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THE EFFECT OF NITRITE AND NITRATE CONCENTRATIONS ON LOW F/M FILAMENT BULKING IN NITROGEN REMOVAL ACTIVATED SLUDGE SYSTEMS

by

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DECLARAT ON BY CANDIDATE

I, MICHAEL EDWARD DE VILLIERS, hereby declare that this thesis is my own work and that it has not been submitted for a d gree at another university.

Signed by candidate

Sep ember 1993

SYNOPSIS

Filamentous bulking and its associated poor sludge settleability is a considerable problem in South African activated sludge plants, as indicated by the two surveys undertaken on these plants in 1985 and 1988 (Blackbeard *et al.*, 1986, 1988). Amelioration of this problem would enable a greater daily flow and load of wastewater to be treated by these plants.

From the surveys it is apparent that bulking in long sludge age activated sludge plants is mainly due to the proliferation of the group of so-called low F/M filaments (as classified by Jenkins *et al.*, 1984). In the course of a 4-year research program investigating specific bulking control methods, Gabb *et al.* (1989) concluded that the selector effect, which was proposed as a method for controlling low F/M filament bulking, was ineffective. This research also indicated that in fully aerobic systems low F/M bulking was ameliorated, but in intermittently aerated anoxic-aerobic systems low F/M filament bulking was promoted.

The research presented in this thesis forms part of a follow-up bulking research program into low F/M bulking which led to the formulation of a bulking hypothesis by Casey *et al.* (1992b) and focuses on the effect of different anoxic-aerobic conditions and their effect on the concentration of nitrate and nitrite entering the aerobic zone/reactor in single intermittently

aerated and multi -reactor nitrification-denitrification systems.

The experimental set-up consisted of a nitrification-denitrification system operated at 15 days sludge age and 20°C which was fed real municipal wastewater, the configuration of which was changed during the 472 day investigation as follows:

(i) An intermittently aerated single reactor anoxic-aerobic system (day 1 to 83 - low ammonium dosing to influent day 1-65; high ammonium dosing, day 66-83)
(ii) A 2-reactor anoxic-aerobic system
(iii) A modified 2-reactor anoxic-aerobic system (with a 2-reactor anoxic-aerobic system run in parallel as a control)(day 254-472) - see Table S.1

The system was initially started up as a single reactor intermittantly aerated system having 3 aeration cycles per day. After this system had been operated for 52 days, an ammonia supplement was added to the feed in order to increase influent TKN concentration. This increased TKN concentration would result in higher concentrations of nitrate and nitrite being generated in the system; - the effect of this increased nitrate and nitrite concentration on low F/M filament proliferation, and its influence on the sludge settleability (DSVI) could thus be observed. Because, in terms of Casey's bulking hypothesis, the proliferation of low F/M filaments is influenced by concentrations of nitrate and nitrite entering the aerobic phase, measurements of effluent nitrate and nitrite concentrations would not be indicative of these initial aerobic concentrations as they are a composite of aerobic and anoxic phase values. In order to

Table S.1 Experimental system operation

Period	System	Operation	Day
1	SRIA	No NH_4^+ dosing	1 - 65
2	SRIA	NH_4^+ dosing	66 - 83
1	2RND	NH_4^+ dosing, recycle 3:1	84 -146
2	2RND	NH_4^+ dosing removed,recycle 3:1	147-205
3	2RND	Recycle 1:1	206-254
1	M2RND	Auxiliary reactor added	254-472
2	M2RND	Auxiliary reactor	254-472
3	M2RND	Auxiliary reactor	254-472

observe the behaviour of the nitrate and nitrite concentrations entering the aerobic zone, two nitrate/nitrite concentration and redox potential profile tests were done on two different occasions.The first of these tests was carried out on a sludge of improving quality (decreasing DSVI), and the second was carried out on a sludge of deteriorating quality (increasing DSVI). These tests showed that in the case of sludge having improving settleability, the nitrate and nitrite concentrations entering the the aerobic phase were significantly lower than those entering the aerobic phase in the case of the sludge having a deteriorating settleability. From the results of these tests a link between nitrate and nitrite concentration entering the aerobic phase, and the tendency of the sludge to bulk due to low F/M proliferation was established.

Due to the difficulties of measuring and controlling nitrate and nitrite concentrations entering the aerobic phase in the single intermittantly aerated reactor, the system was converted to a 2-reactor anoxic-aerobic system, having the same operating parameters. To ensure that complete denitrification did not take place, the ammonium feed supplement was continued. Anoxic nitrate and nitrite concentrations thus remained relatively high (>0.5 mgNO_2-N/l), as did the DSVI (210 ml/g). The ammonium supplement was then removed from the system to lower the influent TKN concentration, so that the effects of the resultant lower nitrate and nitrite concentrations on low F/M filament proliferation could be observed. A decrease in anoxic reactor nitrate and nitrite concentration and a corresponding decrease in the DSVI (from ± 300 ml/g to ± 200 ml/g) was observed. A further decrease in DSVI (to 150 ml/g) was effected by reduction of the inter-reactor recycle ratio, which further reduced the nitrate/nitrite load on the anoxic reactor.

In an attempt to achieve complete denitrification before entering the aerobic zone, the system was modified by the addition of a small auxiliary anoxic reactor located before the aerobic reactor. This reactor received 10% of the daily influent feed directly via a bypass to provide the COD needed to facilitate denitrification. This modification resulted in an initial decline in the DSVI (from 300 ml/g to 190 ml/g) and subsequent fluctuations in DSVI in the modified system followed corresponding variations in the anoxic reactor nitrate and nitrite concentrations quite closely. An identical two-reactor anoxic-aerobic system without the auxiliary anoxic reactor was run in parrallel to the modified system from day 383 to act as a control.

The above investigation seemed to indicate that nitrite and nitrate concentrations exceeding 0.5 mg NO_3-N/l and 0.5 mg NO_2-N/l entering the aerobic reactor of anoxic/aerobic systems leads to the proliferation of low F/M filaments and elevated DSVIs. A statistical analysis of the anoxic nitrate and nitrite concentrations and DSVI for the 2-reactor and modified 2-reactor systems indicated a degree of statistical correlation between these parameters.

The work done on the above systems, led to the development of the bulking hypothesis, as proposed by Casey *et al.* (1992b). This hypothesis is based on the assumption that floc forming organisms in the activated sludge are inhibited in the aerobic zone by denitrification intermediates accumulated during the preceding anoxic conditions, while low F/M filaments do not suffer from the inhibition effect in the aerobic zone, and are thus able to proliferate at the expense of the floc formers.

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CHAPTER 1

INTRODUCTION

Bulking caused by the proliferation of filamentous organisms presents a considerable problem to the efficient operation of nutrient removal activated sludge plants in South Africa (Blackbeard *et al.*, 1988). This filamentous bulking is caused by excessive growth of filamentous organisms in the activated sludge, resulting in a change in floc structure; ie sludge flocs adopt a diffuse or bridged structure. The flocs are thus prevented from approaching each other closely, and the sludge does not settle fast or compact well, resulting in a greater volume of sludge per unit mass (ml/g) in the secondary settling tank, from which the term bulking originates. Bulking sludges can be described in terms of the SVI (Sludge Volume Index) or DSVI (Dilute Sludge Volume Index) as generally having a SVI between 100 and 200 ml/g or a DSVI of greater than 150 ml/g.

The settleability of the activated sludge is adversely affected by filamentous bulking, making the sludge settle more slowly and compact less well as the filaments proliferate, resulting in DSVI's in excess of 200 ml/g. Because sludge settleability governs the flow and load a particular plant can successfully treat, controlling the proliferation of filamentous organisms and keeping the DSVI of the sludge at relatively low values (< 100 ml/g) would permit significantly higher flows (50% - 100%) to be treated in existing plants resulting in huge capital savings. The possibility of achieving these huge savings by controlling bulking is the principal motive behind the research into the causes and control of filament bulking.

From surveys of different full scale activated sludge plants, Jenkins *et al.*, (1984), grouped the different filamentous organisms causing filamentous bulking according to the conditions under which they appeared to proliferate. The following 5 general categories were identified ie (i) low D.O. (dissolved oxygen) , (ii) nutrient deficiency, (iii) low F/M (food to microorganism ratio), (iv) septic wastewater and (v) low pH. The filament species classified by Jenkins *et al.* into the low F/M group are *inter alia:* 0092, *Microthrix parvicella*, 0041, 0581, 0961, 0803 and 0675. Most sludge bulking problems in nutrient removal activated sludge plants in South Africa are caused by filaments belonging to this group.

Various methods have been proposed to ameliorate low F/M filament bulking problems. These methods included intermittent feeding, plug flow conditions, or completely mixed systems including selector reactors. These proposed operating procedures were based on the so-called selector effect. It was hypothesised in the literature that these operating conditions would induce a selector effect in the sludge, whereby floc forming organisms would be able to develop a high readily biodegradable COD (RBCOD) utilisation rate enabling them to successfully compete for soluble substrate against the low F/M filamentous organisms.

From a laboratory investigation, Gabb *et al.* (1989a, 1991) found that the selector effect was not effective for controlling low F/M filament bulking. However it was found in the investigation that intermittently aerated and multi-reactor anoxic-aerobic or anaerobic-anoxic-aerobic systems developed low F/M bulking sludges, but if these sludges were placed under completely aerobic conditions, low F/M filament proliferation ceased and the DSVI decreased to below 80 ml/g within a few sludge ages.

Based on a comprehensive follow-up investigation commenced in 1990, a new hypothesis regarding the proliferation of the filamentous organisms in nutrient removal systems was proposed by Casey *et al.* (1992a,b). This hypothesis is based on metabolic differences between floc-forming and filamentous organisms with regard to their denitrification pathway. This hypothesis maintains that the floc-forming organisms are facultative aerobic denitrifiers, which are able to reduce nitrate to nitrogen gas, while the filamentous organisms are facultative aerobic, which only reduce the nitrate to nitrite. When the sludge is exposed to alternating anoxic and aerobic conditions, where denitrification is not complete at the end of the anoxic period, ie nitrate and nitrite is present at the start of aerobic conditions, an intracellular build-up of nitric oxide (NO) exists in the floc-forming organisms but not in the filamentous organisms. This intracellular nitric oxide is known to cause inhibition of substrate uptake in the floc-forming organisms entering the aerobic conditions. Due to the nature of their denitrification pathway, filamentous organisms do not accumulate nitric oxide, and are not inhibited in their uptake of substrate when entering the aerobic zone. Under anoxic-aerobic conditions with incomplete denitrification the filamentous organisms are afforded a competitive advantage and are able to proliferate at the expense of the floc formers. Under fully anoxic and fully aerobic conditions the filaments have been observed not to proliferate. In terms of the hypothesis this is because the floc formers are not exposed to alternate anoxic and aerobic conditions. Under fully aerobic conditions, denitrification does not take place so that the inhibiting denitrification intermediate NO is not produced. Under fully anoxic conditions, the floc forming organisms denitrification enzymes are fully developed and the aerobic oxidases (for oxygen) are not required with the result that no inhibition of the aerobic oxidases takes place. In contrast under alternating anoxic-aerobic conditions, when denitrification is not complete before conditions become aerobic, the NO denitrification intermediate inhibits the aerobic oxidase and the floc-former needs to generate new oxidase enzymes before it can function normally. The filamentous organism (so it is hypothesized) does not accumulate the NO denitrification intermediate, and thereore upon conditions becoming aerobic can immediately function normally giving it an advantage over the floc formers.

The research presented in this thesis forms part of the comprehensive follow up investigation that led to the development of the new hypothesis for low F/M filament bulking. The effect of various anoxic-aerobic conditions on the concentration of nitrate and nitrite entering the aerobic reactor from the anoxic reactor and their effects on bulking were investigated in various system configurations such as (i) Single reactor intermittently aerated nitrification - denitrification (1RND), (ii) Two reactor nitrification denitrification (2RND) and (ii) Modified two-reactor anoxic-aerobic systems

In these systems anoxic nitrite and nitrate concentrations were measured and their effect on the sludge settleability observed. Under intermittent aeration conditions reactor nitrite, nitrate and redox potential profiles were observed under differing conditions of sludge settleability, while in the two reactor nitrification/denitrification systems the effects of various operating modifications such as high ammonium concentration, and changed recycle ratios on anoxic nitrite and nitrate concentrations and DSVI were investigated.

The results observed on these systems contributed to the experimental evidence supporting the hypothesis proposed by Casey *et al.* (1992a, b), and the batch tests conducted by Casey *et al.* (1992b, c) demonstrating the presence of the inhibition were done on sludge harvested from these systems.

This thesis comprises a literature review in which the development of specific bulking control is laid out(Chapter 2). A detailed description of experimental procedure and results and the significance of the research in the development of the bulking hypothesis as formulated by Casey *et al*, (1992) is given in Chapter 3. The conclusions reached by this investigation are described in Chapter 4.

CHAPTER 2

LITERATURE REVIEW

Preamble

The review presented in this chapter is an adapted version of a comprehensive literature review into specific bulking control compiled by Casey *et al.* (1992a), and presented here to place the objectives of the investigation in context with the present position of bulking research.

2.1 INTRODUCTION

There are two approaches to bulking control, (1) non-specific and (2) specific. With non-specific control some toxicant, usually chlorine although ozone and hydrogen peroxide also can be used, is dosed into the activated sludge system. Because the filamentous organisms causing the bulking extend beyond the flocs into the liquid, they are more exposed to the toxicant and therefore are selectively killed; in contrast the floc-formers are not seriously affected by the toxicant because they find protection inside the sludge flocs. Due to the selective killing of the filaments, their numbers and lengths are reduced and the bulking is ameliorated. The toxicant affects all filaments irrespective of type and for this reason this method of ameliorating bulking is called non-specific.

The principal non-specific bulking control procedure is by chlorination. This procedure is well documented in the literature such as in the bulking control manual of Jenkins *et al.* (1984). The method has been tested for biological N & P removal systems (Lakay *et al.*, 1988) and found to be satisfactory provided the guidelines set down by Jenkins *et al.*(1984) are followed.

However chlorination has rather serious shortcoming in that undesirable compounds such as trihalomethanes and chlorinated hydrocarbons tend to form which pose a potential health risk in the event the treated effluents are reclaimed for potable water supplies. To avoid this problem van Leeuwen (1988) and van Leeuwen and Pretorius (1988) investigated the use of ozone for bulking control in an N & P removal pilot plant. They concluded that ozonation successfully controls filamentous bulking and imparts additional benefits i.e. (1) improves the removal of organic substances, (2) aids nitrification and to some degree biological excess P removal (BEPR) and (3) produces an effluent that is more suitable for reuse than effluent from activated sludge treatment plants without ozonation.

The problem with non-specific bulking control is that as soon as toxicant dosing is terminated, the filaments regrow and, inexorably, bulking conditions return. This is because non-specific bulking control deals with the symptoms of bulking, i.e. reduces the filaments, but does not remove the causes of the filament proliferation on a permanent basis. With specific bulking control the causes of filament proliferation are sought to be eliminated on a permanent basis.

2.2 SPECIFIC BULKING CONTROL

Specific control of bulking focuses on identifying and eliminating the conditions that promote the proliferation of the specific nuisance filaments causing the bulking problem. Once these conditions are identified, through the types of filaments present in the sludge, it may be possible to create environmental conditions in the activated sludge plant which would inhibit or suppress the growth of the filamentous organisms. If successful, the method would provide a permanent solution to the particular bulking situation.

Five conditions in activated sludge systems have been identified that lead to filamentous organism proliferation (Jenkins *et al.*, 1984), viz. low DO, low Food to Micro-organism ratio (low F/M or equivalently long sludge age), nutrient deficiency, septic influent and low pH; each condition favours the growth of certain filamentous organism types. From surveys of activated sludge plants in South Africa (Blackbeard *et al.*, 1986, 1988) the majority of which are long sludge age biological N or N & P removal plants, it was found that the six most frequently dominant filamentous organisms in these plants are types 0092, 0675, 0041, *Microthrix parvicella*, 0914 and 1851. The first four of these filaments belong to the low F/M group. Blackbeard *et al.* (1986,1988) suggested that due to the frequent occurrence of the last two filaments with the low F/M ones, these two also be included into the low F/M filament group. This paper examines some of the research undertaken to delineate the causes and control of low F/M filament bulking in N and N & P removal plants.

2.3 LOW F/M BULKING CONTROL - SOME BACKGROUND

2.3.1 Chudoba's selection criterion

In 1973 Chudoba *et al.* (a,b) proposed an organism selection criterion as an explanation for the occurrence or non-occurrence of filamentous bulking. This criterion is based on competition between the floc-formers and the filaments for the mutually limiting soluble substrate, as follows: In the Monod formulation for the specific rate of growth of organisms, filamentous organisms have lower values for both the maximum specific growth rate (μ_H) and the half saturation coefficient (K_s) than floc-formers. Consequently at low substrate concentrations the filamentous organisms have a higher specific growth rate than floc-formers and at high substrate concentrations, a lower specific growth rate.

Over the past 15 years the selection criterion has provided a framework for research into the causes of bulking and its control by specific methods. Results, reported by a number of investigators who have measured the Monod constants of various filaments and floc- formers, appear to fit within the structure of the selection criterion: Van den Eynde *et al.* (1982a,b) showed that in general, organisms with high μ_H rates have high K_s values and ones with low μ_H rates have low K_s values. Slijkhuis (1983) measured the μ_H of *Microthrix parvicella* (one of the principal filaments causing low F/M bulking) as 1,66/d; this is considerably lower than a μ_H of 4,33/d measured by Richard *et al.* (1982) for a floc- former isolated from activated sludge.

Palm *et al.* (1980) extended the selection criterion to incorporate limiting nutrients: For some filaments (the low D.O. ones), the limiting nutrient apparently is oxygen whereas for others, the limiting nutrient is the soluble substrate concentration surrounding the organism, as originally conceived by Chudoba *et al.* (1973a,b). With regard to low D.O. bulking, Hao *et al.* (1983) and Lau *et al.* (1984) confirmed the work of Palm *et al.* (1980). From dual species studies they showed that low D.O. filaments (*Sphaerotilus natans*, Type 1701) and floc- formers can be selectively grown by manipulating the D.O. concentration; if high, the floc-former dominates; if low, the filament dominates.

With regard to bulking in long sludge age (low F/M) systems, Chudoba *et al.* (1973a,b) tested the selection criterion with pure soluble substrates: They controlled the substrate concentration surrounding the organism by having different configurations for the activated sludge system. For example, in a single reactor completely mixed system, the substrate concentration would be low throughout the reactor whereas in a multi-reactor plug flow system the substrate concentration would be high in the upstream section and low in the downstream section. They found that in aerobic single reactor completely mixed systems filamentous organisms proliferated causing bulking whereas in aerobic multi- reactor plug flow systems filamentous organisms did not proliferate and a good settling sludge was maintained. From this work, Chudoba *et al.* (1973b) developed the selector reactor for bulking control. The selector reactor is a small aerated reactor upstream of the main aeration reactor and receives the influent and underflow recycle. In the selector reactor, the substrate concentration is high and, in terms of the selection criterion, the floc-formers should grow faster than the filaments and, will utilize practically all of the soluble substrate. The mass of soluble substrate that passes through the selector is a very small fraction of that available to the floc- formers in the selector and so filament growth will be restricted and insufficient to cause bulking.

Although the filament categorization into 5 causative groups was not yet developed (this only emerged in 1984 with the work of Jenkins *et al.*). it should be noted that even though the systems operated by Chudoba *et al.* (1973a,b) were long sludge age or low F/M ones, the dominant filament causing the bulking was not a low F/M one but was one of the low D.O. filaments, i.e. *S. natans*. This will be referred to again later.

The work of Chudoba *et al.* (1973a,b) stimulated research into the control of bulking in low F/M (long sludge age) systems. Most of this research was conducted on fully aerobic systems at laboratory scale with real or synthetic sewage as influent. In this research it was found that good settling (non- bulking) sludges were produced in systems with:

(1) compartmentalization of the aeration reactor while maintaining continuous feeding of waste water (Chudoba *et al.*, 1974; Rensink *et al.*, 1982; Wu *et al.*, 1984);

(2) batch or intermittent feeding to completely mixed aeration basins (Houtmeyers, 1978; Houtmeyers *et al.*, 1980; Verachtert *et al.*, 1980; van den Eynde *et al.*, 1982a,b; Eikelboom, 1982; Rensink *et al.*, 1982; Goronszy, 1979; Goronszy and Barnes, 1979; Barnes and Goronszy, 1980; Chiesa and Irvine, 1985; Jenkins *et al.*, 1983; Ekama and Marais, 1986b; Still *et al.*, 1986; van Niekerk, 1985; van Niekerk *et al.*, 1987);

(3)small aerated mixing reactors (aerobic selectors) ahead of the main completely mixed aeration reactor, receiving the influent and underflow streams (Grau *et al.*, 1982; Lee *et al.*, 1982; Jenkins *et al.*, 1983; Daigger *et al.*, 1985; Still *et al.*, 1986; van Niekerk *et al.*, 1987).

As in the investigation of Chudoba *et al.* (1973a,b), in a large number of the investigations cited above, bulking in long sludge age (low F/M) systems was not caused by low F/M filaments. In most cases bulking was caused by *S. natans* which is a low D.O. filament. This raised the question of the appropriateness of the system modification approach for controlling low F/M filaments. It appears that in the bulking research, controlling bulking in low F/M systems was the focus rather than controlling bulking by low F/M filaments. These are two distinctly different objectives because bulking in a low F/M system is not necessarily caused by low F/M filaments. As a result of this difference, a point must be made to clearly distinguish between the two terms in the remainder of this review; low F/M system bulking is bulking in a low F/M system with the filaments causing the bulking unspecified, i.e. could be *S. natans* , whereas low F/M filament bulking is bulking caused specifically by the low F/M filaments, but this condition need not necessarily be in a low F/M system.

2.3.2 Stimulation of the selector effect - aerobic conditions

A common characteristic of the three types of systems outlined above is that a soluble COD (<0,45 m) concentration gradient is induced either in time (i.e. in batch or intermittently fed systems), or in space (i.e. in compartmentalized or selector reactor systems). Some of the investigators concluded that Chudoba's selection criterion does not completely account for the suppression of filamentous organism proliferation and that other factors also play an important role. For example:

(1)Many investigators (Houtmeyers, 1978; Houtmeyers *et al.*, 1980; Verachtert *et al.*, 1980; van den Eynde *et al.* , 1982a,b; Eikelboom, 1982; Jenkins *et al.*, 1983; Daigger *et al.* , 1985; Ekama and Marais, 1986b; Still *et al.* , 1986; van Niekerk, 1985; van Niekerk *et al.*, 1987), using real or synthetic sewages, provided experimental evidence that systems incorporating the 3 modifications cited above stimulate in the sludge soluble biodegradable COD, or more correctly, readily biodegradable COD (RBCOD), and oxygen uptake rates that were much higher than in sludge grown in single reactor completely mixed systems with a constant flow and load. They speculated that the soluble COD (RBCOD) concentration gradient induced by the 3 modifications stimulated the growth of floc-forming organisms with high substrate uptake rates. This stimulation did not supposedly occur in the growth of the filamentous organisms with the result that the filamentous organisms were unable to compete successfully for substrate under these conditions. In this review, a sludge which has had stimulated in it a high RBCOD uptake rate will be referred to as a sludge which exhibits a selector effect.

(2)Chiesa and Irvine (1982,1985) proposed that the alternating feed/starve conditions induced by the three modifications stimulated development of floc- formers with a higher starvation resistance than filamentous organisms.

The significance of these factors in bulking control in low F/M (long sludge age) systems is not yet clear but in any event is not really of much consequence. From a practical point of view, provided the system modification controls the bulking problem, it can be implemented for this purpose; detailed explanation and mechanism will follow hand in hand with practical experience. The urgency is in controlling the bulking problems in many activated sludge plants, in particular the low F/M filament bulking problems so common in biological N and N & P removal plants, not only in South Africa but also in other countries.

2.3.3 Application of selector effect under anoxic conditions

The system modification approach for bulking control in low F/M systems was also applied by incorporating initial anoxic selectors into N removal activated sludge systems. The need for this arose out of the desirability of denitrification for N removal. If an aerobic selector receiving the influent and underflow recycle streams is placed ahead of a nitrification-denitrification system, the influent RBCOD will be utilized in the aerobic selector. This will result in a significant loss in denitrification; as much as 50%; in that the influent RBCOD will be utilized with oxygen rather than with nitrate in the primary anoxic reactor. If the selector effect can be stimulated in an anoxic selector, the RBCOD will be utilized with nitrate (provided sufficient nitrate is recycled to the anoxic selector) and no loss in denitrification will occur, and if the anoxic selector functions, then the conditions for good N removal and selector bulking control can be simultaneously met.

In laboratory, pilot and full scale work, Heide and Pasveer (1974); Bailey and Thomas (1975); Cooper *et al.* (1977); Tomlinson and Chambers (1979); Wagner (1982); Price (1982); Cooper and Boon (1983) and Shao (1986) reported that in nitrifying activated sludge systems, incorporation of initial anoxic mixing zones/selectors ahead of the main aeration reactor improved sludge settleability. However in this work, the filaments either were not specified, or where specified, were not low F/M types. In evaluating anoxic selectors for bulking control in laboratory scale low F/M systems receiving real sewage, Lee *et al.* (1982), reported that incorporation of two anoxic selectors in series, each 1/74th of the total system volume, did not control bulking. Lee and his co-workers sized the selectors in accordance with the volume that would be required to control bulking in aerobic selectors. Based on measurements of soluble COD through the system, they found that not all the soluble biodegradable COD (RBCOD) was taken up in the selectors. The leakage of soluble biodegradable COD (RBCOD) into the aerobic zone was thought to be the cause for the ineffectiveness of the anoxic selectors. In follow-up laboratory research, Shao (1986) concluded that (1) anoxic selectors controlled bulking in low F/M systems provided that they removed practically all the RBCOD, (2) RBCOD and nitrate uptake rates were significantly higher in the systems incorporating anoxic selectors than systems without anoxic selectors, and (3) uptake rate of RBCOD is slower under anoxic conditions than under aerobic conditions so that anoxic selectors should be sized larger than aerobic selectors. From the research reviewed above, it would appear that anoxic selectors are effective also for controlling bulking in low F/M systems, but it needs to be pointed out that the filaments present in the laboratory systems operated by Lee *et al.* (1982) and Shao (1986) were not low F/M filaments but 021N, *Thiothrix* and *S. natans* just as with the system of Chudoba *et al.* (1973a,b) incorporating aerobic selectors, the filaments were not low F/M ones, but *S. natans*. Consequently it was not clear whether or not aerobic or anoxic selectors would control the low F/M filaments. In work on denitrification, Bailey and Thomas (1975) and Arkley and Marais (1981) found that as the hydraulic retention time of an initial (primary) completely mixed anoxic reactor increased, so sludge settleability in long sludge age systems (20 days) deteriorated. In Arkley and Marais' work the anoxic zone had sizes zero (completely aerobic), 39, 50 and 70% of the total system volume. These large anoxic zones cannot be considered selectors in that even though they probably did remove virtually all the RBCOD they would unlikely have stimulated a rapid RBCOD uptake rate. Instead of a single large completely mixed primary anoxic reactor Cooper and Boon (1983) installed a channel type anoxic zone by replacing the surface aerators with stirrers in 25% of the aeration basin (nominal anoxic hydraulic retention time 2,5h) and a good settling sludge (SVI < 100 ml/g) was maintained. In this work on denitrification, the filamentous organisms were not identified so it is difficult to make any conclusions regarding the effect of the different anoxic conditions on the low F/M filaments.

2.3.4 Conclusion - Influence of selector effect on low F/M filaments uncertain

From the evidence presented in this review so far, it appears that a conclusion widely held is that the selector effect, i.e. the stimulation (or adaptation) of floc-formers to develop a rapid RBCOD uptake rate by system modification so that a RBCOD concentration gradient is introduced, enables floc-formers to successfully compete against the filaments for RBCOD. While this may be the mechanism of control over certain filamentous organisms, and from the literature it appears that *S. natans*, *Thiothrix* and 021N are controlled by this mechanism, there was no conclusive evidence that the low F/M filaments were controlled by this mechanism. Because this mechanism had gained considerable credibility as a means of controlling bulking in low F/M systems, its influence on sludge settleability and the low F/M filaments (which are so common in long sludge age biological N and N & P removal systems) was thoroughly investigated at laboratory scale at the University of Cape Town by Gabb *et al.* (1989a) to determine its efficacy.This research was conducted as part of a programme initiated in 1985 at the University of Cape Town for investigation of bulking in N and N & P removal systems. The path of research of this programme can be divided into 3 phases and it is necessary to review the work conducted in each phase so that the experimental work conducted in this thesis (which forms part of the work of Phase 3) can be placed in perspective.

2.4 UNIVERSITY OF CAPE TOWN INVESTIGATION - PHASE 1

2.4.1 Experimental investigation

In this investigation (Gabb *et al.*, 1989a see also Gabb *et al.*, 1991), which extended over a period of 4 years, many types of laboratory scale activated sludge systems were operated. As a starting point (phase 1), the type of experiments reported in the literature reviewed above were repeated to see if the same results could be obtained. The types of systems operated were:

- fully aerobic constant feed single reactor completely mixed (O/CFCM) and intermittently fed fill and draw (O/IFFO) systems

- fully aerobic constant feed completely mixed systems with (O/CFCM/SEL) and without (O/CFCM) aerobic selector reactors.

The need for denitrification required the stimulation of the selector effect in anoxic selectors to be investigated. This was done by operating and evaluating anoxic- aerobic constant feed single reactor completely mixed (AO/CFCM) and intermittently fed fill and draw (AO/IFFD) systems that are similar to the fully aerobic O/CFCM and O/IFFD systems cited above except that alternating aeration/non-aeration periods were imposed on the systems.

These systems were all operated at long sludge ages (15-30 days) and at 20°C. They were all fed Mitchell's Plain raw sewage and started up with low F/M filament bulking sludges (DSVI > 250 ml /g) containing *M. parvicella*, 0675, 0041, 0092 and *Norcardia*.

Conclusions drawn from these first phase experiments were:

2.4.1.1. *Stimulation of the selector effect*

The alternating feed- starve conditions imposed by (i) intermittent feeding to completely mixed reactor systems, either fully aerobic (O/IFFD) or anoxic- aerobic (AO/IFFD) and by (ii) aerobic selector reactors incorporated in fully aerobic continuously fed completely mixed systems (O/CFCM/SEL), stimulated in the mixed liquor a selector effect, i.e. a high readily biodegradable (or dissolved $< 0{,}45\ \mu m$ filtered) COD (RBCOD) uptake rate. The RBCOD uptake rates were 2 to 3 times higher than in systems that did not incorporate selector reactors or alternating feed- starve conditions (O/CFCM and AO/CFCM). If conditions during which the RBCOD was taken up were aerobic, the high RBCOD uptake rate gave rise to an associated high initial oxygen utilization rate (OUR) under batch conditions and if the conditions were anoxic, a high initial nitrate uptake rate (NUR) under batch conditions was observed. The relationship between the RBCOD uptake rate and OUR or NUR was found to be closely proportional and as a result of this, soluble COD concentration profiles were no longer regularly measured due to the ease of measuring the OUR or NUR compared to the soluble COD measurements.

The selector effect could be stimulated or lost in a sludge over a period less than a sludge age in long sludge age (< 15d) systems by introducing or eliminating respectively, alternating feed- starve conditions or through the addition or removal respectively of a selector reactor. Acquisition of a selector effect by a sludge in IFFD and CFCM/SEL systems was in agreement with the literature reviewed above.

2.4.1.2. *Purely aerobic conditions appear to ameliorate bulking by low F/M filaments*

Low F/M filament bulking sludges (DSVI > 250 ml/g) usually containing, in varying proportions, types 0092, 0041, 0914, 0675, 1851 and *M. parvicella*, from long sludge age full scale (N removal) plants, when used to start up the laboratory scale long sludge age activated sludge systems under fully aerobic conditions and the particular anoxic- aerobic conditions, i.e. 1h anoxic 3h aerobic, invariably ceased bulking (DSVI < 80 ml/g) within a month. This occurred irrespective of whether or not the system incorporated an aerobic selector or the system was intermittently fed or continuously fed, i.e. irrespective of whether the selector effect was stimulated in the system or not. It would appear that, in long sludge age fully aerobic systems, and in the particular alternating anoxic- aerobic systems described here, the selector effect did not play a role in the control of low F/M filament bulking; low F/M filament proliferation was suppressed both when the selector effect was present or absent.

2.4.1.3. *Bulking caused by* Sphaerotilus natans (S.natans).

It is important to note that when bulking was observed in fully aerobic systems and in the particular alternating anoxic- aerobic systems in which there was no selector effect (i.e. O/CFCM and AO/CFCM), it was not due to low F/M filaments but due to *S. natans* and *Thiothrix*. According to Jenkins *et al.* (1984) *S. natans* sorts into the low DO group and *Thiothrix* into the septic sewage or nutrient deficient groups. However in the surveys of South African full scale N and N & P removal plants, most of which are operated at long sludge ages, the problematic organisms are those of the low F/M filament group and *S. natans* was never, and *Thiothrix* was only rarely, observed to cause bulking in these plants.

2.4.1.4 S.natans *bulking apparently caused by seeding.*

Regular and thorough cleaning of the influent feed lines eliminated the *S. natans* bulking problems in the laboratory systems (Gabb *et al.* , 1989b). From this it was concluded that *S. natans* proliferation in the laboratory systems was caused by seeding from *S. natans* attached growth on the influent feed line walls. This artifact may also have been present in the many laboratory scale studies throughout the world cited above because numerous investigators have reported the proliferation of *S natans* in their low F/M (long sludge age) laboratory systems under a wide range of operating conditions and that the selector effect controls *S. natans* and *Thiothrix*. As a result of the finding above, it can be concluded that aerobic selectors and intermittent feeding conditions, which induce the selector effect, controlled the proliferation of *S. natans* and *Thiothrix* . This finding is in conformity with results reported in the literature cited above. Because of the importance of the laboratory artifact concerning growth of *S. natans* and *Thiothrix* , it is necessary to discuss the implications of these results as they relate to bulking by low F/M filament bulking.

2.4.2 Implications of the results

2.4.2.1 S.natans,Thiothrix *and* 021N *bulking contrasted with low F/M bulking.*

Up to this point in the investigation, the results obtained were in conformity with those reported in the literature - in particular, a general absence of low F/M filaments in the systems, and, when bulking did take place, it was caused by *S. natans*, *Thiothrix* or 021N and occurred only in the systems which did not stimulate a selector effect. However, the experiments showed that *S. natans* in particular, proliferated in the systems as a result of seeding from the influent feed lines, and that when the feed lines were regularly cleaned (chlorinated twice weekly) *S. natans* no longer proliferated in the systems (Gabb *et al.*, 1989b). In the systems which stimulated a selector effect, *S natans*, *Thiothrix* and 021N did not proliferate indicating that the selector effect, stimulated under either aerobic or anoxic conditions, controlled bulking by *S. natans* and *Thiothrix*. This observation is in conformity with the results published in the literature. The success of the selector effect in controlling bulking by *S. natans* and *Thiothrix* in laboratory scale low F/M systems may have contributed to the notion in the literature that the selector effect also controls bulking by low F/M filaments. In the laboratory systems operated by Gabb *et al.* (1989a), the low F/M filaments did not proliferate; indeed from conclusion (2) above the low F/M filament bulking problems in the starter sludge were ameliorated in all the systems operated. However in marked contrast to these findings was the observation that in biological N & P removal systems which comprise anaerobic- anoxic- aerobic zones usually in single or multi reactors in series and incorporating an appreciable (50%) unaerated sludge mass fraction operated in the laboratory at the time of these experiments, the low F/M filaments did proliferate and cause bulking problems. Indeed, of the laboratory systems operated at the time (which were those cited above and the N & P removal ones) the N & P removal systems were the only ones in which the filament populations were similar to their full scale counterparts i.e. low F/M filaments proliferated, *S. natans* was absent and *Thiothrix* filaments were not commonly observed, even when the feed lines were not regularly cleaned.

2.4.2.2. *Metabolic and kinetic selection*

From the absence of *S. natans* and *Thiothrix* in N & P removal systems, it was hypothesized that the anaerobic reactor in these systems operates as a selector reactor against *S. natans* (and possibly *Thiothrix*) proliferation. This hypothesis finds support from the laboratory experiments of Wanner *et al.* (1987a,b) who calls this type of selection, metabolic selection (as opposed to competitive or kinetic selection in aerobic selectors) which operates as follows: *S natans* is an obligate aerobe (Mulder and Deinema, 1981) and only capable of metabolism in the fully aerobic reactor. However in the anoxic reactor, the RBCOD is utilized by denitrifiers and in the anaerobic reactor, RBCOD is converted to volatile fatty acids (VFA) which together with the VFA from the influent, is taken up by polyphosphate accumulating organisms such as *Acinetobacter spp.* (Wentzel *et al.* , 1985). Consequently with anaerobic and/or anoxic reactors in the system very little RBCOD enters the aerobic reactor for growth of *S. natans*. In terms of this explanation, selectors, whether aerobic, anoxic or anaerobic, control *S. natans* proliferation either by (i) removing RBCOD under conditions in which *S. natans* cannot function (anaerobic or anoxic selectors i.e. metabolic selection) or (ii) stimulating high RBCOD uptake in floc- formers which then can compete successfully against *S. natans* (aerobic selectors i.e. kinetic selection). With regard to *Thiothrix*, this organism is reported to be a facultative aerobe. If it is a facultative organism, anaerobic reactors, anoxic and aerobic selectors should control its proliferation. The literature supports this conclusion in that *Thiothrix* is controlled by anaerobic reactors (Wanner *et al.* , 1987b), anoxic selectors (Shao, 1986) and aerobic selectors (van Niekerk, 1985).

2.4.2.3. *Low F/M filaments appear not to require RBCOD for proliferation*

From the above discussion it can be seen that with respect to the filaments *S. natans*, *Thiothrix* and 021N there is consistency of behaviour in the anaerobic reactor as metabolic selector and aerobic and anoxic selectors as competitive selectors in that in all three, RBCOD is taken up preferentially by floc-formers at the expense of the filaments. The observation that the anaerobic reactor in its function as a metabolic selector, does not control the proliferation of low F/M filaments in N and N & P removal systems because these types of plants so frequently have low F/M filament bulking, raises the question of whether or not aerobic and anoxic selectors will be able to control low F/M filament proliferation through competitive selection. Aerobic and anoxic selectors and anaerobic reactors permit removal of influent RBCOD by floc- formers through kinetic or metabolic selection, but despite this the low F/M filaments continue to proliferate in N & P removal systems. Therefore it would appear that the low F/M filaments do not require RBCOD for growth to the same extent as *S. natans*, *Thiothrix* and 021N. If the low F/M filaments are able to grow on COD other than RBCOD, i.e. the particulate biodegradable COD (PBCOD), then because the PBCOD passes through the aerobic/anoxic selectors and anaerobic reactors, the proliferation of these filaments would not be controlled by aerobic and anoxic selectors. Based on this reasoning the second phase of the investigation by Gabb *et al.* (1989a) focused on ascertaining whether or not aerobic selectors could suppress low F/M filament proliferation.

2.5 UNIVERSITY OF CAPE TOWN INVESTIGATION - PHASE 2

2.5.1 Confirmation that the selector effect is not important in amelioration of low F/M filament bulking

As a preliminary step to ascertaining whether or not aerobic (or anoxic) selectors could ameliorate low F/M filament bulking, it was considered necessary first to confirm that the presence or absence of a selector effect does not play a role in controlling low F/M filament bulking but that the presence of unaerated/aerated conditions do play an important role.

To do this, three single reactor systems were started up with low F/M filament bulking sludge harvested from a laboratory scale N & P removal (Modified UCT) system. All three systems were operated at the same sludge age (20d) and received the same sewage as the parent MUCT system (Mitchell's Plain unsettled). Two of the systems were intermittently fed once daily while the third was continuously fed. One of the intermittently fed systems was anaerobic for the first 6h after feeding, aerobic for 16h, then settled for 2h. The other intermittently fed system, and the continuously fed system, were maintained fully aerobic for 24 h. In the two fully aerobic systems, the DSVI declined steadily from a start-up value of around 200 ml/g to below 60 ml/g over a period of 2 to 3 sludge ages. Over the same period, the DSVI in the intermittently fed anaerobic-aerobic system and in the parent MUCT system remained high, between 180 and 200 ml/g.

These experiments demonstrated that (1) continuous aeration inhibits the growth of most of the low F/M filaments, in particular , 0092 and 0914, irrespective of whether or not alternating feed/starve conditions prevail (intermittently or continuously fed), and (2) an initial anoxic- anaerobic period of 6h during which all the RBCOD is removed from the liquid phase, followed by an aerobic period of 16h, at a DO of 6 mgO/l and the anaerobic (9,6h), anoxic (11,2h), aerobic (14,4h) sequence of the parent MUCT system, allows low F/M filaments to proliferate and cause bulking. However, it was not clear how the continuation of bulking by low F/M filaments in the intermittently fed anaerobic/aerobic system fitted in with the amelioration of low F/M filament bulking observed in the anoxic-aerobic (AO/IFFD) and continuously fed (AO/CFCM) systems operated in phase 1 of the investigation. Nevertheless it was concluded from these experiments, and from the survey of filamentous organisms in full scale plants, that low F/M filaments proliferate in plants that have alternating aeration and non-aeration either in different reactors or at different times or stages in the same reactor.

2.5.2 Proliferation of low F/M filaments in laboratory scale intermittent aeration systems

Before the efficacy of aerobic (or anoxic) selectors in suppressing low F/M filament proliferation through kinetic selection could be determined, it was necessary to devise a laboratory system other than an N & P removal one, wherein low F/M filaments proliferated.

To do this, attention was focussed on unaerated/aerated systems, because it was evident from the experiments so far and from the bulking surveys, that low F/M filaments proliferate in full scale unaerated/aerated systems, irrespective of whether these were biological N & P removal systems or N removal only systems. Accordingly in this second phase of the investigation, unaerated/aerated N removal systems were operated.

In an attempt to grow low F/M filaments in laboratory systems other than N & P removal ones, long sludge age single reactor continuously fed completely mixed systems with intermittent aeration - 1 minute aeration resulting in a peak DO concentration of 2 mgO/l followed by 9 minutes no-aeration i.e. 3 minutes aerobic and 7 minutes anoxic) - and fed real sewage were set up to mimic the conditions of full-scale Carousel or Orbal type N removal plants in which low F/M filaments proliferate. It was found that in these intermittently aerated laboratory systems, most of the low F/M filaments proliferated, in particular, *M. parvicella* and 0092 but also 0914, 0041, 0675 and 1851. Switching these systems from intermittent to continuous aeration invariably caused a sharp decline in DSVI with a concomitant reduction in low F/M filaments and amelioration of the bulking. Switching back to intermittent aeration caused slow but inexorable regrowth of the low F/M filaments and associated bulking, confirming that the low F/M filaments respond very strongly to the presence or absence of unaerated periods in the system.

2.5.3 Aerobic selectors do not control low F/M filament proliferation

Having established that low F/M filaments proliferated in laboratory scale intermittent aeration systems, it became possible to check whether or not aerobic selectors control low

F/M filaments. This was done by setting up an experimental and a control system. Both were long sludge age single reactor, continuously fed completely mixed intermittently aerated systems. With a correctly sized multi- compartment aerobic selector installed on the experimental system, it was found that the selector effect did not control most of the low F/M filaments . The DSVI remained above 250 ml/g in both systems for more than 5 sludge ages (100 days). The presence of the selector effect in the experimental system sludge was verified by doing (i) batch tests to check that rapid RBCOD and oxygen uptake rates had been stimulated, (ii) soluble COD profiles in the selector reactors to see that all the RBCOD was taken up in the selectors and (iii) microscopic examinations to confirm that numerous Zoogloeal colonies had formed.

Switching the control system to continuous aeration caused the DSVI to decrease sharply in 10 days (< 100 ml/g), with a concomitant decline in low F/M filaments, while the DSVI in the experimental system with the selector reactors remained high (> 200 ml/g).

2.5.4 Implications of the results

2.5.4.1 Consistency of effect of aerobic selectors and anaerobic reactors on low F/M filaments

The observation that aerobic selectors did not control bulking by low F/M filaments, in particular types 0092, 0041, 0675 and *M. parvicella*, resolved the inconsistency with respect to the low F/M filaments in the behaviour between metabolic selection in anaerobic reactors (in N & P removal plants) and kinetic selection in aerobic selectors. In N & P removal plants, anaerobic reactors which stimulated preferential removal of influent RBCOD by floc-formers (Wentzel *et al.* , 1985) did not control low F/M filament proliferation. Aerobic (and by implication presumably also anoxic) selectors which promoted preferential removal of influent RBCOD by stimulating the selector effect also did not control low F/M filament proliferation.

2.5.4.2 *Confirmation that low F/M filaments do not require RBCOD for proliferation*

It would appear that the influent RBCOD does not play an important role in the growth of low F/M filaments in long sludge age systems. It would seem then that the low F/M filaments utilize particulate biodegradable COD (or its hydrolysis products) which originate either from the influent or are self generated within the system by death and lysis of organisms (Ekama and Marais, 1986).

2.5.4.3 *Conditions promoting nutrient removal appear to promote low F/M filament bulking.*

Low F/M filaments appear to proliferate in laboratory systems that expose the sludge mass to alternating anoxic-aerobic periods as in anaerobic-anoxic-aerobic multi reactor N & P removal systems and completely mixed intermittently aerated N removal systems (ditch type plants). When these systems or sludge harvested from these systems were exposed to purely aerobic conditions by continuous aeration, the low F/M filament bulking was ameliorated and sludge settleability improved (DSVI < 80 ml/g). From this it would appear that the anaerobic/anoxic conditions that are required to stimulate biological N or N & P removal could also stimulate proliferation of low F/M filaments in long sludge age systems, and unfortunately fully aerobic conditions which inhibit low F/M filament proliferation are not conducive to biological N or N & P removal. Consequently to effect specific control over the low F/M filaments, an environmental condition needs to be found that will lead to exclusion of the filaments but retention of the organisms and conditions that effect biological nutrient removal. At the conclusion of the investigation of Gabb *et al.* (1989a) such an environmental condition was not known.

2.5.4.4 *Anoxic-aerobic conditions apparently stimulate low F/M filament proliferation*

It was considered most likely that it is the anoxic-aerobic alternation that leads to the low F/M filament proliferation because this is a common feature in N & P removal and completely mixed ditch type N removal systems. No answers were offered as to the effects of the magnitude of the anoxic mass fraction, the length of the anoxic retention time (actual or nominal), the duration of the anoxic aerobic cycles in intermittent aeration systems, the concentration of nitrate during the anoxic periods, the frequency of alternation between anoxic and aerobic periods or the effect of the low D.O. concentrations which arise during the " lead- in" to anoxic conditions.

2.6 NEW RESEARCH DIRECTIONS

The finding that the selector effect did not control low F/M filament bulking placed this research back into an exploratory stage. As a consequence a central task of the bulking research programme since 1989 was to establish and pursue new directions of research. By considering the implications of the research reviewed above, investigations were initiated to determine the influence of the following factors on low F/M filament bulking:

1. Which components in the influent wastewater are responsible for bulking by the low F/M filaments? Because the influent RBCOD apparently does not play an important role in the sense that they can proliferate without it, can the low F/M filaments utilize the influent particulate biodegradable COD (PBCOD)? It is anticipated that the influent PBCOD does play a role in the growth of the low F/M filaments because this COD is not significantly reduced in selector reactors (whether aerobic or anoxic) and anaerobic reactors and therefore passes through to the anoxic and aerobic zones of the system. For the purpose of identifying the role of the influent PBCOD and RBCOD, it may be necessary to develop and refine an artificial sewage of known composition, which supports the growth of the low F/M filaments. The artificial sewage can be fed to the nutrient removal and completely mixed intermittent aeration systems to compare the filament populations that develop with the artificial sewage with those in similar systems receiving real sewage. The constituents of the artificial sewage can be manipulated to observe the influence of the RBCOD and PBCOD on the low F/M filaments. Additional to developing an artificial sewage, real sewage can be readily separated into its RBCOD and PBCOD constituents by modern ultra-filtration techniques. The RBCOD and PBCOD, appropriately reconstituted to its original volume with tap water, can be fed to various laboratory scale N and N&P removal systems to observe the effect of the substrate on low F/M filaments and system performance.

2. If PBCOD only supports the growth of the low F/M filaments, do the filaments utilize hydrolysis products of PBCOD in the liquid generated by other organisms or are they able to hydrolyse and utilize PBCOD directly themselves? Are low F/M filaments able to utilize

(either directly or indirectly) the substrate originating from the lysis of dead organisms in the biomass (Ekama and Marais, 1986b)? If influent PBCOD, or its hydrolysis derivatives, can be utilized by the low F/M filaments, what causes the filaments to proliferate under unaerated-aerated conditions but not purely aerated conditions?

3. Due to the strong influence of the periodic unaerated-aerated conditions in biological N and N&P removal plants - most likely the anoxic conditions because this is common to both N and N&P removal plants - Investigate the influence of the characteristics of the anoxic reactor on low F/M filament bulking, such as;

(i) *size* -because low F/M filaments proliferate (DSVI > 300 ml/g) in anoxic-aerobic systems with large anoxic fractions (50-70%) and not (DSVI < 80 ml/g) in purely aerated systems (0% unaerated) is there a trend that the greater the anoxic fraction, the higher the DSVI? From Arkley and Marais (1981), this would appear to be the case; unfortunately in their work the filaments were not identified, but probably these were low F/M ones because *S.natans, Thiothrix* or 021N are rarely found in laboratory multi reactor anoxic-aerobic (N removal) or anaerobic-anoxic-aerobic (N&P removal) systems in which all the influent is discharged into the anoxic or anaerobic reactors. Can the low F/M filaments proliferate under fully anoxic conditions?

(ii) *position* -i.e. as a primary anoxic reactor receiving the influent flow and before the aerobic reactor or as a secondary anoxic reactor after the aerobic reactor.

(iii) *type* - i.e. anoxic reactors in compartments separated from the aerobic reactor or forming part of single intermittent aeration ditch type reactors which are anoxic where the DO is close to zero.

(iv) *nitrate* - investigate the effect of the nitrate concentration in the anoxic zone on the proliferation of low F/M filaments.

(v) *frequency of alternation* between anoxic and aerobic conditions - in intermittent aeration systems the aeration cycle establishes the number of times the sludge is switched between anoxic and aerobic conditions, and in multi reactor anoxic-aerobic systems this is established by the recycle ratios; does this frequency of alternation between the anoxic and the aerobic conditions have an influence on the low F/M filament proliferation?

(vi) *low DO conditions* - in intermittent aeration systems do the low DO conditions leading to anoxic conditions promote the low F/M filament proliferation?

4. Because the low F/M filaments appear to proliferate in long sludge age systems, at what sludge age is their proliferation suppressed so that sludge settleability is at most a DSVI of 100 ml/g? Is N and N&P removal possible at this sludge age?

5. Attempt to control bulking by low F/M filaments in different system configurations which incorporate biological N&P removal. For example;
(i) a system configuration which minimizes utilization of influent PBCOD under anoxic conditions (but not generated by organism death and lysis) is the Johannesburg system, with anaerobic and aerobic zones following sequentially and an anoxic zone in the underflow

recycle stream for denitrification of the return sludge to the anaerobic reactor. If such a system inhibits proliferation of low F/M filaments compared to a MUCT system, it would indicate that the filaments utilize influent PBCOD, under anoxic conditions.

(ii) sludge ages in N and N&P removal plants are long (>20 days) principally to ensure nitrification. Wanner *et al.* (1988) investigated the influence of fixed media in the aerobic zone of N and N&P removal plants on the nitrification rate. With this approach it may be possible to maintain a long aerobic sludge age on the fixed media nitrification while the suspended sludge has a sludge age sufficiently short to suppress low F/M filament proliferation.

2.7 UNIVERSITY OF CAPE TOWN INVESTIGATION-PHASE 3

The above research areas are clearly wide ranging and in order to investigate them a second comprehensive laboratory research investigation was commenced in 1989. The research presented in this thesis forms part of this phase 3 investigation and in order to place it in the context of this investigation, a brief review of its progress relevant to this thesis is given below.

2.7.1 The development of artificial sewage feed supporting low F/M filament growth by Gabb (1988)

This work followed three steps:

(1) Chemical Composition: Nutritional requirements insofar as readily (RBCOD) and particulate (PBCOD) biodegradable COD constituents were concerned were established from the literature for many of the activated sludge bacteria. In addition the chemical constituent analyses of domestic sewage reported in the literature were examined. The composition of Mitchell's Plain raw sewage was important because this was the sewage fed to the laboratory scale activated sludge systems which were compared with systems fed artificial sewage. From this information and measured principal constituents of Mitchell's Plain raw sewage (COD, Organic N, $NH4^+$, fats and oils, RBCOD and PBCOD), an artificial sewage was formulated which was progressively refined after experimentation on activated sludge systems in steps (2) and (3) below.

(2) Kinetic response: The correct proportions of RBCOD and PBCOD were determined by comparing the batch test results with artificial sewage and with Mitchell's Plain raw sewage. RBCOD and PBCOD proportions were varied until they matched those of raw sewage.

(3) Microbiological Response: The ability of the low F/M filaments to proliferate in the systems fed the artificial sewage was evaluated. For this purpose experimental laboratory systems were operated receiving the artificial sewage, both systems receiving Mitchell's Plain raw sewage. It was found that an unaerated-aerated (6 hours unaerated, 16 hours aeration, 2 hours settling) intermittently fed fill and draw (IFFD) system receiving artificial sewage feed on occasion promoted the abundant growth of the following filaments; types 0092, 0914, 0041, 0675, 0803, *Haliscomenobacter hydrossis* and *Nostocoida limicola ll.* In the surveys of Blackbeard *et al.* (1986, 1988), all of these filaments had been observed in bulking sludge of full scale plants (the first four named more common than the last three). During these

experiments the inorganic nutrient concentrations of the artificial sewage were adjusted to prevent these being growth limiting.

2.7.2 The work of Casey *et al.* (1990) with artificial sewage

The artificial sewage developed by the procedure above was later used in experiments by Casey *et al.* (1990) with only the RBCOD and PBCOD proportions being varied. The following observations were made on intermittently aerated single completely mixed reactor systems:

(1) low F/M filaments, in particular *H.hydrossis* and 1851 but also 0092, 0041 and 0675, proliferated to exceptionally high DSVI's (>600 m*l*/g) irrespective of whether the feed comprised only RBCOD or PBCOD. The only difference was that with RBCOD their proliferation was more explosive and rapid than with PBCOD.

(2) Changing the aeration pattern from intermittent (anoxic/aerobic) to continuous (aerobic) caused amelioration of bulking by the low F/M filaments - specifically *H.hydrossis* and 1851.

(3) Changing the systems from continuous to intermittent aeration caused proliferation of low F/M filaments specifically *H.hydrossis* and 1851.

(4) *M.parvicella* did not grow in systems irrespective of whether or not fats and oils were excluded from the artificial sewage. In similar intermittent aeration systems receiving real sewage, *M.parvicella* is often the dominant one (Warburton *et al.*, 1991 see below).

(5) Reducing the amount of nitrate added to the systems so that the effluent nitrate concentration was <5,0 mgN/*l* caused an amelioration of bulking (DSVI down from 680 m*l*/g to 150 m*l*/g) and a reduction of low F/M filaments, specifically *H.hydrossis*.

Casey *et al.* (1990) also found that artificial sewage fed to a MUCT system containing low F/M filaments developed on real sewage, caused the DSVI of the sludge to decrease from 191 ml/g to 83 ml/g in 51 days. In an attempt to reseed the system with low F/M filaments, 10% of the MLSS mass in the system was replaced daily with mixed liquor from MUCT systems fed real sewage containing low F/M filament bulking sludge for 5 consecutive days. This caused a temporary increase in the DSVI, but when seeding ceased the DSVI decreased again indicating the low F/M filaments were unable to grow in a typical MUCT system receiving the artificial sewage feed. The same conclusions were arrived at by Gabb (1988).

2.7.3 The work of Warburton *et al* (1991) with intermittently aerated systems fed real sewage

Warburton *et al.* (1991) investigated the effect of (1) nitrate concentration during the anoxic period, (2) varying the anoxic mass fraction , and (3) varying the sludge age on low F/M filament bulking in continuously fed intermittently aerated single reactor completely mixed reactor systems receiving real sewage as feed. The following conclusions were drawn:

(1) the nitrate concentration during the anoxic period did influence the DSVI; high nitrate levels (effluent nitrate concentrations between 30 and 50 mgN/l) were associated with increases in the DSVI whereas low nitrate concentrations (effluent nitrate concentrations <

5.0 mgN/l) led to a decrease in the DSVI. However even under low nitrate conditions the low F/M filaments, particularly 0092 and *M. parvicella,* were able to proliferate to the extent of causing bulking (i.e. DSVI 200ml/g and higher).

(2) Increasing the aerobic mass fraction from 30% to 70% (reducing the anoxic mass fraction from 70 to 30 %) led to a decrease in the DSVI from 200-400 ml/g down to 120-150 ml/g. The low F/M filaments present in the systems were *M.parvicella, H.hydrossis,* 0092 and 0041.

(3) Sludge age did influence the DSVI: at short sludge ages (< 10 days) the DSVI was lower than at long (> 10 days) sludge ages. However the low F/M filaments still proliferated sufficiently abundantly even at very short sludge ages (5d) to cause bulking (DSVI > 150 ml/g).

(4) While low anoxic nitrate concentrations, short sludge ages and small anoxic mass fractions tend to discourage proliferation, the only factor to date which ameliorated the low F/M filament bulking and yielding DSVI's < 100 ml/g was continuous aeration.

2.7.4 The work of Ketley *et al.* (1991) with intermittently aerated systems fed artificial and real sewage

With artificial sewage feed, Ketley *et al.* (1991) examined the effect on the low F/M filaments of

(1) fully anoxic conditions, and

(2) the magnitude of the nitrate concentration during the anoxic period

and with real sewage feed examined the effect of

(1) fully anoxic conditions, and

(2) the frequency of exposure to alternating anoxic-aerobic conditions.

All the experimental systems operated were long sludge age (15 days) continuously fed single completely mixed reactor N removal systems, either intermittently aerated or fully anoxic. The single reactor form avoided the complexity of biological excess P removal in multi reactor systems and, as was demonstrated earlier, intermittently aerated single reactor systems were found to consistently promote the proliferation of low F/M filaments in the activated sludge with artificial and real sewage.

From their work with artificial sewage Ketley *et al.* (1991) concluded that:

(1) In intermittently aerated systems (70% anoxic mass fraction), low nitrate concentrations during the anoxic period led to amelioration of bulking by filaments 1851 and 1701 (of which only the former is a low F/M one). However the production of polymeric material in the sludge could have played a role in the reduction of the DSVI.

(2) Under fully anoxic conditions, only *H.hydrossis* was able to proliferate to the extent of causing bulking; other low F/M filaments declined.

Because *H.hydrossis* is a filament of little consequence in full scale systems, Ketley *et al.* (1991) repeated the experiments with real sewage. From these experiments it was concluded that:

(1) Low F/M filaments were unable to proliferate under fully anoxic conditions to cause bulking and the excessive growth of *H.hydrossis* with artificial sewage was not a true reflection of that filament's growth under the same conditions when fed real sewage.

(2) Increasing the frequency of alternation between anoxic and aerobic conditions from 48 cycles/d (30 minute cycles) to 1 cycle every 3 days (3 day cycles) had no ameliorating effect on the lw F/M filament bulking.

(3) Stimulation or suppression of low F/M filament proliferation could be reproduced repeatedly by switching from intermittent aeration (stimulation) to either fully aerobic or fully anoxic conditions (suppression) respectively, with fully aerobic conditions leading to more rapid decreases in DSVI than fully anoxic conditions.

2.7.5 The work of Hulsman *et al.* (1992) with compartmentalized N removal systems fed artificial and real sewage.

Hulsman *et al.* (1992) with artificial and real sewage fed to two reactor anoxic-aerobic systems, examined the effect on low F/M filament proliferation of:

(1) the *type* of anoxic zone i.e. compartmentalised into a separate reactor as distinct from

single reactor intermittent aeration systems,

(2) the *size* of the anoxic mass fraction,

(3) the *position* of the anoxic reactor relative to the aerobic reactor i.e. as primary anoxic reactor receiving influent and underflow recycle streams (predenitrification, MLE) or as a secondary anoxic reactor receiving effluent from the aerobic reactor (post denitrification, Wuhrmann),

(4) the frequency of anoxic-aerobic alternation per day and

(5) the system MLVSS concentration.

The following conclusions were made by Hulsman *et al.* (1992)

(1) Filamentous organism proliferation in these systems was much less severe than in intermittently aerated systems operated under similar conditions, but was more severe than in fully anoxic or fully aerobic systems operated under similar conditions.

(2) Changing the size of the anoxic reactor of the MLE system from 70 to 54% and back again from 54 to 74% did not significantly affect the low F/M proliferation and the sludge settleability remained below 125 m*l*/g under both conditions.

(3) Positioning the anoxic reactor after the aerobic reactor did, but only to a small degree, decrease the DSVI for artificial sewage from 200 to 150 m*l*/g and for real sewage from 130 to 100 m*l*/g.

2.7.6 The work of Casey *et al.* (1992c) on MUCT systems using real sewage

Using real sewage feed it was found possible to manipulate the sludge settleability (DSVI) to high and low values, hence low F/M proliferation in MUCT systems by:

(1) Manipulating the anoxic sludge mass fraction. In a pair of MUCT systems, one with a high anoxic mass fraction (15% anaerobic, 20% first anoxic and 32% second anoxic and 33% aerobic), the other, with a low anoxic mass fraction (15% anaerobic, 20% first anoxic and 32+33=65% aerobic) the DSVI was high (200-250 m*l*/g) in the former and low (100-150 m*l*/g) in the latter.

(2) Manipulating the influent TKN concentration with ammonium dosing to the influent. At low TKN/COD ratio (no ammonium dosing) the concentration of nitrate generated to be denitrified by the anoxic reactors was very low (<5 mgN/*l*). At high TKN/COD ratios (with ammonium dosing) complete denitrification in the anoxic reactors (mainly the second anoxic) was no longer possible leading to high concentrations of nitrate and nitrite leaving the anoxic reactor (>10 mgN/*l*). With ammonium dosing the DSVI increased (from 100 to 280 m*l*/g) and without ammonium dosing the DSVI decreased (from 250 to 170 m*l*/g).

2.8 THE BULKING HYPOTHESIS PROPOSED BY CASEY *et al.* (1992b)

From all of the above experiments it was proposed that a possible cause for the low F/M proliferation lay in the requirement for the sludge mass to switch between aerobic and anoxic metabolic pathways, this switching providing some competitive advantage to the filamentous organisms at the expense of the floc-forming organisms. Attention was thus focussed on denitrification pathways.

Payne (1973) proposed the general denitrification pathway

NO_3^- -> NO_2^- -> NO -> N_2O -> N_2

nitrate nitrite nitric oxide nitrous oxide dinitrogen

Initially denitrification was considered a strictly anoxic process, occurring only in the total absence of oxygen. However, subsequently it has been demonstrated quite convincingly in pure cultures that denitrification can continue under aerobic conditions, albeit at a lower rate [Pichinoty and d'Ornano (1961), Showe and De Moss (1968), Krul and Veeningen (1977), Robertson and Kuenen (1984)]. Pure culture studies have also demonstrated that one or more of the intermediates in the denitrification pathway have an inhibitory effect on the aerobic utilisation of a substrate with oxygen as a terminal electron acceptor. Krul (1976) in pure culture studies on a denitrifying organism isolated from activated sludge, cultured under anoxic conditions and tested under aerobic conditions, concluded that the accumulation of the intermediate nitric oxide (NO) during denitrification caused a measurable and prolonged inhibition of oxygen utilisation under subsequent aerobic conditions. Curiously, this inhibition could be demonstrated for pure culture of an isolate from activated sludge but not for a mixed culture of activated sludge.

Some controversy arose as to whether the inhibitory effect was due to NO_2^- or NO, but recent work has concluded that the inhibitory effect is due to NO and not NO_2^-. However, the degree of inhibition is exacerbated by the presence NO_2^- and NO_3^- (Kucera *et al.*, 1987; Carr and Ferguson, 1990).

With this basis Casey *et al.* (1992a,b) proposed the following explanation for the proliferation of low F/M filaments in N and N & P systems: Floc-formers are inhibited under aerobic conditions by denitrification intermediates accumulated under the preceding anoxic conditions; the denitrification intermediate causing the inhibition is NO. For this explanation to be valid requires the low F/M filaments to denitrify only as far as NO_2^- and therefore not accumulate NO, and the floc-formers to denitrify completely to N_2 and thereby accumulate NO under certain conditions. Under this hypothesis, low F/M filament bulking can be expected to take place if nitrate and nitrite removal in the anoxic reactor is incomplete. In this event, the floc-forming organisms would still have NO accumulated in their enzyme systems causing oxygen uptake inhibition in them on entering the aerobic reactor. If nitrate and nitrite reduction is complete, then low F/M filament bulking would not be expected because then the NO intermediate will have been denitrified also and oxygen uptake inhibition in the floc-formers will not take place.

2.9 SCOPE OF THIS THESIS

The research presented in this thesis was commenced in 1989 and was done concurrently with that of Warburton *et al.* (1991), Ketley *et al.* (1991), Hulsman *et al.* (1992) and Casey *et al.* (1992a,b) reviewed above; it therefore contributed to the formulation of the bulking hypothesis in much the same way as did that of Warburton, Ketley, Hulsman and Casey.

Indeed, the systems in which inhibition was first noted and measured by Casey *et al.*, (1992b) was in the two reactor nitrification-denitrification system operated in the investigation presented in this thesis. The experiments in which nitrate and nitrite concentrations and redox potential were determined during the anoxic-aerobic cycles of the intermittent aeration systems operated in this investigation also provided a valuable clue for the bulking hypothesis of Casey *et al.* (1992b). Therefore the research presented in this should not be viewed as work done subsequent to the development of Casey's bulking hypothesis, it was done during and formed part of the experimental evidence from which the hypothesis emerged. In the next chapter (3) the experimental investigation and the results obtained are described in detail.

CHAPTER 3

EXPERIMENTAL INVESTIGATION

3.1 INTRODUCTION

In activated sludge plants, bulking due to the proliferation of filamentous organisms is a considerable problem. Filamentous bulking results in poor settleability of the mixed liquor and, in the event of secondary clarifier overload as a consequence of this, a dramatic deterioration in effluent quality over the peak flow periods of the day. This poor settleability of the sludge limits the wastewater flow which may be treated in the activated sludge plant, at values often considerably lower than the expected design capacity which results in premature plant extensions.

If filamentous bulking could be controlled, considerably higher wastewater flows could be treated in existing plants - between 50 to 100% more (Ekama and Marais, 1986a). The savings this promises to bring is the principal driving force motivating the research into the cause and control of filamentous bulking.

From surveys of long sludge age nutrient removal plants in South Africa, it was determined that most of the filamentous bulking which occurred in these plants was caused by a limited number of filamentous organism species, this group of species all sorting into a category called low food / microorganism ratio (F/M). (Jenkins *et al.*, 1984)

A comprehensive review on bulking was set out in Chapter 2, which concluded with a statement of the hypothesis for low F/M filament bulking of Casey *et al.* (1992b). This hypothesis states that some intermediate nitrogen compounds in the denitrification pathway inhibit floc formers in the aerobic zone of anoxic - aerobic activated sludge systems . This principal inhibiting intermediate is nitric oxide, produced and retained intracellularly by the floc formers in the anoxic zone while denitrifying nitrate to nitrogen gas . The NO forms stable compounds with some of the electron transport cytochromes to oxygen and blocks the electron transport chain via these cytochromes with the result that the floc - formers are inhibited in oxygen uptake in the aerobic zone. The low F/M filaments (it is hypothesized) denitrify NO_3^- only as far as NO_2^- with the result that they do not accumulate NO and therefore are not subject to aerobic NO inhibition. A corollary of this hypothesis is that if all the nitrate and nitrite in the anoxic reactor are completely denitrified prior to return to the aerobic reactor, ie a "zero" nitrate and nitrite concentration in the outflow of the anoxic to the aerobic reactor, then there should be no intracellular NO in the floc - formers. As a result the inhibition would not occur and consequently a non - bulking sludge should be produced in the system. On the other hand, if denitrification is not complete in the anoxic reactor then intracellular NO would be present in the floc formers and bulking would occur.

3.2 EXPERIMENTAL SET-UP

In the literature review, which evaluated various different fully anoxic, fully aerobic and anoxic-aerobic systems it was concluded that the intermittently aerated conditions of the anoxic - aerobic systems appeared to be the principal cause of the low F/M filament bulking - fully anoxic and fully aerobic systems were observed not to bulk, whereas the anoxic aerobic systems did bulk. What was not clear, although some work had been done by Hulsman *et al.* (1992), was the effect of the form in which the anoxic - aerobic conditions prevail in the system ie. in separated anoxic and aerobic reactors with recycles (Modified Ludzack-Ettinger or Wuhrmann systems) or in single reactor intermittently aerated systems. While both these systems have anoxic - aerobic conditions, the form of these conditions are distinctly different even if operated at the same anoxic mass fraction. For instance, with separated reactors the feed is introduced only under the anoxic (MLE) or aerobic (Wuhrmann) conditions and the change from anoxic and aerobic conditions is rapid, with minimal low DO conditions. In contrast in the single reactor intermittently aerated system, the feed is introduced to both anoxic and aerobic periods and the change from aerobic to anoxic conditions is slow, passing through a period of low DO concentration. In this investigation the effect of these different anoxic-aerobic conditions on the low F/M bulking problem, as reflected by the nitrate and nitrite concentrations, is examined in the light of the low F/M filament bulking hypothesis outlined above.

Experiments to determine the influence of nitrogen compounds in the mixed liquor on filamentous bulking

In order to test the above corollary of the low F/M filament bulking hypothesis, intermittently aerated and 2 reactor anoxic aerobic systerns were set up and operated. In these systems, *inter alia*, the concentrations of nitrate and nitrite (the latter as indicator of the presence of nitric oxide) were measured in the various reactors and the effluent. The DSVI was measured to assess the sludge settleability and regular microscopic examinations were conducted to identify the filamentous organisms. The overall plan of the experimental investigation is given in Table S.1 (see Synopsis).

3.2.1 Intermittent aeration system

Initially one intermittently aerated single reactor completely mixed system was started up. The sludge age was set at 15 days by wasting 667 ml of mixed liquor per day (including samples drawn) from the 10 l reactor. The daily aeration pattern of the system was divided into three aeration cycles per day. Within each cycle of 480 minutes the system was aerobic for 140 minutes at a dissolved oxygen concentration between 2-3 mgO/l and unaerated ie. anoxic for a period of 340 minutes, thus giving an aerobic mass fraction of 30% and an anoxic fraction of 70%. The system received a feed volume of 10 l/day at a concentration of 500 mg COD/l, which was obtained by appropriate dilution of stronger, real Mitchell's Plain raw sewage, stored in stainless steel tanks at 4°C.

After 52 days of operation an ammonium supplement was added to the influent feed, at an initial concentration of 10 mgN/l per litre of influent feed (later on day 65 increased to 20 mgN/l) to increase the influent TKN concentration. Through nitrification this increased the concentration of nitrate generated and hence there was more nitrate to be denitrified. At the fixed anoxic mass fraction of around 70% the additional nitrate was expected to increase nitrate and nitrite concentrations in the reactor during the aerobic periods. A schematic layout

3.5

SINGLE INTERMITTENTLY AERATED REACTOR

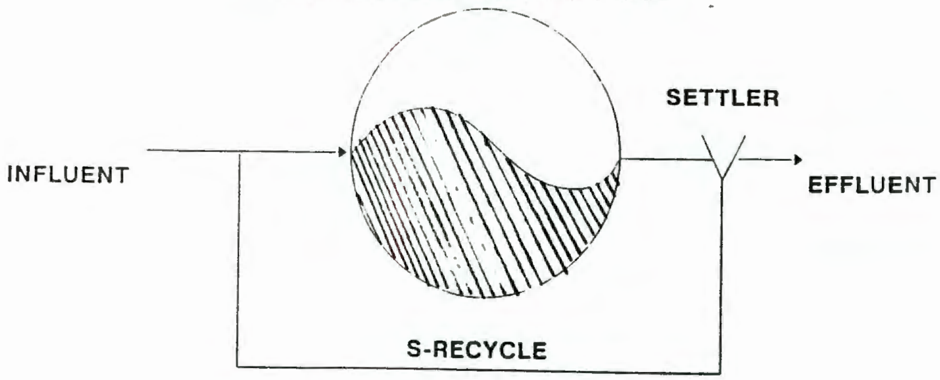


Fig 3.1 Schematic layout of Intermittent aeration system

Table 3.1 Intermittent aeration system operating parameters

SYSTEM	EXPERIMENTAL(intermittent)
Operating conditions	Continuously Fed Intermittently aerated single reactor system
Aeration	25 - 30% aerobic, 70 - 75% anoxic
Sewage Source	Mitchell's Plain Raw
Mass COD fed/d	4000 - 5000
Volume of Feed(l/d)	10 l
Concentration (mg COD/d)	500
Influent TKN (mg N/l)	35 - 50
Ammonium dose:	
day 1 - 52	zero
day 53 - 64	10 mg NH_4-N/l influent
day 65 - 82	20 mg NH_4-N/l influent
Sludge Age (days)	15
Reactor Volume (l)	10
pH of mixed liquor	7.2 - 7.8
Operating temperature	20°C
Recycle ratio (s)	1:1
MLSS Concentration	2000
VSS Concentration	1500
Mass Fractions:	
aerobic	25 - 30%
anoxic	70 - 75%

of the system is given in Fig 3.1 and the system design and operating parameters are listed in Table 3.1. The system was operated in this configuration for a period of 83 days.

Over the 83 day period the following parameters were measured daily on the system.

1. Influent (unfiltered) and effluent (filtered) COD concentrations (Fig 3.2)
2. Influent (unfiltered) and effluent (filtered) TKN concentrations (Fig 3.3)
3. Effluent nitrate and nitrite concentrations (both filtered) (Fig 3.4)
4. Reactor MLSS and MLVSS concentration (Fig 3.5)
5. Oxygen utilization rate (OUR) during aerobic period (Fig 3.6)
6. Sludge settleability as DSVI (Fig 3.7)

Filament identification was carried out every 2 to 4 weeks. (Table 3.2, Fig 3.7)

The results of the above measurements are listed in Appendix 1 and shown graphically in Figs 3.2 to 3.7 (see list of measured parameters above).

Discussion of Results

System behaviour - COD balance

To check the reliability of the experimental results a COD mass balance check was conducted. In intermittently aerated systems a N balance cannot be conducted because it is not possible to calculate the mass of nitrate denitrified per day. Consequently a 100% N balance is assumed to obtain the mass of nitrate nitrified and denitrified required for the COD balance. Details of the N and COD mass balance calculations are given in Appendix 2.

To facilitate the mass balance calculations the experimental data need to be divided into steady state periods to take account of different sewage characteristics (principly the TKN concentration) and system operating conditions. Generally for the 83 days the system was operated, the influent TKN concentration for the different sewage batches varied between 45 and 50 mgTKN-N/l (Fig 3.3). Over the 83 days 6 sewage batches were fed, the commencement of which is indicated in Figs 3.2-3.7 by a * near the upper horizontal axis. The small variation in the influent TKN concentration allows the 83 days of data to be divided into only 2 steady state periods (SSP, indicated by vertical lines in Figs 3.2-3.7) ie. one without ammonium dosing (day 1 - 64) and one with ammonium dosing (day 65 - 83). Averages of the measured data for the two steady state periods are given in Table 3.3. From this data for the 2 steady state periods, the COD mass balances are 84% and 76% respectively (based on a 100% N balance in the system - see Appendix 2 for details). These values seem reasonable when compared to the COD balances obtained by Warburton *et al.* (1991) which varied between 85 and 105% for similar systems. (see Table 3.4)

System behaviour - COD removal

For the first 64 days of operation (steady state period 1 - no ammonium added) the average COD removal was 83%, for the average influent COD of 441 mgCOD/l, and an average effluent COD concentration for this period of 76 mgCOD/l. During steady state period 2 (ammonium added) the average influent COD was slightly higher, 475 mgCOD/l, with an average effluent COD concentration of 92 mgCOD/l giving an average COD removal of 81% (see Table 3.3). This gives an average unbiodegradable soluble COD fraction (f_{us}) of 0.18, which although fairly high, compares favourably with that obtained by Warburton *et al.*

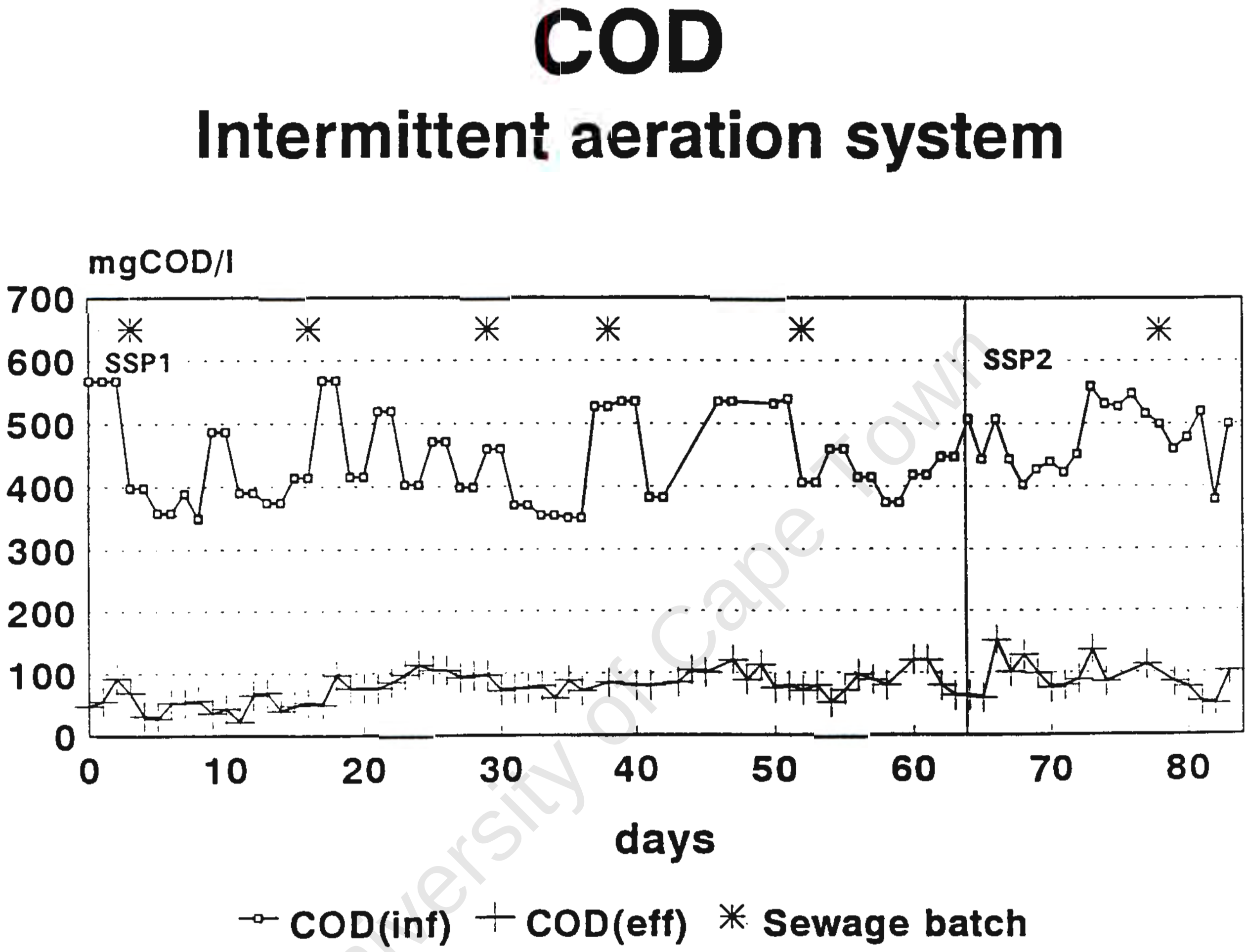


Fig 3.2 Intermittent aeration system influent and effluent COD concentrations

(SSP = steady-state period)

(1991) for the same wastewater.

The unbiodegradable particulate COD fraction (f_{up}) for the wastewater was also calculated. This was done by accepting the estimated f_{us} values of 0.17 and 0.2 for steady state periods 1 and 2 respectively and equating the measured concentrations of VSS in the system to the calculated value from the steady state model WRC(1984):

$$M(X_v) = M(S_{ti})\, R_s \{ \frac{(1 - f_{us} - f_{up})\, Y_h}{(1 + b_h R_s)} (1 + f b_h R_s) + \frac{f_{up}}{f_{cv}} \}$$

Where X_v = measured VSS concentration and the other symbols have the usual designation (see WRC, 1984) and values for 20°C operational temperature (ie. $b^h = 0.24$).

On this basis the f_{up} values for the two steady state periods are 0.188 and 0.102 for steady state periods 1 and 2 respectively. These values are of a similar magnitude compared with those obtained by Hulsman *et al.* (1992) and Warburton *et al.* (1991) for the same wastewater and similar N removal systems. However it is curious that for the same wastewater but Modified UCT system Musvoto *et al.* (1992), Clayton *et al.* (1989) and Casey *et al.* (1992c) all obtained higher f_{up} values in the range 0.20 to 0.30! This indicates that MUCT N & P removal plants produce significantly more sludge (20 - 25%) than N removal systems for the same sewage COD mass load and sludge age. No explanation for this can be advanced at this stage.

System behaviour - Nitrification and denitrification

The influent sewage TKN fed to the system over the 64 day period (Steady state period 1) varied in concentration between 45 and 50 mgTKN-N/l. Sewage from the initial batch (day 0 to 4) had a high TKN concentration (60 - 80 mgTKN-N/l). The effluent TKN concentration of the system during these first four days is thus high (>20 mgTKN-N/l) as well, due to incomplete nitrification. On day 5 when the second sewage batch was commenced with a TKN of about 50 mgTKN-N/l, nitrification improved and was virtually complete - low (6 mgTKN-N/l) effluent TKN concentration by day 7. The effluent TKN concentration remained low (< 17 mgTKN-N/l) for the next 10 days to day 17.

For the period between day 17 and day 60 the effluent TKN concentration was relatively high, ranging between 5 and 24 mgTKN-N/l, indicating variable degrees of nitrification. Around day 25 it was at its highest of 24 mgTKN-N/l, but thereafter gradually decreasing to around 5 mgTKN-N/l, by day 60, when virtually complete nitrification was again achieved. This variability in nitrification probably arose because the sludge age (15 days), and the aerobic mass fraction (30%) placed the system close to the limit for nitrification. A system in this state is very sensitive to small changes in operating conditions such as differences in (1) influent wastewater batches which influence the maximum specific growth rate of the nitrifiers and (2) peak oxygen concentration attained in the air-on cycle and (3) OUR in the initial part of the air-off cycle, the latter two causing variability in the aerobic mass fraction.

With the addition of ammonium to the influent feed on day 64 the measured influent TKN

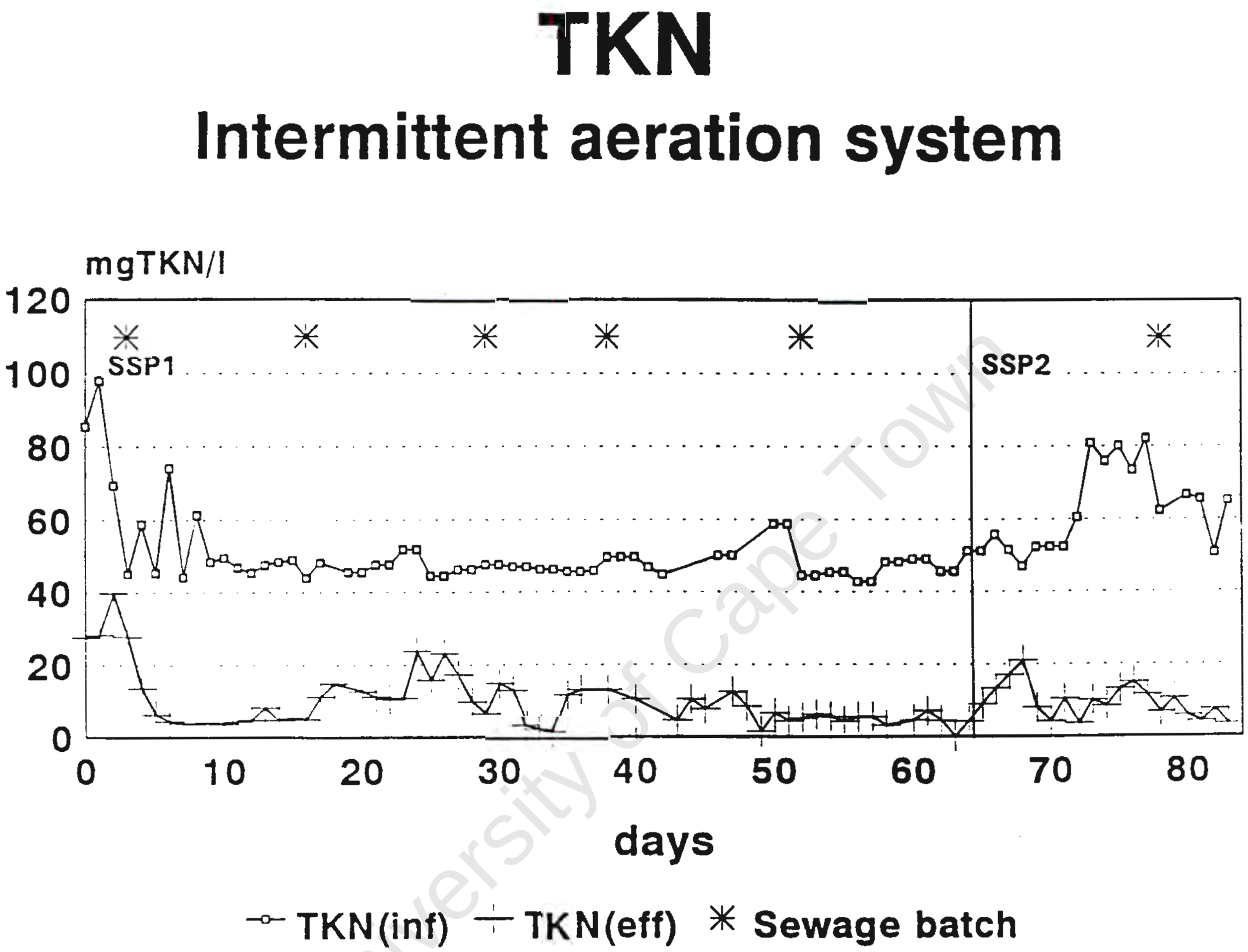
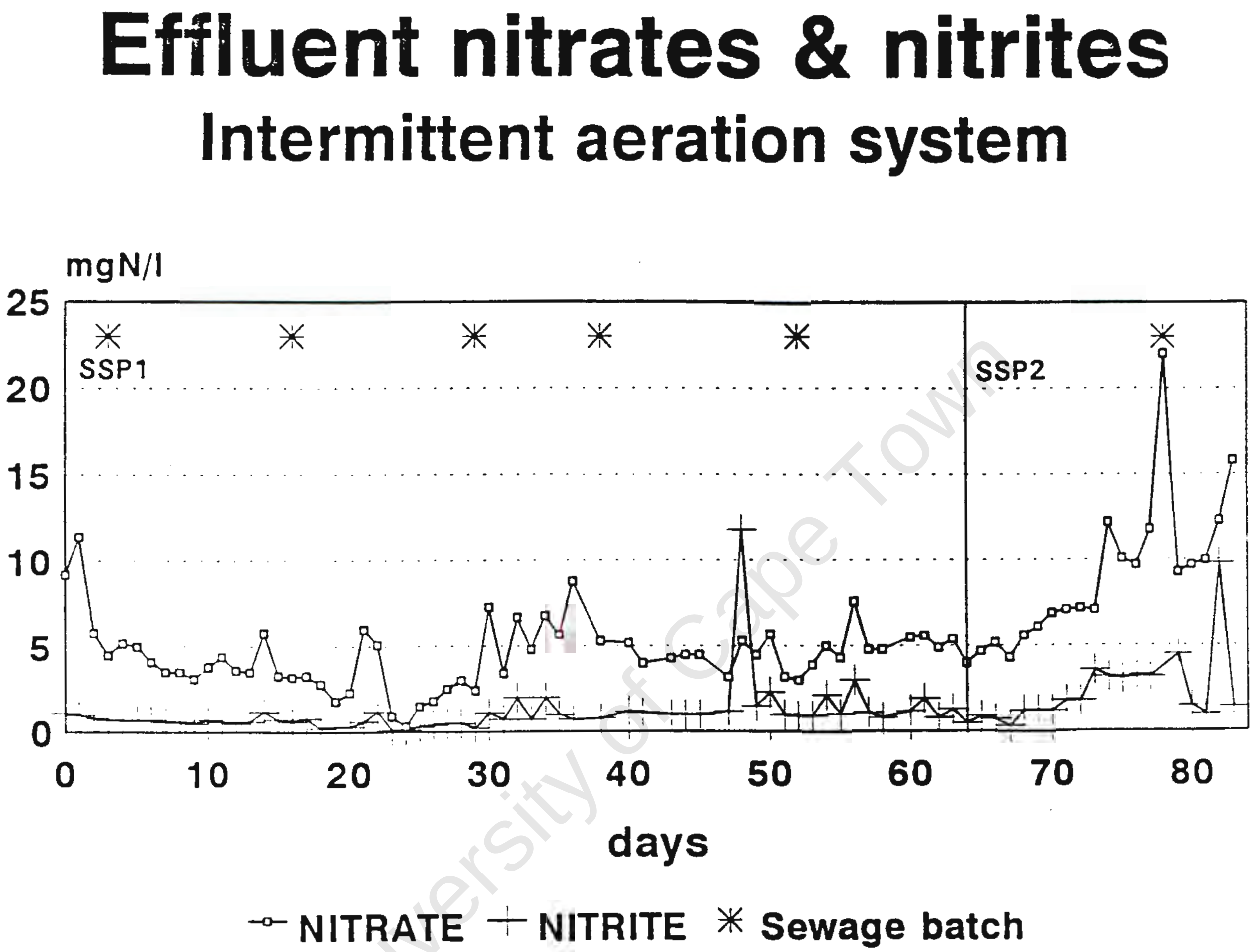


Fig 3.3 Intermittent aeration system influent and effluent TKN concentrations

(SSP = steady-state period)



ig 3.4 Intermittent aeration system effluent nitrates and nitrites

(SSP = steady-state period)

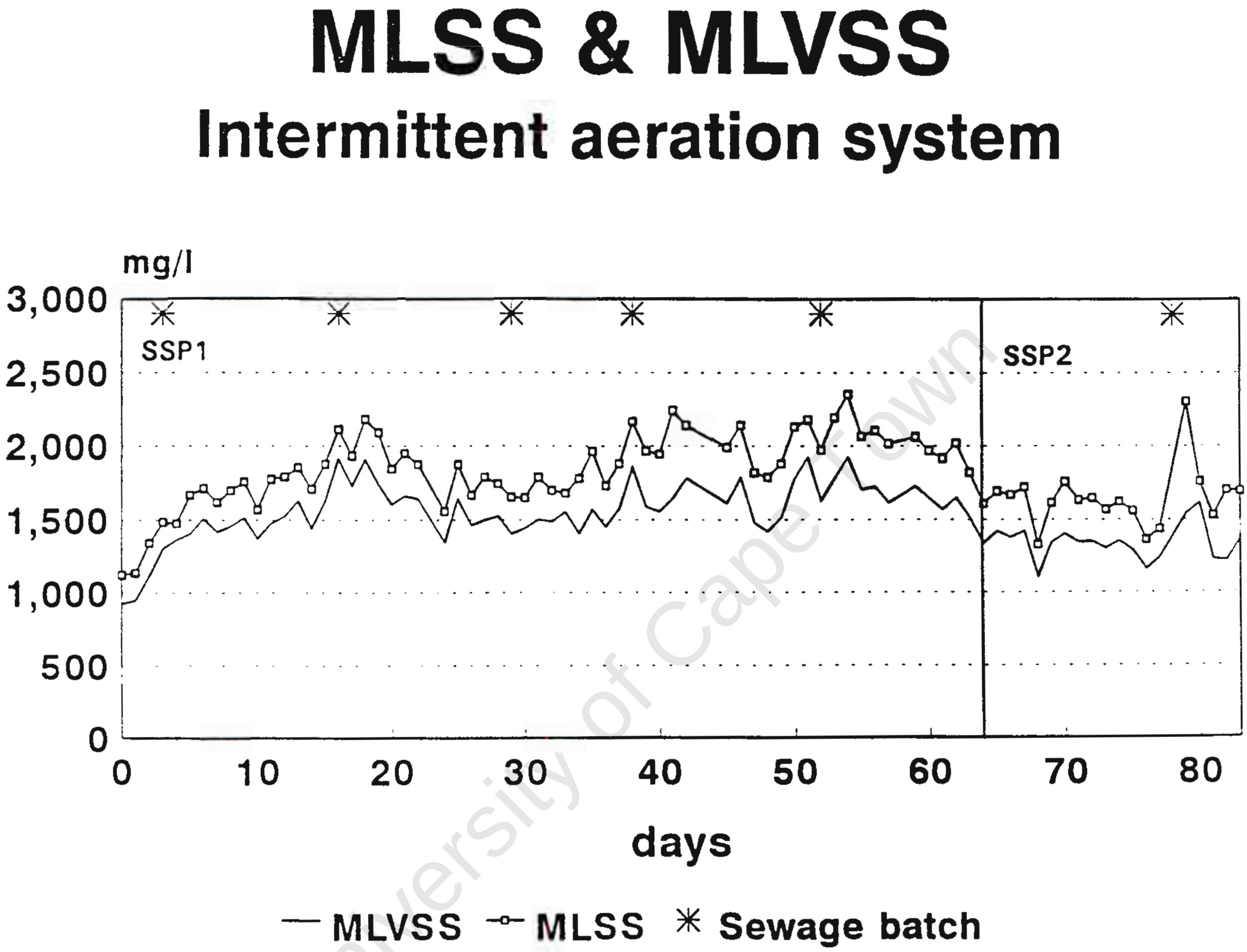


Fig 3.5 Intermittent aeration system MLSS and MLVSS concentrations

(SSP = steady-state period)

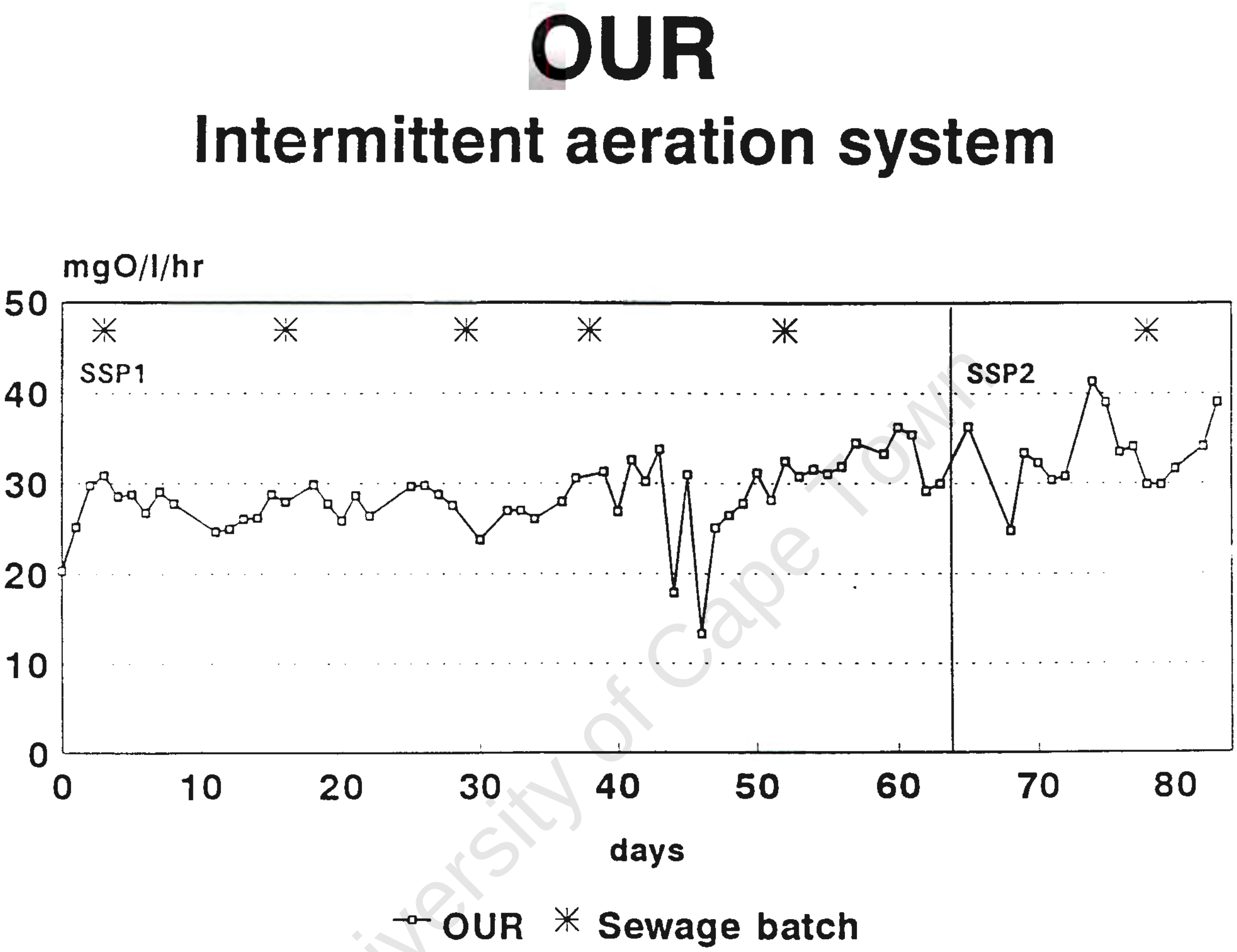


Fig 3.6 Intermittent aeration system OUR during aerobic period

(SSP = steady-state period)

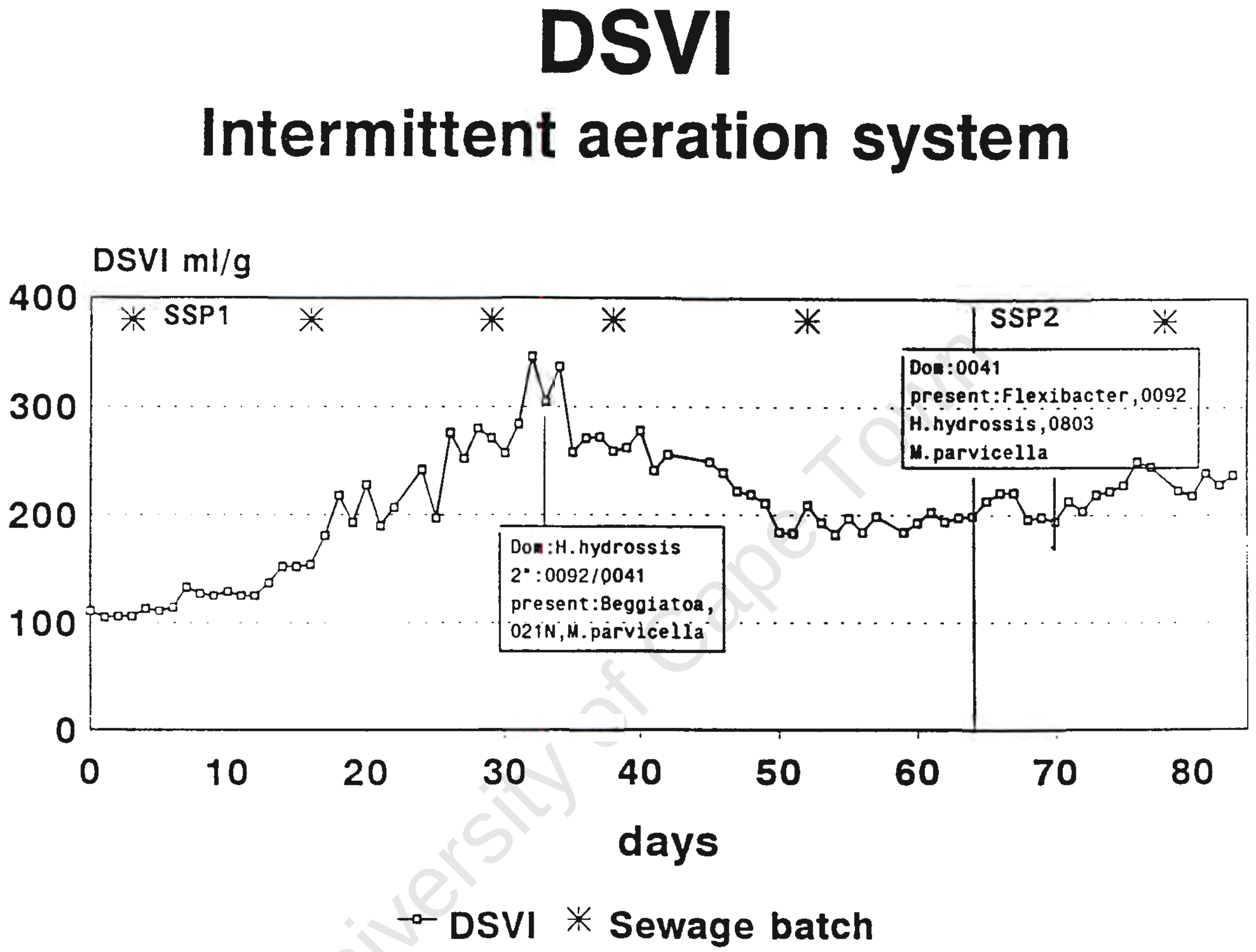


Fig 3.7 Intermittent aeration system DSVI

(SSP = steady-state period)

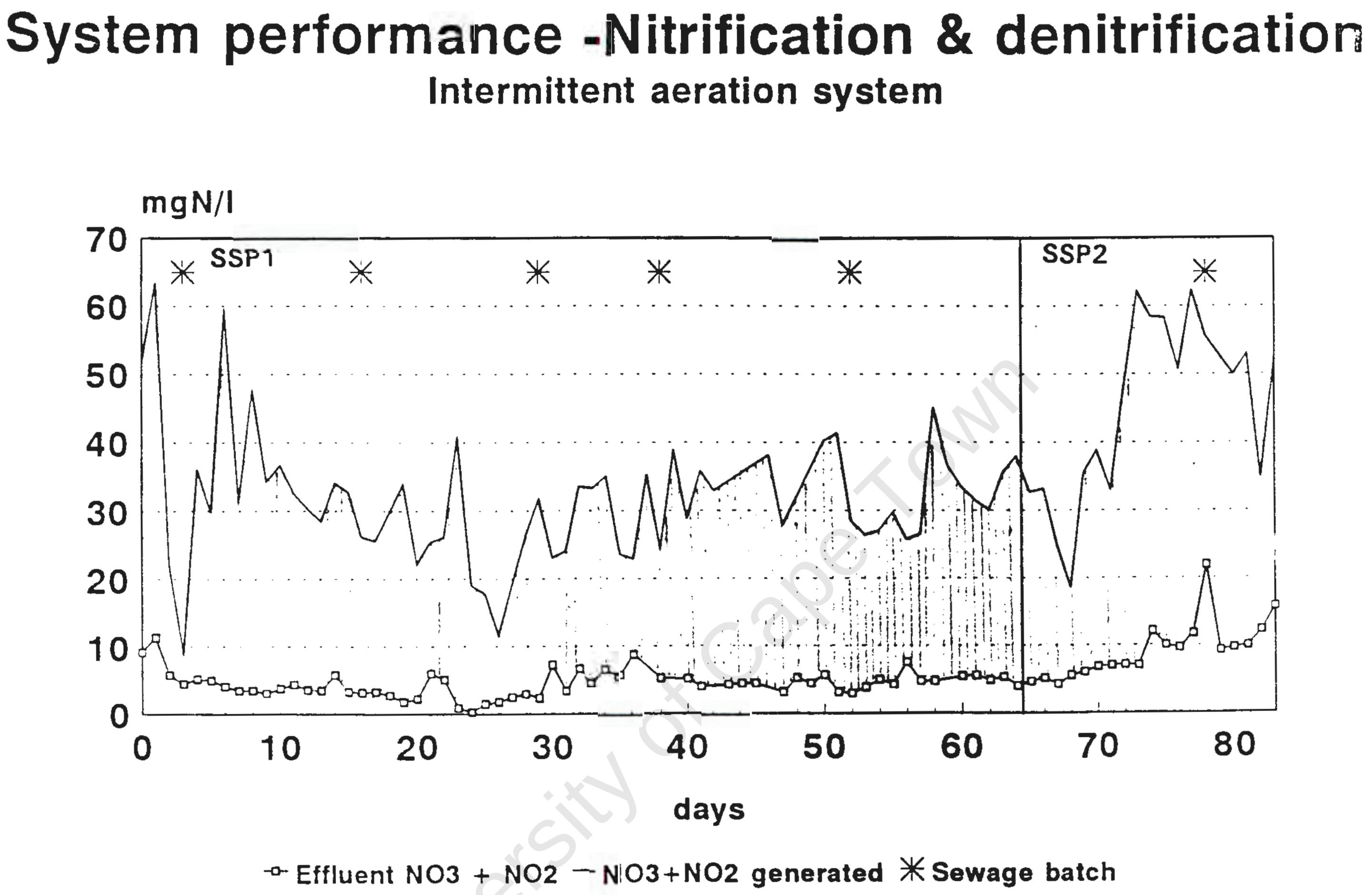


Fig 3.8 Intermittent aeration system performance - Nitrification and Denitrification
Shaded area represents NOx (NO3 + NO2) denitrified
(SSP = steady state period)

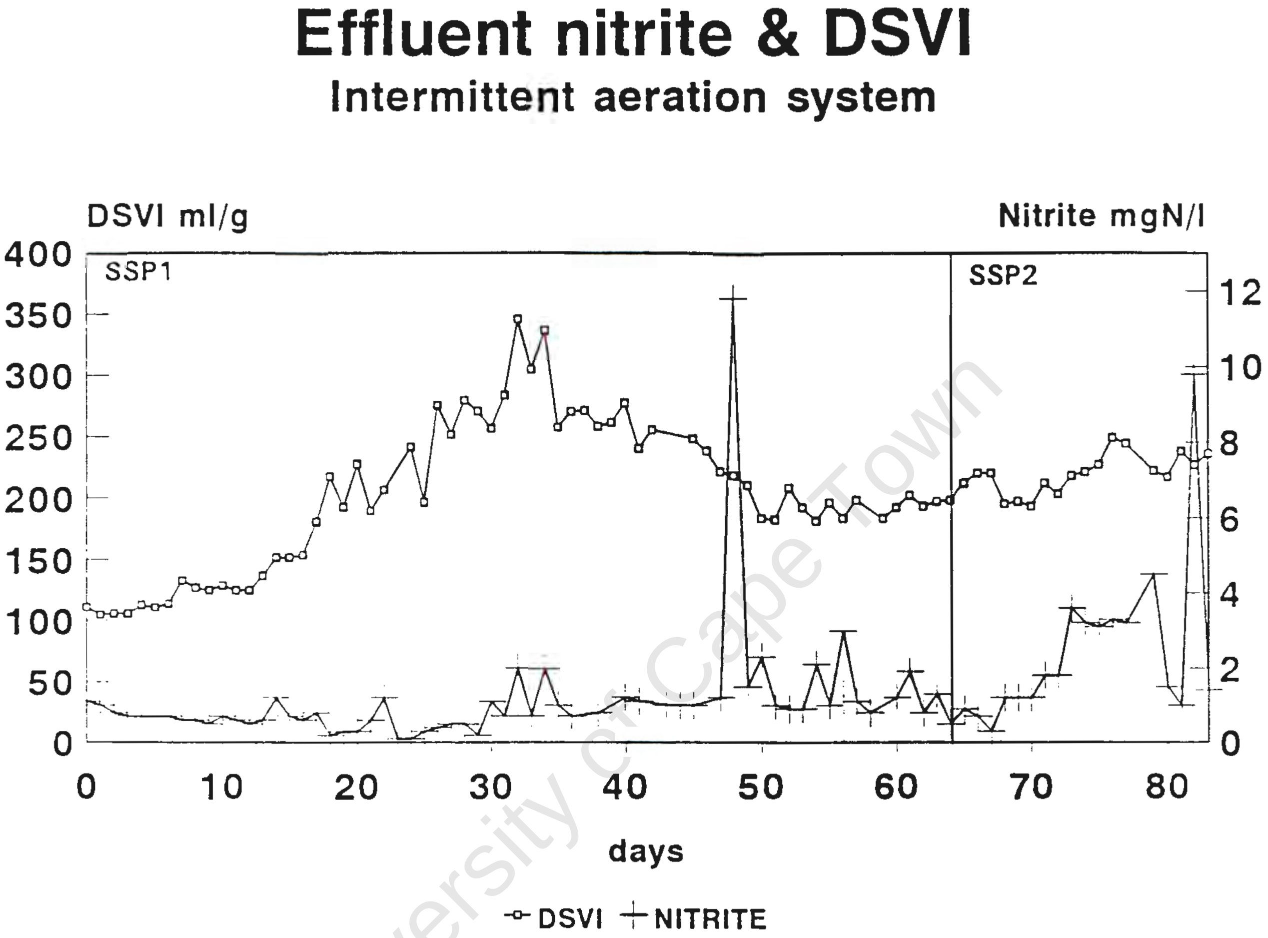


Fig 3.9 Intermittent aeration system DSVI and effluent nitrite concentration

(SSP = steady-state period)

Table 3.2 Filamentous organism identifications

Date	Day	Dominant	Secondary	Other
12/12/90	30	*H.hydrossis*	0092, 0041	*Beggiatoa*, 021N, *M.parvicella*
21/1/91	69	0041	-	021N, *H.hydrossis*, *M.parvicella*,0803, *Flexibacter*
20/2/91	99	0041	-	*M.parvicella* *S.natans*, 1851,0092
2/4/91	140	0041	*M.parvicella*	*S.natans* 021N,0092
10/5/91	178	0803	0041	*H.hydrossis* 0092
11/6/91	210	0961	1851,0041	*Thiothrix*, 0092,0803, *H.hydrossis*
26/7/91	254	0092	*H.hydrossis*	1851,021N 0041,0961
4/12/91	386	0041	0092, *H.hydrossis*	0041,1851, *H.hydrossis*
6/2/91	446	0041	*H.hydrossis*	0092, *M.parvicella*

concentration rose to levels of between 50 and 80 mgTKN-N/l. The ammonium was added step wise with the dose being 10 mgTKN-N/l for the first 4 days (day 64 to 68), then, after noting that this dose did not significantly influence the effluent nitrate and nitrite concentrations, the dose was increased to 20 mgTKN-N/l. Initially the effluent TKN concentration increased from the time of dosing (to a value of 21 mgTKN-N/l) due to the higher ammonium load, probably due to incomplete nitrification in the system, but as more nitrifiers grew in the system in response to the higher load, nitrification again improved so that by day 83 nitrification was again complete and low ($<$ 10 mgTKN-N/l) effluent TKN concentrations were again achieved.

The nitrate concentrations during the 64 day period (steady state period 1) before ammonium addition fluctuated between 2 mgNO_3-N/l and 17 mgNO_3-N/l. The nitrate concentration in the system decreased from 11.4 to 5.8 mgNO_3-N/l the first 3 days of operation due to the change of sewage batch with a lower TKN concentration and improvement in denitrification. From day 3 to 17 the nitrate concentration varied between 3 and 6 mgNO_3-N/l, decreasing to concentrations of 0.9 and 0.3 mgNO_3-N/l on days 23 and 24. This decrease corresponds to an increase in effluent TKN over the same period. The concentration of the nitrate denitrified varied between 20 and 35 mgNO_3-N/l influent with an average of 25.7 mgNO_3-N/l. (Fig 3.8 and Table 3.4).

After commencing ammonium dosing, on day 64 there is a steady increase in the effluent nitrate concentration, to concentrations of 8 - 15 mgNO_3-N/l. This is due to the generation of additional nitrate by nitrification (increase from 30.1 to 43.5 mgNO_3-N/l) in the system due to the availability of the added 20 mgN/l ammonium in the feed. From this it can be seen that on average only 13.4 mgN/l ammonium dosed was nitrified, the remaining 6.6 contributing to the effluent TKN concentration. The nitrate concentration denitrified also increased with the additional nitrate generated, the increase being from 25.7 mgNO_3-N/l to 34.5 mgNO_3-N/l ie by 8.8 mgNO_3-N/l. Therefore on average the effluent nitrate concentration increased by 13.4 - 8.8 = 4.6 mgNO_3-N/l. ie from 4.5 to 9.1 mgNO_3-N/l. (see Table 3.4).

During steady state period 1 (day 1 - 64) the concentrations of effluent nitrite varied between 0.1 to 9.8 mgNO_2-N/l but averaged at a value of 1.1 mgNO_2-N/l. During the second steady state period (with ammonium dosing - day 65 to 83) the effluent nitrite concentration ranged between 1 to 5 mgNO_2-N/l with the average of 2.5 mgNO_2-N/l. This is an increase of 1.4 mgNO_2-N/l over the steady state period 1 average of 1.1 mgNO_2-N/l, indicating that one or both of two possible sources of nitrite had increased as a result of ammonia dosing.

It should be noted that the nitrite concentration in the effluent arises from two sources ie partial nitrification ie nitrite not completely nitrified to nitrate under aerobic conditions and partial denitrification ie nitrite not fully denitrified to nitrogen gas under anoxic conditions. Unless the nitrate and nitrite concentration profiles are measured during the aeration cycle it is not possible to state which of the two sources is the principle contributor to the effluent nitrite concentration. Nitrate and nitrite concentration profiles were measured on two occasions (days 58 and 78), one during each steady state period; the results of these tests are discussed below. However, in general it was noted that the fluctuations in concentrations of effluent nitrite roughly correspond to the fluctuations in concentration of the influent TKN.

Table 3.3 Steady state period averages for the single reactor intermittent aeration system.

STEADY STATE PERIOD AVERAGES	PERIOD 1 (day 0-64)	PERIOD 2 (day 65-82)
VSS	1554 mg/l	1336 mg/l
TSS	1845 mg/l	1640 mg/l
COD(inf)	441.1 mg/l	474.8 mg/l
COD(eff)	76.1 mg/l	92.4 mg/l
f_{up}	0.188	0.102
f_{us}	0.173	0.195
TKN(inf)	50.2 mg/l	62.4 mg/l
TKN(eff)	9.6 mg/l	10.0 mg/l
Nitrate(eff)	4.47 mg/l	8.96 mg/l
Nitrite(eff)	1.09 mg/l	2.49 mg/l
DSVI	202 ml/g	219 ml/g
OUR	28.44 mg/l/hr	24.96 mg/l/hr

Table 3.4 Nitrogen and COD mass balance calculations for different steady state periods during complete investigation

PARAMETER		Intermittent System STEADY STATE PERIOD Day 1-83		Two-reactor N/D System STEADY STATE PERIOD Day 84-254			Modified two-reactor N/D System STEADY STATE PERIOD Day 255-471			Control System STEADY STATE PERIOD Day 383-472	
		1	2	1	2	3	1	2	3	2	3
Day		1-64	65-83	84-146	147-205	206-254	255-360	361-447	448-472	383-447	448-472
Duration (d)		64	19	63	59	49	106	87	23	65	23
Effluent TKN concentration	Nte	9.633	10.01	11.43	6.699	6.827	7.015	6.393	7.9	4.946	6.153
Effluent TKN mass	MNte	96.33	100.1	114.3	66.99	68.27	70.15	63.93	79	49.46	61.53
Effluent Nitrate concentration	Nne	4.477	8.961	11.1	10.28	10.8	12.66	9.847	4.677	8.506	4.905
Effluent Nitrate mass	MNne	44.77	89.61	111	102.8	108	126.6	98.47	46.77	85.06	49.05
Effluent Nitrite concentration		1.09	2.488	1.047	0.475	0.75	0.843	0.525	0.605	0.622	0.981
Effluent Nitrite mass		10.9	24.88	10.47	4.75	7.5	8.43	5.25	6.05	6.22	9.81
Anoxic Nitrate concentration				3.138	1.45	1.291	0.965	1.374	0.708	0.432	0.13[illegible]
Anoxic Nitrite concentration				0.516	0.256	0.375	0.501	0.228	0.133	0.181	0.117
a-recycle ratio				3:1	3:1	1:1	1:1	1:1	1:1	1:1	1:1
Average VSS concentration	VSS	1554	1336	1388	1342	1589	1030	1378	1177	1401	1440
N in waste sludge	MNs	103.7	89.11	92.58	89.51	106	68.7	91.91	78.51	93.45	96.05
Influent TKN concentration	Nti	50.16	62.38	66.42	44.35	44.37	46.99	39.23	35.24	37.73	33.46
Influent TKN mass	MNti	501.6	623.8	664.2	443.5	443.7	469.9	392.3	352.4	377.3	334.6
Mass Nitrate generated	Mng	301.6	434.6	398.1	441.5	285.3	350.9	254.2	119.1	242.2	143.1
Mass Nitrate denitrified	Mno	256.8	345	287.1	338.7	177.3	224.3	155.7	72.3	157.2	94.02
Mass Oxygen for Nitrate	MOn	1378	1986	1819	2018	1304	1603	1162	544.1	1107	653.8
Effluent COD concentration	Ste	76.07	92.4	83.48	63.72	72.63	54.9	65.02	73.5	80.6	52.76
Effluent COD mass	MSte	760.7	924	834.8	637.2	726.3	549	650.2	735	806	527.6
Mass of COD in waste sludge	MSxw	1534	1319	1370	1325	1569	1017	1360	1162	1383	1422
Influent COD concentration	Sti	441.1	474.8	560.6	482.1	522.9	512.3	510.7	426.6	515.7	434.7
Influent COD mass	MSti	4411	4748	5606	4821	5229	5123	5107	4266	5157	4347
OUR		28.44	32.96	38.66	27.16	34.92	22.38	26.74	18.82	27.07	26.11
Measured Oxygen consumed daily		2048	2373	2784	1956	2514	1611	1925	1355	1949	1880
O2 recovered(denitrification)	MOd	734.6	986.6	821.1	968.7	507	641.4	445.4	206.8	449.5	268.9
	MOc	669.3	387	964.2	-62.1	1211	7.975	763.6	810.9	842.1	1226
%N Balance		‡	‡	91.08	134.8	103.6	104.2	104.5	78.48	102.1	89.85
%COD Balance		83.85	76.17	71.18	59.5	76.73	43.24	63.04	68.32	67.49	79.23

‡ Assumed 100% for intermittently aerated system

Nitrate and nitrite concentration profiles

In order to examine the nitrate and nitrite concentration profile during the aeration cycle, on days 58 and 78 samples were taken every 20 minutes over a complete 8 hr (480 minute) aeration cycle and tested for nitrate and nitrite. Simultaneously the redox potential was also measured. The results of these tests are shown graphically in Figs 3.10 and 3.11. Day 58 is midway in a 15 day period when the DSVI was stable at 190 ml/g with no dosing of ammonia (Fig 3.7). In Fig. 3.10 over the aerobic period (approx. 1.83 h or 170 minutes) the reactor nitrate concentration increased from 0.5 mgNO_3-N/l to 9 mgNO_3-N/l. The nitrite concentration over the corresponding period rose from 0.5 mgNO_2-N/l to a maximum of just over 2 mgNO_2N/l, but dropped back to a concentration of 0.5 mgNO_2-N/l by the end of the aerobic period. The redox potential increased from -127 to +42 mV. During the anoxic period (5.16 h or 310 minutes) the nitrate concentration dropped steadily from 9 mgNO_3-N/l to 0.2 mgNO_3-N/l within 3 h. Over this period, the concentration of nitrite increased from 0.5 to 1 mgNO_2-N/l. When the nitrate concentration reached a near zero value, the nitrite concentration started to decrease from 1 mgNO_2-N/l and reached a near zero value ($<$ 0.1 mgNO_2-N/l) at 3.5 h (210 minutes). While nitrate and nitrite were present the redox potential dropped from +40 to -40 mV. After both nitrate and nitrite reached near zero concentrations at 3.6 h, the redox potential rapidly declined further from -40 to -200 mV.

From the time ammonium dosing was increased from 10 to 20 mgN/l, the DSVI increased from 190 to 210 ml/g. This was the same time as the effluent nitrate and nitrite concentrations increased as a result of the ammonium dosing, demonstrating a link between the effluent nitrate and nitrite concentrations and the DSVI. To examine the nitrate and nitrite

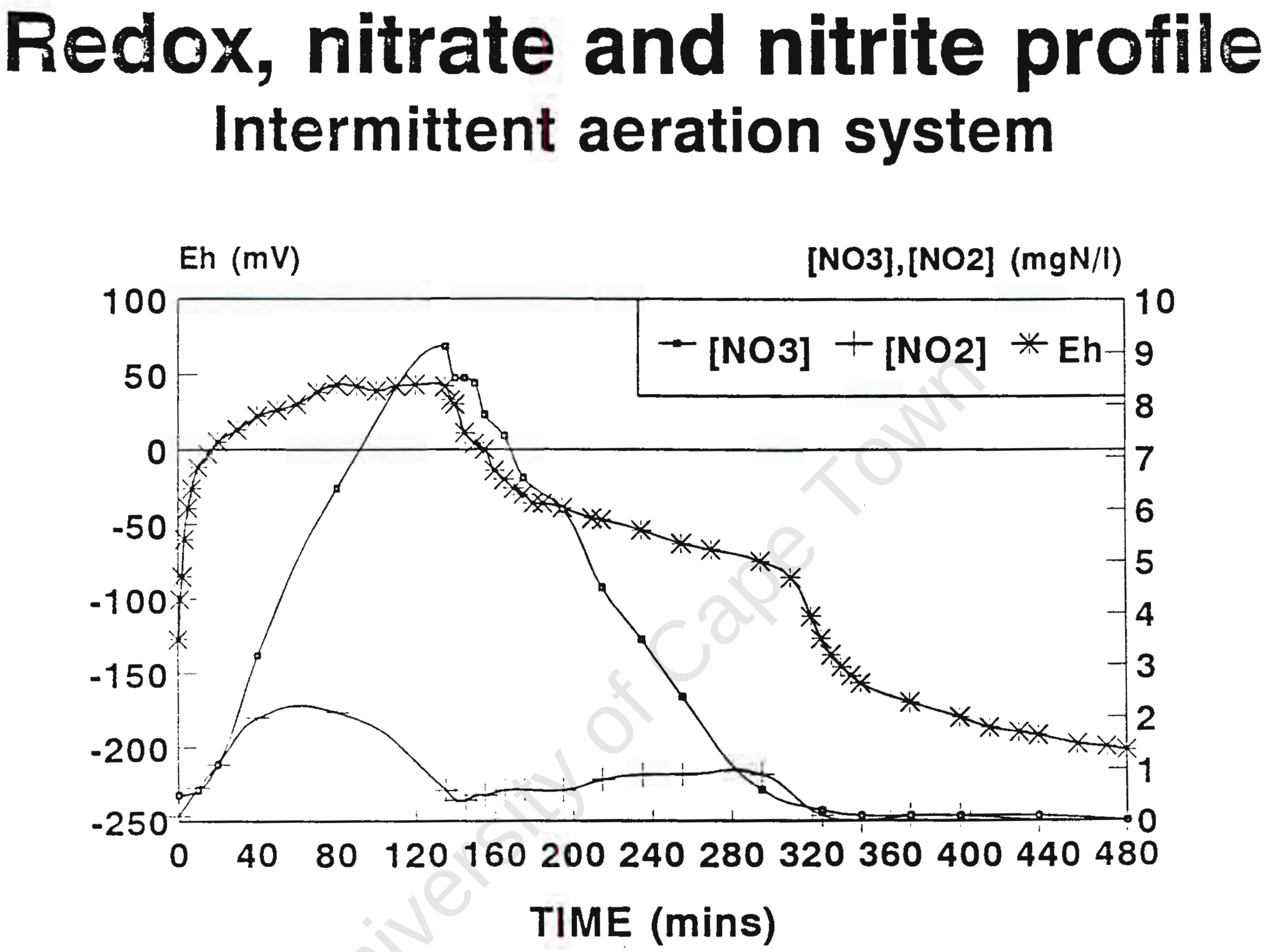
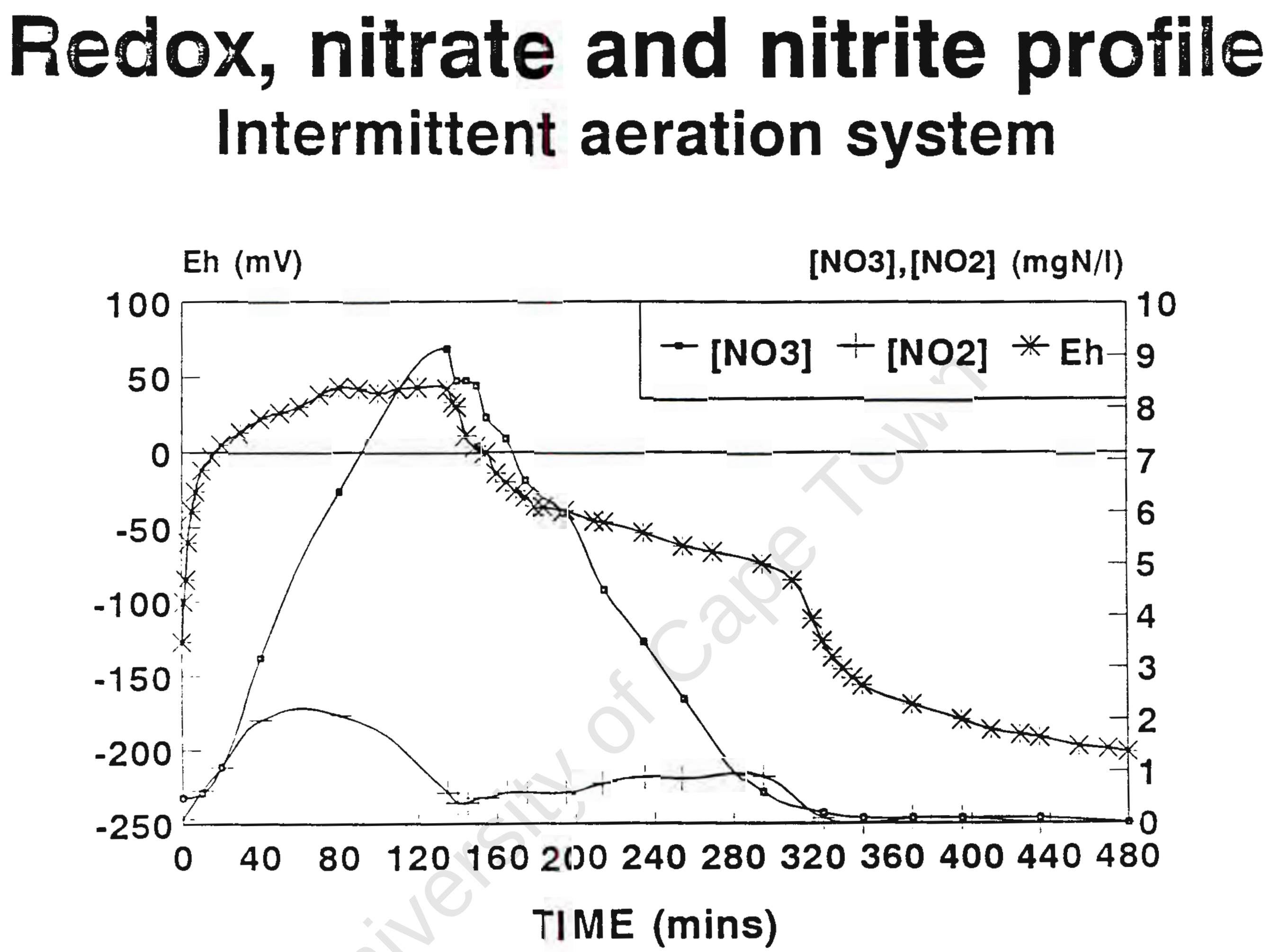


Fig 3.10 8-hr aeration cycle nitrate and nitrite profiles, intermittent aeration system, day 58



Redox, nitrate and nitrite profile
Intermittent aeration system

Fig 3.10 8-hr aeration cycle nitrate and nitrite profiles, intermittent aeration system, day 58 concentration profile with ammonium dosing (steady state period 2) a second profile test was done on day 78 (Fig. 3.11). During the aerobic period (140 mins), the reactor nitrate concentration increased from 1.5 $mgNO_3$-N/l to 11 $mgNO_3$-N/l, while the nitrite concentration increased from 1.5 to 6 $mgNO_2$-N/l. The redox potential, initially measured at -110 at the start of the aerobic period, increased to +30 mV.

During the anoxic period the nitrate concentration dropped steadily from 11 at the start to 0.5 $mgNO_3$-N/l at the end of the 5.5 h (330 minute) anoxic period. The nitrite concentration declined gradually over the first 4 hours, from 6 $mgNO_2$-N/l to 5 $mgNO_2$-N/l and then over the last 1.5 hours declined further to 2.5 $mgNO_2$-N/l. At the commencement of the anoxic period, the measured redox potential showed an initial sharp drop from +30 to +10 mV and thereafter decreased gradually over the 5.5 h anoxic period to a value of -70 mV, following approximately the nitrite concentration decline.

System behaviour - DSVI

The DSVI in the intermittently aerated system showed a tendency to increase over the first 35 day period of operation. The DSVI was initially below 150 ml/g, but increased beyond this level within 28 days. By day 34 the DSVI had increased to 337 ml/g. The dominant filament species present in the system during this time (observed on day 31) was *H. hydrossis,* and the secondary and incidental filaments were 0041 and *Beggiatoa*, 021N, *M. parvicella*, (see Table 3.2 and Fig. 3.7) all of these filaments being of the group commonly observed in intermittent aeration N removal systems fed real or artificial sewages (Warburton *et al.* 1991, Ketley *et al.* 1991, Casey *et al.* 1990). The DSVI then decreased gradually from

337 to around 200 ml/g from day 33 to day 50, and remained near this value until day 64 when steady state period 2 commenced. Filament identification on day 69, (although strictly speaking 6 days after commencement of steady state period 2, ammonia dosing but probably still is indicative of the filament population over the 15 day period preceding the filament identification test) indicated that 0041 was the dominant filament, having supplanted *H. hydrossis*. Secondary filaments were 0092, *M. parvicella*, and 0803. *H. hydrossis* remained present in the system as a secondary filament (see Table 3.2, Fig. 3.7).

From the nitrate, nitrite and redox potential profile measurements over the aeration cycle, it appears that high concentrations of nitrate and nitrite, and a high redox potential at the commencement of the aerobic period (-70 mV compared to -200 mV), as a consequence of incomplete denitrification during the anoxic period, is conducive to low F/M filament proliferation and increasing DSVI. In contrast when nitrate and nitrite concentrations at the commencement of the aerobic period are low (as a consequence of low influent TKN/COD ratio with no ammonium dosing) due to complete denitrification during the anoxic period, the DSVI of the sludge, and hence the low F/M filament proliferation, seems reduced.

Although the DSVI results are somewhat variable over the 83 days the intermittently aerated system was operated, the two nitrate and nitrite concentration profiles during the aeration cycle provide some support for the low F/M filament hypothesis of Casey *et al.* (1992). Variability of the DSVI results arises from the increasing and decreasing DSVI during the first and second 32 day periods respectively - why the DSVI should have increased from days 15 to 32 in the absence of ammonium dosing is not clear - in the absence of nitrate and nitrite concentration aeration profiles during the aeration cycle it is difficult to establish what the nitrate and nitrite concentrations would have been at the start of the aeration period. It is possible that despite the low influent TKN ratio these were high from day 20 to day 32 due to poor denitrification causing an increase in the DSVI. Then acclimatization improved its denitrification performance in the second 32 day period. From Figs 3.8 and 3.9, which show the effluent nitrate and TKN concentrations and denitrification performance of the system over the 83 day period it appears that this may have been the case . This conclusion is supported from the research of Warburton *et al.* (1991) who also operated intermittently aerated N removal systems with and without nitrate or ammonium dosing - while the systems always bulked, the DSVI did not increase exactly in conformity with N dosing or not. However in their experiments, Warburton *et al.* (1991) did not measure the nitrate and nitrite aeration cycle profiles so that in these experiments the concentration of these at the beginning of the aeration period are also not known.

The measurement of the nitrate and nitrite concentration profile has provided some valuable insight into a possible cause for bulking by low F/M filaments, *ie* incomplete denitrification at the commencement of aerobic conditions. The investigation continued by examining the validity of this finding in two reactor nitrification - denitrification systems.

3.2.2 Two reactor anoxic-aerobic system

After 83 days of intermittently aeration operation, the system was converted to an equivalent two reactor completely mixed continuously fed anoxic-aerobic pre-denitrification (MLE) system. All the system parameters such as sludge age, aerobic fraction, mass and type of COD fed daily, total reactor volume, and the anoxic mass fraction remained unchanged. The single 10 l intermittently aerated reactor was replaced by two reactors with a combined volume of 10 l, the first a 7 l anoxic reactor receiving the influent and underflow recycle and the second a 3 l aerobic reactor, giving the same aerobic mass fraction of 30%. In the aerobic reactor, the dissolved oxygen (DO) level was regulated between 2 - 3 mgO/l by an electronic DO controller/OUR meter (Randall *et al.*, 1991) which also measured the OUR. A mixed liquor recycle from the aerobic to the anoxic reactor at a recycle ratio (a) of 3:1 returned nitrate (and mixed liquor) to the anoxic reactor for denitrification. A schematic layout of the system is given in Fig 3.12 and a list of its design and operating parameters are given in Table 3.5.

The system initially (from day 83 to 146) received the same 20 mgN/l supplementary ammonia feed (in the form of NH_4Cl) that was dosed to the intermittently aerated system. This ensured generation of sufficient nitrate in the aerobic reactor so that complete denitrification would not occur in the anoxic reactor. The ammonia dosing was stopped on day 146, 63 days after the 2 - reactor system was set up. From day 147 to day 254, the only source of NH_4^+ to the system was the ammonia component of the influent TKN. On day 206 the a-recycle ratio was reduced from 3:1 to 1:1 in an attempt to reduce the nitrate load on the anoxic reactor and thereby reduce the anoxic reactor nitrate and nitrite concentrations. On day 254, a major change was made to the system, so that the period day 84 to 254, ie 170 days, constituted the second investigation period, ie operation of the 2-reactor system with and without influent ammonia supplementation and high and low a-recycle ratio. The changes made to the system during this second investigation period are listed in Table 3.6.

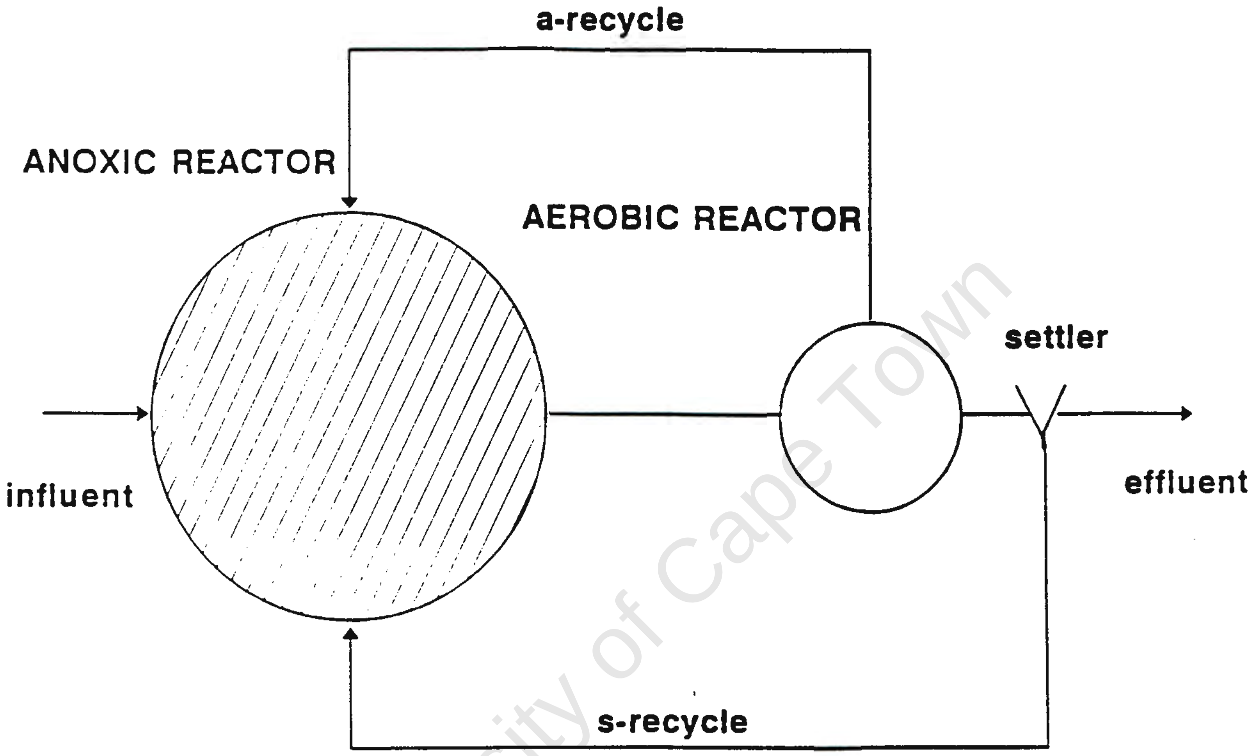


Fig 3.12 Schematic diagram of the 2-reactor anoxic aerobic system

Table 3.5 Operating parameters for the 2-reactor anoxic-aerobic and Control systems.

SYSTEM	SF (EXPERIMENTAL)	CONTROL
Operating conditions	Continosly Fed Anoxic - Aerobic 2 reactor system	Continuously Fed Anoxic - Aerobic 2 reactor system
Aeration	30% aerobic , 70% anoxic	30% aerobic , 70% anoxic
Sewage Source	Mitchell's Plain Raw	Mitchell's Plain Raw
Mass COD fed/d	4000 - 5000	4000 - 5000
Volume of Feed(l/d)	10 l	10 l
Concentration (mg COD/l)	400 - 550	400 - 550
Influent TKN (mgN/l)	35 - 50	35 - 50
Sludge Age (days)	15	15
Reactor Volumes(l)		
R1 (anoxic)	7	7
R2 (aerobic)	3	3
Mass Fractions:		
aerobic	30%	30%
anoxic	70%	70%
pH of mixed liquor	7.2 - 7.8	7.2 - 7.8
Recycle ratios:		
a	3:1	1:1
s	1:1	1:1
MLSS Concentration	2000	2000
VSS Concentration	1500	1500
Operated from:	day 84-472	383-472
* with ammonia dosing	84-146	
* without ammonia dosing	147-254	
* high a-recycle(3:1)	84-205	
* low a-recycle(1:1)	206-254	383-472
* auxilliary reactor	254-472	

Table 3.6 Operational changes and problems for 2-reactor system

DATE	DAY NO	CHANGE	PROBLEM/REASON
12/11	0	Operated as an intermittent aeration system	
15/3	64	Ammonium added to feed	
4/2	83	Operated as 2-reactor anox.-aero. system	
14/3	121	System cleaned, no wasting	
15/3	122	No wasting	
22-23/3	129-130	No wasting	
8/4	146	Ammonia feed stopped	
11/4	149	1l sludge drawn off	Batch test
12-13/4	150-151	No wasting	
16/4	154	1l sludge drawn off	Batch test
17-18/4	155-156	No wasting	
27/4	165	Sludge sifted	Sludge loss (6l) due to blockage
29/4	167	System cleaned, no wasting	
30/4	168	No wasting	
1-5/5	169-173	No wasting	
12-13/5	180-181	No wasting	Spill 1l
15/5	183	Sludge in settler	Pump failure
17/5	185	Sludge sifted	Prevent blockages
21/5	189	Overflow,100ml loss	Blockage
23/5	191	System cleaned, no wasting	
27/5	195	2l sludge drawn off	Batch test
31/5	199	1.2l sludge drawn off	Batch test
1-3/6	200-202	No wasting	
4/6	203	1l sludge drawn off	Batch test
5/6	204	1l sludge drawn off	
7/6	206	A recycle change from 3:1 to 1:1	
9/6	208	Oxygen off overnight	OUR control box failure
13/6	212	1l sludge drawn off	Batch test
14/6	213	1l sludge drawn off	Batch test
15-17/6	215-216	No wasting	
20/6	219	Sludge sifted	Prevent blockages
12/7	241	Sludge sifted	Prevent blockages
17/7	246	System cleaned, no wasting	
18/7	247	Oxygen off overnight	OUR control box failure

During the 170 day period the following parameters were measured daily in the system:

1. Influent (unfiltered) and effluent (filtered) COD concentrations (Fig 3.13)
2. Influent (unfiltered) and effluent (filtered) TKN concentrations (Fig 3.14)
3. Effluent nitrate and nitrite concentrations

(Fig 3.16)

4. Anoxic reactor nitrate and nitrite concentrations (Fig 3.17)
5. Aerobic reactor MLSS and MLVSS concentration (Fig 3.18)
6. Oxygen utilization rate (OUR) in the aerobic reactor (Fig 3.19)
7. Sludge settleability as DSVI (Fig 3.20)

Filament identification was carried out every 2 to 4 weeks.

The day to day results of the above measurements are given in Appendix 1 and shown graphically in Figs 3.13 to 3.20.

Results

N and COD mass balances

To check the reliability of the experimental data nitrogen(N) and COD mass balances were conducted. To facilitate these calculations the data was divided into 3 steady state periods (SSP, indicated by vertical lines in Figs 3.13 - 3.20) taking into account changes in system operating conditions and different sewage characteristics (principally the TKN concentration). For the 7 sewage batches (marked on the horizontal axis by a *) used over the 170 day period, the influent TKN concentration varied between 40 mgTKN-N/l and 45 mgTKN-N/l (excluding the dosing of 20 mgN/l ammonium). This small variability in influent TKN concentration allows the steady state period boundaries to be defined at the times when changes to the system operation conditions were made ie. ammonium dosing and changing a-recycle ratio. As a consequence only 3 steady state periods need be recognized.

In the two reactor system it is possible to calculate the nitrogen mass balance because the masses of nitrate (and nitrite) generated by nitrification and removed by denitrification can be calculated from a nitrate (and nitrite) mass balance over the anoxic and aerobic reactors respectively - ie for the two reactor system it is not necessary to assume a 100% N balance as needs to be done for the intermittently aerated single reactor system. Details of the N and COD mass balance calculations are given in Appendix 2 and the results are listed in Table 3.4.

The nitrogen mass balances calculated for the 3 steady state periods are 91.8, 134.8 and 103.6% respectively giving an average N mass balance for the system of 110%. These N balances appear to be acceptable, except for the N balance of steady state period 2, which is most likely caused by disturbances when 5 batches of sludge were harvested from the system for batch tests over 7 days during this period.

The COD mass balances calculated for the 3 steady state periods are 71.2%, 59.5% and 76.7%, giving an average COD balance for the 3 steady state periods of 70.6%. The COD balances are low, partly due to the abstraction of sludge for batch tests, but not significantly lower than the COD balances obtained by Warburton *et al.* (1992) and Hulsman *et al.* (1992) on N removal systems with large anoxic mass fraction (70%).

System performance - COD removal

For the 2-reactor system the average influent and effluent COD concentrations over steady state period 1 , 2 and 3 were respectively 560, 482, and 523 mgCOD/l and 83, 64, and 73 mgCOD/l (see Table 3.7). The average COD removal for steady state periods 1, 2 and 3 are thus 85%, 87% and 86% respectively. The average unbiodegradable soluble COD fraction (f_{us}) for the 3 steady state periods is 0.141 mgCOD/mg COD. The average unbiodegradable particulate COD fraction (f_{up}) over the 3 steady state periods was also calculated. This was done in the same way as described earlier for the intermittent aeration system. In terms of this approach the f_{up} values for the 3 steady state periods are 0.043, 0.077 and 0.114 giving an average value of 0.078. This value compares favourably with that measured in similar N removal systems fed Mitchell's Plain raw wastewater by Hulsman *et al.* (1992), and Warburton *et al.* (1991).

System behaviour - Nitrification and denitrification

The influent TKN concentration during the first steady state period of operation (day 83 - 146 - ammonium added), varied between 60 mgTKN-N/l and 80 mgTKN-N/l, with an average of 66 mgTKN-N/l. During this period the average effluent TKN concentration was 11.4 mgTKN-N/l (see Table 3.7). There are two occasions when there was a sharp rise in effluent TKN concentration ie around days 105 and 143 (see Fig 3.14). On the first occasion (day 106 to 110), the effluent TKN concentration increased to a value of 35 mgTKN-N/l, with a corresponding drop in nitrate concentration (from 20 to 5 mg NO_3-N/l), due to incomplete nitrification. During this time the DO controller/OUR meter had broken down

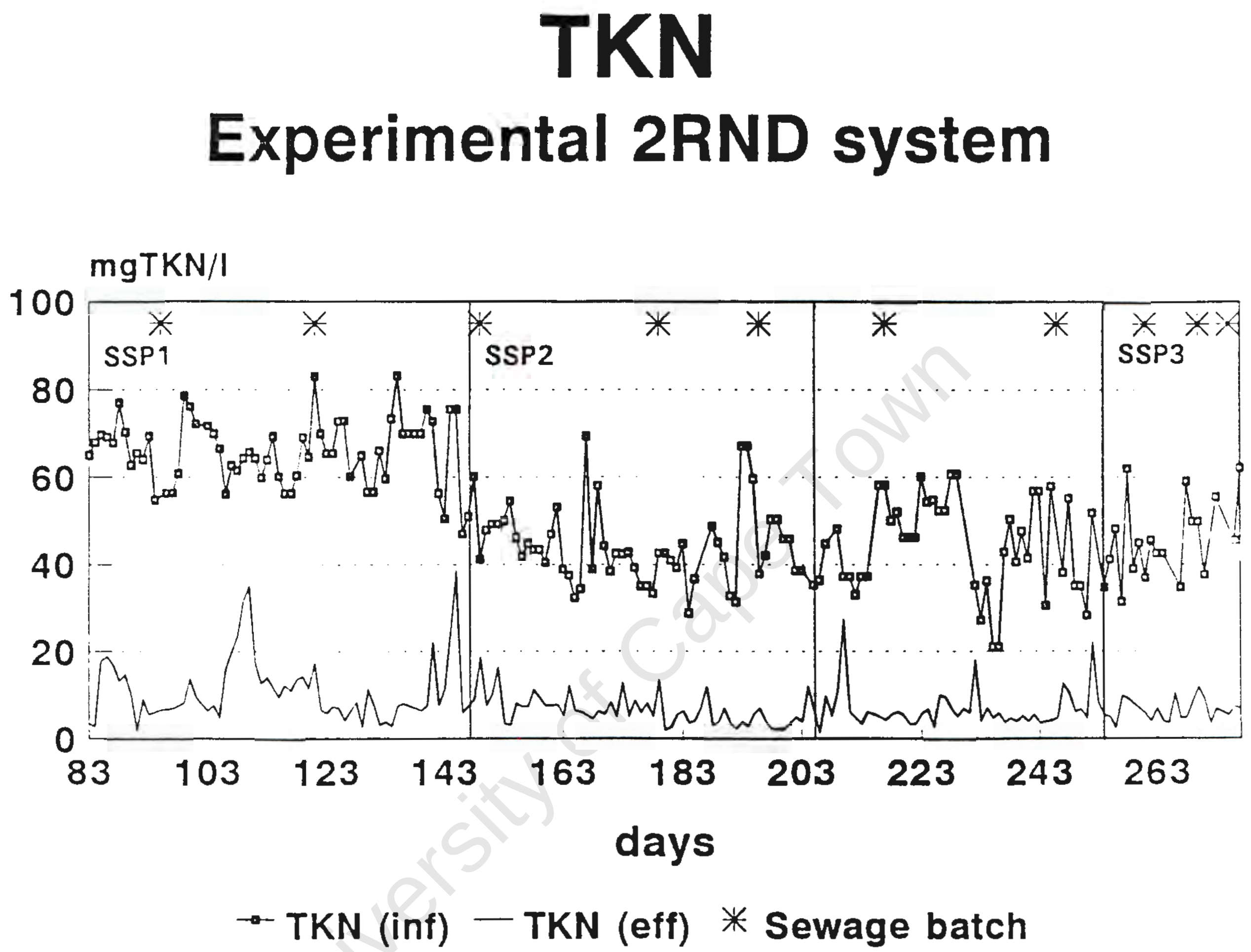
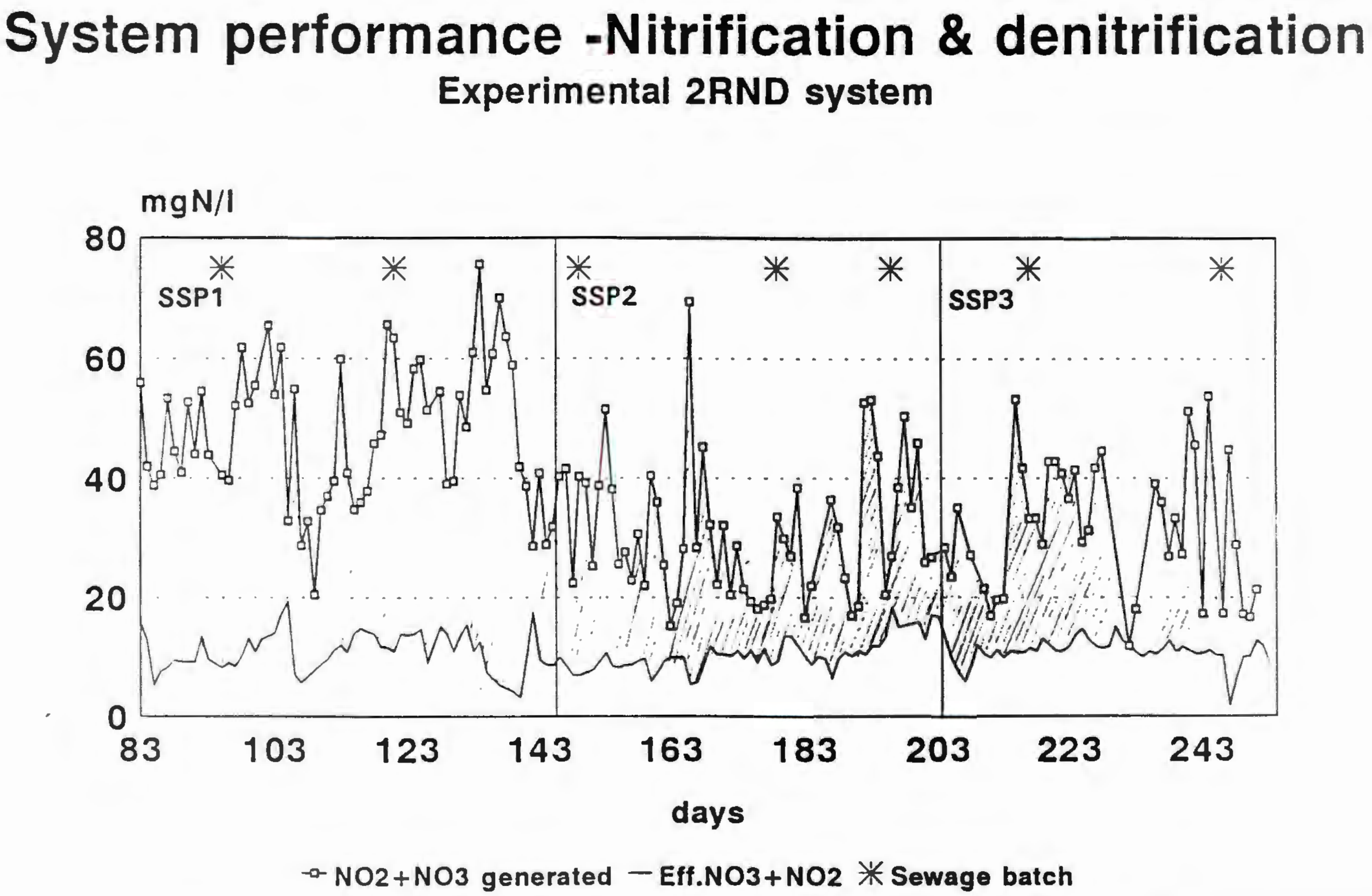


Fig 3.14 2RND system influent and effluent TKN concentration

(SSP = steady state period)



System performance -Nitrification & denitrification

Experimental 2RND system

Fig 3.15 Nitrate and nitrite (NOx) generated and effluent nitrate and nitrite (NOx) concentrations
Difference between the two (shaded area) is nitrate + nitrite denitrified
(SSP = steady-state period)

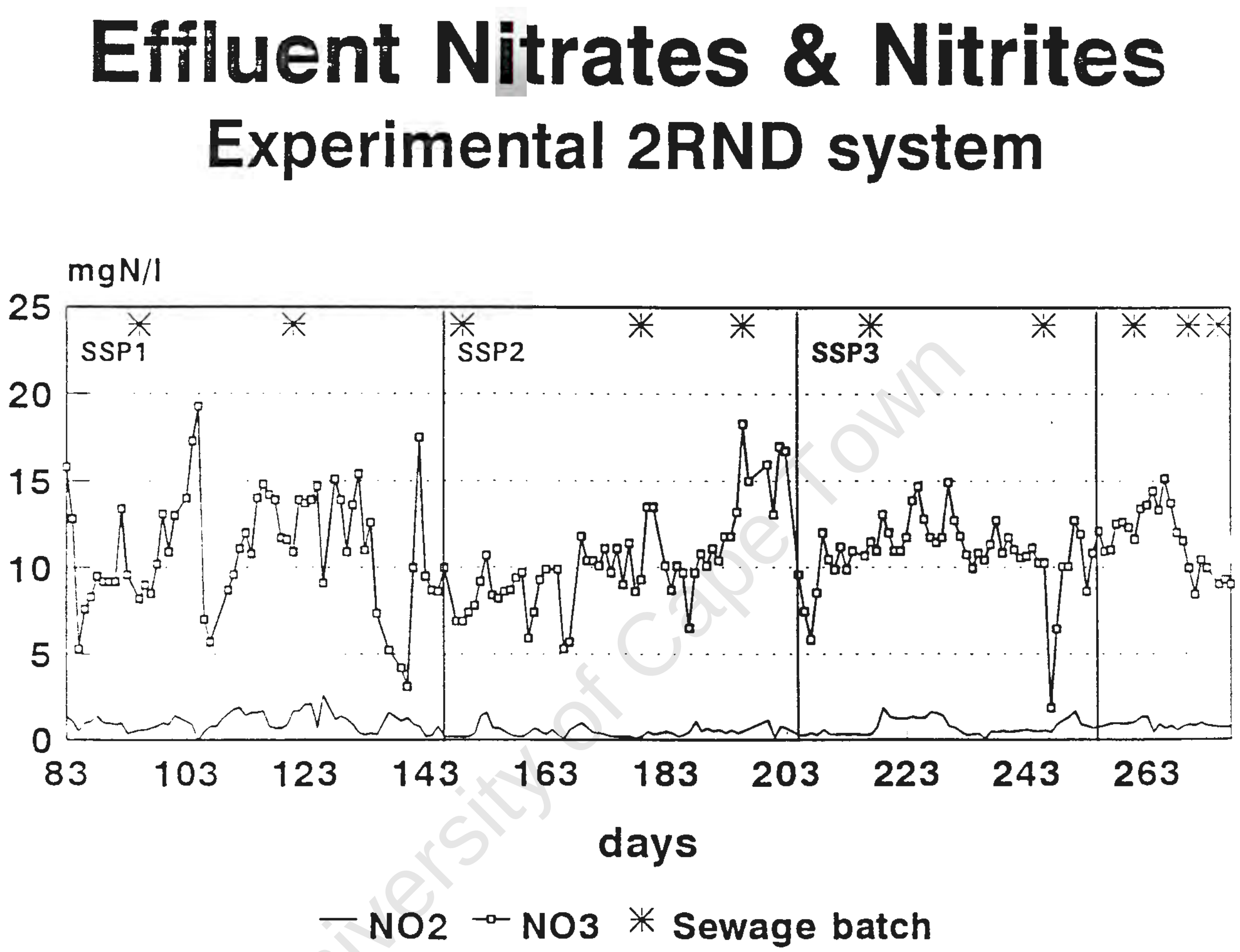


Fig 3.16 2RND system effluent nitrate and nitrite concentrations

(SSP = steady-state period)

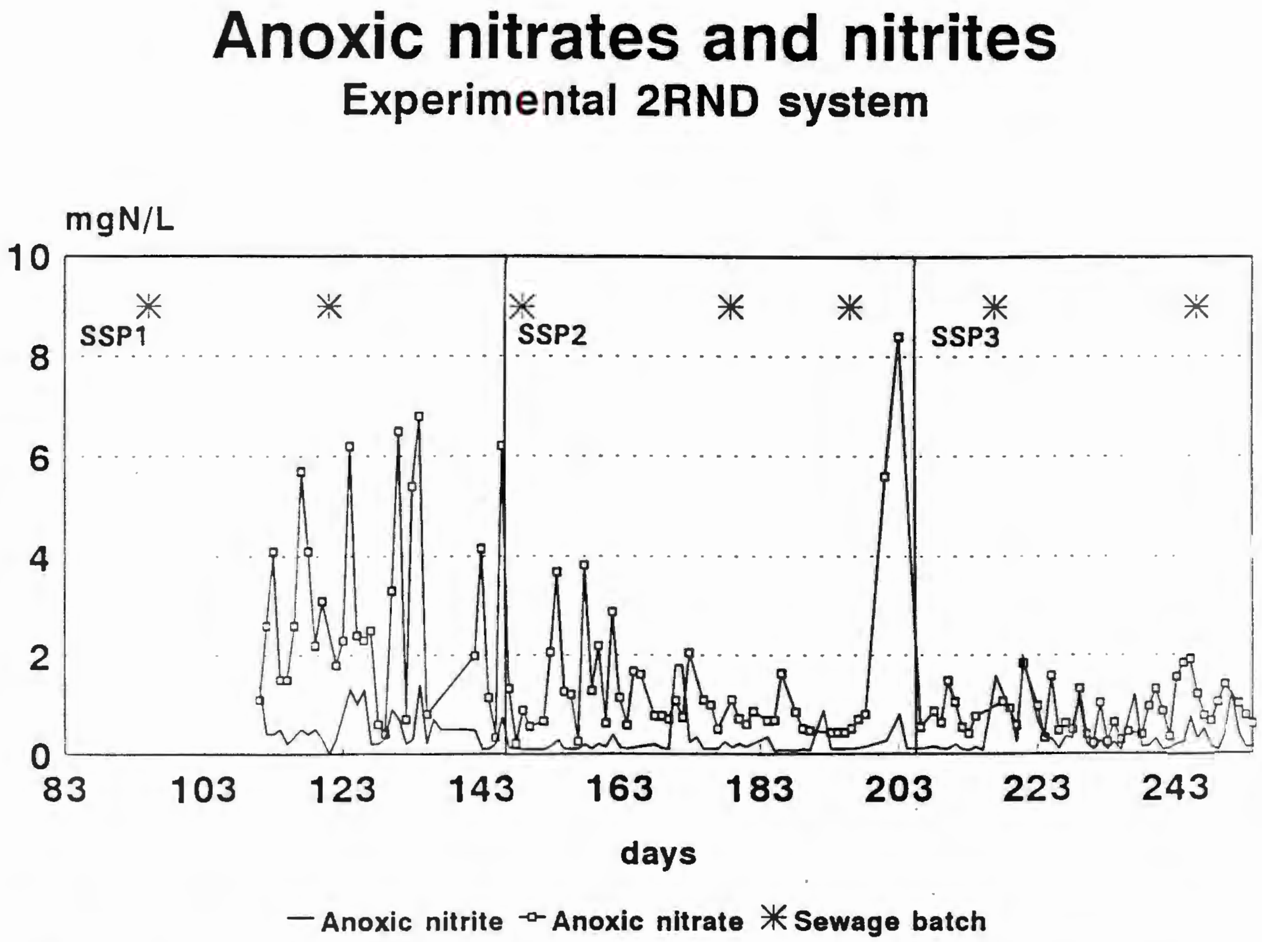


Fig 3.17 2RND system anoxic reactor nitrate and nitrite concentrations

(SSP = steady-state period)

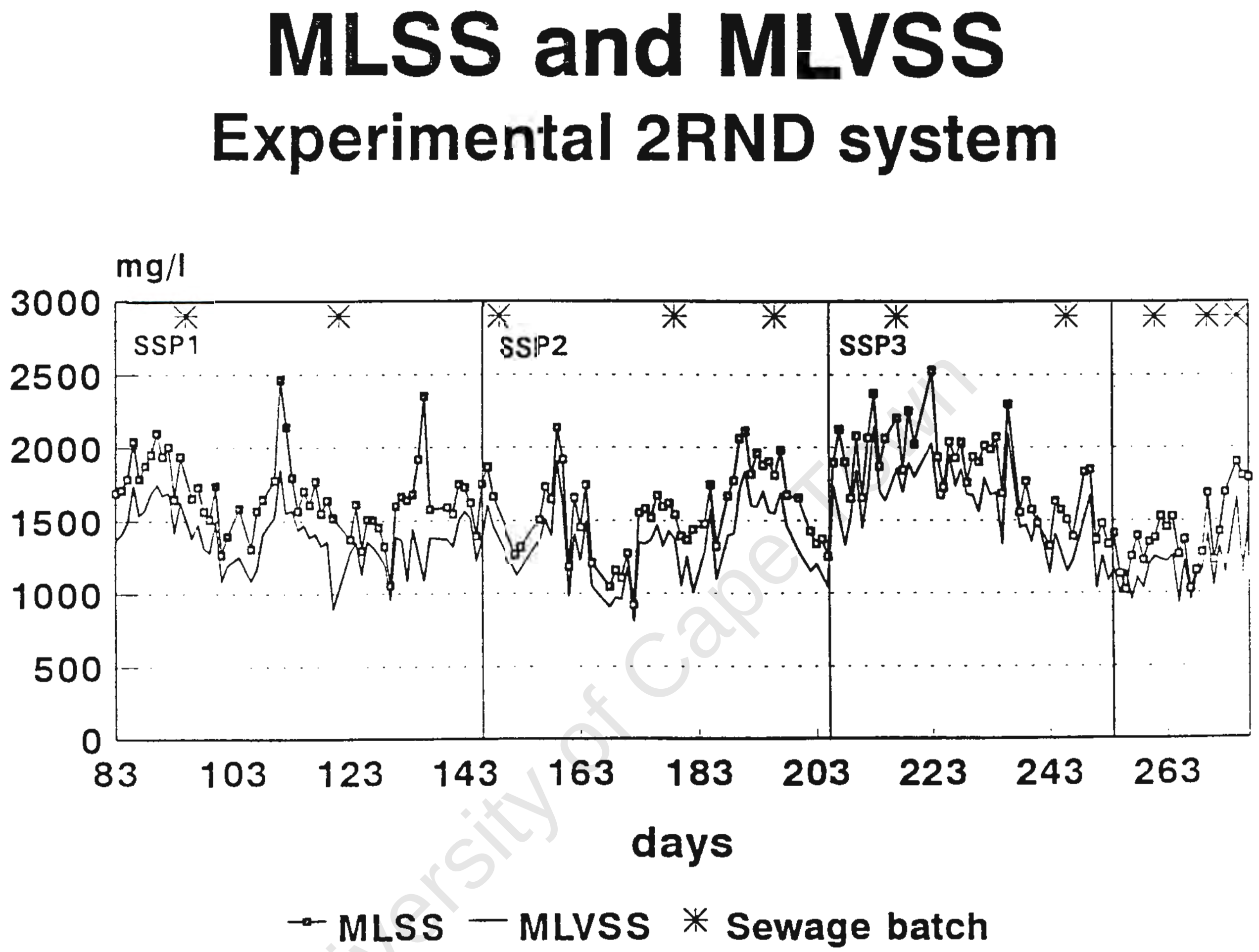


Fig 3.18 2RND system MLSS and MLVSS concentrations

(SSP = steady-state period)

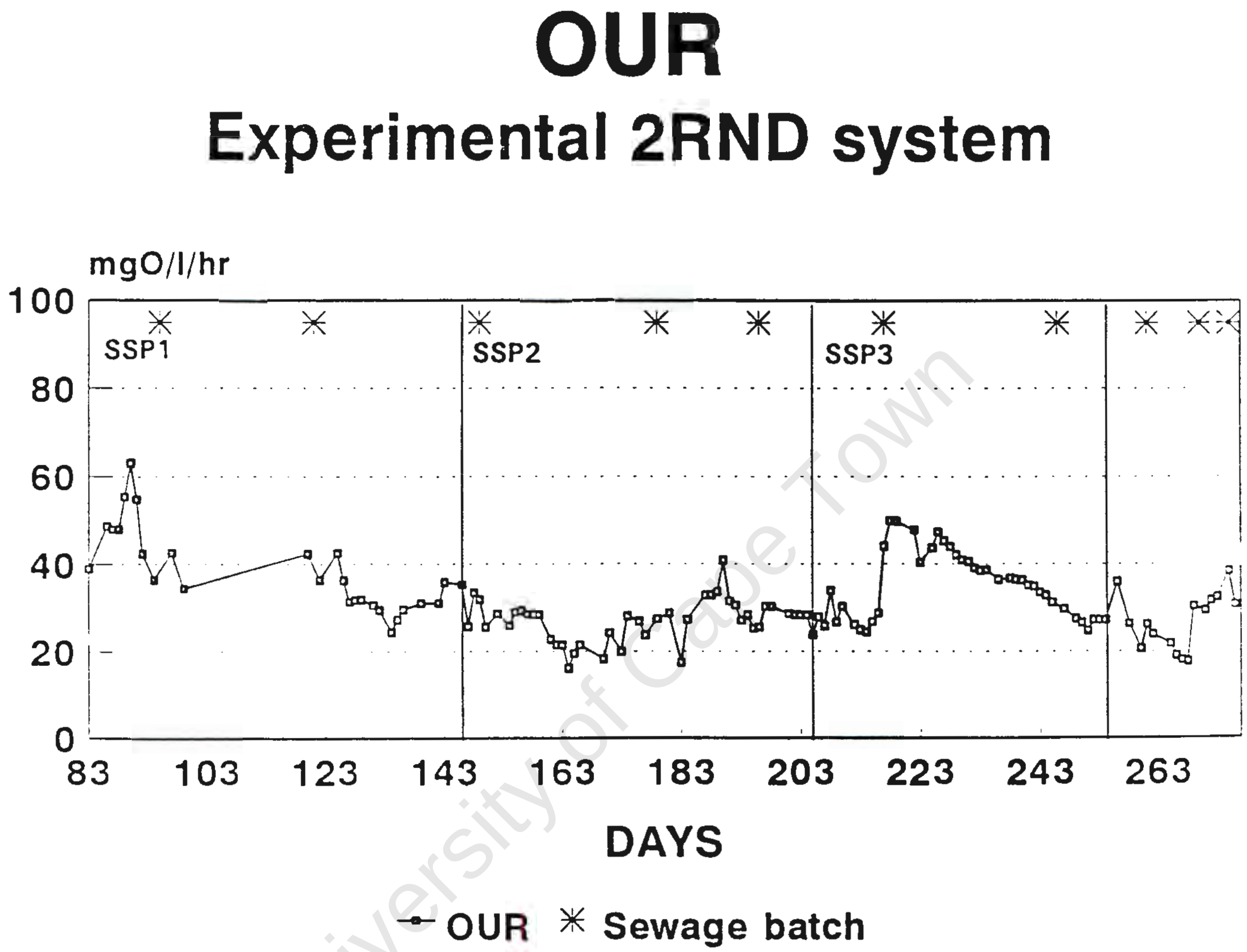


Fig 3.19 2RND system oxygen utilization rate (aerobic reactor)

(SSP = steady-state period)

and as a consequence the DO in the aerobic reactor needed to be manually controlled. This proved quite difficult and DO concentration tended to be below 1.0 mg O/l. This was too low to achieve complete nitrification. The same problem arose also on the second occasion (days 144 and 145) when the effluent TKN concentration also increased to above 25 mgTKN-N/l and the effluent nitrate concentration decreased to around 8.7 mgNO_3-N/l as a result of the incomplete nitrification. Although low on these two occasions the effluent nitrate concentration generally varied between 8 and 15 mgNO_3-N/l and had an average of 11.1 mg NO_3-N/l. The effluent nitrite concentrations varied between 0.4 and 2.6 mgNO_3-N/l, with an average concentration of 1.0 mgNO_3-N/l. The average concentrations of nitrate and nitrite generated (by nitrification) and removed by denitrification during steady state period 1 were 39.8 and 28.7 mgNO_x-N/l respectively (see Table 3.4).

During steady state periods 2 and 3 (no ammonia dosing) the influent TKN concentration varied between 20 and 60 mgTKN-N/l, with an average of 44 mgTKN-N/l. The average TKN concentration for steady state periods 2 and 3 are much lower than during steady state period 1, due to a cessation of the 20 mgTKN-N/l ammonia dosing to the influent. The effluent TKN concentration remained fairly steady between 5 and 10 mgTKN-N/l over these 2 steady state periods, except for sharp increases in concentrations around days 210 and 252 to 27 and 22 mgTKN-N/l respectively. These high TKN concentrations in the effluent were caused by overnight aeration equipment failures inhibiting nitrification in the aerobic reactor. The average effluent TKN concentrations for steady state periods 2 and 3 are 6.7 and 6.8 mgTKN-N/l respectively (see Table 3.7), these values being about 4 mgTKN-N/l lower than the steady state period 1 average (11.4 mgTKN-N/l).

During steady state period 2 the measured effluent nitrate concentration increased from around 7 to 8 mgNO_3-N/l to around 14 to 16 mgNO_3-N/l. Effluent nitrite concentrations remained below 1 mgNO_2-N/l and averaged at 0.48 mgNO_2-N/l. The average concentration of nitrate generated and denitrified over this period are 44.1 and 33.9 mgNO_x-N/l respectively (see Table 3.4).

At the start of steady state period 3, the a-recycle ratio was reduced from 3:1 to 1:1 to reduce the nitrate load on the anoxic reactor so that the nitrate and nitrite concentrations in the inflow to the aerobic reactor would decrease. The effluent nitrate concentrations remained steady between 10 and 15 mgNO_3-N/l (except for the decreases in concentration on days 208 and day 247, when overnight aeration failures took place), with an average for the period of 10.8 mgNO_3-N/l. The effluent nitrite concentration was somewhat higher for this period, with an average concentration of 0.75 mgNO_2-N/l. The concentration of nitrate generated and denitrified for this steady state period was lower, compared to those for steady state period 2 and had an averages of 28.5 and 17.7 mgN/l respectively (see Table 3.4).

System Behaviour - Anoxic reactor nitrate and nitrite concentrations

Samples for anoxic reactor nitrate and nitrite concentration analysis were taken from the system from day 111 (during steady state period 1 - ammonium addition). Nitrate concentrations measured in the anoxic reactor during this steady state period (up to day 146, - cessation of ammonium addition) show erratic variation of between 0.5 and 7 mgNO_3-N/l. The average concentration of anoxic nitrate during this period is 3.1 mgNO_3-N/l. (Table 3.7). The anoxic reactor nitrite concentrations varied between 0.1 and 2.5 mgNO_2-N/l.

Between days 112 and 120 the nitrite concentration remained between 0.2 and 0.5 mgNO_2-N/l. From around day 120 to day 126 the nitrite concentration increased to above 1 mgNO_2-N/l. For the remainder of steady state period 1 (day 121-146) the anoxic reactor nitrite concentration varied between 0.1 and 1.6 mgNO_2-N/l. The average anoxic reactor nitrite concentration for steady state period 1 is 0.52 mgNO_2-N/l.

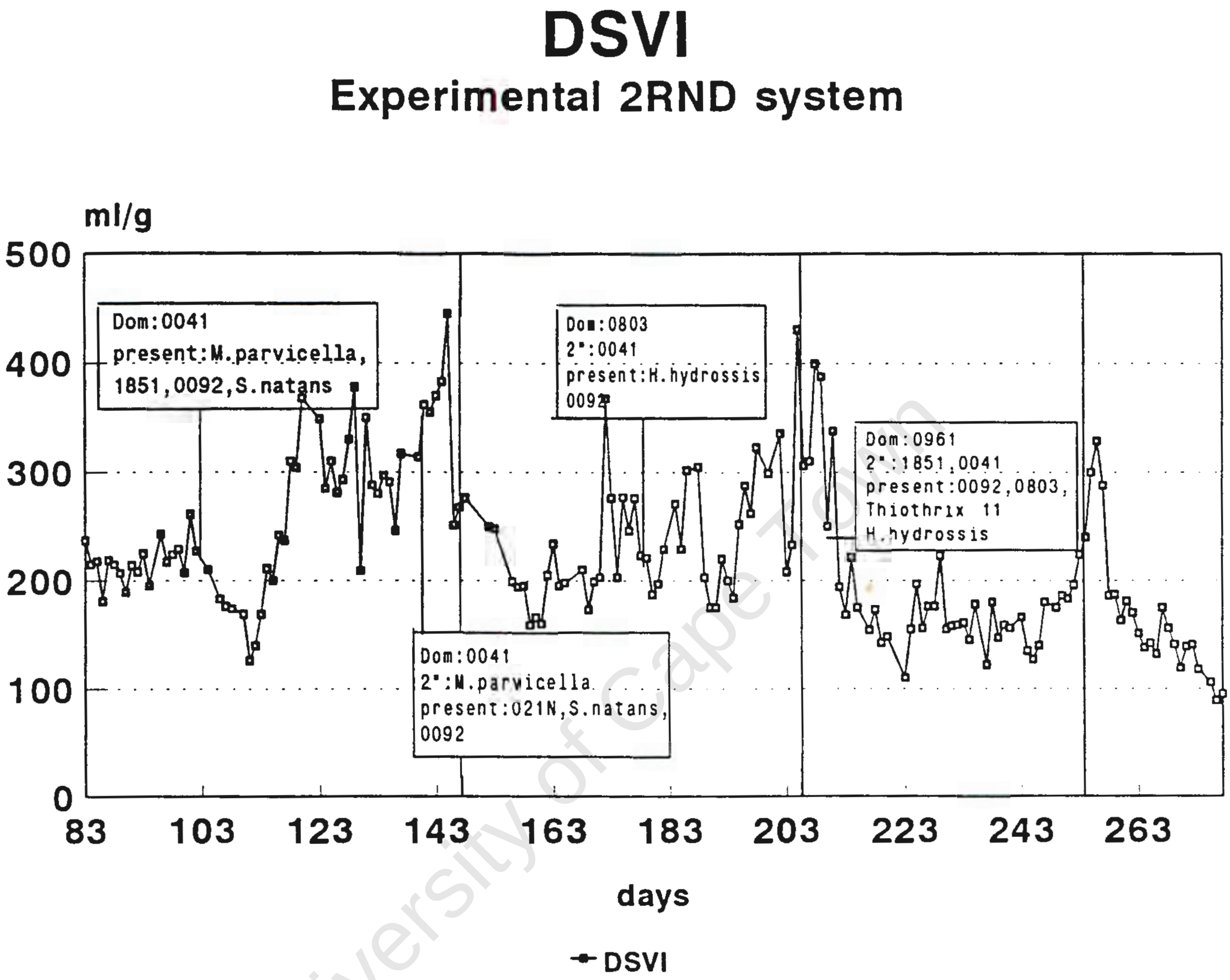
During steady state period 2 (day 146- 206) the daily anoxic nitrate concentration showed less fluctuation. From day 146 to day 170 the anoxic nitrate concentration varied between 0.2 and 4 mgNO_3-N/l. From day 170 to the end of the steady state period (day 206), the fluctuations in anoxic nitrate concentrations were smaller, and the concentrations lower, between 0.4 and 2 mgNO_3-N/l. The average reactor nitrate concentration for steady state period 2 is 1.5 mgNO_3-N/l, lower than for period 1, due to removal of the ammonium supplement from the feed. The anoxic nitrite concentration generally remained below 0.2 mgNO_2-N/l from day 147 to 170, only on days 154 and 162 the anoxic nitrite concentration increased to above 0.2 mgNO_2-N/l. The anoxic nitrite concentration increased slightly from day 165 to day 168, and showed a sharp increase from around day 171-172, but decreased again on days 173 - 177. There were small increases in anoxic nitrite concentration from day 178 to day 183, but by day 185 the concentration had again dropped to below 0.1 mgNO_2-N/l. The anoxic nitrite concentration increased to 0.8 mgNO_2-N/l on day 192, but decreased again to below 0.1 mgNO_2-N/l on the following day. Thereafter up to day 203 a gradual increase in the concentration took place, to 0.8 mgNO_2-N/l. The anoxic nitrite concentration then decreased and was low for the rest of the steady state period (day 203 - 205). The average of anoxic reactor nitrite concentration during this steady state period was 0.26 mgNO_2-N/l.

During steady state period 3 (day 206-254) anoxic nitrate concentrations remained low during steady state period 3, with concentrations varying between 0.2 and 2 mgNO_3-N/l. The average anoxic nitrate concentration for period 3 is 1.3 mgNO_3 -N/l. The anoxic nitrite concentration also remained low, generally below 0.2 mgNO_2-N/l. However, on 5 of the 48 days of steady state period 3 (ie days 217, 221, 229, 236, 249) the anoxic nitrite concentration increased above 1.0 mgNO_2-N/l. The average anoxic reactor nitrite concentration for steady state period 3 is 0.38 mgNO_2-N/l, somewhat higher than the average concentration for steady state period 2.

In conclusion the average anoxic reactor nitrate and nitrite concentrations for steady state periods 1, 2 and 3 are 3.2, 1.45, and 1.29 mgNO_3-N/l and 0.52, 0.25 (including the high values of 1.8 mgNO_2-N/l on day 163 and 164) and 0.38 mgNO_2-N/l respectively; ie each progressively decreasing (albeit very slightly) with removal of the ammonium supplement (steady state period 1 to 2) and reduction of a-recycle ratio (steady state period 2 to 3).

System behaviour - DSVI

When the system configuration was changed to a two reactor anoxic-aerobic system on day 84, with addition of ammonia to the feed, the DSVI initially increased gradually over 29 days from 215 to 243 (Fig.3.20). Thereafter the DSVI decreased over a period of 19 days of operation to a value of 126 ml/g on day 111. During this period the dominant filament present in the system was 0041 (see Fig 3.20 and Table 3.8). After day 111 the DSVI increased sharply over 10 days to between 280 and 320 ml/g (see Fig 3.21 with DSVI and anoxic reactor nitrite concentration versus time). From day 120 to 135 the DSVI fluctuated



DSVI

Experimental 2RND system

Fig 3.20 2RND system daily sludge settleability as DSVI

Table 3.7 Steady state period averages for the 2-reactor Experimental system

STEADY STATE PERIOD AVERAGES	PERIOD 1 (day 83-146)	PERIOD 2 (day 147-205)	PERIOD 3 (day 206-254)
VSS	1388 mg/l	1342 mg/l	1589 mg/l
TSS	1675 mg/l	1573 mg/l	1834 mg/l
COD(inf)	560.6 mg/l	482.1 mg/l	522.9 mg/l
COD(eff)	83.5 mg/l	63.7 mg/l	72.6 mg/l
f_{up}	0.043	0.077	0.114
f_{us}	0.148	0.132	0.139
TKN(inf)	66.4 mg/l	44.4 mg/l	44.4 mg/l
TKN(eff)	11.4 mg/l	6.7 mg/l	6.8 mg/l
Nitrate(eff)	11.10 mg/l	10.28 mg/l	10.80 mg/l
Nitrate(anox)	3.14 mg/l	1.45 mg/l	1.29 mg/l
Nitrite(eff)	1.047 mg/l	0.475 mg/l	0.75 mg/l
Nitrite(anox)	0.516 mg/l	0.256 mg/l	0.375 mg/l
DSVI	256 ml/g	236 ml/g	191 ml/g
OUR	38.7 mg/l/hr	27.2 mg/l/hr	34.92 mg/l/hr

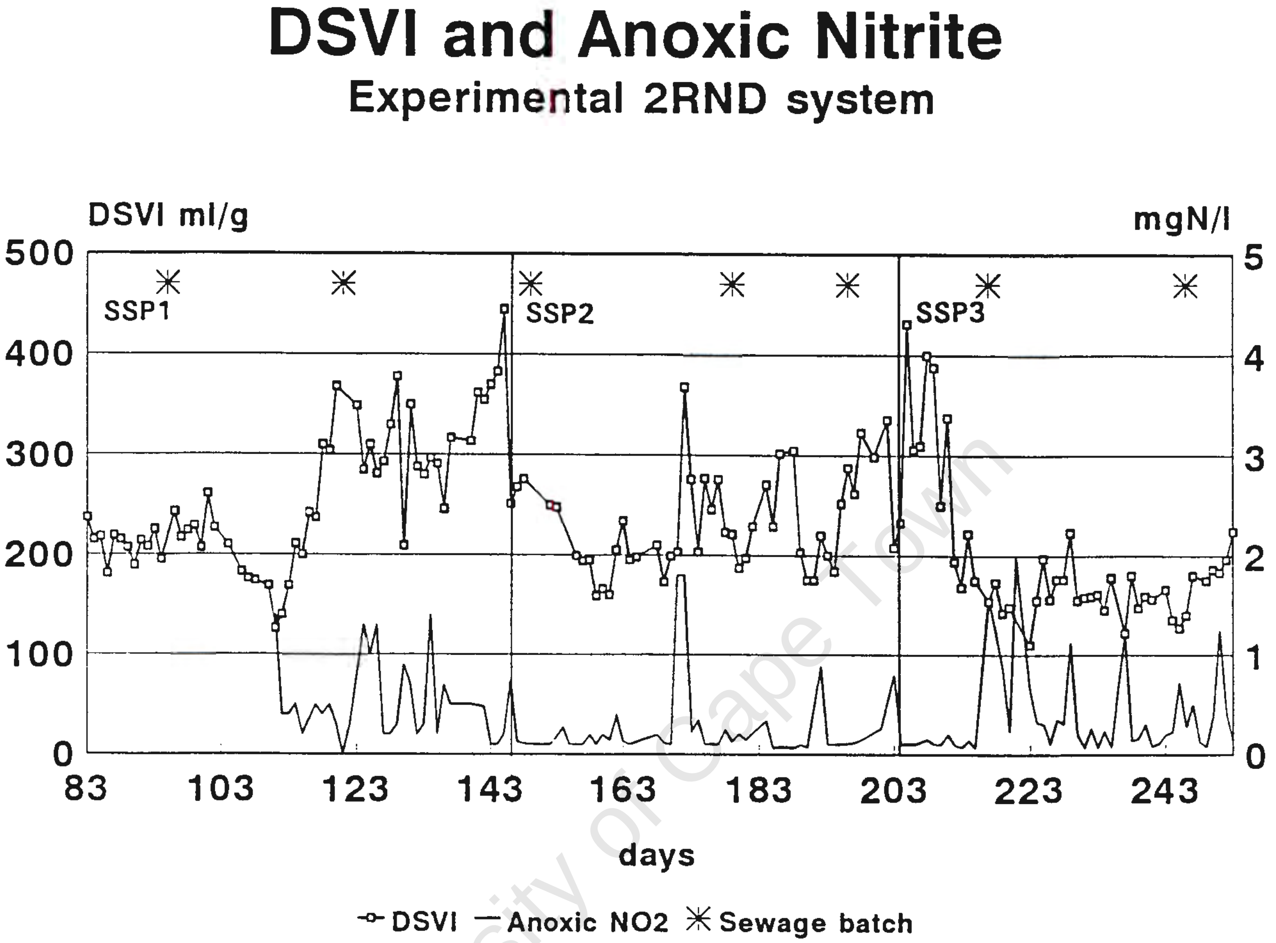


Fig 3.21 2RND system DSVI and anoxic nitrite concentration

(SSP = steady-state period)

Table 3.8 Filamentous organism identifications - day 83 to 247

Date	Day	Dominant	Secondary	Other
20/2/91	99	0041	-	*M.parvicella* *S.natans*, 1851,0092
2/4/91	140	0041	*M.parvicella*	*S.natans* 021N,0092
10/5/91	178	0803	0041	*H.hydrossis* 0092
11/6/91	210	0961	1851,0041	*Thiothrix*, 0092,0803, *H.hydrossis*

around 300ml/g and from day 134 to the end of steady state period 1 (day 146) the DSVI increased to 445 ml/g. Filament analyses done during this period indicated the presence of the low F/M filaments 0041 as the dominant filament in the system and *M. parvicella* as the secondary filament (see Table 3.8). During this period the anoxic reactor nitrite concentration was generally above 0.5 mgNO_2-N/l.

On day 146 the ammonium supplement to the feed was terminated (steady state period 2). This resulted in lower anoxic reactor nitrite concentrations. The DSVI dropped from a value of 445 ml/g to a value of 251 on day 147. Thereafter the DSVI continued to decrease, reaching a value of 160 ml/g on day 161. From day 161 to day 209 the DSVI increased gradually, remaining above the level of 200 ml/g. At this stage the dominant filament species was 0803, with 0041 as secondary filament (see Table 3.8). From day 164 to day 173 the DSVI increased to 234 ml/g, which seemed to correspond to slight increases in anoxic reactor nitrite concentration during this time. The sharp increase in DSVI on days 171-172 (to 368 ml/g) also seems to correspond to a sharp increase in anoxic nitrite concentration on these days. Small increases in anoxic nitrite concentration on days 178-183 coincide with a increase in DSVI over these days. Subsequent decreases and increases in anoxic reactor nitrite concentration for the rest of the steady state period, such as the increase from day 193 to day 203, seem to be mirrored in the performance of the DSVI, where the DSVI increased from around 180 to over 300 ml/g.

In an attempt to reduce the anoxic reactor nitrite concentration, the inter- reactor recycle ratio (a-recycle) was decreased from 3:1 to 1:1 on day 206 thereby reducing the nitrate /nitrite load on the anoxic reactor. The DSVI initially increased from 233 to 400 ml/g on day 208.

By day 216 the DSVI had decreased to below 200 ml/g. At the start of this steady state period till day 215 the anoxic reactor nitrite concentration remained fairly low, around 0.1 $mgNO_2$-N/l. From day 225 the DSVI again showed a tendency to increase, reaching a value of 240 ml/g on day 229. This increasing tendency in DSVI may have been caused by high anoxic reactor nitrite concentrations between day 216 and day 230. From day 244 the DSVI decreased, from 166 to 127 ml/g on day 245. From day 246 until the end of steady state period 3 (day 254) the DSVI increased to 240 ml/g. The anoxic reactor nitrite concentrations during this steady state period displayed a somewhat erratic fluctuation from day to day, probably due to perturbations in system operation during this steady state period, making it difficult to see much correlation between anoxic nitrite concentration and DSVI here. Figure 3.21 shows the DSVI and nitrite concentrations versus time during the 3 steady state periods, with filament identification indicated as well.

3.2.3 Modified two reactor anoxic-aerobic system

In the above experiments, although removing the influent ammonium dose and reducing the mixed liquor a-recycle ratio, had the desired effect of reducing the anoxic reactor nitrate and nitrite concentrations (by reducing the nitrate load on the anoxic reactor to be denitrified), some nitrate and, more importantly, nitrite remained. These remaining concentrations of nitrate and nitrite were quite low (around 1.0 $mgNO_3$-N/l and 0.5 $mgNO_2$-N/l) but possibly not quite low enough to control the bulking by the low F/M filaments. It is somewhat perplexing also that the reduction in mixed liquor a-recycle ratio did not reduce the nitrate and nitrite concentrations to lower values than recorded. In view of this, it was decided to introduce into the system a strong denitrification reaction just prior to entry to the aerobic zone. This was done by introducing a small anoxic reactor receiving a 10% influent feed bypass between the anoxic and aerobic rectors. It was hoped that with this reactor and feed, the nitrate and nitrite concentrations entering the aerobic zone could be reduced to below detectable levels and lead to a stronger influence of low F/M filament and DSVI control.

On day 254 an auxiliary reactor was added to the system between the anoxic and aerobic reactors, this reactor having a volume of 500 ml and receiving a bypass of 1 l/d of the 10l/d daily influent feed (see Fig 3.22). Later, on day 384, to compare the effect of this anoxic auxiliary reactor, a second 2 reactor anoxic - aerobic system without the auxiliary reactor was started up to serve as a Control system. This Control system had the identical system parameters and operating conditions as the experimental system, but did not include the auxiliary reactor.

Over the 216 day period from day 255 to 471 the following parameters were measured daily in the Experimental and Control systems:

1. Influent (unfiltered) and effluent (filtered) COD concentrations (Fig 3.23 and 3.33)
2. Influent (unfiltered) and effluent (filtered) TKN concentrations (Fig 3.24 and 3.34)
3. Effluent nitrate and nitrite concentrations (Fig 3.26 and 3.35)
4. Anoxic reactor nitrate and nitrite concentrations(Fig 3.27 and 3.36)
5. Auxiliary anoxic reactor nitrate and nitrite concentrations on Experimental system(Fig 3.28)
6. Aerobic reactor MLSS and MLVSS concentrations (Fig 3.29 and 3.37)
7. Oxygen utilization rate (OUR) in the aerobic reactor (Fig 3.30 and 3.38)
8. Sludge settleability as DSVI (Fig 3.31 and 3.39)

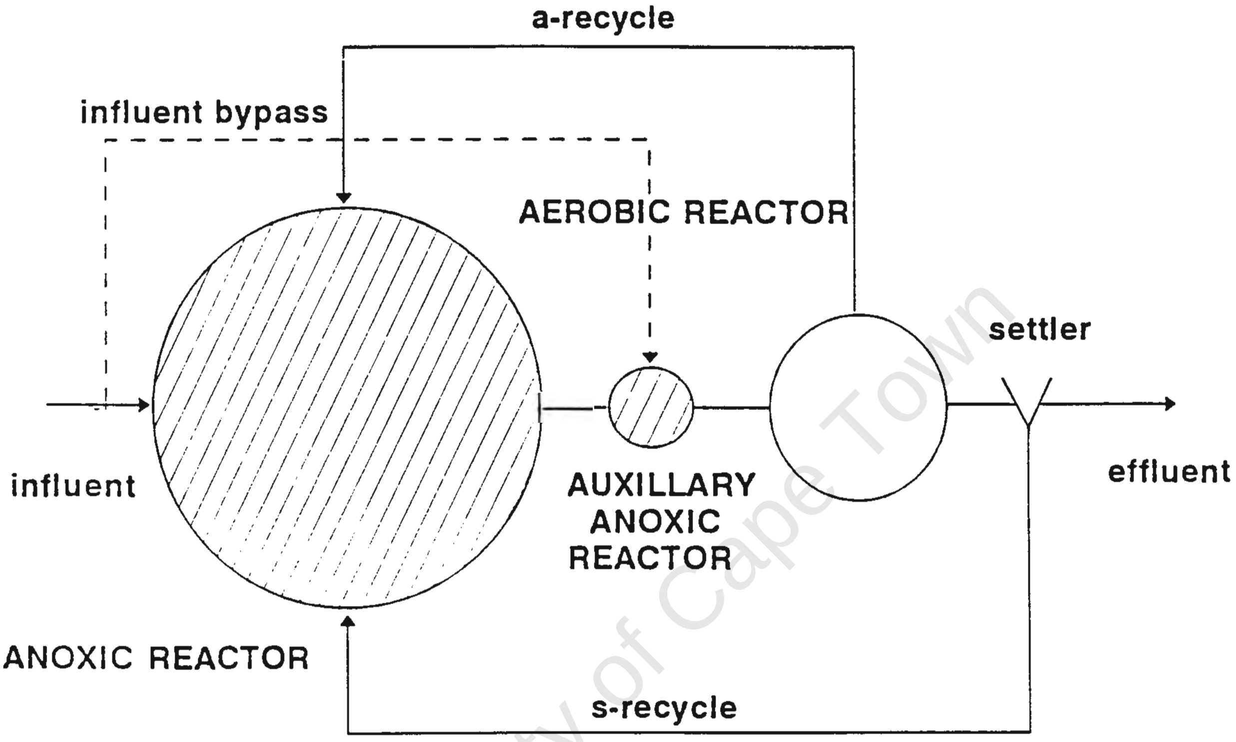


Fig 3.22 Schematic diagram of the Modified 2-reactor anoxic aerobic system

Table 3.9 Operational changes and problems for modified 2-reactor system

DATE	DAY NO.	CHANGE	PROBLEM/REASON
25/7	254	500ml Auxiliary anoxic reactor receiving 1l of daily feed added to system	Improve denitrification
30/8	290	System cleaned, no wasting	
31/8	291	500ml sludge loss	Blockage
1-2/9	292-293	No wasting	
5/9	296	No feed and aeration	Power failure
19/9	310	1l sludge drawn off	Batch test
26/9	317	Aeration off overnight,sludge in settler	OUR control box failure, settler motor failure
1/10	322	1l sludge drawn off	Batch test
10/10	331	1l sludge drawn off	Batch test
12/10	333	1l sludge drawn off	Batch test
17/10	338	Algae in system	
18/10	339	Sludge in settler	
22/10	343	1l sludge drawn off	Batch test
25/11	377	No feed	Pump relay burnt out
2/12	384	Sludge mixed with control system sludge	Ensure both systems have same mass of sludge
3/12	385	System cleaned, no wasting	
7/1	420	System cleaned, no wasting	
14/2	458	Sludge sifted	
26/2	470	Sludge loss - 4l	Blockage
27/2	471	System cleaned	

Filament identification was carried out every 2 to 4 weeks.

The results of the above measurements are listed in Appendix 1 and shown graphically in Figs 3.23 to 3.39. The changes made to the systems and operational problems encountered are listed in Table 3.9.

System behaviour - Nitrogen and COD mass balances

To check the reliability of the experimental results N and COD mass balance checks were conducted. Details of the N and COD mass balance calculations are given in Appendix 2.

To facilitate the mass balance calculations the experimental data need to be divided into steady state periods to take account of different sewage characteristics (principally the TKN concentration) and system operating conditions. For the 216 days the Experimental system was operated, the influent TKN concentration for the different sewage batches varied between 30 and 70 mgTKN-N/l (Fig 3.24). 17 sewage batches were fed, the commencement of which is indicated in Figs 3.23 - 3.39 by a * near the top horizontal axis. The variations in the influent TKN concentration allow division of the data into 3 steady state periods (SSP, indicated by vertical lines in Figs 3.23 - 3.39), ie steady state period 1 day, 254 to day 360; steady state period 2, day 360 to 347; and steady state period 3, day 447 to day 472. For the 3 steady state periods the N balances are (1) 104.2%, (2) 104.5% and (3) 78.5% respectively (average for the 3 periods-95.7%). The COD mass balances for the three steady state periods are 43.2%, 63.0% and 68.3% respectively, giving an average for the three periods of 58.2 %. These COD mass balances are very low, and can be attributed to variations in the volume and sludge concentration caused by blockages and in particular for the first steady state period, abstraction of sludge for batch test purposes, which was not returned to the system. (see Table 3.4)

The data of the Control system were divided into two steady state periods which corresponded to the steady state periods of the Experimental system because both systems received the same influent sewage ie period 2 day 360 to day 447 and period 3 day 448 to day 472 (Note, no steady state period 1 exists because the Control system steady state periods were numbered the same as the corresponding Experimental steady state periods). The N balances for the two steady state periods are 102.1%, and 89.9% for steady state periods 2 (day 383 to 447) and 3 (day 448 to 472) respectively, giving an average N balance for the system of 95%. The N balances of the control system for steady state periods 2 and three are very similar to those of the Experimental system for the same periods. The COD balances of the Control system for the two steady state periods 2 and 3 are 67.5%, and 79.2% giving an average of 73.3%. These values for the COD balance also are very low, but are better than those obtained for the Experimental system (see Table 3.4).

System behaviour - COD removal

On average COD removal for the modified 2-reactor(or Experimental) system remained high. For the three steady state periods the average COD removals were 89% (average influent COD - 512 mgCOD/l; average effluent COD 55 mgCOD/l), 87% (average influent COD 511 mgCOD/l; average effluent COD 65 mgCOD/l) and 82% (average influent COD of 427 and average effluent COD of 76 mgCOD/l) respectively. The f_{us} values for the three steady state periods are 0.107, 0.127 and 0.172 respectively. Calculation of the f_{up} fraction

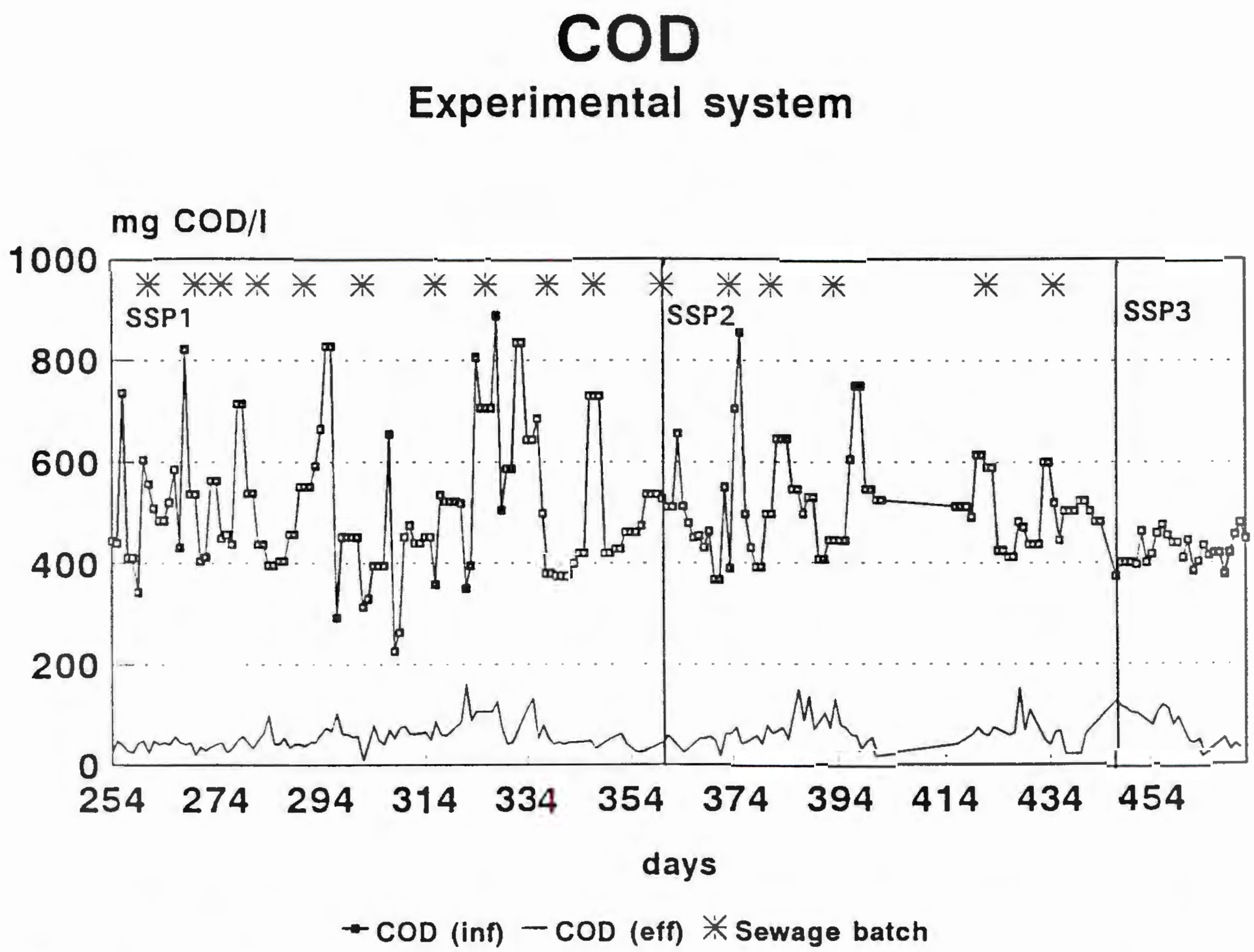


Fig 3.23 Modified anoxic-aerobic system influent and effluent COD concentrations

(SSP = steady state period)

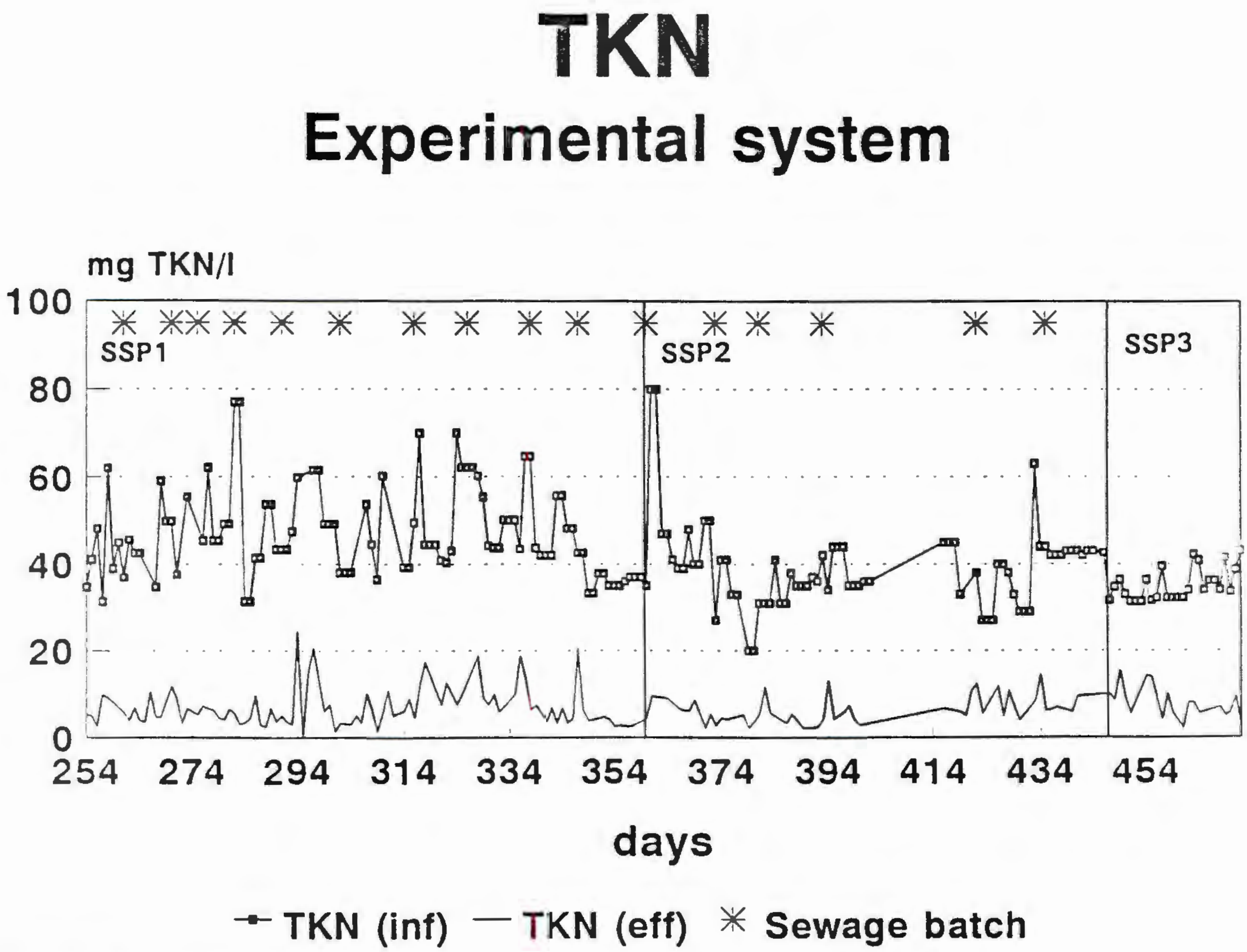


Fig 3.24 Modified anoxic-aerobic system influent and effluent TKN concentration

(SSP = steady-state period)

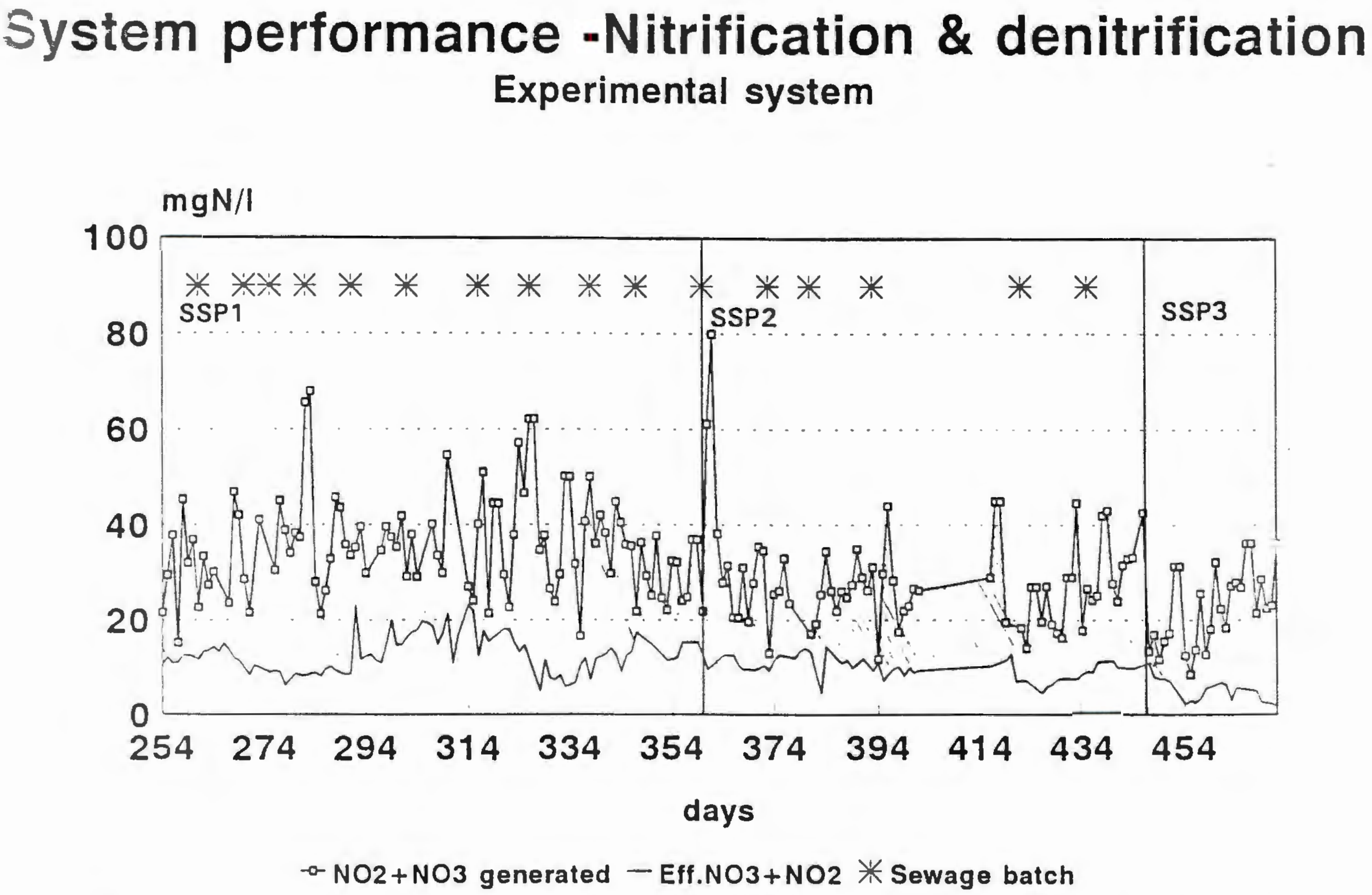


Fig 3.25 Modified 2-reactor system nitrification and denitrification performance
Shaded area indicates nitrate + nitrite (NOx) denitrified
(SSP = steady-state period)

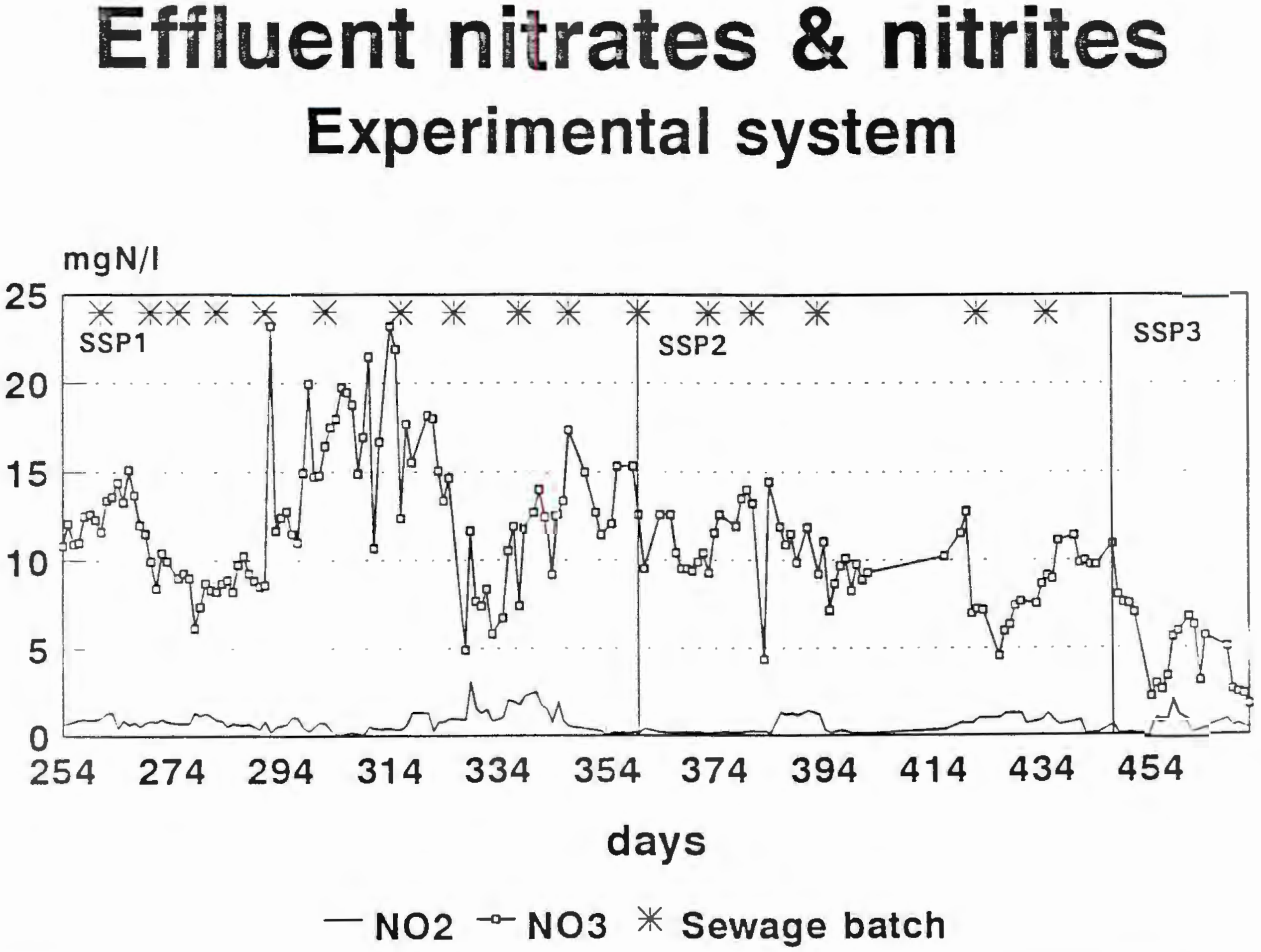


Fig 3.26 Modified anoxic-aerobic system daily effluent nitrate and nitrite concentrations (SSP = steady-state period)

for the 3 steady state periods (see Appendix 1) gives values of (1) 0.0, (2) 0.065 and (3) 0.088 respectively (see Table 3.11). Interestingly, these f_{up} values are very low compared to modified UCT nutrient removal systems operated on the same wastewater in the laboratory at the time of these experiments; in the MUCT systems the f_{up} were between 0.20 to 0.40 depending on whether the systems bulked or not (Clayton *et al.*, 1989; Musvoto *et al.*, 1992).

The average influent and effluent COD's (Fig 3.33) for the Control system for steady state periods 2 and 3 are 515.7, and 434.7 mgCOD/l and 80.6, and 52.8 mgCOD/l respectively. This gives average COD removals for steady state periods 2 and 3 of 84%, and 88% respectively. The average unbiodegradable soluble COD fractions (f_{us}) were 0.156 (steady state period 2) and 0.121 (steady state period 3), and the unbiodegradable particulate fractions (f_{up}) were 0.077 (steady state period 2) and 0.144 (steady state period 3), these values being fairly similar to those obtained in the Experimental system during the steady state period 2, but differing in steady state period 3, the f_{us} value in the Control system being slightly lower, and the f_{up} being higher than those values in the experimental system.

System behaviour - Nitrification and denitrification

Experimental System

The average influent TKN concentration for steady state period 1 was 47.0 mgTKN-N/l, and the average effluent TKN concentration was 7.0 mgTKN-N/l. There are sharp increases in effluent TKN concentration from days 294 to 297, probably due to a corresponding sharp increase in influent TKN concentration these days. The influent TKN on these days was about 60 mgTKN-N/l, while the effluent TKN concentration was just over 20 mgTKN-N/l. On days 328, 336 and 337 the effluent TKN concentrations also increased to around 20 mgTKN-N/l. These high effluent TKN concentrations seem to correspond mostly with high influent TKN concentrations on these days which is indicative of the system operating close to the minimum sludge age for nitrification (exacerbated by small variations in aeration and hence DO concentration in the aerobic reactor). This is confirmed in that for $u_{nm20} = 0.36/d$ and a 70% anoxic mass fraction the minimum system sludge age for nitrification at 20°C is 14.7 d, where the system sludge age is 15 d.

From day 254, the effluent nitrate concentration increased to 15 mgNO_3-N/l on day 266, thereafter declining to a concentration of 6 mgNO_3-N/l on day 278. The nitrate concentration then increased to a concentration of 21.5 mgNO_3-N/l. During this time the nitrite concentration decreased from 0.75 mgNO_2-N/l on day 270 to 0.07 mgNO_2-N/l on day 304. The nitrite concentration then increased to a relatively high concentration of between 0.8 and 3.2 mgNO_2-N/l from day 318 to day 346. The concentrations of nitrate plus nitrite(NO_x) generated and denitrified for steady state period 1 are 35.1 and 22.4 mgNO_x^-N/l respectively.

Influent TKN concentrations during steady state period 2 varied between 20.0 and 50.0 mgTKN-N/l, with an average for the period of 39.2 mgTKN-N/l. At the start of the steady state period 2 unusually high TKN values were recorded (80 mgTKN-N/l, days 361-362), but can probably be regarded as outliers, as can the low values of around 20.0 mgTKN-N/l on days 379-380. From around day 432 influent TKN values remained fairly steady above 40.0 mgTKN-N/l to the end of the steady state period. Effluent TKN concentrations were

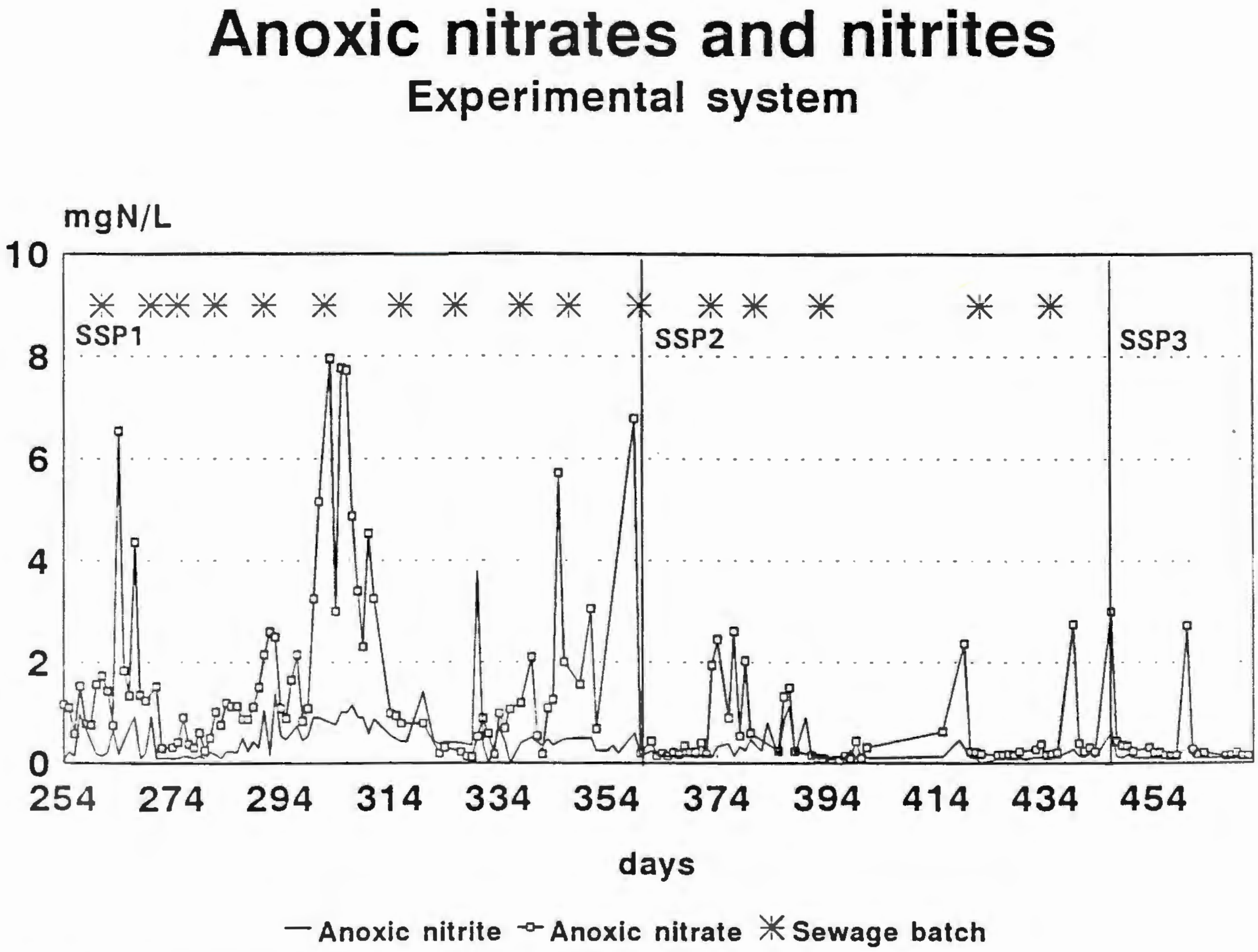


Fig 3.27 Modified anoxic aerobic system daily anoxic reactor nitrate and nitrite concentrations

(SSP = steady-state period)

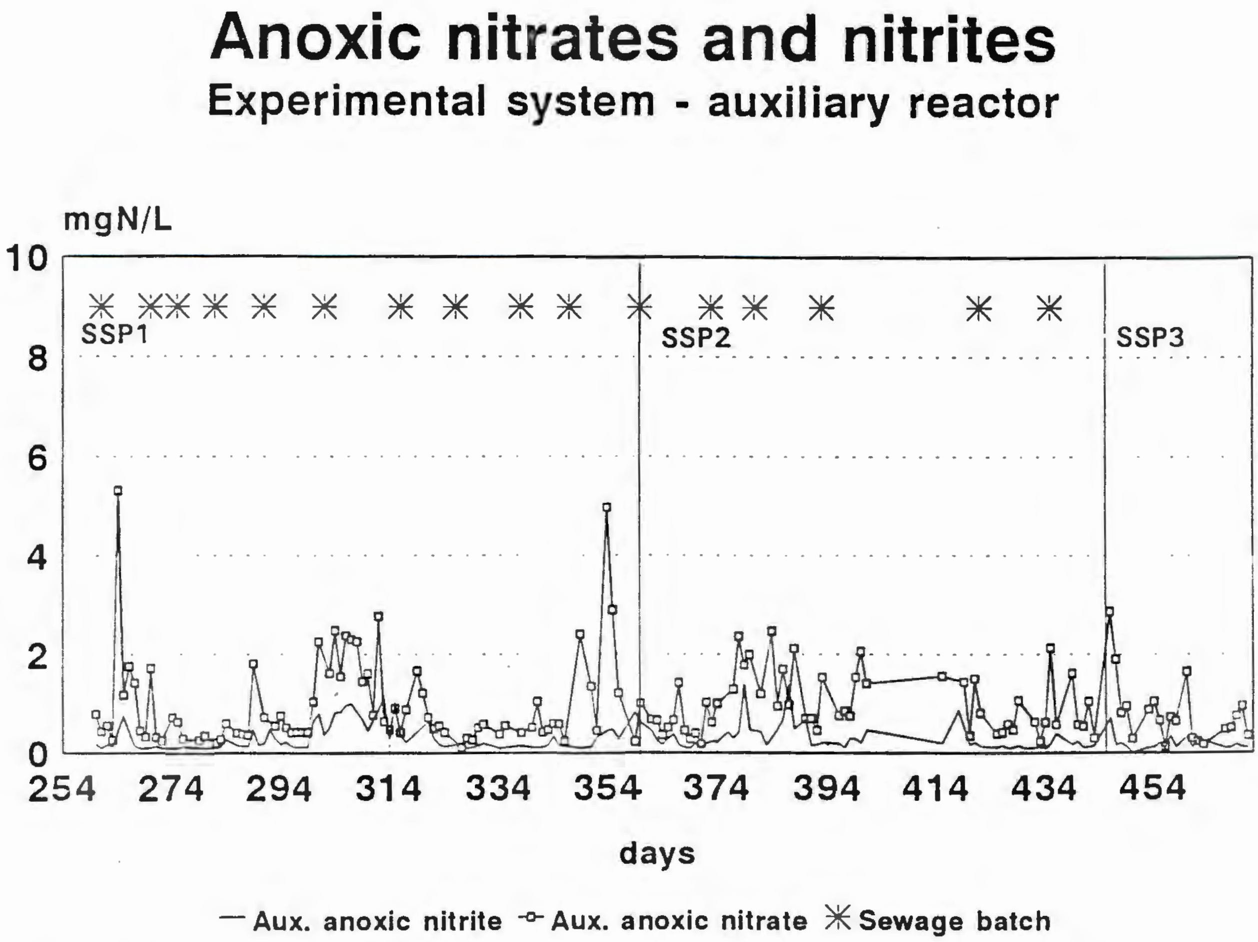


Fig 3.28 Modified anoxic-aerobic system daily auxiliary anoxic reactor nitrate and nitrite concentrations
(SSP = steady-state period)

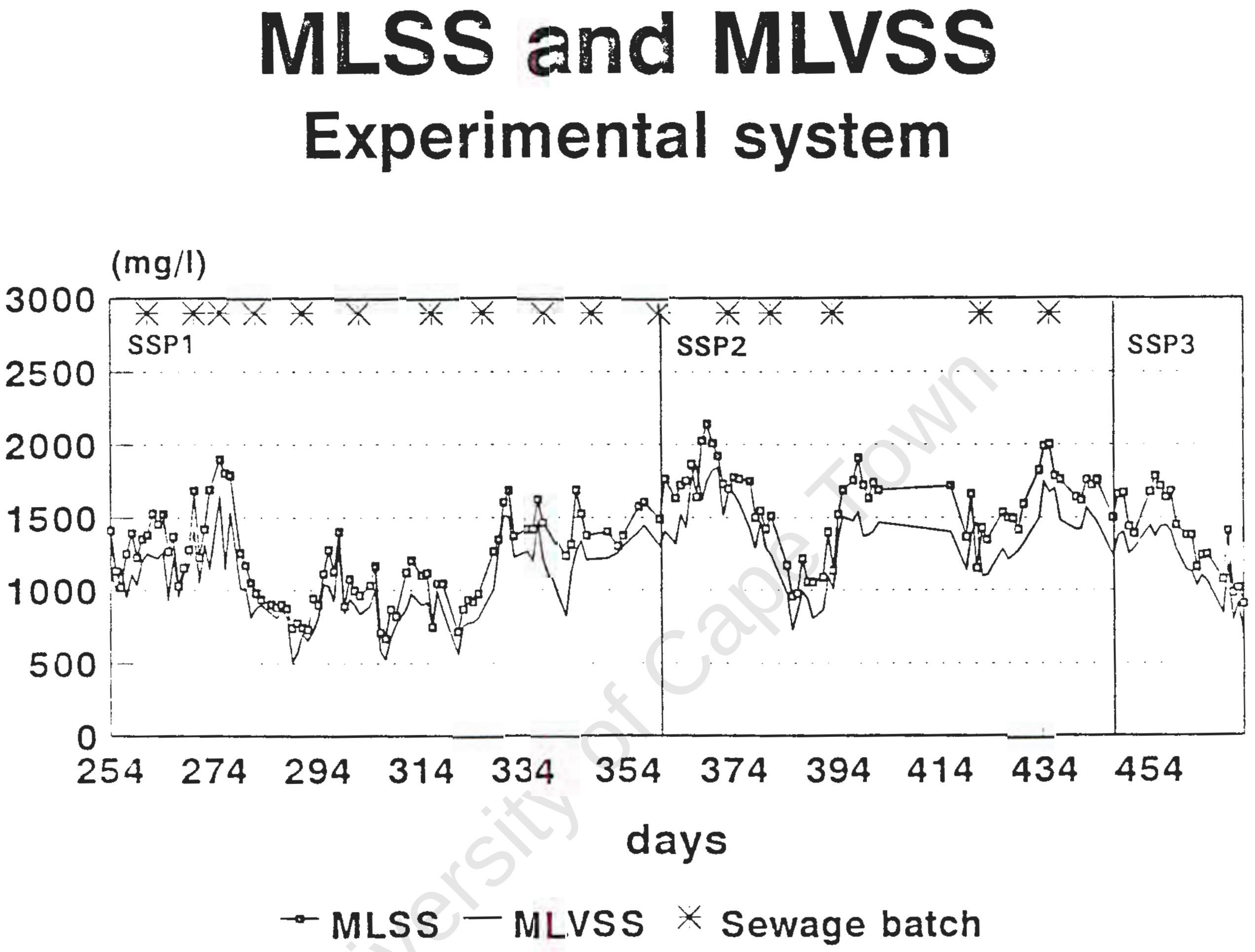


Fig 3.29 Modified anoxic-aerobic system MLSS and MLVSS concentrations (SSP = steady-state period)

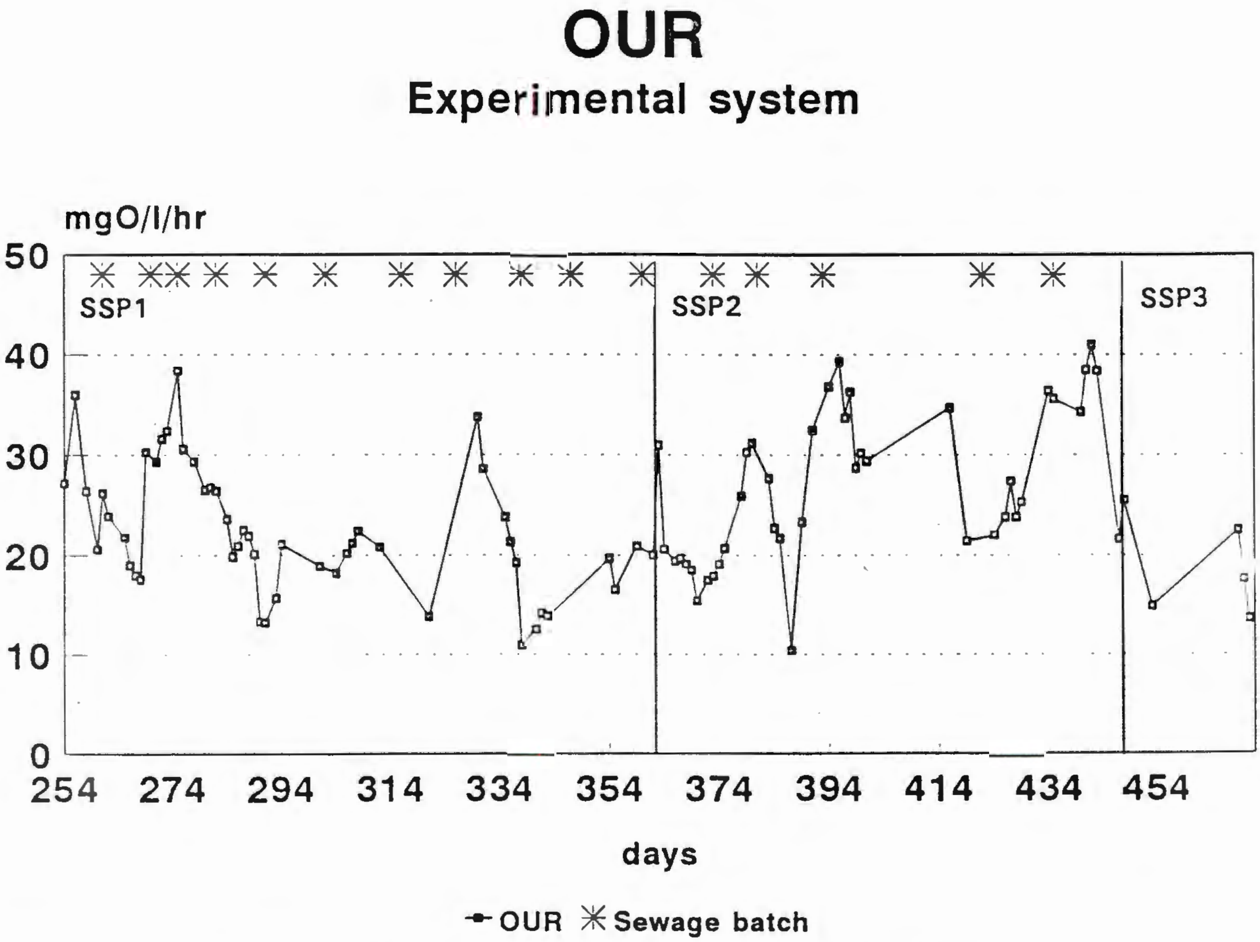


Fig 3.30 Modified anoxic-aerobic system OUR (aerobic reactor)

(SSP = steady-state period)

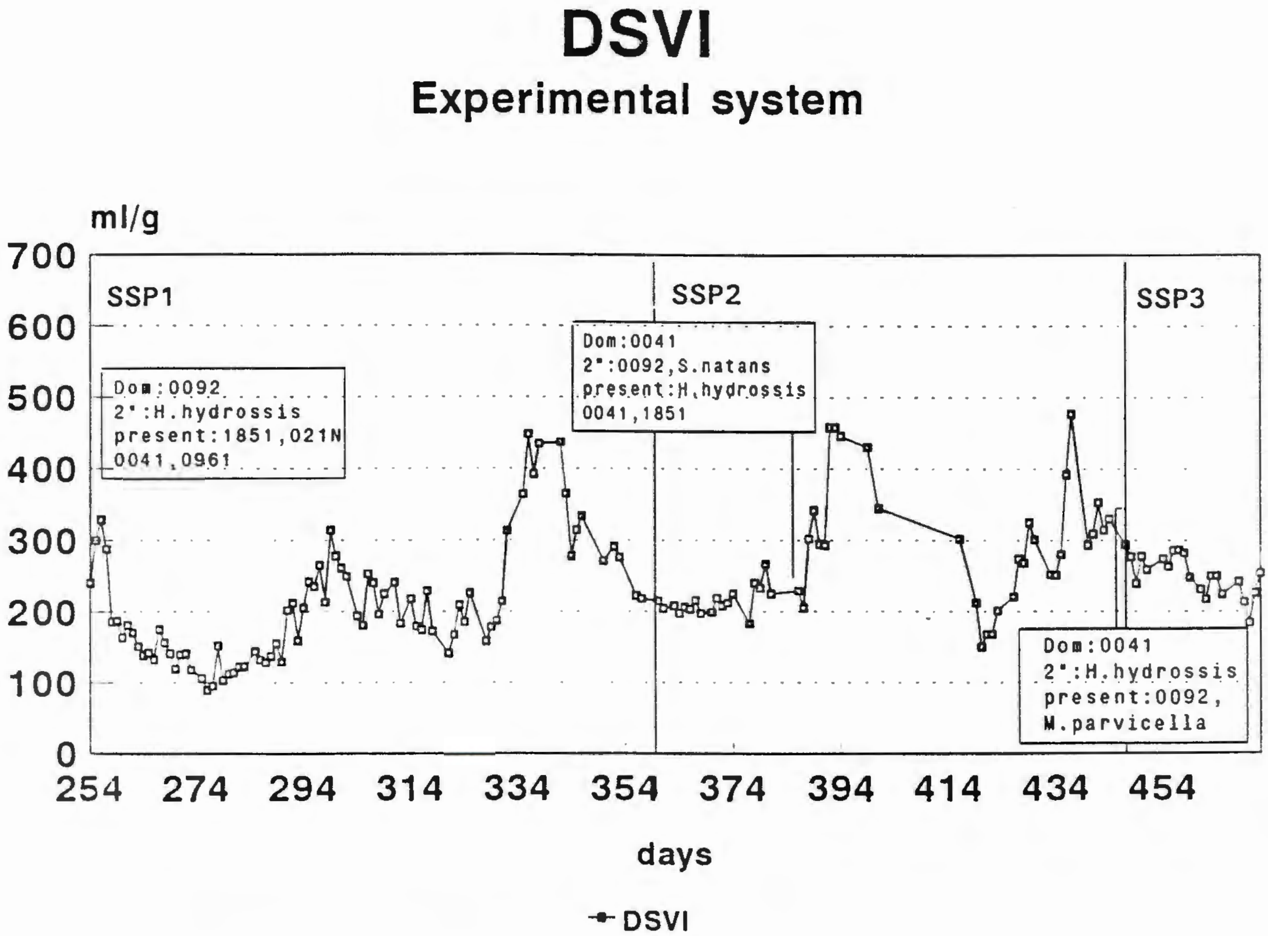


Fig 3.31 Modified anoxic- aerobic system DSVI

(SSP = steady-state period)

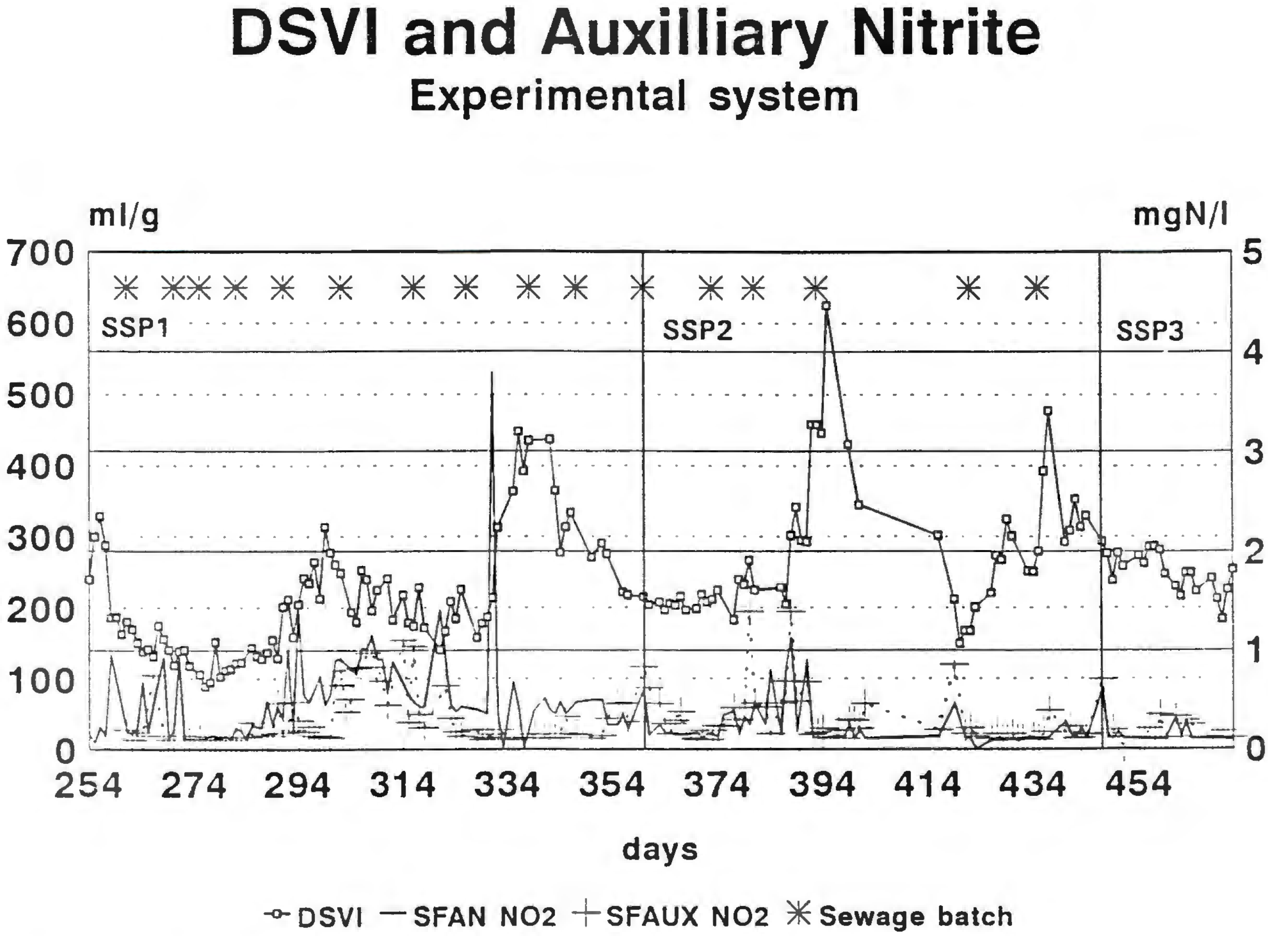


Fig 3.32 Modified 2 reactor system DSVI and auxiliary anoxic nitrite concentration

(SSP = steady-state period)

mostly below 10 mgTKN-N/l with an average for the period of 6.4 mgTKN-N/l.

Effluent nitrate concentration during steady state period 2 varied between concentrations of 15.0 and 5.0 mgNO_3-N/l (average 9.9 mgNO_3-N/l for period 2). From day 385 to day 402 the nitrate concentration declined from 14.4 to 9.3 mgNO_3-N/l. The nitrite concentration increased briefly to levels of above 1 mgNO_3-N/l from day 386 to day 393 (average nitrite concentration for period 2: 0.5 mgNO_2-N/l). From day 420 to 426 the effluent nitrate concentration declined from 12.8 to 4.5 mgNO_3-N/l. Over this period the effluent nitrite concentration increased from 0.7 to 1 mgNO_2-N/l. The concentration of nitrate then increased from day 427 to day 440 to a concentration of 11.4 mgNO_3-N/l on day 440. The average nitrate/nitrite concentrations generated and denitrified for this steady state period are 25.4 and 15.6 mgNO_x-N/l, influent respectively.

A lower influent TKN concentration during steady state period 3 (average 35 mgTKN-N/l), and a high effluent TKN concentration during this period seem to indicate incomplete nitrification, especially from day 454 to day 457, where the effluent TKN was high (above 10 mgTKN-N/l), and the effluent nitrate concentration low (below 5 mgNO_3-N/l). The mass of nitrate generated during this period (Table 3.4) shows a much lower value (12.9 mgNO_3-N/l influent) than that for period 2 (26.0 mgNO_3-N/l influent). The average effluent nitrate concentration was 4.7 mgNO_3-N/l, which also is lower than the average for period 2. The average concentration of nitrate plus nitrite denitrified for steady state period 3 was 8.3 mgNO_x-N/l influent, with an average of 11.9 mgNO_x-N/l generated

Control system

Influent TKN concentration (Fig 3.34) during steady state period 2 of the Control system (day 383 to day 447)varied between 27.0 and 47.0 mgTKN-N/l with an average of 37.7 mgTKN-N/l for the period. Effluent TKN concentrations were generally low (< 10 mgTKN-N/l) during this steady state period, giving an average effluent TKN of 4.9 mgTKN-N/l. Effluent nitrate concentrations varied between 10 and 4 $mgNO_3$-N/l with an average for the period of 8.5 $mgNO_3$-N/l. Effluent nitrite concentrations were generally low, having an average concentration of 0.62 mg NO_2-N/l for steady state period 2. The average concentrations of nitrate plus nitrite (NO_x-N/l) generated and denitrified for steady state period 2 are 24.2 $mgNO_x$-N/l and 15.7 $mgNO_x$-N/l respectively.

The influent TKN concentration was lower during steady state period 3 of the Control system - around 32 to 36 mgTKN-N/l giving an average of 34.2 mgTKN-N/l. The average effluent TKN concentration for this steady state period is 6.2 mgTKN-N/l. Effluent nitrate concentrations varied between 1.4 and 8 $mgNO_3$-N/l, with an average effluent concentration of 4.91 $mgNO_3$-N/l. The nitrite concentration in the effluent varied between 0.3 and 1.5 $mgNO_2$-N/l, with an average of 0.98 $mgNO_2$-N/l for steady state period 3. The average concentration of nitrate plus nitrite (NO_x-N/l) generated and denitrified for this steady state period was 14.3 $mgNO_x$-N/l and 9.4 $mgNO_x$-N/l respectively.

The nitrification performance of the Control system thus appears to be similar to that of the Experimental system for the same periods of operation.

System behaviour - Anoxic reactor nitrate and nitrite concentrations

Experimental System

Nitrate concentrations in the anoxic reactor (Fig 3.27) remained mostly between 0.5 and 2 mgNO_3-N/l during the first steady state period (steady state period 1 - day 254 to day 360). Between days 301 and 312 the anoxic nitrate concentration was high (> 2 mgNO_3-N/l), due to losses of sludge caused by blockages and abstractions of sludge for batch test purposes during this time.

From day 360, the start of steady state period 2, the anoxic nitrate concentration remained relatively low, below 0.5 mgNO_3-N/l, except for some days between days 374 and 390 and single high values on days 417, 440, and 447, where the anoxic reactor nitrate concentration increased to over 2 mgNO_3-N/l.

The pattern of anoxic reactor nitrate concentrations of steady state period 3 was similar to that of steady state period 2. The concentration of nitrate in the anoxic reactor did not increase above 0.45 mgNO_3-N/l, except on day 461, where it was measured at 2.72 mgNO_3-N/l. The average concentrations of nitrate in the anoxic reactor for steady state periods 1, 2, and 3 are 1.9, 1.2, and 0.4 mgNO_3-N/l respectively.

The nitrate concentration in the auxiliary anoxic reactor was generally low during steady state period 1, usually not exceeding 1.1 mgNO_3-N/l. Around days 300-311, the concentration increased to about 1.8 mgNO_3-N/l, exceeding 2 mgNO_3-N/l on some days, due to lower sludge concentrations caused by sludge abstractions and losses due to blockages. The average auxiliary anoxic reactor nitrate concentration for steady state period 1 was 0.97 mgNO_3-N/l.

During steady state period 2 the auxiliary anoxic nitrate concentration tended to fluctuate between 0.4 and 2 mgNO_3-N/l. The average nitrate concentration in the auxiliary anoxic reactor during this steady state period was 1.37 mgNO_3-N/l. For most of the third steady state period the auxiliary anoxic reactor nitrate concentration fluctuated between 0.1 and 1.7 mgNO_3-N/l, the average being 0.71 mgNO_3-N/l.

Anoxic reactor nitrite concentrations during the first steady state period varied between 0.1 and 0.5 mgNO_2-N/l, with some high values between 0.5 and 1.0 mgNO_3-N/l between day 300 and day 314 due to disturbances in unit operation (see Table 3.9). Nitrite concentrations in the anoxic reactor were low at the start of steady state period 2 - around 0.2 mgNO_2-N/l. From around day 374 the nitrite concentration in the anoxic reactor showed an irregular increasing tendency, generally being higher than 0.5 mgNO_2-N/l from day 384 to day 393. Thereafter for the remaining part of steady state period 2 (day 394 - 447), the nitrite concentration remained below 0.2 mgNO_2-N/l with specific high values greater than 0.5 mgNO_2-N/l on days 418 and 445. During steady state period 3 (day 448 - 464) the nitrite concentration remained below 0.2 mgNO_2-N/l. The average nitrite concentration in the anoxic reactor for steady state periods 1, 2, and 3 were 0.50, 0.23, and 0.13 mgNO_2-N/l respectively.

Nitrite concentrations in the auxiliary anoxic reactor (measured from day 261 in steady state period 1) remained relatively low, around 0.15 mgNO_2-N/l, but increased sharply from around day 300, showing a similar trend toward higher concentrations between days 300 to 320 as the main anoxic reactor nitrite concentration. The nitrite concentration in the auxiliary anoxic reactor varied between 0.2 mgNO_2-N/l to 1.4 mgNO_2-N/l during the second steady state period. During the third steady state period the auxiliary reactor nitrite concentration

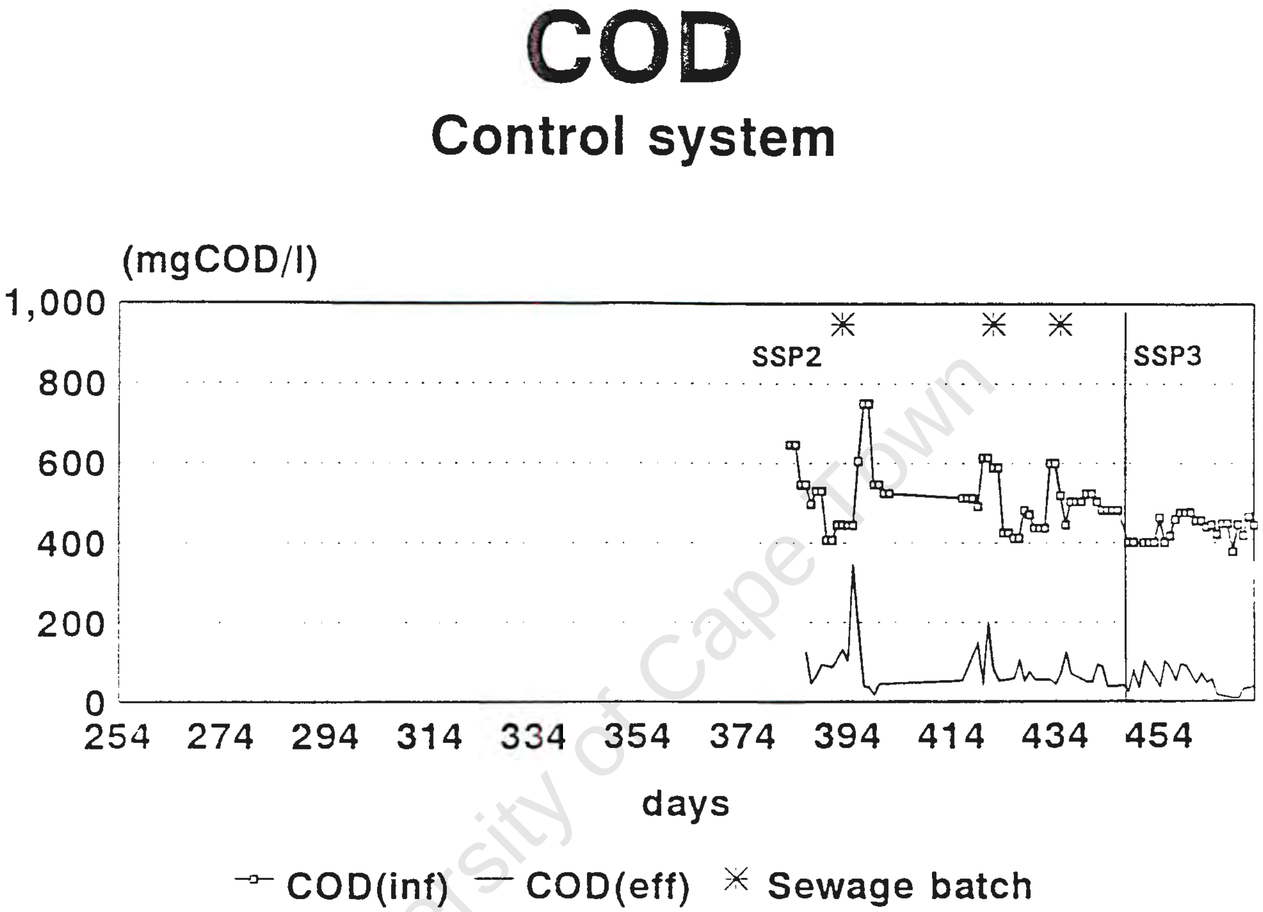
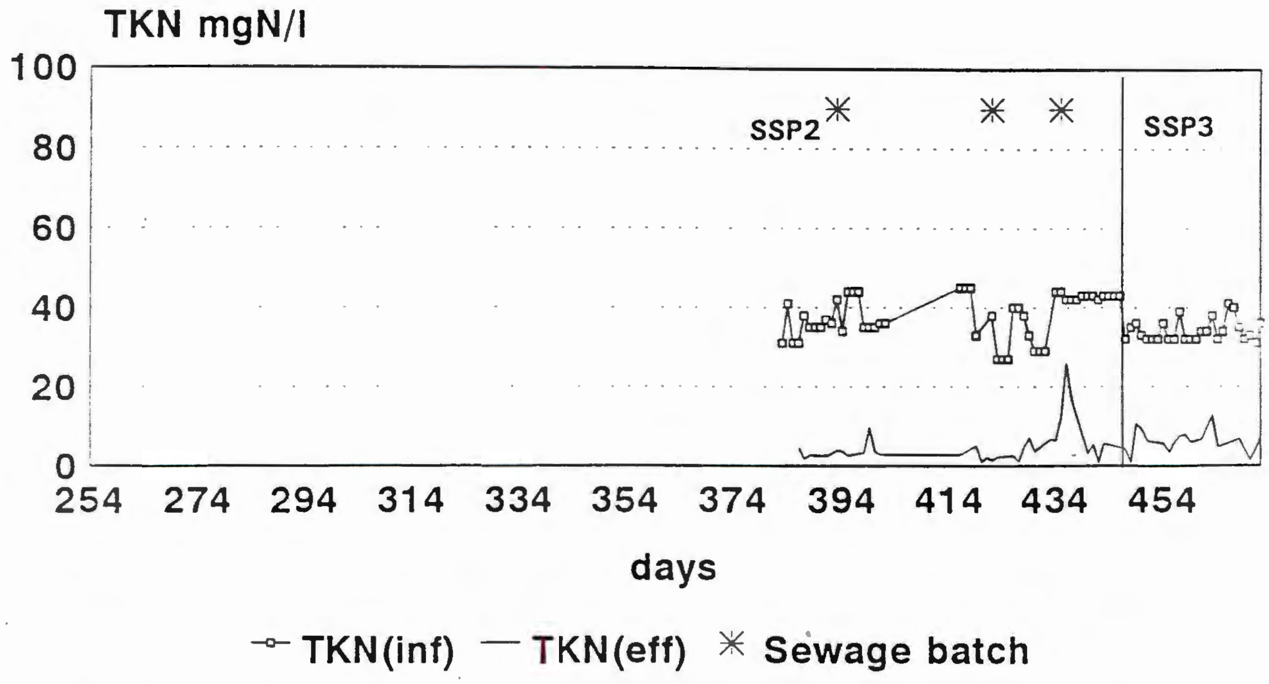


Fig 3.33 Control system influent and effluent COD concentrations

(SSP = steady-state period)



TKN

Control system

Fig 3.34 Control system influent and effluent TKN concentrations

(SSP = steady-state period)

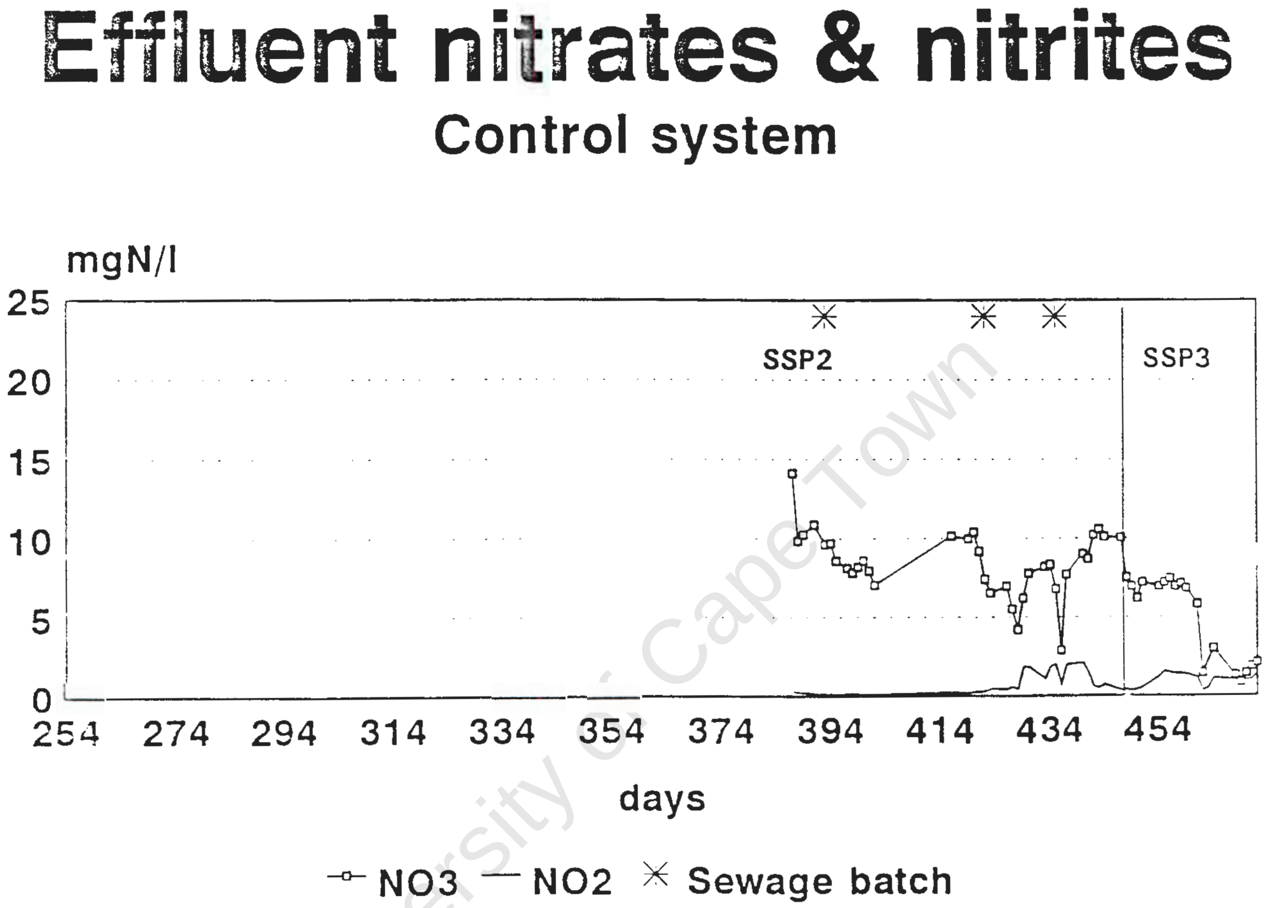


Fig 3.35 Control system effluent nitrate and nitrite concentrations

(SSP = steady-state period)

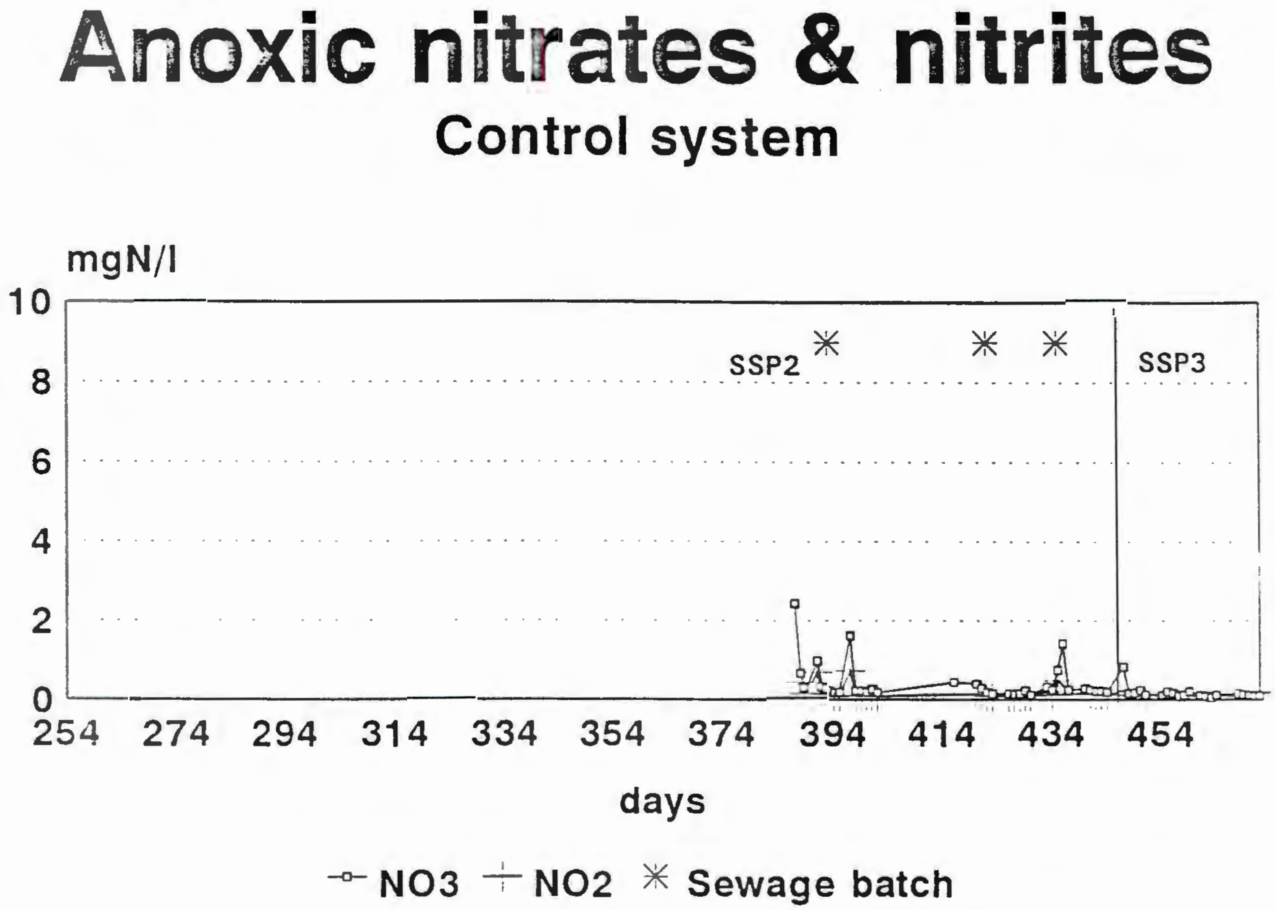


Fig 3.36 Control system anoxic reactor nitrate and nitrite concentrations

(SSP = steady-state period)

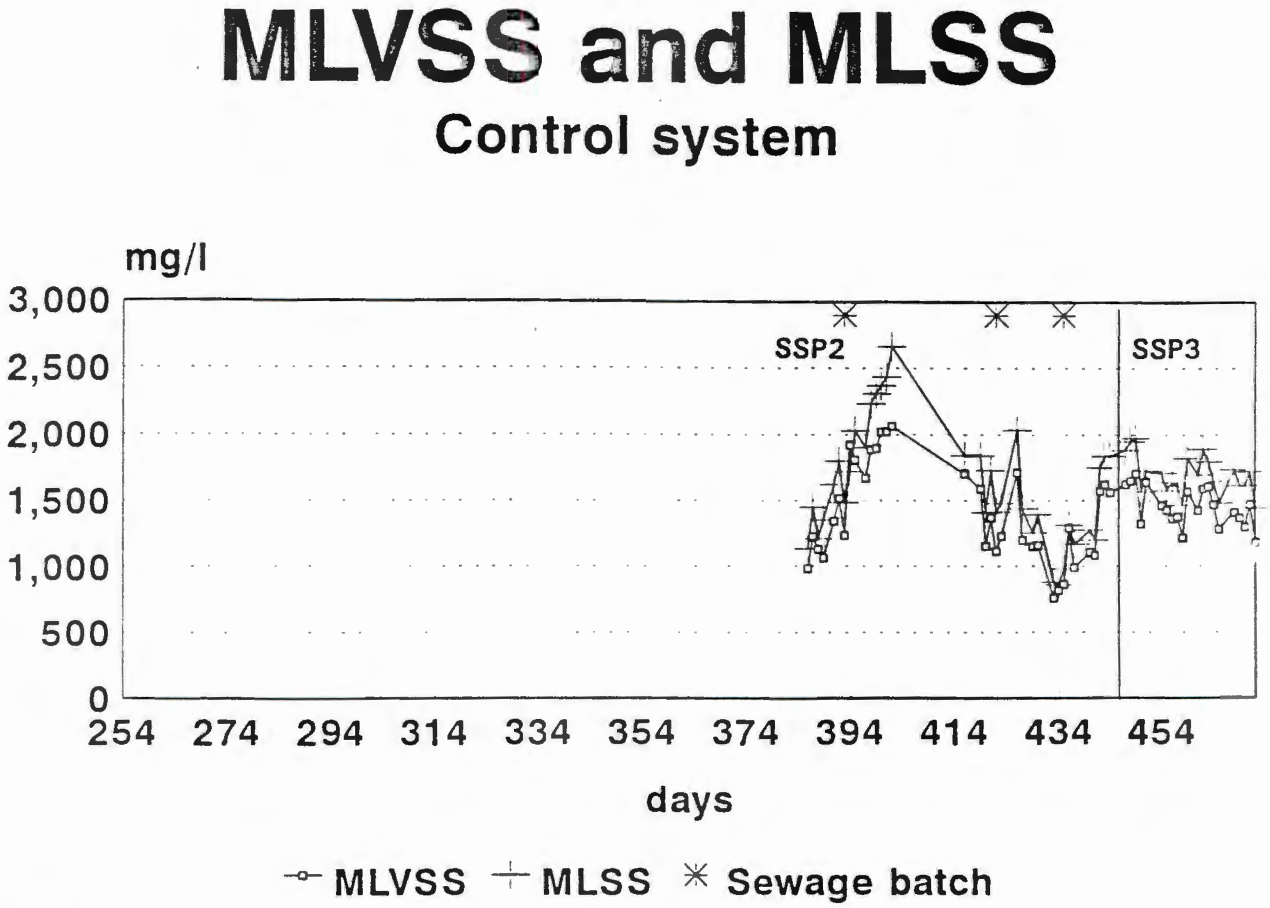


Fig 3.37 Control system MLVSS and MLSS concentrations

(SSP = steady-state period)

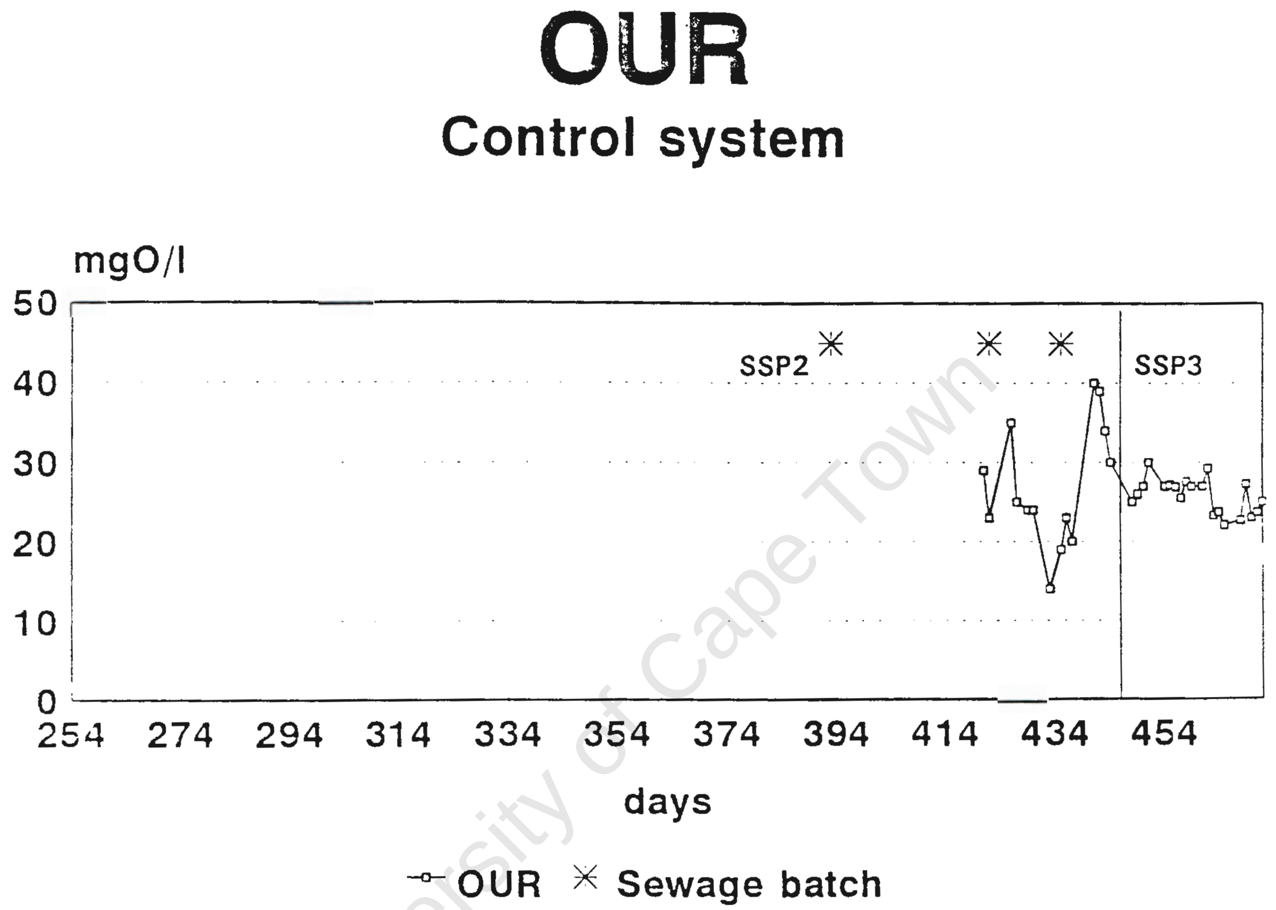


Fig 3.38 Control system oxygen utilization rate (OUR)

(SSP = steady-state period)

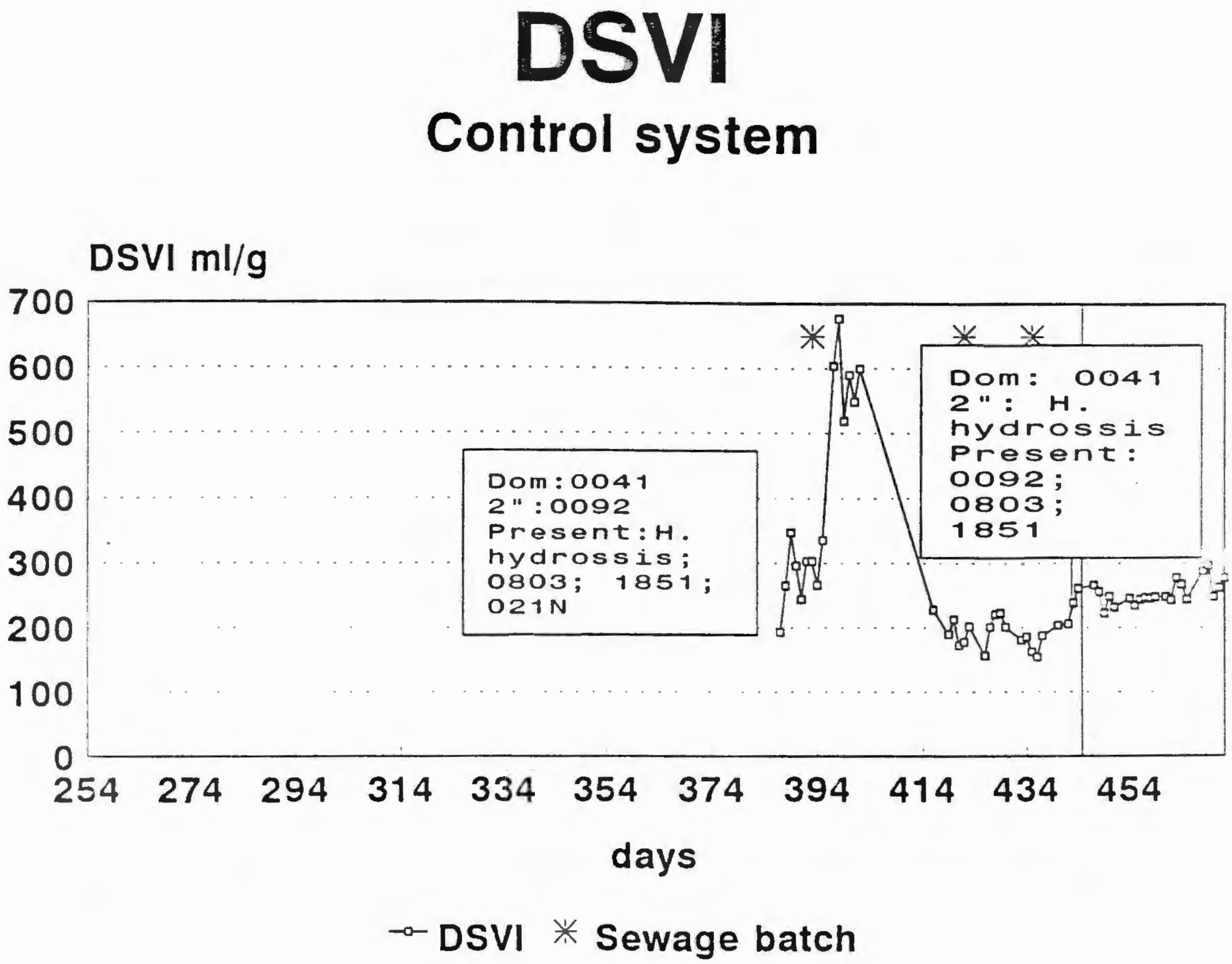


Fig 3.39 Control system DSVI

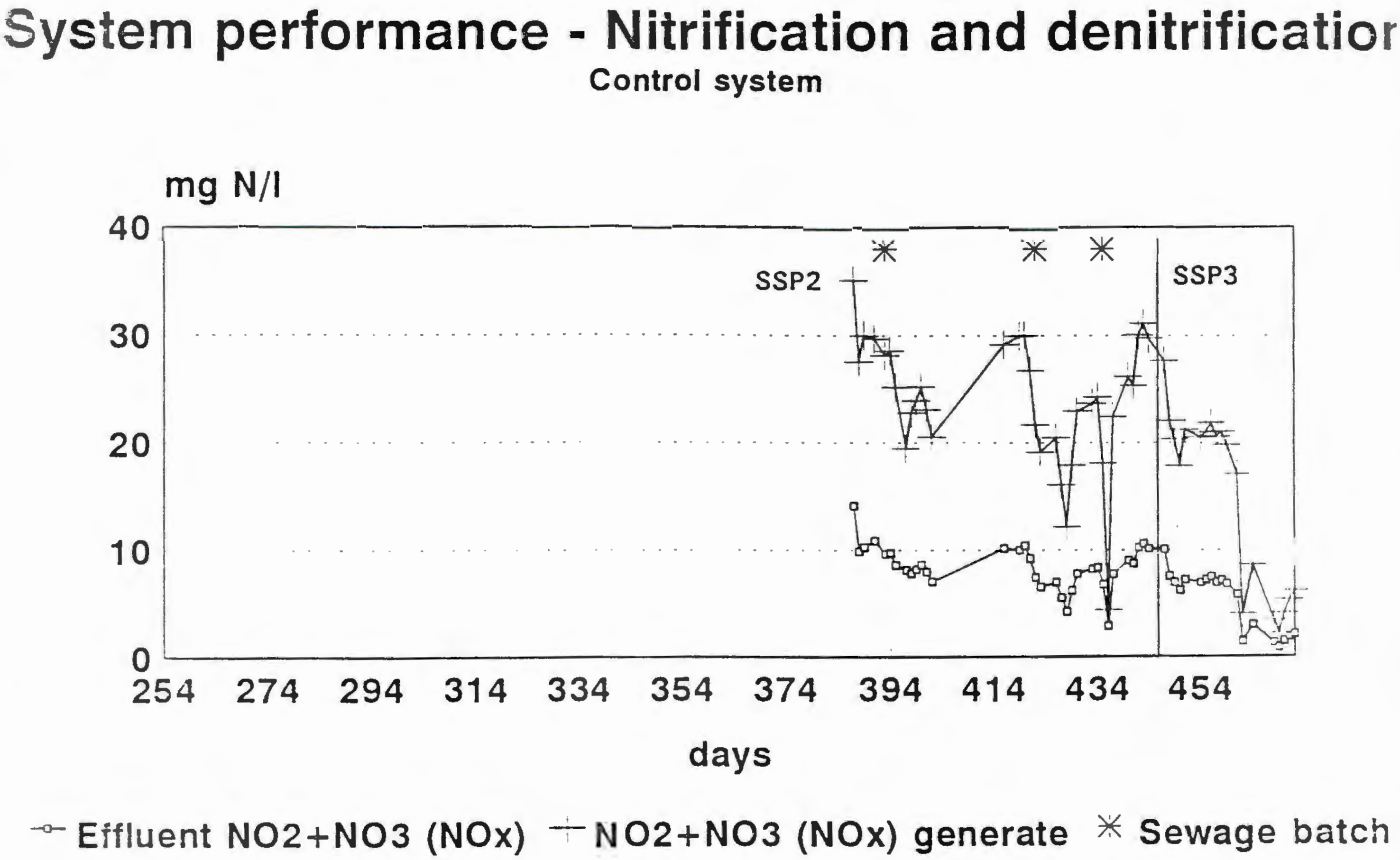


Fig 3.40 Control system nitrate + nitrite generated (NOx) and effluent nitrate + nitrite (NOx) concentrations (SSP = steady-state period)

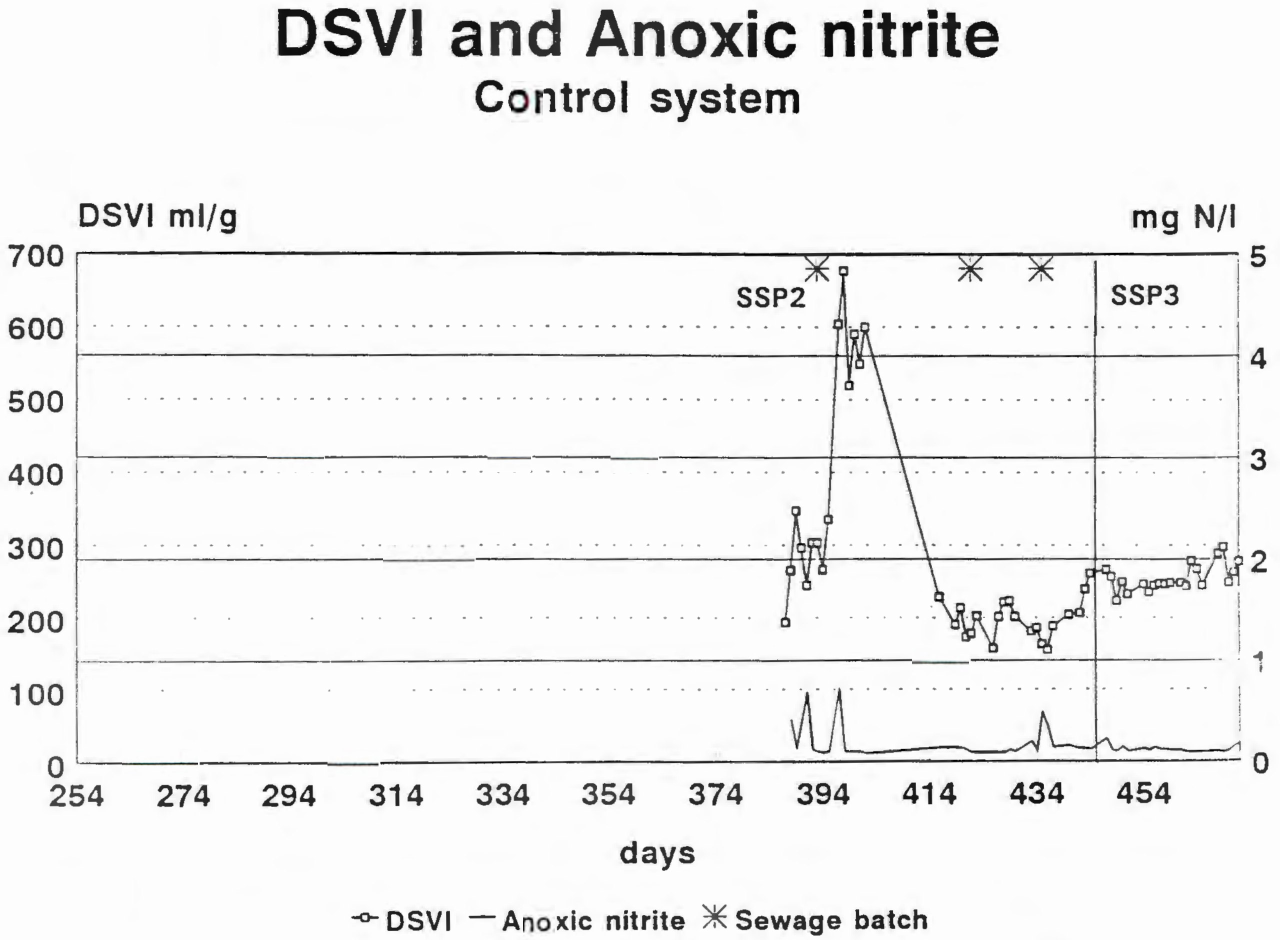


Fig 3.41 Control system DSVI and anoxic reactor nitrite concentration

(SSP = steady-state period)

Table 3.10 Filamentous organism identifications - Control system

Date	Day	Dominant	Secondary	Other
1/12/91	383	0041	0092	*H.hydrossis*, 1851, 1851, 021N
30/1/92	443	0041	*H. hydrossis*	0092, 0803, 1851

showed no significant increase, and remained relatively low between 0.1 and 0.3 mgNO_2-N/l. The average auxiliary reactor nitrite concentrations for steady state periods 1, 2 and 3 were 0.30 , 0.31, and 0.16 mgNO_2-N/l respectively.(see Table 3.11a)

Control System

Anoxic reactor nitrate concentrations (Fig 3.36) in the Control system were generally low, below 0.5 mgNO_3-N/l for both steady state periods, except for a few single values. The average concentrations of anoxic nitrate were 0.43 and 0.14 mgNO_3-N/l for steady state periods 2 and 3 respectively, lower than those values in the experimental system at the same times.

Anoxic reactor nitrite concentrations (Fig 3.36) were also low in the Control system, generally around 0.1 mgNO_2-N/l for all three steady state periods. The average anoxic reactor nitrite concentrations were 0.18 and 0.12 mgNO_2-N/l for steady state periods 2 and 3 respectively.

Concentrations of both nitrate and nitrite in the anoxic reactor of the Control system seemed to be slightly lower than those of the Experimental system during the same period.

System behaviour - DSVI

Experimental System

Although the DSVI (Fig 3.31 and 3.32) showed a sharp increase to 329 ml/g (day 256) immediately after the installation of the auxiliary anoxic reactor in the system on day 254, the DSVI declined from 240 ml/l to below 90 ml/g over 25 days to day 276. The dominant filament species present at this time was 0092, with *H hydrossis* present as secondary filament. From day 279 the DSVI increased reaching 300 ml/g on day 299, and thereafter until day 319, fluctuated between 250 and 210 ml/g. During the same time the auxiliary anoxic reactor nitrite concentration increased significantly from 0.1 to 1.0 mgNO_2-N/l. From day 320 to day 342 the DSVI increased sharply from around 200 ml/g to 437 ml/g while the auxiliary reactor nitrite concentration decreased from around 1 to 0.1 mgNO_2-N/l. From day 342 to day 354 the DSVI decreased from 437 to 200 ml/g while the auxiliary anoxic nitrite concentration showed an increasing tendency from 0.1 to 0.6 mgNO_2-N/l.

From day 356 to 376 the DSVI remained stable at around 200 ml/g and the auxiliary anoxic reactor nitrite concentration decreased from 0.8 to 0.2 mgNO_2-N/l. Thereafter both the DSVI and the auxiliary anoxic reactor nitrite concentration increased simultaneously over a period of 20 days, the DSVI from 200 ml/g to over 600 ml/g and the nitrite concentration from 0.2 to around 1 mgNO_2-N/l. In the following 22 day period (day 396 to 418), the DSVI and auxiliary anoxic reactor nitrite concentration also behaved similarly, both declining, the former from 600 ml/g to 150 ml/g and the latter from 1.6 to 0.1 mgNO_2-N/l. The DSVI then increased again from 150, reaching 300ml/g on day 448, although the anoxic nitrite

Table 3.4 Nitrogen and COD mass balance calculations for different steady state periods during complete investigation

PARAMETER		Intermittent System STEADY STATE PERIOD Day 1-83		Two-reactor N/D System STEADY STATE PERIOD Day 84-254			Modified two-reactor N/D System STEADY STATE PERIOD Day 255-471			Control System STEADY STATE PERIOD Day 383-472	
		1	2	1	2	3	1	2	3	2	3
Day		1-64	65-83	84-146	147-205	206-254	255-360	361-447	448-472	383-447	448-472
Duration (d)		64	19	63	59	49	106	87	23	65	23
Effluent TKN concentration	Nte	9.633	10.01	11.43	6.699	6.827	7.015	6.393	7.9	4.946	6.153
Effluent TKN mass	MNte	96.33	100.1	114.3	66.99	68.27	70.15	63.93	79	49.46	61.53
Effluent Nitrate concentration	Nne	4.477	8.961	11.1	10.28	10.8	12.66	9.847	4.677	8.506	4.905
Effluent Nitrate mass	MNne	44.77	89.61	111	102.8	108	126.6	98.47	46.77	85.06	49.05
Effluent Nitrite concentration		1.09	2.488	1.047	0.475	0.75	0.843	0.525	0.605	0.622	0.981
Effluent Nitrite mass		10.9	24.88	10.47	4.75	7.5	8.43	5.25	6.05	6.22	9.81
Anoxic Nitrate concentration				3.138	1.45	1.291	0.965	1.374	0.708	0.432	0.156
Anoxic Nitrite concentration				0.516	0.256	0.375	0.501	0.228	0.133	0.181	0.117
a-recycle ratio				3:1	3:1	1:1	1:1	1:1	1:1	1:1	1:1
Average VSS concentration	VSS	1554	1336	1388	1342	1589	1030	1378	1177	1401	1440
N in waste sludge	MNs	103.7	89.11	92.58	89.51	106	68.7	91.91	78.51	93.45	96.05
Influent TKN concentration	Nti	50.16	62.38	66.42	44.35	44.37	46.99	39.23	35.24	37.73	33.46
Influent TKN mass	MNti	501.6	623.8	664.2	443.5	443.7	469.9	392.3	352.4	377.3	334.6
Mass Nitrate generated	Mng	301.6	434.6	398.1	441.5	285.3	350.9	254.2	119.1	242.2	143.1
Mass Nitrate denitrified	Mnd	256.8	345	287.1	338.7	177.3	224.3	155.7	72.3	157.2	94.02
Mass Oxygen for Nitrate	MOn	1378	1986	1819	2018	1304	1603	1162	544.1	1107	653.8
Effluent COD concentration	Ste	76.07	92.4	83.48	63.72	72.63	54.9	65.02	73.5	80.6	52.76
Effluent COD mass	MSte	760.7	924	834.8	637.2	726.3	549	650.2	735	806	527.6
Mass of COD in waste sludge	MSxw	1534	1319	1370	1325	1569	1017	1360	1162	1383	1422
Influent COD concentration	Sti	441.1	474.8	560.6	482.1	522.9	512.3	510.7	426.6	515.7	434.7
Influent COD mass	MSti	4411	4748	5606	4821	5229	5123	5107	4266	5157	4347
OUR		28.44	32.96	38.66	27.16	34.92	22.38	26.74	18.82	27.07	26.11
Measured Oxygen consumed daily		2048	2373	2784	1956	2514	1611	1925	1355	1949	1880
O2 recovered(denitrification)	MOd	734.6	986.6	821.1	968.7	507	641.4	445.4	206.8	449.5	268.9
	MOc	669.3	387	964.2	-62.1	1211	7.975	763.6	810.9	842.1	1226
%N Balance		*	*	91.08	134.8	103.6	104.2	104.5	78.48	102.1	89.85
%COD Balance		83.85	76.17	71.18	59.5	76.73	43.24	63.04	68.32	67.49	79.23

* Assumed 100% for intermittently aerated system

Table 3.11 Steady state period averages for the Modified 2-reactor Experimental system and Control system

STEADY STATE PERIOD AVERAGES(EXP. SYSTEM)	PERIOD 1 (day 255-360)	PERIOD 2 (day 361-447)	PERIOD 3 (day 448-472)
VSS	1030 mg/l	1378 mg/l	1177 mg/l
TSS	1201 mg/l	1594 mg/l	1394 mg/l
COD(inf)	512.9 mg/l	510.7 mg/l	426.6 mg/l
COD(eff)	54.9 mg/l	65.0 mg/l	73.5 mg/l
f_{up}	0.0	0.065	0.088
f_{us}	0.107	0.127	0.172
TKN(inf)	46.99 mg/l	39.23 mg/l	35.24 mg/l
TKN(eff)	7.02 mg/l	6.39mg/l	7.9 mg/l
Nitrate(eff)	12.66 mg/l	9.85 mg/l	4.68 mg/l
Nitrate(anox)	1.86 mg/l	1.17 mg/l	0.357 mg/l
Nitrite(eff)	0.84 mg/l	0.53 mg/l	0.61 mg/l
Nitrite(anox)	0.50 mg/l	0.23 mg/l	0.13 mg/l
DSVI	209 ml/g	324.7 ml/g	249 ml/g
OUR	22.4 mg/l/hr	26.7 mg/l/hr	18.8 mg/l/hr
(CON.SYSTEM)		PERIOD 2 (day 383-447)	PERIOD 3 (day 448-472)
VSS		1401 mg/l	1440 mg/l
TSS		1626 mg/l	1683 mg/l
COD(inf)		515.7 mg/l	434.5 mg/l
COD(eff)		80.6 mg/l	52.8 mg/l
f_{up}		0.077	0.144
f_{us}		0.156	0.121
TKN(inf)		37.7 mg/l	34.2 mg/l
TKN(eff)		4.9 mg/l	6.2 mg/l
Nitrate(eff)		8.51 mg/l	4.91 mg/l
Nitrate(anox)		0.43 mg/l	0.14 mg/l
Nitrite(eff)		0.62 mg/l	0.98 mg/l
Nitrite(anox)		0.18 mg/l	0.12 mg/l
DSVI		287 ml/g	254 ml/g
OUR		27 mg/l/h	26 mg/l/h

Table 11a Steady state period averages for the Modified 2-reactor Experimental system(anoxic reactor nitrate and nitrite concentrations).

STEADY STATE PERIOD AVERAGES(EXP. SYSTEM)	PERIOD 1 (day 255-360)	PERIOD 2 (day 361-447)	PERIOD 3 (day 448-472)
Nitrate (aux)	0.965 mgN/l	1.374 mgN/l	0.708 mgN/l
Nitrite (aux)	0.302 mgN/l	0.308 mgN/l	0.163 mgN/l

concentration showed no significant increase above 0.20 $mgNO_2$-N/l. During steady state period 3 (day 448 - 472) the DSVI declined from 300 ml/g to 225 ml/g while the auxiliary anoxic nitrate and nitrite concentration was low, between 0.1 and 0.3 $mgNO_2$-N/l. With the lower TKN concentration in the feed the anoxic nitrite concentration was low, and the DSVI decreased to below 250 ml/g.

Control System

The DSVI in the Control system (Fig 3.39) was high during the first half of the steady state period 1, increasing around 500 - 700 ml/g. During steady state period 2 the DSVI increased to around 250 ml/g, and remained between 250 - 300 ml/g during steady state period. The DSVI of the Control system behaved in much the same manner as that of the experimental system during the same time period of operation.

3.3 STATISTICAL ANALYSIS OF EXPERIMENTAL RESULTS

3.3.1 Introduction

In order to clarify the relationship between anoxic nitrite and nitrate concentrations and DSVI, a statistical analysis was carried out on the relevant data. This took the form of a correlation analysis on the DSVI and nitrate/nitrite results in the anoxic reactor preceding the aerobic reactor. For interest correlations between DSVI and effluent nitrate and nitrite conentrations were also done, as well as between nitrite and nitrate concentrations themselves

3.3.2 Procedure

The correlation analysis was carried out by computer, using the Statgraphics software package, (Statistical Graphics Corporation, 1988). Using the correlation function, ie.

$$r=\frac{\Sigma (X-\overline{X})(Y-\overline{Y})}{\sqrt{\Sigma (X-\overline{X})^2 \Sigma (Y-\overline{Y})^2}}$$

the correlation coefficient (r) is calculated, X being the value of the independent variable (eg. NO_2) and Y being the value of the dependent variable (ie. DSVI). The results are presented as a matrix cross correlating the input variables. To test the validity of the correlation coefficient, the significance, s, is determined using Student's t distribution by computing the t statistic for the correlation using the equation:

$$t=\frac{r}{\sqrt{\frac{1-r^2}{n-2}}}$$

where t= number of standard deviations r (correlation coefficient) is from 0, and n is the sample size. This t-statistic is then inserted into the formulation for the t-distribution from which the significance level s is calculated. An s value of < 0.05 indicates a significantly non-zero correlation, (ie. in only 5% of similar samples from the population will the correlation not be significant) assuming that both dependent and independent variables are normally distributed.(ie. bivariate)

3.3.3 Results

The correlation analysis was carried out on DSVI and both the anoxic and effluent nitrate and nitrite data, the results being presented as a correlation matrix. The correlation analysis was carried out for each steady state period of both the 2RND and M2RND systems and the pooled data of all the steady state periods of each system. For the M2RND system, the correlation included nitrite and nitrate concentrations in both anoxic reactors, ie the main and auxiliary - insofar as the bulking hypothesis is concerned, only the concentration in the auxiliary anoxic, which precedes the aerobic, would be important. Significantly deviant initial values in each steady state period resulting from possible disturbances in the system were eliminated, the pooled data of each system also being without these significantly deviant values.

For the steady state periods of the 2RND system (Table 3.12), correlations of DSVI with anoxic nitrite and nitrate concentration are generally not very significant ($s > 0.05$), except for strong negative (ie. inverse) correlations with anoxic nitrite concentration in steady state period 1 and anoxic nitrate concentration in steady state period 2. Negative correlations are contrary to expectation - according to the hypothesis, high nitrite and possibly nitrate concentrations should cause high DSVIs. There are some strong positive correlations amongst the various nitrate and nitrite data (marked on Table 3.12), which are expected, indicating that on average denitrification was not complete in these steady state periods - nitrite denitrification only commences once nitrate concentrations reach low (< 1 mg NO_3-N/l)

Table 3.12 Correlations of the Steady State periods of the 2RND system

System	DSVI Correlation	Other possible correlations	$(s<0.05)$
2RND SSP1	An.NO_2 -0.5149 s=0.049 (n=15) An.NO_3 -0.3385 s=0.2172 (n=15)	Eff.NO_2 An.NO_2 0.5731 s=0.0255 (n=15)	An.NO_3 An.NO_2 0.5853 s=0.0219 (n=15)
2RND SSP2	An.NO_2 0.3103 s=0.1311 (n=25) An.NO_3 -0.0470 s=0.8233 (n=25)	Eff.NO_3 An.NO_3 0.4450 s=0.0258 (n=25)	
2RND SSP3	An.NO_2 -0.0407 s=0.8340 (n=29) An.NO_3 -0.1073 s=0.5794 (n=29)	An.NO_2 Eff.NO_2 0.4481 s=0.0148 (n=29)	An.NO_3 An.NO_2 0.4170 s=0.0244 (n=29)

Table 3.13 Correlations of the pooled data of the 2RND system

System	DSVI Correlation	Other possible correlations	($s<0.05$)
2RND **POOL**	An.NO_2 0.1875 s=.1228 (n=69)	Eff.NO_2 Eff.NO_3 0.2679 s=.0261 (n=69)	Eff.NO_3 An.NO_3 0.2283 s=.0592 (n=69)
	An.NO_3 0.2684 s=.0258 (n=69)	Eff.NO_2 An.NO_2 0.3493 s=.0033 (n=69)	An.NO_2 An.NO_3 0.4447 s=.0001 (n=69)

values (Musvoto *et al.*, 1992).

The pooled 2RND system data (Table 3.13) shows a significant positive correlation between anoxic nitrate concentration and DSVI (r=0.2684; s=0.0258), but no significant (s = 0.123) (positive) correlation between anoxic nitrite concentration and DSVI. A significant (s=0.0001) positive correlation between anoxic nitrate and nitrite concentration is obtained for the pooled data indicating that when the nitrate concentration is low so is the nitrite. Therefore, although there is not a significant correlation between the anoxic nitrite concentration and DSVI, (s=0.123) a positive correlation is implied via the nitrate concentration.

For the M2RND system, for which the auxiliary anoxic nitrate and nitrite concentrations are more important because these are discharged to the aerobic reactor, steady state periods 1 and 2 of the M2RND system show positive (but not significant) correlations of DSVI and auxiliary anoxic nitrate and nitrite concentrations (Table 3.14). Positive but not significant correlations with DSVI and nitrate and nitrite concentrations of the anoxic reactor are also obtained. For the pooled results of the M2RND system (Table 3.15), there are also positive correlations between auxiliary anoxic reactor nitrite and nitrate concentrations but only the nitrate concentration - DSVI approach a significant correlation (s=0.059). As earlier with the steady state periods of the 2RND system, a significant positive correlation between auxiliary anoxic reactor nitrate and nitrite concentrations is also obtained, indicating that, although not significant a correlation between auxiliary anoxic nitrite concentration and DSVI is implied via the nitrate concentration.

Table 3.14 Correlations of the Steady State periods of the M2RND system

System	DSVI Correlation	Other			$s<0.05$
M2RND SSP1	An.NO_2 0.1890 s=0.1592 (n=57)	Eff.NO_3 Eff.NO_2 -0.4280 s=.0009 (n=57)	Eff.NO_3 An.NO_3 0.4678 s=.0002 (n=57)	Eff.NO_3 Aux.NO_2 0.4508 s=.0004 (n=57)	Eff.NO_3 Aux.NO_3 0.4863 s=.0001 (n=57)
	An.NO_3 0.0412 s=0.7610 (n=57)	Eff.NO_2 An.NO_3 -0.3651 s=.0052 (n=57)	Eff.NO_2 Aux.NO_2 -0.5123 s=0 (n=57)	Eff.NO_2 Aux.NO_3 -0.4520 s=.0004 (n=57)	An.NO_2 Aux.NO_2 0.2859 s=.0311 (n=57)
	Aux.NO_2 0.851 s=0.5292 (n=57)	An.NO_2 Aux.NO_2 0.2859 s=.0275 (n=57)	An.NO_3 Aux.NO_2 0.5872 s=0 (n=57)	An.NO_3 Aux.NO_3 0.5495 s=0 (n=57)	Aux.NO_2 Aux.NO_3 0.6928 s=0 (n=57)
	Aux.NO_3 0.1665 s=0.2157 (n=57)				

Table 3.14 cont.

M2RND SSP2	An.NO_2 0.0534 s=0.7642 (n=34) An.NO_3 0.0964 s=0.5875 (n=34) Aux.NO_2 0.0840 s=0.6367 (n=34) Aux.NO_3 0.1230 s=0.4885 (n=34)	Eff.NO_3 Eff.NO_2 -0.3458 s=.0452 (n=34) Eff.NO_2 An.NO_2 0.3486 s=.0433 (n=34)	Eff.NO_3 An.NO_2 0.3711 s=.0307 (n=34) An.NO_2 An.NO_3 0.5830 s=.0003 (n=34)	Eff.NO_3 Aux.NO_2 0.5123 s=.0020 (n=34) An.NO_2 Aux.NO_2 0.6984 s=0 (n=34)	Eff.NO_3 Aux.NO_3 0.3523 s=.0410 (n=34) An.NO_2 Aux.NO_3 0.4047 s=.0176 (n=34)
M2RND SSP3	An.NO_2 -0.0851 s=0.7371 (n=18) An.NO_3 -0.1404 s=0.5785 (n=18) Aux.NO_2 0.0285 s=.9106 (n=18) Aux.NO_3 0.0656 s=0.7977 (n=18)	An.NO_2 An.NO_3 0.7200 s=.0008 (n=18) Aux.NO_3 An.NO_2 0.5616 s=.0153 (n=18)	An.NO_2 Aux.NO_2 0.6061 s=.0077 (n=18)	Ef.NO_2 Aux.NO_2 0.4672 s=.0506 (n=18)	An.NO_3 Aux.NO_2 0.4734 s=.0472 (n=18)

Table 3.15 Correlations of the pooled data of the M2RND system

System	DSVI Correlation	Other			$(s<0.05)$
M2RND POOL	An.NO$_2$ 0.0092 s=0.9244 (n=109)	Eff.NO$_3$ Eff.NO$_2$ 0.3625 s=.0001 (n=109)	Eff.NO$_3$ An.NO$_3$ 0.4707 s=0 (n=109)	Eff.NO$_3$ Aux.NO$_2$ 0.4150 s=0 (n=109)	Eff.NO$_3$ Aux.NO$_3$ 0.3271 s=.0005 (n=109)
	An.NO$_3$ -0.0400 s=.6781 (n=109)	Eff.NO$_2$ Aux.NO$_2$ -0.1949 s=.0423 (n=109)	Eff.NO$_2$ Aux.NO$_3$ -0.2641 s=.0055 (n=109)	An.NO$_2$ An.NO$_3$ 0.3783 s=0 (n=109)	An.NO$_2$ Aux.NO$_3$ 0.3456 s=.0002 (n=109)
	Aux.NO$_2$ 0.0857 s=.0591 (n=109)	An.NO$_2$ Aux.NO$_3$ 0.2174 s=.0231 (n=109)	An.NO$_3$ Aux.NO$_2$ 0.4761 s=0 (n=109)	An.NO$_3$ Aux.NO$_3$ 0.3430 s=.0003 (n=109)	Aux.NO$_2$ Aux.NO$_3$ 0.5615 s=0 (n=109)
	Aux.NO$_3$ 0.1814 s=.0591 (n=109)				

One of the requirements of the correlation statistical analysis is that the data sets being compared eg. DSVI and anoxic nitrite concentration or anoxic nitrate and nitrite concentrations, each should be normally distributed. To check that this requirement of the data was met, the distributions of the data were examined with the Statgraphics package.

The individual steady state period DSVI data, as well as the pooled data, (see Fig 3.42 and Fig 3.43 for the pooled data sets of 2RND and M2RND systems) for the 2RND and M2RND systems were found to be normally distributed. However none of the anoxic or auxiliary (and effluent) nitrate and nitrite concentrations data sets, whether individually by steady state period or pooled, showed normal distribution. All of them showed strongly curved lines (instead of linear for normal distribution), steep at low concentrations and flattening out as the concentration increased (see Figs 3.42-3.43). The non-normality creates uncertainty in the statistical analyses based on the t- distribution.

Three approaches were explored in an attempt to deal with the non-normality of nitrite and nitrate data sets; approach (1) repeated the above evaluation but on the basis of log DSVI and log nitrate and nitrite concentrations; approach (2) repeated the evaluation using the Spearman rank correlation rather than the data correlation; and approach (3) eliminated low values from the nitrate (< 1 mg NO_3-N/l) and nitrite < 0.1 mg NO_2-N/l) data sets - many data were concentrated in his lower range as a consequence of the analytical detection limit for nitrite and nitrate. The results of these trials are discussed below.

Approach (1)

No improvement in correlations were obtained on the basis of logs, ie. those correlations that

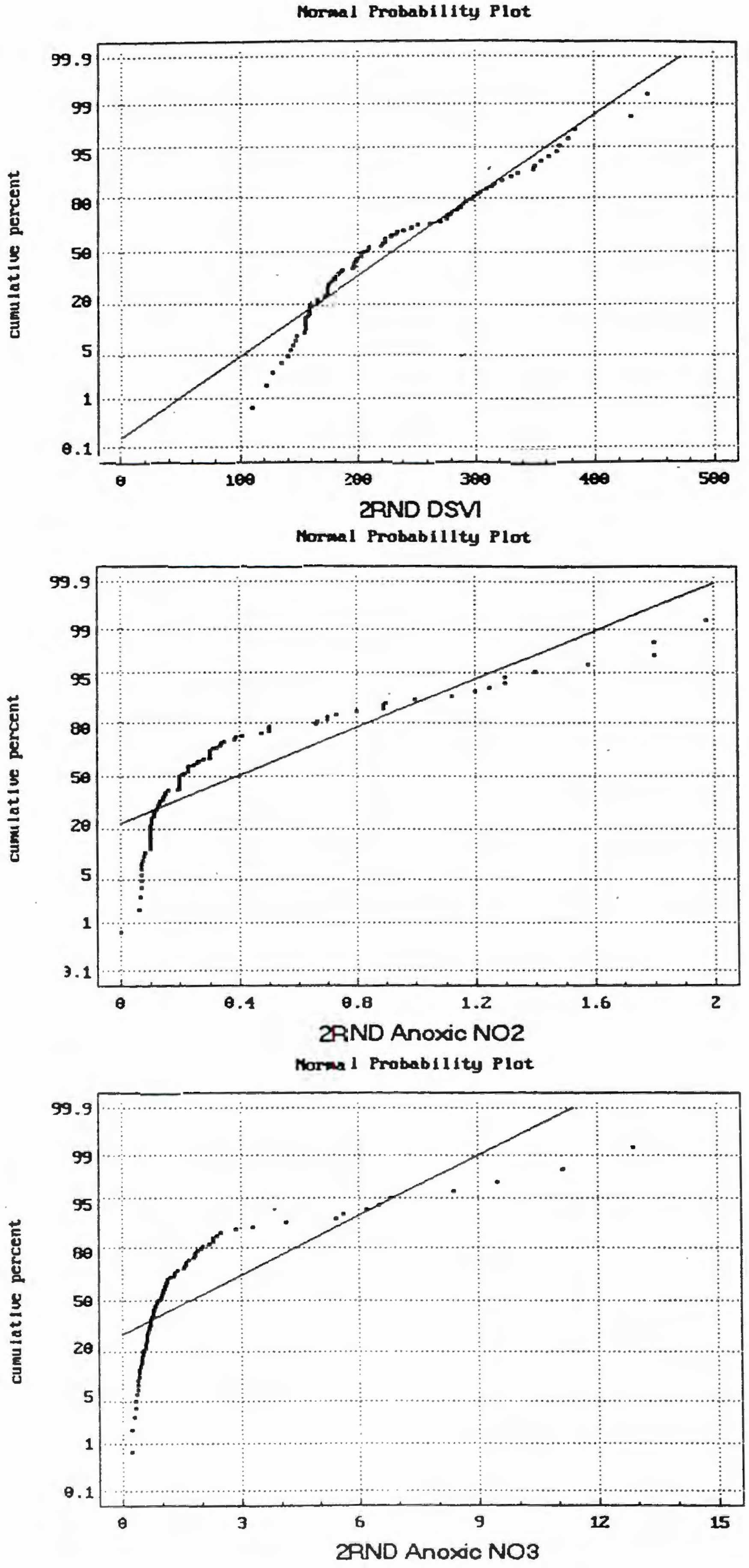


Figure 3.42 Distribution of DSVI, anoxic nitrite and anoxic nitrate for the 2RND system

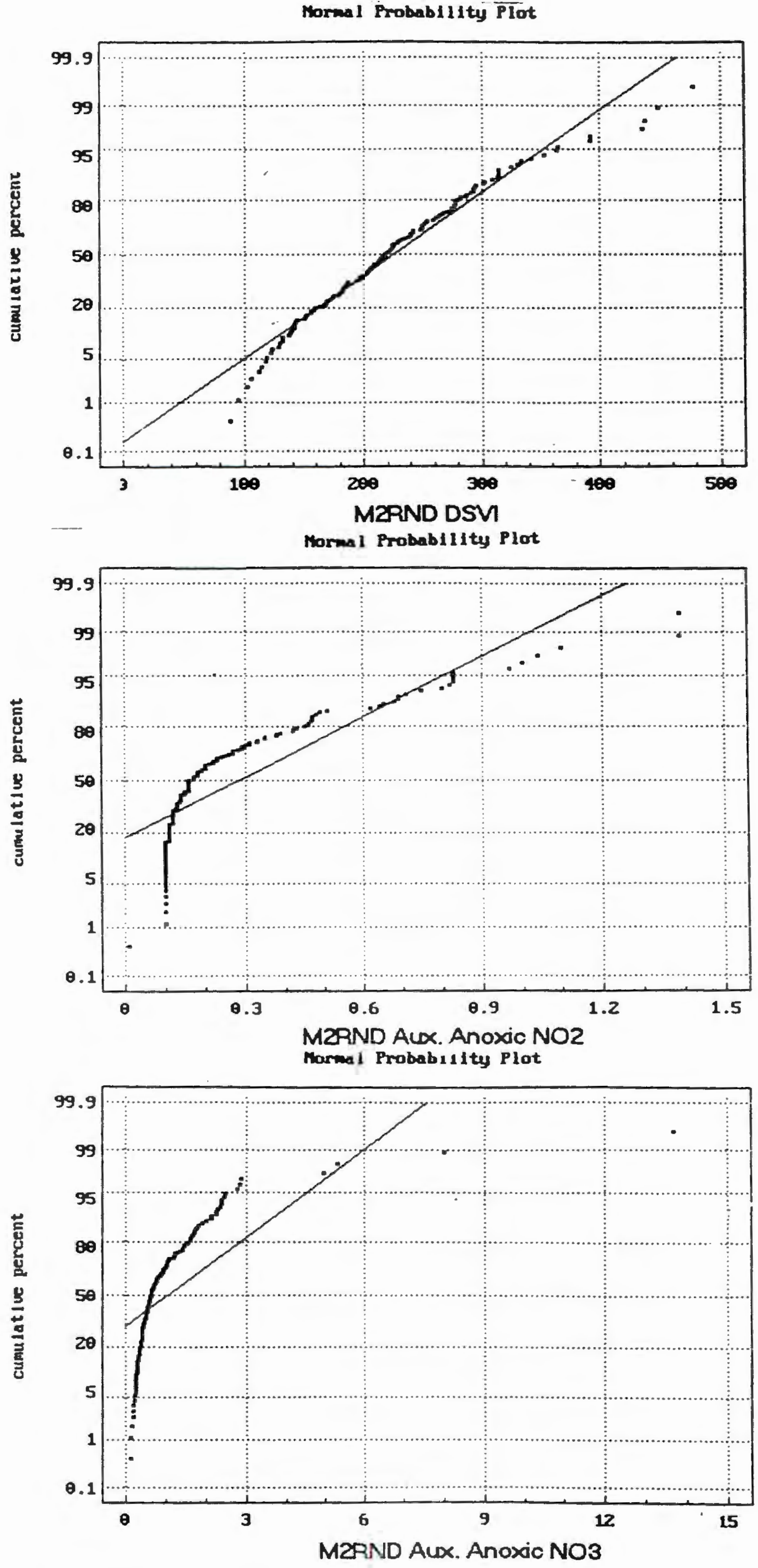


Figure 3.43 Distribution of DSVI, auxiliary anoxic nitrite and auxiliary anoxic nitrate for the M2RND system

were significant remained so and those that were not significant remained so also (see Tables 3.16 and 3.17 for the pooled results of the 2RND and M2RND systems). The only difference was that now both the DSVI and the nitrate and nitrite concentration data sets were both normally distributed (bivariate) and therefore statistically conform to the normal distribution required for the t-distribution. Because the outcome did not differ the same conclusions as the evaluation on natural numbers apply.

Approach (2)

The Spearman rank analysis does the correlation on the rank number of the data, and not on the values of the data themselves. The rank number is the position of the data in the set ranked from smallest (lowest) to largest (highest). This has the effect of reducing the influence of high values in the data set that tend to cause deviations from normality. (Groebner and Shannon,1988).

As with the log-normal analysis no improvement or deterioration was found with the Spearman-ranked analysis - the data sets that were significantly and positively correlated remained so and *vice versa* - no steady state period or pooled data set correlation changed from not significant to significant or from significant to not significant (see Tables 3.18 and 3.19 for the pooled data set results of the 2RND and M2RND systems).

Approach (3)

To reduce the curvature of the nitrite and nitrite data sets low values of nitrate and nitrite concentrations (< 1 and 0.1 mgN/l respectively) were removed. This reduced the number of values in data sets for the individual steady state periods to only a few, eg. for steady state

Table 3.16 Log correlations of the DSVI and Anoxic nitrate and nitrite concentrations for the 2RND system

System	DSVI Correlation	Other possible correlations	($s<0.05$)
2RND POOL	LOGAn.NO_2 0.1071 s=0.3813 n=69	LOGAn.NO_2 LOGAn.NO_3 0.5236 s=0.0000 n=69	LOGEf.NO_2 LOGEf.NO_3 0.2619 s=0.0297 n=69
	LOGAn.NO_3 0.2620 s=0.0297 n=69	LOGAn.NO_2 LOGEf.NO_2 0.4101 s=0.0005 n=69	

Table 3.17 Log correlations of the DSVI and Anoxic nitrate and nitrite concentrations for the M2RND system

System	DSVI Correlation	Other possible correlations	($s<0.05$)
M2RND POOL	LOGAn.NO$_2$ 0.0849 s=0.3802 n=109	LOGAn.NO$_2$ LOGAn.NO$_3$ 0.6787 s=0.0000 n=109	LOGAux.NO$_2$ LOGAux.NO$_3$ 0.5760 s=0.0000 n=109
	LOGAn.NO$_3$ -0.0910 s=0.3468 n=109	LOGAn.NO$_2$ LOGAux.NO$_2$ 0.4923 s=0.0000 n=109	LOGAux.NO$_2$ LOGEf.NO$_2$ -0.1898 s=0.0481 n=109
	LOGAux.NO$_2$ 0.1396 s=0.1476 n=109	LOGAn.NO$_2$ LOGAux.NO$_3$ 0.3010 s=0.0015 n=109	LOGAux.NO$_2$ LOGEf.NO$_3$ 0.3912 s=0.0000 n=109
	LOGAux.NO$_3$ 0.2925 s=0.0020 n=109	LOGAn.NO$_2$ LOGEf.NO$_3$ 0.5504 s=0.0000 n=109	LOGAn.NO$_3$ LOGAux.NO$_3$ 0.3733 s=0.0001 n=109
		LOGAn.NO$_3$ LOGAux.NO$_2$ 0.4999 s=0.0000 n=109	LOGAn.NO$_3$ LOGEf.NO$_3$ 0.5800 s=0.0000 n=109
		LOGAuxNO$_3$ LOGEfNO$_2$ -0.2985 s=0.0048 n=109	LOGAuxNO$_3$ LOGEfNO$_3$ 0.2777 s=0.0035 n=109

Table 3.18 Spearman ranked correlations for the pooled data of the 2RND system

System	DSVI Correlation	Other possible correlations	($s<0.05$)
2RND POOL	An.NO_2 0.0644 s=0.5954 (n=69)	Ef.NO_3 Ef.NO_2 0.3884 s=0.0014 (n=69)	Ef.NO_2 An.NO_2 0.4030 s=0.0009 (n=69)
	An.NO_3 0.2407 s=0.0471 (n=69)	Ef.NO_3 An.NO_2 0.0291 s=0.2646 (n=69)	An.NO_2 An.NO_3 0.4648 s=0.0001 (n=69)

Table 3.19 Spearman ranked correlations for the pooled data of the M2RND system

System	DSVI Correlation	Other possible correlations	($s<0.05$)
M2RND POOL	An.NO_2 0.0302 s=0.7533 (n=109)	Ef.NO_3 An.NO_2 0.0.6559 s=0.0000 (n=109)	Ef.NO_2 Aux.NO_2 0.4422 s=0.0131 (n=109)
	An.NO_3 -0.1025 s=0.2869 (n=109)	Ef.NO_3 An.NO_3 0.6104 s=0.0000 (n=109)	Ef.NO_2 Aux.NO_3 -0.2329 s=0.0155 (n=109)
	Aux.NO_2 0.1305 s=0.1750 (n=109)	Ef.NO_3 Aux.NO_2 0.4422 s=0.0000 (n=109)	AnNO_3 AuxNO_3 0.3228 s=0.0008 (n=109)
	Aux.NO_3 0.2826 s=0.0033 (n=109)	Ef.NO_3 Aux.NO_3 0.3073 s=0.0014 (n=109)	Aux.NO_2 Aux.NO_3 0.5771 s=0.0000 (n=109)

period 1 of the 2RND system only 9 DSVI NO_3-N and NO_2-N triplets remained after elimination of the low values. This made the individual steady state data sets too small for meaningful analysis so that (a) the pooled data were analysed and (b) the analysis was conducted on DSVI and NO_2 or NO_3 pairs even though many pairs may have had a low corresponding NO_3 or NO_2 value eliminated.

The results obtained using this approach were worse than the results obtained by other methods for both the 2RND and M2RND systems. This is probably in part due to the smaller number of available data pairs after deletion, which must lead to a poorer correlation.

3.4 CONCLUSION

3.4.1 Introduction

From comparative plots of DSVI and nitrite concentrations in the anoxic reactor outflow, (Fig 3.32), there appears to be some link between concentrations of nitrite and the tendency of the system to bulk. Increases in DSVI roughly correspond to increased anoxic nitrite concentrations in all three phases of the experimental investigation, where the system was allowed to operate without disruption.

3.4.2 Intermittent aeration system

During the period that the system was operated intermittently (day 0 to 83), the DSVI showed little correlation with the concentration of nitrite in the daily composite effluent samples from the system (the only daily sample taken for nitrite analysis). A single daily composite nitrite sample taken from the effluent of an intermittently aerated system does not give a good indication of the nitrite concentration entering the aerobic phase. Unfortunately samples for nitrate and nitrite concentrations were not taken at the end of the anoxic cycle at this early stage of the investigation because their importance was not yet realized. The DSVI measured in the system and the effluent nitrite concentrations showed little connection because the DSVI increased as the effluent nitrite decreased. However, the nitrite concentration profile tests done on days 58 and 78 gave a fairly good indication of the link between nitrite concentration built up during the anoxic period and DSVI in the system. The addition of the ammonium supplement to the feed of the system which resulted in an increase of both the nitrate and nitrite concentration throughout the anoxic and aerobic periods but notably those at the onset of aerobic conditions, also led to an increase in DSVI. The nitrite profile test done on day 78 showed the effect of this quite clearly.

3.4.3 Two reactor anoxic-aerobic system

After conversion to a two reactor system on day 84 (with ammonium added to the feed), the DSVI increased significantly but because nitrite concentrations in the anoxic reactor were measured only from day 111, the cause for this increase cannot be established. The DSVI showed a tendency to increase from day 111, and remained fairly high until around day 145, while the average nitrite concentration between days 111 and 145 was just above 0.5 mgNO_2 -N/l. The cessation of the ammonium dosing (on day 146) to the feed caused a decline in anoxic reactor nitrite concentrations and a drop in DSVI. However, the sludge continued to bulk, with the DSVI remaining above 200 ml/g for most of the time during the 65 day period between termination of ammonium addition and reduction of the a-recycle ratio from 3:1 to 1:1 on day 206. During this period the anoxic reactor nitrite concentration remained fairly low (mostly below 0.5 mgNO_2-N/l) with an average of 0.25 mgNO_2-N/l. The average DSVI for this period was 236 ml/g, compared to an average of 256 ml/g for the period of ammonium addition. Immediately after the end of this steady state period (steady state period 2) the DSVI was high, between 300 and 400 ml/g. At this stage (steady state period 3) the nitrite concentration in the anoxic reactor was low, and the DSVI decreased. Although difficult to ascertain due to fluctuations caused by disturbances to the system (due to sludge abstraction for batch tests to demonstrate aerobic floc former inhibition, see Casey *et al.* 1992), some connection is noticeable between anoxic nitrite concentrations and DSVI fluctuations in steady state period 3 (changed recycle ratio) of the 2 reactor system. The average DSVI was lower during this steady state period (192 ml/g) than for the previous two steady state periods.

Considering the system overall, there are stronger indications that the anoxic reactor nitrite concentration does cause the change in DSVI, because the former is high in the first steady state period (ammonium dosing), 0.52 mgNO_2-N/l average, with a corresponding average DSVI of 256 ml/g. For steady state period 2 (no ammonium dosing) the average anoxic nitrite concentration is 0.25 NO_2-N/l, with a corresponding average DSVI of 236 ml/g, and for steady state period 3 (low a-recycle) the average anoxic nitrite concentration is 0.38 mg NO_2-N/l with an average DSVI of 191.5 ml/g. In this system concentrations of above 0.5 mgNO_2 -N/l in the anoxic reactor seemed to indicate proliferation of low F/M filaments and caused the system to bulk.

3.4.4 Modified two reactor system

The link between anoxic reactor nitrite concentration as an indicator of inhibition of floc forming organisms entering the aerobic reactor is fairly clearly discernable when the system was operated as a 3 reactor system, with a small anoxic, separately fed reactor added between the anoxic and aerobic reactors to aid reduction of nitrite (and intracellular denitrification intermediates such as NO and N_2O) before entry to the aerobic reactor. The plot of increases and decreases in anoxic nitrite concentration in both these anoxic reactors corresponds roughly to the plot of DSVI, and indicates that high concentrations of anoxic reactor nitrite(> 0.5 mgNO_2-N/l) tend to promote AA (low F/M) filament bulking in a nitrification/denitrification system. In anoxic-aerobic systems there thus seems to be some support for the nitrite inhibition hypothesis of Casey *et al.* (1992).

In the Control system (started on day 383) the DSVI increased to around 600-700 ml/g from day 385 to day 403. By day 413 the DSVI had decreased to around 230 ml/g, and remained between 200 and 300 ml/g until the end of the investigation period. The average anoxic reactor nitrite concentrations for the 2 steady state periods during which the system was operated were 0.18 and 0.12 mgNO_2-N/l for steady state periods 2 and 3 respectively. The anoxic reactor nitrite concentrations for the Modified 2-reactor system for the same steady state periods were 0.23 and 0.13 mgNO_2-N/l. The average DSVI for steady state periods 1 and 2 of the Modified 2-reactor and Control systems are fairly similar, being 325 and 249 ml/g (Modified 2-reactor system) and 287 and 254 ml/g (Control system) for steady state periods 2 and 3 respectively.

From these results of the Modified 2-reactor and Control systems it can be concluded that concentrations of anoxic reactor nitrite in the vicinity of 0.5 mgNO_2-N/l can be associated with an increasing DSVI, while concentrations below this can be associated with a decreasing DSVI.

3.4.5 Statistical correlation analysis

From the results obtained by the correlation analysis, it can be concluded that (i) in the separate steady state periods there are not enough points to give meaningful results, (ii) the relationship between anoxic reactor nitrite and DSVI is not apparent in terms of satistical correlation. However, such a correlation is implied through the significant correlations between nitrate and DSVI in the 2RND and M2RND systems and between nitrate and nitrite. Therefore the statistical analysis provides supporting evidence for Casey's bulking hypothesis.

The conclusions of this investigation are discussed in Chapter 4.

CHAPTER 4

CONCLUSIONS

Filamentous bulking resulting in poor sludge settleability is a major problem in the operation of activated sludge plants. This is indicated clearly in the two surveys on 122 activated sludge plants in South Africa by Blackbeard *et al.* (1986, 1988). Most of the bulking which occurred was caused by the group of low F/M filaments, namely 0092, *M. parvicella,* 0041, 0675, 0914 and 1851.

A 4 year research program conducted by Gabb *et al.* (1989) into, amongst others, the effectiveness of the selector effect, concluded that the selector effect was not effective in controlling low F/M bulking. It was found however that fully aerobic conditions ameliorated low F/M filament bulking and that alternating anoxic aerobic conditions appeared to promote low F/M filament bulking. To identify the causes of low F/M bulking a second follow up research programme was undertaken to examine the influence of various system parameters such as sludge age, anoxic mass fraction, frequency of exposure to alternating anoxic-aerobic conditions, type of anoxic-aerobic system and nitrate/nitrite concentrations in the anoxic and aerobic reactors/zones. During the course of this research it was confirmed that intermittent aeration N removal systems having anoxic mass fractions >30% but less than 80% tended to bulk, while low F/M filament bulking was ameliorated in completely aerobic and completely anoxic single reactor systems. (Warburton *et al.*, (1991); Casey *et al.* (1990,1991); Ketley *et al.* (1991) and Hulsman *et al.*, (1992).

The research presented in this thesis forms part of the follow-up comprehensive research investigation into low F/M bulking and examines the effects of various nitrification-denitrification system configurations on low F/M bulking. Three types of experimental systems were operated during a 472 day investigation period. The 3 experimental systems were obtained by modifying a single laboratory scale nitrification-denitrification system at different stages viz.

(1) A single reactor intermittently aerated system (day 1 - 83)

(2) A 2-reactor anoxic-aerobic nitrification/denitrification system (day 84 - 253)

(3) A modified 2-reactor anoxic-aerobic system with a small separately fed auxiliary anoxic reactor placed between the anoxic and aerobic reactors to facilitate additional denitrification (day 254 - 472)

All these systems were operated at 20°C, 15 day sludge age, 30% aerobic mass fraction and received real unsettled sewage as influent.

The results obtained in the different configurations are as follows:

(i) Intermittent aeration system (day 1 - 83)

For the first 51 days of operation of this system, no ammonium was dosed into the influent. To increase the concentrations of nitrate and nitrite in the system, from day 52 ammonium was dosed to the influent first at 10 mg/l (to day 65) and then on day 65 this was increased to 20 mgN/l and maintained at this value for the remaining period (to day 83) of operation of this system. During the 83 days of operation, two nitrate and nitrite concentration profile tests were carried out on the reactor during the 8h aeration cycle (2.5 h aerobic, 5.5 h anoxic) in order to observe the changes in these concentrations. When the test was conducted during the period of low ammonium dosing, denitrification of the nitrate and nitrite generated in the aerobic period was complete by the end of the anoxic period. At the time of this test (day 58) it was noted that the DSVI was low, 190 ml/g, and decreasing, with the following low F/M filaments present: *H. hydrossis,* 0092, 0041, 021N, *Beggiatoa* and *M parvicella.*

The second profile test was done during the period with 20 mgN/l ammonium dosing (day 78). The profile indicated that now the nitrate and nitrite concentrations at commencement of the aerobic period were high. This was in part due to a slower denitrification rate compared to the previous test. During the time that this second test was carried out it was observed that the DSVI was 210 mg/l and increasing with the following filamentous organisms occurring in the sludge: 0041, 0092, *H. hydrossis,* 0803, and *M. parvicella.* These reactor nitrate/nitrite concentration profile tests (Figs. 3.10 and 3.11) gave a clue to the connection between nitrite entering the aerobic period at the end of the anoxic period and the tendency of the system to bulk. This led to the conclusion that nitrite (or nitrate) present at the start of the aerobic period due to incomplete denitrification has a stimulating effect on the proliferation of low F/M filaments.

(ii) 2-reactor anoxic-aerobic system

On day 84 while continuing dosing ammonium to the influent, the system configuration was changed from that of a single reactor intermittent system to a 2-reactor anoxic-aerobic system (Modified Lutzack-Ettinger system), where concentrations of nitrate and nitrite entering the aerobic reactor from the anoxic reactor could be more readily controlled and observed. Anoxic nitrate and nitrite concentrations were measured from day 111. During the period of ammonium dosing to day 145, the DSVI was high (210 ml/g), and the average anoxic reactor nitrite and nitrate concentration was above 0.5 mg NO_2-N/l and ammonium dosing was terminated on day 145. The removal of the influent ammonium dose on day 145 resulted in a decrease in the nitrate and nitrite (NO_x) load on the anoxic reactor and as a consequence both the anoxic reactor nitrate and nitrite concentrations decreased. During this time the DSVI decreased from a very high value ($>$ 300 ml/g) to around 200 ml/g. The DSVI remained at ±200 ml/g for 20 days but later commenced increasing again slowly. However, even after 30 days it never increased above the value at which the ammonium dose was stopped.(see Fig. 3.20). The filaments present in the sludge during this time were 0041, *M. parvicella, S. natans*, 021N and 0092.

In an attempt to further reduce the anoxic reactor nitrate and nitrite concentrations, and therefore those entering the aerobic reactor, the a-recycle ratio was reduced from 3:1 to 1:1 on day 206. Both the anoxic reactor nitrite concentration and the DSVI were then observed to decrease from 400 ml/g to 150 ml/g. As in the previous period, after 35 days the DSVI began to increase slowly over 10 days to 250 ml/g (Fig. 3.20).

Statistical analysis of this 2RND system's data over the 3 different periods (ie with and without influent ammonium dosing and a low a-recycle ratio) showed no significant positive correlation between DSVI and anoxic reactor nitrite concentration, although a significant positive correlation was obtained between the DSVI and anoxic reactor nitrate concentration. A significant positive correlation was also obtained between anoxic reactor nitrate and nitrite concentration data, from which a positive correlation between anoxic reactor nitrite concentration and DSVI can be inferred.

From the behaviour of the 2-reactor nitrification-denitrification system, viz. the system's DSVI response to removal of the ammonium supplement and the reduction in the recycle ratio, both resulting in decreases in the anoxic reactor nitrate and nitrite concentrations and the statistical correlation between them, it seems reasonable to conclude that there is a connection between low F/M filament proliferation and nitrite/nitrate concentration entering the aerobic reactor in this type of system configuration.

This experimental information, together with that measured in the bulking research program by Warburton *et al.* (1991), Ketley *et al.* (1992), Hulsman *et al.* (1992), and Casey *et al.* (1991, 1992), formed the basis for developing the hypothesis for low F/M filament bulking (Casey *et al.* 1992).

This hypothesis is based on a proposal that a possible cause for low F/M proliferation lay in the necessity for the activated sludge mass to switch between aerobic and anoxic metabolic pathways, this switching providing some competitive advantage to the filamentous organisms at the expense of the floc-forming organisms. It has been fairly convincingly demonstrated in pure cultures that denitrification can continue (at a lower rate) under aerobic conditions, and need not take require purely anoxic conditions. It has been proposed that a denitrification pathway intermediate (or intermediates) is responsible for the inhibition of aerobic utilisation of substrate. The inhibitory intermediate is thought to be NO, with the presence of NO_2^- and NO_3^- exacerbating the degree of inhibition.

Based on the above principles, Casey *et al.* (1992) proposed a bulking hypothesis as follows: Floc-formers are inhibited under aerobic conditions by denitrification intermediates accumulating under the preceding anoxic conditions, and the denitrification intermediates causing the inhibition is NO. Low F/M filaments are thought to denitrify only as far as NO_2^-, and therefore do not accumulate NO, while the floc-formers are thought to be able to denitrify completely to N_2, and will therefore be able to accumulate NO under certain conditions. Low F/M filament bulking is thus expected to occur under conditions of incomplete denitrification in the anoxic reactor. If this occurred, the floc-forming organisms would still have internal accumulations of NO, causing inhibition of their aerobic metabolism when they enter the aerobic reactor, thus bestowing a competitive advantage on the low F/M filaments, which, due to the nature of their anoxic metabolism, do not suffer from inhibition caused by NO when entering the aerobic reactor. If complete denitrification takes place in the anoxic reactor, then low F/M filament bulking is not expected to occur, as the inhibitory intermediate is no longer present when the organisms enter the aerobic reactor.

(iii) Modified 2-reactor anoxic-aerobic system

In terms of the hypothesis described above, and the tests done by Casey *et al.* (1992), on sludge harvested from the 2-reactor ND system, it appears that the presence of RBCOD under aerobic conditions resulted in an alleviation of the inhibition of the floc-formers and a progressive increase in specific OUR under batch test conditions as the test proceeded. From this it was hypothesized that if RBCOD could be dosed to the activated sludge after the anoxic period, but before the aeration period it might lead to an alleviation of the contribution of the floc-formers and therefore an amelioration of bulking. This presence of RBCOD at the intra-anoxic-aerobic stage would also improve denitrification just prior to the aerobic reactor, consequently a small auxiliary anoxic reactor receiving a bypass influent flow of 10% of the system's influent flow was placed after the main anoxic reactor on day 254. (For comparative purposes, a Control 2-reactor system identical to the Experimental system but without the auxiliary reactor was set up on day 383.) After the addition of this auxiliary reactor, the DSVI was initially observed to decline from 300 ml/g to 190 ml/g. For the rest of the period this system was operated, increases and decreases in concentration of anoxic reactor nitrite showed a fairly close correlation with fluctuations of the DSVI. The system showed little difference in performance in terms of anoxic nitrite and nitrate and DSVI when compared to the values of these parameters for the Control system. However in both systems it seems that anoxic reactor nitrite concentrations above 0.4-0.5 mg NO_2^--N/l favour proliferation of low F/M filaments. Fairly brief increases in anoxic reactor nitrite concentration in these systems also seemed to effect an increased DSVI for a relatively long period of time, the DSVI declining much more gradually than anoxic nitrite concentration. A statistical correlation analysis of the DSVI and auxiliary anoxic reactor nitrate and nitrite concentration data for the M2RND system yielded similar results to those obtained earlier for the 2RND sytem. Here too there was a significant positive statistical correlation between the DSVI and auxiliary anoxic reactor nitrate concentration, as well as a significant positive correlation between auxiliary anoxic reactor nitrate and nitrite concentrations. From this a correlation between auxiliary anoxic reactor nitrite concentration and DSVI can be inferred, and provides further supporting evidence for the bulking hypothesis. Further details on the bulking hypothesis and its verification are given by Casey *et al* (1993).

The results obtained in the above experimental systems indicate that high concentrations of nitrate and especially nitrite entering the aerobic zone or phase of an anoxic - aerobic nitrification - denitrification system due to incomplete denitrification will favour proliferation of low F/M filaments in the sludge. Therefore it could be concluded that nitrate, nitrite and/or some intermediate denitrification compound inhibits the growth of the floc - forming organisms in the aerobic zone/phase of these systems.

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APPENDIX 1

Experimental data for Experimental and Control systems

APPENDIX 1

EXPERIMENTAL UNIT (Intermittant Aeration)

DATE	DAY NO	VSS (mg/l)	TSS (mg/l)	DSVI ml/g	OUR mg/l/h	COD (mg/l) inf	COD (mg/l) eff	TKN (mg/l) inf	TKN (mg/l) eff	Eff NO3	Eff NO2	Anox NO2	Anox NO3	Aux NO2	Aux NO3
12	0	928	1126	111	20.4	568	48	85.5	27.6	9.2	1.1				
13	1	950	1138	105	25.2	568	56	98	28.3	11.4	1				
14	2	1116	1342	106	29.8	568	93	69.4	39.9	5.8	0.8				
15	3	1304	1484	106	30.9	398	69	45.1	27.8	4.5	0.7				
16	4	1362	1474	113	28.6	398	32	58.8	13.6	5.2	0.7				
17	5	1404	1668	111	28.8	358	28	45.4	6.3	5	0.7				
18	6	1508	1714	114	26.8	358	53	74.2	4.5	4.1	0.7				
19	7	1416	1618	133	29.1	389	53	44.2	3.8	3.5	0.6				
20	8	1456	1698	127	27.8	349	57	61.3	3.8	3.5	0.6				
21	9	1514	1758	125		487	36	48.4	4	3.1	0.5				
22	10	1370	1568	129		487	44	49.5	3.7	3.8	0.7				
23	11	1472	1774	125	24.7	390	23	46.9	4.5	4.4	0.6				
24	12	1522	1792	125	25	390	65	45.4	4.8	3.6	0.5				
25	13	1628	1856	137	26.1	374	69	47.6	8.3	3.5	0.6				
26	14	1436	1708	152	26.2	374	40	48.4	4.7	5.8	1.2				
27	15	1624	1878	152	28.8	414	48	49	5.4	3.3	0.7				
28	16	1916	2114	154	28	414	53	44	5	3.2	0.6				
29	17	1730	1934	181		568	49	48.2	11.1	3.3	0.8				
30	18	1908	2182	218	29.9	568	97		14.8	2.8	0.2				
1/12	19	1744	2090	193	27.8	415	76	45.6		1.8	0.3				
2	20	1604	1844	228	25.9	415	76	45.6	12.7	2.3	0.3				
3	21	1660	1952	190	28.7	519	76	47.6	11.1	6	0.6				
4	22	1640	1876	207	26.4	519	85	47.6	10.5	5.1	1.2				
5	23					402	97	51.8	10.8	0.9	0.1				
6	24	1346	1556	242		402	113	51.8	23.8	0.3	0.1				
7	25	1642	1876	197	29.7	471	105	44.5	15.7	1.5	0.3				
8	26	1462	1666	276	29.8	471	105	44.5	23.1	1.8	0.4				
9	27	1498	1792	252	28.8	398	93	46.2	17.2	2.5	0.5				
10	28	1526	1744	280	27.6	398	96	46.2	9.8	3	0.5				
11	29	1402	1652	271		459	97	47.6	6.5	2.4	0.2				
12	30	1442	1648	257	23.8	459	72	47.6	14.8	7.3	1.1				
13	31	1500	1788	284		370	76	47	12.9	3.4	0.7				
14	32	1486	1698	346	27	370	76	47	3.5	6.7	2				
15	33	1554	1680	305	27	354	80	46.2	2.5	4.8	0.7				
16	34	1406	1780	337	26.1	354	60	46.2	1.7	6.8	2				
17	35	1570	1964	258		350	89	45.6	11.6	5.7	1				
18	36	1450	1730	271	28	350	72	45.6	13	8.8	0.7				
19	37	1574	1880	272	30.6	527		45.9							
20	38	1858	2166	259		527	85	49.6	13	5.3	0.8				
21	39	1586	1966	262	31.3	535		49.6							
22	40	1554	1944	278	26.9	535	80	49.6	10.4	5.2	1.2				
23	41	1646	2242	241	32.6	382	80	46.8		4	1.1				
24	42	1780	2142	256	30.2	382		44.8							
25	43				33.8		85		4.6	4.3	1				
26	44				17.9		105		10.4	4.5	1				
27	45	1610	1990	249	31		101		7.8	4.5	1				
28	46	1788	2140	239	13.3	535		50.1							
29	47	1478	1818	222	25.1	535	121	50.1	12.5	3.2	1.2				
30	48	1414	1788	219	26.5		89		8.5	5.3	11.8				
1	49	1512	1880	211	27.8		113		1.5	4.5	1.5				
2	50	1776	2132	184	31.2	531	76	58.7	6.6	5.7	2.3				
3	51	1924	2180	183	28.2	539	80	58.7	4.5	3.2	1				
4	52	1626	1972	209	32.5	406	72	44.5	5.2	3	0.9				
5	53	1778	2194	193	30.8	406	80	44.5	6.2	3.9	0.9				
6	54	1924	2350	182	31.6	459	52	45.4	5.5	5	2.1				
7	55	1702	2066	197	31.1	459	72	45.4	4.3	4.3	1				

8	56	1722	2104	184	31.9	414	97	42.9	5.5	7.6	3				
9	57	1614	2014	199	34.5	414	89	42.9	5.5	4.8	1.1				
10	58					374	80	48.2	3.1	4.8	0.8				
11	59	1728	2062	184	33.3	374		48.2							
12	60	1642	1968	193	36.2	418	121	49	4.6	5.5	1.2				
13	61	1566	1916	203	35.4	418	121	49	7.1	5.6	1.9				
14	62	1648	2020	194	29.2	447	80	45.6	4.5	4.9	0.8				
15	63	1512	1820	198	30	447	64	45.6	0	5.4	1.3				
16	64	1336	1606	199		507	64	51.2	4.3	4	0.5				
Period 1 av		1554	1845	202.1	28.44	441.1	76.07	50.16	9.633	4.477	1.09				
17	65	1422	1692	213	36.3	443	60	51.2	9	4.7	0.9				
18	66	1378	1668	221		507	153	55.7	13.3	5.2	0.7				
19	67	1422	1720	221		443	101	51.5	17.1	4.3	0.3				
20	68	1108	1328	196	24.8	402	129	47	21	5.6	1.2				
21	69	1340	1614	198	33.4	427	99	52.4	8	6.1	1.2				
22	70	1404	1756	194	32.3	439	76	52.4	4.2	6.9	1.2				
23	71	1344	1632	213	30.4	422	80	52.4	10.5	7.1	1.8				
24	72	1348	1646	204	30.8	451	89	60.5	3.6	7.2	1.8				
	73	1302	1566	219		559	137	80.9	10.1	7.1	3.6				
26	74	1354	1620	222	41.3	531	85	75.9	8.5	12.2	3.2				
27	75	1288	1558	228	39	527		80.1	13.2	10.1	3.1				
28	76	1160	1360	250	33.5	547		73.4	15	9.7	3.3				
29	77	1240	1436	245	34.1	515	113	82.1	11.5	11.8	3.2				
30	78				29.9	499		62.2	6.7	22					
31	79	1542	2302	223	29.9	459	85		10.6	9.3	4.5				
1	80	1614	1759	218	31.7	478	77	66.6	5.9	9.7	1.5				
2	81	1226	1526	239		519	53	65.5	4.3	10	1				
3	82	1218	1699	228	34.1	378	49	50.6	7.6	12.3	9.8				
Period 2 av		1336	1640	219.5	32.96	474.8	92.4	62.38	10.01	8.961	2.488				

EXPERIMENTAL UNIT (Anoxic-aerobic 2 reactor system)

DATE	DAY NO	VSS (mg/l)	TSS (mg/l)	DSVI ml/g	OUR mg/l/h	COD (mg/l) inf	eff	TKN (mg/l) inf	eff	Eff NO3	Eff NO2	Anox NO2	Anox NO3	Aux NO2	Aux NO3
4	83	1364	1690	237	39	499	102	65	3.5	15.8	1.4				
5	84	1410	1710	215		402	77	68	2.7	12.8	1.1				
6	85	1486	1756	218		539	73	69.7	17.8	5.3	0.5				
7	86	1738	2044	181	48.7	535	73	69.2	18.8	7.6	1				
8	87	1576	1784	219	48	515	73	67.8	17	8.3	1.1				
9	88	1572	1876	215	48	559	53	77	13.2	9.5	1.4				
10	89	1582	1952	207	55.4	551	53	70.3	14.6	9.2	1				
11	90	1748	2100	189	63.1	551	53	62.7	10.1	9.2	1				
12	91	1672	1938	214	54.8	583	102	65.5	1.6	9.2	0.9				
13	92	1694	2006	208	42.4	684	77	64.1	8.8	13.4	1				
14	93	1412	1646	225		563	79	69.4	5.5	9.6	0.4				
15	94	1632	1938	195	36.4	583		54.8							
16	95						53		6.6	8.2	0.6				
17	96	1378	1652	243		515	56	56.4	6.7	9	0.6				
18	97	1476	1730	217	42.6	507	61	56.4	6.9	8.5	0.7				
19	98	1314	1564	224		539	61	60.9		10.2	0.8				
20	99	1232	1508	229	34.4	543	73	78.6	8.3	13.1	1				
21	100	1498	1740	207		583	98	76.2	13.7	10.9	0.9				
22	101	1082	1266	261		555	61	72.2	9.5	13	1.4				
23	102	1196	1394	227											
24	103					571	49	71.8	6.4	14	1.1				
25	104	1254	1584	210		523	53	70	7.7	17.3	0.9				
26	105						61	66.6	4.8	19.3	0				
27	106	1086	1308	183			150	56.2	16.1	7	0.5				
28	107	1174	1566	176				62.7		5.7	0.8				
29	108	1408	1646	174		523	93	61.6	23.5		0.8				
30	109					573	89	64.4	31.6		1.2				

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3	110	1522	1776	169		619	71	65.8	35.2	8.7	1.5				
4	111	1856	2464	126		538	71	64.4	17.4	9.6	1.8	1.4	1.1		
5	112	1548	2142	140		546	80	59.9	12.6	11.1	1.9	0.4	2.6		
6	113	1570	1794	169		686	93	64	14	12	1.4	0.4	4.1		
7	114	1436	1566	211		583	97	69.4		10.8	1.6	0.5	1.5		
8	115	1470	1704	200		579	80	60.2	9.5	14	1.6	0.2	1.5		
9	116	1384	1608	242		558	97	56.3	12.3	14.8	1.7		2.6		
10	117	1410	1770	237		559	124	56.3	10.9	14.2	0.8	0.5	5.7		
11	118	1324	1550	310		527	105	60.4	13.7	13.9	0.7	0.4	4.1		
12	119	1360	1640	304		636	76	69.2	14.3	11.7	0.7	0.5	2.2		
13	120	890	1518	368	42.4	583	64	64.7	11.5	11.6	0.9	0.3	3.1		
14	121					579	72	83	17.4	10.9	1.7	0	12.9		
15	122				36.4	511	52	70	6.6	13.9	1.7	0.3	1.8		
16	123	1286	1372	349		495	68	65.5	5.9	13.7	2.1		2.3		
17	124	1346	1612	285		495	129	65.5	7.3	13.9	2.1	1.3	6.2		
18	125	1130	1292	310	42.6	664	60	72.8	7	14.7	0.7	1	2.4		
19	126	1354	1508	281	36.3	660	89	73	4.2	9.1	2.6	1.3	2.3		
20	127	1316	1502	293	31.4	628	97	60.2				0.2	2.5		
21	128	1262	1452	330	31.8		76		8.4	15.1	1.2	0.2	0.6		
22	129	1196	1322	378	31.9	556	125	65	2.5	13.9	1.4	0.3	0.4		
23	130	954	1054	209		535	77	56.6	11.2	10.9	1.2	0.9	3.3		
24	131	1388	1598	350	30.6	535	65	56.6	7.8	13.6	0.9	0.7	6.5		
25	132	1370	1664	288	29.5	514	73	66.1	3.1	15.4	0.4	0.2	0.7		
26	133	1086	1638	280		571	162	59.7	3.9	11	0.3	0.3	5.4		
27	134	1442	1678	297	24.4	611	89	73.4	2.8	12.6	0.4	1.4	6.8		
28	135		1918	291	27.3	611	73	83.2	7.6	7.35	0.3	0.2	0.8		
29	136	1090	2352	246	29.6	563		70	8			0.7			
30	137	1382	1576	317		563	77	70		5.22	1.6	0.5			
31	138					526		70							
1/4	139				31.1	526	158	70	6.4	4.2	1.1				
2	140	1370	1590	314		676	61	75.6	7.6	3.1	1.3	0.5			
3	141	1320	1544	362		457	158	72.8	22.1	10	0.9				
4	142	1502	1748	355	31.1	551	67	56.3	7.6	17.5	0.8	0.475	1.99		
5	143	1560	1726	370	35.9	526	100	50.5	11.5	9.5	0.2	0.1	4.15		
6	144	1516	1620	383		597	75	75.6	24.6	8.7	0.3	0.1	1.14		
7	145	1220	1392	445		597	108	75.6	38.6	8.6	0.8	0.2	0.33		
Period 1 av'		1388	1675	256.1	38.66	560.6	83.48	66.42	11.43	11.1	1.047	0.516	3.138		
8	146	1366	1750	251	35.3	451	63	47	6	10	0.2	0.74	6.21		
9	147	1608	1866	268	25.7	581	75	51				0.125	1.32		
10	148	1458	1662	276	33.5	508	61	60.2	8.9	6.9	0.2	0.105	0.21		
11	149				31.9	475	75	41.2	18.8	6.9	0.2	0.1	0.88		
12	150				25.5	378	71	47.9	7.6	7.4	0.2	0.1	0.55		
13	151					549	79	49.3	10.1	7.8	0.4				
14	152	1126	1280	250	28.6	549	40	49.3	16.5	9.2	1.4	0.1	0.66		
15	153	1128	1330	248		445	65	50.1	3.4	10.7	1.6	0.175	2.06		
16	154				26	608	20	54.6	3.1	8.4	0.7	0.27	3.68		
17	155				29.1	502	39	46.3	8.1	8.2	0.7	0.1	1.26		
18	156	1366	1504	199	29.5	490	61	42	7.3	8.6	0.5	0.1	1.2		
19	157	1506	1730	194	28.6		20	45	7.3	8.7	0.3	0.1	0.25		
20	158	1392	1644	195	28.5	477	28	43.4	11.2	9.4	0.2	0.195	3.82		
21	159	1908	2138	159	28.4	477	98	43.4		9.7	0.2	0.1	1.28		
22	160	1618	1912	166		616	51	40.4	7.6	5.9	0.4	0.2	2.19		
23	161	984	1184	160	22.8	612	41	47		7.4	0.7	0.14	0.62		
24	162	1412	1656	205	21.5	506	41	53.2	7.8	9.3	0.5	0.385	2.88		
25	163	1224	1454	234	21.4	403	49	38.9	5.3	9.9	0.3	0.13	1.14		
26	164	1496	1744	195	16.1	427	37	37.5	12.3		0.6	0.1	0.58		
27	165	1046	1208	198	19.5	458	45	32.4	6.4	9.9	0.3		1.67		
28	166				21.5	458		34.4	6.2	5.3	0.1	0.15	1.61		
29	167					548	37	69.4		5.7	0.6				
30	168	906	1046	210		492	28	38.9	4.5			0.195	0.77		
1/5	169	972	1158	173		637	77	58.1	6.4	11.8	1	0.11	0.77		
2	170	958	1106	199	18.2	681	101	44.2	5.6	10.4	0.7	0.1	0.7		
3	171	1178	1276	203	24.2	609	36	38.4	8.4	10.4	0.4	1.8	1.08		

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4	172	808	922	368		540	40	42.5	5	10.1	0.4	1.8	0.74		
5	173	1346	1554	276	19.9	512	93	42.3	12.9	11.1	0.3	0.23	2.05		
6	174	1340	1580	203	28.1	324	109	42.8	5.2	9.7	0.2	0.34			
7	175	1368	1518	277		336	145	39.2	8.7	11.1	0.2	0.1	1.09		
8	176	1466	1670	246	26.9	328		35	5.9	9	0.2	0.105	0.99		
9	177	1324	1594	276	23.7	328	68	35	8.1	11.4	0.2	0.1	0.5		
10	178	1428	1618	223		468	50	33.3	5	8.6	0.1	0.245			
11	179	1370	1540	221	27.4	448	76	42.6	13.7	9.3	0.2	0.125	1.1		
12	180	1052	1392	187		448	64	42.6	2	13.5	0.5	0.205	0.71		
13	181	1258	1366	197	28.8	452	68	40.9	2.6	13.5	0.3	0.144	0.6		
14	182	1002	1438	229		452		39.2	5.6			0.216	0.86		
15	183				17.4	512	68	44.8	6.4	10.1	0.5				
16	184	1288	1476	271	27.3	484	48	28.8	3.6	8.7	0.4	0.336	0.67		
17	185	1576	1744	229		456	80	36.7	4.2	10.1	0.2	0.06	0.68		
18	186	1096	1322	302		492	56		7	9.7	0.3	0.076	1.63		
19	187				32.9	492	80		12	6.5	0.5				
20	188	1400	1668	305	33	436	80	48.8	3	9.7	1.1	0.064	0.85		
21	189	1408	1772	203	33.7	464	60	45.1	3.9	10.8	0.5	0.1	0.51		
22	190	1708	2062	175	40.9	500	52	41.7	7	10.1	0.7	0.07	0.47		
23	191	1840	2112	175	31.5	440	65	32.8	3.6	11.1	0.5				
24	192	1600	1818	220	30.6	576	76	31.4	2.2	10.4	0.6	0.89			
25	193	1592	1962	200	27.1	468	61	67.2	3.9	11.8	0.4	0.1	0.43		
26	194	1696	1879	184	28.4	468	98	67.2	2.8	11.8	0.6	0.1	0.43		
27	195	1556	1906	252	25.3	427	82	59.7	5.6	13.2	0.4		0.43		
28	196	1546	1808	288	25.6	470	90	37.8	7	18.3		0.105	0.51		
29	197	1690	1982	262	30.3	506	65	42.1	3.9	15		0.12	0.7		
30	198	1462	1674	323	30.3	470	74	50.4	2.2			0.145	0.8		
31	199					470		50.4							
1/8	200	1290	1652	299		455	77.67	45.92	2.24	15.94	1.15				
2	201				28.6	455	53.14	45.72		13.06	0.135	0.265	5.6		
3	202	1152	1426	336	28.3	459	73.58	38.64	5.04	16.98	0.79				
4	203	1200	1340	208	28.3	459	53.13	38.64	3.92	16.72	0.675	0.8	8.4		
5	204	1122	1372	233	28.3	459			12.04			0.105			
6	205	1040	1252	431	23.8	451	61.32	35.28		9.6	0.275				
Period 2 av		1342	1573	236.4	27.16	482.1	63.72	44.35	6.699	10.28	0.475	0.256	1.45		
7	206	1734	1894	306	27.9	463	74.54	36.4	1.4	7.45	0.28	0.105	0.54		
8	207		2124	310	25.8	573	87.39	44.8	9.8	5.82	0.4				
9	208	1324	1858	400	34	573	51.1		5.32	8.53	0.275	0.15	0.86		
10	209	1524	1650	388	26.7	524	87.89	48.16	10.92	12	0.625	0.1	0.63		
11	210	1850	2078	250	30.3	463	75.65	37.24	27.44	10.46	0.335	0.1	1.48		
12	211	1442	1652	338		463	83.8	37.24	6.16	9.87	0.3	0.2	1.05		
13	212	1712	2064	194	26.1	508	75.6	33.04	4.76	11.2	0.35	0.095	0.54		
14	213	2146	2370	168	24.9	487	116.6	37.24	3.36	9.87	0.3	0.07	0.41		
15	214	1696	1868	222	24.4	487	71.54	37.24	6.16	10.94	0.335	0.14	0.76		
16	215	1632	2062	175	26.8	487						0.07	11.1		
17	216				28.8	610	67.45	58.24	5.04	10.67	0.27		9.5		
18	217	1852	2202	154	44.1	610	75.63	58.24	4.2	11.47	0.345	1.575			
19	218	1692	1842	173	49.9	600	128.8	50.12	5.6	10.94	0.76		1.06		
20	219	1294	2252	142	49.8	650	87.89	52.08	6.16	13.06	1.85	0.89	0.92		
21	220	1796	2022	148		447	91.98	46.2	5.32	12	1.35	0.23	0.57		
22	221					442	79.72	46.2	3.36	10.94	1.25	1.975	1.83		
23	222				47.8	589	79.72	46.2	3.36	10.94	1.25				
24	223	2026	2528	110	40.4	589	55.19	60.2	5.88	11.73	1.25	0.66	0.97		
25	224	1654	1934	155		638	91.98	54.32	6.72	13.85	1.35	0.325	0.32		
26	225	1650	1724	197	43.7	638	75.63	54.88	2.52	14.68	1.25	0.3	1.58		
27	226	1950	2040	156	47.4		47.01	52.36	10.04	12.79	1.3	0.11	0.47		
28	227	1740	1928	176	45.3	573	63.36	52.36	9.52	11.73	1.625	0.35	0.62		
29	228	1846	2038	176	44	573	47.48	60.76	6.72	11.44	1.55	0.31	0.49		
30	229	1676	1758	223	42.1	682	55.19	60.76	5.04	11.73	1.4	1.125	1.32		
31	230	1672	1936	155	41	524.3	73.15		7	14.92	0.775	0.2	0.39		
1	231	1554	1898	158	40.6	548.6	77.22		5.88	12.71	0.7	0.067	0.21		
2	232	1786	2012	159	39.1	463.3	109.7	35.28	18.2	11.81	0.465	0.26	1.03		
3	233	1674	1988	161	38.5	512.1	101.6	27.16	4.2	10.74	0.265	0.07	0.23		

5	234	1588	2072	145	38.7	561	81.28	36.24	7	9.96	0.34	0.23	0.63		
6	235	1336	1684	178		504	69.09	21	4.76	10.83	0.365	0.07	0.28		
7	236	2098	2298		36.5	504	60.96	21	5.88	10.44	0.07	0.665	0.45		
8	237			122		504	154.4	42.84	3.78	11.32	0.51	1.2			
9	238	1450	1554	180	36.8	512	126	50.4	4.76	12.71	0.45	0.135	0.39		
10	239	1466	1766	147	36.5	471	73.15	40.6	3.92	10.83	0.545	0.16	0.96		
11	240	1356	1572	159	36.3	516	81.28	47.64	5.32	11.72	0.44	0.3	1.31		
12	241	1534	1478	156	35.2	548	69.09	41.44	3.92	11.03	0.5	0.08	0.86		
13	242				34.9		81.28	56.84	5.6	10.54	0.55	0.11	0.34		
14	243	1134	1324	166	33.5		81.28	56.34	3.64	10.64	0.6	0.2	1.55		
15	244	1407	1630	135	32.8	455	65.02	30.54	3.92	11.13	0.5	0.23	1.83		
16	245		1572	127	31.2	439	28.4	57.96	4.2	10.25	0.48	0.73	1.91		
17	246	1150	1502	140		467	20.32		4.76	10.25	0.56	0.28	1.2		
18	247	1226	1388	180	29.7	467	40.6	38.08	12.6	1.86	0.44	0.5	0.76		
19	248					423	36.6	55.16	10.36	6.44	0.91	0.125	0.65		
20	249		1832	175	27.5	472	40	35	6.16	10.06	1.14	0.075	1.04		
21	250	1670	1848	186	26.6	472	28	35	6.72	10.06	1.34	0.39	1.4		
22	251	1032	1364	183	24.7	392	52	28.28	4.76	12.71	1.67	1.25			
23	252	1256	1476	196	27.2		44	51.8	22.12	11.91	0.885	0.41	1.01		
24	253	1084	1334	224	27.2	664	96		8.12	8.61	0.815	0.115	0.77		
25	254	1164	1412	240	27.2	444	24	34.72	5.32	10.83	0.675	0.15	0.59		
Period 3 av		1589	1834	191.5	34.92	522.9	72.63	44.37	6.827	10.8	0.75	0.375	1.291		

EXPERIMENTAL UNIT (Modified anoxic-aerobic 2 reactor system)

DATE	DAY NO	VSS	TSS	DSVI	OUR	COD (mg/l)		TKN (mg/l)		Eff	Eff	Anox	Anox	Aux	Aux
		(mg/l)	(mg/l)	ml/g	mg/l/h	inf	eff	inf	eff	NO3	NO2	NO2	NO3	NO2	NO3
26	255	994	1134	300		440	48	41.16	5.04	12.11	0.75	0.065	1.16		
27	256	1156	1022	329	36	736	40	48.16	2.52	10.93	0.85	0.226	1.1		
28	257	950	1252	288		411	28	31.36	9.8	11.03	0.96	0.14	0.58		
29	258	1104	1394	186	26.4	411	24	62	9.24	12.51	0.98	0.965	1.53		
30	259	1037	1226	187		343	44	39		12.61	0.91		0.78		
31	260	1192	1352	163	20.6	604	48	45		12.31	0.96		0.76		
1	261	1252	1380	181	26.2	556	24	36.96	5.88	11.62	1.06	0.18	1.56	0.2	0.79
2	262	1232	1528	170	23.9	508	48	45.64	3.92	13.4	1.34	0.15	1.73	0.1	0.44
3	263	1218	1454	151		484	40.8	42.56	7	13.61	1.34	0.22	1.43	0.17	0.56
4	264	1264	1526	138		484	44.8	42.56	3.92	14.41	0.445	0.68	0.75	0.14	0.26
5	265	922	1266	142	21.8	520	40.8		3.64	13.3	0.915	0.18	6.54		5.31
6	266	1234	1368	132	19	585	57.12		10.64	15.12	0.65		1.83	0.75	1.13
7	267	950	1028	175	18	431	44.88	34.72	4.76	13.71	0.795	0.7	1.34		1.76
8	268	1110	1154	156	17.6	822	40.8	59.08	4.76	12	0.54	0.93	4.36	0.14	1.42
9	269	1162	1278	141	30.3	536	44.88	49.84		11.52	0.79	0.1	1.35	0.1	0.46
10	270	1416	1624	119		536	20.4	49.84	11.76	9.96	0.875	0.2	1.24	0.1	0.33
11	271	1048	1224	139	29.3	404	36.72	37.52	6.96	8.42	0.815	0.93			1.72
12	272	1220	1420	141	31.6	412	28.56		3.36	10.44	1	0.1	1.52	0.135	0.32
13	273	1140	1670	118	32.4	563	36.72	55.44	6.72	9.96	0.8	0.1	0.3	0.1	0.25
14	274					563									
15	275	1654	1898	106	38.4	449	44.88		5.32	9	0.725	0.1	0.32	0.1	0.72
16	276	1132	1804	89	30.6	457	24.48	45.36	7.28	9.28		0.1	0.42	0.1	0.63
17	277	1544	1788	95		437	32.64	62.12	6.72	9	0.725	0.14	0.91	0.13	0.29
18	278			152	29.3	714	48.96	45.36	6.44	6.16	1.36	0.135	0.38	0.1	0.2
19	279	1008	1254	103		714	57.12	45.36	4.48	7.38	1.16	0.1	0.31	0.1	0.19
20	280	1010	1170	112	26.5	538	44.88	49.28	4.2	8.71	1.32	0.14	0.61	0.1	0.26
21	281	812	1052	114	26.8	538	32.64	49.28	6.44	8.33	1.14	0.1	0.25	0.1	0.35
22	282	876	980	122	26.4	437	50	77	5.6	8.23	0.92	0.22	0.5	0.1	
23	283	902	936	123		437	62.4	77	3.08	8.71	0.875	0.185	1.02	0.1	0.21
24	284				23.6	396	99.8	31.36	3.36	8.9	0.55	0.1	0.76	0.125	0.28
25	285	838	902	144	19.8	396	41.6	31.36	4.48	8.23	0.73	0.24	1.2	0.27	0.6
26	286	810	982	132	20.9	404	41.6	41.44	9.8	9.77	0.655		1.13		
27	287	860	902	128	22.5	404	54.08	41.44	2.8	10.25	0.64	0.218	1.13	0.16	0.41
28	288	818	876	137	21.9	457	33.28	53.76	2.52	9.28	0.7	0.5	0.87	0.14	0.38
29	289	508	740	155	20.1	457	41.6	53.76	6.72	8.86	0.53	0.24	0.87	0.135	0.36
30	290	568	776	129	13.3	551	41.6	43.4	3.68	8.52	0.37	0.43	1.117	0.465	1.81
31	291	702	744	202	13.2	551	37.44	43.4	5.04	8.61	0.875	0.31	1.51	0.17	

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1/9	292	654	732	212		551	45.76	43.4	3.7	23.21	0.2	1.05	2.15	0.2	0.72
2	293	720	946	159	15.7	592	45.76	47.52	3.02	11.67	0.53	0.13	2.6	0.48	
3	294	808	900	205	21.1	665	62.4	59.92	24.64	12.43	0.61	1.41	2.5	0.29	0.55
4	295	1042	1116	242		828	74.88		0	12.77	0.68	0.55	1.09	0.18	0.76
5	296	1032	1278	235		828	66.56		15.12	11.49	1.09	0.48	0.89	0.23	0.52
6	297	924	1130	265		292	104	61.6	20.72	11.01	1.04	0.6	1.65	0.14	0.42
7	298	1318	1402	213		452	61.2	61.6	13.16	14.94	0.5	0.74	2.15	0.12	0.43
8	299	842	890	314		452	61.2	49.28	6.16	19.97	0.27	0.45	0.84	0.13	0.43
9	300	940	1078	278		452	55	49.28	7.56	14.73		0.55	1.09	0.12	0.42
10	301	900	998	261	18.9	452	57.12	49.28	1.4	14.81	0.79	0.91	3.25	0.65	1.04
11	302	836	964	249		313	8.15	38.08	3.36	16.45	0.73	0.91	5.15	0.8	2.26
12	303					330		38.08		17.51	0.38			0.38	
13	304	888	1032	194	18.2	395	77.52	38.08	3.08	17.98	0.11	0.81	7.96	0.51	1.62
14	305	1004	1168	180		395	48.96		5.04	19.77	0.07	0.76	3	0.82	2.48
15	306	582	710	253	20.2	395	40.8		3.36	19.49	0.17	1.03	7.77	0.83	1.55
16	307	524	668	240	21.2	655	69.36	53.76	10.08	18.79	0.2	1.01	7.73	0.97	2.38
17	308	668	868	196	22.4	226	53.04	44.52	6.44	14.89	0.13	1.15	4.87	1	2.3
18	309	760	820	225		263	73.4	36.4	1.4	16.95	0.05	0.91	3.4	0.83	2.26
19	310					452	77.52	60.2	5.6	21.49	0.54	0.91	2.3	0.69	1.45
20	311	872	1122	241		475	61.6		10.92	10.68	0.43	0.59	4.53	0.45	1.61
21	312	974	1204	183	20.8	440	61.2		5.04	16.68	0.38	0.88	3.25		0.77
22	313					440									2.77
23	314	894	1100	218		452	65.28	39.2	6.16	23.21	0.43			1.1	0.64
24	315	918	1118	179		452	48.96	39.2	8.96	21.92	0.38	0.55	0.99	0.27	0.47
25	316	731	746	174		358	85.68	49.56	4.48	12.37	0.36	0.48	0.94	1.04	0.91
26	317	990	1046	229		535	61.2	70	12.38	17.69	0.63	0.43	0.79	0.35	0.42
27	318	864	1044	172		522	57.12	44.52	17.36	15.52	1.33	0.43	11.44	0.22	0.88
28	319					522		44.57							
29	320					522		44.52							1.67
30	321	560	712	141	13.8	518	85.68	40.88	7.56	18.18	1.31	1.41	0.79		1.22
1/10	322	752	868	167		350	161	40.32	12.6	18	0.254			0.64	0.72
2	323	774	932	209		395	89	43.12		15.05	0.8	0.45		0.31	0.5
3	324	780	918	185		806	106	70	7.64	13.35	0.8	0.38	0.2	0.18	0.55
4	325	810	974	226		706		62.16	10.08	14.65	1	0.43	0.32	0.13	0.42
5	326					706		62.16							
6	327					706	106	62.16							
7	328	1006	1266	158		888	125	60.2	18.74	4.89	0.93	0.4	0.23	0.2	0.12
8	329	1292	1346	178		505	69	55.4	8.96	11.67	3.13	0.38	0.14	0.1	0.31
9	330	1510	1604	187	33.3	586	41	44.24	7.56	7.68	1.55	0.36	0.13	0.12	0.27
10	331	1504	1684	214	28.6	586	45	43.68	9.8	7.4	1.31	3.79	0.53	0.15	0.52
11	332	1226	1372	313		835		43.68	5.88	8.35	1.53	0.37	0.89	0.2	0.59
12	333					835		50.2		5.82	0.84	0	0.59		
13	334					643	115	50.12					0.18		
14	335	1274	1428	364	23.8	643	131	50.12	7.82	6.72	1.05	0.69	0.99	0.1	0.38
15	336	1206	1428	448	21.3	685	53	43.44	18.76	10.53	2.04	0.43	0.69	0.12	0.56
16	337	1486	1630	392	19.2	498	78	64.68	14	11.92	1.94	0	1.07		
17	338	1218	1472	435	10.9	383	53	64.68	6.44	7.4	1.77				
18	339					383	41	43.68	7.56	11.84	2.26	0.4	1.19	0.15	0.41
19	340					378	45	42							
20	341				12.7	378	41	42	3.64	12.77	2.51	0.52	2.1	0.11	0.52
21	342	820	1236	437	14.3	378	45	42	6.72	14.08	1.72	0.4	0.54	0.11	1.04
22	343	1120	1314	365	14	400	45	55.72	3.36	12.52	1.53	0.36	0.18	0.12	0.42
23	344	1270	1688	278		420		55.72	6.72	9.18	0.77	0.48	1.09	0.16	0.47
24	345	1336	1524	314		420	46	48.16	3.36	12.6	1.91	0.36	1.26	0.33	0.6
25	346	1210	1376	334		730	49	48.16	4.48	13.35	0.82		5.71	0.16	0.59
26	347					730	33	42.56	20.72	17.36	0.54	0.48	2	0.15	0.24
27	348					730		42.56	6.16						
28	349					420		33.32	3.92						
29	350	1220	1402	271		420	53	33.32		14.97	0.41	0.5	1.56	0.11	2.4
30	351					428		37.8							
31	352	1256	1302	291		428	62	37.8	4.76	12.71		0.5	3.05	0.14	1.35
1/11	353	1300	1374	276		461	41	35	4.2	11.45	0.28	0.25	0.68	0.31	0.45
2	354				19.7	461	33	35	2.52		0				
3	355				16.5	461	25	35	2.8	12.07	0.2	0.25		0.46	4.96
4	356	1400	1574	222		474	25	36	2.52	15.32	0.17	0.36		0.47	2.89

5	357	1444	1604	218		536		37	2.52		0.18	0.2		0.28	1.215
6	358					536		37							
7	359				20.9	536		37		15.32					
8	360	1302	1486	215		527	45	35	4.48	12.58	0.2	0.6	6.78	0.83	0.23
Period 1 av		1030	1201	209	22.38	512.3	54.9	46.99	7.015	12.66	0.843	0.501	1.886	0.302	0.965
9	361	1404	1764	204		511	58	80	9.52	9.52	0.4	0.14	0.2	0.62	1.01
10	362				20	511		80							
11	363	1320	1632	208	31	656		47				0.25	0.44	0.46	0.68
12	364	1526	1722	197	20.6	513	25	47	8.96	12.58	0.2	0.16	0.15	0.26	0.66
13	365	1426	1750	206		480		41				0.17	0.2	0.16	0.36
14	366	1762	1866	203	19.4	451		39	6.72	12.58	0.17	0.14	0.15	0.29	0.52
15	367	1856	1640	216	19.7	455	53	39	6.16	10.41	0.16	0.15	0.22	0.38	0.67
16	368	1616	2026	197	19.1	431	53	48	6.16	9.52	0.19	0.15	0.18	0.16	1.42
17	369	1756	2142		18.5	463	57	40	8.6	9.52	0.2	0.16	0.35	0.11	0.46
18	370	1826	2008	199	15.4	368	48	40		9.39	0.17	0.17	0.22	0.1	0.28
19	371	1848	1922	219		368	17	50	2.24	9.9	0.19	0.11	0.23	0.19	0.4
20	372	1512	1730	208	17.5	550	62	50	5.32	10.41	0.15	0.16	0.41	0.1	0.18
21	373	1688	1698	212	17.9	390	62	27	2.8	9.27	0.15	0.15	0.19	0.19	1.03
22	374	1660	1778	225	19.1	705	74	41	4.48	11.55	0.17	0.13	1.94	0.24	0.62
23	375	1596	1764		20.7	856	41	41	4.2	12.58	0.2	0.34	2.45	0.23	1
24	376					497	45	33							
25	377	1420	1750	183		431		33				0.39	0.9	0.42	8.01
26	378	1298	1500	240	25.9	392	57		5.32	11.94	0.16	0.14	2.61	0.3	1.29
27	379	1472	1546	233	30.3	392	40	20	2.24	13.49	0.2	0.33	0.54	0.42	2.36
28	380	1290	1422	267	31.2	497	77	20	3.64	14.01	0.25	0.25	2.03	1.39	1.78
29	381	1242	1512	225		497	61	31	5.6	13.23	0.25	0.47	0.6	0.47	1.99
30	382					646		31	11.76		0.21				
1/12	383				27.7	646	73	31	5.6	4.35	0.28	0.25	12.52	0.43	1.2
2	384	979	1172		22.7	546	49	41		14.44	0.11	0.81	8.63	0.16	13.68
3	385	728	958		21.7	546		31							2.47
4	386	860	979	229		546	150	31	3.36	11.89	1.35	0.15	0.25	0.47	0.95
5	387	958	1218	205	10.4	497	86	38	5.32	10.88	1.19	0.84	1.32	0.69	1.7
6	388	738	1058	302		530	135	35	3.92	11.5	1.29	1.14	1.5	1.39	0.78
7	389	804	1054	342	33.3	530	69	35	2.24	9.86	1.14	0.17	0.24	0.49	2.12
8	390			295		407		35							
9	391	868	1090	293	32.5	407	102	37	2.24	11.86	1.43	0.91	12.59	0.68	0.7
10	392	1094	1400	458		445	73	36	2.52			0.12	0.16	0.15	0.7
11	393	1004	1136	468		445	131	42	4.2	9.21	1.19	0.12	0.11	0.17	0.46
12	394	1364	1522	446	36.8	444	78	34	13.16	11.05	0.35	0.11	0.09	0.21	1.54
13	395	1502	1658	625		444	73	44	4.2	7.15	0.16				
14	396				39.3	605	58	44		8.66					
15	397	1474	1754	1026	33.7	750	57	44	5.88	9.7	0.3	0.14	0.06	0.19	0.76
16	398	1540	1908	911	36.3	750	29	35	7.28	10.11	0.24	0.11	0.15	0.11	0.55
17	399	1370	1722	430	28.7	546	45	35	3.92	8.27	0.14	0.27	0.09	0.3	0.75
18	400	1330	1632	980	30.2	546	53	35	2.8	9.78	0.15	0.1	0.44	0.29	1.54
19	401	1404	1740	345	29.4	524	20	36		8.89	0.13	0.2	0.11	0.21	2.06
20	402	1464	1668	735		524		36		9.29	0.14	0.11	0.31	0.46	1.41
21	403														
22	404														
23	405														
24	406														
25	407														
26	408														
27	409														
28	410														
29	411														
30	412														
31	413														
1/1	414														
2	415														
3	416	1400	1719	302	34.7	512	41	45	6.72	10.22	0.35	0.13	0.63	0.2	1.56
4	417					512		45							
5	418					512		45							

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6	419	1140	1366	212	21.4	491	61	33	5.88	11.53	0.73	0.47		0.86	
7	420	1386	1662	150		614	74		5.04	12.76	0.71		2.37		1.44
8	421	1468	1152	168		614	61		10.92	6.97	0.69	0.11	0.23	0.16	0.35
9	422	1098	1428	168		589	57	38	12.32	7.22	0.99	0.11	0.22	0.22	1.51
10	423	1106	1346	201		589	74	27	5.6	7.16		0	0.19	0.13	0.81
11	424				22	425		27							
12	425					425		27							
13	426	1284	1536	221	23.8	412	59	40	11.76	4.54	1	0.09	0.17	0.11	0.39
14	427	1212	1498	274	27.4	412	64	40	4.76	5.96	1.27	0.1	0.17	0.16	0.42
15	428	1242	1492	268	23.8	462	153	38	10.64	6.34		0.09	0.19	0.1	0.59
16	429	1272	1414	325	25.3	471	68	33	7.28	7.42		0.1	0.16	0.11	0.47
17	430	1324	1592	301		437	109	29	3.92	7.66	1.26	0.12	0.24	0.16	1.06
18	431					437		29			0.67	0.08		0.1	
19	432					437		29						0.1	
20	433	1494	1824	252		600	47	63	8.4	7.53	0.8	0.13	0.28		0.63
21	434	1744	1992	251	36.4	600	39	44	14.56	8.64	0.91	0.12	0.38	0.12	0.24
22	435	1670	2002	280	35.6	519	64	44	6.16	9.13	1.27	0.1	0.17	0.1	0.62
23	436	1700	1784	392		445	68	42	6.44	8.95	0.91	0.1	0.19	0.18	2.13
24	437	1476	1760	477		503	21	42	7	11.11	0.6	0.13	0.21	0.39	0.57
25	438					503		42							
26	439					503		43							
27	440	1416	1640	293	34.3	523	21	43	5.88	11.41	0.79	0.28	2.75	0.17	1.61
28	441	1420	1616	309	38.5	523	62	43	9.52	9.86	0.87	0.14	0.4	0.23	0.57
29	442	1566	1758	353	41	503		42		9.98	0.1	0.12	0.24	0.11	0.54
30	443	1514	1722	314	38.4	482		43		9.74	0.14	0.22	0.31	0.12	1.04
31	444	1468	1756	330		482		43		9.74	0.12	0.11	0.23	0.15	0.3
1	445														
2	446							42.6							
3	447	1234	1498	294	21.6	374	127	31.64	10.08	10.93	0.62	0.62	3	0.71	2.86
Period 2 av.		1378	1594	324.7	26.74	510.7	65.02	39.23	6.393	9.847	0.525	0.228	1.166	0.308	1.374
4	448	1376	1658	277	25.5	401	115	34.72	8.68	8.04	0.13	0.13	0.43	0.16	1.9
5	449	1400	1672	239		401	111	36.4	15.4	7.59	0.11	0.11	0.35	0.2	0.81
6	450	1248	1438	278		401	102	33.04	9.24	7.53	0.17	0.17	0.34	0.12	0.95
7	451	1234	1328	259		397	102	31.36	5.6	7.02	0.11	0.11	0.24	0.01	0.29
8	452					463		31.36							
9	453				14.9	401		31.36							
10	454	1446	1676	274		418	78	36.4	14.28	2.22	0.11	0.11	0.31	0.11	0.88
11	455	1370	1784	263		459	106	31.64	14	2.93	0.98	0.11	0.21	0.12	1.05
12	456	1436	1716	286		476	118	32.22	8.98	2.61	0.85	0.11	0.22	0.21	0.66
13	457	1446	1638	287		455	111	39.48	4.2	3.38	0.91	0.1	0.16	0.1	0.17
14	458	1404	1682	282		440	78	32.2	10.08	5.62	2	0.11	0.15	0.35	0.73
15	459	1262	1448	248		440	94	32.2	5.6	5.94	1.16	0.1	0.16	0.12	0.65
16	460					410		32.2							
17	461	1140	1380	231		445	45	32.2	2.24	6.77	0.82	0.32	2.72	0.33	1.65
18	462	1128	1380	217		384	41	34	8.12	6.28	0.29	0.13	0.28	0.12	0.3
19	463	1032	1158	250		402	49	42.2	8.12	3.11	0.37	0.29	0.18	0.29	0.24
20	464	1098	1240	250		435	20	40.8	5.6	5.67		0.1	0.21	0.25	0.17
21	465	1064	1248	224		415		34							
22	466					420		36.2							
23	467					420		36.2							
24	468	838	1074	242		378	52	34	7	5.04	0.9	0.12	0.15	0.1	0.48
25	469	1180	1408	213	22.5	421	29	41.5	5.04	2.59	0.51	0.11	0.16	0.11	0.51
26	470	792	978	184	17.6	456	40	33.6	5.88	2.44	0.63	0.11	0.21	0.18	0.75
27	471	916	1016	226	13.6	480	32	38.7	9.52	2.34	0.48	0.09	0.15	0.11	0.95
28	472	650	904	254		448		43	2.52	1.74	0.36	0.1	0.15	0.11	0.35
Period 3 av.		1177	1394	249.2	18.82	426.6	73.5	35.24	7.9	4.677	0.605	0.133	0.357	0.163	0.708

UNIT CONF (CONTROL)

DATE	DAY NO.	VSS mg/l	TSS mg/l	DSVI	OUR mg/l/d	COD(mg/l) inf.	COD(mg/l) eff.	TKN (mg/l) inf.	TKN (mg/l) eff.	(mg/l) (eff) NO2	(mg/l) (eff) NO3	Anox. NO2	Anox. (mg/l) NO3
1/12	383					646		31					
2	384					646		41					
3	385					546		31					
4	386	982	1136			546	125	31	4.48				
5	387	1228	1450	193		497	45	38	1.68	0.32	14.13		2.43
6	388	1130	1208	265		530	65	35	2.8	0.23	9.86	0.42	0.67
7	389	1064	1354	347		530	94	35	2.52	0.22	10.26	0.12	0.26
8	390			296		407		35					
9	391	1344	1620	244		407	86	37	2.52	0.15	10.88	0.69	0.98
10	392	1516	1800	303		445		36		0.09		0.12	0.29
11	393	1234	1483	303		445	131	42	3.92	0.15	9.62	0.1	0.24
12	394	1920	1720	266		444	102	34	3.64	0.1	9.7	0.09	0.18
13	395	1806	2030	335		444	346	44	2.52	0.09	8.58	0.1	0.19
14	396					605		44					
15	397	1670	1904	604		750	37	44	3.08	0.13	8.12	0.73	1.62
16	398	1886	2230	677		750	37	35	3.36	0.16	7.82	0.1	0.22
17	399	1896	2310	519		546	16	35	9.8	0.12	8.2	0.1	0.22
18	400	2020	2372	590		546	45	35	3.64	0.11	8.6	0.1	0.19
19	401	2022	2438	549		524	45	36	2.8	0.11	7.96	0.1	0.26
20	402	2064	2666	600		524		36		0.1	7.03	0.08	0.18
21	403												
22	404												
23	405												
24	406												
25	407												
26	408												
27	409												
28	410												
29	411												
30	412												
31	413												
1	414												
2	415												
3	416	1702	1840	228		512	53	45	2.8	0.22	10.16	0.14	0.44
4	417					512		45					
5	418					512		45					
6	419	1588	1842	190		491	147	33	5.04	0.2	9.98		
7	420	1154	1410	213		614	41		0.9	0.29	10.4	0.14	0.4
8	421	1370	1728	173	29	614	201		2.24	0.26	9.19	0.12	0.29
9	422	1116	1416	178	23	589	82	38	1.4	0.31	7.41	0.09	0.19
10	423	1230	1482	202		589	53	27	2.24	0.45	6.53	0.09	0.15
11	424					425		27					
12	425					425		27					
13	426	1712	2036	157	35	412	59	40	2.52	0.42	6.97	0.09	0.15
14	427	1198	1440	201	25	412	105	40	1	0.61	5.51	0.09	0.14
15	428			221		482	51	38	4.76	0.41	4.21	0.09	0.15
16	429	1150	1254	223	24	471	76	33	7	1.84	6.21	0.12	0.23
17	430	1160	1394	201	24	437	55	29	3.64	1.84	7.78	0.1	0.12
18	431					437		29					
19	432					437		29					
20	433	758	882	181	14	600	55		6.72	1.08	8.21	0.2	0.32
21	434	818	860	186		600	43	44	6.44	1.9	8.34	0.11	0.24
22	435	864	982	163	19	519	72	44	12.32	2	6.78	0.5	0.75
23	436	1290	1310	155	23	445	125	42	26	0.73	2.89	0.36	1.42
24	437	990	1168	188	20	503	71	42	16.8	2	7.72	0.14	0.24
25	438					503		42					
26	439					503		43					
27	440	1106	1274	204		523	49	43	3.1	2.1	9.01	0.16	0.28
28	441	1082	1200		40	523	49	43	5.32	1.5	8.7	0.14	0.24
29	442	1570	1748	206	39	503	94	42	1	0.57	10.22	0.13	0.21

30	443	1620	1838	239	34	482	88	43	5.6	0.5	10.58	0.13	0.21
31	444	1560	1836	261	30	482	37	43	5.32	0.72	10.1	0.12	0.19
1/2	445					482		43					
2	446					482		43					
3	447	1622	1862	266			41	32	4.2	0.35	10.05	0.23	0.83
Period 2 avg.		1401	1626	286.9	27.07	515.7	80.6	37.73	4.946	0.622	8.506	0.181	0.432
4	448	1648	1950	256	25	401	25	35	0.68	0.41	7.53	0.11	0.16
5	449	1704	1974	223	26	401	78	36	10.64	0.33	6.96	0.1	0.17
6	450	1322	1566	249	27		33	33	9.24	0.43	6.2	0.15	0.24
7	451	1642	1726	232	30	400	102	32	6.16	0.55	7.21	0.1	0.12
8	452					401		32					
9	453					401		32					
10	454	1462	1710	246	27	463	37	36	5.6	1.23	6.96	0.13	0.11
11	455	1426	1574	235	27.14	401	102	32	3.36	1.56	7.21	0.11	0.2
12	456	1362	1638	244	26.91	418	86	32	5.88	1.47	7.47	0.14	0.16
13	457	1376	1614	247	25.5	459	53	39	7.56	1.4	6.96	0.12	0.09
14	458	1216	1466	246	27.6	476	94	32	7.84	1.4	7.15	0.12	0.12
15	459	1568	1820	248	27	476	90	32	5.88	1.35	6.83	0.11	0.21
16	460					476		32					
17	461	1422	1692	248	27	455	45	34	6.72	1.18	5.82	0.11	0.11
18	462	1590	1890	243	29.22	455	70	34		0.33	1.44	0.09	0.09
19	463	1606	1796	278	23.3	440	48.1	38	12.6	0.49		0.09	
20	464	1468	1648	267	23.71	445	56.1	32	4.76	1.1	3		0.12
21	465	1282	1478	244	22.06	421	16	34					
22	466					448		41					
23	467					448		40					
24	468	1408	1732	288	22.66	377	8	35	6.72	1	1.3	0.1	0.15
25	469	1366	1614	297	27.23	445	8	32	4.2	1	0.86	0.09	0.12
26	470	1356	1610	248		417	32	33	1.4	1	1.42	0.11	0.1
27	471	1462	1720	262	25	465	32	31	3.92	1	1.87	0.15	0.09
28	472	1178	1442	277	26.7	443	40	36	7.6	1.4	2.1	0.18	0.09
Period 3 avg.		1440	1683	253.9	26.11	434.7	52.76	34.2	6.153	0.981	4.905	0.117	0.136

APPENDIX 2

Calculation of mass balances

To test the reliability of the data COD and N balances were carried out over the steady state periods of operation of the systems. The N balance is calculated from the sum of the masses of N leaving the system and the amount of N entering the system . The N balance is calculated from the following components:

(i) Mass of N present as influent TKN (Total Kjeldahl Nitrogen) in the influent per day $M(N_{ti})$.

(ii) Mass of N added to the influent per day $M(M_{ni})$.

(iii) Mass of N in the waste sludge per day $M(N_w)$.

(iv) Mass of N denitrified per day $M(N_{nd})$.

(v) Mass of TKN in the effluent per day $M(N_{te})$.

(vi) Mass of NO_3^- and NO_2^- in the effluent per day $M(N_{ne})$.

These are calculated as follows:

(i) Mass of N as TKN in the influent: $M(N_{ti}) = Q.N_{ti}$ where N_{ti} is the influent TKN concentration. For steady state period 3 of the modified 2-reactor system:

$M(N_{ti}) = 10$ l/d*35.2 mgN/l = 352 mgN/d

(ii) Mass of N added: $M(N_{ni}) = Q.N_{ni}$ where N_{ni} is the concentration of N added (either as ammonium or nitrate or nitrite) (mgN/l)

Q = influent feed volume/day (l/d)

(iii) The mass of N in the waste sludge is given by:

$$M(N_w) = f_n.M(X_v)/R_s = f_n.V_p.X_n/R_s$$

where

$M(X_v)$ = VSS mass in the reactor

= VSS concentration in reactor * volume of reactor

f_n = nitrogenous fraction of the sludge = 0.1 mgN/mgVSS

R_s = sludge age (days)

For steady state period 3 of the modified 2 reactor system:

$M(N_w)$ = 0.10 mgN/mgVSS*10 l/d*1177 mgVSS/l/15d = 78.5 mgN/d

(iv) For the 2 reactor system the mass of N denitrified is calculated by subtracting the mass of nitrate + nitrite in the anoxic reactor effluent from the mass of nitrate entering the anoxic reactor. For the intermittent aeration system, a nitrate and nitrite mass balance cannot be conducted with the result that the mass of nitrate and nitrite denitrified cannot be calculated. For these systems the N balance therefore cannot be checked. However, as will become apparent below, the mass N/d nitrate generated by nitrification (MN_{ng}) and removed by denitrification (MN_{nd}) are required for the COD balance. Consequently for the intermittently aerated systems these MN_{ng} and MN_{nd} are calculated assuming a 100% N balance.

For 2 reactor ND systems:

$M(N_{nd})$ = mass NO_x in - mass of NO_x out

= $(s+a).Q.N_{ne} - (1+s+a).Q.N_{anox}$

s = sludge return recycle ratio

a = mixed liquor recycle ratio

N_{ne} = aerobic reactor (equivalent to effluent) nitrate + nitrite concentration (mgN/l)

N_{anox} = anoxic reactor NO_x concentration (mgN/l)

Therefore for steady state period 3 (modified 2 reactor system) with a = 1:1, s = 1:1, Q = 10l/day and N_{anox} and N_{ne} 0.4 and 4.7 mgN/l respectively:

$M(N_{nd})$ = 2*10 l/d*4.7 mgN/l - 3*10 l/d*0.7 mgN/l = 72.3 mgN/d

(v) The mass of N as TKN in the effluent: $M(N_{tc}) = Q.(N_{tc})$

N_{te} = measured effluent TKN concentration (mgN/l)

For steady state period 3 (modified 2 reactor system) with N_{te} = 7.9 mgN/l:

$M(N_{tc})$ = 10 l/d*7.9 mgN/l = 79 mgN/d

(vi) The mass of effluent NO_3: $M(N_{nc}) = Q.(N_{nc})$

N_{ne} = measured effluent nitrate concentration (mgN/l)

For steady state period 3 (modified 2 reactor system) with N_{ne} = 4.7 mgN/l:

$M(N_{ne})$ = 10 l/d*4.7 mgN/l = 47 mgN/l

The N balance is therefore:

$$\text{N balance} = \frac{M(N_{nd})+M(N_{ne})+M(N_{tc})+M(N_{w})}{M(N_{ti})+M(N_{ni})} *100$$

$$= 100*(72.3 + 47 + 79 + 78.5)/352$$

$$= 78.6\%$$

For the intermittently aerated system:

Assuming a 100% N balance the mass of nitrate denitrified ($M(N_d)$) is calculated as the difference between total N entering the system in the form of TKN (and dosed N) and total N leaving the system, in the form of (i) Effluent TKN (MN_{te}); (ii) Effluent nitrate (MN_{nc}); and (iii) N in the waste sludge (MN_w) ie.

$$M(N_d) = M(N_{ti}) + M(N_{ni}) - M(N_{tc}) - M(N_{nc}) - M(N_w) \quad \text{(mgN/d)}$$

The mass of nitrate denitrified $M(N_d)$ is required to calculate the mass of oxygen recovered via denitrification $M(O_d)$ which is needed for the COD mass balance ie.

$$MO_d = 2.86.MN_{nd} \text{ (mgO/d)}$$

Also required for the COD mass balance is the oxygen demand for nitrification which is obtained from the mass of nitrate generated (MN_{ng}) calculated in the N balance. The MN_{ng} is given by the sum of the masses of nitrate denitrified and in the effluent viz.

$MN_{ng} = MN_{nd} + MN_{ne}$ (mgN/d)

Normally in nitrification-denitrification systems the nitrite concentrations are very low in which case it is reasonable to accept without significant error that the total analyzed nitrogen (nitrate and nitrite) is all in the form of nitrate. However, separate mass balances on nitrate and nitrite concentrations allows masses of these concentrations generated and denitrified to be calculated. This is demonstrated below.

The nitrate and nitrite balances for the anoxic and aerobic reactors can be calculated :

For aerobic reactor:

NO_2/NO_3 out - NO_2/NO_3 in = NO_2/NO_3 generated, ie.

Nitrite: $(1+s+a).Q.NO_2^-{}_{eff} - NO_2^-{}_{anox}.(1+s+a).Q = NO_2^-$ nitrified

Nitrate: $(1+s+a).Q.NO_3^-{}_{eff} - NO_3^-{}_{anox}.(1+s+a).Q = NO_3^-$ produced

For anoxic reactor:

NO_2/NO_3 in - NO_2/NO_3 out = NO_2/NO_3 denitrified

Nitrite: $(a+s).Q.NO_2^-{}_{eff} - NO_2^-{}_{anox}.(a+s+1).Q = NO_2^-$ denitrified

Nitrate: $(a+s).Q.NO_3^-{}_{eff} - NO_3^-{}_{anox}.(a+s+1).Q = NO_3^-$ denitrified

where: $NO_2^-{}_{eff}$ = system effluent nitrite concentration (mgN/l)

$NO_2^-{}_{anox}$ = anoxic reactor nitrite concentration (mgN/l)

$NO_3^-{}_{eff}$ = system effluent nitrate concentration (mgN/l)

$NO_3^-{}_{anox}$ = effluent nitrate concentration (mgN/l)

See Tables A.1 and A.2 for nitrite and nitrate balances

Table A.1 Nitrite and nitrate balances for the anoxic reactor(s) of the Experimental 2RND, M2RND, and Control systems.

Steady State Period	NO_2in (mgN/l)	NO_2out (mgN/l)	NO_2 Denit (mgN/l)	NO_3in (mgN/l)	NO_3out (mgN/l)	NO_3 Denit (mgN/l)
2RND 1	4.19	2.58	1.61	40.21	13.12	27.09
2	1.90	1.28	0.62	39.22	5.97	33.25
3	1.50	1.13	0.38	20.10	2.75	17.36
M2RND 1	1.68	0.91	0.77	23.64	1.99	21.65
2	1.05	0.92	0.13	18.65	3.198	15.45
3	1.22	0.49	0.73	8.14	1.64	6.51
CONT. 1	1.24	0.54	0.70	15.78	0.75	15.03
2	1.96	0.36	1.60	7.86	0.06	7.80

Table A.2 Nitrite and nitrate balances for the aerobic reactor of the Experimental 2RND. M2RND, and Control systems.

Steady State Period	NO_2out (mgN/l)	NO_2in (mgN/i)	NO_2 Gener (mgN/l)	NO_3out (mgN/l)	NO_3in (mgN/l)	NO_3 Gener. (mgN/l)
2RND 1	2.54	2.58	2.66	50.27	13.12	37.15
2	2.38	1.28	1.10	49.03	5.97	43.06
3	2.25	1.13	1.13	30.15	2.75	27.41
M2RND 1	2.52	0.91	1.61	35.46	1.99	33.47
2	1.58	0.92	0.65	27.98	3.198	27.78
3	1.83	0.49	1.34	12.21	1.64	10.58
CONT. 1	1.86	0.54	1.32	23.67	0.75	22.92
2	2.94	0.36	2.58	11.79	0.06	11.73

The COD Balance

With the COD mass balance a reconciliation is made of the influent COD mass with the COD masses of the waste sludge and effluent and of the oxygen required for COD utilisation.

These components are calculated as follows:

(i) Mass of COD in the influent: $M(S_{ti}) = Q.S_{ti}$

S_{ti} = COD concentration of the influent feed (mgCOD/l)

Q = feed volume/day (l)

For steady state period 3 (modified 2-reactor system) with S_{ti} = 426.6 mgCOD/l and Q = 10l:

10l*426.6 mgCOD/l = 4266 mgCOD/d

(ii) Mass of COD in the waste sludge: $M(S_{xw}) = f_{cv}.MX_v/R_s$

f_{cv} = 1.48 mgCOD/mgVSS (COD/VSS ratio)

MX_v = mass of VSS in the system

R_s = sludge age (days)

For steady state period 3 (modified 2-reactor system) with R_s = 15 days, X_v = 1177 mgVSS/l, and Q = 10l:

1.48 mgCOD/mgVSS*1177 mgVSS/l*10l/15d = 1161 mgCOD/d

(iii) Mass of COD in the effluent: $M(S_{tc}) = Q.S_{tc}$

S_{te} = concentration of COD in the effluent (mgCOD/l)

For steady state period 3 (modified 2-reactor system) with Q = 10 l/d and S_{te} = 73.5 mgCOD/l:

10 l/d*73.5 mgCOD/l = 735 mgCOD/d

(iv) Mass of oxygen required for COD utilisation:

$M(O_c) = M(O_{tm}) + M(O_d) - M(O_n)$

$M(O_{tm})$ = measured mass of oxygen consumed daily

= OUR.24.%aerobic V_p/100

OUR = measured oxygen utilisation rate (mgO/l/hr)

V_p = volume of reactor (l)

For steady state period 3 (modified 2-reactor system):

18.8 mgO/l/hr*30%*24hr*10/100 = 1353.6 mgO/d

$M(O_n)$ = mass of oxygen used for nitrification

= $4.57.MN_{ng}$

4.57 = oxygen demand for nitrification (4.57 mgO/mgN times mass of nitrate generated)

For steady state period 3 (modified 2 reactor system):

4.57*119 mgN/d = 544 mgO/d

As given above the oxygen recovered via denitrification MO_d is given by:

$M(O_d) = 2.86\ M(N_d)$

2.86 = oxygen equivalent of nitrate (mgO/mgNO_x^- N denitrified)

For steady state period 3 (modified 2-reactor system):

2.86*72.3 mgNO_x/d = 206.8 mgO/d

Hence the COD mass balance is:

$$\text{COD balance} = \frac{M(S_{te}) + M(S_{xw}) + M(O_c) * 100\%}{M(S_{ti})}$$

For steady state period 3 (modified 2-reactor system):

$100*(764.1 + 1161 + 1016.4)/4266 = 68.9\%$