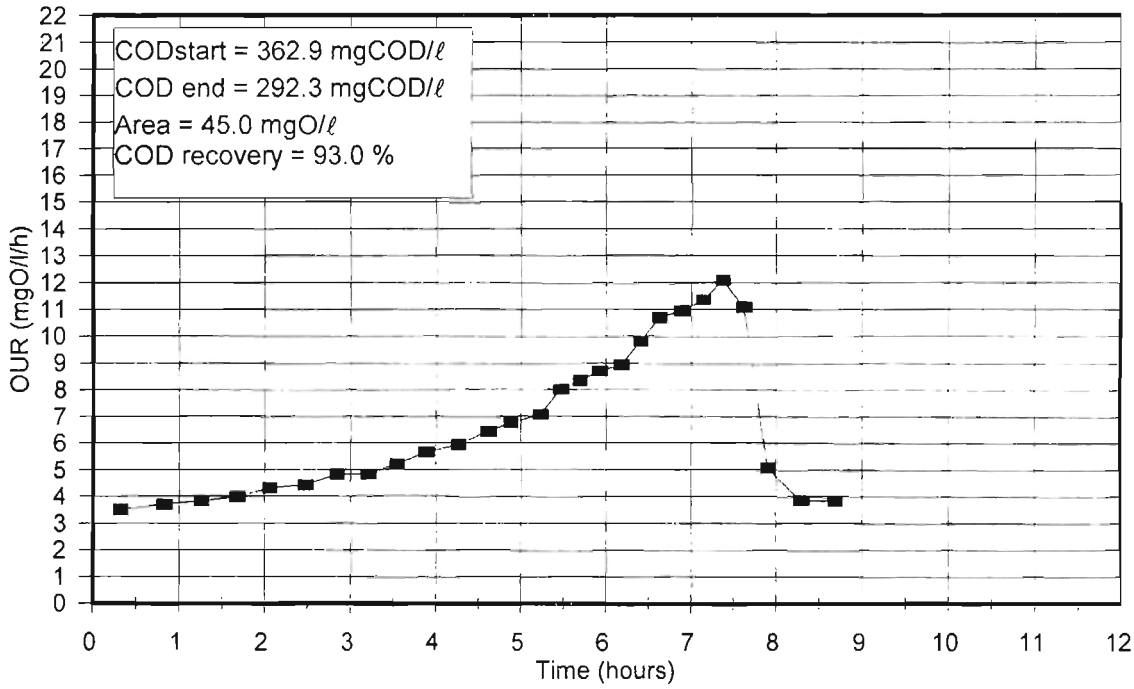
NO_3/NO_2 graph for for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 46, 15-05, Sewage Batch No. 23B



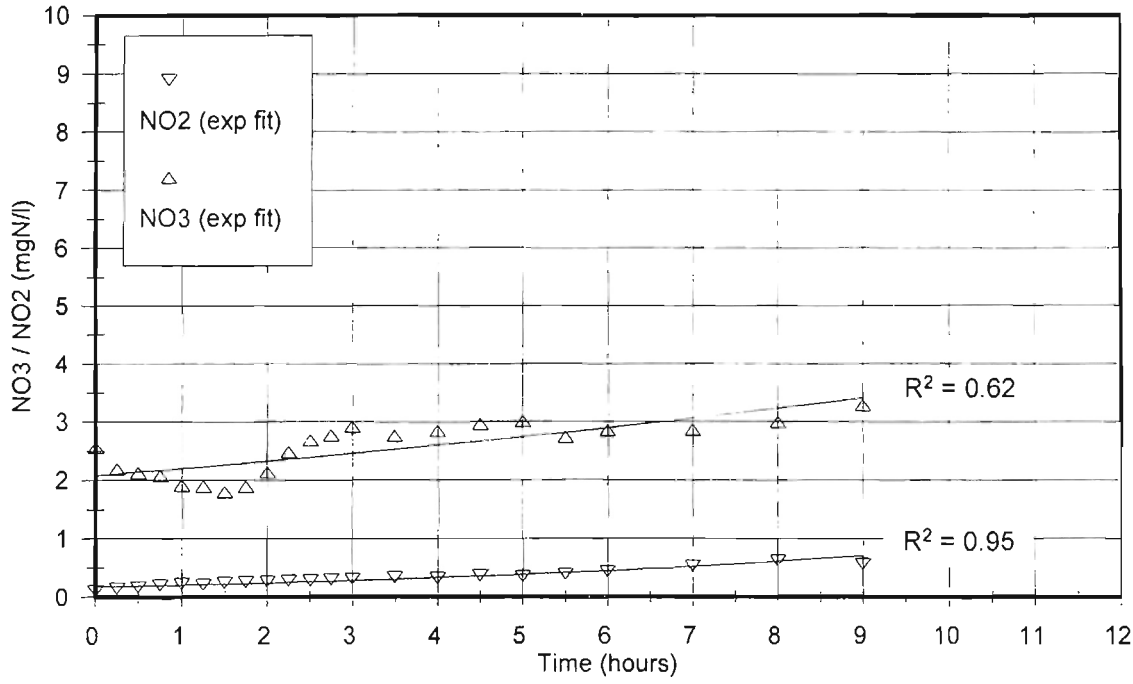
OUR-h graph for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 46, 15-05, Sewage Batch No. 23B



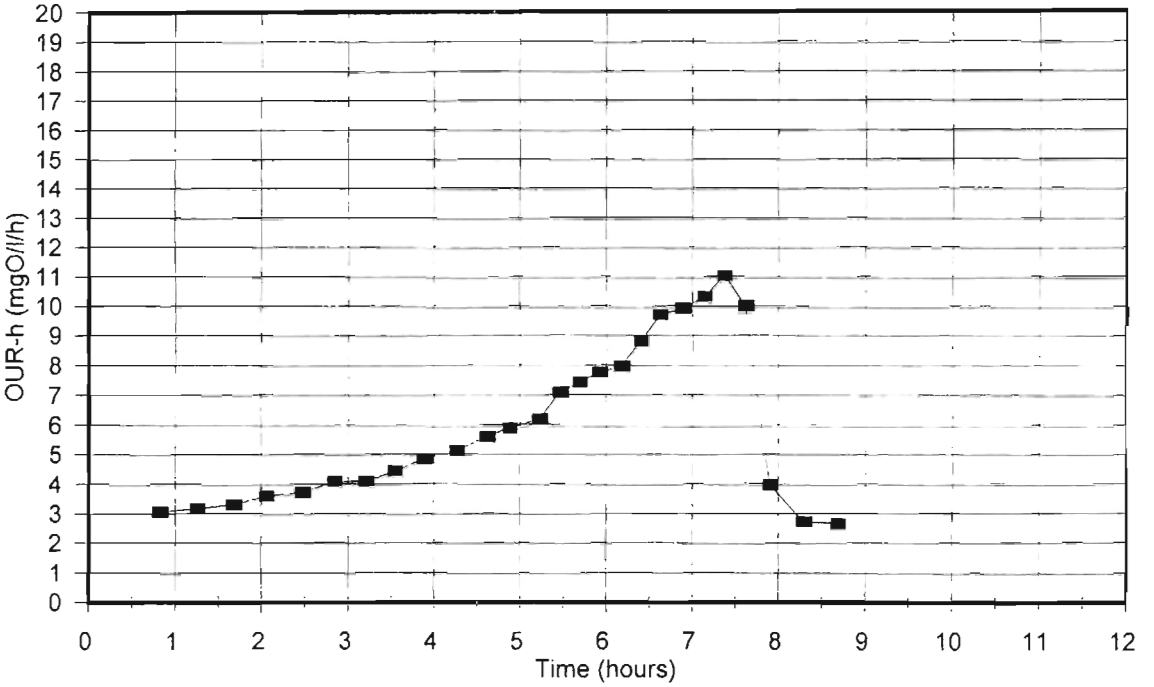
ℓn(OUR-h) graph for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 46, 15-05, Sewage Batch No. 23B



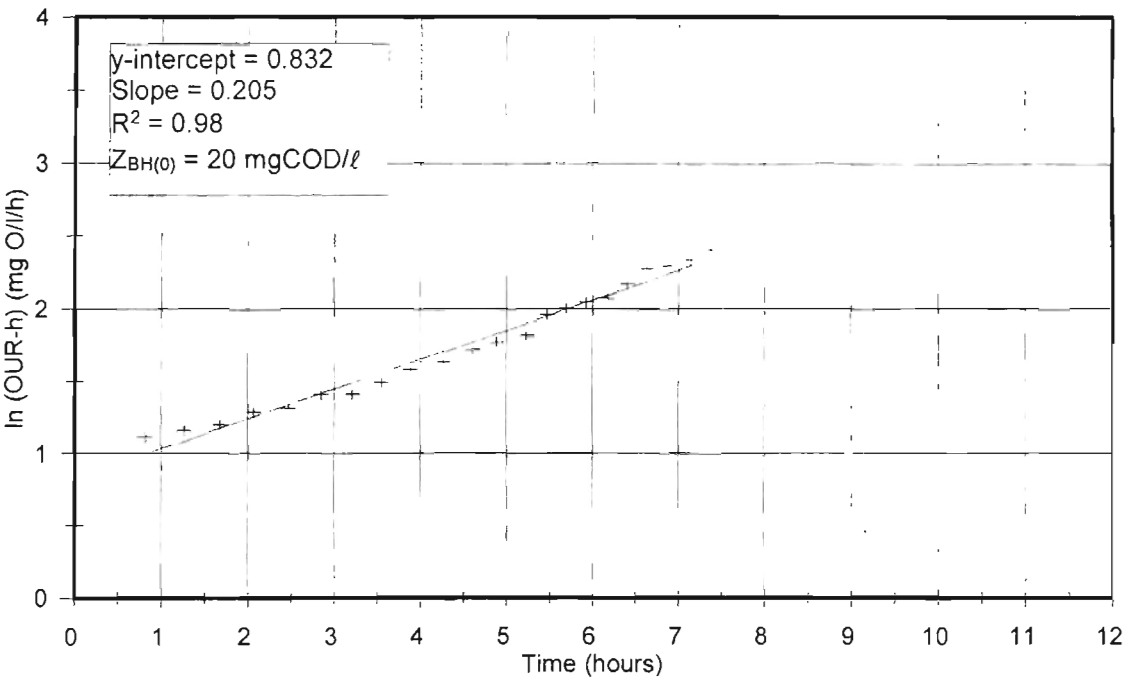
OUR graph for filtered wastewater (2.88ℓ) plus mixed liquor (0.12ℓ)
Batch Test No. 47, 16-05, Sewage Batch No. 23B



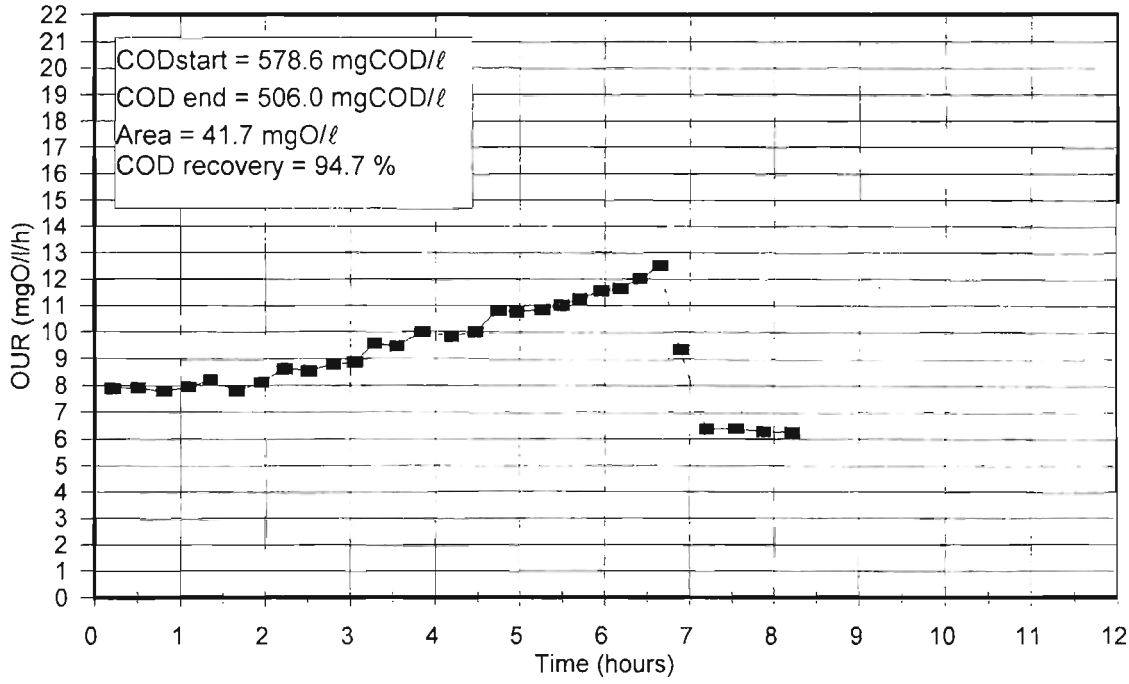
NO_3/NO_2 graph for for filtered wastewater (2.88ℓ) plus mixed liquor (0.12ℓ)
Batch Test No. 47, 16-05, Sewage Batch No. 23B



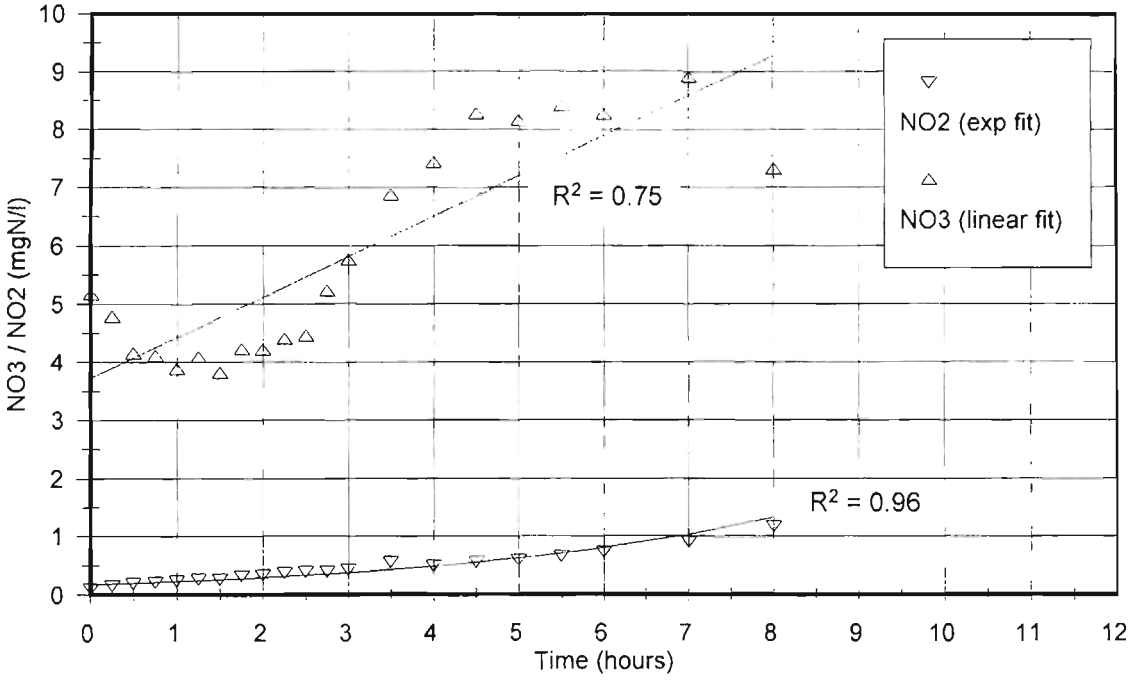
OUR-h graph for filtered wastewater (2.88ℓ) plus mixed liquor (0.12ℓ)
Batch Test No. 47, 16-05, Sewage Batch No. 23B



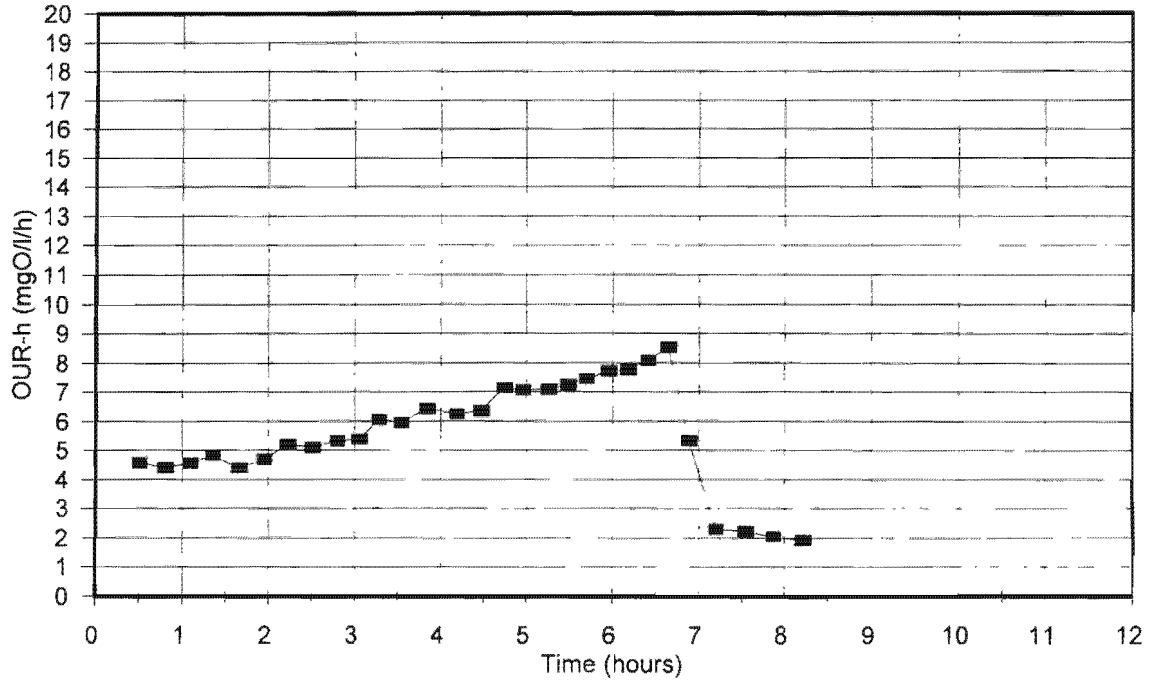
ℓn(OUR-h) graph for filtered wastewater (2.88ℓ) plus mixed liquor (0.12ℓ)
Batch Test No. 47, 16-05, Sewage Batch No. 23B



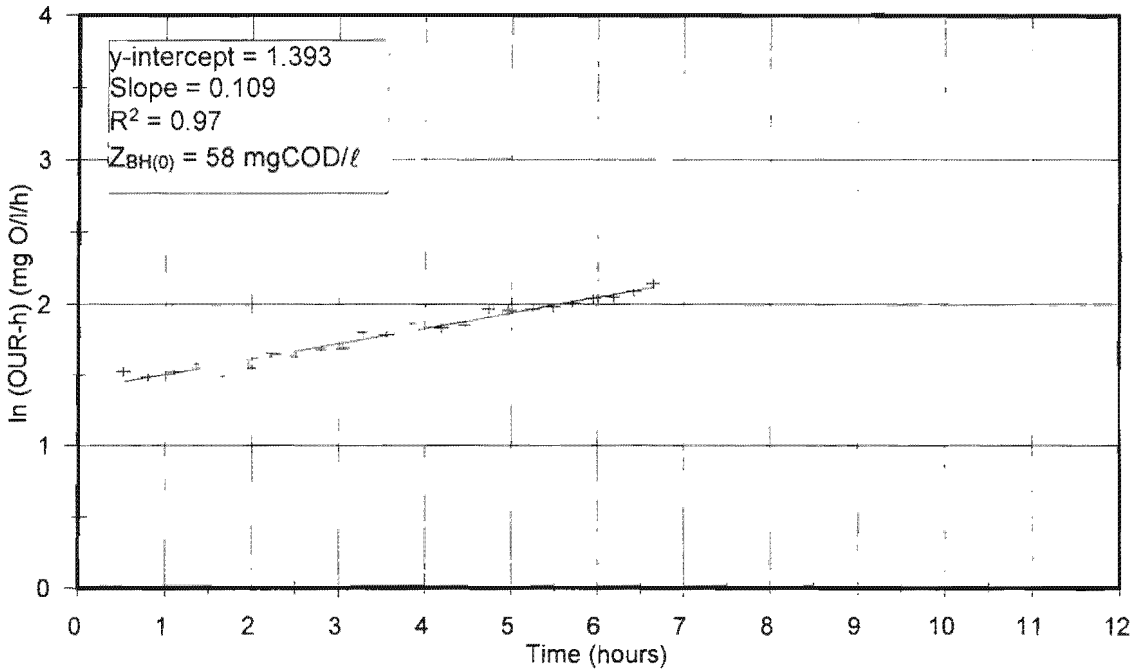
OUR graph for filtered wastewater (2.72ℓ) plus mixed liquor (0.28ℓ)
Batch Test No. 48, 16-05, Sewage Batch No. 23B



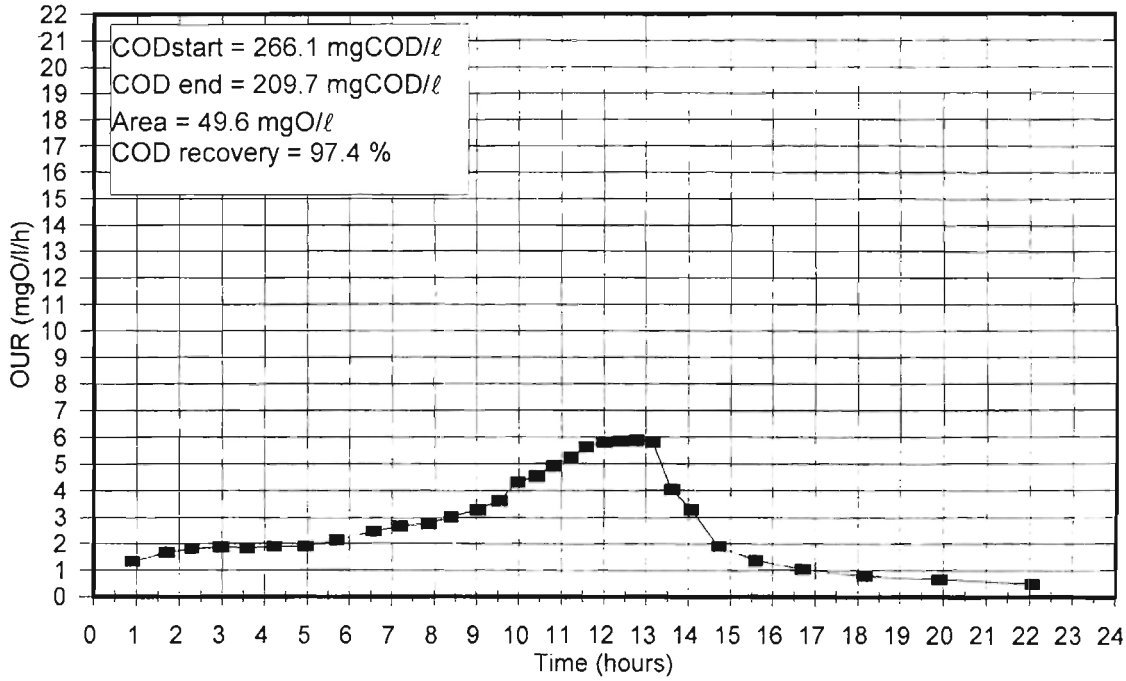
NO_3/NO_2 graph for for filtered wastewater (2.72ℓ) plus mixed liquor (0.28ℓ)
Batch Test No. 48, 16-05, Sewage Batch No. 23B



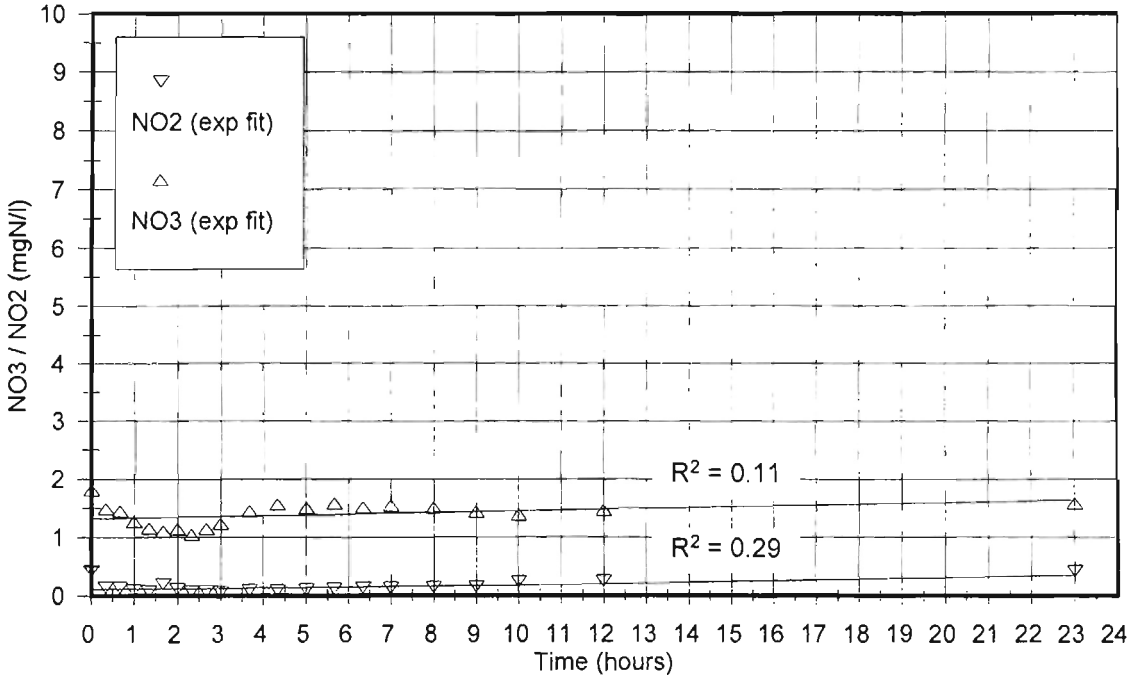
OUR-h graph for filtered wastewater (2.72ℓ) plus mixed liquor (0.28ℓ)
Batch Test No. 48, 16-05, Sewage Batch No. 23B



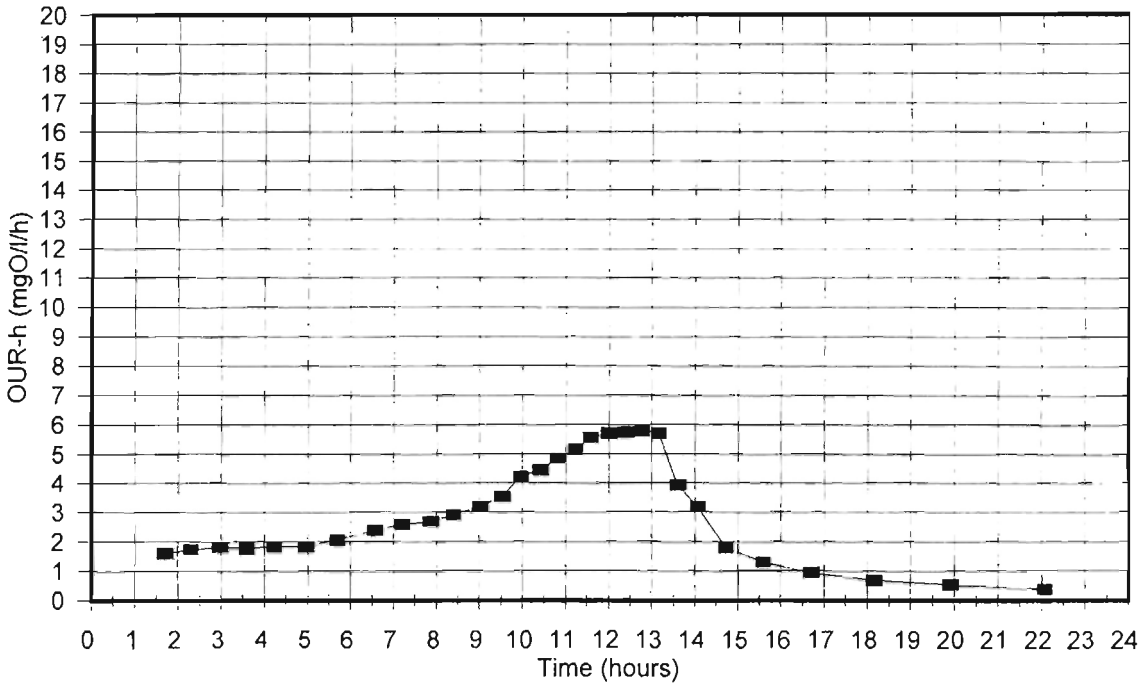
ℓn(OUR-h) graph for filtered wastewater (2.72ℓ) plus mixed liquor (0.28ℓ)
Batch Test No. 48, 16-05, Sewage Batch No. 23B



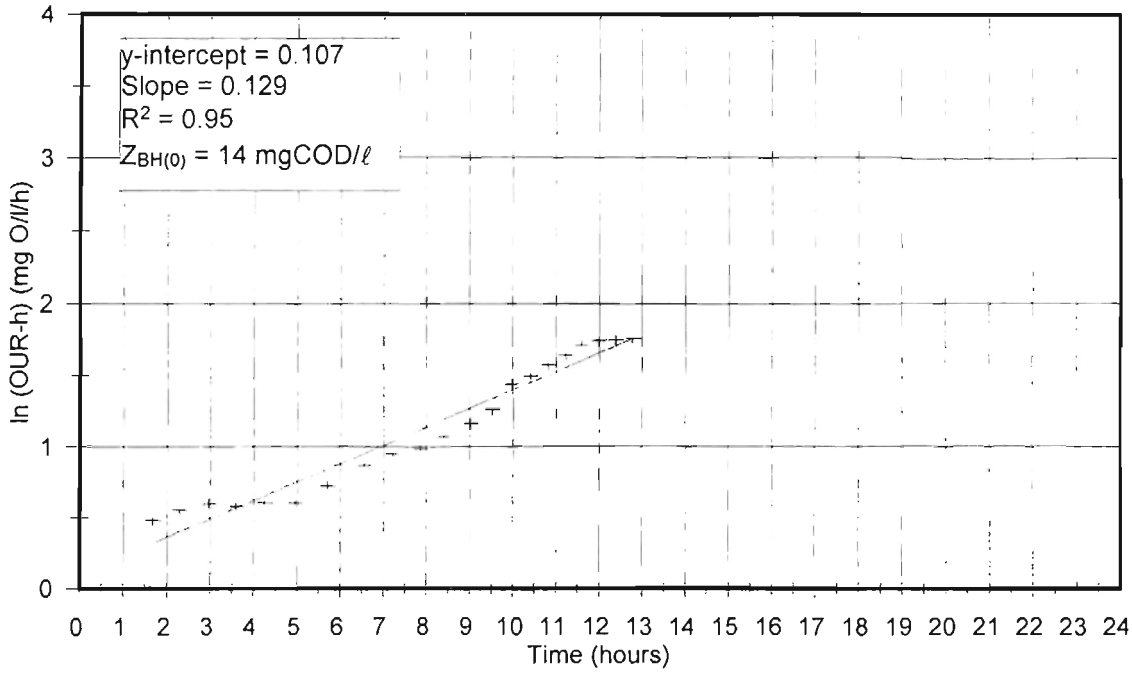
OUR graph for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 49, 21-05, Sewage Batch No. 24



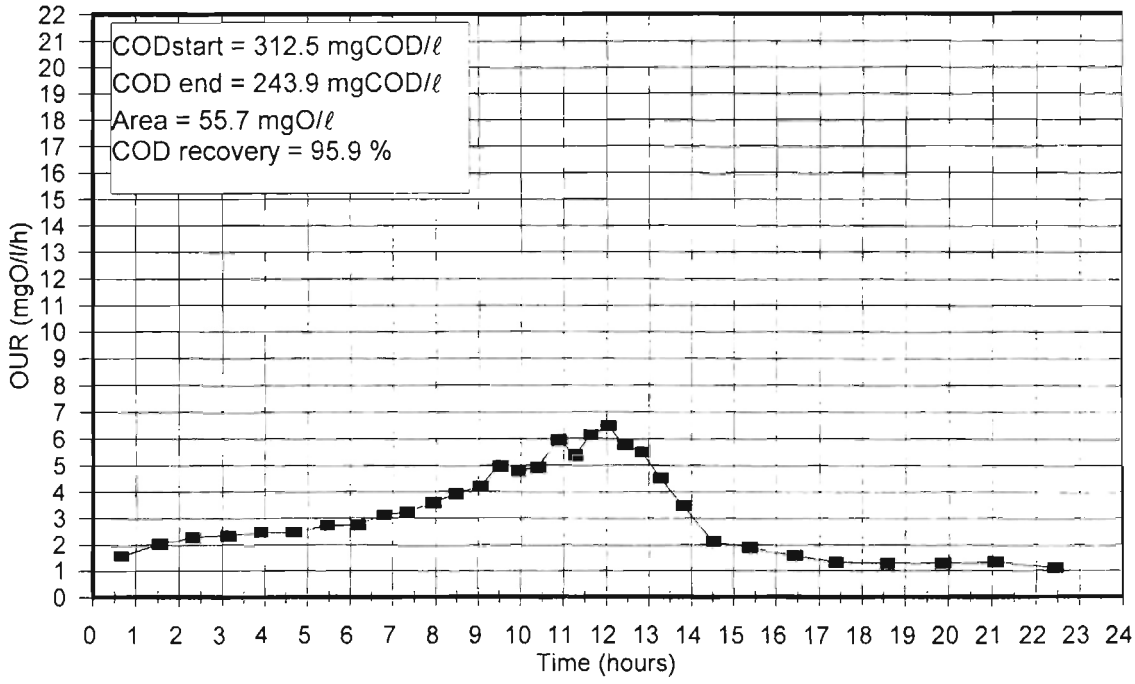
NO_3/NO_2 graph for for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 49, 21-05, Sewage Batch No. 24



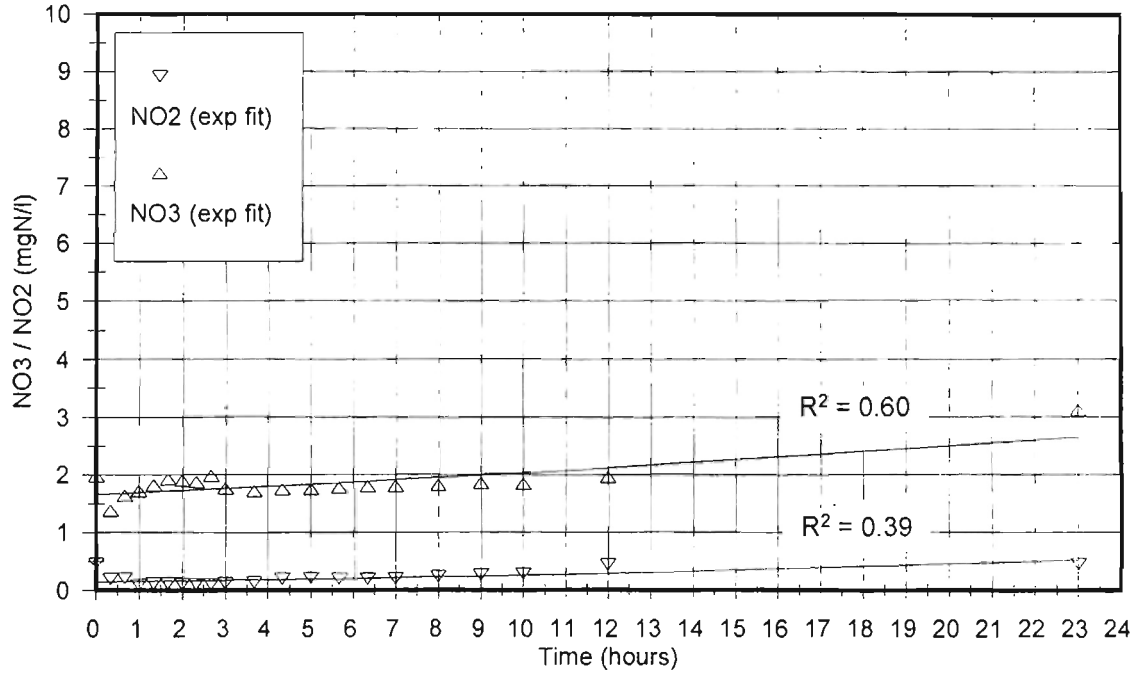
OUR-h graph for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 49, 21-05, Sewage Batch No. 24



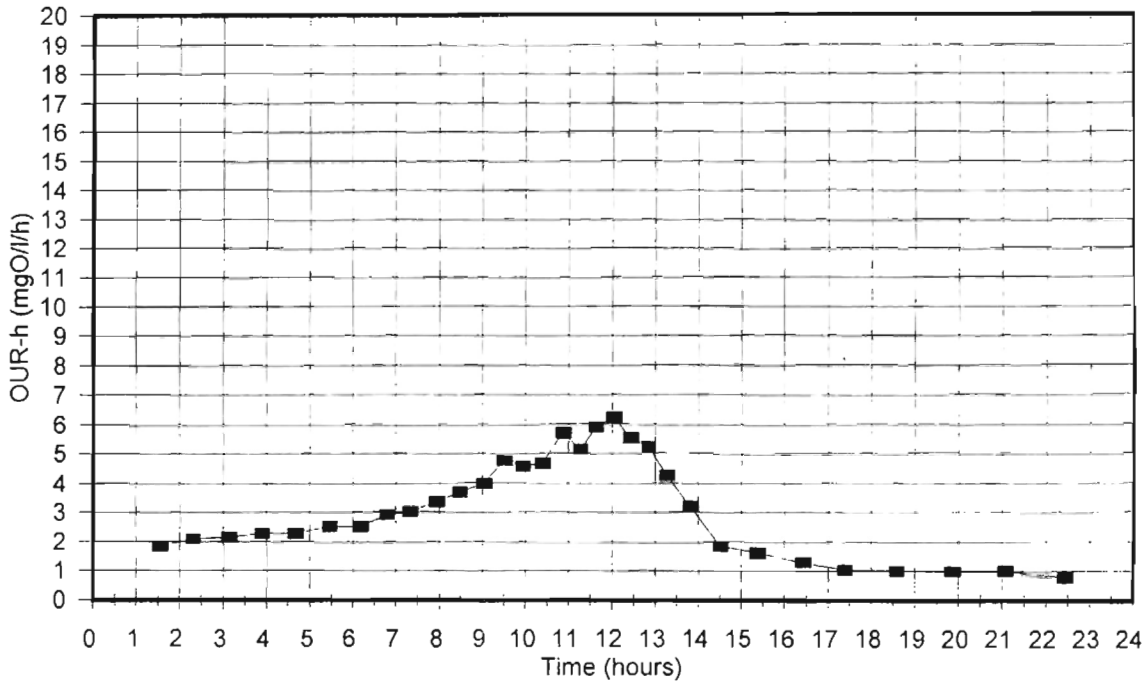
ℓn(OUR-h) graph for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 49, 21-05, Sewage Batch No. 24



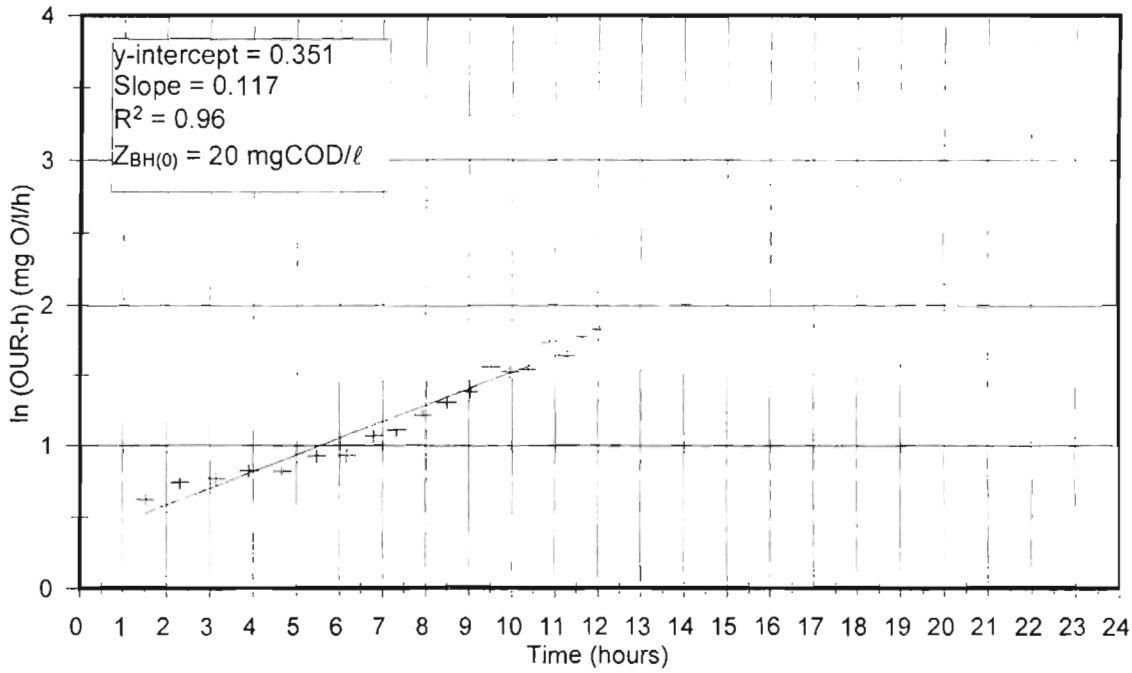
OUR graph for filtered wastewater (2.88ℓ) plus mixed liquor (0.12ℓ)
Batch Test No. 50, 21-05, Sewage Batch No. 24



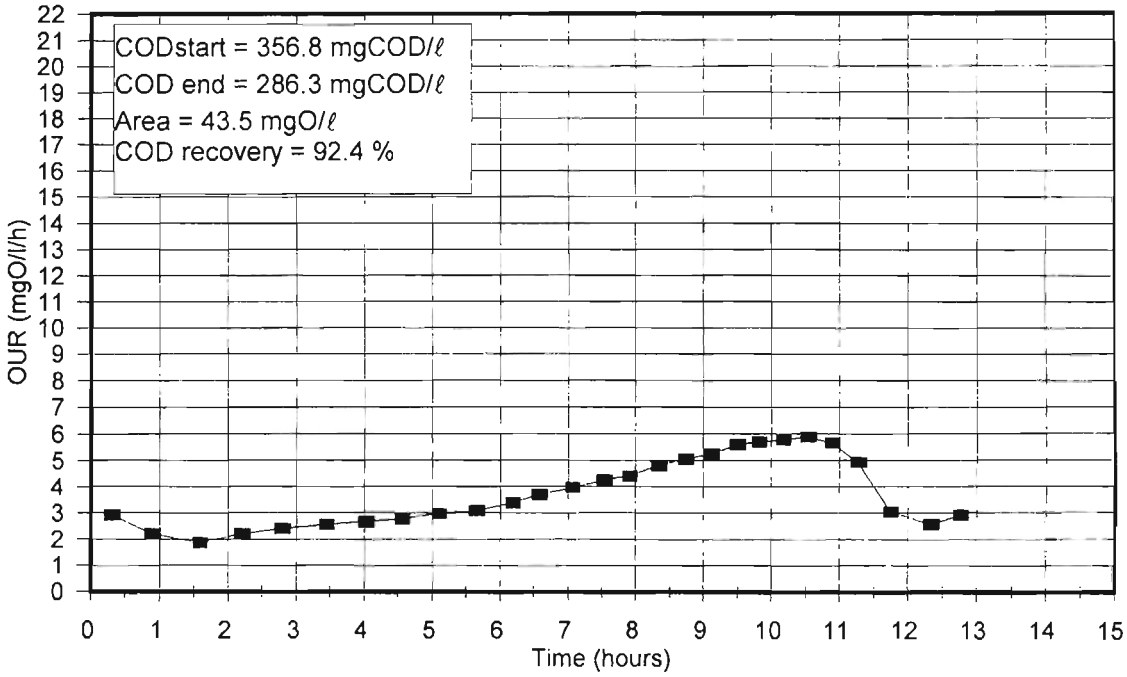
NO_3/NO_2 graph for for filtered wastewater (2.88ℓ) plus mixed liquor (0.12ℓ)
Batch Test No. 50, 21-05, Sewage Batch No. 24



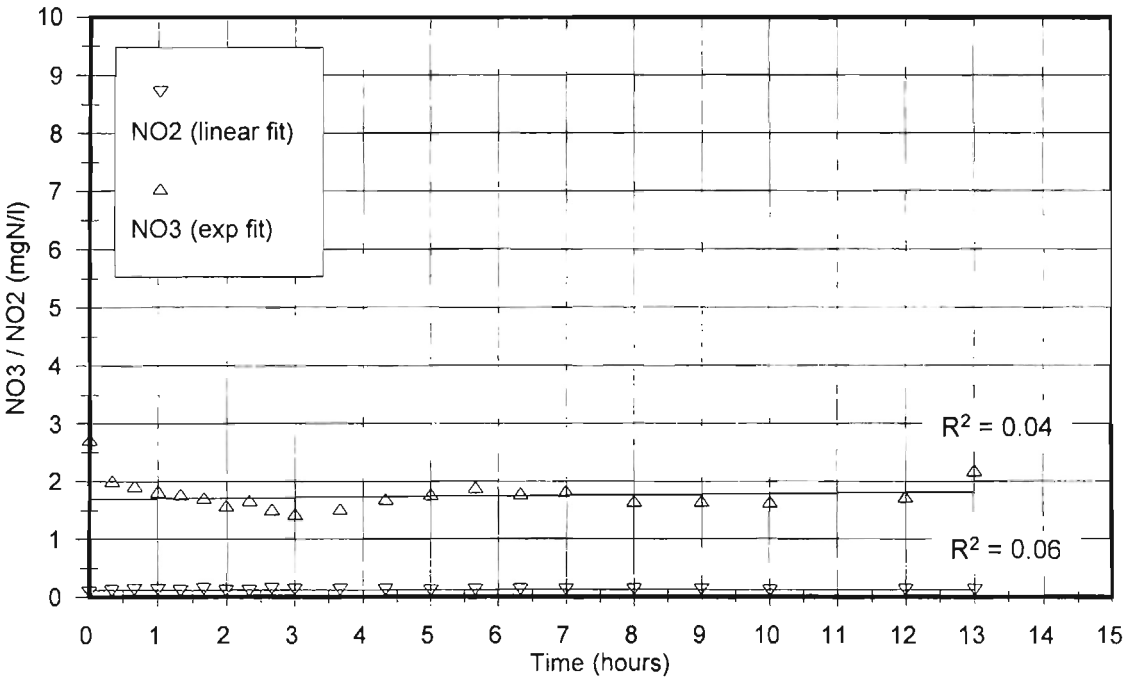
OUR-h graph for filtered wastewater (2.88ℓ) plus mixed liquor (0.12ℓ)
Batch Test No. 50, 21-05, Sewage Batch No. 24



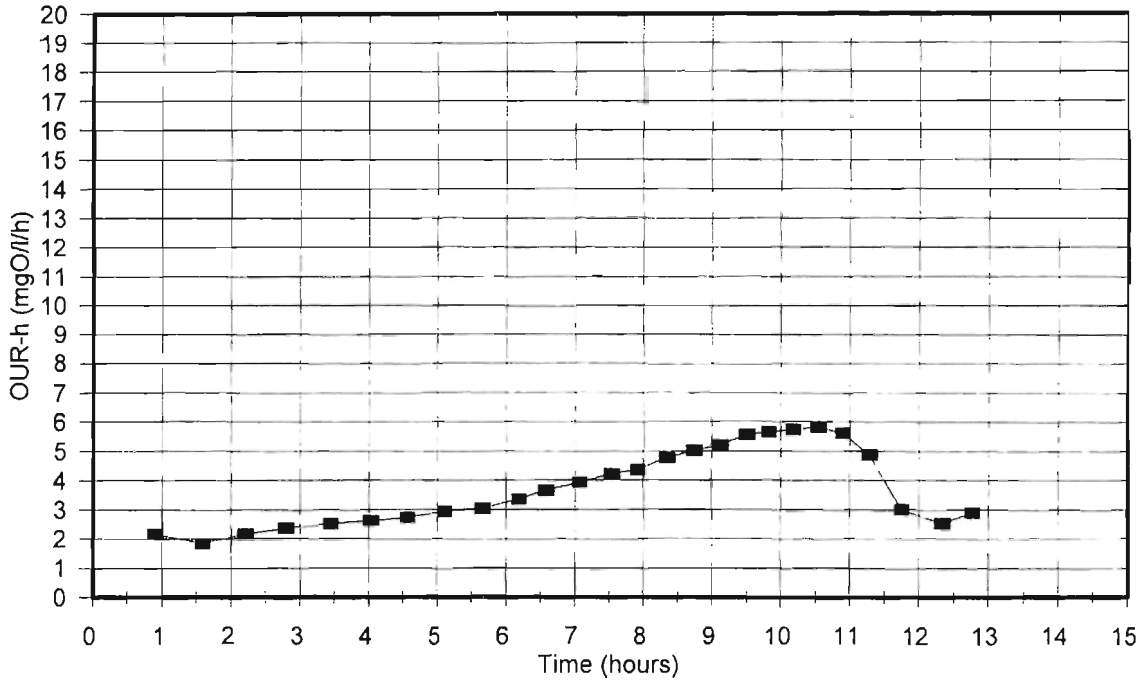
ℓn(OUR-h) graph for filtered wastewater (2.88ℓ) plus mixed liquor (0.12ℓ)
Batch Test No. 50, 21-05, Sewage Batch No. 24



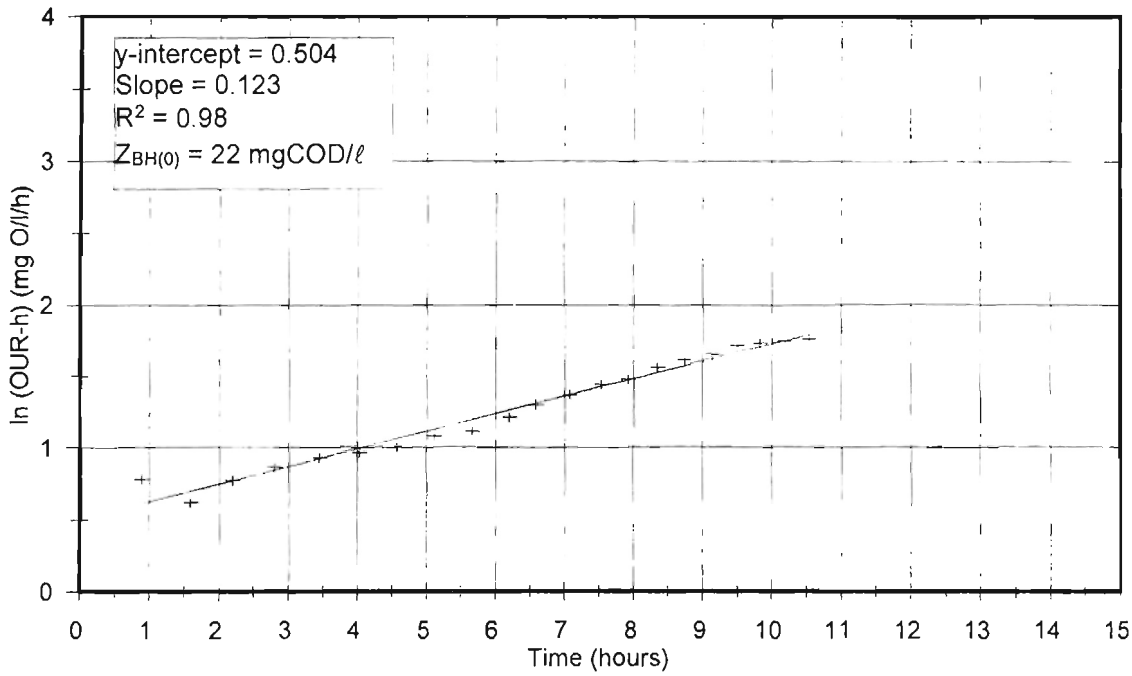
OUR graph for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 51, 22-05, Sewage Batch No. 24



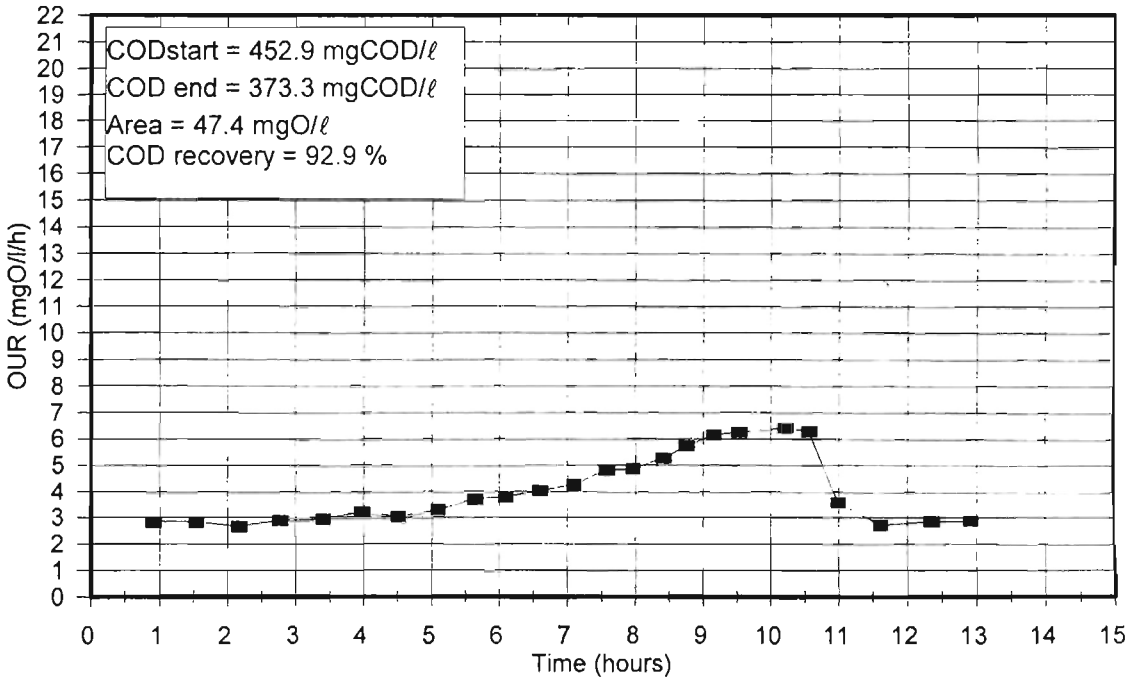
NO_3/NO_2 graph for for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 51, 22-05, Sewage Batch No. 24



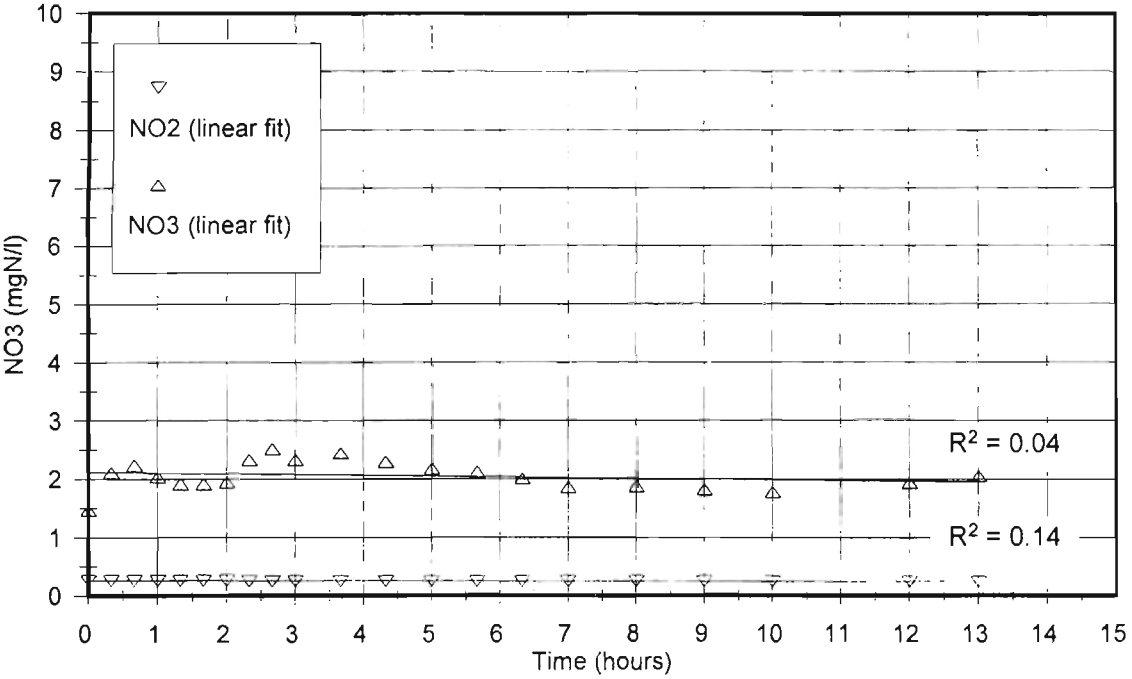
OUR-h graph for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 51, 22-05, Sewage Batch No. 24



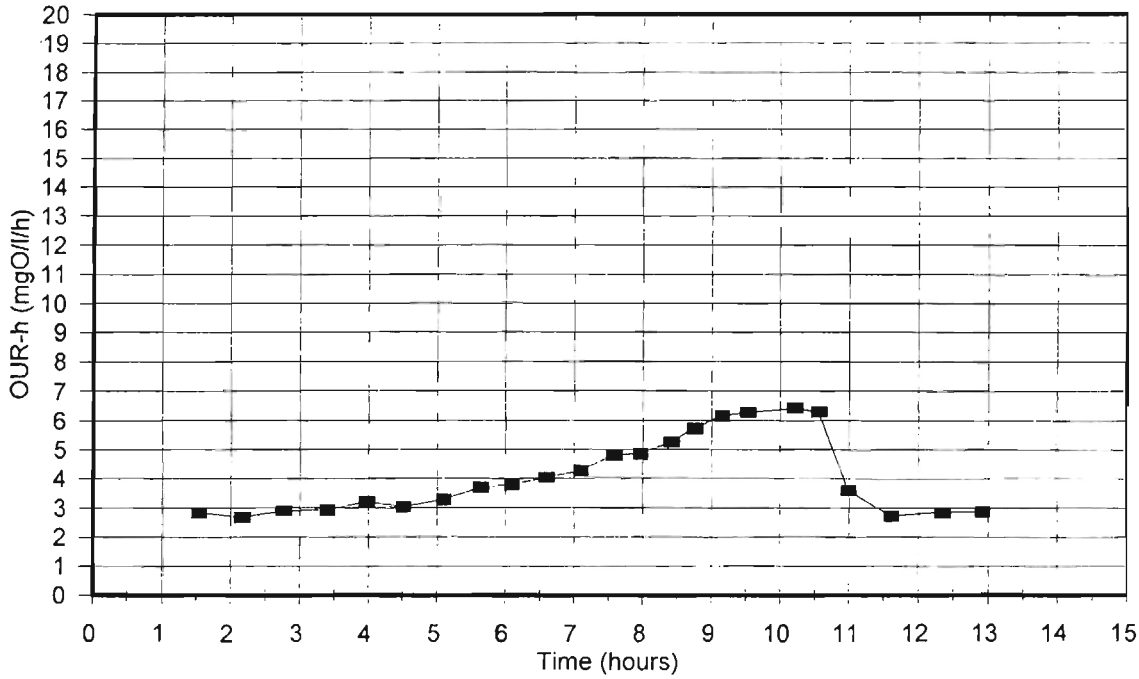
ℓn(OUR-h) graph for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 51, 22-05, Sewage Batch No. 24



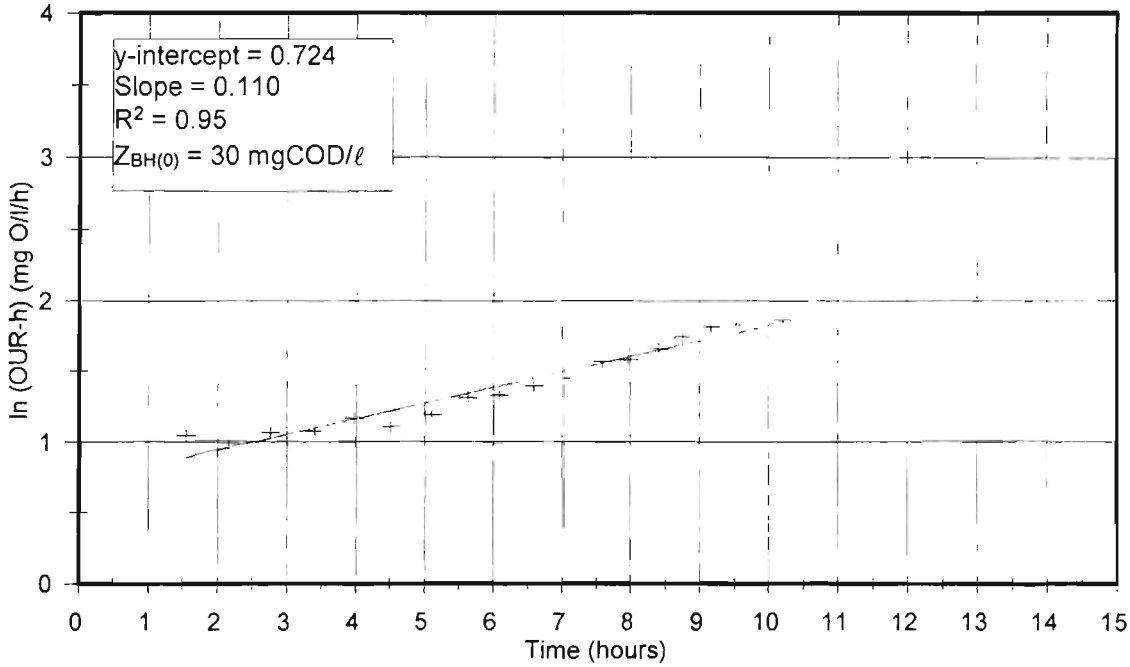
OUR graph for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 52, 22-05, Sewage Batch No. 24



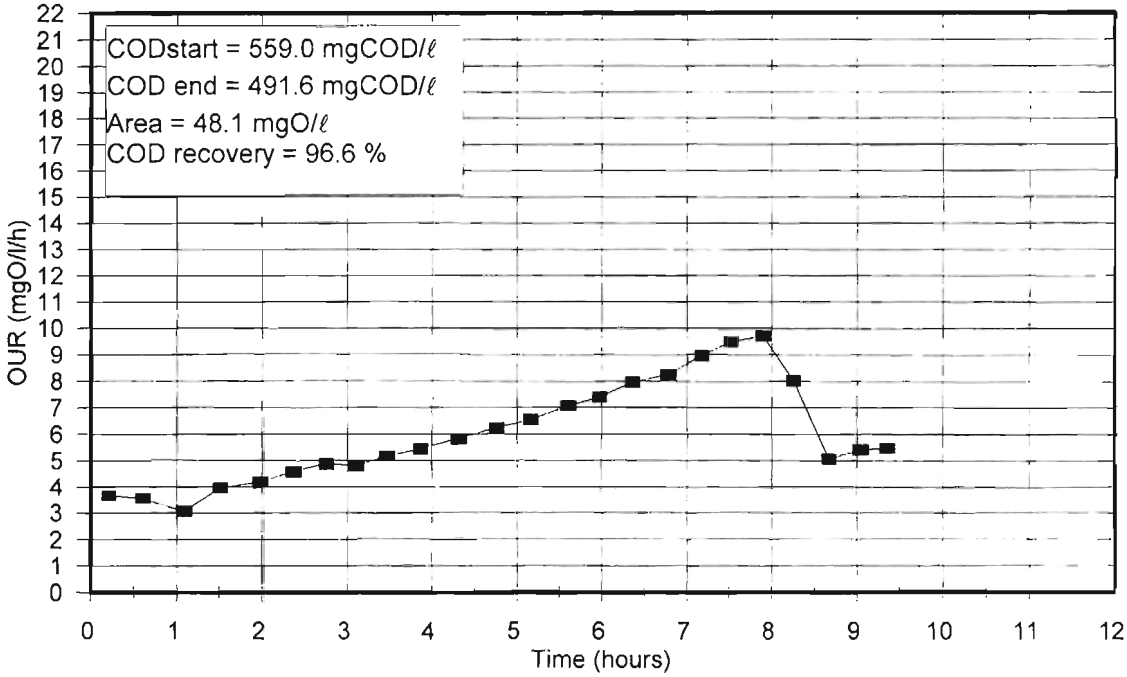
NO_3/NO_2 graph for for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 52, 22-05, Sewage Batch No. 24



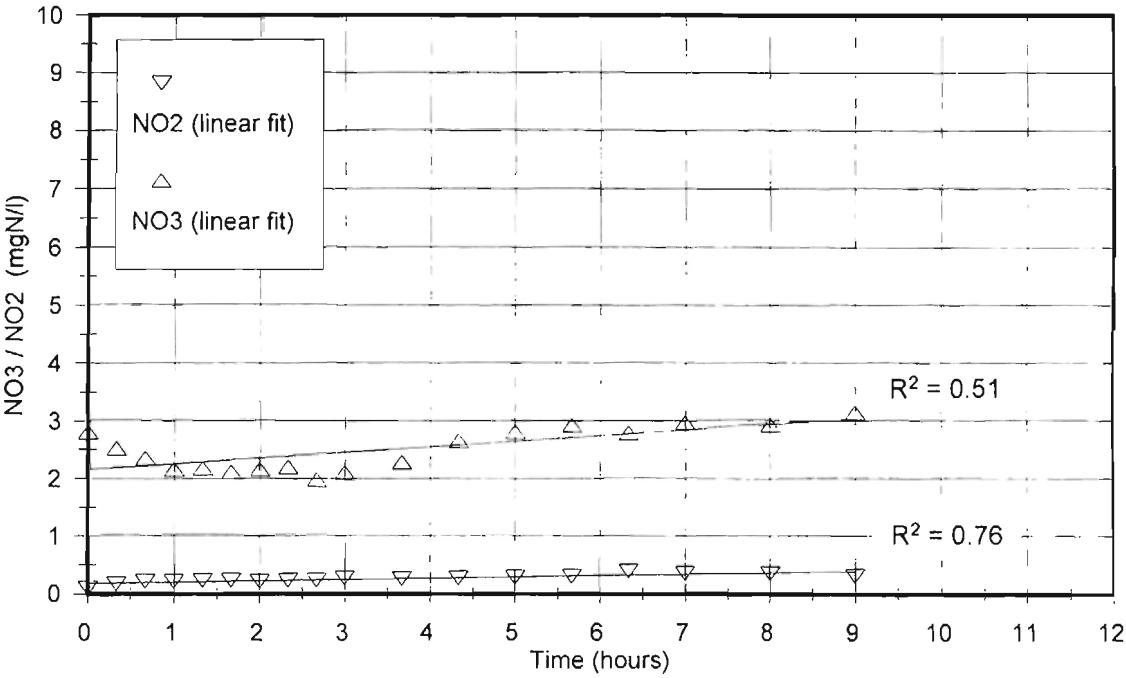
OUR-h graph for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 52, 22-05, Sewage Batch No. 24



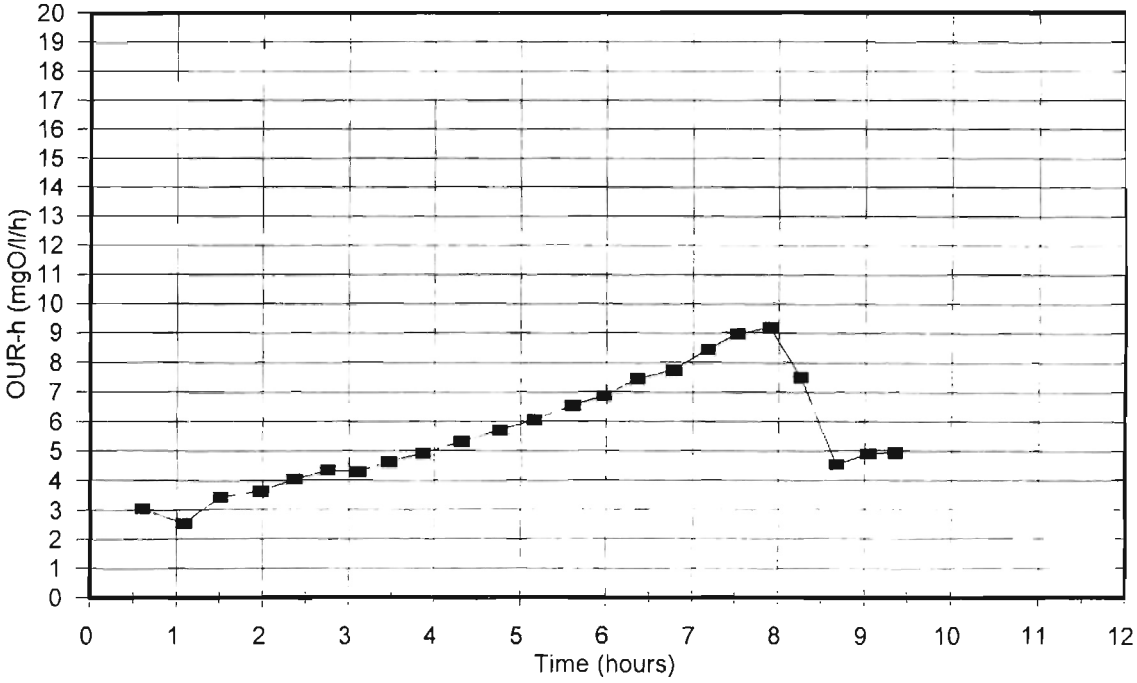
ℓn(OUR-h) graph for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 52, 22-05, Sewage Batch No. 24



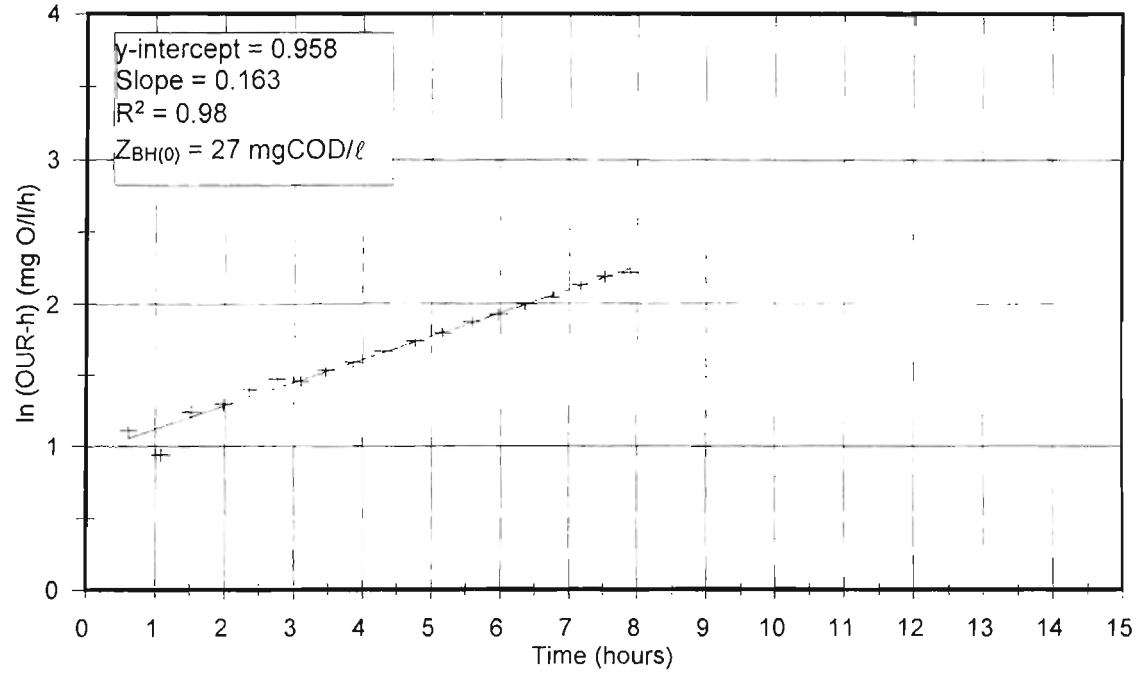
OUR graph for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 53, 23-05, Sewage Batch No. 24



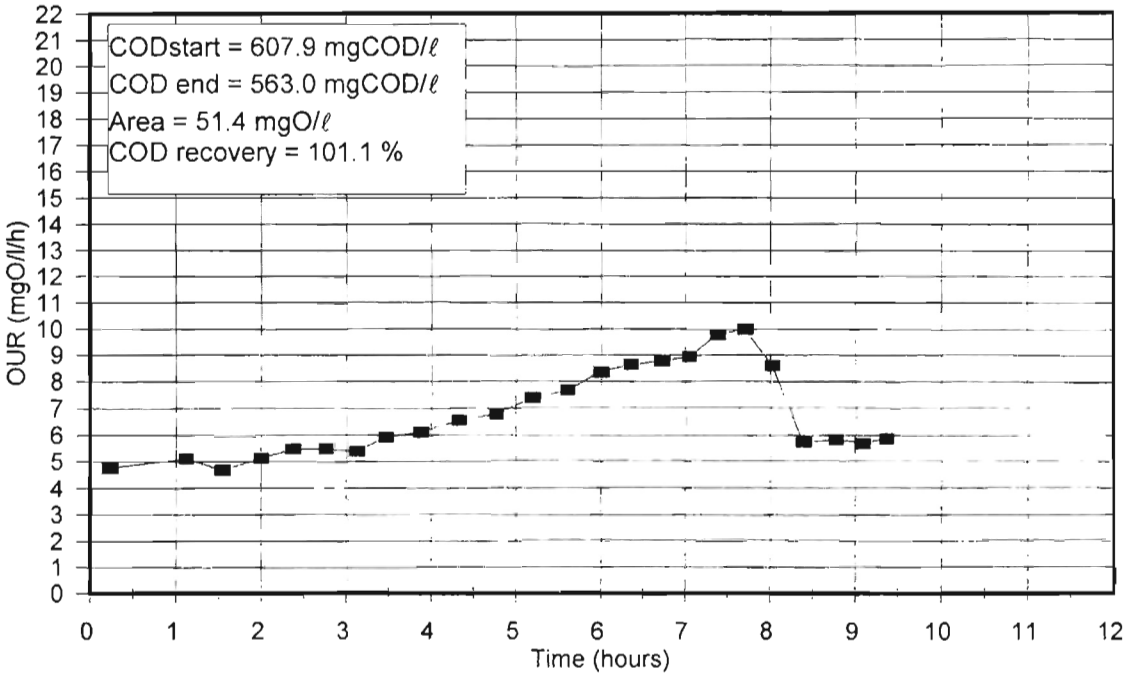
NO_3/NO_2 graph for for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 53, 23-05, Sewage Batch No. 24



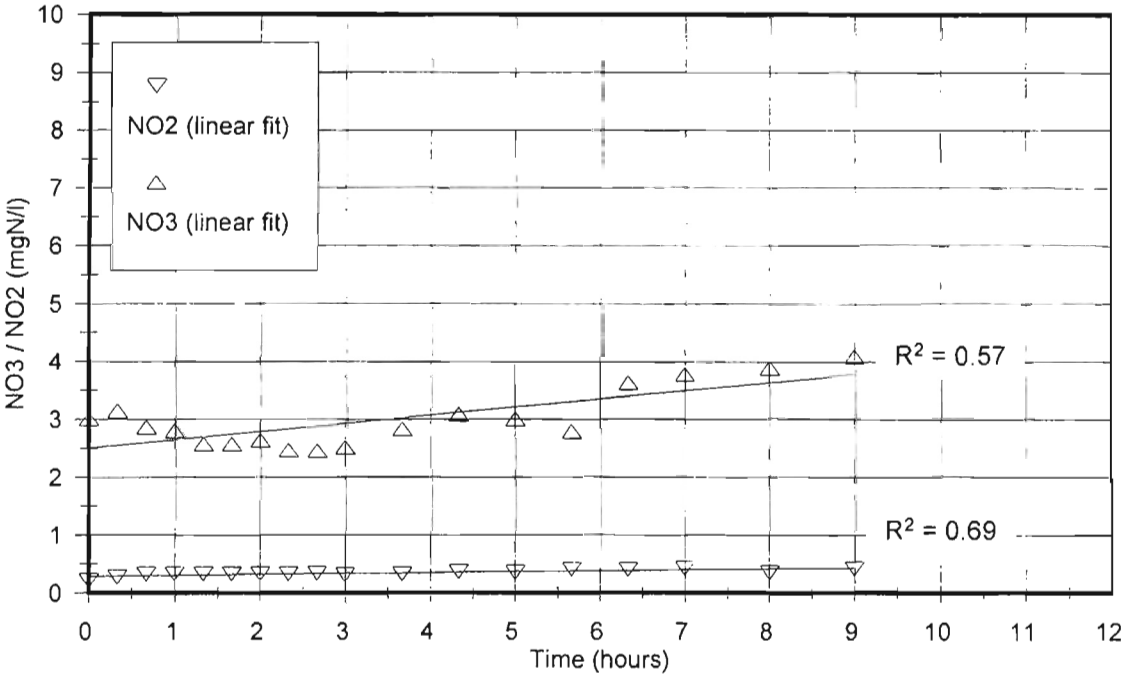
OUR-h graph for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 53, 23-05, Sewage Batch No. 24



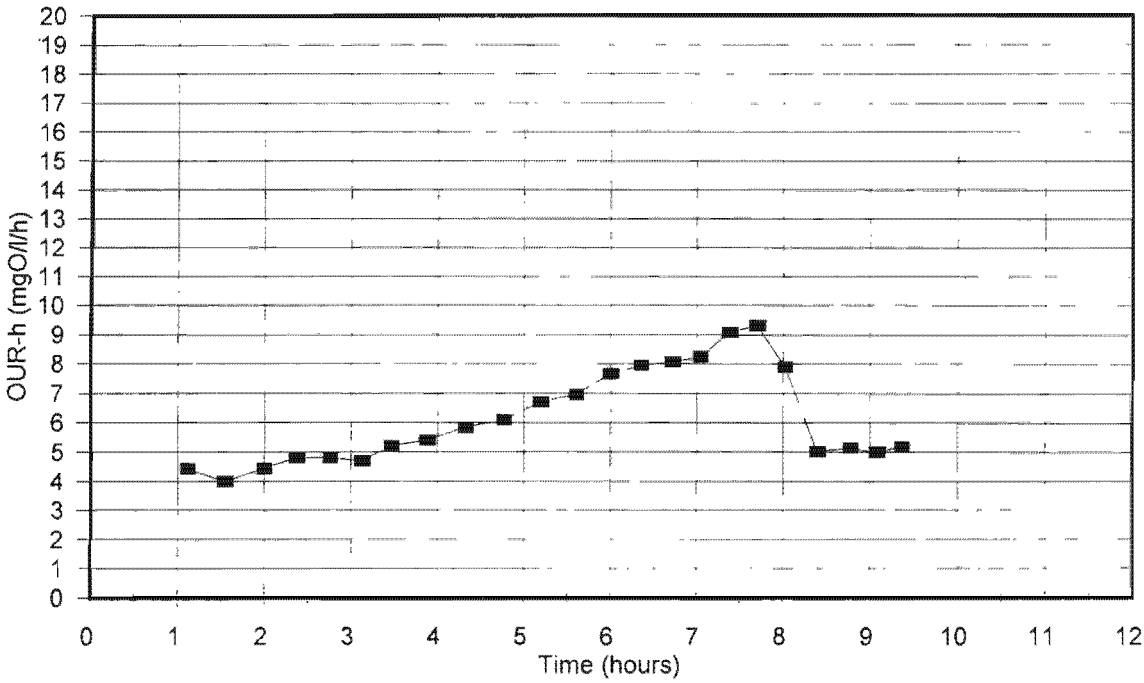
ℓn(OUR-h) graph for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 53, 23-05, Sewage Batch No. 24



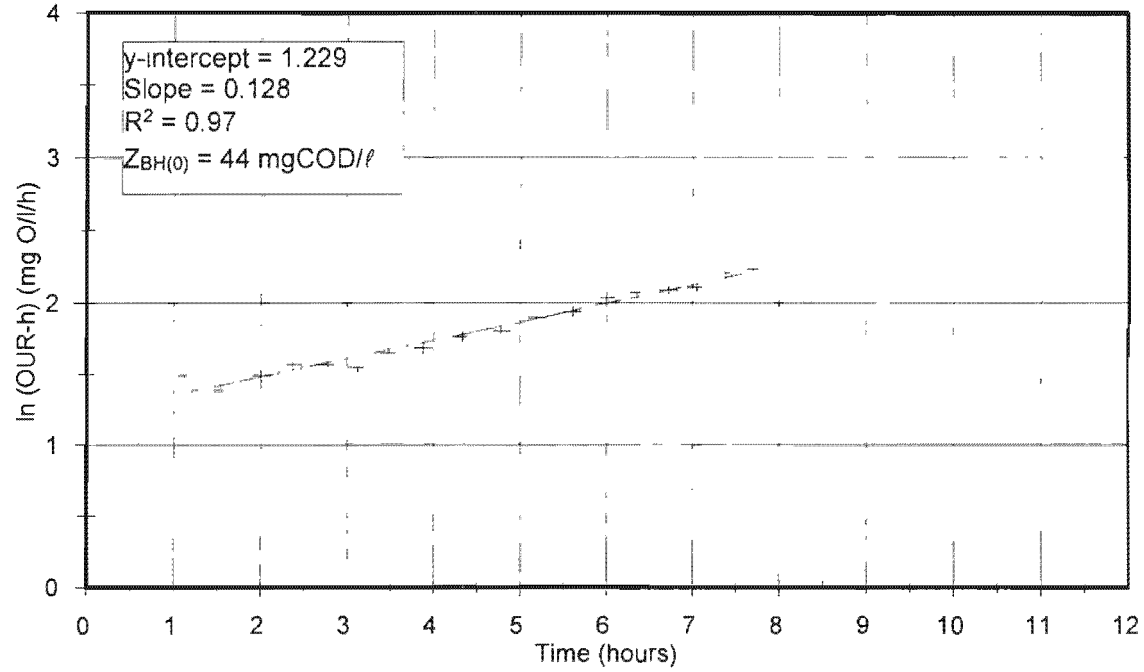
OUR graph for filtered wastewater (2.72ℓ) plus mixed liquor (0.28ℓ)
Batch Test No. 54, 23-05, Sewage Batch No. 24



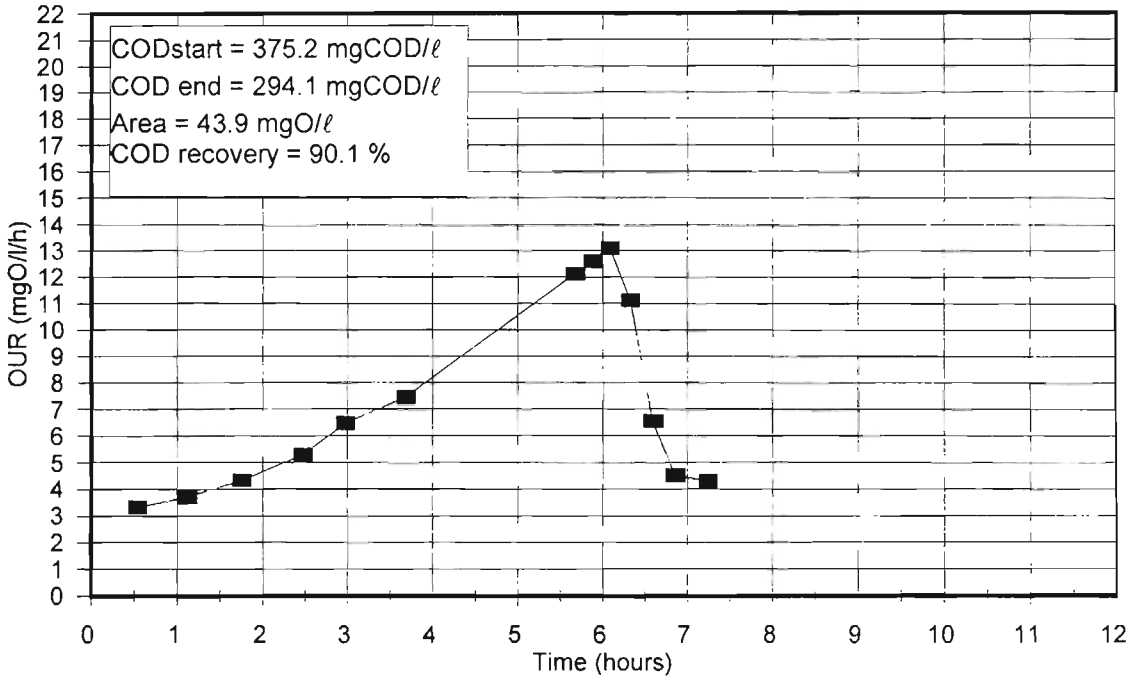
NO_3/NO_2 graph for for filtered wastewater (2.72ℓ) plus mixed liquor (0.28ℓ)
Batch Test No. 54, 23-05, Sewage Batch No. 24



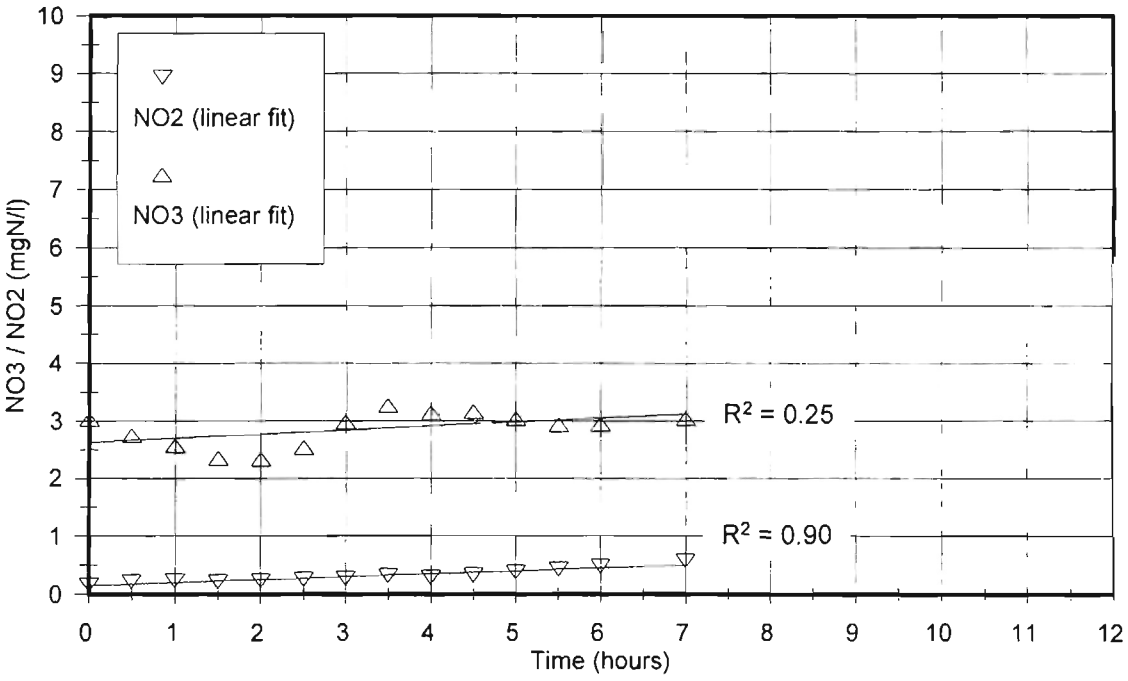
OUR-h graph for filtered wastewater (2.72ℓ) plus mixed liquor (0.28ℓ)
Batch Test No. 54, 23-05, Sewage Batch No. 24



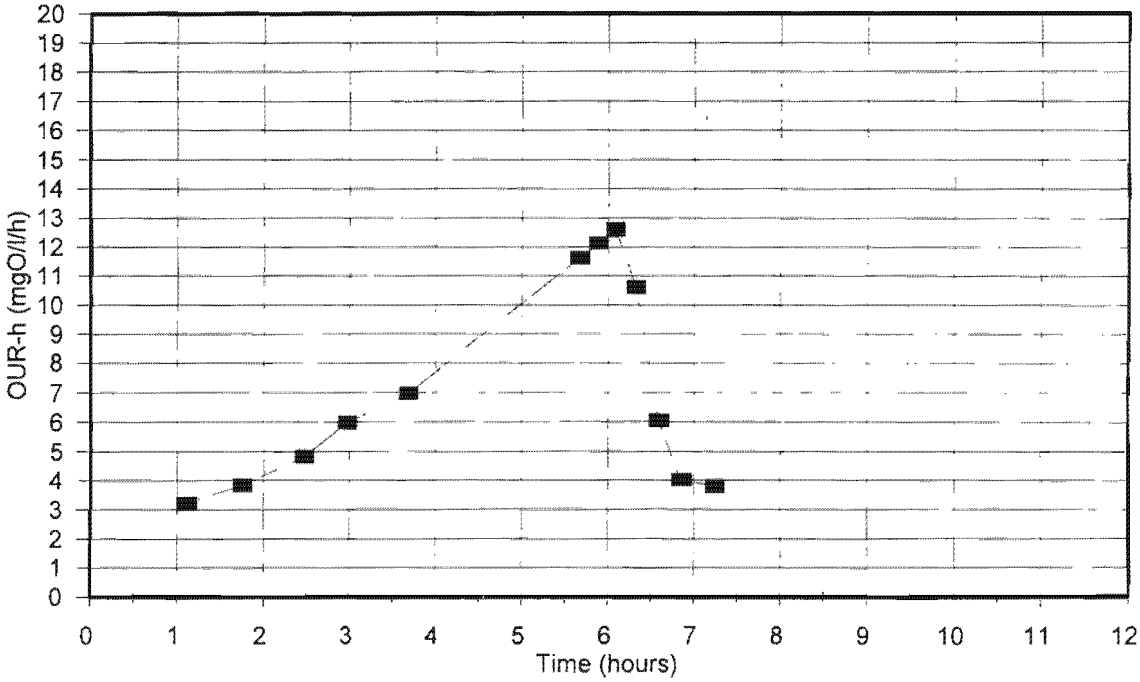
ℓn(OUR-h) graph for filtered wastewater (2.72ℓ) plus mixed liquor (0.28ℓ)
Batch Test No. 54, 23-05, Sewage Batch No. 24



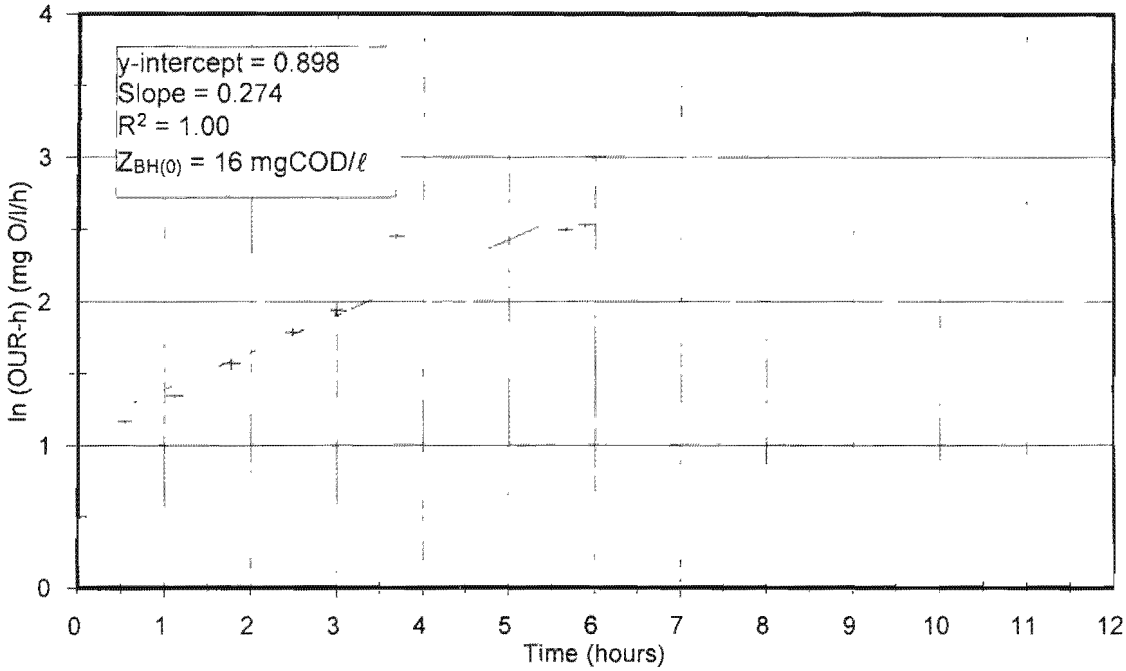
OUR graph for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 55, 06-06, Sewage Batch No. 25



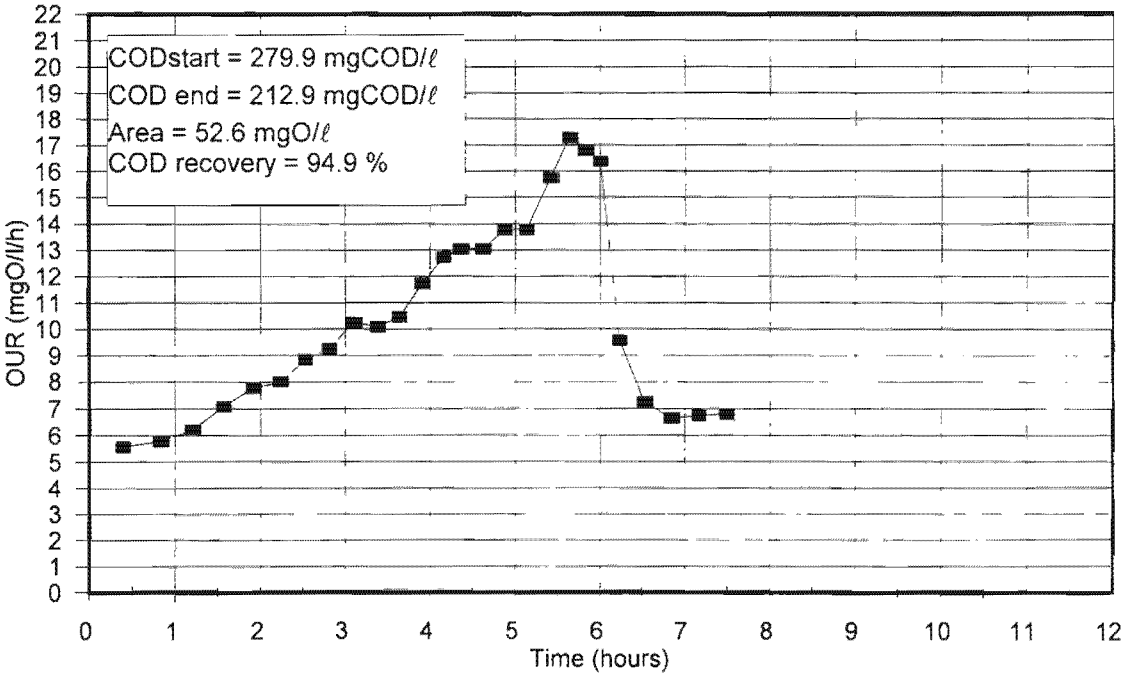
NO_3/NO_2 graph for for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 55, 06-06, Sewage Batch No. 25



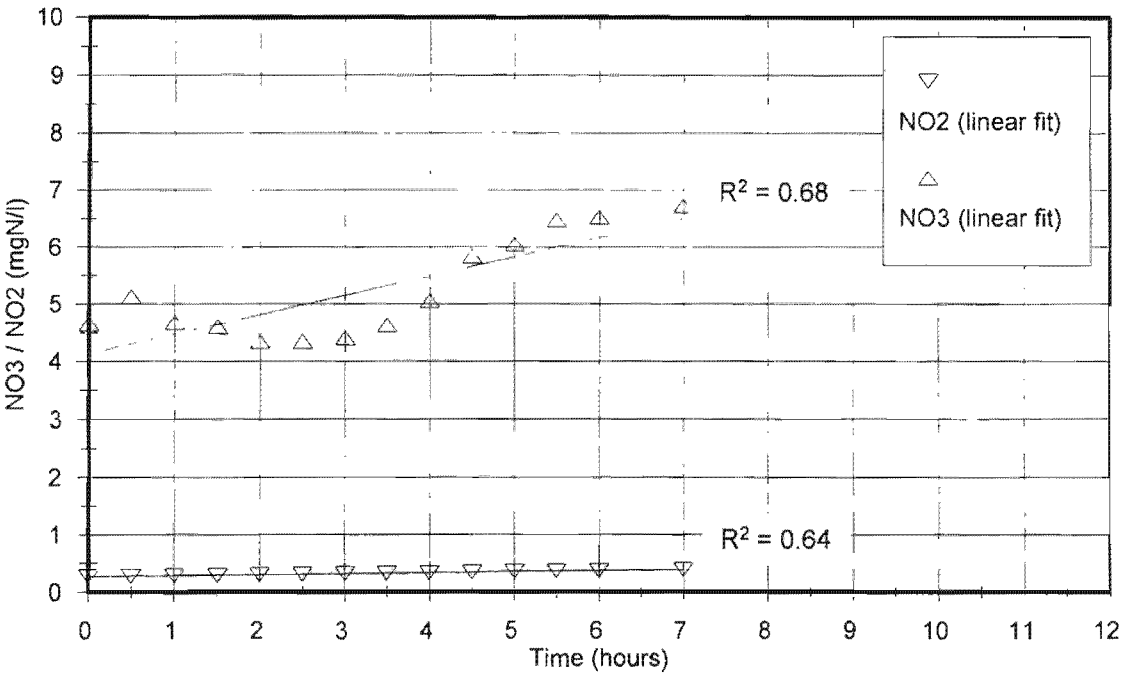
OUR-h graph for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 55, 06-06, Sewage Batch No. 25



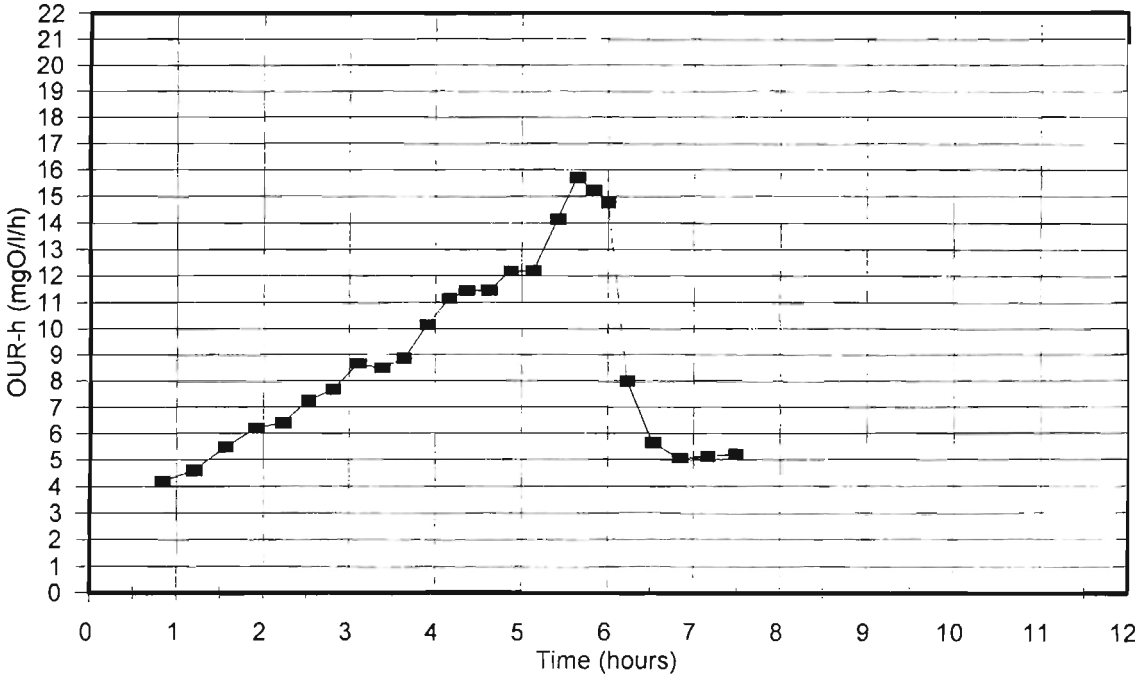
ℓn(OUR-h) graph for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 55, 06-06, Sewage Batch No. 25



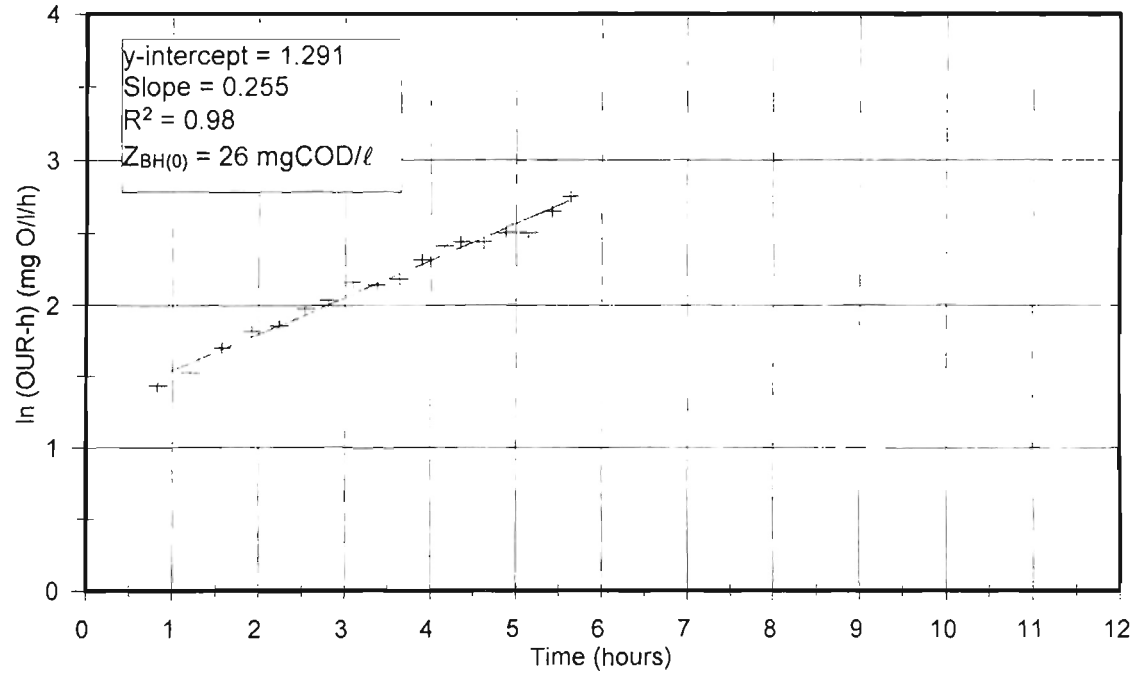
OUR graph for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 56, 06-06, Sewage Batch No. 25



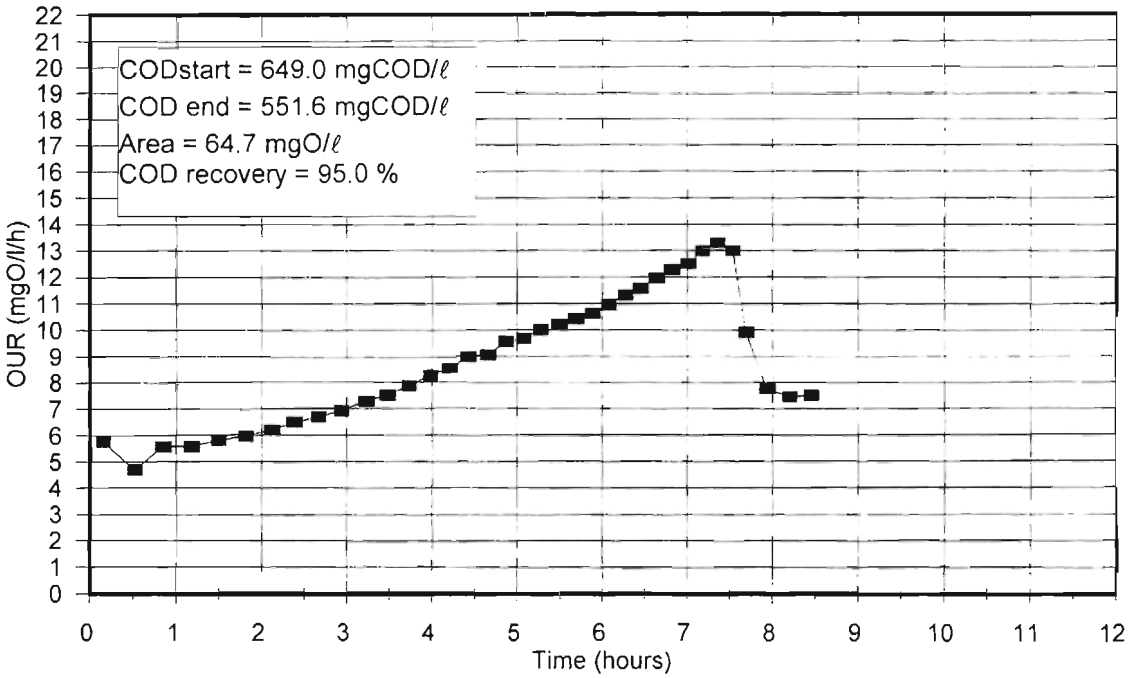
NO_3/NO_2 graph for for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 56, 06-06, Sewage Batch No. 25



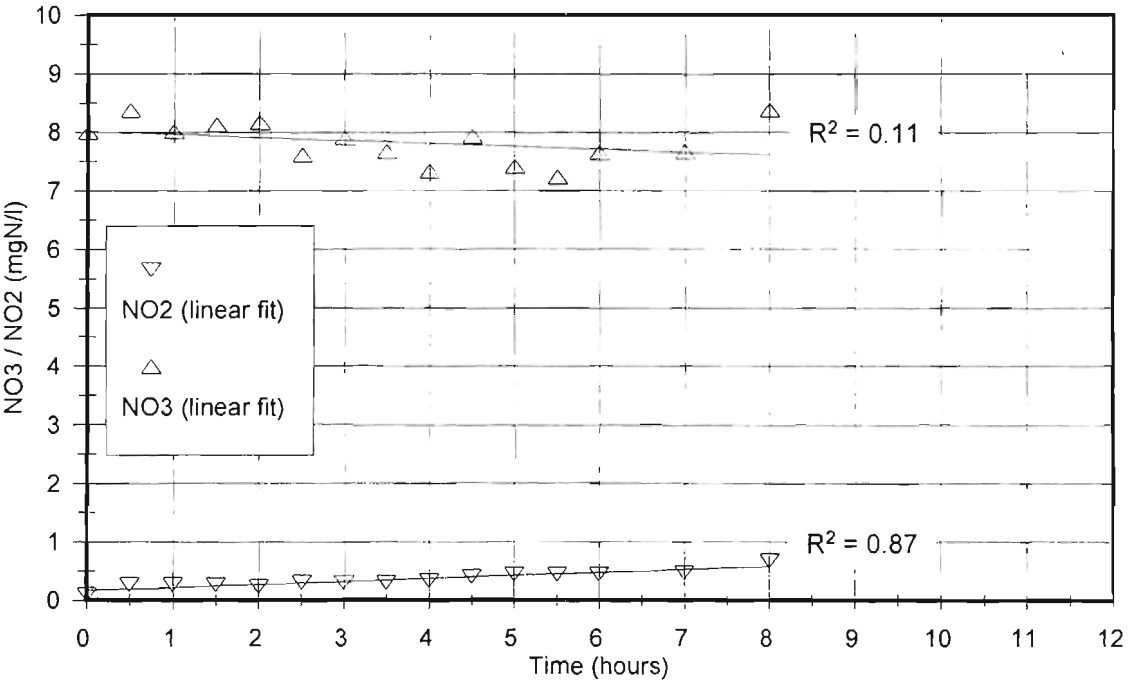
OUR-h graph for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 56, 06-06, Sewage Batch No. 25



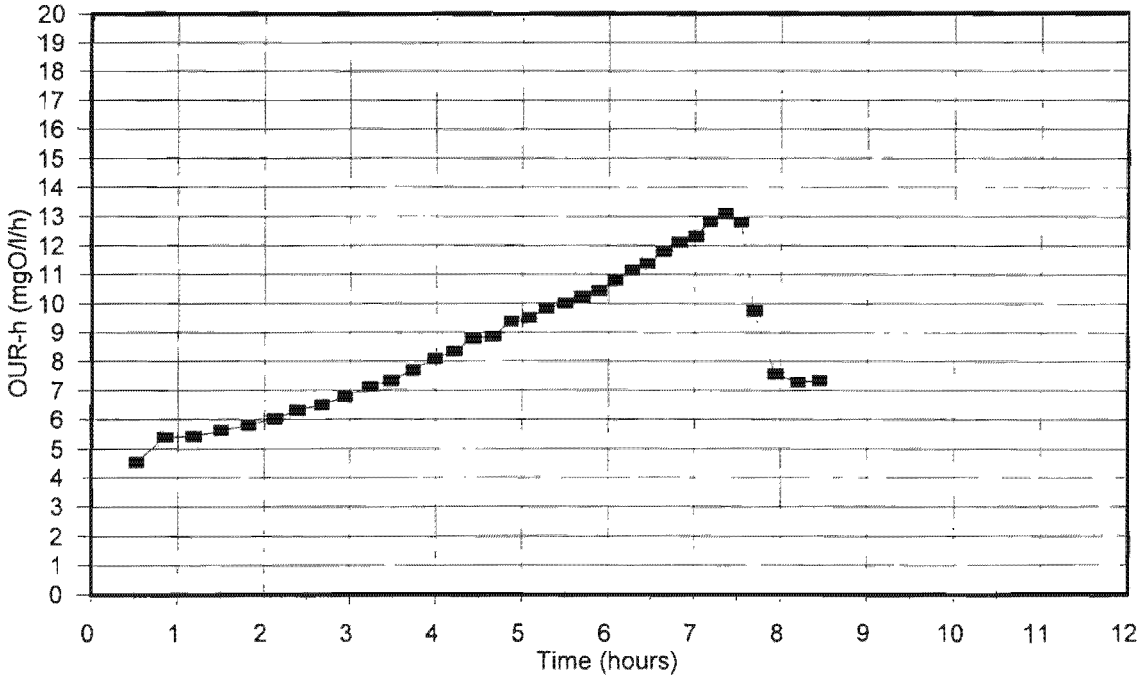
ℓn(OUR-h) graph for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 56, 06-06, Sewage Batch No. 25



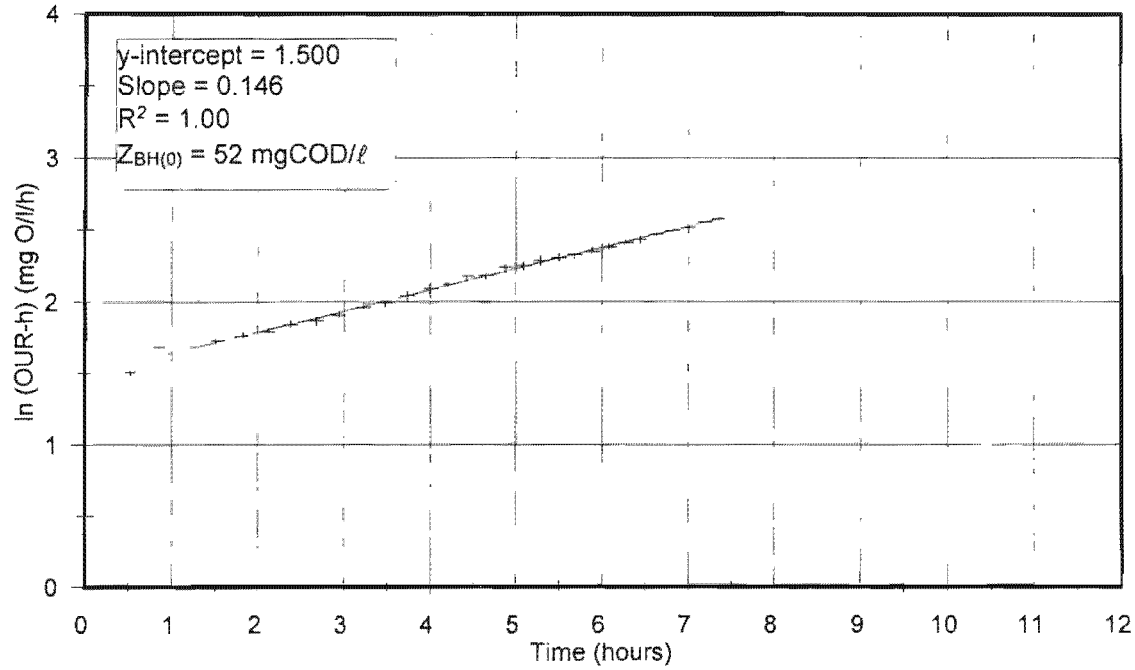
OUR graph for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 57, 07-06, Sewage Batch No. 25



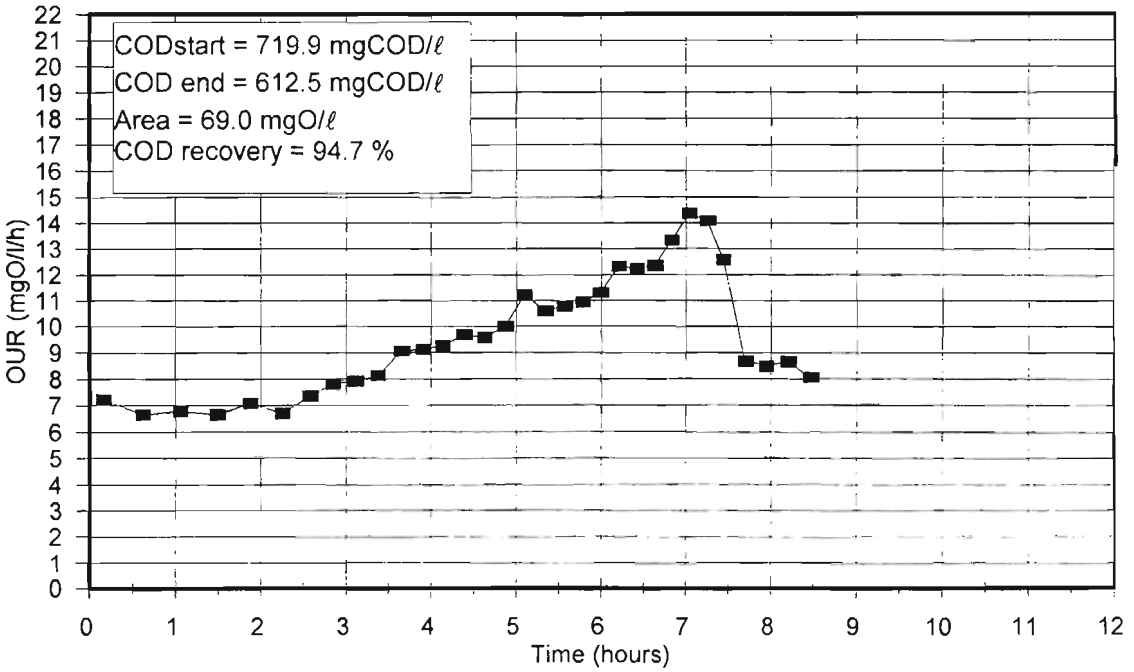
NO_3/NO_2 graph for for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 57, 07-06, Sewage Batch No. 25



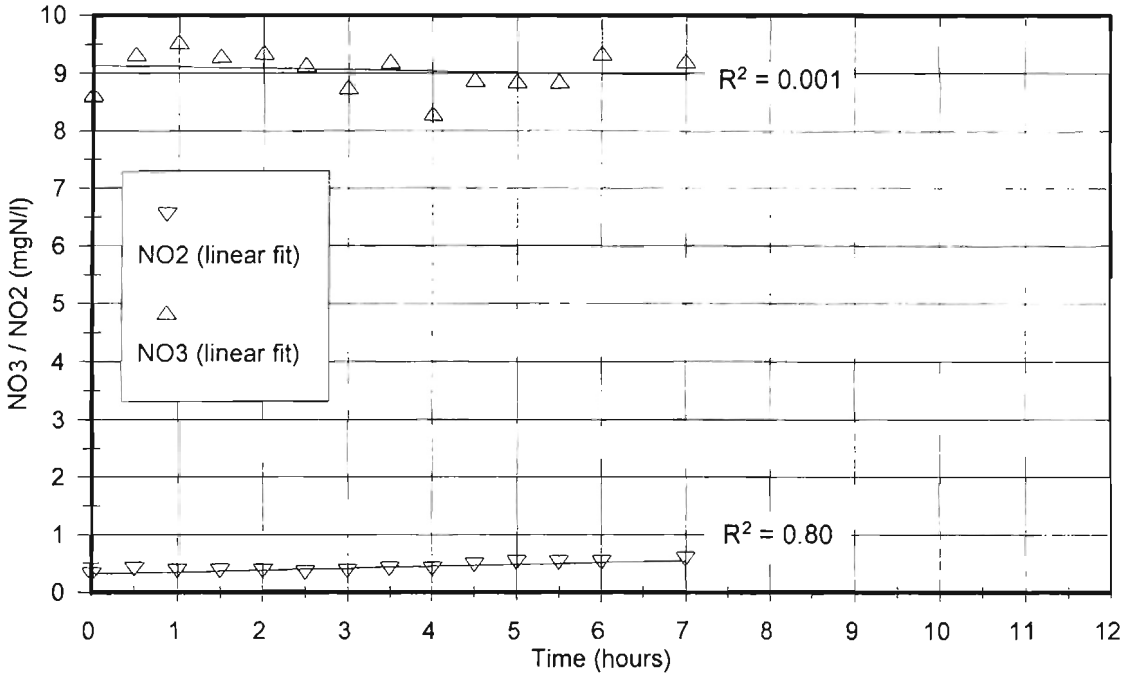
OUR-h graph for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 57, 07-06, Sewage Batch No. 25



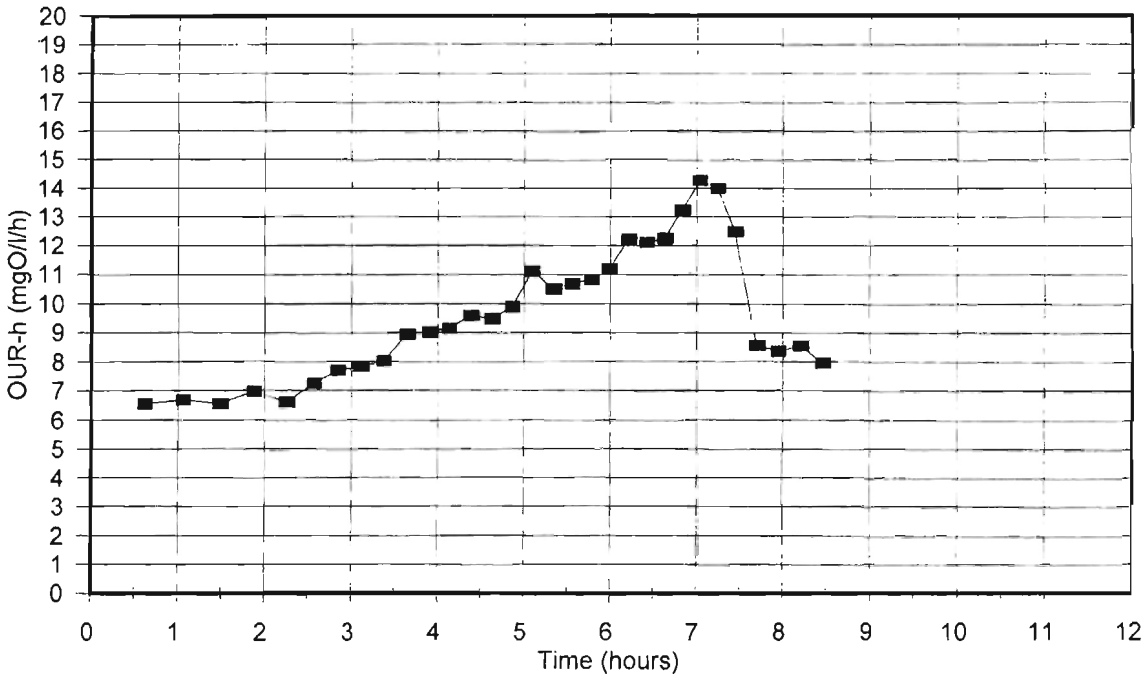
ℓn(OUR-h) graph for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 57, 07-06, Sewage Batch No. 25



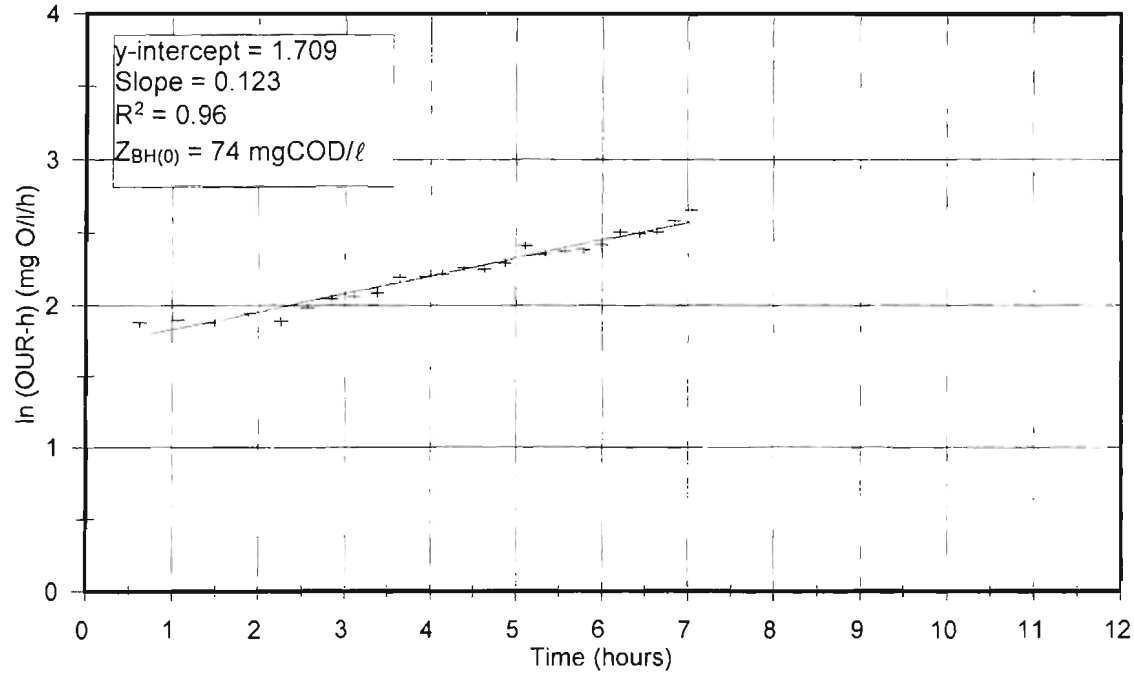
OUR graph for filtered wastewater (2.72ℓ) plus mixed liquor (0.28ℓ)
Batch Test No. 58, 07-06, Sewage Batch No. 25



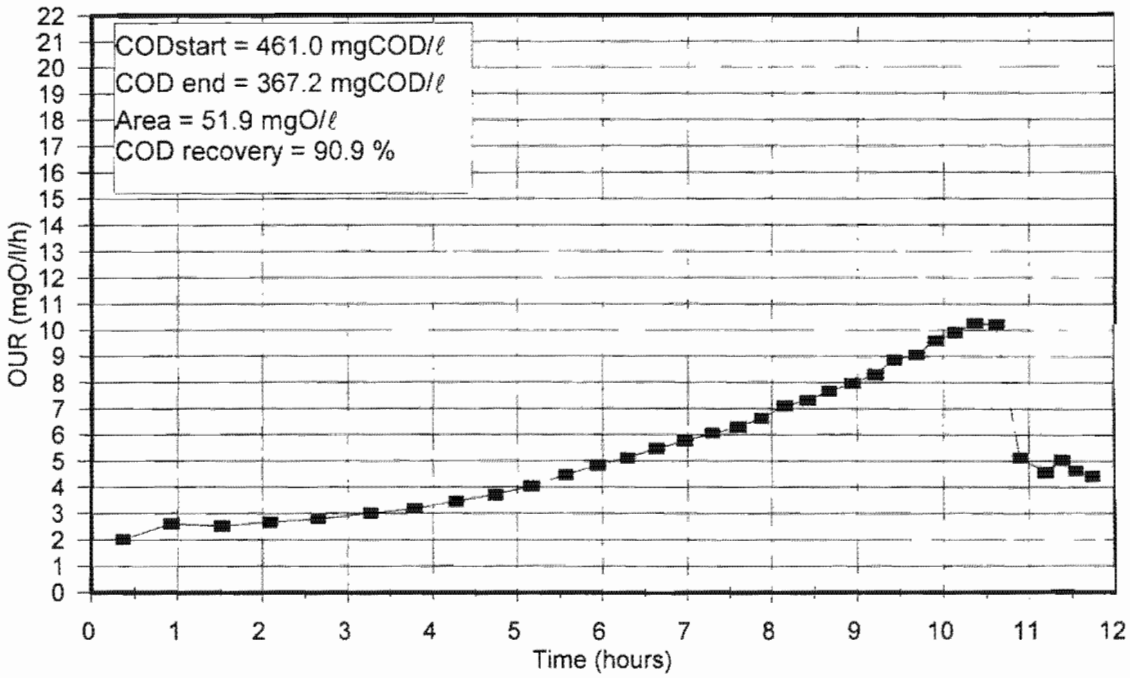
NO_3/NO_2 graph for for filtered wastewater (2.72ℓ) plus mixed liquor (0.28ℓ)
Batch Test No. 58, 07-06, Sewage Batch No. 25



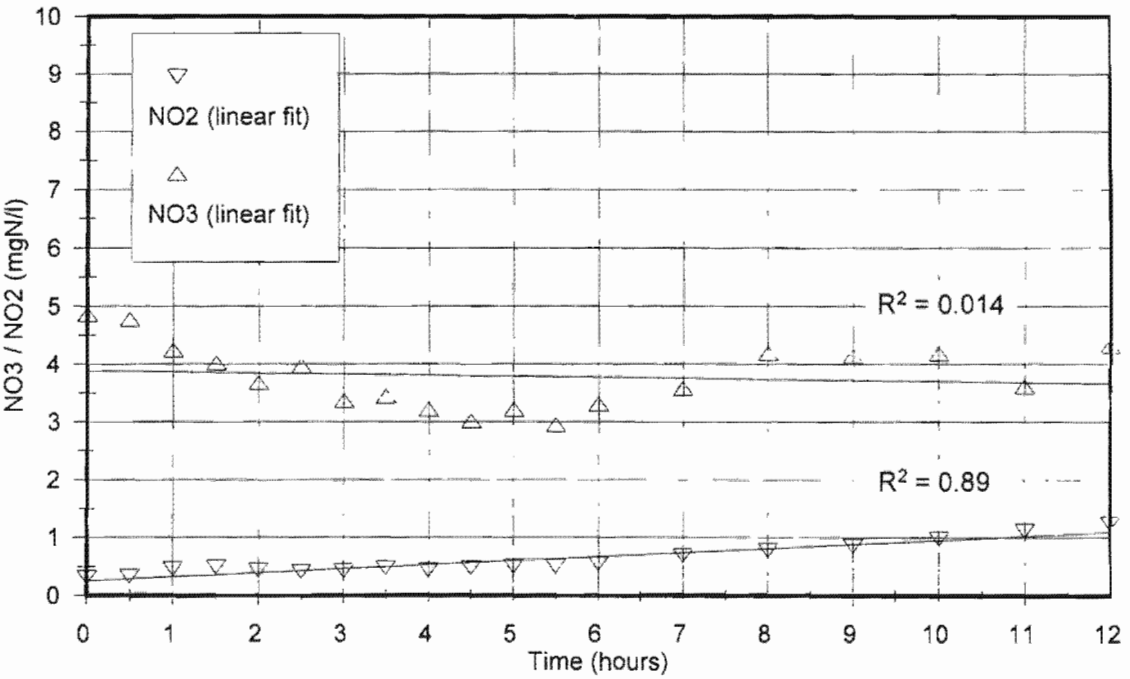
OUR-h graph for filtered wastewater (2.72ℓ) plus mixed liquor (0.28ℓ)
Batch Test No. 58, 07-06, Sewage Batch No. 25



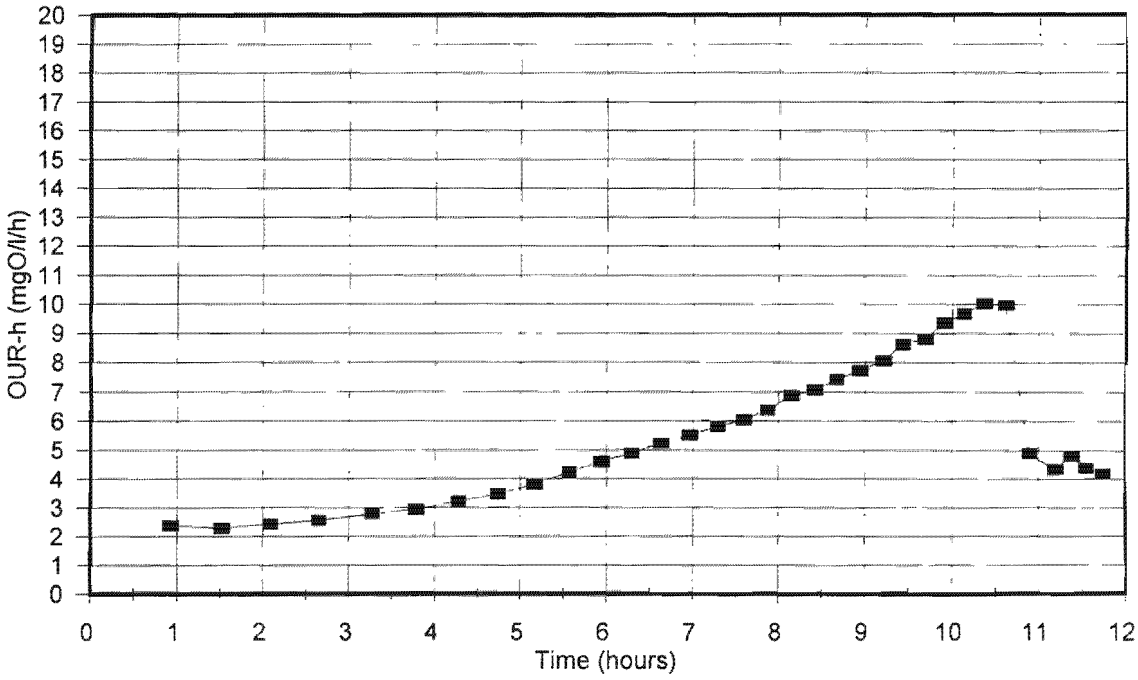
ℓn(OUR-h) graph for filtered wastewater (2.72ℓ) plus mixed liquor (0.28ℓ)
Batch Test No. 58, 07-06, Sewage Batch No. 25



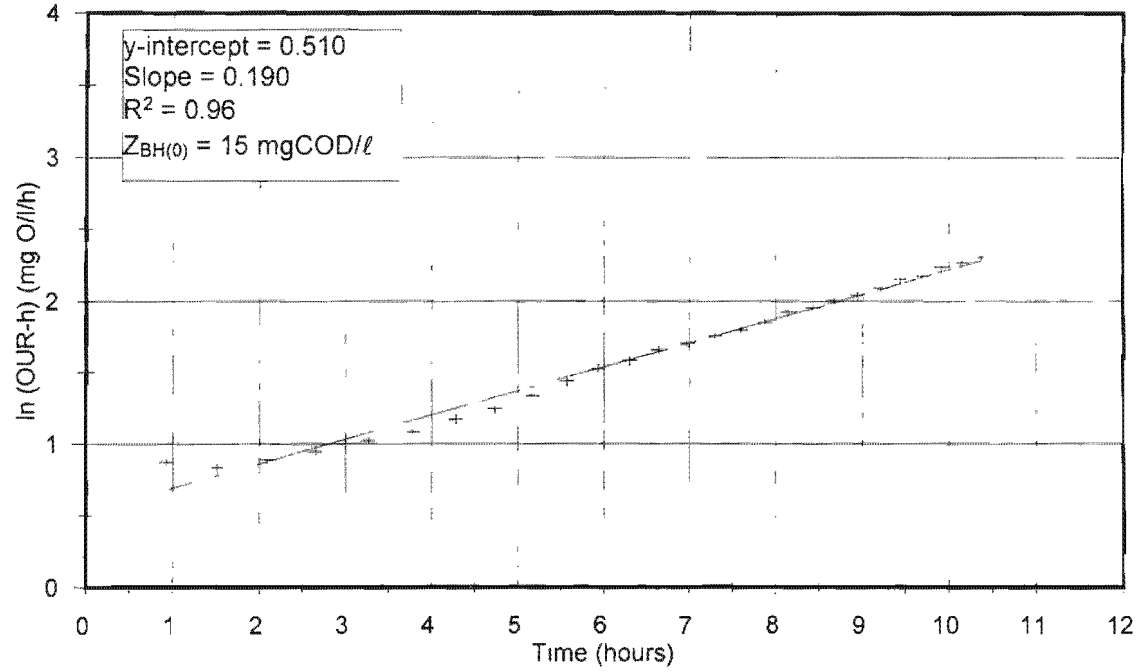
OUR graph for filtered wastewater (2.88ℓ) plus mixed liquor (0.12ℓ)
Batch Test No. 59, 09-06, Sewage Batch No. 25



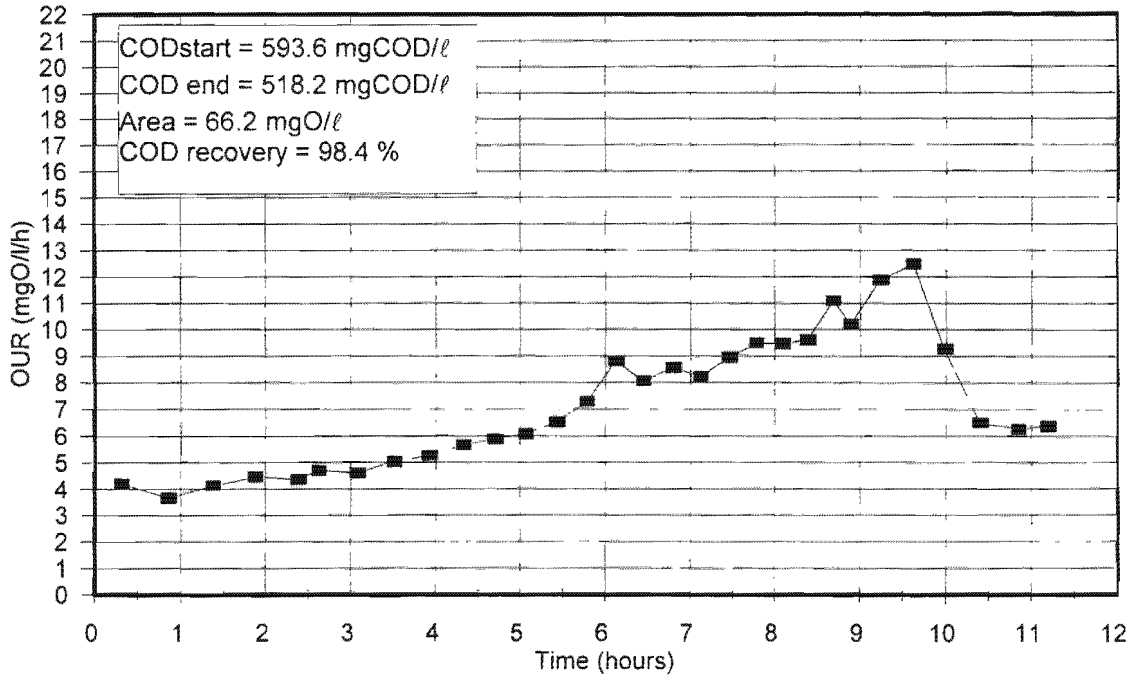
NO_3/NO_2 graph for for filtered wastewater (2.88ℓ) plus mixed liquor (0.12ℓ)
Batch Test No. 59, 09-06, Sewage Batch No. 25



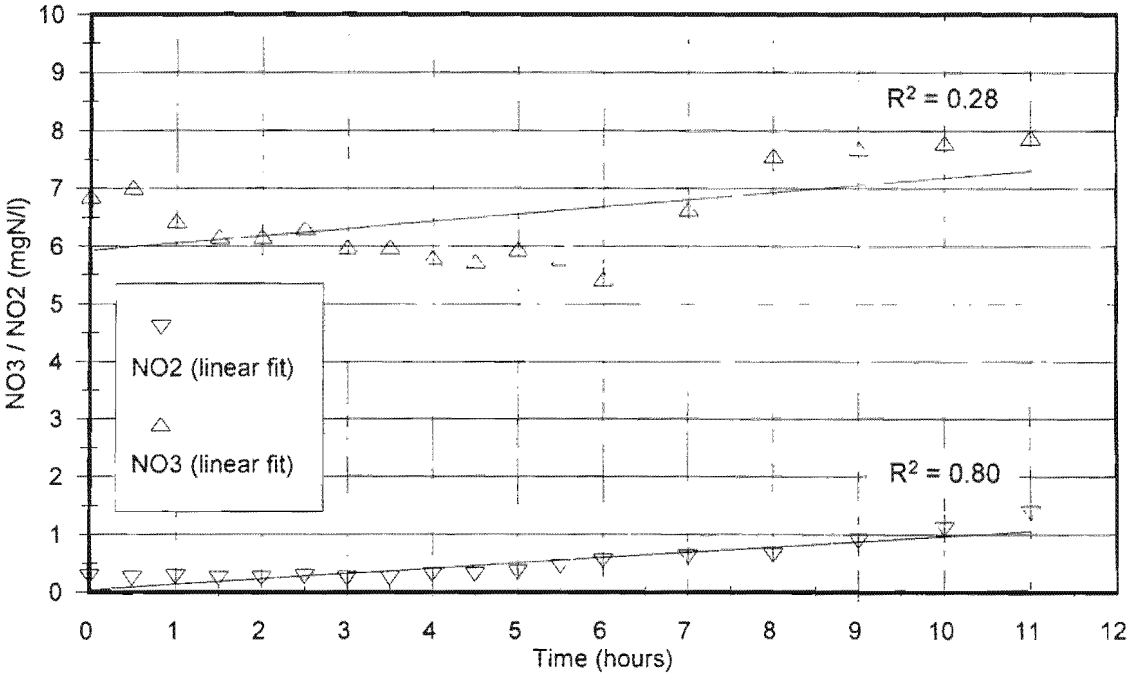
OUR-h graph for filtered wastewater (2.88ℓ) plus mixed liquor (0.12ℓ)
Batch Test No. 59, 09-06, Sewage Batch No. 25



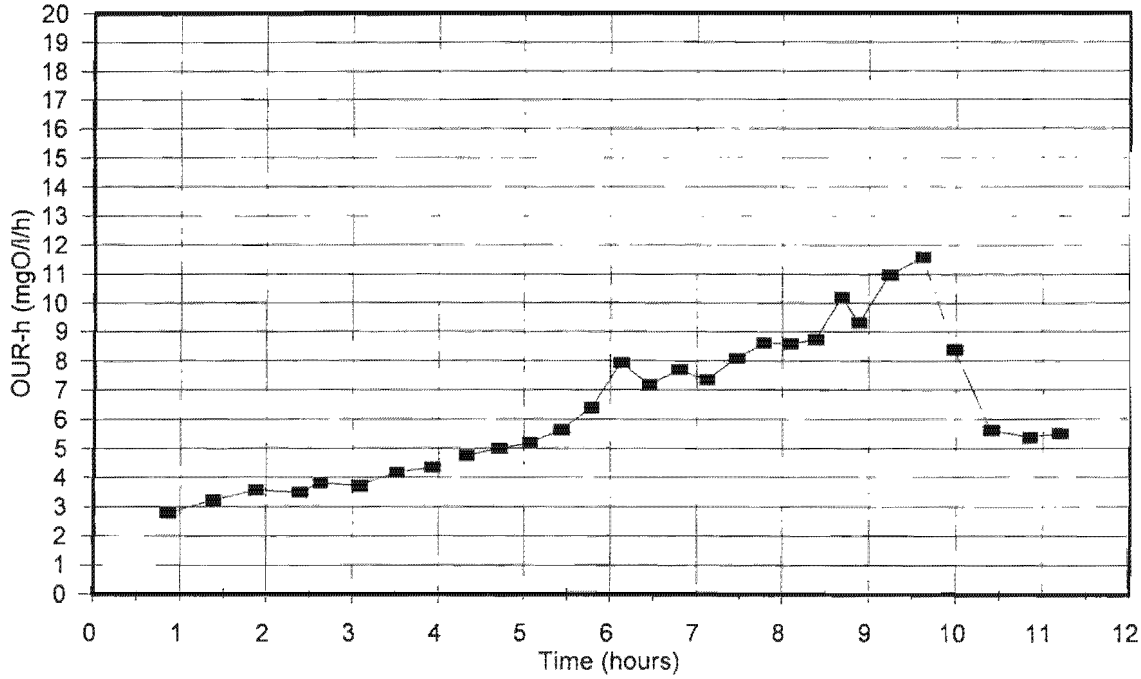
ℓn(OUR-h) graph for filtered wastewater (2.88ℓ) plus mixed liquor (0.12ℓ)
Batch Test No. 59, 09-06, Sewage Batch No. 25



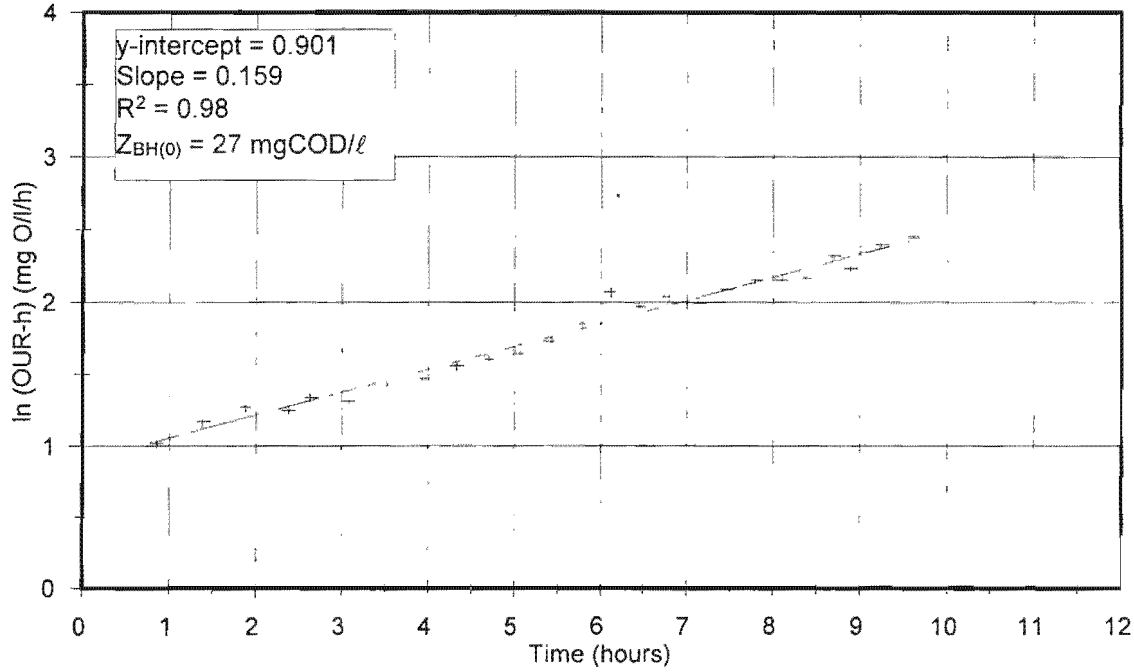
OUR graph for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 60, 09-06, Sewage Batch No. 25



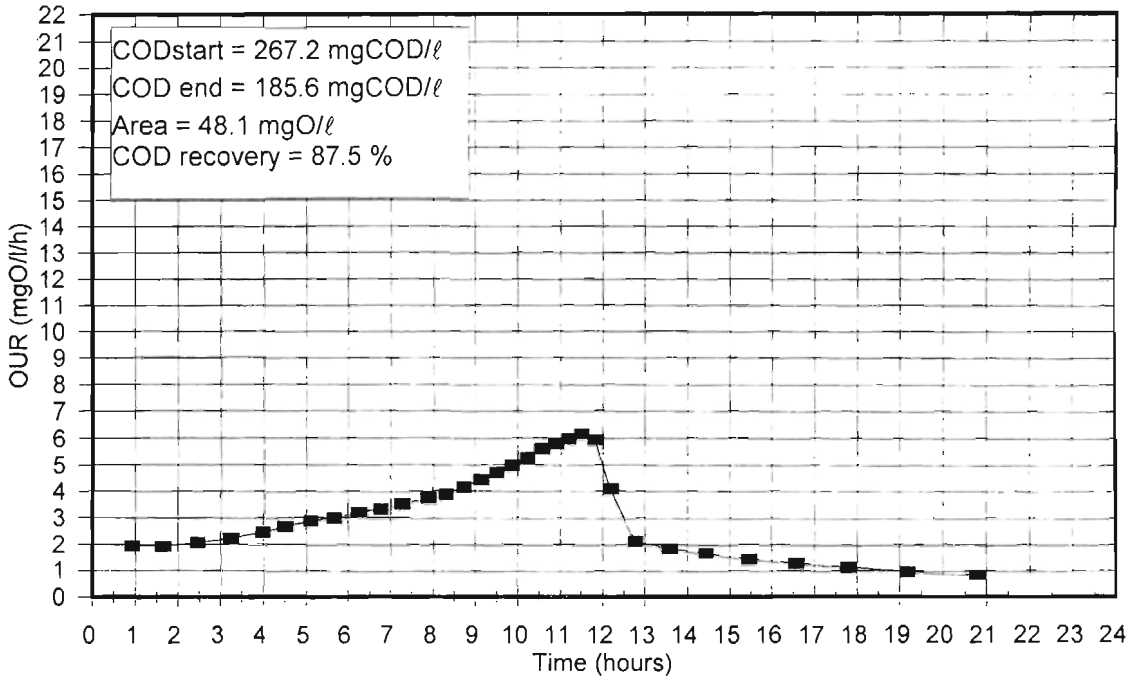
NO_3/NO_2 graph for for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 60, 09-06, Sewage Batch No. 25



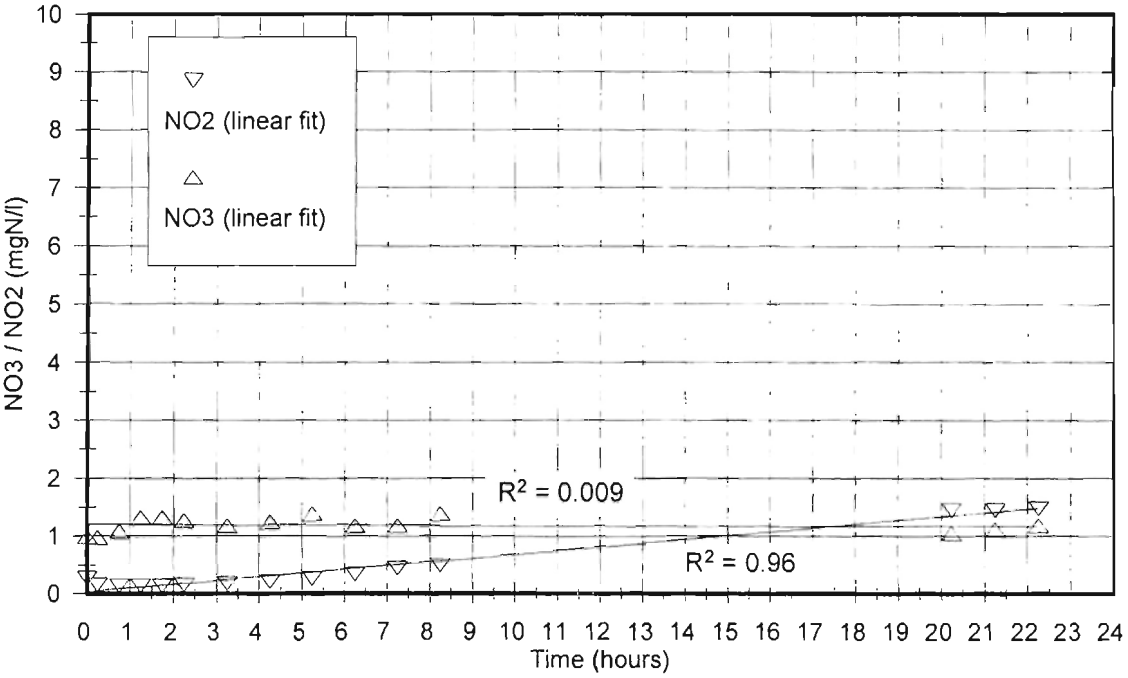
OUR-h graph for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 60, 09-06, Sewage Batch No. 25



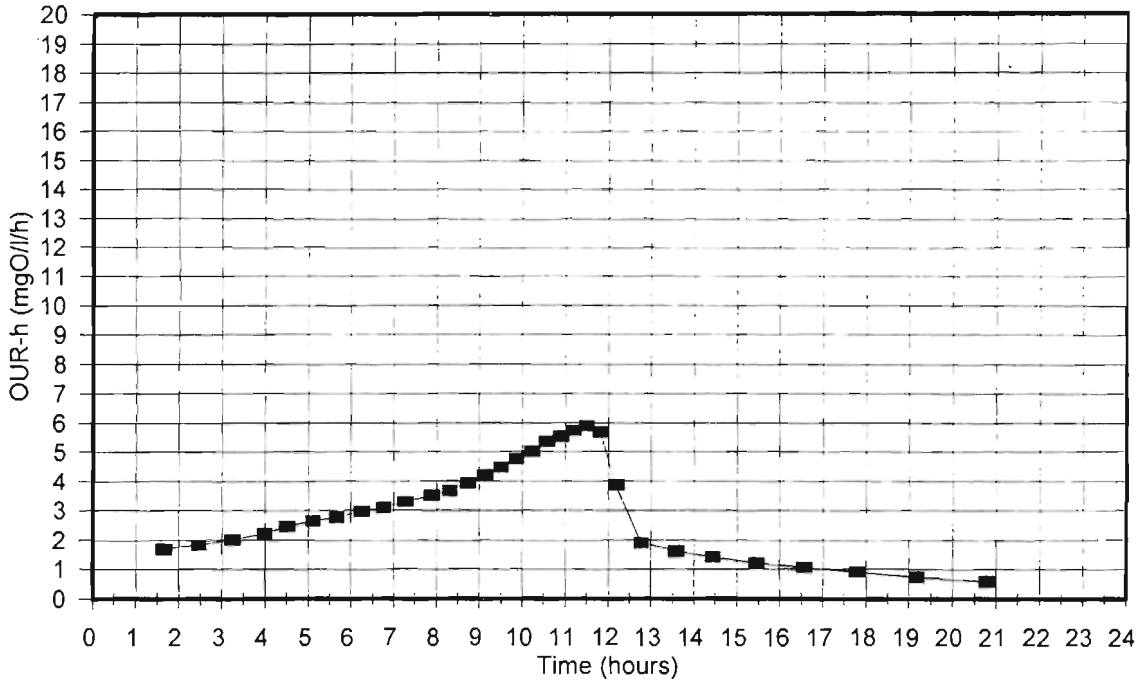
ℓn(OUR-h) graph for filtered wastewater (2.80ℓ) plus mixed liquor (0.20ℓ)
Batch Test No. 60, 09-06, Sewage Batch No. 25



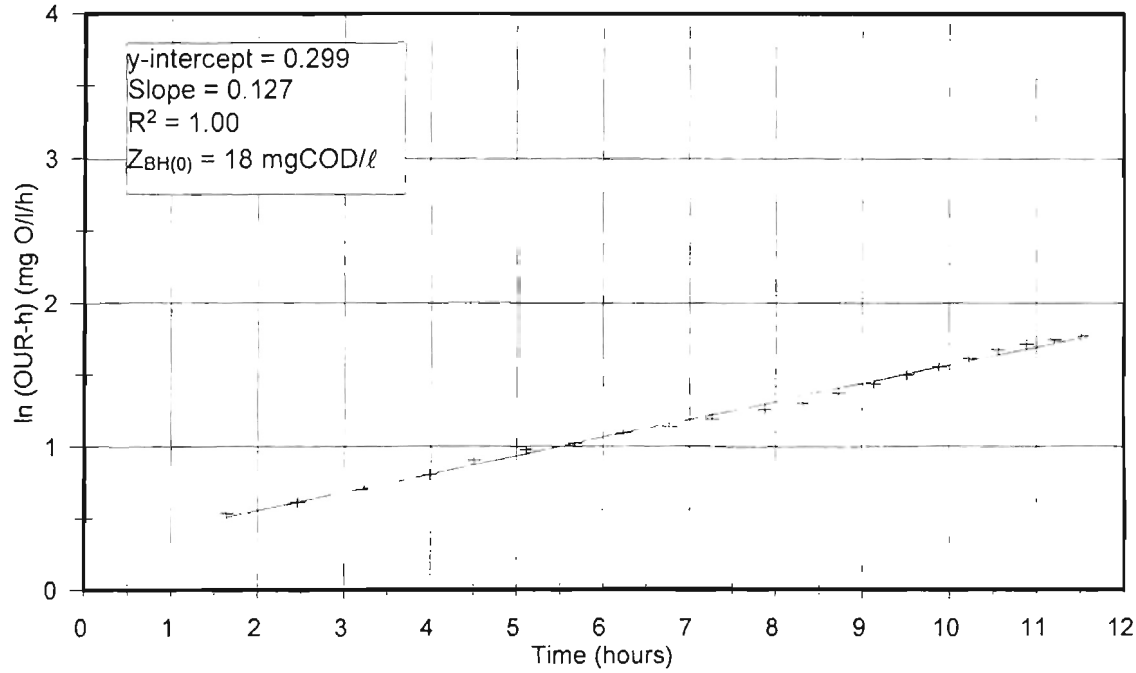
OUR graph for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 61, 15-06, Sewage Batch No. 26



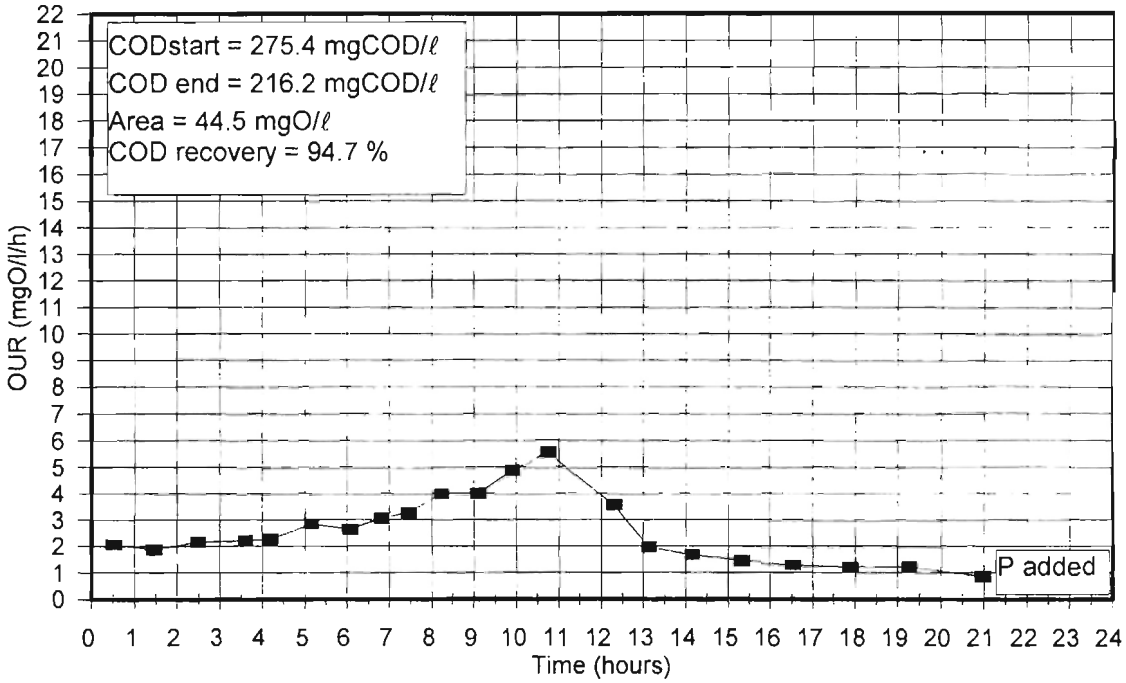
NO_3/NO_2 graph for for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 61, 15-06, Sewage Batch No. 26



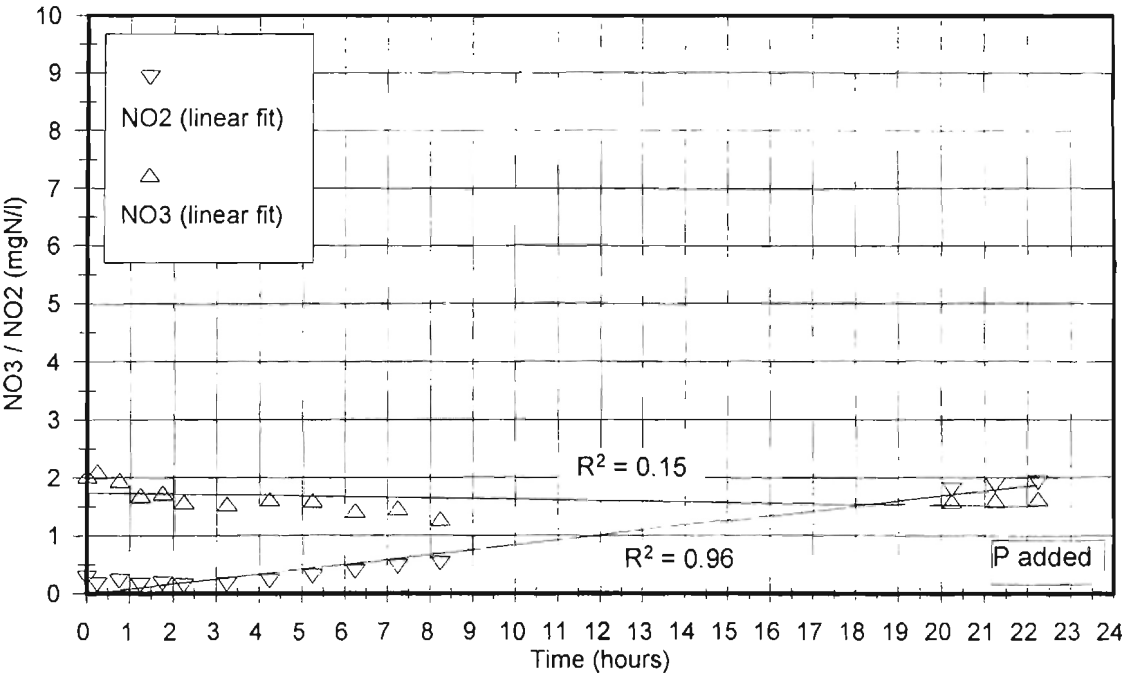
OUR-h graph for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 61, 15-06, Sewage Batch No. 26



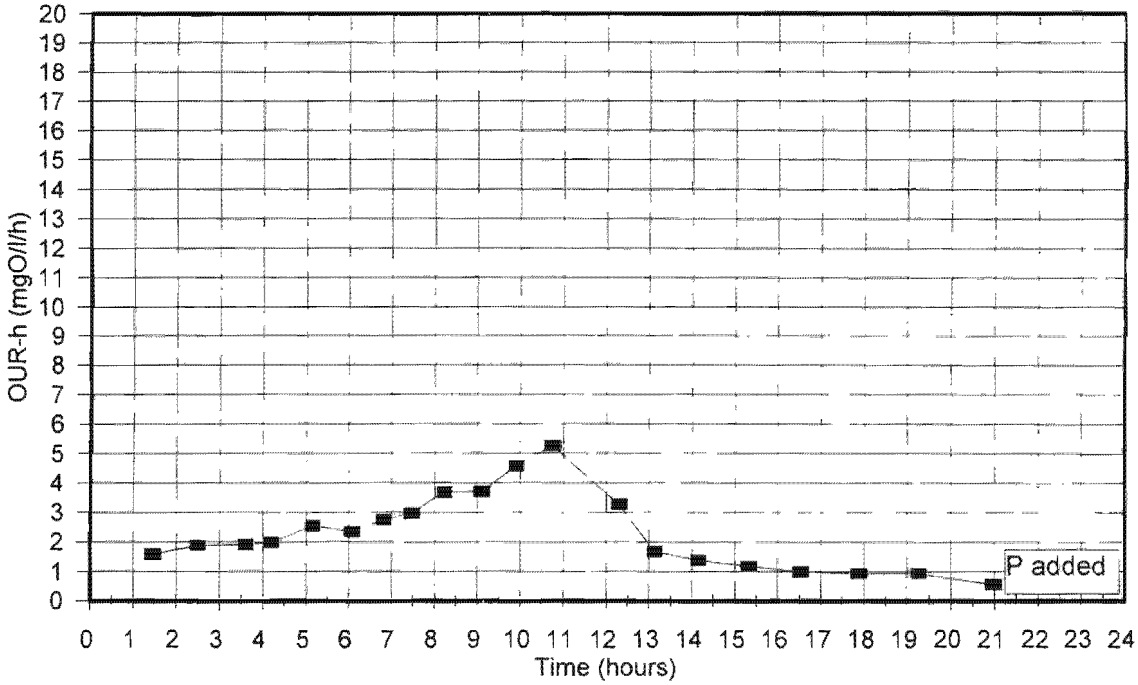
ℓn(OUR-h) graph for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 61, 15-06, Sewage Batch No. 26



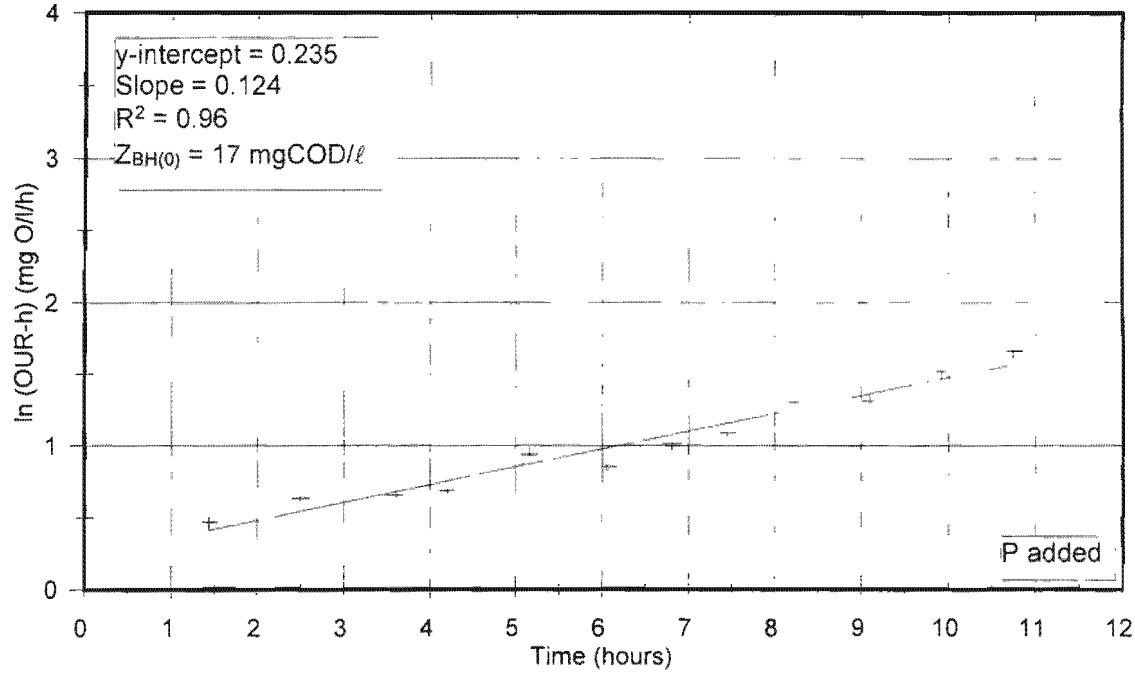
OUR graph for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 62, 15-06, Sewage Batch No. 26



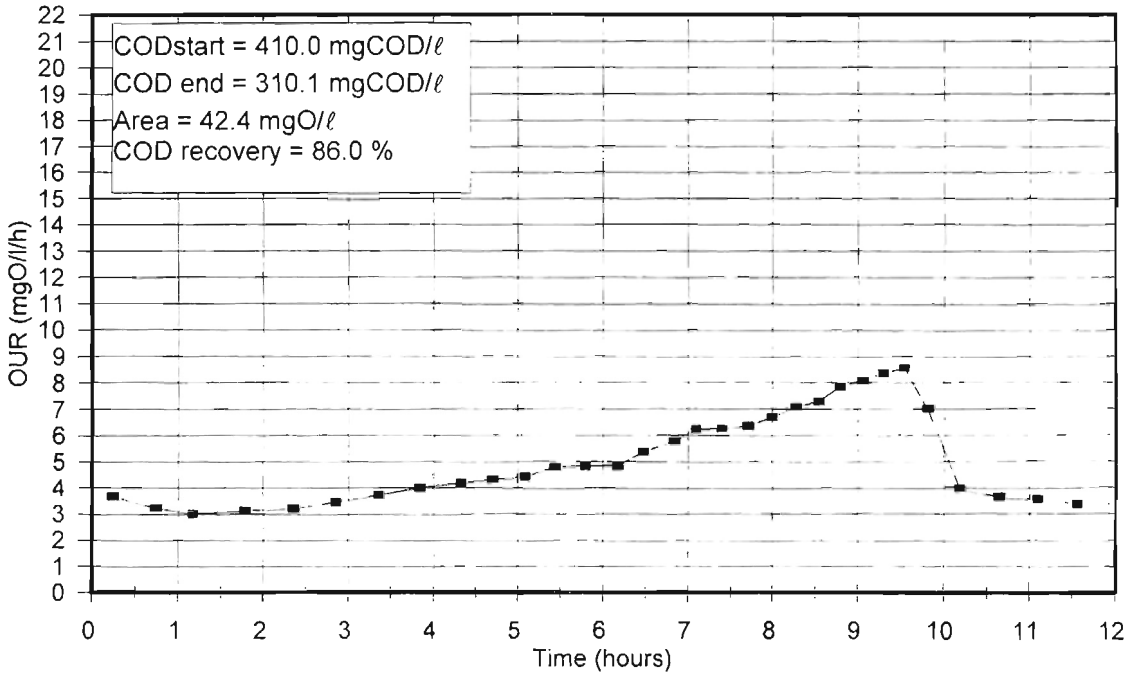
NO_3/NO_2 graph for for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 62, 15-06, Sewage Batch No. 26



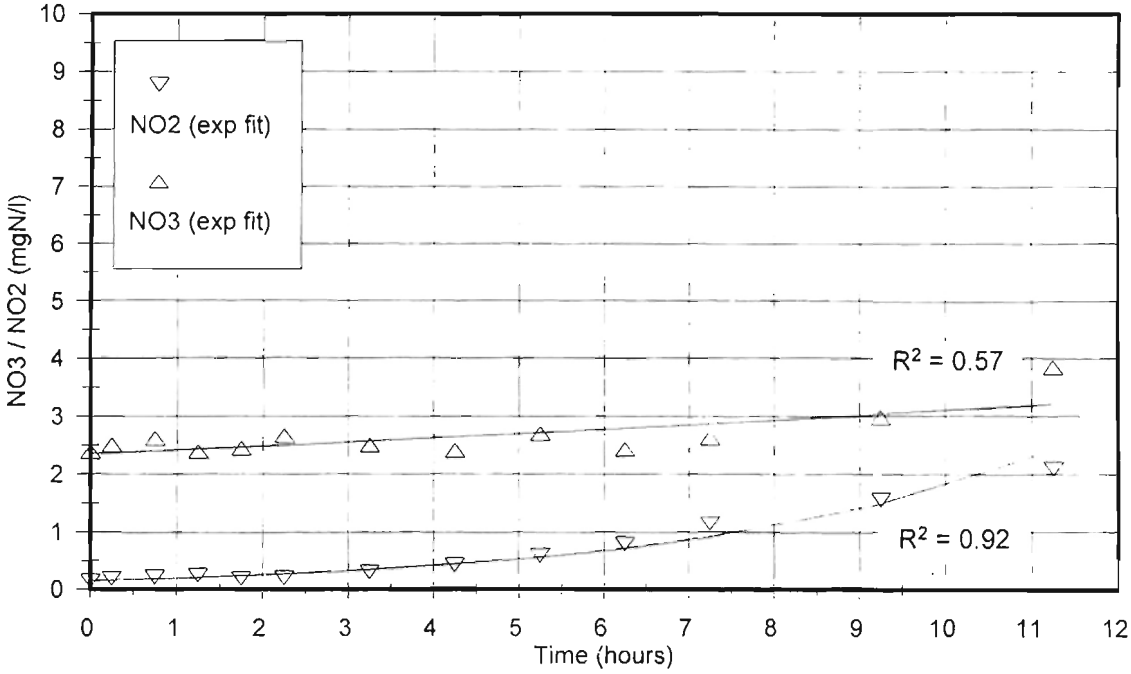
OUR-h graph for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No. 62, 15-06, Sewage Batch No. 26



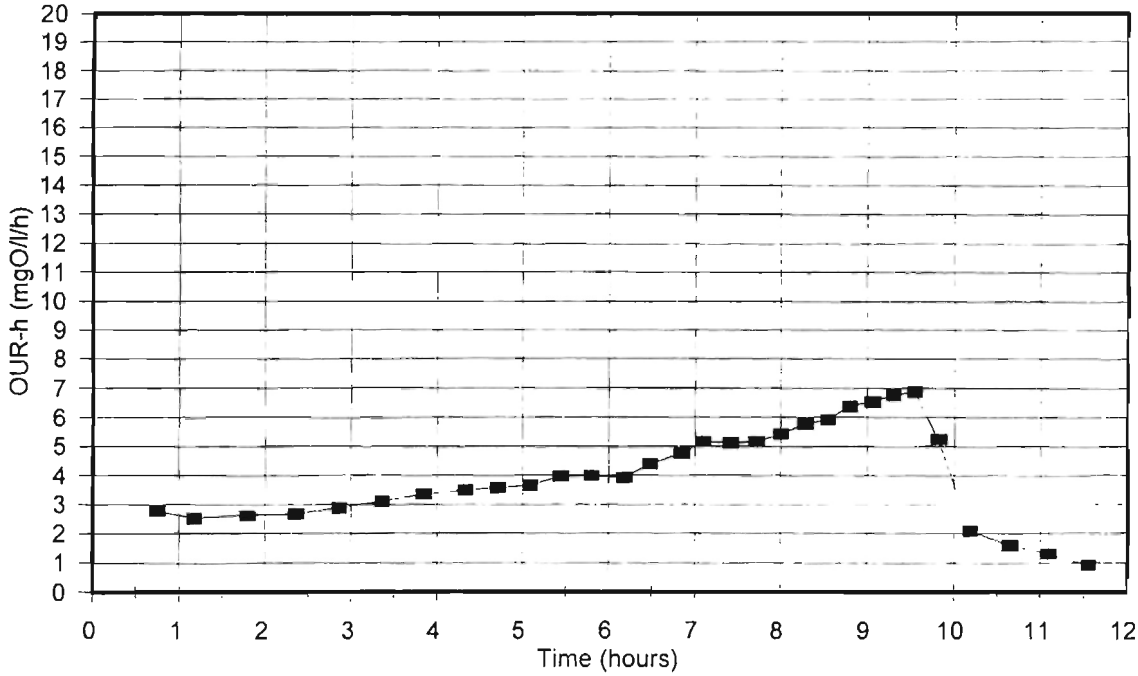
ℓn(OUR-h) graph for filtered wastewater (2.92ℓ) plus mixed liquor (0.08ℓ)
Batch Test No 62, 15-06, Sewage Batch No. 26



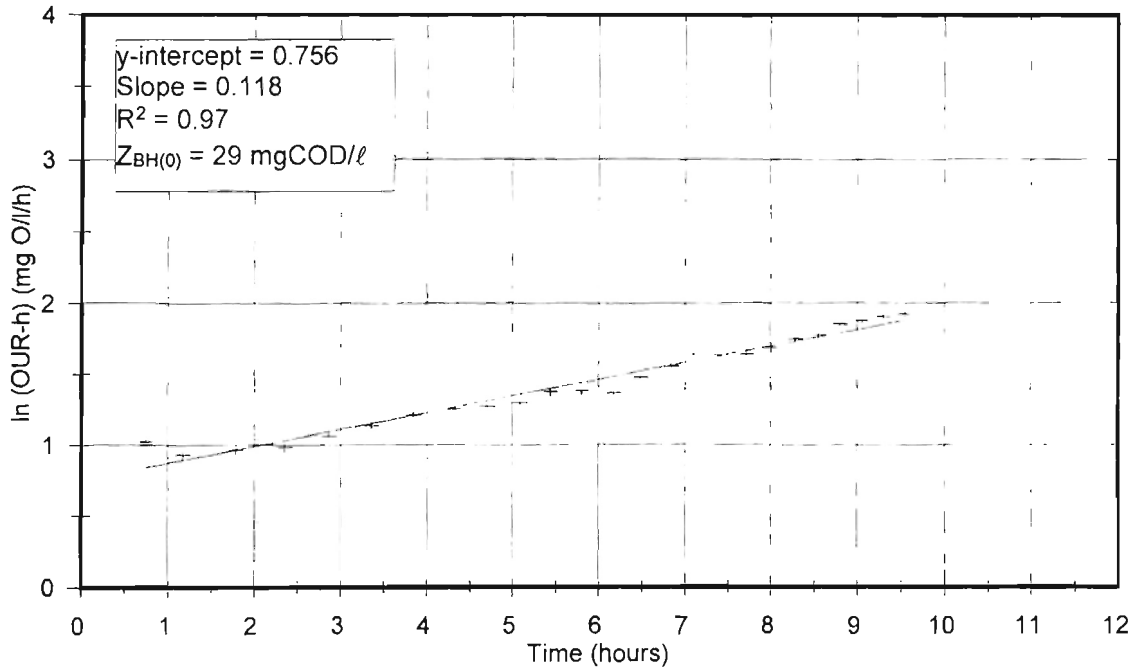
OUR graph for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 63, 16-06, Sewage Batch No. 26



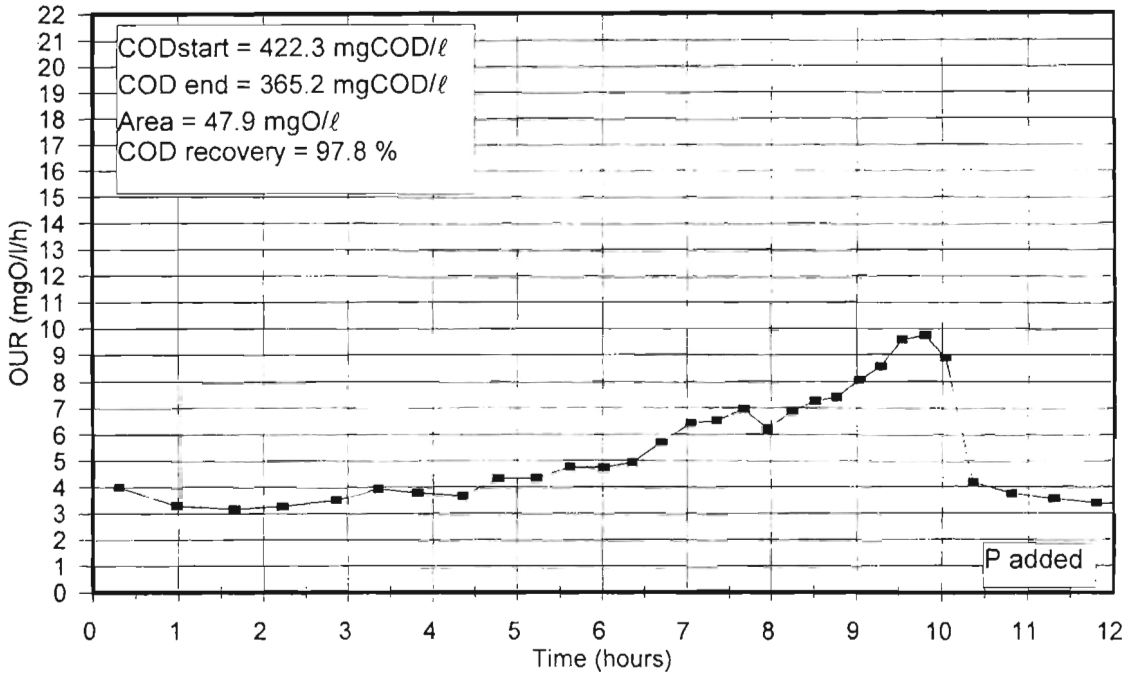
NO_3/NO_2 graph for for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 63, 16-06, Sewage Batch No. 26



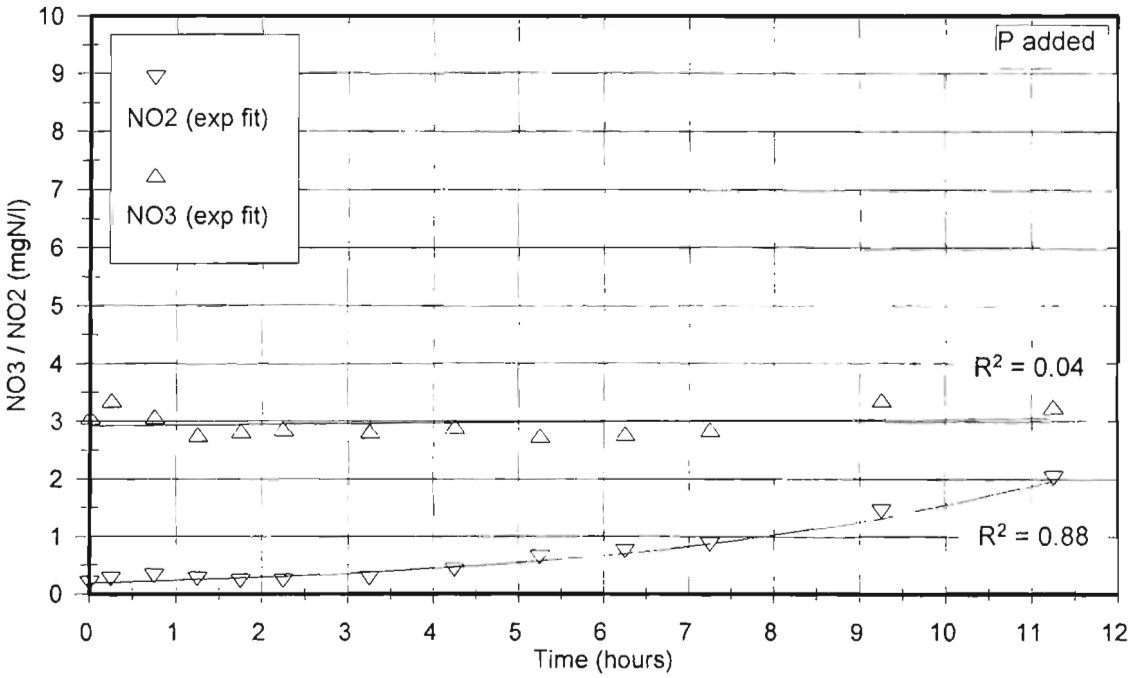
OUR-h graph for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 63, 16-06, Sewage Batch No. 26



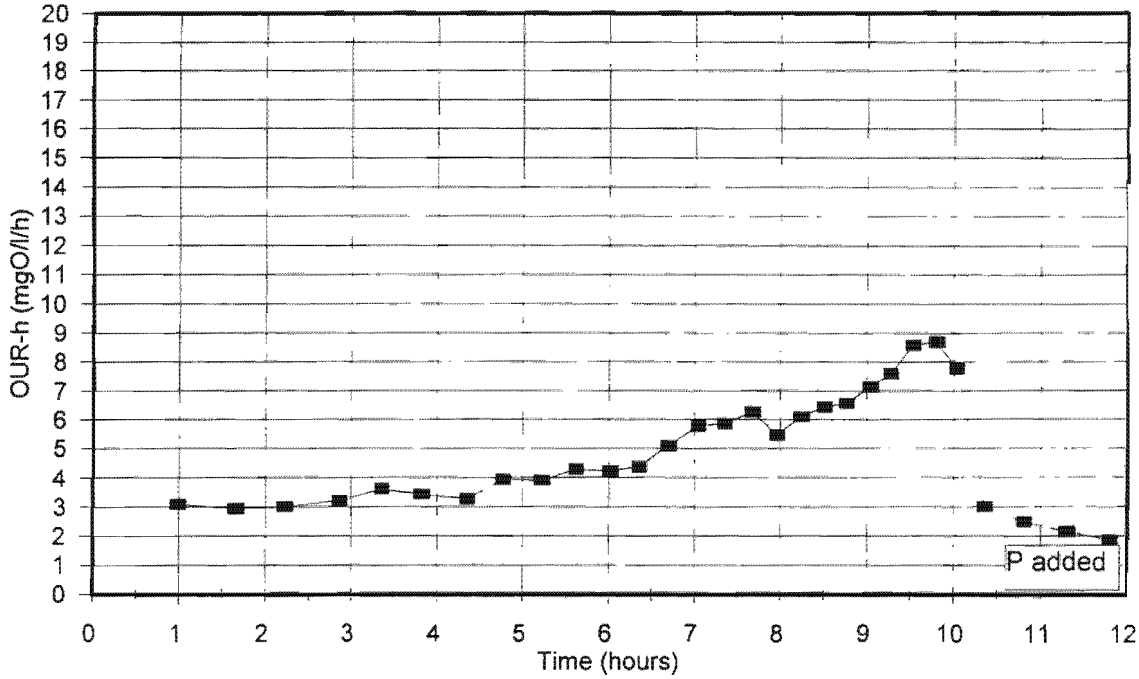
ℓn(OUR-h) graph for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 63, 16-06, Sewage Batch No. 26



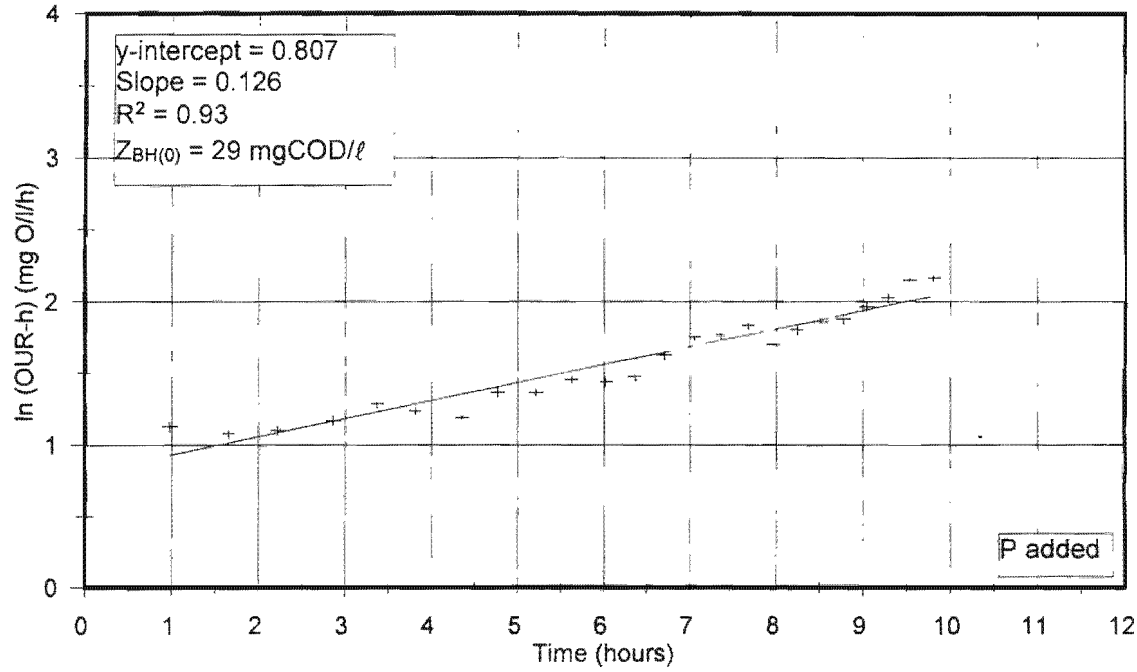
OUR graph for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 64, 16-06, Sewage Batch No. 26



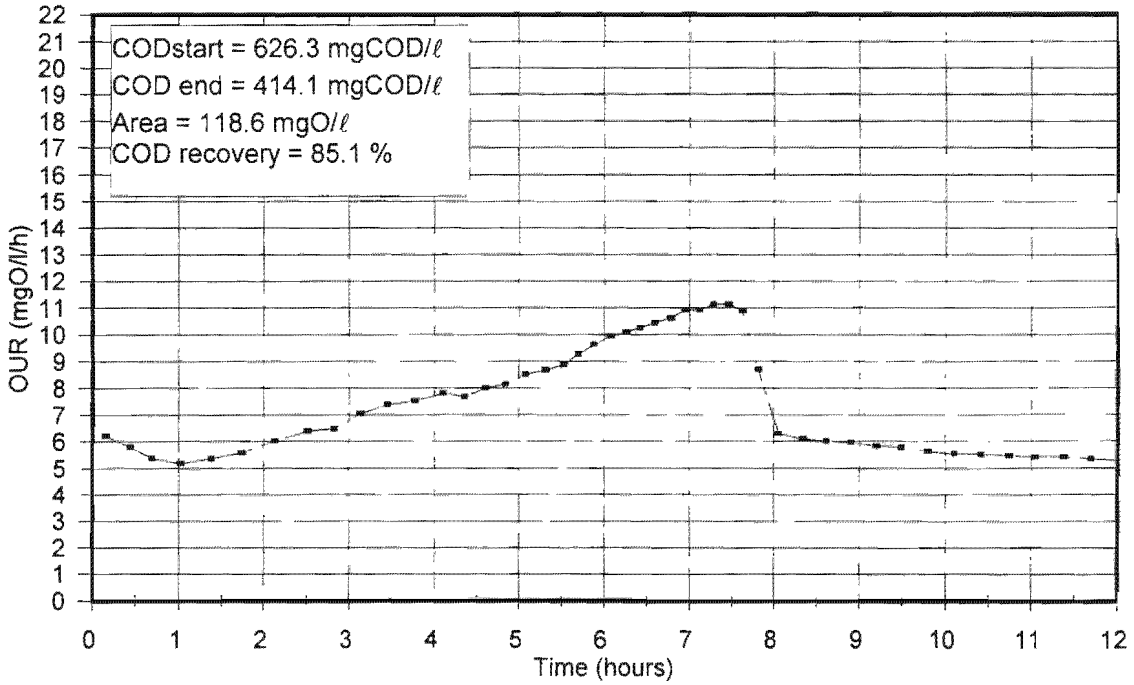
NO_3/NO_2 graph for for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 64, 16-06, Sewage Batch No. 26



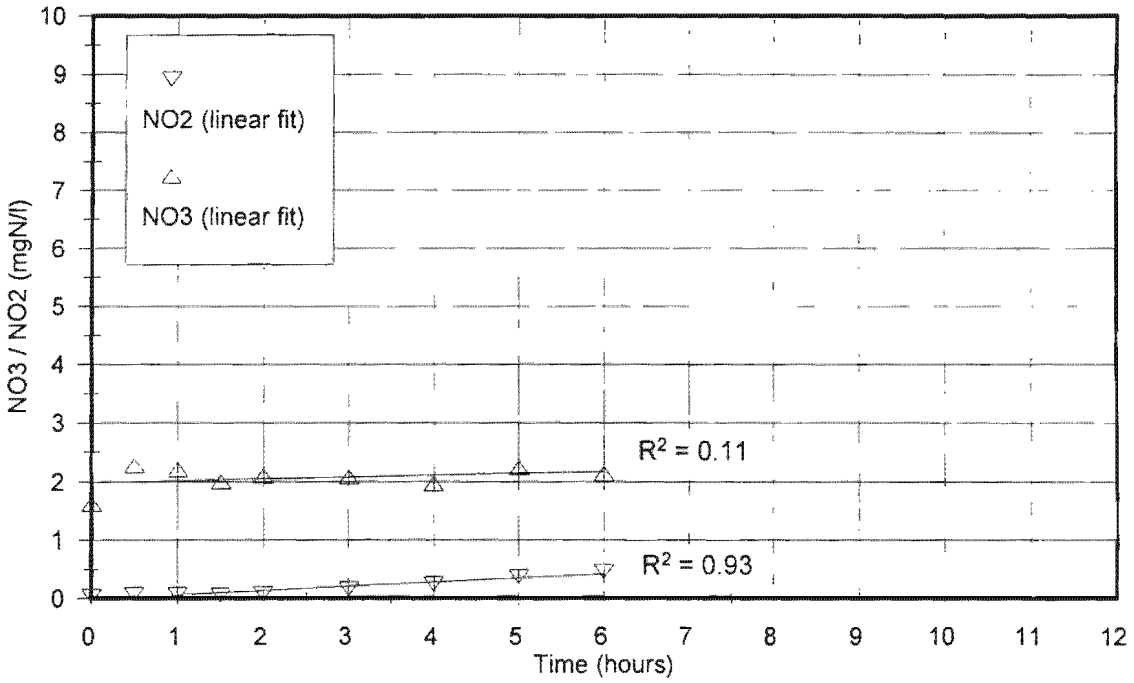
OUR-h graph for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 64, 16-06, Sewage Batch No. 26



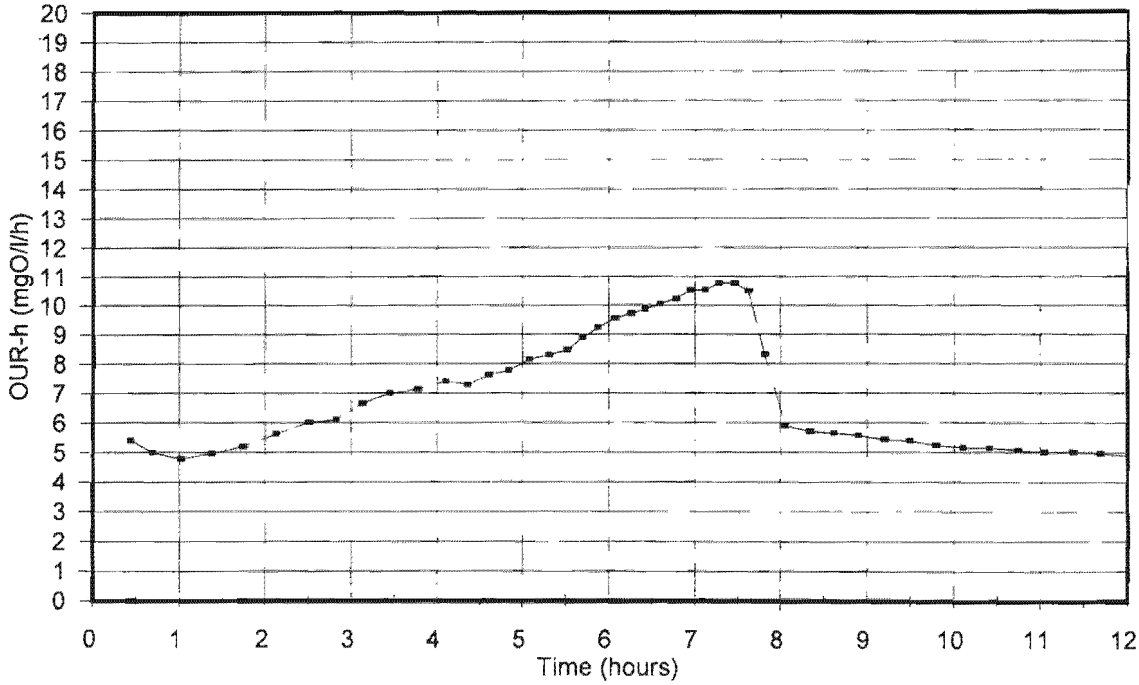
ℓn(OUR-h) graph for filtered wastewater (2.84ℓ) plus mixed liquor (0.16ℓ)
Batch Test No. 64, 16-06, Sewage Batch No. 26



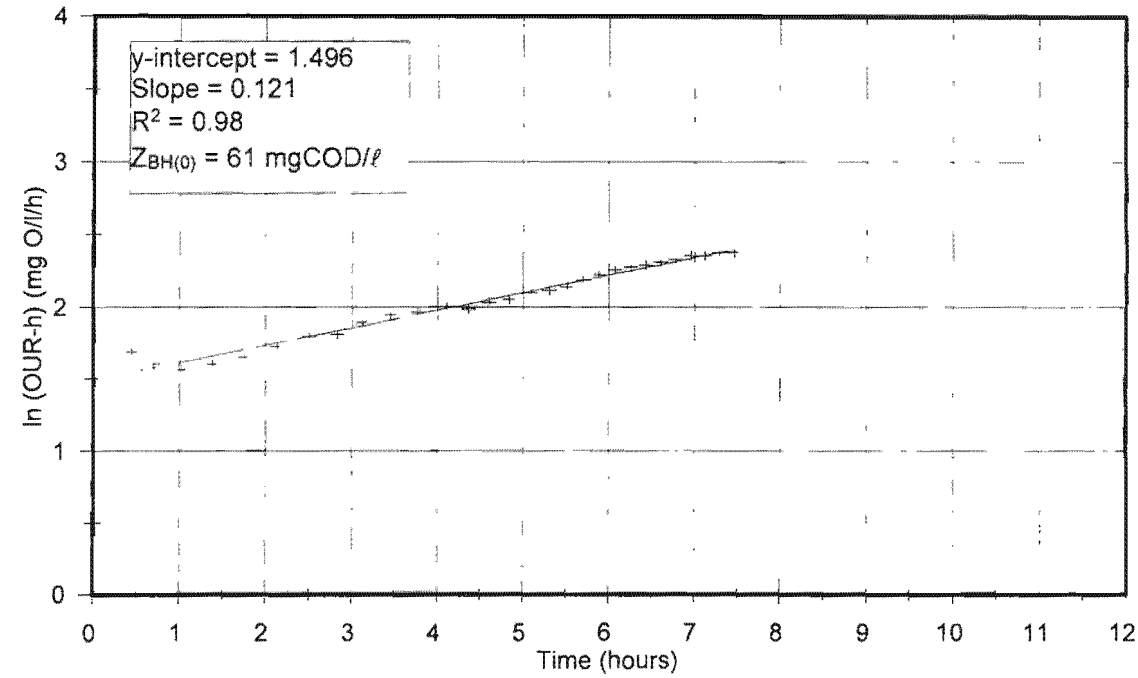
OUR graph for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 65, 17-06, Sewage Batch No. 26



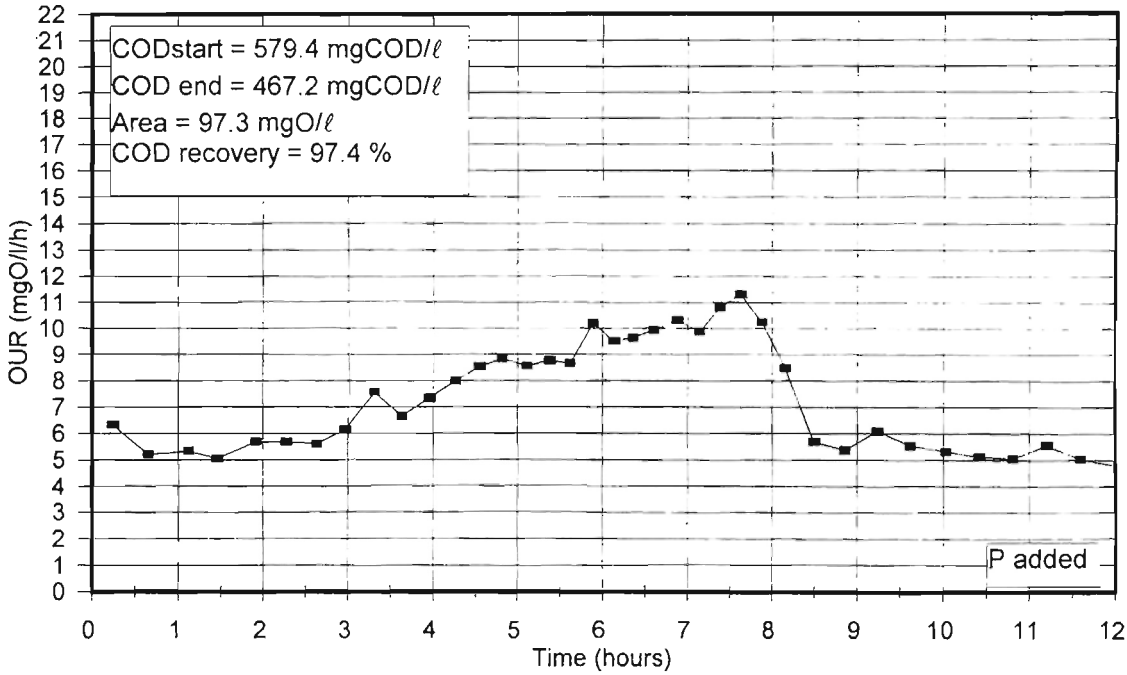
NO_3/NO_2 graph for for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 65, 17-06, Sewage Batch No. 26



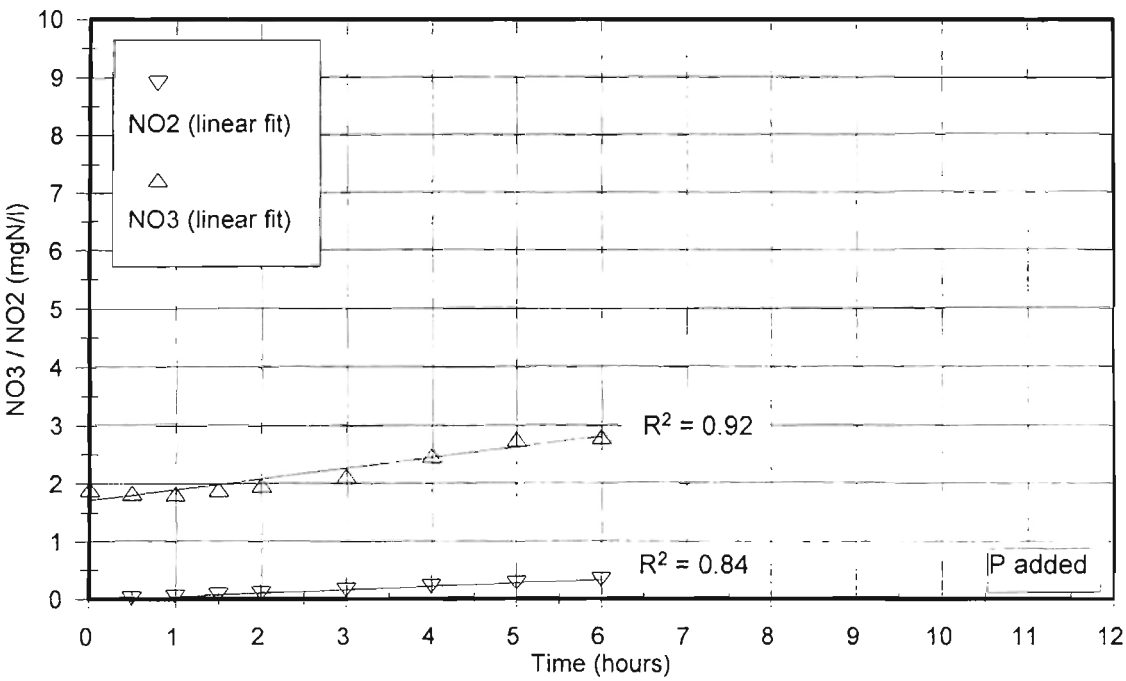
OUR-h graph for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 65, 17-06, Sewage Batch No. 26



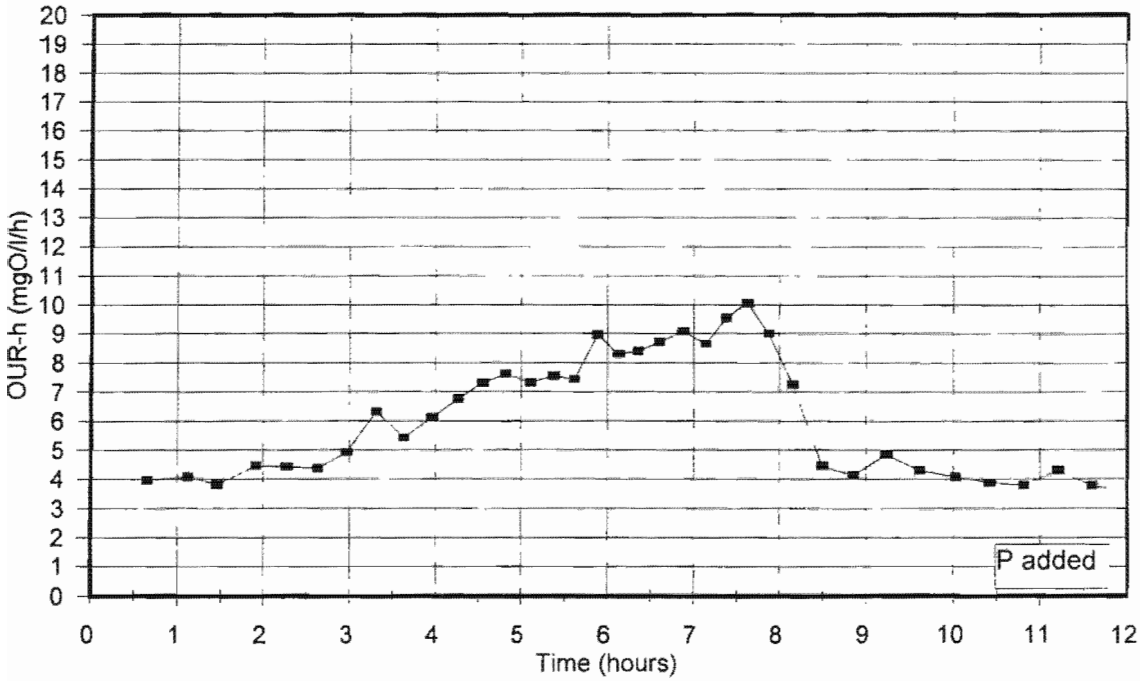
ℓn(OUR-h) graph for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 65, 17-06, Sewage Batch No. 26



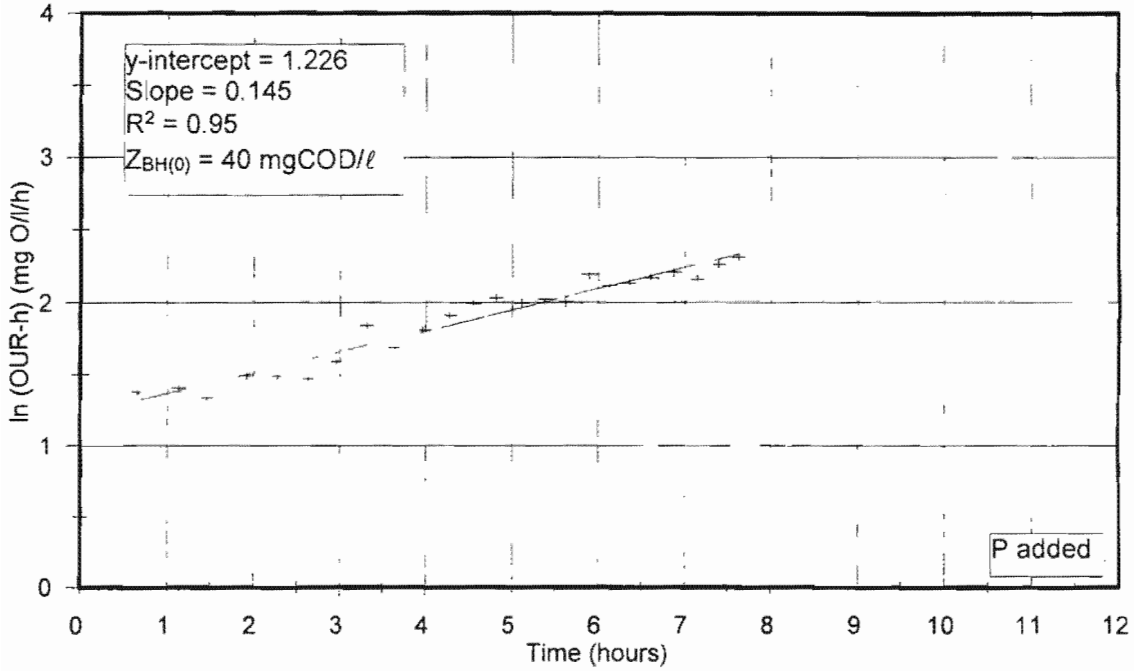
NO_3/NO_2 graph for for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 66, 17-06, Sewage Batch No. 26



NO_3/NO_2 graph for for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 66, 17-06, Sewage Batch No. 26



OUR-h graph for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No 66, 17-06, Sewage Batch No. 26



ℓn(OUR-h) graph for filtered wastewater (2.76ℓ) plus mixed liquor (0.24ℓ)
Batch Test No. 66, 17-06, Sewage Batch No. 26

APPENDIX B

COMPREHENSIVE DATA FOR THE PARENT LABORATORY-SCALE *CONTROL* NITRIFICATION / DENITRIFICATION AND FULLY AEROBIC ACTIVATED SLUDGE SYSTEM

TABLE OF CONTENTS

Table B1: Summary of the parent control system TKN and Nitrate statistical data for the various sewage batches.

Sewage Batch No.	TKN (mgN/ℓ)									NITRATES (mgN/ℓ)								
	INFLUENT			UNFIL.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	42	2	7	4.6	2.8	7	194	10	7	14.8	1.6	7	20.0	2.1	7	21.9	2.0	7
2	46	2	10	6.0	1.2	10	160	23	10	13.5	2.1	10	19.4	1.8	10	21.8	3.8	10
3	61	2	8	5.0	1.1	8	265	5	8	12.8	1.6	8	18.0	0.9	8	23.8	1.8	8
4	59	1	6	2.1	0.9	6	294	12	6	12.7	1.5	6	25.7	0.8	6	25.8	1.9	6
5	49	3	6	2.4	1.0	6	255	20	6	7.0	1.3	6	15.2	3.1	6	17.2	3.1	6
6	46	3	12	3.6	1.2	12	224	9	12	9.6	3.0	12	17.0	3.0	12	17.5	1.7	12
7	63	2	4	3.5	0.3	4	215	24	4	24.5	0.7	4	36.0	1.2	4	36.8	1.8	4
8A	74	3	7	3.6	0.8	7	216	12	7	22.7	1.7	7	24.9	1.5	7	34.2	1.1	7
8B	106	0	4	4.4	2.3	4	227	7	4	16.6	2.4	4	36.7	2.6	4	36.3	1.9	4
9	97	1	11	5.1	0.9	11	222	13	11	3.6	2.2	11	21.7	1.0	11	21.9	1.8	11
10	74	8	13	3.7	1.6	13	199	23	13	0.3	0.2	13	12.1	0.9	13	11.3	0.9	13
11	81	3	10	8.3	1.2	10	238	16	10	1.6	1.7	10	10.4	2.6	10	11.0	1.4	10
12	93	5	10	6.7	0.8	10	256	17	10	5.6	2.1	10	19.4	2.4	10	20.3	1.9	10
13	73	6	11	5.1	1.2	11	255	10	11	0.3	0.1	11	11.5	1.1	11	11.5	1.3	11
14	79	2	9	6.2	0.8	9	227	20	9	7.7	1.3	9	19.6	1.7	9	20.7	2.2	9
15	64	3	10	6.8	1.2	10	230	13	10	2.4	1.5	10	11.1	2.0	10	11.1	1.3	10
16	73	2	9	7.9	0.9	9	253	7	9	1.6	0.6	9	10.6	1.5	9	10.9	1.6	9
17	78	6	5	8.2	1.5	5	213	30	5	2.2	0.4	5	14.3	1.0	5	15.5	1.7	5
18	63	3	8	8.1	0.7	8	208	14	8	1.6	0.4	8	9.2	1.1	8	8.6	0.9	8
19	85	4	8	6.2	1.8	8	243	14	8	4.8	1.9	8	17.3	1.5	8	15.2	1.2	8
20	70	4	8	5.1	0.9	8	239	7	8	5.4	3.5	8	14.6	5.2	8	12.6	3.0	8
21	70	3	9	5.6	1.3	9	241	11	9	5.7	1.4	9	14.7	1.4	9	11.5	1.8	9
22	73	2	10	8.3	0.6	10	224	16	10	3.3	1.5	10	11.5	2.1	10	9.1	1.3	10
23A	86	5	3	5.5	1.4	3	252	11	3	24.8	0.3	3	36.4	0.3	3	35.6	1.1	3
23B	80	3	5	6.1	1.4	5	257	11	5			5	60.4	3.1	5	58.8	4.7	5
24	57	6	6	7.1	1.6	6	258	21	6			6	28.8	3.9	6	30.0	6.0	6
25	104	2	5	4.3	0.4	5	277	7	5			5	69.3	3.0	5	73.1	1.6	5
26	72	5	5	4.5	0.5	5	250	6	5			5	50.0	1.0	5	52.6	0.3	5

Table B2: Summary of the parent control system COD, OUR and VSS statistical data for the various sewage batches.

Sewage 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	495	35	7	62	17	7	3309	229	7	37.8	0.8	7	2320	133	7
2	514	20	10	144	81	10	2706	221	10	37.8	1.5	10	1878	255	10
3	525	51	8	51	5	8	4242	173	8	37.0	0.6	8	3405	491	8
4	494	9	6	41	5	6	5137	267	6	41.5	2.3	6	3528	145	6
5	500	10	6	42	5	6	4344	117	6	36.2	1.4	6	3044	77	6
6	498	70	12	40	12	12	3538	257	12	32.7	2.7	12	2622	186	12
7	420	19	4	30	8	4	3058	131	4	35.5	0.7	4	2348	88	4
8A	509	18	7	43	11	7	3378	208	7	41.7	2.0	7	2480	177	7
8B	701	39	4	52	11	4	3533	214	4	43.7	4.3	4	2638	270	4
9	744	85	11	52	16	11	3411	255	11	39.0	2.5	11	2584	291	11
10	756	46	13	54	10	13	3452	147	13	34.5	2.5	13	2464	169	13
11	730	27	10	61	11	10	3070	166	10	31.2	2.2	10	2548	237	10
12	751	71	10	49	20	10	3769	274	10	36.9	1.8	10	2651	185	10
13	766	67	11	43	11	11	4089	213	11	34.8	4.0	11	2622	74	11
14	735	23	9	44	7	9	3413	354	9	38.3	0.9	9	2408	77	9
15	728	48	10	48	18	10	3722	105	10	34.6	1 9	10	2483	132	10
16	766	22	9	48	7	9	3764	95	9	38.2	1.1	9	2700	126	9
17	759	92	5	57	13	5	3335	276	5	42.4	4.1	5	2406	127	5
18	655	60	8	36	14	8	3119	301	8	37.5	1.4	8	2409	138	8
19	728	37	8	52	10	8	4073	260	8	41.6	0.9	8	3042	80	8
20	741	56	8	65	14	8	3936	158	8	40.9	2.3	8	3760	106	8
21	774	30	9	40	10	9	3908	91	9	36.4	1.3	9	2890	164	9
22	749	37	10	46	18	10	3862	216	10	37.1	2.6	10	2736	143	10
23A	795	23	3	57	7	3	4410	128	3	41.4	0.9	3	3111	88	3
23B	785	30	5	66	26	5	4429	66	5	47.9	1.3	5	3220	69	5
24	798	16	6	52	21	6	5083	125	6	43.0	2.1	6	3625	160	6
25	815	25	5	54	12	5	5313	64	5	43.7	0.9	5	3726	128	5
26	787	29	5	31	13	5	5112	173	5	45.9	1.6	5	3526	72	5

Table B3: Daily COD, TKN and Nitrates concentration results for the parent laboratory-scale MLE control activated sludge system.

YEAR 2000

Sew. Batch No.	Dates of Test	Day No.	COD(mgCOD/ℓ)			TKN(mgN/ℓ)			NITRATES(mgN/ℓ)		
			Unfil. Inf.	Unf. Eff.	Fil. Eff.	Unfil. Inf.	Unf. Eff.	Fil. Eff.	Anoxic	Aerobic	Eff.
1	28.04	1	541	92	40	42	3.5	0.7	15.0	20.3	23.4
	29.04	2	495	73	23	41	3.8	1.1	16.3	21.7	22.8
	01.05	4	465	48	28	39	2.4	1.1	15.8	20.1	23.1
	02.05	5	469	44	22	44	2.9	2.1	15.3	21.8	21.7
	03.05	6	485	69	36	41	10.6*	4.1	15.2	21.3	21.2
	04.05	7	465	48	46	44	4.9	2.8	14.7	19.2	23.5
	05.05	8	548	62	50	39	3.8	2.4	11.3*	15.8*	17.8*
MEAN			495	62	35	42	4.6	2.0	14.8	20.0	21.9
Std. Deviation			35	17	11	2	2.8	1.2	1.6	2.1	2.0
2	08.05	11	468*	66	52	42	4.9	3.1	12.2	17.8	18.1
	09.05	12	516	54	46	45	4.5	2.8	14.4	20.3	22.2
	10.05	13	512	84	42	45	5.5	2.2	12.9	18.4	19.4
	11.05	14	519	75	45	46	5.6	2.9	11.3	18.2	18.7
	12.05	15	535	97	39	46	5.5	1.7	10.5	17.0	16.7
	13.05	16	507	136	49	47	6.0	2.4	11.8	19.2	20.0
	15.05	18	523	197	32	49	6.3	2.1	15.6	22.6	25.0
	16.05	19	539	215	59	46	5.9	2.1	16.9	21.3	28.3
	17.05	20	500	248	63	45	6.7	2.7	15.0	20.9	26.1
	18.05	21	524	268	75	46	9.0*	3.2	14.6	18.4	23.4
MEAN			514	144	50	46	6.0	2.5	13.5	19.4	21.8
Std. Deviation			20	81	13	2	1.2	0.5	2.1	1.8	3.8
3	02.06	36	557	45	30	61	5.3	3.4	11.0	18.0	22.4
	05.06	39	581	53	30	63	6.6	4.3	10.4	17.4	21.3
	06.06	40	549	53	41	60	5.2	2.5	13.7	16.7	24.3
	07.06	41	528	57	51*	60	6.3	3.4	14.6	18.4	26.7
	08.06	42	570	56	32	63	3.8	1.5	13.9	17.8	23.4
	09.06	43	510	52	30	66	3.2	1.0	12.4	19.6	22.6
	10.06	44	442	46	32	62	5.3	3.1	11.8	17.3	23.5
	12.06	46	462	46	26	58	4.6	2.0	14.6	18.5	25.8
MEAN			525	51	34	61	5.0	2.6	12.8	18.0	23.8
Std. Deviation			51	5	8	2	1.1	1.1	1.6	0.9	1.8
4	15.06	49	478	40	30	58	1.7	0.6	11.3	25.2	23.7
	16.06	50	496	34	18	59	2.4	0.6	11.1	25.0	24.8
	17.06	51	492	38	26	60	2.9	1.1	15.2	26.9	28.2
	18.06	52	496	40	26	60	2.5	0.8	12.6	26.1	28.0
	19.06	53	500	40	26	60	0.6	0.3	12.6	26.1	25.6
	20.06	54	504	50	30	59	2.5	1.0	13.1	24.9	24.3
MEAN			494	41	26	59	2.1	0.7	12.7	25.7	25.8
Std. Deviation			9	5	4	1	0.9	0.3	1.5	0.8	1.9

Table B3 (cont.): Daily COD, TKN and Nitrates concentration results for the parent laboratory-scale MLE control activated sludge system.

Sew. Batch No.	Dates of Test	Day No.	COD(mgCOD/ℓ)			TKN(mgN/ℓ)			NITRATES(mgN/ℓ)		
			Unfil. Inf.	Unf. Eff.	Fil. Eff.	Unfil. Inf.	Unf. Eff.	Fil. Eff.	Anoxic	Aerobic	Eff.
5	23.06	57	506	45	41	51	0.7	0.4	7.8	17.3	21.6
	24.06	58	506	41	31	53	2.4	0.4	7.0	15.7	18.3
	25.06	59	510	41	16	50	2.1	1.3	4.9	10.5	17.3
	27.06	61	481	35	24	48	2.8	1.5	6.1	12.2	16.7
	29.06	63	498	49	31	45	3.8	0.4	8.2	17.7	17.2
	30.06	64	500	44	32	47	2.5	1.0	8.1	17.5	11.9
MEAN			500	42	29	49	2.4	0.8	7.0	15.2	17.2
Std. Deviation			10	5	8	3	1.0	0.5	1.3	3.1	3.1
6	11.07	75	572	46	26	41	2.9	1.3	8.4	17.7	16.8
	12.07	76	680*	54	30	49	1.3	0.6	6.0	14.0	16.8
	13.07	77	532	38	24	48	4.6	2.9	6.1	13.5	15.6
	14.07	78	500	58	38	44	1.7	0.7	7.2	14.4	15.1
	17.07	81	440	42	26	46	4.5	2.0	9.8	18.1	17.5
	18.07	82	460	36	30	48	4.8	3.8	6.8	13.8	15.9
	19.07	83	456	38	30	47	5.2	4.3	8.3	14.6	16.5
	20.07	84	484	46	36	47	3.9	3.1	10.1	16.3	17.4
	22.07	86	472	34	24	48	4.3	2.8	11.4	19.3	19.3
	23.07	87	435	41	18	45	3.2	1.7	12.4	19.8	18.5
	24.07	88	496	10*	4*	48	3.6	2.8	14.3	21.1	19.8
	25.07	89	451	43	16	42	3.6	1.3	14.5	21.5	20.6
MEAN			498	40	25	46	3.6	2.3	9.6	17.0	17.5
Std. Deviation			70	12	9	3	1.2	1.2	3.0	3.0	1.7
7	27.07	91	410	33	35	60	3.6	2.7	24.0	36.8	37.7
	28.07	92	415	18	14	62	3.6	1.1	24.4	35.7	37.8
	29.07	93	406	33	28	63	2.9	0.8	25.6	34.5	37.5
	31.07	95	447	37	41	66	3.6	1.5	24.1	37.0	34.1
MEAN			420	30	29	63	3.5	1.5	24.5	36.0	36.8
Std. Deviation			19	8	11	2	0.3	0.8	0.7	1.2	1.8
8A	03.08	98	494	27	23	68*	3.9	2.2	23.7	35.0	35.7
	04.08	99	519	56	45	76	3.9	2.8	23.8	35.6	35.2
	07.08	102	507	33	25	76	3.2	0.3	24.9	34.9	34.9
	08.08	103	544	58	47	76	3.6	1.0	23.3	32.2	34.1
	09.08	104	503	41	27	73	4.5	2.2	21.2	34.2	32.8
	10.08	105	490	39	23	75	3.9	3.5	20.3	35.1	33.7
	11.08	106	507	45	31	75	2.1	0.4	21.6	37.3	33.2
MEAN			509	43	31	74	3.6	1.8	22.7	34.9	34.2
Std. Deviation			18	11	11	3	0.8	1.2	1.7	1.5	1.1

Table B3 (cont.): Daily COD, TKN and Nitrates concentration results for the parent laboratory-scale MLE control activated sludge system.

Sew. Batch No.	Dates of Test	Day No.	COD(mgCOD/ℓ)			TKN(mgN/ℓ)			NITRATES(mgN/ℓ)		
			Unfil. Inf.	Unf. Eff.	Fil. Eff.	Unfil. Inf.	Unf. Eff.	Fil. Eff.	Anoxic	Aerobic	Eff.
8B	12.08	107	647	41	39	106	3.9	0.4	15.0	36.9	33.4
	14.08	109	737	65	38	106	7.8	5.2	19.8	40.2	37.6
	15.08	110	721	59	53	106	2.7	2.2	14.4	35.3	36.7
	16.08	111	700	45	43	106	3.4	0.8	17.1	34.3	37.3
MEAN			701	52	43	106	4.4	2.2	16.6	36.7	36.3
Std. Deviation			39	11	7	0	2.3	2.2	2.4	2.6	1.9
9	18.08	113	611	43	40	79	4.2	2.9	6.5	21.2	22.6
	19.08	114	611	40	28	80	5.3	1.0	7.1	22.8	19.4
	21.08	116	680	45	36	87	4.3	4.2	5.8	21.0	19.7
	22.08	117	741	55	34	96	4.9	3.4	4.3	20.9	21.2
	23.08	118	777	53	36	100	5.3	1.1	2.9	21.9	21.9
	24.08	119	782	46	42	98	5.0	4.3	0.4	20.4	20.2
	25.08	120	786	44	40	101	3.8	3.5	0.6	20.4	20.2
	26.08	121	790	44	35	107	5.6	3.1	3.1	23.1	23.8
	28.08	123	807	46	35	112	4.5	4.2	2.8	22.7	23.8
	30.08	125	711	92*	52	103	6.6	5.9	3.1	22.7	24.7
	31.08	126	890	71	50	109	6.3	4.9	2.6	21.6	23.0
MEAN			744	52	39	97	5.1	3.5	3.6	21.7	21.9
Std. Deviation			85	16	7	11	0.9	1.5	2.2	1.0	1.8
10	04.09	130	803	48	40	74	2.9	1.5	0.5	11.6	10.6
	05.09	131	803	37	15*	72	3.9	2.9	0.5	12.5	11.5
	06.09	132	807	52	42	83	1.7	0.8	0.3	11.1	9.5*
	07.09	133	824	67	25	69	1.5	1.0	0.5	12.5	11.3
	08.09	134	757	60	39	64	2.8	1.3	0.1	12.1	12.3
	09.09	135	757	56	37	62	3.4	2.7	0.2	10.7	10.7
	11.09	137	699	43	33	68	2.7	1.4	0.5	14.2*	12.3
	12.09	138	703	49	47	67	3.1	2.5	0.2	12.8	12.5
	13.09	139	761	41	29	73	4.2	2.8	0.2	11.8	10.8
	14.09	140	773	66	41	80	4.5	2.5	0.2	11.3	10.7
	15.09	141	724	64	49	83	6.9*	5.5*	0.2	11.7	11.1
	18.09	144	729	51	41	79	4.3	2.9	0.3	12.1	11.6
	19.09	145	684	66	45	84	6.2	5.0	0.6	12.8	12.2
MEAN			756	54	37	74	3.7	2.5	0.3	12.1	11.3
Std. Deviation			46	10	10	8	1.6	1.4	0.2	0.9	0.9
11	22.09	148	721	55	41	80	7.7	4.8	1.6	10.6	10.4
	23.09	149	713	53	43	78	9.2	6.0	0.2	7.1	10.9
	25.09	151	741	68	53	78	8.0	6.4	0.8	8.7	9.9
	26.09	152	737	59	49	78	8.3	5.7	0.6	9.1	12.2
	27.09	153	713	78	49	83	7.3	5.6	1.0	8.4	9.1
	28.09	154	758	59	31	84	8.3	7.4	0.4	9.1	10.8
	29.09	155	736	54	28	86	9.0	6.7	0.4	9.7	9.8
	02.10	158	700	80	56	84	8.5	6.6	2.1	12.5	11.3
	03.10	159	696	44	34	79	9.1	6.9	3.9	13.5	11.7
	04.10	160	784	60	60	81	7.8	7.0	5.4*	15.6	14.2*
MEAN			730	61	44	81	8.3	6.3	1.6	10.4	11.0
Std. Deviation			27	11	11	3	0.6	0.8	1.7	2.6	1.4

Table B3 (cont.): Daily COD, TKN and Nitrates concentration results for the parent laboratory-scale MLE control activated sludge system.

Sew. Batch No.	Dates of Test	Day No.	COD(mgCOD/ℓ)			TKN(mgN/ℓ)			NITRATES(mgN/ℓ)		
			Unfil. Inf.	Unf. Eff.	Fil. Eff.	Unfil. Inf.	Unf. Eff.	Fil. Eff.	Anoxic	Aerobic	Eff.
12	07.10	163	652	50	34	86	8.7	5.7	4.1	16.5	17.6
	08.10	164	660	44	46	89	6.3	5.9	4.3	17.8	19.8
	09.10	165	756	102*	39	86	7.7	5.2	9.4	22.4	22.3
	10.10	166	813	43	41	96	5.5	5.2	5.9	22.6	23.6
	11.10	167	703	47	37	100	7.8	5.9	7.4	21.3	22.1
	12.10	168	719	41	30	93	5.9	5.3	4.6	19.3	20.4
	13.10	169	870	55	53	98	5.9	5.3	4.3	18.3	20.5
	14.10	170	802	31	25	97	6.6	5.3	3.7	16.0	18.0
	16.10	172	810	39	31	95	7.0	5.0	8.6	21.5	18.7
	17.10	173	728	35	33	90	5.2	4.5*	4.2	18.0	19.9
MEAN			751	49	37	93	6.7	5.3	5.6	19.4	20.3
Std. Deviation			71	20	8	5	1.1	0.4	2.1	2.4	1.9
13	20.10	176	803	54	36	77	4.9	4.2	0.4	11.2	12.4
	21.10	177	739	50	24	77	5.9	4.9	0.2	10.3	10.3
	23.10	179	799	19*	17	75	7.1	5.5	0.3	11.6	10.3
	24.10	180	766	46	33	71	3.4	2.5	0.5	10.9	11.6
	25.10	181	736	40	38	68	5.2	3.1	0.3	10.2	11.2
	26.10	182	699	25	23	70	4.5	2.1	0.5	12.2	9.9
	27.10	183	676	45	30	62	3.5	3.1	0.5	11.0	9.9
	28.10	184	842	47	34	79	3.8	2.8	0.3	13.4	12.8
	30.10	186	903*	53	47	81	5.6	2.9	0.2	13.0	13.6
	31.10	187	757	47	38	79	5.7	4.3	0.2	11.9	13.1
	01.11	188	700	51	40	68	6.2	4.1	0.4	10.7	10.9
MEAN			766	43	33	73	5.1	3.6	0.3	11.5	11.5
Std. Deviation			67	11	9	6	1.2	1.1	0.1	1.1	1.3
14	04.11	191	777	47	30	82	6.7	3.4	8.3	21.0	22.0
	06.11	193	745	45	30	81	6.4	4.8	5.9	17.8	18.7
	07.11	194	727	55	43	77	6.7	6.0	5.8	16.0*	17.0
	08.11	195	740	43	39	81	6.2	4.3	9.3	20.1	20.3
	09.11	196	703	41	33	78	5.6	4.6	8.8	19.3	24.0
	10.11	197	715	51	35	78	6.0	5.0	6.7	21.0	19.4
	11.11	198	740	45	28	77	6.9	5.5	7.2	19.6	20.1
	12.11	199	715	39	26	79	7.0	6.2	8.9	20.6	21.8
	13.11	200	756	33	26	79	4.5*	2.9	8.6	21.1	22.8
MEAN			735	44	32	79	6.2	4.7	7.7	19.6	20.7
Std. Deviation			23	7	6	2	0.8	1.1	1.3	1.7	2.2

Table B3 (cont.): Daily COD, TKN and Nitrates concentration results for the parent laboratory-scale MLE control activated sludge system.

Sew. Batch No.	Dates of Test	Day No.	COD(mgCOD/ℓ)			TKN(mgN/ℓ)			NITRATES(mgN/ℓ)		
			Unfil. Inf.	Unf. Eff.	Fil. Eff.	Unfil. Inf.	Unf. Eff.	Fil. Eff.	Anoxic	Aerobic	Eff.
15	18.11	205	654	53	41	61	6.0	4.8	2.0	10.3	10.3
	19.11	206	699	63	55	63	7.3	6.0	2.3	8.9	9.7
	20.11	207	691	65	37	63	6.3	5.5	1.6	10.2	11.0
	21.11	208	719	16	8	65	6.0	5.7	1.9	11.4	10.4
	22.11	209	700	61	39	64	6.6	6.3	1.1	11.0	10.3
	23.11	210	770	47	37	68	8.4	6.4	1.1	8.1	9.3
	24.11	211	807	61	41	69	7.1	4.9	2.0	10.6	11.3
	25.11	212	791	29	16	65	4.5	2.9*	1.9	12.3	12.3
	27.11	214	721	57	41	64	7.1	5.3	4.0	13.2	12.9
	28.11	215	725	25	23	63	8.3	7.7	6.0*	14.8	13.2
MEAN			728	48	34	64	6.8	5.6	2.4	11.1	11.1
Std. Deviation			48	18	14	3	1.2	1.3	1.5	2.0	1.3
16	30.11	217	754	57	43	71	7.8	6.7	1.0	10.6	9.2
	01.12	218	729	43	43	71	6.7	5.0	1.4	8.6	10.6
	02.12	219	766	49	39	70	8.7	7.3	0.7	9.7	11.7
	04.12	221	774	47	37	78*	8.8	7.4	1.2	11.6	9.9
	05.12	222	795	52	38	72	6.3	5.6	1.6	11.8	8.4
	06.12	223	771	56	42	72	8.5	6.9	2.2	12.1	12.9
	07.12	224	735	36	20*	73	8.4	7.0	2.6	8.8	13.1
	08.12	225	783	40	38	74	7.6	6.4	1.6	9.7	10.6
	09.12	226	783	50	30	75	8.0	5.7	2.3	12.6	11.8
MEAN			766	48	37	73	7.9	6.5	1.6	10.6	10.9
Std. Deviation			22	7	7	2	0.9	0.8	0.6	1.5	1.6

Table B3 (cont.): Daily COD, TKN and Nitrates concentration results for the parent laboratory-scale MLE control activated sludge system.

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Sew. Batch No.	Dates of Test	Day No.	COD(mgCOD/ℓ)			TKN(mgN/ℓ)			NITRATES(mgN/ℓ)		
			Unfil. Inf.	Unf. Eff.	Fil. Eff.	Unfil. Inf.	Unf. Eff.	Fil. Eff.	Anoxic	Aerobic	Eff.
17	09.02	288	888	44	40	81	6.3	5.9	2.1	15.5	17.5
	10.02	289	827	54	20	85	7.4	6.4	2.4	14.9	[illegible]
	12.02	291	687	64	60	80	9.4	8.1	2.8	14.0	[illegible]
	13.02	292	695	76	24	70	9.9	7.6	1.8	14.4	[illegible]3
	14.02	293	699	48	44	74	8.0	6.7	1.9	12.8	14.4
MEAN			759	57	38	78	8.2	6.9	2.2	14.3	15.5
Std. Deviation			92	13	16	6	1.5	0.9	0.4	1.0	1.7
18	16.02	295	600	41	26	61	7.7	7.0	2.4	8.0	7.4
	17.02	296	559	11	6	63	8.0	6.3	1.7	7.7	8.2
	19.02	298	611	20	19	61	8.3	7.1	1.3	8.5	9.0
	20.02	299	643	36	18	65	7.3	6.0	1.5	9.0	7.7
	21.02	300	715	50	40	65	9.4	7.1	1.8	9.3	8.2
	23.02	302	691	40	32	56*	8.1	6.9	1.1	10.5	8.9
	24.02	303	707	48	44	65	8.8	6.9	1.8	10.5	9.6
	25.02	304	715	44	40	66	7.3	6.2	1.4	10.3	9.8
MEAN			655	36	28	63	8.1	6.7	1.6	9.2	8.6
Std. Deviation			60	14	13	3	0.7	0.5	0.4	1.1	0.9
19	12.03	319	762	62	43	85	7.3	6.0	3.2	17.3	14.7
	13.03	320	758	58	41	92	6.9	5.2	4.5	16.0	16.9
	14.03	321	655	47	60	78	8.0	6.9	2.4	16.2	14.2
	15.03	322	758	49	62	86	2.4*	0.7*	5.6	17.1	14.4
	16.03	323	729	52	60	82	6.2	5.5	2.8	15.5	14.0
	17.03	324	725	39	41	84	6.3	5.0	7.0	20.1	15.1
	18.03	325	742	66	31	88	7.3	6.7	7.7	18.8	17.2
	19.03	326	692	39	47	87	5.2	3.9	4.9	17.2	15.0
MEAN			728	52	48	85	6.2	5.0	4.8	17.3	15.2
Std. Deviation			37	10	11	4	1.8	2.0	1.9	1.5	1.2
20	24.03	331	753	51	57	74	5.6	4.3	9.5	17.1	15.4
	26.03	333	725	55	77	68	7.1*	5.5	6.8	11.6	13.9
	27.03	334	761	69	81	67	4.8	2.9	1.4	10.4	9.2
	28.03	335	822	51	59	64	5.2	4.8	1.2	8.6	8.8
	30.03	337	793	61	57	68	5.0	3.9	1.4	9.2	9.8
	31.03	338	644	69	81	70	4.3	0.7	6.7	19.8	12.8
	02.04	340	737	77	65	74	4.1	1.5	8.6	21.3	14.6
	04.04	342	693	92	2*	73	5.0	4.6	7.7	18.9	16.5
MEAN			741	65	60	70	5.1	3.5	5.4	14.6	12.6
Std. Deviation			56	14	26	4	0.9	1.7	3.5	5.2	3.0

Table B3 (cont.): Daily COD, TKN and Nitrates concentration results for the parent laboratory-scale MLE control activated sludge system.

Sew. Batch No.	Dates of Test	Day No.	COD(mgCOD/ℓ)			TKN(mgN/ℓ)			NITRATES(mgN/ℓ)		
			Unfil. Inf.	Unf. Eff.	Fil. Eff.	Unfil. Inf.	Unf. Eff.	Fil. Eff.	Anoxic	Aerobic	Eff.
21	09.04	347	774	24	28	67	5.6	3.8	7.0	16.2	12.2
	10.04	348	741	30	20	69	4.9	3.9	4.1	14.2	9.3
	11.04	349	798	52	22	72	8.5*	6.3	5.6	15.2	14.1
	12.04	350	782	32	28	63	5.2	3.1	8.0	15.6	12.2
	13.04	351	733	46	40	72	6.3	3.9	4.4	12.9	10.6
	14.04	352	749	34	28	75	4.8	2.5	6.2	16.2	13.4
	16.04	354	766	46	36	69	3.9	1.5	3.7	12.1	8.6
	17.04	355	810	48	40	68	5.5	4.1	5.7	14.8	11.1
	18.04	356	818	46	36	72	6.2	5.0	6.3	15.0	12.3
MEAN			774	40	31	70	5.6	3.8	5.7	14.7	11.5
Std. Deviation			30	10	7	3	1.3	1.4	1.4	1.4	1.8
22	23.04	361	714	38	8	71	7.7	4.2	2.2	10.3	8.7
	24.04	362	762	62	30	71	9.2	4.9	3.4	12.4	9.2
	25.04	363	709	32	26	70	8.0	5.5	2.4	10.2	7.9
	26.04	364	722	32	20	78*	8.3	2.5	3.8	11.3	10.2
	27.04	365	738	32	10	72	7.3	3.1	4.0	14.3	11.3
	30.04	368	734	30	24	72	8.3	2.1	2.4	9.2	8.3
	01.05	369	823	48	46	73	9.0	3.1	1.5	9.6	7.6
	02.05	370	802	48	42	74	8.5	2.8	5.3	13.2	9.7
	03.05	371	742	50	22	75	9.1	2.9	5.8	15.1	10.2
	04.05	372	742	89*	52	75	7.8	4.3	1.8	9.8	7.6
MEAN			749	46	28	73	8.3	3.5	3.3	11.5	9.1
Std. Deviation			37	18	15	2	0.6	1.1	1.5	2.1	1.3
23A	09.05	377	776	59	30	87	6.4	3.6	24.5	36.7	35.0
	10.05	378	789	63	42	90	6.0	3.1	25.1	36.1	36.8
	11.05	379	820	48	32	81	3.9	1.1	24.7	36.4	34.9
MEAN			795	57	35	86	5.5	2.6	24.8	36.4	35.6
Std. Deviation			23	7	6	5	1.4	1.3	0.3	0.3	1.1

Table B3 (cont.): Daily COD, TKN and Nitrates concentration results for the parent laboratory-scale Fully Aerobic control activated sludge system.

Sew. Batch No.	Dates of Test	Day No.	COD(mgCOD/ℓ)			TKN(mgN/ℓ)			NITRATES(mgN/ℓ)		
			Unfil. Inf.	Unf. Eff.	Fil. Eff.	Unfil. Inf.	Unf. Eff.	Fil. Eff.	Anoxic	Aerobic	Eff.
23B	12.05	380	812	46	32	80	6.2	3.6		58.6	55.6
	14.05	382	766	107	22	80	3.9	2.9		60.8	52.5
	15.05	383	778	44	44	83	5.9	3.9		57.1	62.6
	16.05	384	750	54	20	82	7.1	2.8		65.4	63.5
	17.05	385	818	77	42	76	7.6	4.5		60.0	59.7
MEAN			785	66	32	80	6.1	3.6		60.4	58.8
Std. Deviation			30	26	11	3	1.4	0.7		3.1	4.7
24	21.05	389	802	67	18	57	8.3	2.5		33.0	36.5
	22.05	390	790	20	4	68	9.5	6.2		33.2	35.9
	23.05	391	808	49	29	59	6.4	4.5		29.1	31.7
	24.05	392	800	35	27	51	5.0	2.8		25.2	29.3
	25.05	393	771	69	27	55	6.9	5.0		28.6	25.9
	26.05	394	816	69	18	53	6.3	4.5		23.8	20.8
MEAN			798	52	20	57	7.1	4.2		28.8	30.0
Std. Deviation			16	21	9	6	1.6	1.4		3.9	6.0
25	04.06	403	803	47	18	105	3.9	2.7		72.3	75.6
	05.06	404	827	49	8	105	4.5	3.4		71.3	73.4
	06.06	405	848	49	32	103	4.2	2.9		70.5	72.7
	07.06	406	815	51	22	101	4.8	2.7		65.3	71.4
	09.06	408	783	75	8	106	3.9	0.4		67.0	72.3
MEAN			815	54	18	104	4.3	2.4		69.3	73.1
Std. Deviation			25	12	10	2	0.4	1.1		3.0	1.6
26	14.06	413	767	41	20	68	5.0	3.2		49.6	52.5
	15.06	414	820	41	31	69	4.6	4.3		49.8	53.1
	16.06	415	755	16	6	68	4.9	3.5		49.0	52.3
	17.06	416	816	39	31	80	4.1	3.6		50.2	52.4
	18.06	417	779	18	14	73	3.9	2.9		51.6	52.8
MEAN			787	31	20	72	4.5	3.5		50.0	52.6
Std. Deviation			29	13	11	5	0.5	0.5		1.0	0.3

Table B4: Daily MLTSS, MLVSS, MLCOD, MLTKN, DSVI and OUR results for the parent laboratory-scale MLE control activated sludge system.

YEAR 2000

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)
1	28.04	1	2815	2436	3744	207	79	38.7
	29.04	2	2741	2325	3224	198	88	37.2
	01.05	4	2491	2263	3139	202	113	37.3
	02.05	5	2473	2148	3030	198	129	36.9
	03.05	6	2734	2430	3414	186	121	39.2
	04.05	7	2607	2278	3331	183	134	37.4
	05.05	8	2696	2360	3280	184	135	38.1
MEAN			2651	2320	3309	194	114	37.8
Std. Deviation			136	133	229	10	23	0.8
2	08.05	11	2619	2311	3140	206	237	38.6
	09.05	12	2437	2110	2920	183	260	39.1
	10.05	13	2308	1980	2840	172	269	38.0
	11.05	14	2283	2019	2718	166	267	38.5
	12.05	15	2366	2042	2758	164	271	37.2
	13.05	16	2121	1757	2697	157	308	38.3
	15.05	18	2039	1783	2555	153	329	34.3*
	16.05	19	1917	1630	2535	138	430	37.9
	17.05	20	1835	1600	2479	134	477	36.2
	18.05	21	1850	1551	2418	131	553	39.5
MEAN			2177	1878	2706	160	340	37.8
Std. Deviation			270	255	221	23	108	1.5
3	02.06	36	4553	3994	4308	259	75	36.8
	05.06	39	4108	3518	4389	267	84	37.6
	06.06	40	4797	4109	4125	264	66	37.2
	07.06	41	4104	3561	4389	265	93	36.0
	08.06	42	3828	3315	4257	265	91	37.4
	09.06	43	3305	2878	4357	277*	106	36.7
	10.06	44	3468	3000	4237	263	99	37.7
	12.06	46	3317	2867	3875*	263	110	36.5
MEAN			3935	3405	4242	265	90	37.0
Std. Deviation			572	491	173	5	15	0.6
4	15.06	49	4311	3719	5422	305	91	40.3
	16.06	50	4232	3661	5322	286	92	40.1
	17.06	51	4149	3550	5262	299	90	39.1
	18.06	52	3996	3428	5181	304	91	41.1
	19.06	53	3991	3416	4717	273	90	44.8
	20.06	54	3872	3394	4919	298	96	43.9
MEAN			4092	3528	5137	294	92	41.5
Std. Deviation			173	145	267	12	2	2.3

Table B4 (cont.): Daily MLTSS, MLVSS, MLCOD, MLTKN, DSVI and OUR results for the parent laboratory-scale MLE control 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)
5	23.06	57	3511	3026	4427	263	101	37.7
	24.06	58	3582	3087	4468	279	96	37.7
	25.06	59	3585	3145	4345	258	102	36.9
	27.06	61	3513	3044	4182	267	96	35.4
	29.06	63	3347	2945	4223	235	98	34.4
	30.06	64	3444	3020	4420	227	101	35.2
MEAN			3497	3044	4344	255	99	36.2
Std. Deviation			104	77	117	20	3	1.4
6	11.07	75	3254	2794	3760	224	95	36.8
	12.07	76	3391	2939	3960	223	85	36.2
	13.07	77	3208	2766	3720	233	87	35.5
	14.07	78	3000	2632	3880	242	102	34.0
	17.07	81	3016	2647	3580	224	114	28.5
	18.07	82	2891	2446	3460	226	120	33.6
	19.07	83	2803	2371	3420	227	139	32.3
	20.07	84	2915	2526	3480	220	143	30.1
	22.07	86	2941	2523	3520	235	152	33.6
	23.07	87	2979	2579	3129	209	154	31.7
	24.07	88	3107	2715	3190	222	133	31.1
	25.07	89	2884	2520	3353	209	122	28.9
MEAN			3032	2622	3538	224	120	32.7
Std. Deviation			204	186	257	9	25	2.7
7	27.07	91	2762	2372	3231	245	118	36.3
	28.07	92	2679	2336	2946	218	124	35.1
	29.07	93	2573	2248	3089	202	135	35.7
	31.07	95	2772	2434	2967	189	138	34.8
MEAN			2697	2348	3058	214	129	35.5
Std. Deviation			105	88	131	24	9	0.7
8A	03.08	98	2704	2375	3069	209	170	40.2
	04.08	99	2485	2175	3275	192	173	39.7
	07.08	102	2782	2424	3255	214	181	39.7
	08.08	103	2958	2608	3729	221	249	44.9
	09.08	104	2947	2665	3399	224	257	43.2
	10.08	105	2983	2617	3440	228	252	42.9
	11.08	106	2873	2494	3481	223	187	41.5
MEAN			2819	2480	3378	216	210	41.7
Std. Deviation			187	177	208	12	41	2.0

Table B4 (cont.): Daily MLTSS, MLVSS, MLCOD, MLTKN, DSVI and OUR results for the parent laboratory-scale MLE control 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)
8B	12.08	107	2983	2597	3749	232	167	37.7
	14.08	109	2702	2345	3562	219	299	47.7
	15.08	110	3225	2776	3582	234	197	44.1
	16.08	111	3237	2832	3238	224	224	45.3
MEAN			3037	2638	3533	227	221	43.7
Std. Deviation			310	270	214	7	56	4.3
9	18.08	113	3163	2696	3360	228	219	34.4
	19.08	114	2848	2503	3643	242	191	36.6
	21.08	116	2829	2469	3198	218	209	36.7
	22.08	117	2814	2190	3502	228	193	40.2
	23.08	118	2711	2356	3805	211	178	43.5
	24.08	119	3101	2750	3058	199	195	40.7
	25.08	120	3184	2746	3390	211	190	39.6
	26.08	121	3213	2766	3536	222	201	38.5
	28.08	123	3073	2652	3224	218	177	39.6
	30.08	125	2954	2517	3078	219	174	37.7
	31.08	126	3119	2780	3723	245	155	41.0
MEAN			3001	2584	3411	222	189	39.0
Std. Deviation			289	291	255	13	18	2.5
10	04.09	130	2779	2370	3619	183	198	33.5
	05.09	131	2693	2251	3536	183	203	32.1
	06.09	132	2648	2305	3474	169	207	32.8
	07.09	133	2578	2270	3349	179	226	34.3
	08.09	134	2925	2576	3742	177	173	36.0
	09.09	135	2805	2455	3577	192	219	35.6
	11.09	137	2977	2544	3290	184	177	33.9
	12.09	138	2942	2595	3577	214	167	36.1
	13.09	139	3095	2686	3290	197	176	35.8
	14.09	140	2916	2526	3290	218	170	36.2
	15.09	141	2898	2531	3372	241	172	37.3
	18.09	144	2930	2583	3400	215	164	36.8
	19.09	145	2741	2335	3359	233	158	27.9*
MEAN			2841	2464	3452	199	185	34.5
Std. Deviation			184	169	147	23	22	2.5
11	22.09	148	2889	2493	3154	230	178	27.1
	23.09	149	2727	2389	3011	224	180	31.2
	25.09	151	2811	2371	3031	239	161	34.0
	26.09	152	2914	2561	3482*	249	167	30.3
	27.09	153	3188	2320	3133	277*	146	33.1
	28.09	154	3488	2952	3031	235	178	28.9
	29.09	155	3301	2900	3040	225	162	33.9
	02.10	158	2921	2560	2920	240	155	29.7
	03.10	159	2820	2448	2880	235	144	32.2
	04.10	160	2901	2485	3020	230	119*	31.9
MEAN			2996	2548	3070	238	159	31.2
Std. Deviation			267	237	166	16	19	2.2

Table B4 (cont.): Daily MLTSS, MLVSS, MLCOD, MLTKN, DSVI and OUR results for the parent laboratory-scale MLE control 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)
12	07.10	163	3084	2678	3880	265	124	33.5
	08.10	164	2980	2583	3680	273	129	35.8
	09.10	165	2647	2303	3231	218*	145*	35.2
	10.10	166	2955	2562	3475	249	138	38.2
	11.10	167	3052	2599	3820	243	132	38.0
	12.10	168	3041	2612	3637	253	132	38.5
	13.10	169	3100	2683	4023	265	119	39.6
	14.10	170	3124	2720	3783	274	130	36.4
	16.10	172	3179	2745	4112	259	128	37.4
	17.10	173	3471	3026*	4050	257	125	36.7
MEAN			3063	2651	3769	256	130	36.9
Std. Deviation			211	185	274	17	7	1.8
13	20.10	176	3210	2668	4268	257	131	35.6
	21.10	177	3180	2623	3858	252	137	33.4
	23.10	179	3134	2602	3870	251	155	31.1
	24.10	180	3090	2659	4121	250	176	32.4
	25.10	181	3181	2676	4351	262	144	33.1
	26.10	182	3136	2551	4247	265	138	32.1
	27.10	183	3218	2636	4149	240	132	30.7
	28.10	184	3130	2547	4331	277*	129	32.2
	30.10	186	3180	2674	3886	247	114	40.8
	31.10	187	3138	2610	3744	249	106	41.7
	01.11	188	3253	2591	4149	256	95	39.3
MEAN			3168	2622	4089	255	133	34.8
Std. Deviation			72	74	213	10	22	4.0
14	04.11	191	2839	2464	3724	232	98	39.1
	06.11	193	2866	2474	3623	238	99	37.6
	07.11	194	2860	2439	3556	245	92	38.1
	08.11	195	2776	2395	3536	248	94	37.4
	09.11	196	2678	2403	2560*	183*	75	39.7
	10.11	197	2914	2334	3170	211	76	38.1
	11.11	198	2937	2467	3576	223	84	39.1
	12.11	199	2799	2276	3434	230	73	36.9
	13.11	200	2913	2424	3536	229	90	38.5
MEAN			2842	2408	3413	227	87	38.3
Std. Deviation			87	77	354	20	10	0.9

Table B4 (cont.): Daily MLTSS, MLVSS, MLCOD, MLTKN, DSVI and OUR results for the parent laboratory-scale MLE control 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)
15	18.11	205	2911	2470	3678	235	111	33.1
	19.11	206	2998	2536	3759	237	102	32.6
	20.11	207	2941	2574	3678	223	103	35.1
	21.11	208	2956	2531	3576	243	107	35.8
	22.11	209	3021	2600	3850	234	115	34.5
	23.11	210	2866	2463	3666	243	110	36.2
	24.11	211	2922	2492	3686	235	109	36.8
	25.11	212	3015	2584	3604	229	108	36.5
	27.11	214	2698	2242	3850	202*	118	30.9
	28.11	215	2845	2369	3871	214	107	34.2
MEAN			2917	2483	3722	230	109	34.6
Std. Deviation			125	132	105	13	5	1.9
16	30.11	217	3019	2642	3727	246	114	38.2
	01.12	218	3020	2576	3656	251	106	36.5
	02.12	219	2956	2582	3809	251	113	37.2
	04.12	221	3049	2626	3809	253	107	37.1
	05.12	222	3028	2583	3614	251	102	39.0
	06.12	223	3294	2846	3755	270*	101	39.5
	07.12	224	3223	2801	3755	258	99	39.4
	08.12	225	3246	2807	3815	246	107	39.0
	09.12	226	3343	2837	3936	253	99	37.8
MEAN			3131	2700	3764	253	105	38.2
Std. Deviation			151	126	95	7	6	1.1

Table B4 (cont.): Daily MLTSS, MLVSS, MLCOD, MLTKN, DSVI and OUR results for the parent laboratory-scale MLE control activated sludge system.

YEAR 2001

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)
17	09.02	288	2630	2307	3434	193	126	43.2
	10.02	289	2680	2367	3474	239	118	44.8
	12.02	291	2661	2323	2922	239	127	45.8
	13.02	292	2739	2577	3213	171	131	42.8
	14.02	293	2764	2456	3634	224	115	35 4
MEAN			2695	2406	3335	213	123	42.4
Std. Deviation			74	127	276	30	7	4.1
18	16.02	295	2809	2487	2709	206	110	37 5
	17.02	296	2707	2393	2778	235	114	35.7
	19.02	298	2833	2534	2917	216	115	37 0
	20.02	299	2642	2298	3153	188	113	37.1
	21.02	300	2879	2546	3534	215	94	36.6
	23.02	302	2644	2284	3474	195	114	40.3*
	24.02	303	2744	2399	3173	204	97	37.8
	25.02	304	2614	2328	3213	204	107	38 1
MEAN			2734	2409	3119	208	108	37.5
Std. Deviation			137	138	301	14	8	1 4
19	12.03	319	3473	3065	4099	237	207	40.6
	13.03	320	3444	3055	4202	253	207	41 7
	14.03	321	3372	2992	3502*	249	229	42.1
	15.03	322	3415	3052	4285	225	227	43.1
	16.03	323	3571	3157	4314	233	281	40.6
	17.03	324	3396	3019	4192	231	257	40.6
	18.03	325	3365	2972	4017	263	250	42.3
	19.03	326	3428	3025	3976	256	231	41.7
MEAN			3433	3042	4073	243	236	41.6
Std. Deviation			78	80	260	14	25	0.9
20	24.03	331	3288	2799	3785	242	311	36.6
	26.03	333	3299	2850	3805	235	333	38.2
	27.03	334	3255	2759	4149	255	281	41.1
	28.03	335	3290	2641	3906	242	283	41.8
	30.03	337	3289	2793	4163	237	247	43.0
	31.03	338	3180	2634	3967	231	262	43.2
	02.04	340	3297	2867	3967	238	220	41.6
	04.04	342	3253	2737	3747	236	243	42.0
MEAN			3269	2760	3936	239	272	40.9
Std. Deviation			76	106	158	7	37	2.3

Table B4 (cont.): Daily MLTSS, MLVSS, MLCOD, MLTKN, DSVI and OUR results for the parent laboratory-scale MLE control 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)
21	09.04	347	3311	2667	3848	233	215	38.6
	10.04	348	3482	2987	3968	238	215	35.9
	11.04	349	3485	3045	3988	237	228	34.5
	12.04	350	3413	2944	3968	239	232	35.7
	13.04	351	3247	2845	3828	226	262*	36.2
	14.04	352	3444	3020	3988	238	243	35.2
	16.04	354	3254	2794	3747	264	241	38.2
	17.04	355	3391	2939	3988	256	220	36.7
	18.04	356	3208	2766	3848	242	229	37.0
MEAN			3359	2890	3908	241	232	36.4
Std. Deviation			154	164	91	11	15	1.3
22	23.04	361	2909	2587	3629	232	218	36.7
	24.04	362	2887	2573	3972	238	200	33.4
	25.04	363	3079	2736	3568	230	201	38.6
	26.04	364	2973	2648	3951	235	192	37.7
	27.04	365	3156	2731	3931	183*	188	32.7
	30.04	368	3186	2765	4133	211	177	38.6
	01.05	369	3166	2763	4012	223	192	35.6
	02.05	370	3201	2840	3911	230	199	37.9
	03.05	371	3309	2937	3498	229	208	41.2
	04.05	372	3201	2785	4012	228	205	38.6
MEAN			3107	2736	3862	224	198	37.1
Std. Deviation			172	143	216	16	12	2.6
23A	09.05	377	3716	3050	4484	244	183	40.6
	10.05	378	3740	3072	4262	265	171	41.3
	11.05	379	3866	3212	4484	248	186	42.3
MEAN			3774	3111	4410	252	180	41.4
Std. Deviation			81	88	128	11	8	0.9

Table B4 (cont.): Daily MLTSS, MLVSS, MLCOD, MLTKN, DSVI and OUR results for the parent laboratory-scale MLE control 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)
23B	12.05	380	3856	3224	4424	242	166	46.3
	14.05	382	3752	3134	4415	267	160	48.3
	15.05	383	3878	3318	4455	263	155	47.6
	16.05	384	3760	3180	4334	265	149	47.6
	17.05	385	3842	3246	4516	247	156	49.8
MEAN			3818	3220	4429	257	157	47.9
Std. Deviation			58	69	66	11	6	1.3
24	21.05	389	3974	3444	5020	246	141	44.4
	22.05	390	4064	3492	5080	298	148	45.9
	23.05	391	4294	3750	5324	255	140	43.7
	24.05	392	4358	3804	4978	236	147	42.9
	25.05	393	4010	3508	5018	261	150	40.2
	26.05	394	4206	3754	5080	251	143	41.2
MEAN			4151	3625	5083	258	145	43.0
Std. Deviation			158	160	125	21	4	2.1
25	04.06	403	4546	3896	5293	271	123	54.3
	05.06	404	4474	3830	5273	271	116	53.6
	06.06	405	4526	3650	5415	277	106	54.7
	07.06	406	4422	3606	5334	278	100	53.7
	09.06	408	4310	3648	5253	288	93	52.3
MEAN			4456	3726	5313	277	108	53.7
Std. Deviation			95	128	64	7	12	0.9
26	14.06	413	4360	3580	4957	251	92	47.1
	15.06	414	4220	3480	5161	258	95	46.1
	16.06	415	4324	3624	4937	244	83	44.7
	17.06	416	4282	3476	5365	249	84	47.8
	18.06	417	4356	3468	5141	247	73	43.9
MEAN			4308	3526	5112	250	85	45.9
Std. Deviation			58	72	175	6	8	1.6

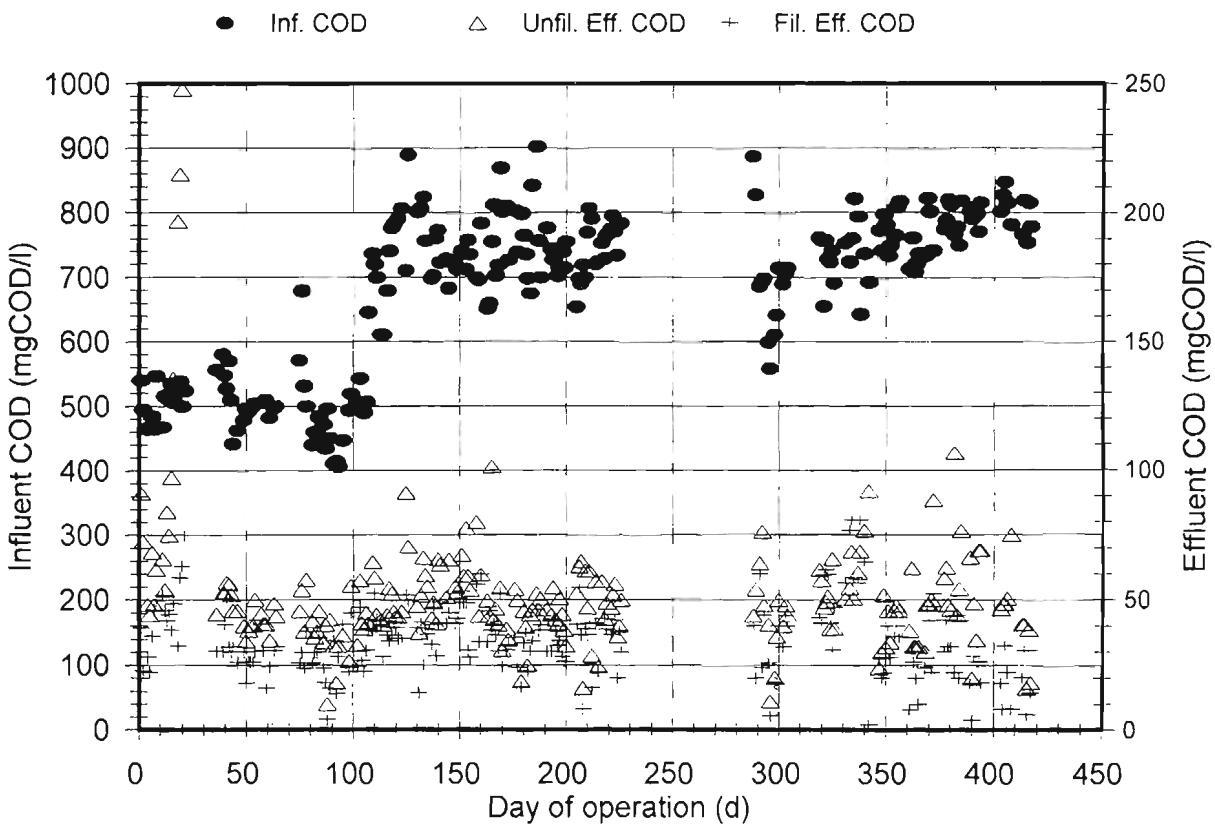

Figure B1: Graphical representation of the daily influent, unfiltered effluent and filtered effluent COD concentrations for the parent control laboratory-scale activated sludge system.

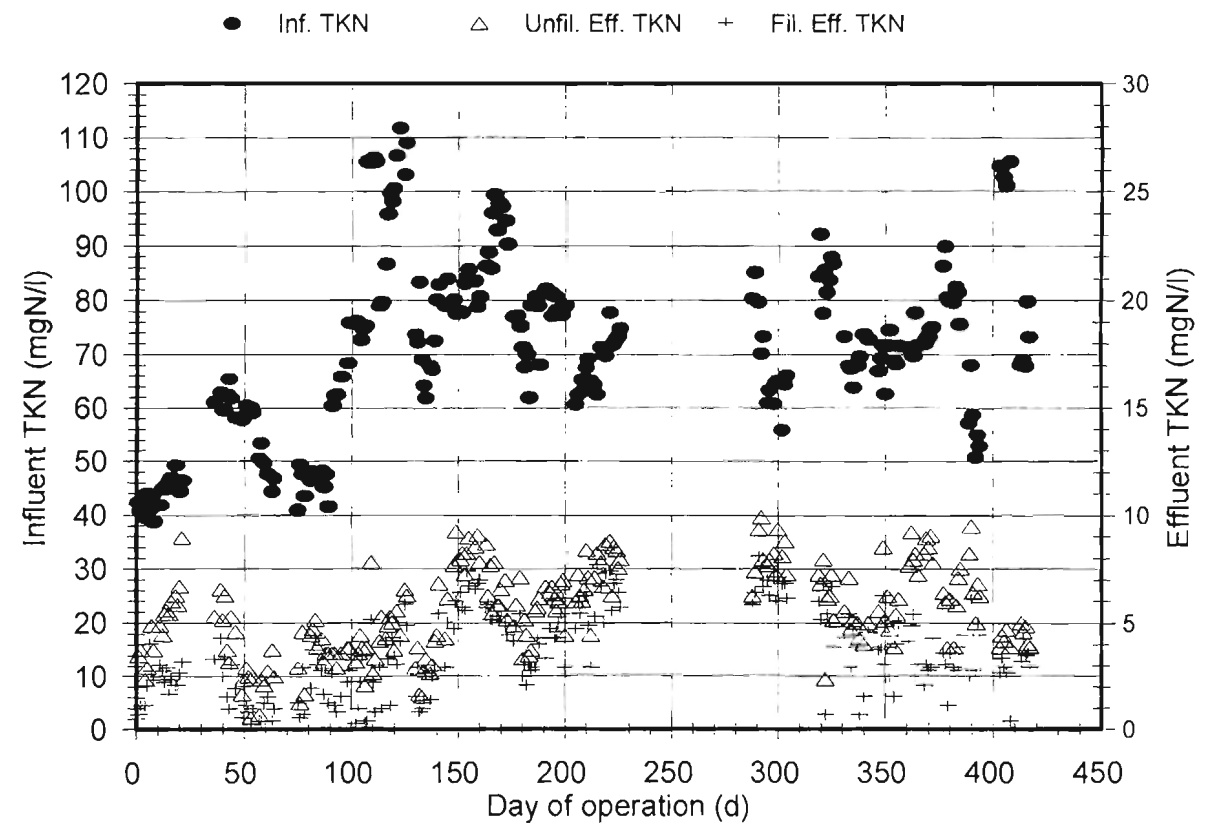

Figure B2: Graphical representation of the daily influent, unfiltered effluent and filtered effluent TKN concentrations for the parent control laboratory-scale activated sludge system.

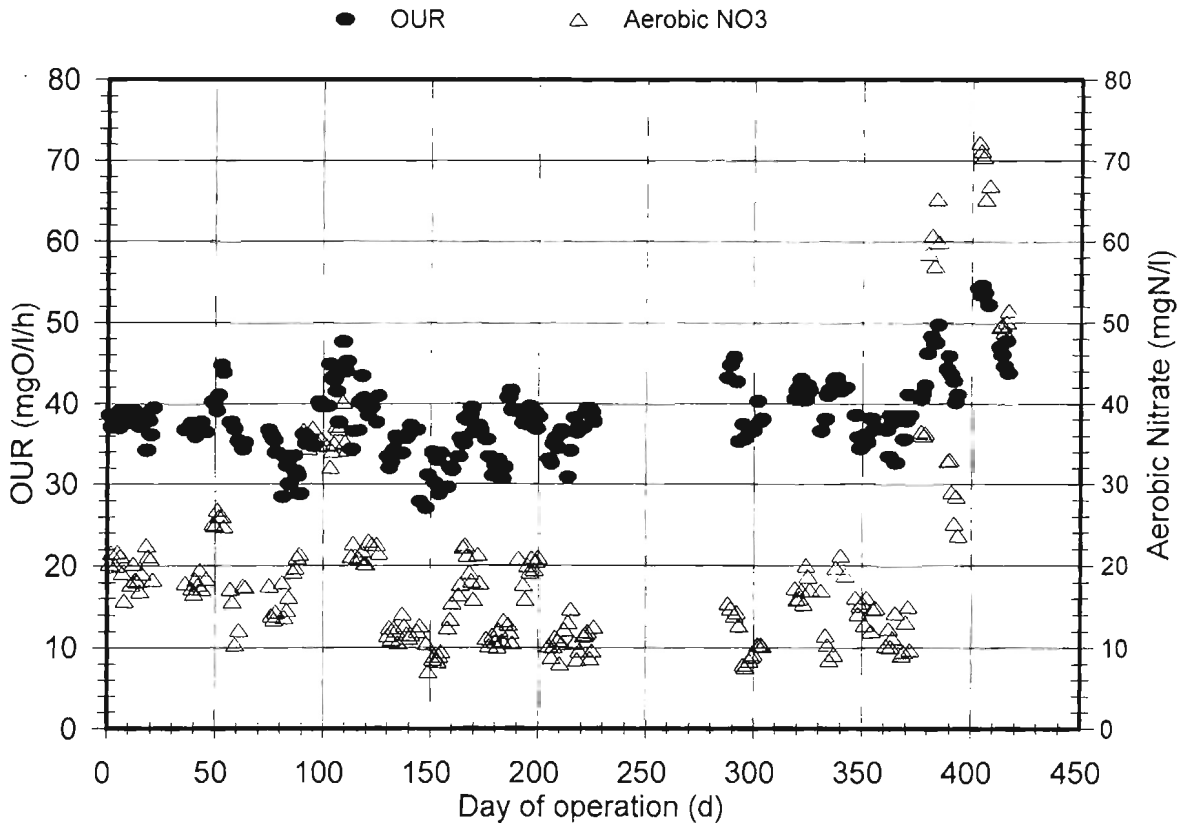

Figure B3: Graphical representation of the daily oxygen utilization rate (OUR) and nitrate (NO_3) co for the aerobic reactor of the parent control laboratory-scale activated sludge system.

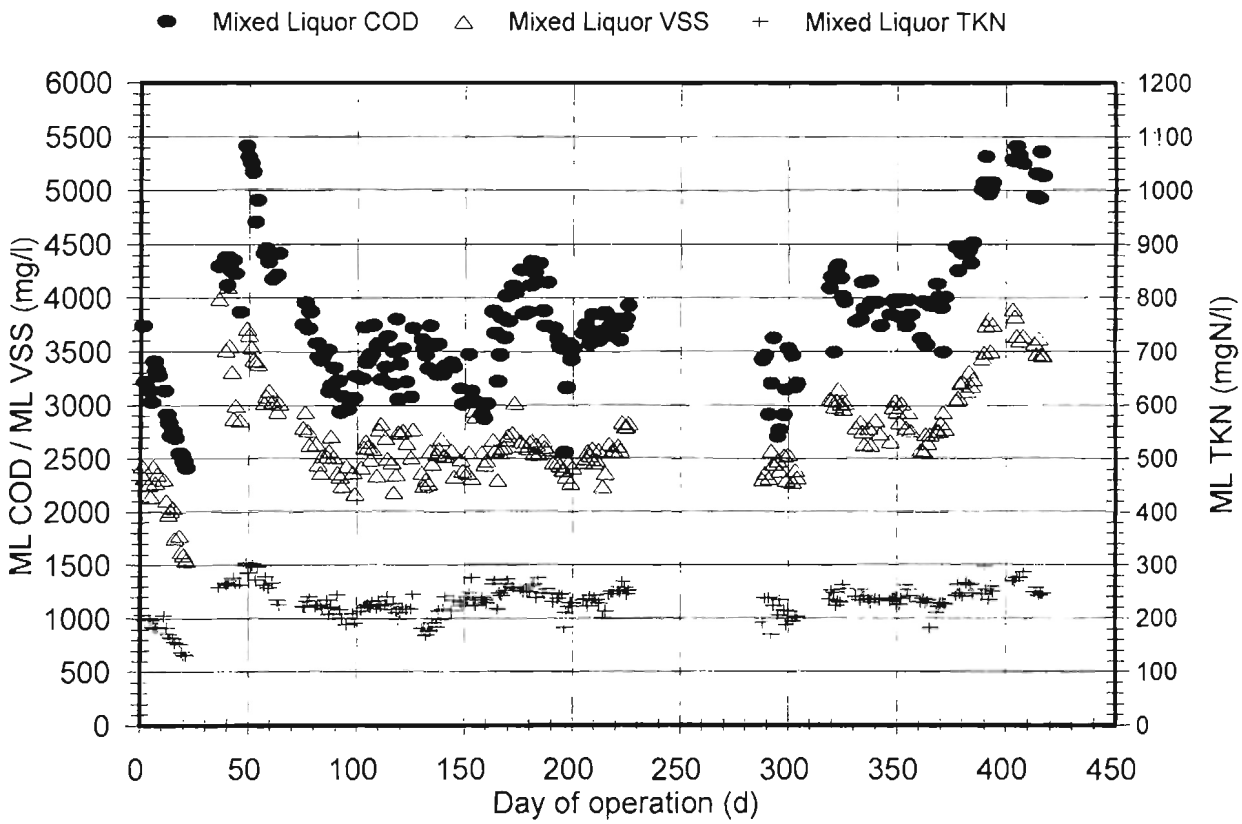

Figure B4: Graphical representation of the daily mixed liquor COD, VSS and TKN concentrations for the parent control laboratory-scale activated sludge system.

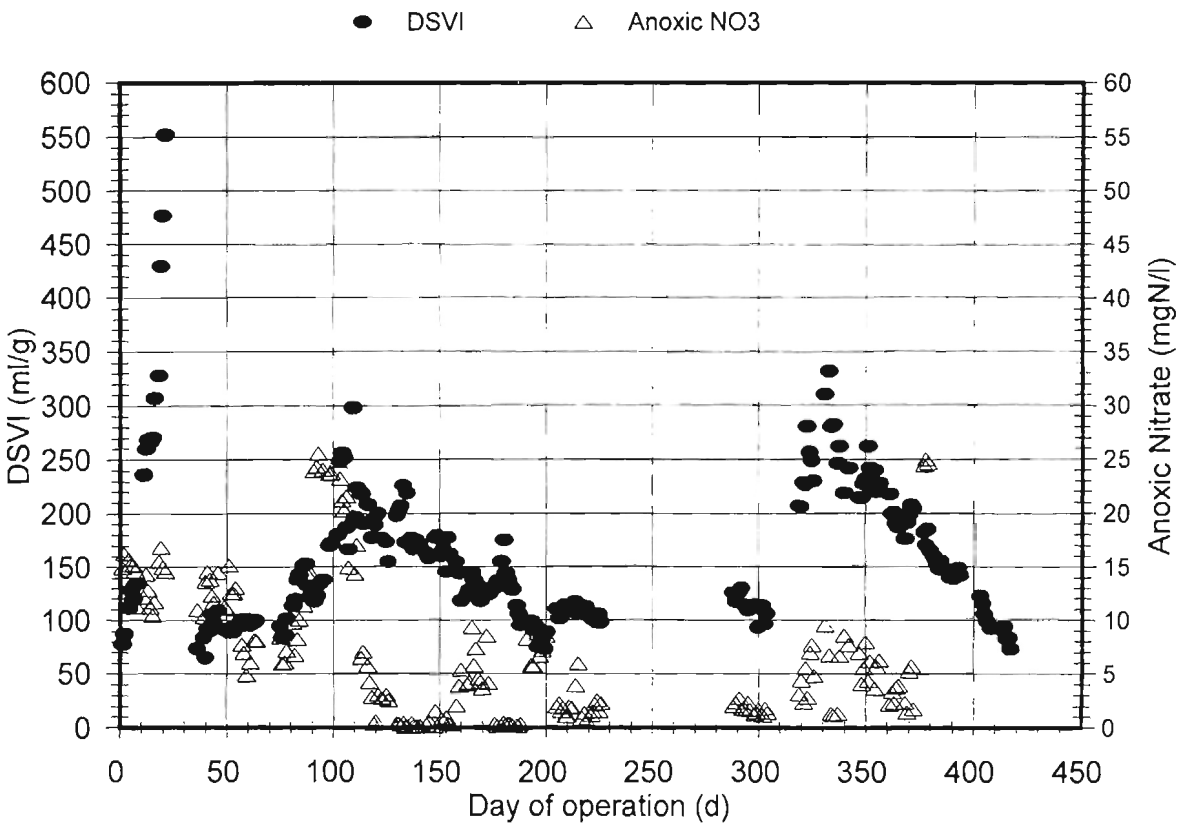

Figure B5: Graphical representation of the daily Diluted Sludge Volume Index (DSVI) and anoxic nitrate (NO_3) concentration for the parent control laboratory-scale activated sludge system

APPENDIX C

COMPREHENSIVE DATA FOR THE PARENT LABORATORY-SCALE *EXPERIMENTAL* NITRIFICATION / DENITRIFICATION ACTIVATED SLUDGE SYSTEM

TABLE OF CONTENTS

Table C1: Summary of the parent experimental system TKN and Nitrate statistical data for the various sewage batches.

Sewage Batch No.	TKN (mgN/ℓ)									NITRATES (mgN/ℓ)								
	INFLUENT			UNFIL.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
3	61	2	8	4.8	1.2	8	303	10	8	12.5	1.2	8	20.7	1.1	8	22.6	1.5	8
4	59	1	6	2.4	1.1	6	318	19	6	11.6	0.7	6	20.3	1.0	6	23.4	2.3	6
5	49	3	6	3.1	0.9	6	265	19	6	9.0	1.7	6	17.4	2.0	6	20.1	2.9	6
6	46	2	12	4.5	1.3	12	231	13	12	9.5	2.0	12	16.4	1.8	12	16.8	1.4	12
7	61	4	4	6.4	5.1	4	214	2	4	22.1	0.5	4	32.0	1.2	4	33.9	0.4	4
8A	74	1	7	6.2	1.8	7	226	7	7	20.1	1.4	7	30.6	2.0	7	32.6	0.8	7
8B	105	5	4	7.2	1.6	4	225	5	4	12.4	2.1	4	30.4	2.3	4	32.2	2.1	4
9	95	10	11	11.0	9.9	11	234	15	11	0.5	0.6	11	15.5	0.7	11	14.8	1.7	11
10	71	8	13	4.8	2.5	13	198	44	13	0.1	0.0	13	10.4	2.3	13	10.5	1.4	13
11	81	4	10	8.0	1.7	10	248	38	10	0.4	0.7	10	7.7	1.5	10	7.9	1.0	10
12	91	5	10	8.5	2.1	10	239	27	10	0.5	0.4	10	12.0	1.1	10	11.7	0.5	10
13	73	7	11	6.8	2.8	11	281	12	11	0.1	0.0	11	9.3	1.0	11	9.6	1.1	11
14	79	2	9	6.3	0.9	9	270	6	9	8.2	1.5	9	17.2	1.0	9	20.9	2.2	9
15	63	3	10	6.6	1.2	10	294	8	10	2.5	0.6	10	10.6	0.9	10	9.7	0.9	10
16	75	1	9	8.0	0.9	9	302	10	9	2.2	0.5	9	11.3	1.4	9	12.0	1.4	9
17	75	8	5	8.2	2.1	5	240	21	5	2.3	1.4	5	12.0	0.8	5	13.7	1.4	5
18	63	3	8	9.2	1.8	8	227	13	8	0.7	0.1	8	7.7	1.7	8	8.0	0.7	8
19	83	5	8	7.8	0.9	8	266	15	8	2.3	1.2	8	15.8	1.6	8	14.8	1.6	8
20	71	2	8	6.6	1.4	8	247	13	8	3.2	1.1	8	11.3	2.5	8	[illegible]	2.5	8

Table C2: Summary of the parent experimental system COD, OUR and VSS statistical data for the various sewage batches.

Sewage 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
3	525	51	8	56	7	8	4381	122	8	40.2	1.5	8	3045	78	8
4	494	9	6	39	7	6	5399	233	6	41.3	2.5	6	3745	169	6
5	500	10	6	39	7	6	4362	198	6	36.3	1.9	6	3122	86	6
6	515	68	12	58	11	12	3771	152	12	35.1	2.1	12	2792	165	12
7	483	20	4	68	60	4	3673	104	4	43.2	0.5	4	2805	89	4
8A	609	25	7	74	36	7	4126	201	7	45.4	2.1	7	3115	155	7
8B	827	39	4	72	29	4	3838	106	4	46.2	4.3	4	2934	69	4
9	886	81	11	56	10	11	4178	121	11	45.0	3.3	11	3085	136	11
10	1045	38	13	62	10	13	3944	488	13	42.8	4.2	13	2689	378	13
11	981	43	10	83	28	10	4347	929	10	44.3	5.4	10	2807	552	10
12	1070	87	10	98	32	10	4265	453	10	48.4	4.1	10	3033	355	10
13	1012	86	11	63	20	11	5268	299	11	43.9	4.8	11	3653	156	11
14	935	30	9	48	11	9	4827	106	9	44.4	1.8	9	3493	82	9
15	963	37	10	46	14	10	5200	87	10	40.7	1.6	10	3966	113	10
16	992	13	9	64	10	9	5335	174	9	43.0	0.7	9	3861	93	9
17	957	107	5	69	37	5	4657	193	5	44.7	6.0	5	3147	107	5
18	799	62	8	66	26	8	4011	251	8	41.8	2.4	8	3103	178	8
19	807	34	8	66	12	8	4720	268	8	49.0	4.4	8	3482	72	8
20	904	23	8	81	19	8	4778	289	8	42.6	1.1	8	3359	92	8

Table C3: Daily COD, TKN and Nitrates concentration results for the parent laboratory-scale MLE experimental activated sludge system.

YEAR 2000

Sew. Batch No.	Dates of Test	Day No.	COD(mgCOD/ℓ)			TKN(mgN/ℓ)			NITRATES(mgN/ℓ)		
			Unfil. Inf.	Unf. Eff.	Fil. Eff.	Unfil. Inf.	Unf. Eff.	Fil. Eff.	Anoxic	Aerobic	Eff.
3	02.06	36	557	61	39	61	4.5	3.8	12.2	19.6	22.6
	05.06	39	581	63	22	63	4.9	4.5	10.9	20.4	24.4
	06.06	40	549	59	33	60	4.3	2.5	11.3	21.8	20.1
	07.06	41	528	65	43	60	3.9	2.1	12.7	20.6	23.0
	08.06	42	570	50	32	63	5.0	3.4	11.8	22.0	21.8
	09.06	43	510	56	34	66	3.5	0.7	13.8	21.5	23.2
	10.06	44	442	50	30	62	7.6*	5.3	14.0	18.7	24.6
	12.06	46	462	44	24	58	4.6	1.3	13.5	20.8	21.6
MEAN			525	56	32	61	4.8	2.9	12.5	20.7	22.6
Std. Deviation			51	7	7	2	1.2	1.6	1.2	1.1	1.5
4	15.06	49	478	38	30	58	0.4	0.1	12.8	21.4	24.6
	16.06	50	496	30	16	59	3.6	0.8	11.7	20.9	26.3
	17.06	51	492	44	30	60	2.5	1.1	11.1	20.5	25.2
	18.06	52	496	32	22	60	2.8	0.7	11.7	20.5	21.6
	19.06	53	500	40	34	60	2.1	1.4	10.9	19.0	20.3
	20.06	54	504	48	28	59	2.9	1.7	11.4	19.2	22.4
MEAN			494	39	27	59	2.4	1.0	11.6	20.3	23.4
Std. Deviation			9	7	7	1	1.1	0.5	0.7	1.0	2.3
5	23.06	57	506	49	24	51	2.4	2.1	12.2	20.4	25.6
	24.06	58	506	33	24	53	2.4	2.2	7.8	15.9	20.4
	25.06	59	510	35	22	50	2.5	1.7	8.5	17.7	18.1
	27.06	61	481	33	31	48	3.4	2.9	9.3	18.7	19.8
	29.06	63	498	47	35	45	2.9	2.5	8.6	17.2	18.2
	30.06	64	500	36	26	47	4.8	2.2	7.4	14.7	18.4
MEAN			500	39	27	49	3.1	2.3	9.0	17.4	20.1
Std. Deviation			10	7	5	3	0.9	0.4	1.7	2.0	2.9
6	11.07	75	572	38	26	41*	3.6	1.7	7.4	14.2	18.4
	12.07	76	680*	58	44*	49	2.1	1.5	7.4	13.7	16.5
	13.07	77	532	68	26	48	4.8	2.7	7.4	15.6	16.3
	14.07	78	500	70	24	44	4.3	4.1	6.7	14.2	14.4
	17.07	81	440	64	26	46	5.3	3.1	8.6	17.2	16.3
	18.07	82	460	68	20	48	4.5	3.9	9.2	16.3	14.8
	19.07	83	456	56	28	47	4.9	3.6	9.7	15.4	15.4
	20.07	84	484	70	30	47	3.9	2.8	12.2	16.7	17.7
	22.07	86	472	50	20	48	5.2	1.3	12.4	18.3	18.2
	23.07	87	545	43	10	46	5.0	1.0	9.8	19.5	18.7
	24.07	88	565	63	4	46	7.6*	2.8	10.9	17.8	18.0
	25.07	89	480	53	18	43	3.1	2.5	11.7	17.8	17.2
MEAN			515	58	23	46	4.5	2.6	9.5	16.4	16.8
Std. Deviation			68	11	10	2	1.3	1.0	2.0	1.8	1.4

Table C3 (cont.): Daily COD, TKN and Nitrates concentration results for the parent laboratory-scale MLE experimental activated sludge system.

Sew. Batch No.	Dates of Test	Day No.	COD(mgCOD/ℓ)			TKN(mgN/ℓ)			NITRATES(mgN/ℓ)		
			Unfil. Inf.	Unf. Eff.	Fil. Eff.	Unfil. Inf.	Unf. Eff.	Fil. Eff.	Anoxic	Aerobic	Eff.
7	27.07	91	471	61	22	62	4.3	2.2	22.0	33.0	34.1
	28.07	92	463	6	2	56	4.5	2.0	21.4	33.1	34.1
	29.07	93	488	55	16	60	2.8	1.7	22.5	31.1	34.0
	31.07	95	508	150	20	65	14.0	2.2	22.5	30.8	33.3
MEAN			483	68	15	61	6.4	2.0	22.1	32.0	33.9
Std. Deviation			20	60	9	4	5.1	0.3	0.5	1.2	0.4
8A	03.08	98	577	43	27	71	5.2	3.1	20.8	31.7	33.6
	04.08	99	606	146*	35	74	9.2	2.8	20.8	27.2	32.0
	07.08	102	639	66	27	74	5.9	2.5	21.3	30.2	32.7
	08.08	103	626	93	52*	73	6.4	2.8	21.7	28.8	31.3
	09.08	104	630	49	33	75	5.3	3.6	19.5	30.9	33.5
	10.08	105	606	58	29	74	3.8	3.1	18.5	32.6	32.6
	11.08	106	577	60	37	74	7.7	3.4	18.0	32.8	32.6
MEAN			609	74	34	74	6.2	3.0	20.1	30.6	32.6
Std. Deviation			25	36	9	1	1.8	0.4	1.4	2.0	0.8
8B	12.08	107	873	111	35	112	8.3	3.6	10.3	29.1	30.8
	14.08	109	785	69	36	104	8.4	6.0	15.0	32.6	32.6
	15.08	110	806	67	45	102	5.0	4.2	13.0	32.0	35.1
	16.08	111	842	43	32	102	7.3	4.8	11.2	27.9	30.4
MEAN			827	72	37	105	7.2	4.7	12.4	30.4	32.2
Std. Deviation			39	29	5	5	1.6	1.0	2.1	2.3	2.1
9	18.08	113	781	57	36	77	5.6	4.2	2.0*	16.3	17.2
	19.08	114	757	53	43	81	5.6	4.5	0.9	14.5	13.2
	21.08	116	822	49	36	87	4.5	4.2	0.7	14.3	11.9
	22.08	117	818	40	26*	86	4.8	2.9	0.3	15.7	12.7
	23.08	118	932	58	46	103	4.6	3.2	0.6	16.1	15.7
	24.08	119	953	46	35	100	5.6	5.2	0.0*	16.0	15.9
	25.08	120	894	60	50	98	17.1	6.0	0.0*	15.0	14.1
	26.08	121	940	56	50	103	4.3	3.8	0.0*	15.7	15.3
	28.08	123	953	62	44	103	9.9	5.2	0.1	16.0	15.9
	30.08	125	886	79*	52	102	32.2*	5.2	0.5	15.5	16.4
	31.08	126	1011	60	50	101	26.6	6.9	0.8	15.4	15.0
MEAN			886	56	43	95	11.0	4.7	0.5	15.5	14.8
Std. Deviation			81	10	8	10	9.9	1.2	0.6	0.7	1.7

Table C3 (cont.): Daily COD, TKN and Nitrates concentration results for the parent laboratory-scale MLE experimental activated sludge system.

Sew. Batch No.	Dates of Test	Day No.	COD(mgCOD/ℓ)			TKN(mgN/ℓ)			NITRATES(mgN/ℓ)		
			Unfil. Inf.	Unf. Eff.	Fil. Eff.	Unfil. Inf.	Unf. Eff.	Fil. Eff.	Anoxic	Aerobic	Eff.
10	04.09	130	1086	62	44	65	2.9	1.5	0.1	9.6	10.2
	05.09	131	1065	46	27	64	3.6	2.4	0.2	8.9	11.4
	06.09	132	1094	56	31	66	1.5	0.6	0.2	10.1	9.5
	07.09	133	1061	62	46	66	2.0	1.5	0.2	10.3	11.7
	08.09	134	1077	49	45	63	2.4	1.8	0.1	17.9*	13.8*
	09.09	135	1044	58	39	70	2.5	1.4	0.1	8.8	11.9
	11.09	137	1036	58	39	65	4.9	3.8	0.1	9.5	9.5
	12.09	138	995	70	53	66	5.9	4.8	0.1	9.3	9.9
	13.09	139	1020	60	29	79	7.6	6.0	0.1	9.9	8.9
	14.09	140	1073	76	49	68	6.7	5.7	0.1	9.2	9.7
	15.09	141	1061	70	47	86*	7.4	6.6	0.1	10.8	9.9
	18.09	144	983	78	49	81	6.4	5.0	0.2	10.1	9.5
	19.09	145	987	66	43	79	8.8	6.7	0.1	10.4	10.0
MEAN			1045	62	42	71	4.8	3.7	0.1	10.4	10.5
Std. Deviation			38	10	8	8	2.5	2.2	0.0	2.3	1.4
11	22.09	148	930	59	43	80	8.0	4.9	0.2	6.4	6.9
	23.09	149	930	70	45	75	6.6	5.9	0.1	6.1	7.6
	25.09	151	995	80	59	77	6.0	4.6	0.2	7.1	7.7
	26.09	152	1053	90	59	80	6.9	4.8	0.3	8.7	8.2
	27.09	153	987	70	53	82	7.3	6.9	0.2	5.5	6.6
	28.09	154	954	92	37	84	9.4	6.6	0.1	7.4	6.9
	29.09	155	964	68	46	88	7.7	6.9	0.3	8.1	8.7
	02.10	158	1032	74	56	87	7.1	5.7	0.4	9.2	8.7
	03.10	159	952	70	52	80	11.2	9.9*	2.5*	10.6	9.9
	04.10	160	1016	158*	56	81	10.2	7.3	0.0*	7.6	7.9
MEAN			981	83	51	81	8.0	6.3	0.4	7.7	7.9
Std. Deviation			43	28	8	4	1.7	1.6	0.7	1.5	1.0
12	07.10	163	940	72	42	85	6.4	1.8*	1.2	11.2	10.9
	08.10	164	968	84	52	84	7.1	6.6	0.5	12.1	12.2
	09.10	165	1012	96	41	86	6.2	5.9	0.8	13.5	11.7
	10.10	166	1057	158	47	92	9.9	6.0	0.4	11.7	11.2
	11.10	167	1065	138	49	92	12.3	6.0	0.6	13.6	12.3
	12.10	168	1044	108	49	92	9.4	5.3	0.3	10.8	11.8
	13.10	169	1126	122	53	95	7.3	5.7	0.3	11.2	11.4
	14.10	170	1135	64	47	96	10.9	9.1	0.3	10.9	11.4
	16.10	172	1234	74	41	101	8.0	5.2	1.0	13.1	11.9
	17.10	173	1118	68	39	88	7.1	4.9	0.0*	12.3	12.1
MEAN			1070	98	46	91	8.5	5.7	0.5	12.0	11.7
Std. Deviation			87	32	5	5	2.1	1.8	0.4	1.1	0.5

Table C3 (cont.): Daily COD, TKN and Nitrates concentration results for the parent laboratory-scale MLE experimental activated sludge system.

Sew. Batch No.	Dates of Test	Day No.	COD(mgCOD/ℓ)			TKN(mgN/ℓ)			NITRATES(mgN/ℓ)		
			Unfil. Inf.	Unf. Eff.	Fil. Eff.	Unfil. Inf.	Unf. Eff.	Fil. Eff.	Anoxic	Aerobic	Eff.
13	20.10	176	1213*	96	52	73	13.9*	4.5	0.1	8.7	8.6
	21.10	177	1053	78	42	73	8.8	6.3	0.2*	10.1	8.5
	23.10	179	992	50	25	74	7.8	6.6	0.1	8.7	9.2
	24.10	180	971	44	21	72	6.9	3.5	0.1	8.5	10.3
	25.10	181	958	94	54	72	4.6	2.7	0.1	8.1	9.8
	26.10	182	958	52	29	65	3.6	1.7	0.2	8.9	8.3
	27.10	183	907	47	30	63	7.8	3.4	0.1	8.7	8.4
	28.10	184	1048	38	28	78	6.2	1.1	0.1	11.3	10.9
	30.10	186	1081	63	40	83	5.7	2.0	0.1	10.9	10.8
	31.10	187	1012	75	36	81	4.2	2.1	0.2	9.9	11.0
	01.11	188	935	51	51	64	5.6	3.4	0.1	9.0	9.7
MEAN			1012	63	37	73	6.8	3.4	0.1	9.3	9.6
Std. Deviation			86	20	12	7	2.8	1.8	0.0	1.0	1.1
14	04.11	191	984	49	40	81	6.7	5.0	9.7	18.0	23.5
	06.11	193	915	51	34	78	6.3	5.0	8.4	16.7	22.8
	07.11	194	939	61	55	78	4.6	4.2	9.9	18.5	23.1
	08.11	195	910	57	41	78	6.7	5.6	10.0	17.2	23.1
	09.11	196	923	39	41	78	6.6	5.9	5.8	15.4	18.9
	10.11	197	939	55	49	81	6.4	4.9	6.6	16.8	20.3
	11.11	198	890	57	39	81	6.9	4.5	8.7	18.2	19.5
	12.11	199	943	28	26	81	7.1	5.9	7.6	16.7	18.6
	13.11	200	975	39	30	78	5.0	4.3	7.2	17.1	18.3
MEAN			935	48	39	79	6.3	5.0	8.2	17.2	20.9
Std. Deviation			30	11	9	2	0.9	0.6	1.5	1.0	2.2
15	18.11	205	996	53	49	64	7.1	5.9	2.1	10.1	9.2
	19.11	206	931	45	37	60	6.4	5.3	2.6	9.7	8.6
	20.11	207	983	55	28	58	5.6	5.3	3.0	9.9	10.1
	21.11	208	963	33	12	66	8.4	5.7	2.0	9.9	8.7
	22.11	209	958	59	51	65	6.3	3.9	1.5	9.9	8.6
	23.11	210	999	31	18	62	5.9	5.2	2.4	10.3	10.5
	24.11	211	938	66	45	64	4.5	2.9	1.9	12.1	9.6
	25.11	212	926	55	51	60	5.9	4.6	3.5	11.7	10.8
	27.11	214	1024	25	8	65	7.1	6.7	3.1	11.4	10.2
	28.11	215	913	43	12	67	8.3	7.1	3.1	11.5	10.5
MEAN			963	46	31	63	6.6	5.3	2.5	10.6	9.7
Std. Deviation			37	14	17	3	1.2	1.2	0.6	0.9	0.9
16	30.11	217	999	74	51	75	7.6	6.7	2.1	10.4	12.2
	01.12	218	975	61	41	74	8.5	5.2	1.6	9.8	14.6
	02.12	219	987	61	43	76	6.6	3.6	3.0	11.8	10.6
	04.12	221	1012	84	55	77	8.0	6.4	1.7	12.4	13.6
	05.12	222	996	64	54	75	8.5	6.3	2.2	14.2*	10.9
	06.12	223	972	66	46	77	9.5	4.3	1.7	10.7	12.6
	07.12	224	1000	52	24	72	7.8	3.1	3.0	11.6	11.6
	08.12	225	996	56	38	74	8.3	6.4	2.4	9.9	10.2
	09.12	226	992	52	24	76	7.1	4.8	1.9	10.7	11.7
MEAN			992	64	42	75	8.0	5.2	2.2	11.3	12.0
Std. Deviation			13	10	12	1	0.9	1.3	0.5	1.4	1.4

Table C3 (cont.): Daily COD, TKN and Nitrates concentration results for the parent laboratory-scale MLE experimental activated sludge system.

YEAR 2001

Sew. Batch No.	Dates of Test	Day No.	COD(mgCOD/ℓ)			TKN(mgN/ℓ)			NITRATES(mgN/ℓ)		
			Unfil. Inf.	Unf. Eff.	Fil. Eff.	Unfil. Inf.	Unf. Eff.	Fil. Eff.	Anoxic	Aerobic	Eff.
17	09.02	288	1088	52	42	81	6.9	5.6	1.1	12.2	13.4
	10.02	289	1028	44	40	85	10.4	8.5	3.2	12.6	13.4
	12.02	291	964	62	34	75	9.0	8.5	3.0	12.4	12.6
	13.02	292	819	135	36	66	5.3	4.5	3.8	12.4	13.0
	14.02	293	888	54	78	70	9.7	7.6	0.6	10.7	16.1
MEAN			957	69	46	75	8.2	6.9	2.3	12.0	13.7
Std. Deviation			107	37	18	8	2.1	1.8	1.4	0.8	1.4
18	16.02	295	852	74	37	58	10.4	6.9	0.9	6.0	7.7
	17.02	296	704	39	31	61	9.4	8.1	0.6	6.1	7.4
	19.02	298	704	48	22	61	8.0	7.7	0.6	6.6	7.1
	20.02	299	791	70	34	64	9.2	5.5	0.8	7.1	7.1
	21.02	300	819	124*	32	65	13.0*	7.4	0.6	6.9	8.5
	23.02	302	827	52	40	61	7.0	4.1	0.8	10.8	8.7
	24.02	303	851	60	52	65	8.1	6.9	0.8	8.6	8.7
	25.02	304	847	62	48	65	8.5	5.7	0.7	9.2	8.5
MEAN			799	66	37	63	9.2	6.5	0.7	7.7	8.0
Std. Deviation			62	26	10	3	1.8	1.4	0.1	1.7	0.7
19	12.03	319	791	82	49	83	7.6	3.9	1.6	15.3	15.5
	13.03	320	824	72	47	88	6.4	4.3	2.7	16.3	14.4
	14.03	321	754	70	60	72*	7.3	4.8	3.8	16.5	13.8
	15.03	322	836	56	68	86	9.0	3.9	2.9	15.8	16.4
	16.03	323	845	74	68	87	8.0	6.4	0.4	13.5	12.6
	17.03	324	824	56	64	84	7.6	6.6	1.1	16.7	12.8
	18.03	325	820	72	58	83	9.1	7.3	3.5	18.5	16.9
	19.03	326	762	45	76	85	7.8	6.7	2.2	14.0	15.8
MEAN			807	66	61	83	7.8	5.5	2.3	15.8	14.8
Std. Deviation			34	12	10	5	0.9	1.4	1.2	1.6	1.6
20	24.03	331	911	53	83	72	4.2	3.4	5.2	7.1	20.1
	26.03	333	854*	91	67	73	6.4	4.8	3.0	8.9	15.2
	27.03	334	911	63	71	68	6.4	4.2	3.4	13.4	16.2
	28.03	335	895	89	81	72	6.6	5.5	2.6	11.5	14.6
	30.03	337	903	93	79	72	9.2	5.2	2.7	10.1	17.7
	31.03	338	907	91	61	69	6.4	3.6	1.5	11.3	12.2
	02.04	340	935	107	65	69	7.7	5.0	3.5	13.7	13.4
	04.04	342	913	64	47	70	6.0	2.4	4.0	14.3	14.6
MEAN			904	81	69	71	6.6	4.3	3.2	11.3	15.5
Std. Deviation			23	19	12	2	1.4	1.1	1.1	2.5	2.5

Table C4: Daily MLTSS, MLVSS, MLCOD, MLTKN, DSVI and OUR results for the parent laboratory-scale MLE experimental activated sludge system.

YEAR 2000

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)
3	02.06	36	3622	3108	4369	311	106	41.1
	05.06	39	3610	3029	4328	297	106	42.3
	06.06	40	3594	3045	4308	298	113	39.0
	07.06	41	3408	2944	4450	316	112	38.4
	08.06	42	3439	2972	4317	308	105	41.7
	09.06	43	3578	3113	4618	299	109	39.3
	10.06	44	3623	3137	4438	307	104	40 9
	12.06	46	3476	3012	4217	286	114	38.6
MEAN			3544	3045	4381	303	109	40.2
Std. Deviation			107	78	122	10	4	1.5
4	15.06	49	4577	3955	5683	350	84	40.7
	16.06	50	4436	3838	5504	295	91	42.7
	17.06	51	4431	3808	5524	311	93	44.3
	18.06	52	4345	3733	5443	326	93	37.7
	19.06	53	4261	3665	5060	323	94	43.0
	20.06	54	4152	3472	5181	303	95	39.6
MEAN			4367	3745	5399	318	92	41.3
Std. Deviation			154	169	233	19	4	2.5
5	23.06	57	3660	3167	4570	275	99	35.8
	24.06	58	3745	3250	4631	284	97	36.8
	25.06	59	3575	3119	4284	262	108	34.6
	27.06	61	3532	3126	4202	273	103	33.9
	29.06	63	3487	2999	4345	269	92	37.4
	30.06	64	3511	3069	4140	230	92	39.1
MEAN			3585	3122	4362	265	98	36.3
Std. Deviation			101	86	198	19	6	1.9
6	11.07	75	3410	2955	4080*	226	84	36.8
	12.07	76	3393	2960	4000	239	86	38.0
	13.07	77	3208	2828	3780	227	100	37.2
	14.07	78	2967	2595	3760	218	91	35.0
	17.07	81	3290	2862	3800	229	97	34.2
	18.07	82	3211	2823	3760	234	105	33.1
	19.07	83	3132	2737	3660	236	112	31.1
	20.07	84	3096	2645	3700	235	121	32.9
	22.07	86	3234	2813	3800	262*	125	36.6
	23.07	87	2908	2566	3597	216	125	36.3
	24.07	88	3346	2919	3536	229	120	36.6
	25.07	89	3183	2805	3780	216	133	33.8
MEAN			3198	2792	3771	231	108	35.1
Std. Deviation			181	165	152	13	17	2.1

Table C4 (cont.): Daily MLTSS, MLVSS, MLCOD, MLTKN, DSVI and OUR results for the parent laboratory-scale MLE experimental 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)
7	27.07	91	3287	2872	3780	214	127	42.5
	28.07	92	3169	2801	3556	215	130	43.5
	29.07	93	3044	2684	3617	217	135	43.5
	31.07	95	3202	2865	3739	212	142	43.4
MEAN			3176	2805	3673	214	133	43.2
Std. Deviation			102	89	104	2	6	0.5
8A	03.08	98	3284	2931	3811	223	156	42.7
	04.08	99	3312	2961	3996	218	147	42.7
	07.08	102	3509	3135	4285	232	148	45.3
	08.08	103	3471	3093	4408	221	146	48
	09.08	104	3607	3215	4058	223	157	[illegible]
	10.08	105	3728	3305	4244	235	172	46.3
	11.08	106	3445	3162	4079	232	164	46.7
MEAN			3479	3115	4126	226	155	45.4
Std. Deviation			164	155	201	7	10	2.1
8B	12.08	107	3372	2998	3976	228	170	42.4
	14.08	109	3147	2873	3866	218	226	51.7
	15.08	110	3323	2963	3765	225	157	43.2
	16.08	111	3116	2902	3744	229	174	47.3
MEAN			3240	2934	3838	225	182	46.2
Std. Deviation			130	69	106	5	30	4.3
9	18.08	113	3407	2969	4048	231	220	[illegible]
	19.08	114	3398	3032	4109	232	220	43.2
	21.08	116	3459	3076	4008	239	214	42.8
	22.08	117	3387	3070	4048	228	233	41.7
	23.08	118	3347	2987	4243	232	250	44.8
	24.08	119	3436	3074	4139	204	228	45.5
	25.08	120	3554	3123	4285	214	248	48.4
	26.08	121	3448	3044	4202	238	215	48.6
	28.08	123	3402	3011	4243	244	246	46.7
	30.08	125	3647	3228	4222	256	188	46.0
	31.08	126	3675	3322	4410	252	174	49.9
MEAN			3469	3085	4178	234	221	45.0
Std. Deviation			150	136	121	15	24	3.3

Table C4 (cont.): Daily MLTSS, MLVSS, MLCOD, MLTKN, DSVI and OUR results for the parent laboratory-scale MLE experimental 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)
10	04.09	130	2420	2070	3307	153	155	41.8
	05.09	131	2445	1996	3162	146	165	41.3
	06.09	132	2440	2182	3162	129	154	33.4*
	07.09	133	2699	2420	3515	151	158	36.5
	08.09	134	3125	2831	4009	178	137	42.0
	09.09	135	3179	2847	4030	193	150	45.1
	11.09	137	3298	2948	4359	206	141	45.1
	12.09	138	3313	2999	4194	221	133	46.4
	13.09	139	3312	2958	3989	212	121	47.4
	14.09	140	3290	2953	4482	222	114	40.5
	15.09	141	3346	2976	4400	259	115	47.8
	18.09	144	3323	2899	4362	250	112	44.2
	19.09	145	3247	2880	4301	253	109	45.0
MEAN			3034	2689	3944	198	136	42.8
Std. Deviation			383	378	488	44	20	4.2
11	22.09	148	3485	3118	4731	274	104	45.8
	23.09	149	3348	3033	4772	260	107	49.6
	25.09	151	3371	3051	4895	260	108	47.2
	26.09	152	3477	3051	5222	270	99	48.8
	27.09	153	3592	3186	4813	267	94	47.2
	28.09	154	3773	3311	5161	299	95	44.6
	29.09	155	3588	3228	4660	259	100	47.5
	02.10	158	2593	2190	3660	223	117*	42.7
	03.10	159	2080	1870	2840	182	94	32.7*
	04.10	160	2277	2033	2720	188	94	37.2
MEAN			3158	2807	4347	248	101	44.3
Std. Deviation			611	552	929	38	8	5.4
12	07.10	163	3068	2763	3880	217	86	44.1
	08.10	164	2967	2666	3840	229	100	44.7
	09.10	165	2953	2679	3759	218	97	44.1
	10.10	166	3294	2980	3840	195	115	47.8
	11.10	167	3150	2804	4389	223	101	45.9
	12.10	168	3478	3106	4288	256	113	46.9
	13.10	169	3466	3140	4491	275	112	49.8
	14.10	170	3707	3333	4626	267	101	52.4
	16.10	172	3744	3371	4338	238	108	52.2
	17.10	173	3893	3491	5202	271	94	56.0
MEAN			3372	3033	4533	239	103	48.4
Std. Deviation			395	355	1065	27	9	4.1

Table C4 (cont.): Daily MLTSS, MLVSS, MLCOD, MLTKN, DSVI and OUR results for the parent laboratory-scale MLE experimental 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)
13	20.10	176	4154	3681	5188	257	115	49.4
	21.10	177	4183	3763	5244	282	112	47.6
	23.10	179	4098	3662	5607	279	117	42.8
	24.10	180	4259	3770	5336	298	113	42.4
	25.10	181	4167	3693	5586	288	119	40.7
	26.10	182	4142	3668	5125	265	127	41.2
	27.10	183	4110	3614	5202	278	121	37.3
	28.10	184	4104	3628	5384	293	117	36.3
	30.10	186	4052	3582	5242	289	117	48.5
	31.10	187	3831	3400	4514*	272	124	48.7
	01.11	188	4156	3721	5505	285	110	48.3
MEAN			4114	3653	5268	281	118	43.9
Std. Deviation			165	156	299	12	5	4.8
14	04.11	191	4004	3561	4878	281	120	48.9*
	06.11	193	3953	3527	4939	270	121	44.3
	07.11	194	3789	3318*	4775	265	117	43.7
	08.11	195	3977	3518	4714	263	116	43.4
	09.11	196	3947	3531	4877	267	120	44.6
	10.11	197	3955	3449	4735	273	120	42.8
	11.11	198	3998	3516	4674	274	114	43.1
	12.11	199	4062	3543	4877	267	123	43.9
	13.11	200	3984	3478	4978	272	123	44.8
MEAN			3963	3493	4827	270	119	44.4
Std. Deviation			88	82	106	6	3	1.8
15	18.11	205	4503	3947	5080	302	123	41.9
	19.11	206	4569	4029	5161	278	126	40.3
	20.11	207	4549	3995	5304	289	132	40.2
	21.11	208	4461	4015	5202	304	141	42.1
	22.11	209	4546	4128	5345	291	137	41.7
	23.11	210	4543	3930	5202	298	136	39.1
	24.11	211	4514	3973	5284	293	133	40.1
	25.11	212	4473	3980	5181	289	135	39.2
	27.11	214	4364	3795	5140	294	126	43.6
	28.11	215	4470	3868	5100	306	120	38.6
MEAN			4499	3966	5200	294	131	40.7
Std. Deviation			70	113	87	8	7	1.6
16	30.11	217	4339	3834	5366	301	124	42.9
	01.12	218	4185	3762	5222	293	120	41.5*
	02.12	219	4220	3830	5345	308	119	42.8
	04.12	221	4333	3847	5673	312	119	43.0
	05.12	222	4394	3872	5181	288	110	42.8
	06.12	223	4325	3817	5361	317	113	43.1
	07.12	224	4361	3878	5261	295	106	43.3
	08.12	225	4429	3928	5100	294	111	44.0
	09.12	226	4562	3977	5502	311	104	43.6
MEAN			4350	3861	5335	302	114	43.0
Std. Deviation			127	93	174	10	7	0.7

Table C4 (cont.): Daily COD, TKN and Nitrates concentration results for the parent laboratory-scale MLE experimental activated sludge system.

YEAR 2001

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)
17	09.02	288	3388	3046	4659	212	101	35.9
	10.02	289	3401	3084	4799	267	100	47.6
	12.02	291	3624	3266	4347	253	102	51.2
	13.02	292	3466	3117	4638	230	103	47.1
	14.02	293	3553	3221	4839	235	100	41.6
MEAN			3486	3147	4657	240	101	44.7
Std. Deviation			114	107	193	21	1	6.0
18	16.02	295	3572	3226	3926	211	96	45.8
	17.02	296	3742	3404	3500*	240	97	44.5
	19.02	298	3377	3085	3834	219	116	41.5
	20.02	299	3457	3119	4197	229	104	40.3
	21.02	300	3395	3014	4163	232	110	39.9
	23.02	302	3300	2889	4157	208	111	42.3
	24.02	303	3273	2944	4257	240	104	38.7
	25.02	304	3609	3140	4056	235	102	41.8
MEAN			3466	3103	4011	227	105	41.8
Std. Deviation			194	178	251	13	7	2.4
19	12.03	319	3922	3527	4511	265	176	47.6
	13.03	320	3865	3447	4779	276	185	46.4
	14.03	321	3835	3443	4202	231*	200	42.7
	15.03	322	3842	3457	5026	271	242	47.5
	16.03	323	3877	3486	4779	266	288	51.7
	17.03	324	3989	3600	5006	268	204	56.9
	18.03	325	3853	3484	4800	278	160	52.2
	19.03	326	3791	3410	4656	274	161	46.9
MEAN			3872	3482	4720	266	202	49.0
Std. Deviation			77	72	268	15	44	4.4
20	24.03	331	3787	3396	4372	256	129	40.7
	26.03	333	3716	3258	4473	236	129	42.0
	27.03	334	3883	3379	4847	270	113	44.0
	28.03	335	3822	3431	4675	256	104	43.2
	30.03	337	3821	3454	4756	249	99	43.7
	31.03	338	3782	3346	4797	235	96	43.1
	02.04	340	3740	3337	5020	242	114	42.4
	04.04	342	3714	3269	5282	236	119	41.7
MEAN			3783	3359	4778	247	113	42.6
Std. Deviation			91	92	289	13	12	1.1

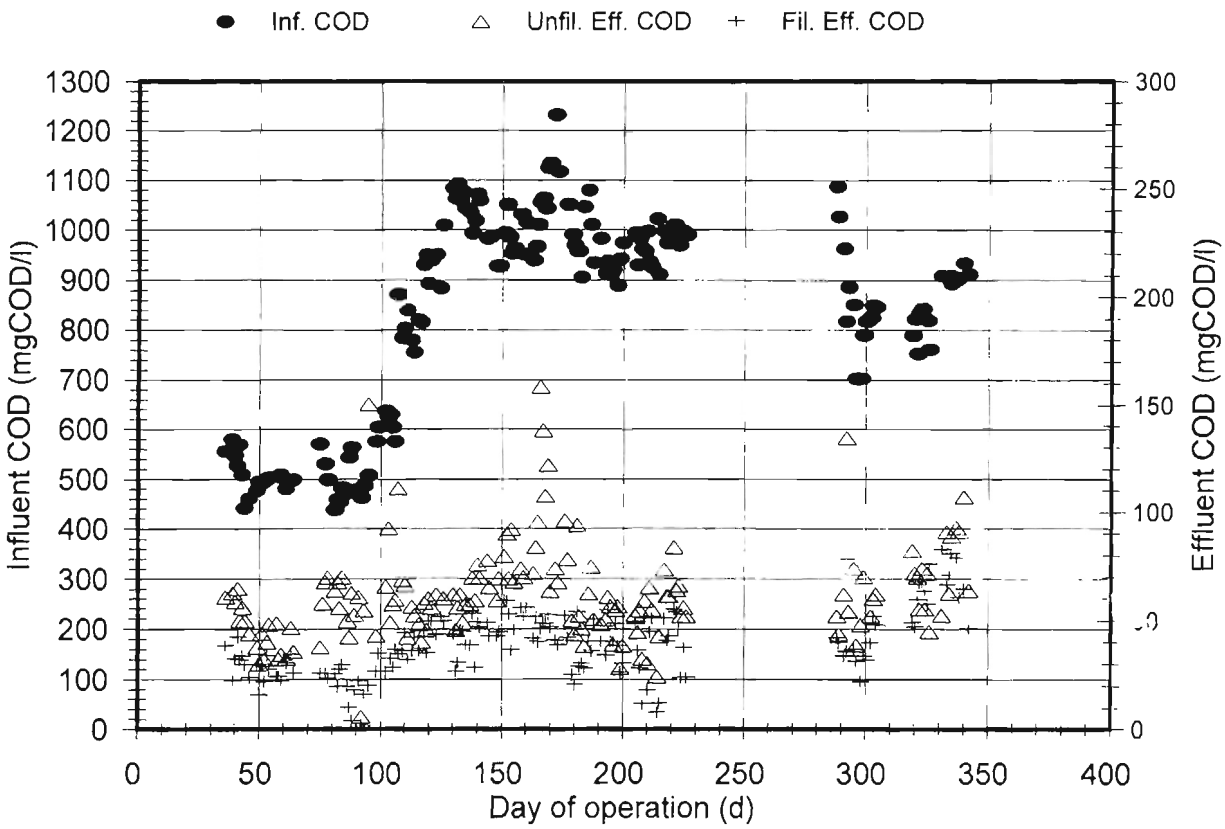

Figure C1: Graphical representation of the daily influent, unfiltered effluent and filtered effluent COD concentrations for the parent experimental laboratory-scale activated sludge system.

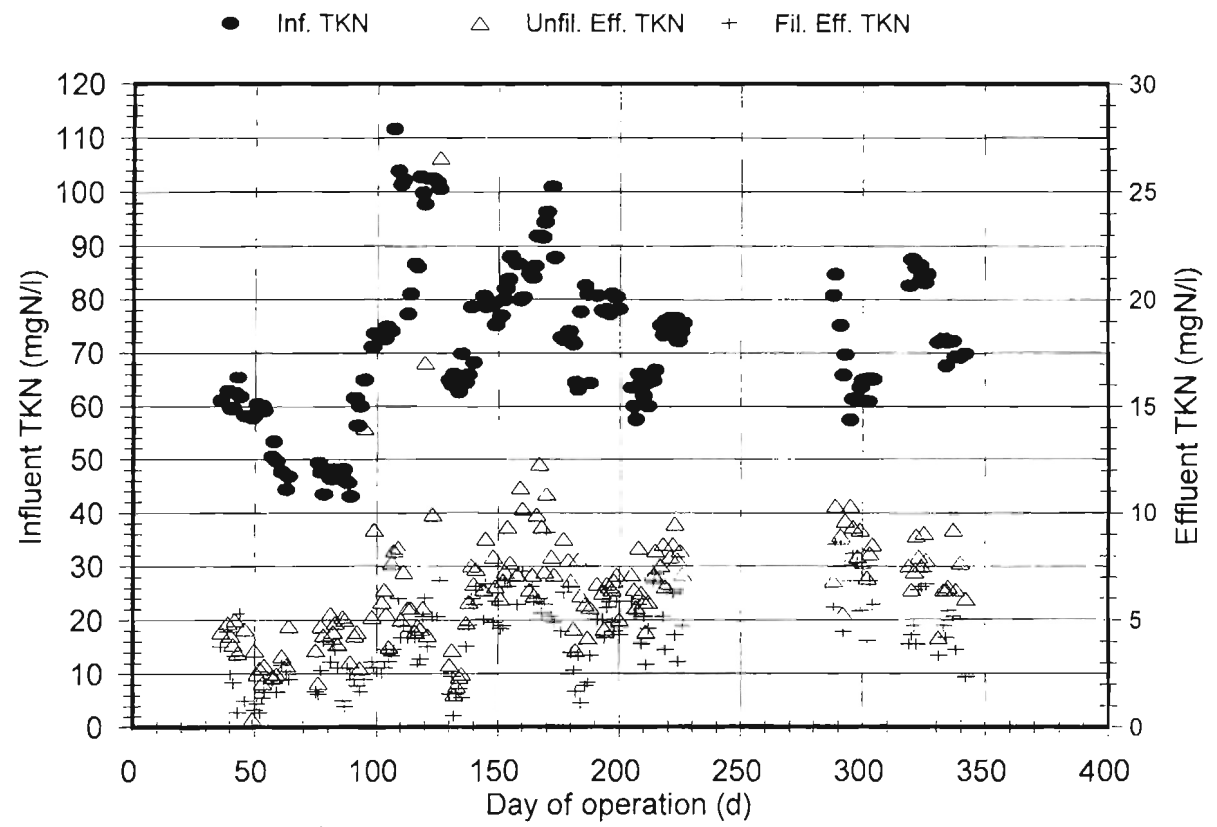

Figure C2: Graphical representation of the daily influent, unfiltered effluent and filtered effluent TKN concentrations for the parent experimental laboratory-scale activated sludge system.

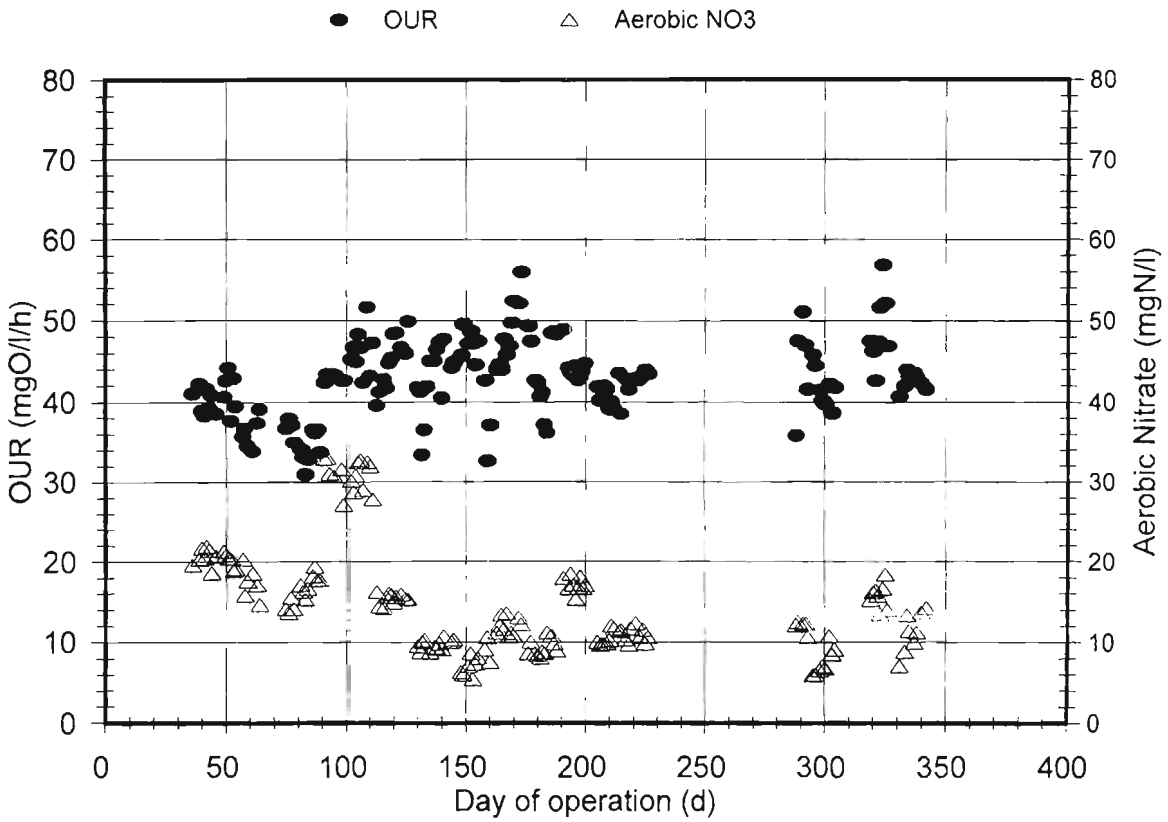

Figure C3: Graphical representation of the daily oxygen utilization rate (OUR) and nitrate (NO_3) concentrat for the aerobic reactor of the parent experimental laboratory-scale activated sludge system.

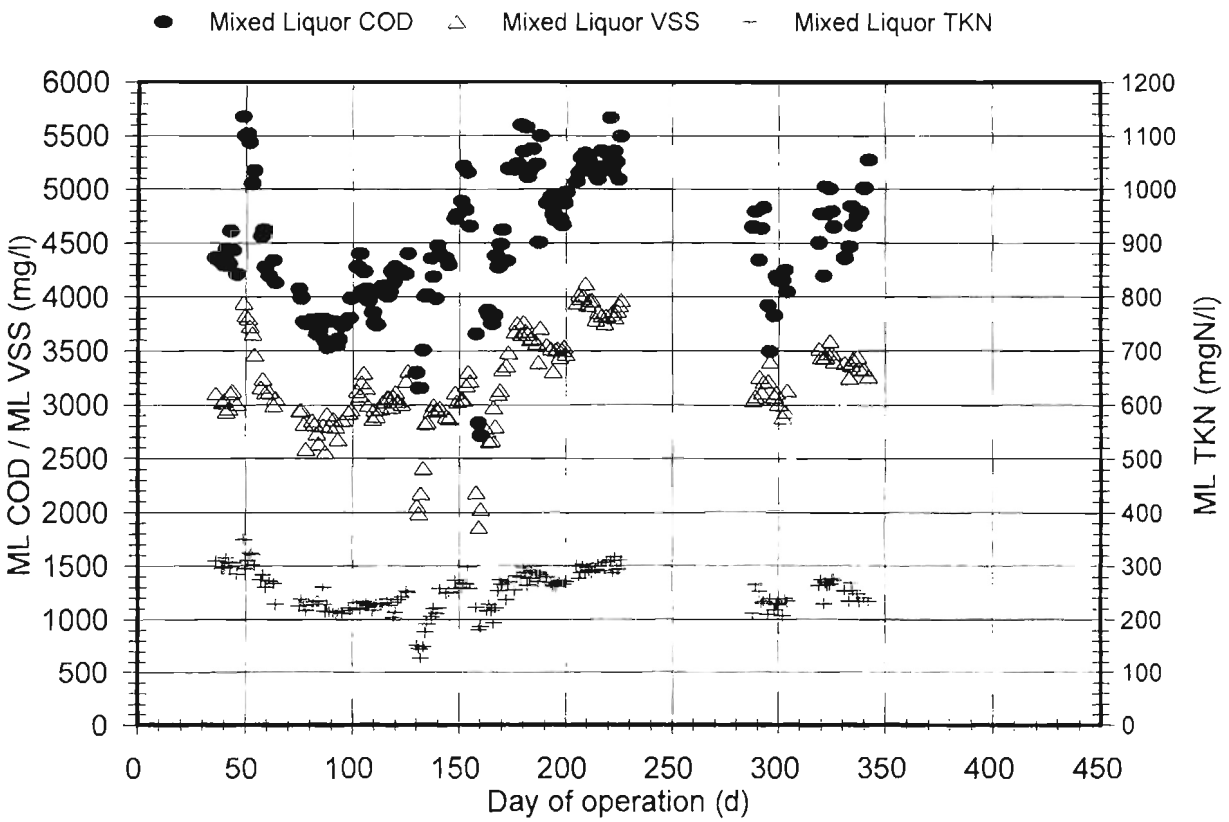

Figure C4: Graphical representation of the daily mixed liquor COD, VSS and TKN concentrations for the parent experimental laboratory-scale activated sludge system.

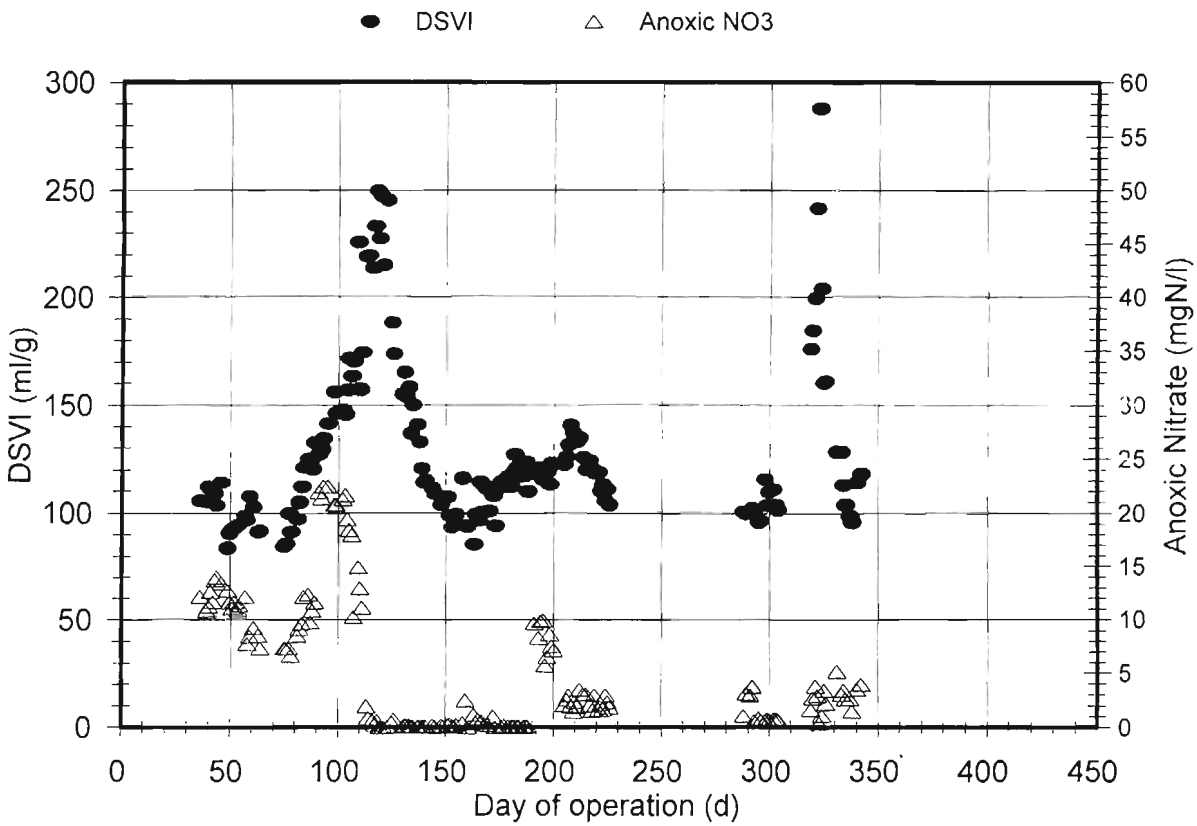

Figure C5: Graphical representation of the daily Diluted Sludge Volume Index (DSVI) and anoxic nitrate (NO_3) concentration for the parent experimental laboratory-scale activated sludge system.

APPENDIX D

CONSTRUCTION AND INTERPRETATION OF STATISTICAL PLOTS FOR DATA ANALYSIS

TABLE OF CONTENTS

CONSTRUCTION AND INTERPRETATION OF STATISTICAL PLOT FOR DATA ANALYSIS

D.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 sewage batch, 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 could then be used to evaluate whether the difference between the means from two data sets is statistically significant at a selected confidence level, or not.

D.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. D1. Alternatively, probability paper can be used on which the y-axis has been linearized.

D.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 cannot 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 the distribution (i.e. the data are normally distributed), it is possible to determine graphically (refer to Fig. D1):

- 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).

D.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.

D.5 ILLUSTRATION BY AN EXAMPLE

An example plot is given in Fig. D1.

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. D1, i.e. from graph:

the x-value at y = 6 = 22.6 mgCOD/ℓ
the x-value at y = 4 = 13.4 mgCOD/ℓ
$\therefore$ 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
$\therefore$ 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:

Test = mean (difference) – 2 • SD (difference)

= 2 – 2 • 2.09

= **-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.

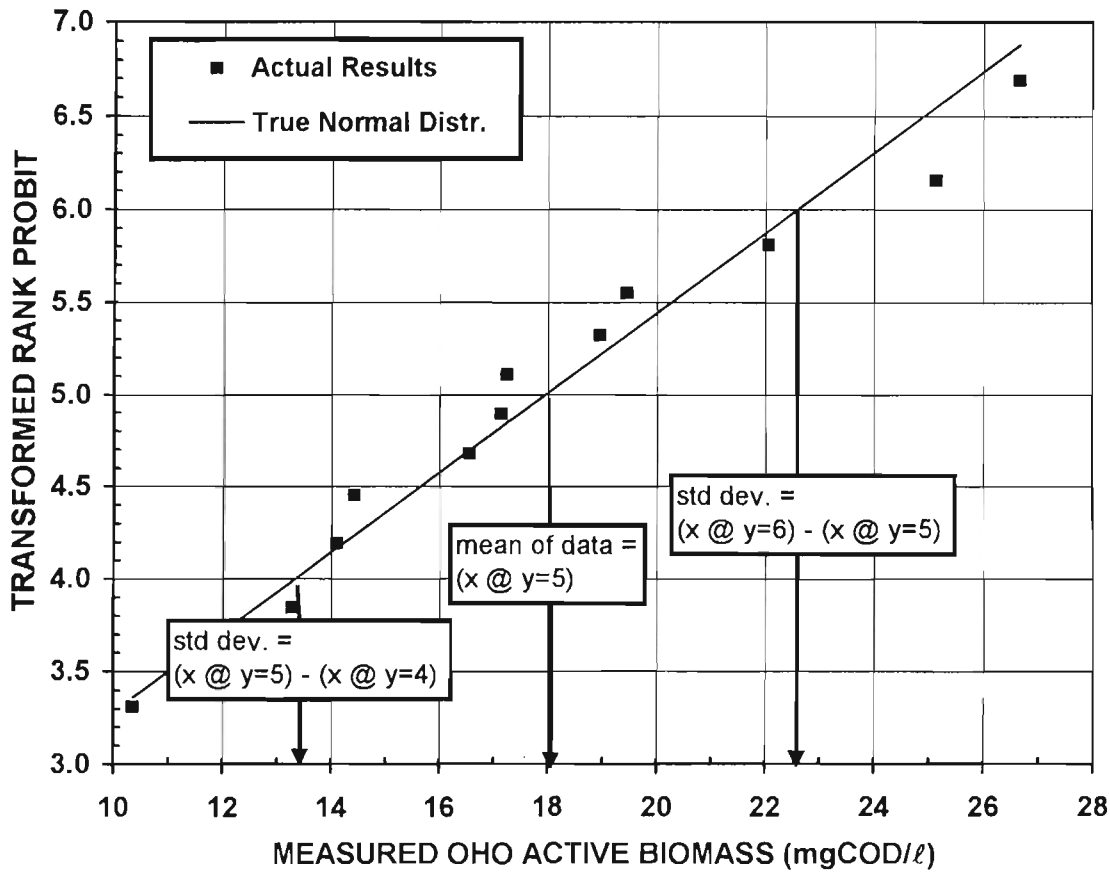

Figure D1 : Example of a statistical probability plot for a number of measured OHO active biomass derived from the batch test.