COD Removal and Nitrification of Piggery Wastewater in a Sequencing Batch Reactor

A thesis submitted in partial fulfilment
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Errata Sheet

Pg		
11	Ln 2	New Zealand (1995) should be New Zealand Ministry of Health (1995)
12	Ln 6	Corrected word from "micro organism" to "microorganisms"
14	Ln 4	Corrected reference from (Blouin *et al*, 1989) to (Blouin *et al.*, 1990)
14	Table 2.1	Corrected references to "Ten Have *et al.* (1994)" ; "Andreottola *et al.* (1997)"
15	Sect. 2.3	Lower case for faecal coliforms; enterococci replaces *Streptococci*
15	Ln 3	Corrected reference to Blouin *et al.* (1990)
15	Lns 5,6,7	Concentration of *Nitrosobacteria* was 990 MPN/ 100 ml, *Nitrobacteria* concentration was 2.8 $*10^5$ MPN/ 100 ml. Inoculations of bacteria were 10^6 - 10^9 MPN/ 100ml
16	Para.4, ln 2	Replace "chemotrophs" with "chemoautotrophs"
17	Sect. 2.7	Equations rewritten as: $6NO_3^- + 2CH_3OH \rightarrow 6NO_2^- + 2CO_2 + 4H_20$ (2.3) $6NO_2^- + 3CH_3OH \rightarrow 3N_2 + 3CO_2 + 3H_20 + 6OH^-$ (2.4) $6NO_3^- + 5CH_3OH \rightarrow 3N_2 + 5CO_2 + 7H_20 + 6OH^-$ (2.5)
23	Para 2, ln 4	Corrected underlined word ...*Nitrobacter* growth rates using nitrite as the limiting substrate.
28	Ln 3	Reference: Bernardes, R.S. and Klapwijk, A. (1996). Biological Nutrient Removal in a Sequencing Batch Reactor Treating Domestic Wastewater. **Water Science and Technology**.33 (3) pp. 29-38
29	Ln 7	Lower case for "faecal coliforms" ; replace "Faecal *Streptococci*" with "enterococci"
30	Table 2.5	Corrected reference to Wong *et al.* (1990)
32	Sect.3.2.4 ln 1	State reference as APHA Standard Methods (1995)
33	Sect.3.2.5.2 ln 1	State reference as APHA Standard Methods (1995)
42	Lns 2 & 17	Replace "whom" with "who"
47	Fig 4.3	Legend for first 9 weeks "SBR cycle time = 24 hours"
54	Last line	Replace reference to Fig 4.8 with Fig 4.7
61	Para.2, ln 4	Add underlined word: " of the successive"
61	Para. 3	1st sentence rewritten: As ammonia oxidation rates increased from the day 160 to 236 batch test, thus in the day 256 batch test, it was predicted that 6 hours for the initial aerobic period was sufficient in removing of most of the ammonia.
61	Para.3, ln 7	Replace the word "were" with "was"
61	Para 4, ln 4	Replace the word "oppose" with "opposed"
64	Table 4.8	Correct μ value to 2 decimal place as "0.1524" becomes "0.15"
65	Sect. 4.12 ln 1	Express μ_{max} as 0.29 hr^{-1}
66	Fig 4.16	x-axis is S (mg/l), y-axis is S/μ (mg.h/l)
69	1st and 2nd sentence	combined 1st and 2nd sentence. ...waste was in the day 256 test, however in day 276...
69	Para 2, ln 3	Correct sentence to "Thus indicating that factors other than reaction rate affected the overall removal of nitrate."
81	Ln 1	Correct reference to New Zealand Ministry of Health (1995)

Abstract

Piggery wastewaters are particularly problematic when released untreated into the environment. They contain high levels of chemical oxygen demand (COD) and also nutrients such as nitrogen and phosphorus which can cause eutrophication in surface waters.

The sequencing batch reactor is a form of biological treatment in a completely mixed reactor with aerobic and anoxic periods to facilitate nutrient removal. In this study nitrogen removal of piggery wastewater in a SBR by nitrification and denitrification was investigated.

Screened raw piggery effluent was used in this study. Average non filtered feed contained a chemical oxygen demand of 12,679 mg /l. The average of the non filtered feed TKN was 1103 mg/l with its largest component being ammonia having an average concentration of 681 mg/l (non filtered feed).

Initial experiments with solids retention time (SRT) of 15 days and the hydraulic retention time (HRT) was 5 and 3.3 days for 9 and 4 weeks respectively during Stage 1. No significant nitrification activity was observed during this period. The reactor cycle time was then increased to 2 days which effectively increased the SRT to 30 days and HRT to 6.7 days (Stage 2). The new environment allowed the nitrifying population to develop and nitrification was observed with the formation of nitrite and nitrate.

The heterotrophic kinetic constants determined the yield coefficient as 0.49. The maximum specific growth rate (μ_{max}) was 6.8 day^{-1} and half saturation constant (K_s) was 293.6 mg/l.

The COD removal of the feed in the SBR started from around 70% in weeks 6-10 during Stage 1 and reached 92.7% in week 29. Ammonia removal was not significant in the first 17 weeks due to no significant nitrification activity during that time. After initiating a 2 day reactor cycle, ammonia removal rates increased to over 90%.

Batch tests indicated that most of the ammonia needed to be remove in the first aerobic period. This allows nitrite and nitrate concentrations to build up and be removed by the subsequent anoxic period. This was when there was enough readily degradable COD as not to inhibit denitrification.

The reactor cycle time which achieved full nitrification and the highest nitrate removal by denitrification was observed in the batch test on day 256. The first 6 hour aerobic period removed 81.1% of the ammonia. Subsequent anoxic periods reduced the nitrate concentration in the effluent to 11.0 mg N/l.

The nitrification rates increased in the reactor over time as the nitrifying population acclimatised to the piggery effluent. In fact the highest nitrate formation and ammonia oxidation rate was 15.5 mg N/l. h and 24.6 mg N/l.h measured during the last test on day 270. Nitrite formation rates peaked at 11.5 mg N/l.h. The SBR biomass population was able to remove nitrate efficiently as batch tests showed that denitrification rates could reach 22.1 mg N/l.h.

The relationship between effluent nitrate levels and COD: ammonia concentration ratio was assessed in order the determine the importance of these chemical characteristics important in controlling the nitrification and denitrification activity in the SBR. Results showed that as the COD: ammonia concentration ratio increases, the effluent nitrate levels decreases.

The study found that the SBR was suitable in removing COD and Nitrogen from piggery wastewater.

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Contents

Chapter 1
Introduction

Wastewaters from animal farms have traditionally been discharged into surface waters or spread onto land to supplement the nutrients in the soil. However such practices can lead to algal blooms resulting in eutrophication in water bodies (Davies-Colley, 1996). Environmental awareness and legislation have been the forces that have pushed for nutrient removal in wastewater treatment. Of all nutrients, nitrogen and phosphorus, are normally the limiting macronutrients for cell growth. Of particular interest is nitrogen removal in piggery waste as it contains high concentrations of nitrogen.

Piggery waste waters consist mainly of pig faeces and urine. The waste also contains bedding and spilled feed material (Littlejohn, 1974). They are usually removed with washwater into a storage tank for further treatment. Nitrogen in the form of ammonia concentrations is particularly high due to urea in urine in the piggery wastes that easily decompose in the process of deamination. Due to the pig's type of food a lot of difficult to biodegrade material is found due to fibrous material and husks.

Due to the high Chemical Oxygen Demand (COD) of the piggery wastes, the treatment of these wastes is normally by anaerobic pond treatment. Anaerobic processing allows some of the COD to be removed without adding energy. It is cheap, needing minimal mixing and no air is required. After primary treatment some farms pump the more stabilised wastes onto land.

Further secondary treatment to remove the remainder of the COD and nutrients can be done by aerobic treatment in a completely mixed reactor. Different methods of aerobic biological treatment have been studied extensively to remove nitrogen.

1.1 Water Quality Standards

In terms of the ammonia concentrations, the drinking water guidelines stated in New Zealand (1995), recommend that it should not exceed 0.01 mg/l. Maximum permitted nitrate and nitrite concentrations in discharges to natural waters are 50 mg/l and 3 mg/l respectively. The Resource Management Act guidelines indicate that dissolved oxygen of the discharging water body should not fall below 5 g/m^3. In terms of clarity, the water parameter used is suspended solids and the guideline given is that discharges should not exceed 4 mg/m^3. In the RMA guidelines, faecal coliforms should not exceed 200 cfu/ 100 mL for bathing water or 1000 cfu/100ml for use in crop irrigation (Davies-Colley, 1996).

Regional councils are in charge of issuing agricultural wastewater discharge permits according to their standards which must not be below the minimum stated in the Resource Management Act. In general any Permitted Activity (i.e. discharges) after reasonable mixing should, according to section 70 of the Act, not produce the following effects:

1) c) Any conspicuous oil and grease films, scums or foams, or floatable or suspended materials
   d) Any conspicuous change in the colour or visual clarity
   e) Any emission of objectionable odour
   f) The rendering of fresh water unsuitable for consumption by farm animals
   g) Any significant adverse effects of aquatic life

The Manawatu- Wanganui regional council indicate that the 5-day Biochemical Oxygen Demand (BOD_5) of receiving water should exceed 2 g/m^3. The ammonia content must not exceed 1.1 g/m^3 at temperatures at or below 15°C, and it must not exceed 0.8 g/m^3 at temperatures over 15°C. The phosphorus levels should not exceed 0.015 g/m^3 (Forsyth, 1996).

1.2 Objectives

The project focuses on the measurement of nitrification rates using piggery effluent in order to assess the ability of biological nutrient removal using a sequencing batch reactor to treat this waste.

To evaluate the literature on sequencing batch reactors, nitrification and related studies on the treatment of piggery waste.

To acclimate a culture of micro organisms in a SBR to a piggery waste feed.

To determine the heterotrophic kinetic parameters.

To treat the piggery waste using a SBR program and to monitor the parameters of the feed, effluent and mixed liquor required in determining the nitrification rate at different sequencing batch programs.

Establish the optimal conditions for piggery waste treatment in a SBR by comparing influent and effluent concentrations of chemical oxygen demand , total kjeldhal nitrogen , ammonia, nitrite and nitrate.

Chapter 2

Literature Research

2 Piggery Waste

2.1 Components of pig waste

Piggery wastewater includes pig faeces and urine that have been washed with water from pig sties. Normally they contain large amounts of total suspended solids. A high percentage of dry matter (24%) is present in faeces. Another significant component is crude fibre constituting 16.5% of the dry matter (Littlejohn, 1974). Crude fibre is insoluble and does not absorb water.

There are also fine particulates produced from undigested food residues, cells from the digestive tract and gut micro-organisms which constitute the other large part of the insoluble solids found in faeces. The fine particulates have a high COD and nitrogen content.

Dissolved solids also have high nitrogen and phosphorus concentrations. Metals such as copper and zinc are often found in the wastewaters, as these are present in food supplements fed to the pig.

Urine, on the other hand, contains low dry matter content and is readily decomposable releasing ammonia during storage. They contribute a large amount of ammonia to pig waste.

2.2 Chemical Components Characteristics

Pig wastes are highly variable and are strong in strength as they command a high amount of Chemical Oxygen Demand (COD). The COD of crude swine waste was measured as 29900 mg/l compared to 15800 mg/l for the supernatant of centrifuged waste and 3300 mg/l for stabilised waste (Blouin *et al*, 1989). Nitrogen concentrations are also high within the waste. The total kjeldahl nitrogen (TKN) concentration was measured as high as 4760 mg/l for crude waste and ammonia of 2856 mg/l for the supernatant of centrifuged waste by the same study.

Andreotolla *et al.* (1997) used pre-screened piggery waste as feed in a sequencing batch reactor. Their reported influent piggery waste values show total COD to be 19140 mg/l and soluble COD to be 6800 mg/l. TKN and ammonia values were on average 2060 and 1165 mg/l respectively. Examples of the large variations in the chemical composition depending on the initial treatment and sources of waste are shown below in Table 2.1.

Table 2.1 Summary of Characterisation of Piggery Waste in Literature

Parameter	Untreated Sow Manure	Screened	Screened by 0.1 mm mesh	Centrifuged (supernatant)	Diluted	Diluted and Screened	Diluted and Anaerobically treated
NH_4^+ (mg/l)	2100	1165	1265	844	330	ND	ND
NO_2-,NO_3-N (mg/l)	ND	ND	6.2	ND	0.56	ND	ND
TKN (mg/l)	3300	2060	2580	1258	ND	ND	ND
P (mg/l)	900	ND	ND	236	ND	ND	ND
BOD (mg/l)	7700	ND	ND	ND	507	1294	1075-1167
COD (mg/l)	29000	6800-19140	31175	3710-10580	2347	7106	2028-2194
TSS (mg/l)	3400	12010	10690	4430	3199	3467	380
Reference	Ten Have *et al.*(994)	Andreottola *et al.*(992)	Fernandes *et al.*(1991)	Bortone *et al.* (1992)	Lo *et al.* (1990)	Wong *et al.* (1990)	Ng (1987)

BOD-Biological oxygen demand
COD-chemical oxygen demand
NH_4^+-Ammonia
NO_2, NO_3-N -Nitrite and Nitrate Nitrogen
TKN-Total Kjeldhal Nitrogen
TSS-Total Suspended Solids
P -Phosphorus
ND- no data

2.3 Bacteria composition

Ginnivan (1983) found high levels of Faecal Coliforms and S*treptococci* in piggery waste. Faecal Coliforms ranged from 1.3 * 10^5 ml^{-1} to 7.2 *10^6 ml^{-1} . The faecal *Streptococci* numbers ranged from 2.6 * 10^5 ml^{-1} to 4.7 * 10^6 ml^{-1}. Blouin *et al.* (1991) indicated that there were low concentrations of nitrifying bacteria in swine waste. The highest concentration of *Nitrosobacteria* was 9.9 *MPN/ml and the highest concentration of *Nitrobacteria* measured was 2800 MPN/ml. These concentrations are low compared to inoculations of 10^6- 10^7 MPN/ml of bacteria in the waste.

2.4 Biological Nutrient Removal

Aerobic oxidation requires bacteria in order to catalyse the break down of the carbonaceous organics in waste. Bacteria assimilate nutrients as shown in the following equation:

organic material + oxygen + nutrients = CO_2 + H_20 + new cells + nutrients + energy

(Mason & Ellwood, 1996)

The nutrients, nitrogen and phosphorus, are macronutrients required for cell growth. However, in excess amounts they cause unsightly algal blooms that clog up water bodies. The degradation of algae results in the depletion of dissolved oxygen. Therefore, wastewater treatment must not only address the removal of carbonaceous organic material but also these nutrients.

[1] * Most Probable Number

2.5 Nitrogen Removal

The main forms of nitrogen in raw piggery wastewater are ammonium and organic nitrogen generally termed total kjeldhal nitrogen (TKN). Lo *et al.* (1990) and Fernandes *et al.* (1991) showed that nitrate and nitrite concentrations were below 7 mg N/l in piggery wastewaters.

Ammonia nitrogen is important in animal wastes due to a high concentration of urine. The ammonia originates from the decomposition of urine, and from organically bound nitrogen by extra-cellular biochemical action.

Nitrogen removal occurs in two processes. Firstly the ammonium nitrogen is oxidised to nitrate during nitrification. Nitrate is then converted to gaseous nitrogen in denitrification in order to remove nitrogen from the system.

Ammonia nitrogen is also used in cell assimilation, but eventually the nitrogen will return to the system during cell decay. Chemotrophs such as nitrifying bacteria need large amounts of energy to change carbon dioxide to cells and have a low cell yield. However heterotrophs have higher biomass yields, thus using more nitrogen in assimilation. It was predicted that $^{1}/_{3}$ of the TKN which is the sum of organic nitrogen and ammonia nitrogen is removed by assimilation of cells in domestic sewage at non-nitrifying loading rates (Barnes & Bliss, 1983).

2.6 Nitrification

The nitrification process is spilt into two parts. The first stage is performed by the group of nitrifiers termed *Nitrosomonas*. In this process called nitritisation these nitrifying bacteria oxidise ammonia into nitrite as described in equation 2.1. The nitrite produced in this reaction does not accumulate under optimum conditions but is oxidised further into nitrate by the bacteria, *Nitrobacter,* in the process of nitratisation (Equation 2.2). The reactions show that the process is aerobic, and acid is produced during nitritisation. Therefore in order to sustain a nitrification system, a high alkalinity environment needs to be maintained by controlling the pH.

$$2NH_4 + 3O_2 \rightarrow 2\ NO_2 + 4H^+ + 2H_20 + \text{Energy} \qquad (2.1)$$

$$2NO_2 + O_2 \rightarrow 2NO_3 + \text{Energy} \qquad (2.2)$$

2.7 Denitrification

Nitrogen is removed from wastewater by denitrification in which nitrate in the wastewater is reduced to nitrogen gas and released into the atmosphere. This occurs without oxygen and the heterotrophic bacteria utilises the nitrate as an electron acceptor instead of oxygen. An organic carbon source is needed for this reaction. The reduction of nitrate ions to nitrite ions using methanol in the equations 2.3 - 2.5 below show how organic carbon is oxidised during this process.

$$NO_3^- + {}^1/_3CH_3OH \rightarrow NO_2^- + {}^1/_3CO_2 + {}^2/_3H_20 \qquad (2.3)$$

$$NO_2^- + {}^1/_2CH_3OH \rightarrow N_2 + CO_2 + {}^1/_2H_20 + OH^- \qquad (2.4)$$

$$NO_3^- + {}^5/_6CH_3 \rightarrow N_2 + {}^5/_6CO_2 + {}^7/_6H_20 + OH^- \qquad (2.5)$$

During biological treatment, if the anoxic or anaerobic phase occurs after the aerobic phase, then carbon levels may have already been depleted and external carbon sources

such as methanol are needed. Molasses has been used as an inexpensive carbon sources for denitrification treatment of sow manure (Ten Have *et al.* 1994).

2.8 Conditions for Biological Nitrification

Nitrifying bacteria are dominantly chemoautotrophs. These are organisms that obtain energy from the chemical oxidation of inorganic molecules such as ammonia or nitrite. A net input of energy is required to convert carbon dioxide to cell tissue which is a reductive process. Therefore they need more energy for cell growth and has a lower yield than heterotrophs that use organic matter as a carbon source (Metcalf & Eddy, 1991).

The growth of nitrifying bacteria is favoured by a moderately high sludge age as it allows the lower yield nitrifying population to accumulate to significant proportions. The SRT often used in a SBR ranges from as little as 7 days to 20 days for treating piggery wastewaters. Fernandes *et al.* (1991) used a 20 day SRT in their sequencing batch reactor treating pig waste. Bortone *et al.* (1992) used a SRT of 11-15 days. Moreover, Bortone *et al.* (1994) also showed that increasing their sludge age from 19.8 to 38.6 days counteracted the effect of some inhibitory compounds in industrial treated wastewater. Nitrification is one of the most sensitive processes to environmental perturbation in the biological treatment process. The factors controlling efficiency of the process are discussed in the following sections.

2.9 Temperature

Studies have shown that nitrification kinetics are affected by temperature. Chudoba and Pannier (1994) showed increases in nitrification rates from 3.08 mg N/g.h at 10 °C to 7.8 mg N/g.h at 20°C. The rates follow an Arrhenius type response according to Hall & Murphy (1985). However, Fernandes (1994) indicated that temperature had minimal effect over normal operating temperature ranges (10-25°C) found at treatment plants. Nevertheless he did indicate that there was a slight increased process efficiency when

increasing temperatures between 5-21°C. One experiment in this study, examined the rate of oxidation, hydrolysis and assimilation of ammonia measured in the variation of ammonia/ ammonium (NH_3-N/NH_4^+-N) concentration at different temperatures. The results showed the rate increased from 7.6 mg/l/h at 5°C to 12 mg/l/h at 21°C. Overall Fernandes (1994) showed that the COD and ammonia removal were 97% and 99.8% at 21°C and only decreased slightly to 96.7% and 97% removal at 10°C. However they did indicate that the process was severely limited at temperatures at and below 5°C.

2.10 Dissolved Oxygen

Dissolved oxygen (DO) values are important in nitrification as the value of half saturation constants (K_s) for oxygen are relatively high, compared to the DO levels in the reactor and low K_s values of ammonia. Therefore oxygen can act as a limiting factor in the process. In heterotrophic systems, the K_s values for oxygen are relatively small compared to the DO in the system thus this is not considered a limiting factor (Grady & Lim, 1980). Barnes and Bliss (1983) summarised that the critical dissolved oxygen values required for normal nitrification was 0.2 mg/l. Mason & Ellwood (1996) using stoichiometric demand of ammonia on oxygen, calculated that a dissolved oxygen level needed to be maintained above 1.0 g/m^3 in order to give a satisfactory nitrification performance.

2.11 pH

Nitrifiers are particularly sensitive to pH concentrations. The optimum values for these micro organisms range from pH 7.5 to 8.5. The pH of the nitrification system needs to be controlled as the process destroys alkalinity which then lowers the pH and destabilises the process. On the other hand, the denitrification system liberates alkalinity. Not only does the pH affect the nitrifiers but it also controls the concentration of inhibitors in the system

for example the non-ionised forms of substrate nitrogen. The amount of other inhibitors such as free ammonia and nitrous acid are also controlled by pH (Barnes & Bliss, 1983).

Often the optimum range of pH values for nitrifier growth are hard to estimate in a plant as the bacteria can acclimatised to lower pH values. Therefore pilot tests are normally done in order to assess the efficiencies of sludge in a particular plant. In the study conducted by Bortone & Piccinini (1991), a pH below 6.5 slowed down the nitrifying activity as the oxygen uptake rates were much lower and they had high concentrations of nitrite (453 mg/l) .

2.12 Substrate effects

The microbes responsible for nitrification are subject to substrate variations. Studies have disagreed on the amount of inorganic nitrogen levels which will inhibit nitrification. Some have quoted 100 mg/l while others have shown a concentration of 1000 mg/l to be non limiting. Blouin *et al.* (1990), indicated that ammonia concentrations as high as 1200 mg/l were not limiting to nitrification. However, calcium carbonate was added to favour the development of nitrifying bacteria thus accelerating the oxidation of ammonia in the wastewater to nitrite. In a study conducted by Mahne *et al.* (1996) the nitrifiers were able to adapt to high ammonium concentrations and nitrification occurred at ammonium concentrations as high as 3000 mg/l. Generally nitrification inhibition is expected from animal wastes that have high ammonia nitrogen due to urine decomposition, but inhibition can also be lessened by acclimatisation of the microbes.

2.13 Toxic Substances

Most of the toxic materials inhibiting nitrification contain nitrogen or sulphur. These include substances such as thiourea and cyanide which have a greater effect on *Nitrosomonas* than *Nitrobacter*. In particular, allylthiourea at 5 mg/l is frequently used as an inhibitor in order to measure rates of ammonia oxidisation (Surmacz-Gorska *et al.*, 1995). Substances that are more toxic to *Nitrobacter* than *Nitrosomonas* include potassium chlorate, sodium cyanate and hydrazine sulphate. The long term effects of these inhibitors cannot be estimated as the sludge again can become accustomed to these compounds to a certain extent (Barnes & Bliss, 1983).

Heavy metals are also major inhibitors of nitrification. Copper was shown to inhibit ammonia oxidation in concentrations as low as 0.5 mg/l (Barnes & Bliss, 1983). Industrial wastewaters frequently have silver, mercury, nickel and other metals which are capable of reducing the rate of this process. Animal wastes often contain small concentrations of heavy metals originating from food supplements given as feed (Littlejohn, 1974).

2.14 Other factors

It has been found that high light intensity in the reactor inhibits nitrification, however this is not a major problem in the case of piggery effluents due to their turbid nature. Nitrifiers have been found attached to surfaces in river beds, suggesting that nitrifiers will attach to surfaces that are available to them (Barnes and Bliss, 1983). Therefore the abundance of surfaces will attract nitrifying activity.

2.15 Nitrite accumulation

The nitritisation reaction is often the rate controlling reaction during nitrification as it is controlled by the amount of ammonia as substrate. However there are instances in biological reactors when the nitrification process is not completed and nitrite accumulation occurs. Picioreani *et al.* (1997) suggested that high pH values and high free ammonia concentrations inhibited *Nitrobacter* activity. Rhee *et al.* (1997) also agreed that the presence of free ammonia at 0.35 mg N/l inhibited the nitrite oxidisers as they are already sensitive to stresses in the environment such as pH or high concentrations of toxic chemicals.

Yang & Alleman (1992) also suggested that limited oxygen supply, the presence of inhibitors, high solids content and acute process loadings would result in nitrite build-up. Their study found that low DO levels of 0.5 mg/l caused nitrite build up when the pH was controlled between 7.3-7.8. High pH of 8.0 and 8.5 also showed nitrite build up when DO levels were low. When the pH was controlled at 7, low DO levels did not affect the formation of nitrate from nitrite in this case. They concluded from their results that DO and pH were not the most important factors in controlling nitrite build up but the amount of unionized hydroxylamine was the cause under low DO levels and high pH conditions.

Other studies such as Ng (1987) indicated that nitrite accumulation occurred in their SBR. Ten Have *et al.* (1994) found that even with over 99% ammonia nitrified in an activated sludge system with separate nitrification and denitrification tanks, nitrite accumulation was observed at the start of their experiment. However after 80 days, the nitrifiers were able to acclimatise themselves to the environment and nitrate formation was completed.

2.16 Nitrification Kinetics

The growth kinetics of nitrifiers are important in the determining the nitrification rates. Nitrification, denitrification and phosphorus removal processes can be defined and controlled in order to obtain optimal operating conditions for nutrient removal in biological treatments. In defining nutrient removal rates, an important factor to consider is the amount of oxygen needed so that costs for aeration can be minimised. Another factor to consider is toxic inhibition meaning a longer reactor time is necessary to complete nitrification (Surmacz-Gorska *et al*, 1995).

Assuming nitrification to be limited by one substrate, it may be described by Monod type kinetics as shown in equation 2.6. In terms of nitrification kinetics, the Monod type equation can be modified to equation 2.7. K_m is defined as *Nitrosomonas* half saturation constant. This equation can also be used for *Nitrobacter* growth rates using nitrate as the limiting substrate.

$$\mu = \frac{\mu_{max}\, S}{K_s + S} \qquad (2.6)$$

$$\mu = \frac{\mu_{max}\, (NH_4^+ \text{-}N)}{K_m + (NH_4^+ \text{-}N)} \qquad (2.7)$$

μ = specific growth rate
μ_{max} = max. specific growth rate
K_s = half saturation constant
S = substrate concentration
NH_4^+-N = ammonia nitrogen concentration
K_m = *Nitrosomonas* half saturation constant

As described in section 2.10 the dissolved oxygen concentration significantly affects nitrification. The above equation can be modified to equation 2.8 to take this into account.

$$\mu = \frac{\mu_{max}\, (NH_4^+ \text{-}N)}{K_m + (NH_4^+ \text{-}N)} * \frac{DO}{K_o + DO} \qquad (2.8)$$

K_o = half saturation constant of Oxygen
DO = Dissolved oxygen concentration

The typical values of kinetic coefficients for nitrifying bacteria are shown in Table 2.2

Table 2.2 Typical Kinetic Coefficients for Nitrifying Bacteria (Barnes & Bliss, 1983,)

Coefficient	*Nitrosomonas*	*Nitrobacter*
(Y) Yield coefficient g cells (g NH_4^+ - N oxidised)$^{-1}$	0.03 - 0.13	0.02 - 0.08
μ_{max} maximum. specific growth rate (d^{-1})	0.46 - 2.2	0.28 - 1.44
K_s (mg/l) (energy substrate) half saturation constant	0.06 - 5.6	0.06 - 8.4
K_o mg/l	0.3 - 1.3	0.25 - 1.3

2.17 Measurement of Nitrification Rates

Nitrification rates have been measured by monitoring oxidised nitrogen levels and ammonia levels. The gradients of ammonia levels can describe ammonia lost to nitrification. However Sozen *et al.* (1996) stated that this method does not account for ammonia used for assimilation of cells.

Batch tests have been done to evaluate nitrification rates. They are simple to perform and are not affected by hydraulic effects. Chudoba & Pannier (1994) performed batch tests using 2 litre beakers and measured biomass, nitrification substrate and product concentration. Table 2.3 shows that they obtained nitrification rates of 5.16-7.14 mg N/g Mixed Liquor Volatile Suspended Solids per hour for municipal waste. Nitrification rates from other studies are summarised in Table 2.3.

In another study, oxidised nitrogen levels were monitored because they are directly a result of the nitrification processes, and can show the rate of this process. Sinkjaer *et al.* (1994) and Harremoes & Sinkjaer (1995) utilised batch tests to gain estimates of maximum nitrification rates of the activated sludge, utilising a known amount of ammonium as substrate. To gain the actual nitrification rates for the process, monthly

cycle tests were done whereby samples were taken directly from the reactor over 24 hours. In order to monitor any changes in nitrification in the day to day running of the reactor, samples were taken at certain aerobic or anoxic periods within the cycle (mini cycle tests). The aerobic period was monitored during these tests in order to monitor nitrification rates to determine if there were nitrification inhibition.

Another method is to use oxygen uptake rates with nitrification inhibitors in order to assess the nitrification activity of a sequencing batch reactor. By measuring differences in the oxygen uptake rates with and without inhibitors, the activity of the two stages of nitrification can be successfully monitored. Surmacz-Gorska *et al.* (1995) indicated high nitritisation activity when ammonia concentrations were high. Consequently the nitratisation activity was low during high nitrite concentrations.

As well as measuring the actual nitrification rates, the kinetics of the biomass in the activated sludge can be investigated. Cech *et al.* (1985) measured the oxygen uptake rate and Orhon *et al.* (1994) utilised the oxygen uptake rate and nitrogen uptake rate to determine the maximum specific growth rate of heterotrophic biomass in the aerobic and anoxic phases. This can also be done for autotrophic growth. By adding inhibitors, the growth due to nitrifiers can be distinguished.

Table 2.3 Summary of Nitrification Rates in Literature

Waste	Nitrification rate	Research
Municipal Waste	5.16 - 7.14 mg N/ g MLVSS. h	Chudoba & Pannier (1994)
Domestic waste	0.85 - 1.18 mg N/ gVSS.h	Harremoes & Sinkjaer (1995)
Synthetic waste	*2.58 mg N/ g MLSS/h	Rhee *et al.* (1997)
Centrifuged piggery effluent	*1.45 mg /g VSS h	Bortone *et al* (1992)
Synthetic waste similar to piggery wastewater	177-218 mg N/l.d	Mahne *et al.* (1996)

*Nitrification Rate Calculated from Ammonia oxidation. Other Rates calculated from NOx formation

2.18 Measurement of Denitrification rates

Bortone *et al.* (1994) evaluated denitrification rates with industrial wastes using batch tests. Their batch experiments were put under anoxic conditions by bubbling pure nitrogen gas through the vessel. Filtered feed was added as the external carbon source. They found that denitrification rates measured by this test were comparable to the rates calculated from the reactor itself.

2.19 Sequencing Batch Reactor

2.19.1 Description

An SBR is a fill and draw type reactor with a single completely mixed vessel. The activated sludge remains in the reactor through all the above cycles (Metcalf & Eddy, 1991). Operation can be modified to perform nitrification, denitrification and even phosphorus removal.

The SBR cycle generally consists of Fill, React, Settle and Idle periods. The fill period is the time when the feed is added to the reactor. The length of this period may govern the efficiency of nutrient removal and reduction of coliforms. However it reduces the time for the react stage (Irvine *et al*, 1987).

The react period, employing aeration and mixing follows the fill period. Carbon removal by the heterotrophs occurs during this period. Nitrification is also an aerobic process as the nitrifiers only use oxygen as a terminal electron acceptor. The main biological reactions components in the SBR react stage are summarised in Table 2.4

Table 2.4 Biological reactions in React Stage in SBR (Fernandes, 1994)

Substances	**Biological Reactions**
NO_x-N Oxidised Nitrogen	Nitrification
NH_4^+ -N Ammonia nitrogen	Hydrolysis and ammonificaiton of organic nitrogen under aerobic conditions ; assimilation ; nitrification
COD Chemical Oxygen Demand	Degradation (oxidation and assimilation)

The react stage is generally followed by a settling period with no aeration or mixing thereby allowing the sludge to settle. The settling phase is frequently between 0.5 - 1.5 hours to allow removal of effluent without affecting the sludge blanket (Mason & Ellwood, 1996). The effluent is drawn out after the settling phase.

There may be an idle period where mixing occurs and the air may be on or off. If only slight mixing occurs this phase is generally an anoxic period. During anoxic periods, denitrification occurs when heterotrophic bacteria use the nitrates formed by nitrification as an electron acceptor instead of free oxygen. Denitrification completes the nitrogen nutrient removal as nitrogen gas is produced and released.

Sludge wasting can occur during the react stage, draw or idle stages. Usually sludge is wasted at the end of the react stage in order not to deplete the biomass needed for the react stage and the reactor is completely mixed at that time for consistent sludge wastage.

In terms of reactor kinetics, a SBR system acts like an ideal plug-flow reactor. Carbon removal, nitrification, denitrification and clarification can be achieved in one reactor. The SBR also allows for a certain amount of flow equalisation and is able to cope with peak loads (Ng, 1987; Mason & Ellwood, 1996). This robustness arises because batch systems do not operate under steady-state conditions. Thus SBR processes can be easily controlled to treat shock loads (Dennis & Irvine, 1979).

2.19.2 Nutrient Removal by SBR from other wastes

Sequencing batch reactors have been successful in treating domestic wastes shown by Irvine *et al.* (1987) and Bernardes & Klapwijk (1996). Irvine *et al.* (1987) indicated nitrogen removal capabilities as high as 80% with an anoxic period. Bernardes & Klapwijk (1996) also showed phosphorus release from the sludge and reduction of phosphate in their double reactor set up.

Sequencing batch reactors are also flexible as the stages of treatment can be easily changed. Surmacz-Gorska *et al.* (1995) found that a sequence of short anoxic and aerobic stages were economically viable as it allows denitrification when there were inherent carbon sources that have not been utilised during a long aerobic reaction time.

2.19.3 Removal of high strength wastes

Mahne *et al.* (1996) showed that high strength pig wastes can be treated by activated sludge in order to remove the nutrients. They demonstrated that the nitrification inhibition of high ammonia concentrations can be overcomed by acclimatising the biomass. Ten Have *et al.* (1994) also demonstrated nutrient removal can be achieved with untreated sow manure in a series of completely mixed reactors. They enhanced denitrification by adding an external carbon source during the anoxic period. Blouin *et al.* (1990) removed inhibition of high ammonia content by adding calcium carbonate to accelerate the oxidation of ammonia into nitrite. These studies indicate that biological nutrient removal using activated sludge is capable of removing high strength wastes under suitably controlled conditions.

2.19.4 Nutrient removal in SBR from Piggery Wastes

The oxidation of carbon can be shown by the decreasing concentration of the COD. For dilute swine waste, Lo *et al.* (1990) demonstrated COD removals of between 32 to 42 percent. Where the influent filtered feed was 2297 mg/l, the resultant effluent after the first sequencing batch reactor showed a COD of 1570 mg/l. Ng (1987) also showed 48% removal for diluted or anaerobically treated pig waste. Systems investigated by Bortone *et al.* (1992) using high strength pig waste showed as much as 93% removal of COD. In addition, more than 98% reduction in the Faecal *Streptococci* and Faecal Coliforms were recorded by Ginnivan (1983).

Wong *et al.* (1990), compared the performance of an aerated lagoon system already in place with a pilot sequencing batch reactor plant. The BOD_5 removal increased from over 85% in the aerated lagoon to over 99% in the sequencing batch reactor. The SBR not only improved effluent quality but also saved space as only a single basin was required for this plant. Similar BOD_5 removal results were obtained from Ng (1987).

Removal of ammonia has been demonstrated by Lo *et al.* (1990). They achieved a reduction in ammonia from 300 mg/l to 43.6 mg/l using three SBRs in series. However there were high nitrite and nitrate nitrogen concentrations (296 mg/l) after the third reactor because the sequencing phases did not allow denitrification to occur.

A 98% removal of ammonia from high strength pig waste was achieved in studies done by Fernandes *et al.* (1991) who used slurry that had been screened with a 0.1 mm mesh, and the inorganic nitrogen removal was above 80%. Bortone *et al.* (1992) also found that 88-93% of the nitrogen present was removed. The results of these removal efficiencies in different studies are summarised in Table 2.5.

Table 2.5 Summary in Removal of Piggery Wastewater Components in SBR Systems

Parameter	Wong *et al.* (1991)	Ng (1987)	Lo *et al.* (1990)	Fernandes *et al.* (1991)	Bortone *et al.* (1992)
Ammonia	ND	ND	ND	98%	ND
Inorganic nitrogen	ND	ND	ND	80.3 - 93.2%	total nitrogen 93%
TKN	ND	ND	ND	81 - 93%	
P	ND	ND	ND	ND	95%
BOD_5	85%	84 - 98%	88	ND	ND
COD	97%	48 - 81%	41	95%	93%
TSS	98%	-57%	19	95%	ND

ND = no data

2.19.5 Further Investigation

The results from the literature review indicate that it is possible for nitrification and denitrification to occur with high strength wastes (Mahne *et al.* 1996). Researchers have also treated liquid swine waste in a sequencing batch reactor with favourable results (Fernandes *et al.* 1991, Bortone *et al.* 1992) .

In order to gain a better understanding and to determine optimal environmental conditions, the nitrification kinetics and heterotrophic kinetic constants need to be determined. The literature review also shows that the nitrification kinetics of piggery waste in a SBR has not been investigated in detail which is done in this study.

Chapter 3
Materials and Methods

3.1 Sample preservation

In order to preserve the samples from the sequencing batch reactor collected in this study for later analysis, sulphuric acid solution was added to keep it below pH 2. This was then kept in a refrigerator at 4°C for a maximum of 28 days. This was done in order to preserve the ammonia concentration in the sample.

3.2 Analytical Methods

3.2.1 Dissolved oxygen

The dissolved oxygen content was monitored with a YSI oxygen probe. Calibration was done every reactor cycle with the air-saturated method water calibration according to the YSI Model 57 Dissolved Oxygen Meter Instructions.

3.2.2 pH control

The pH was measured with a Hach pH probe. If the pH dropped below 7, a 0.1 N sodium hydroxide solution was added manually to the reactor in order to maintain the pH between 7-9, which are optimum conditions for the nitrification process to occur. The pH probe was calibrated with pH buffers every reactor cycle.

3.2.3 Biomass Measurement

Mixed Liquor Volatile Suspended Solids and Mixed Liquor Suspended Solids

Total suspended solids were measured by drying at 103-105°C as stated in the APHA Standard Methods (1995) 2540 D. The samples were left for 24 hours in order to remove all moisture. A 1.2 μm glass fibre filter paper with a diameter of 4 cm paper was used. The mixed liquor suspended solids as defined in the study were measured with this method.

The mixed liquor volatile solids (MLVSS) were ignited at 550°C for 20 minutes as according to APHA Standard Methods (1995) 2540E.

3.2.4 Chemical Oxygen Demand

The method used was the closed reflux colourmetric method as described in the Standard Methods (1995) 5220 D. A Shimadzu UV 1201 spectrophotometer was set at an absorbance of 600 nm. The filtered samples were filtered through GFC filter papers. Standards were produced from a 1000 mg/l stock COD solution. As the relationship between the absorbance and COD concentration of the samples are only linear from 0 to 900 mg/l COD, samples such as feed samples with large amounts of TSS and thus COD were diluted to up to 20 times with distilled water before analysis. The preserved samples were performed in triplicate.

3.2.5 Nitrogen Analysis

3.2.5.1 Ammonia-Nitrogen

Normally 50 ml of the sample was used for analysis. With high concentrations of ammonia or samples with high TSS content, 20 ml was used. The samples were preserved with sulphuric acid solution at pH 2.

The sample was distilled into a solution of 4% boric acid with a screened methyl red indicator by a Macro-kjeldhal apparatus (Buchi 323 Distillation Unit). Ammonia nitrogen was then determined with the Titrimetric method similar to that described in APHA Standard Methods (1995) 4500-NH3 C, using a Mettler DL25 auto titrator. The titrant was 0.1N Hydrochloric acid.

3.2.5.2 Total Kjeldhal Nitrogen

"Kjeldhal nitrogen" is the sum of organic nitrogen and ammonia nitrogen (Standard Methods, 1995).

Digestion of the sample was carried out with 15 ml of concentrated sulphuric acid. Two tablets (Kjeltabs S/3.5) were added as a catalyst. The digestion process took 2 hours or until the entire sample became transparent. As the piggery feed samples contained high concentrations of TSS, a 20 ml sample size was used. With effluent samples, 50 ml were digested and analysed.

The sample was then put through the Kjeldhal distillation unit as done with measuring ammonia nitrogen and titrated in the same way.

3.2.5.3 Nitrate and Nitrite

Samples were filtered through 0.45 μm sterilised filter papers to remove all solids and then injected into the Dionex DX-100 ion chromatograph with an AS 9-HC anion exchange column similar to that used in Bortone *et al.* (1992). Phosphate, sulphate and chloride concentrations were also measured at the same time. Less than 1 ml was needed for the analysis. Samples were analysed immediately or stored at 4°C after filtration without acid preservation for no longer than seven days.

3.3 Reactor

A 14 litre New Brunswick fermenter was used with a pyrex glass cylinder. Ten litres were used as the working volume. In order not to inhibit the sensitive nitrifying population, the pH of the reactor was controlled at 7-9 and the dissolved oxygen in the reactor was kept at or above 2 mg/l. The temperature was kept between 20-25 °C.

The speed of the mixer was adjusted to a lower turning speed of 75 rpm during anoxic cycles and increased to speeds of 120 rpm during aerobic cycles.

3.4 Culture and Feed Stock

The reactor was inoculated at the start with a population from the bottom of an anaerobic pond at Managh piggery farm in Feilding. Fresh feed was collected from the same piggery after preliminary screening and before it enters into the pond. Feed samples were stored below 5 °C and left to reach normal room temperature before input into the SBR. The reactor was run in batch mode for 3 weeks in order to allow for the micro-organisms that treat piggery effluent to develop. During this initial phase there was no wasting of sludge and the hydraulic residence time was 2 days.

3.5 Reactor Cycle for Initial Experiments

Once COD studies showed that the system was capable of removing COD from the waste, initial experiments commenced. The Sludge Retention Time (SRT) was set at 15 days and Hydraulic Retention Time (HRT) at 2 days and after 59 days increased to 3.3 days. The cycle consisted of an 18 hr aerobic period, and a 4 hr anoxic period followed by 1 hr aerobic and 1 hr settling. Reactor cycle batch tests were conducted where COD, ammonia, nitrate and nitrite levels were monitored throughout the cycle time.

3.5.1 Heterotrophic kinetics

The yield coefficient was determined for the heterotrophic population. In order to remove the effects of particulate matter in calculations, sequential culturing with filtered feed was performed in batch studies. The filtered feed was prepared from centrifuging raw feed at 5000 rpm for 20 minutes and then filtering it through GFC 1.2 μm paper.

Three sets of 50 ml of sludge wasted from the SBR was added to 200 ml of filtered feed in 1 litre conical flasks and agitated in a temperature controlled shaker set at 20°C for 24 hours. After twenty four hours, 50 ml of this subculture was added to fresh filtered feed similar to the previous cycle and agitated for 24 hours. This subculturing was repeated a total of 4 times in order to obtain a culture in which the MLVSS reflected the actual biomass present.

In batch experiments, 250 ml of feed were added to 50 ml of the subcultured sludge from the SBR. The Mixed Liquor Volatile Suspended Solids (MLVSS) and the filtered COD were measured at regular intervals. A 15 ml sample was taken every half an hour for the first 4 hours and then every hour until 10 hours after the start of the test. A final sample was taken at the end of twenty four hours. The yield coefficient was calculated from the MLVSS produced per COD utilised.

3.5.2 K_s and μ_{max}

The activated sludge was subcultured as discussed above with the same volume of MLVSS and filtered feed. The feed was then diluted to concentrations of 1:1, 1:2, 1:4, 1:5 and 1:10 in order to obtain feed with different starting substrate concentrations. Most dilutions were duplicated.

Batch experiments were conducted by adding 50 ml of subculture to 250 ml of filtered feed. A 15 ml sample was collected where the MLVSS and COD were measured every half an hour for the first 4 hours and then every hour for up until 10 hours. The specific growth rate μ was calculated from the MLVSS results. Based on the Monod equation, the Hanes-Woolf Plot was used to evaluate the half saturation constant K_s and maximum specific growth rate μ_{max}.

3.6 Reactor Cycle for Nitrification

Since nitrate concentrations remained low in the first 87 days of the experiment, the SRT was increased to 30 days and the HRT was increased to 6.7 days. Various combinations of anoxic and aerobic cycle times were tried in order to achieve full nitrification. Forty-eight hour cycle tests were conducted whereby the initial TKN and final TKN were measured. The ammonia, COD and nitrite and nitrate levels were also monitored throughout the reactor cycle time.

3.7 Nitrate formation

When the SBR was able to maintain full nitrification, the HRT and SRT were kept constant while the anoxic and aerobic periods within the cycle were varied to achieve maximum COD removal, nitrification and denitrification.

In order to facilitate full nitrate formation, a 4 hour anoxic period at the start of the cycle was added to allow the denitrification of the accumulated nitrite from the previous reactor cycle. This was done to ensure that the formation of nitrate would not be inhibited.

As readily degradable COD was used up early in the reactor cycle, methanol was added at the last anoxic period in some batch cycle tests. It acts as an external carbon source to allow denitrification of the nitrate accumulated in the reactor.

3.8 Determining Ammonia Volatilisation

In order to assess the amount of ammonia removed by volatilisation during the reactor cycle, the large reactor was made air tight. Air from the reactor passed through two gas bottles with 4% boric acid containing a screened methyl red indicator in order to capture ammonia gas. The sample was then analysed in the Macro-kjeldhal apparatus (Buchi 323 Distillation Unit) and ammonia concentration determined as discussed in section 3.2.5.1.

3.9 Adjusting the Reactor Cycle Times.

By observing the change in chemical parameters during a reactor cycle. A more optimal reactor cycle was determined. At the beginning of all of the cycles, a 4 hour anoxic cycle was added to reduce any concentration of nitrite and nitrate already formed from the previous batch of feed. The reactor cycle times are indicated in Appendix 3.8.

3.10 Nitrification Kinetics

A series of experiments with several cycle times were conducted with the 10 litre SBR processing piggery wastewater. The rate of nitrification was determined by the gradient when nitrate or nitrite production was directly proportional to time. This was termed the exponential phase of the nitrification process.

Due to the high concentrations of TSS and slowly biodegradable material in the raw piggery wastewater, it was not possible to measure the amount of biomass in the activated sludge in the reactor. Therefore, the specific nitrification rate could not be determined in the 10 litre fermenter.

Later when a satisfactory reactor cycle time was established which could remove ammonia and cause full nitrification, the wasted sludge from the 10 litre reactor was subcultured into a one litre pyrex glass reactor. This was done to remove fibrous material that was not readily degradable. The reactor was operated with the same 48 hour cycle time as the large reactor for 3 runs with filtered piggery wastewater, in a temperature controlled room at 20°C and the pH controlled between 7-9.

As sludge was continually wasted as done in the large reactor, the amount of unbiodegradable and slowly biodegradable material decreased thus a more accurate measure of biomass during the nitrification and denitrification process could be determined. This method allowed for the measurement of the specific rate of nitrification and denitrification.

3.11 Denitrification Test

To show that the reactor was capable of denitrification, wasted mixed liquor (750ml) after each cycle of the SBR with high concentrations of nitrate (around 200 mg N/l) was isolated in a closed 1 litre reactor and was mixed at low speed. The external source of COD added to the reactor was in the form of methanol. The experiment was carried in a temperature controlled room at 20°C.

Samples were taken every 1/2 hour for 2 hours and then once an hour. The length of the time for each batch run was dependant upon the concentration of nitrate in the reactor. The batch run tests conducted in this study ranged from 4 to 10 hours. Fifteen ml samples were collected in order not to remove a large proportion of the reactor's working volume. The sample was filtered through 1.2 μm GFC filter paper to assess for MLVSS and then filtered through 0.45 μm paper and was tested for nitrate and COD. The denitrification rate was assessed by the rate of nitrate removal.

Chapter 4

Results and Discussion

4.1 Feed characteristics

The raw piggery feed varies widely in terms of chemical oxygen demand, ammonia and suspended solids. The results presented below are separated into 2 stages. The first stage was between weeks 1 to 13 (day 1 to 87) during the preliminary experiments in the large SBR where a 1 day reactor cycle was used. No significant nitrification processes were evident during this stage which was useful in the determination of heterotrophic kinetic constants in the SBR. After 13 weeks, stage 2 with a 2 day reactor cycle began. A nitrifying population became established during this stage and nitrification kinetics of the SBR was measured. The reactor cycle times were optimised during stage 2 to achieve maximum nitrogen removal by alternating aerobic and anoxic periods to achieve nitrification and denitrification.

During stage 2 the feed strength was adjusted to achieve a consistent feed for the SBR to minimise shock loads fed to the reactor so that bulking did not occur and to produce a constant effluent quality. This was done by assessing the ammonia concentration with each batch of feed and then diluting the waste with tap water in order to keep the ammonia concentration to about 600 ppm. COD concentrations were normally 7000 - 8000 ppm for non filtered feed. Ammonia concentration was used to determine feed strength because results could be obtained immediately and from past observation it was in proportion with the strength of the waste.

Table 4.1 Stage 1 Feed Characteristics used in large SBR

Parameter	Average	Standard deviation	No. samples
Filtered Feed COD (mg/l)	3,686	1,595	22
Non filtered Feed COD (mg/l)	12,679	7,330	22
Filtered Feed Ammonia (mg N/l)	660	406	50
Non Filtered Feed Ammonia (mg N/l)	681	325	51
Non filtered Feed TKN (mg N/l)	1,103	200	7

Table 4.2 Stage 2 Feed Characteristics used in Large SBR

Parameter	Average	Standard deviation	No. samples
Filtered Feed COD (mg/l)	614	304	19
Non Filtered Feed COD (mg/l)	8,842	311	17
Filtered Feed Ammonia (mg N/l)	472	216	24
Non Filtered Feed Ammonia (mg N/l)	478	200	29
Non filtered Feed TKN (mg N/l)	641	161	7

All piggery feed samples had nitrate and nitrite concentrations below 3 ppm.

The results indicated that the feed was very high strength as shown by the COD and ammonia nitrogen concentrations. They were highly variable too as the standard deviation of the sets of data were large compared to the average values. The COD and ammonia values in stage 2 were markedly less than those in Stage 1 as a result of the dilution. The average ammonia value (681 mg N/l) was not as high as those found in screened fresh liquid swine waste used in the study conducted by Fernandes (1994). The ammonia concentration found by Fernandes (1994) was 1000-1400 mg N/l. TKN values used here were only 53% of that found in same study. The centrifuged piggery wastewater used in a study conducted by Bortone *et al.* (1992) was similar in terms of ammonia concentration to the unadjusted feed used in stage 1 of this work, as their average was 844 mg N/l.

The average filtered COD concentrations of the non diluted piggery wastewater were similar to those found in diluted piggery effluent as in the case of Lo *et al.* (1990) whom had feed with a COD concentration of 2347 mg/l. They were also similar to centrifuged wastewater used by Bortone *et al.* (1992) which had a COD of 3710 mg/l.

Non filtered feed had over 3 times higher COD values than filtered feed in Stage 1. This was mainly due to the large amount of solids (7.0 g/l) in the raw feed. The non filtered feed had high concentrations similar to those found in Fernandes *et al.* (1991) with 31175 mg/l whom used raw pig waste that had been screened and would still contain large amounts of suspended solids.

In batch studies an average of 74.4% of the filtered feed COD was used up in the initial 4 hour anoxic period and 6 hour first aerobic period in day 232, thus indicating that filtered feed contains mostly readily degradable material. However 25.6% of the COD was not readily biodegradable and part of this was due to small fibrous material remaining in the effluent. The colour of the filtered feed also ranged from yellow to green yellow depending on the batch of feed.

Stage 2 piggery feed had lower concentrations of COD, ammonia and TKN than Stage 1 feed. Non filtered feed COD was similar to that found by Wong *et al.* (1990) whom utilised piggery waste that had been diluted 20 times and had a COD concentration of 7106 mg/l. Filtered feed COD during stage 2 was 614 mg/l which was much lower than diluted waste used in Lo *et al.* (1990) although ammonia concentrations measured in Stage 2 (472 mg/l) were similar to those found in the same study. During this stage the non filtered COD concentrations were 14 times that measured for filtered COD.

The average ammonia concentrations of stage 1 were 63% of TKN on a nitrogen basis. The proportion of ammonia was 68% of the TKN values in Stage 2 for non filtered feed. The ammonia to TKN ratio was higher than those found in Fernandes (1994) where only 50% of the TKN was measured as ammonia.

4.2 COD and Ammonia at the Start of Nitrification Batch Tests

Although in Stage 2 the raw feed was diluted, its characteristics were still highly variable. Therefore, the concentrations of soluble COD (as defined by the 0.45 μm filtration) and ammonia concentration at the start of each nitrification batch tests in the reactor conducted during Stage 2 are shown in Table 4.3.

Table 4.3 Chemical Characteristics of the Filtered Fraction of Reactor Contents at Start of Nitrification Batch Tests in SBR

Batch Test (days from start of reactor)	COD Concentration mg/l	Ammonia Concentration mg N/l
160	784	154.3
166	834	117.3
232	2,293	233.8
256	1,345	135.4
276	1,147	166.3
270	701	118.0
281	758	118.0

* Shaded area denotes batch tests done in 1 litre reactors

4.3 COD and Nitrate at the Start of Denitrification Test

During denitrification batch tests conducted in a 1 litre reactor in stage 2, the COD and nitrate concentrations at time zero are shown below in Table 4.4.

Table 4.4 COD and Nitrate Concentrations of the Filtered Fraction of Reactor Contents for Denitrification Batch Tests in 1 Litre Reactor

Batch Tests (days)	COD Concentration (mg/l)	Nitrate Concentration (mg N/l)
230	1,750	19.4
270	758	95.3
275	1,767	45.0
282	701	105.6

Bortone *et al.* (1994) conducted batch denitrification tests which started with 28.2 mg N/l nitrate. Compared to the batch tests conducted in this study, the tests conducted here have sufficient nitrate concentration for determination of a denitrification rate.

4.4 Chemical Oxygen Demand in SBR

The average influent (feed) and effluent COD values from the SBR in Fig. 4.1 show that there were large variations between the filtered feed and non filtered feed due to the amount of total suspended solids in the feed. Therefore even as daily COD levels of the feed and effluent were monitored, weekly averages were determined as they were able to show the trends in the SBR.

Day 1 and the beginning of week 1 denotes when the SBR started wasting 0.75 l of MLSS every reactor cycle. After 5 weeks of a starting period, Fig. 4.1 shows that high COD concentrations in weeks 6 to 13 for filtered and non filtered feed have effluent COD concentrations as high as 4000 mg/l. This was because in stage 1 with a HRT of 3.3 days and SRT of 15 days, the SBR was unable to cope with the high strength loads shown by the high COD values. During week 13 (87 days), stage 2 commenced with a subsequent increase in the SRT to 30 days and HRT to 6.7 days. The COD values of the feed were more stable and nitrification was observed. Overall the performance of the SBR improved with a more reduced and steady effluent COD concentration.

Filtered and non filtered feed removal are discussed separately here as non filtered feed removal would mainly be physical removal by settling in the reactor. Whereas the filtered feed removal will indicate predominantly biological removal. However, there are some colloidal particles in the filtered fraction which may be physically removed by absorption onto particles and are settled out.

Figure 4.1 Average weekly COD values (mg/l) used in SBR

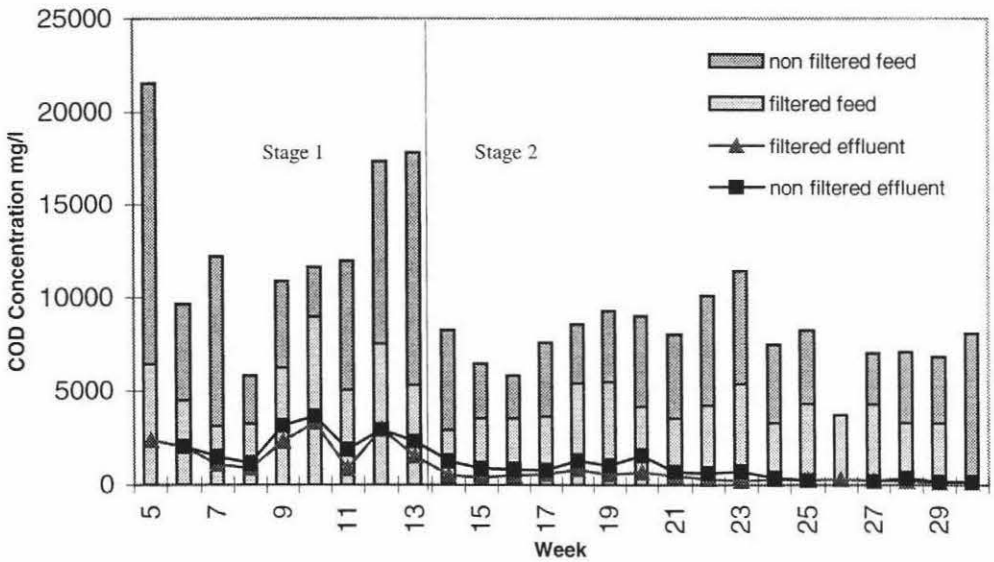


Figure 4.2 Average Weekly COD Removal of Non Filtered Feed in Reactor

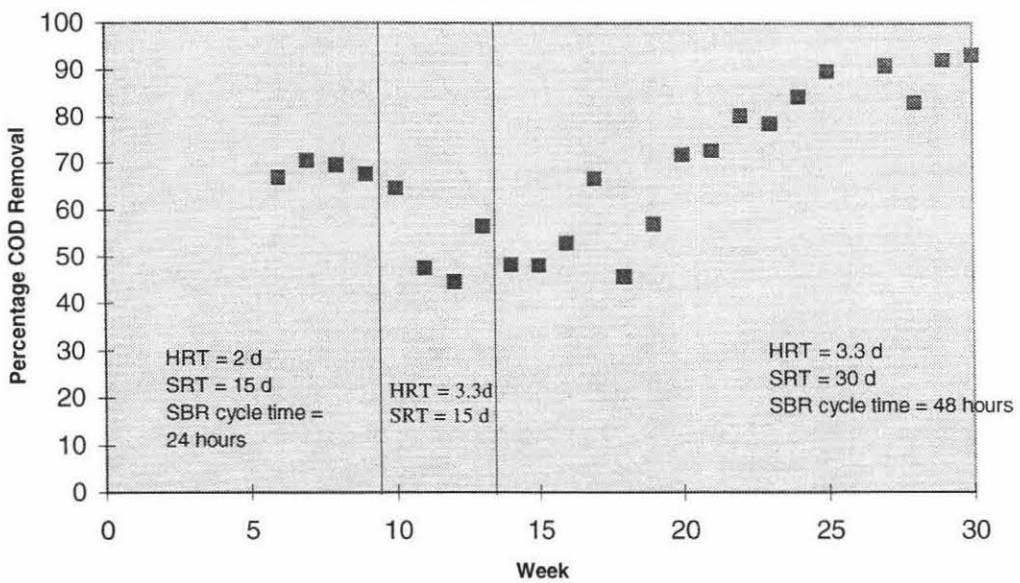


Fig 4.3 Average Weekly COD Removal Percentage of the Soluble Fraction of the Feed (defined by GFC filtration)

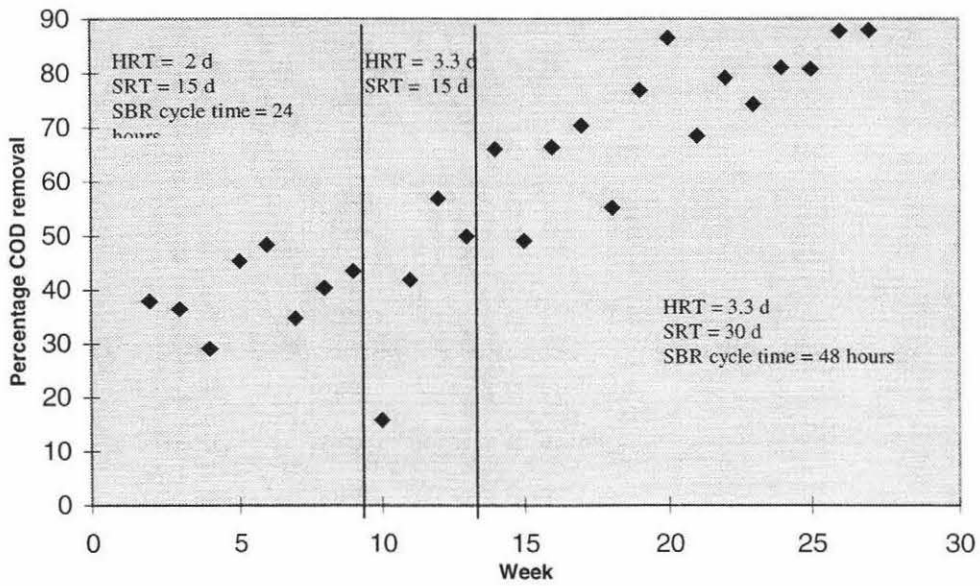


Fig. 4.2 and Fig. 4.3 show the COD removal achieved by the SBR expressed as percentages. They are computed as in example below:

Assuming all solids had settled (no solids the effluent)

COD of non filtered feed = 20000 mg/l

Input to reactor = 3 l

COD non filtered effluent = 3000 mg/l

$$\text{COD removal \%} = \frac{[(20000*3) - (3000*3)]}{(20000*3)} * 100$$

$$= 85\%$$

The average weekly COD concentrations were calculated in order to identify trends in the removal of COD. Fig. 4.2 shows that the percentage COD removal from non filtered feed was over 70% up until week 9 within stage 1 of the experiment. The removal of COD decreased to around 45% during the following weeks until week 17 when the HRT was adjusted from 2 days to 3.3 days. The drop in COD removal was caused by the solids not

settling well and remaining in the effluent. This may have been due to the reduction of feed in the reactor from 5 to 3 litres.

Fig. 4.3 shows that before 59 days (weeks 9), the soluble COD removal rates were low and did not exceed 40%. This was because the HRT was 2 days and the SBR was unable to cope with such a large volume of high strength waste. Often the activated sludge would not settle which indicated that the SBR was bulking. In fact solids were removed from the SBR when bulking was observed in order to remove suspended solids and organisms causing unsettling of the mixed liquor. During this period, the effluent often contained some biomass and increased the COD of the effluent.

During weeks 9 - 13 (days 59 to 87), when the HRT was increased to 3.3 days the removal rates remained low as the COD loads were still high. As the HRT was further increased in week 13 to 6.7 days and SRT to 30 days with a two day reactor cycle time, the COD removal increased to over 60% for both the filtrate fraction and the non filtered liquid. It took four weeks after week 13 for the SBR to acclimatise to the new environment and for significant COD reduction.

After week 17 the SBR was able to acclimatise to the COD loading and removal continued to increase to 88.2% by week 29 in the filtered feed. A similar pattern was observed with non filtered feed with removal increasing to 92.7% in the 29^{th} week.

The removal rates of these SBR studies were similar to those found by Bortone *et al.* (1992) who obtained average COD removals of 93%. Their effluent was a yellow colour containing 300 mg/l of soluble COD. Their study used centrifuged piggery wastewater which would have removed most of the TSS in the waste at the start thus explaining why their removal rates were similar to soluble COD removal rates in this study.

Ng (1987) used anerobically treated piggery effluent in a SBR with a HRT of 1.3 days and with no systematic removal of MLSS as they were left to be removed when decanting the effluent. The SBR in this case removed an average of 81% soluble COD

which is similar to the removal of filtered COD found in this study. Their piggery wastewater had similar characteristics such as the large amounts of TSS as in the raw piggery feed which was diluted and used in this study. Ng (1987) had problems with suspended solids in the effluent as their total COD removal rate was only 48% which they attributed to VSS in the effluent. A better result was obtained from this study with a high removal rate of 92.7% which was observed in week 29 when the SBR had adapted to the COD loading and the MLSS settled well.

Fernandes (1994) used screened liquid swine manure treatment in an SBR with a HRT of 9 days and SRT of 20 days. His sludge age was shorter but the HRT was longer compared to a HRT of 6.7 days in this study. COD removal of non filtered feed ranged from 93 to 97% which was similar to the COD removal of 92.7% measured in this study for non filtered feed.

4.5 Ammonia Removal

The ammonia removal percentages were calculated as shown in the example below:

Ammonia of non filtered feed = 300 mg N/l

Feed input = 3 l

Ammonia non filtered effluent = 5 mg N/l

$$\text{Ammonia removal \%} = \frac{[(300*3) - (5*3)]}{900} * 100$$

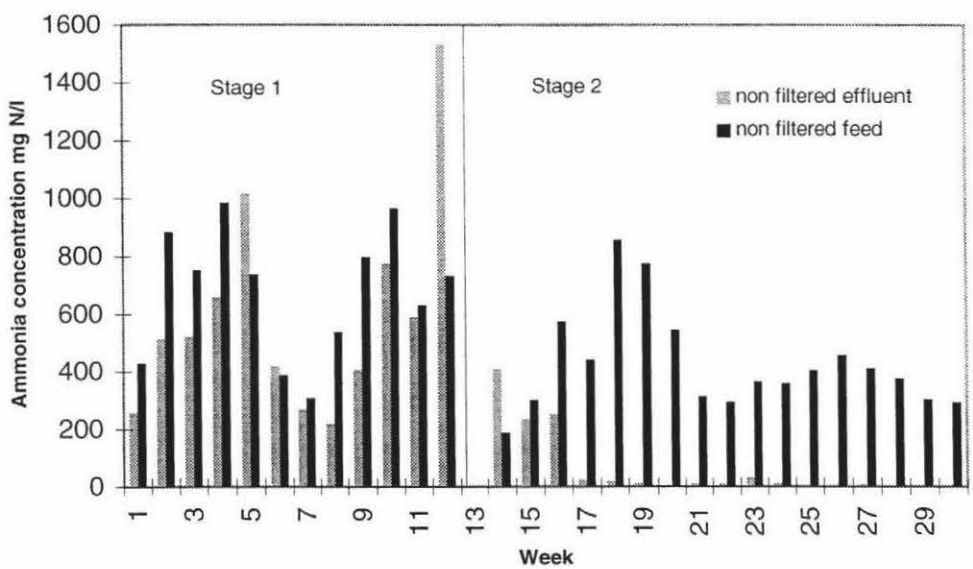
$$= 98.3\%$$

Ammonia volatilisation was assessed by directing the air stream from the reactor to a 4% boric acid solution in gas bottles. However no volatilisation was detected and all loss of ammonia was attributed to nitrification or cellular synthesis.

During the one day reactor cycle times of Stage 1, there was very little ammonia removal and no significant nitrification activity. Fig. 4.4 indicates that some effluent concentrations were higher than the influent concentrations during weeks 1 to 13. The increase in ammonia concentration could be due to decay of organic nitrogen substances.

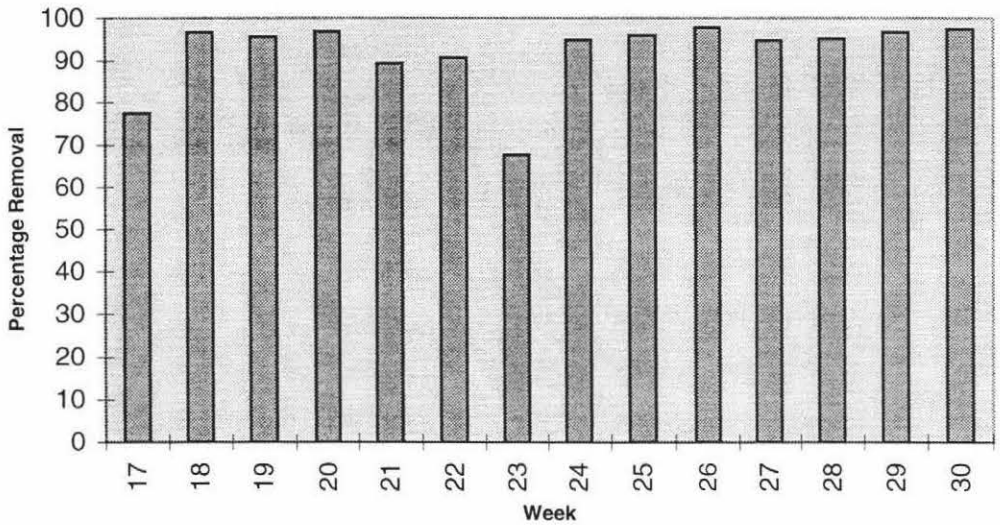
After the cycle was changed to a two day cycle (Stage 2) in week 13. The nitrifying population needed about 2 weeks to become established and there was a decrease in the ammonia concentration after the 15th week. In week 17 more than 70% of the non filtered ammonia was removed. In later sections it shows that after changing to the two day cycle in week 13, nitrite and nitrate were formed and that most of the ammonia was effectively utilised during the nitrification process by week 17 . Fig. 4.5 shows that in most weeks the average ammonia removal was over 90% during this period.

Figure 4.4 Average Weekly Ammonia Concentration in SBR



Very little ammonia was removed during Stage 1 due to lack of nitrification activity. Ammonia concentration in the effluent rapidly decreases after the experiment was changed to stage 2 (Week 13) by the nitrification process.

Figure 4.5 Percentage Ammonia Removal of Non Filtered Feed in SBR



Once nitrification commenced in stage 2 the ammonia removal rates were fairly consistent.

The ammonia removal rates measured in this study were comparable to those found in literature as Fernandes *et al.* (1991) measured 98% ammonia removal when using screened piggery effluent in their SBR experiments. Another study conducted by Fernandes (1994) also showed 99.8% removal of ammonia in their SBR.

4.6 TKN removal in SBR

Results from samples indicate a 73.2% removal of TKN from filtered feed. This removal rate was measured in week 18 to week 25 when the SBR had acclimatised to a two day cycle. Ammonia removal was high and nitrification processes were evident as nitrate and nitrite was formed. The removal of TKN was a little lower than those found in Fernandes *et al.* (1991) (80.3-93.2%) but they had a higher average TKN concentration of 2580 mg N/l . They would also have calculated the total TKN removal which includes cells and organic detritus. Thus their TKN removal would be greater as physical settling will also remove some of the TKN.

4.7 Cycle Tests in SBR

The SBR operation in Stage 1 is shown in Table 4.5.

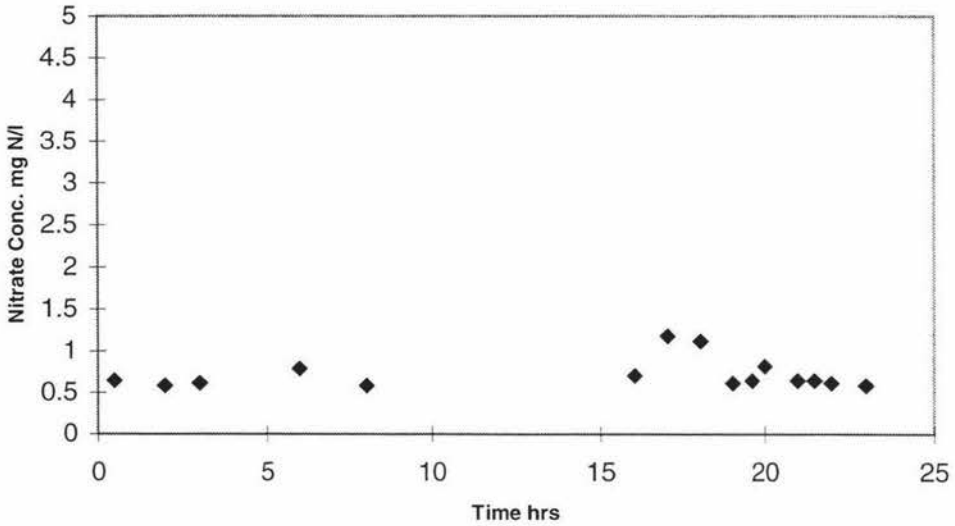
Table 4.5 SBR operation during week 9 to 13 in Stage 1.

Cycle	Time
Aerobic	0 - 18 hours
Anoxic	18 - 22 hours
Aerobic	22 - 23 hours
Settling	23 - 24 hour

The initial aerobic cycle was 18 hours to allow for COD and ammonia reduction. Then there was a 4 hour anoxic period to allow for denitrification. The SBR was operated under a twenty four hour cycle with a solids retention time (SRT) of 15 days and hydraulic retention time (HRT) of 3.3 days.

Initial reactor cycle tests with the twenty four hour cycle showed a COD removal of 63% with the largest drop within the first 6 hours of aeration from 895 to 398 mg/l or 56% COD removal. The nitrification process was not significant as nitrate concentrations remained below 1.2 ppm as shown in Fig 4.6. The ammonia concentration reflect this as it dropped on average from 300 ppm to 250 ppm. The ammonia which disappeared could have been used in cell synthesis.

Figure 4.6 Nitrate Concentration in 24 hour Reactor Cycle Time



4.8 Nitrite accumulation

It was proposed that the SRT was not long enough for a nitrifying population to develop. Thus the reactor cycle time was increased to two days, increasing the SRT to 30 days and HRT to 6.7 days. As a result a nitrifier population was established and improved settling was observed with the consistent feed stream and the 2 day cycle.

The reactor cycle times used (Fig. 4.8) were the same as 1 day cycle times shown above with the cycle repeated for another 24 hours and where the 24th hour used as settling time in stage 1 was added to the following aerobic period. After the reactor was allowed to acclimatise to the new conditions for 2 weeks, cycle tests on the reactor were conducted.

Figure 4.7 Nitrite Build up in the Reactor without Initial Anoxic Cycle

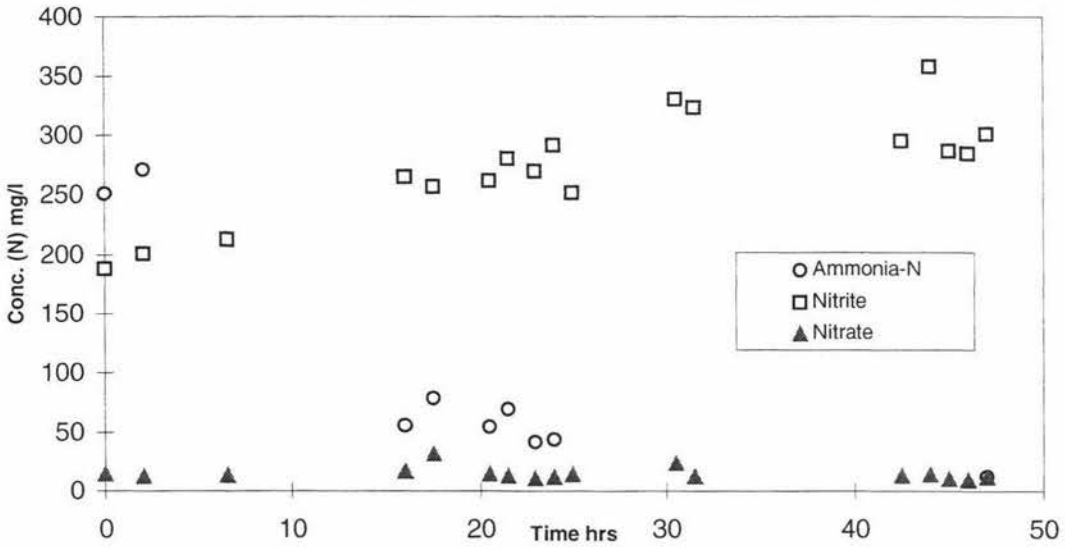
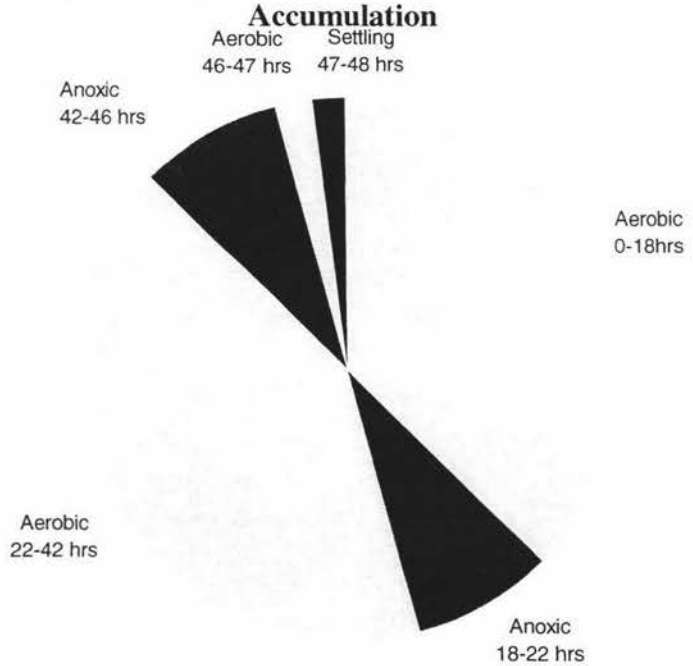


Figure 4.8 Cycle Time in Reactor with Nitrite Accumulation



Results of the full cycle test showed that ammonia concentrations rapidly decreased during the first aerobic cycle time as shown in Fig. 4.8. In the 18 hour period the

ammonia concentration was reduced from 251 to 56 mg N/l (78% removal). The SBR effluent concentrations show that 95.2 % of the ammonia was removed.

Fig. 4.7 indicates that the nitrification process occurred but was only half completed as nitrite concentration increased from 187.98 mg N/l from build up from the previous cycle to 264.95 mg N/l during the reactor cycle shown in Fig. 4.8. There were slight decreases in nitrite concentration during the anoxic periods but overall the concentration remained high. The nitrate concentrations fluctuated but did not exceed 12 mg N/l. Thus a nitrifying population was established which was capable of ammonia oxidation but nitratisation was inhibited for 6 weeks after the start of stage 2 when this reactor cycle time was utilised.

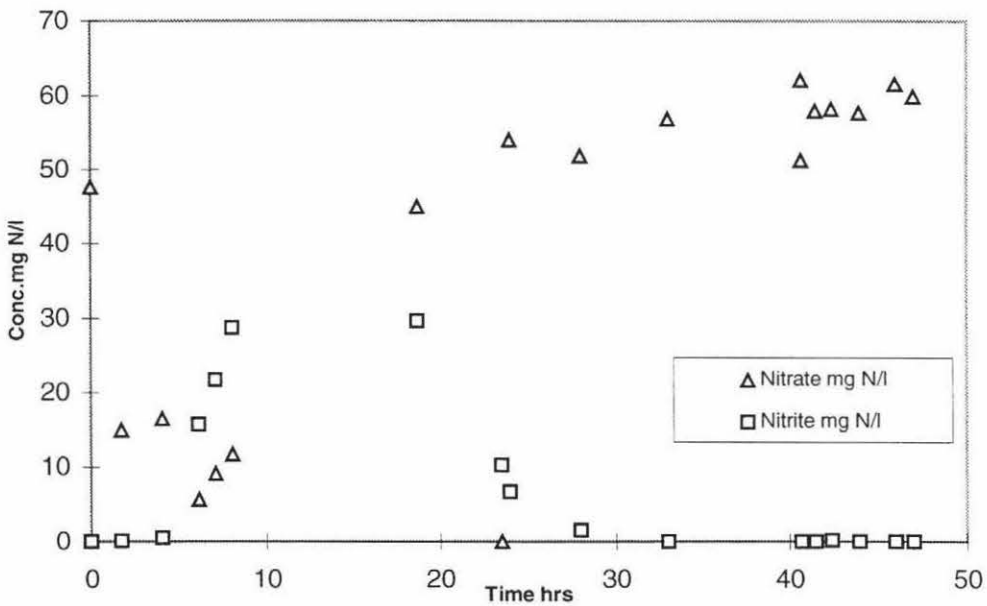
4.9 Nitrate formation

There was no evidence to suggest that nitrite accumulation was due to pH as the SBR was operated at pH 7 to 9 during the reactor cycle time. High concentrations of free ammonia or unionised hydroxylamine as stated by Yang & Alleman (1992) and Rhee *et al.* (1997) were not ruled out in this case. However, nitrate was able to form in small amounts and there was the possibility that the nitrite carried over from the previous cycle inhibited nitrate formation.

An anoxic period was added at the start of the cycle allowing denitrification of the accumulated nitrite. Results of the full cycle tests indicated that nitrite and nitrate levels decreased to below 10 ppm after the first 4 hour anoxic period. For example, in the day 232 batch test, the nitrate concentrations decreased from 17.8 to 0.2 mg N/l in the first 4 hour anoxic period. The rate of nitrate and nitrite (NO_X) removal was high because of the abundance of available carbon in the feed.

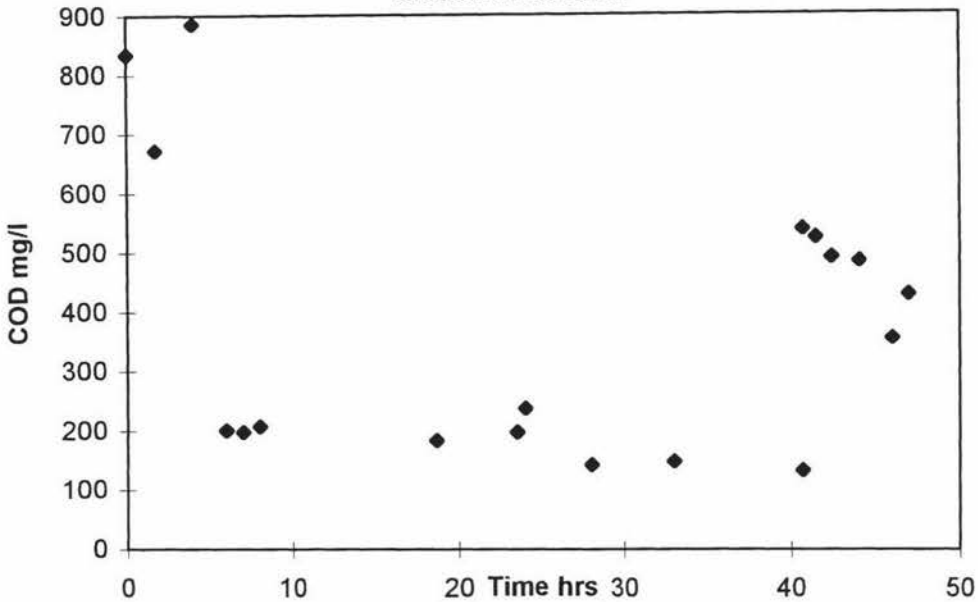
The results shown in Fig 4.9 indicated that nitrate and nitrite formation occurred simultaneously in the first aerobic period of the reactor cycle (Fig. 4.11) . In fact nitrate concentrations persisted at high levels as subsequent anoxic periods after 24 hours did not show significant denitrifying activity. As the pH was above 7 and dissolved oxygen levels was 0 mg/l, the most possible factor affecting denitrification was the lack of readily degradable COD.

Figure 4.9 Nitrification Batch Test on Day 166



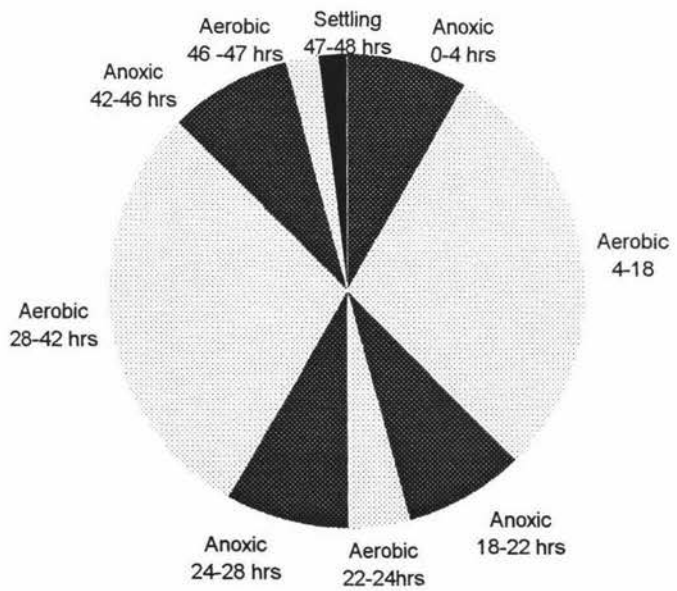
The nitrification process was completed with nitrate formation. However there was a build up of nitrate in the effluent. Adding methanol at the last anoxic period remove very little of the nitrate.

Figure 4.10 COD of Nitrification Batch Test Day 166 with Added Methanol



Methanol was added at 41 hours in order to facilitate dentrification.

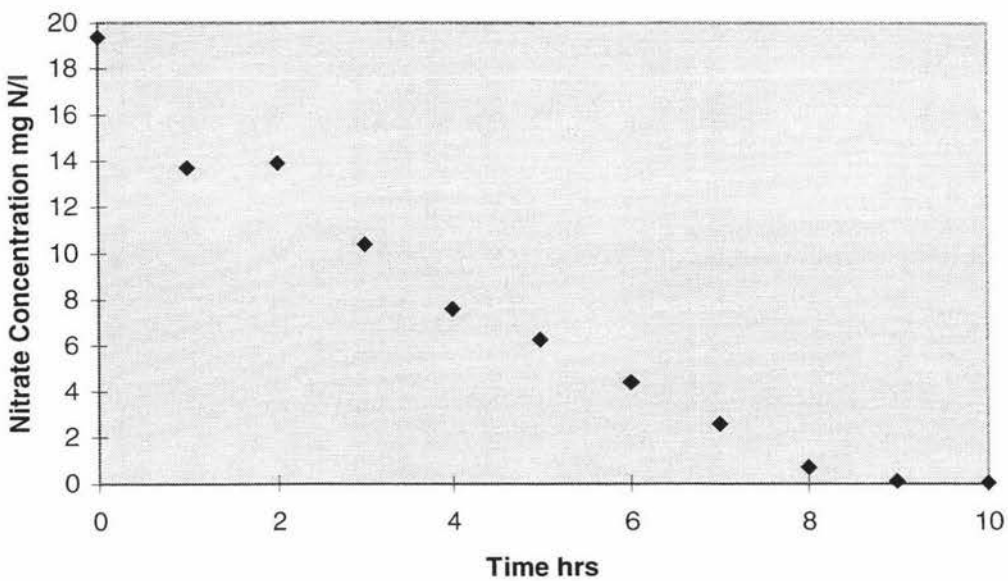
Figure 4.11 Reactor cycle time for batch study shown in Figure 4.9



The first 4 hours of the cycle removed nitrate and nitrite carried over from previous cycles. The long 18 hour aerobic period facilitated full nitrification and there was nitrate build up in the effluent since most of the readily degradable COD was used up in the first 10 hours of the cycle.

Methanol as an external source of COD was added at the start of the last anoxic cycle, shown by the increase in COD at 41 hours (Fig 4.10). In the large SBR this method of encouraging denitrification did not show any improvement in nitrate removing activity (Fig. 4.9). However batch studies performed in 1 litre reactors such as the one done at 230 days after reactor start up confirmed that there were denitrification activity over a period of 10 hours when all the nitrate concentration was removed indicated by Fig. 4.12. The batch test also suggests that the last 4 hour anoxic period may not be long enough for the large SBR to remove all the nitrate concentration.

Figure 4.12 Denitrification Batch Test



The external carbon source was methanol during a denitrification batch test with a 1 litre reactor on day 230. The denitrification rate was measured as 2.2 mg N/l.h and all nitrate was removed after 10 hours.

4.10 Adjusting the Anoxic and Aerobic Periods

A series of tests of cycle variations were performed. The aerobic and anoxic periods were varied and the performance was monitored in terms of COD and ammonia removal and effluent concentrations of nitrite and nitrate. During this time the biomass was also becoming better accumulated. Table 4.6 shows the trends during this period of operation.

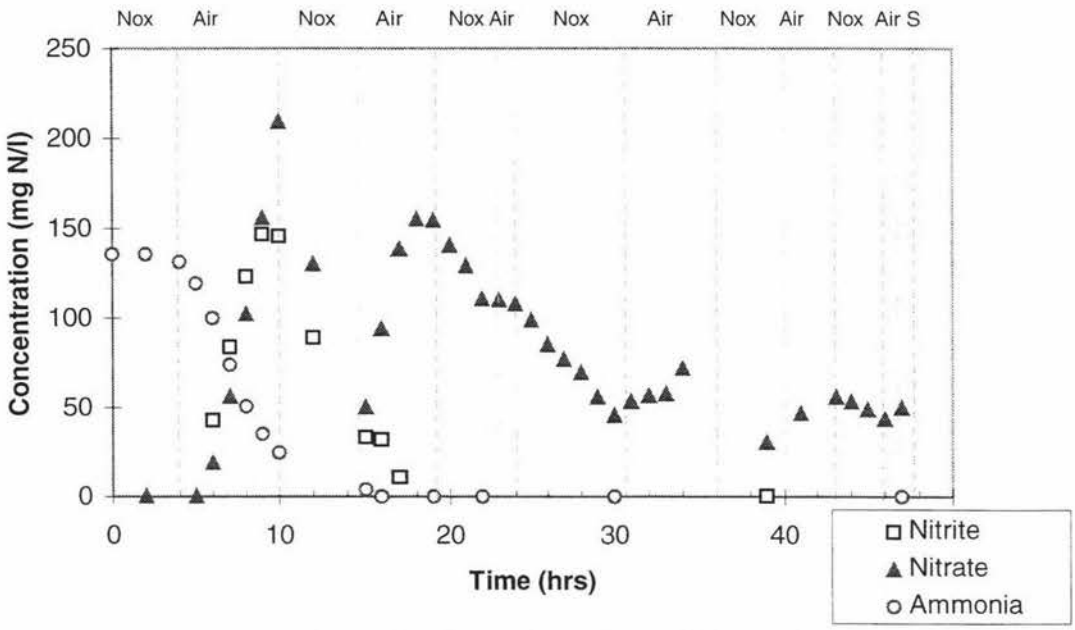
Table 4.6 Summary of Stage 2 Reactor Cycle batch Tests

Cycle test day	COD removal	Ammonia removal	Effluent nitrate conc. mg N/l	Effluent nitrite conc. mg N/l
160	81.5%	94.5%	60.2	0.7
166	84.1%	94.0%	59.9	0
232	87.2%	99.3%	38.9	0
256	88.0%	100%	11.00	0
276	87.8%	100%	111.4	0

It was observed that most of the ammonia needs to be used up during the first aerobic cycle. This was due to the COD reducing rapidly during this period and then stabilises as the readily degradable matter was utilised. By the time maximum nitrate levels were reached there was no available COD left for denitrification. Ammonia removal of the batch test on day 256 was 82% after the first aerobic period. The subsequent anoxic period was able to remove 39% nitrite and 76% of the nitrate already formed in day 256. A similar ammonia removal was observed on day 270 and 35% nitrite and 28% nitrate was removed in the subsequent anoxic period.

Starting from week 22 (day 160) the cycle length was maintained at 2 days, and the HRT and SRT were kept constant while the anoxic and aerobic periods within the cycle were varied to achieve maximum COD removal, nitrification and denitrification. This was done following Surmacz-Gorska *et al.* (1995) who found that a sequence of short anoxic and aerobic periods promoted COD removal, nitrification and denitrification, as suitable carbon sources were present in both aerobic and anoxic periods.

Figure 4.13 Nitrification Batch Test on Day 256



Nox = Anoxic period Air = Aerobic period S = Settling period

Full nitrification and best nitrate removal was achieved during this cycle. Most of the ammonia was removed during the first aerobic period. The subsequent anoxic period reduced the nitrate and nitrite concentrations already formed. The remaining nitrite was converted to nitrate in the second aerobic period.

These cycle tests showed nitrate formation during the 48 hour reactor cycle time. The COD and ammonia removal showed consistent results between the runs with different aerobic and anoxic periods. However there were differences in the effluent nitrate concentration and differences in the nitrogen removal capacity of the SBR during these runs. The first two runs on day 160 and 166 started off with high nitrate concentrations from the previous reactor cycle with 292 mg N/l in day 160 and 47.6 mg N/l in the day 166 test. The nitrate was denitrified in the initial anoxic cycle when there were sufficient available COD as a carbon source. However in the day 160 test, the first aerobic period was only 5 hours thus not giving enough time all the ammonia removal. Thus nitrate gradually built up in later aerobic periods and there was a lack of COD needed for denitrification.

During the day 166 batch test, the first aerobic period was increased to 14 hours in order to allow enough time to remove all the ammonia. This was achieved but again there was a lack of readily degradable carbon which inhibited denitrification in later anoxic periods. The effluent nitrate concentration was 59.9 mg N/l therefore increasing to a very long aerobic period did not facilitate nitrogen removal.

On day 232 there was a lower nitrate build up in the effluent but the DO in the first aerobic period was low thus no significant ammonia was nitrified. The second aerobic period was when all the nitrate accumulated. By that time the readily degradable COD concentration had decreased significantly and denitrification of successive anoxic cycle was less effective.

As the ammonia oxidation rate was observed to increase from the day 160 to 232 batch test. The first aerobic period was then set at 6 hours for the batch test at day 256. The reactor started with very little nitrate at the start of the reactor. The results showed that the first aerobic period reduced ammonia levels from 131.21 to 24.71 mg N/l which was 81.1% removal. This allowed time for nitrite and nitrate concentration to build up during this period. Thus it allowed denitrification of part of the oxidised nitrogen in the reactor in the following anoxic period when there were still sufficient COD for the process. During the second aerobic period all the remaining nitrite was converted to nitrate. In order to encourage denitrification, in the middle of the 48 hours reactor cycle, there was a long anoxic period of 7 hours as indicated in Table 4.7 . The batch test was able to achieve full nitrification and remove most of the nitrate as only 11.0 mg N/l was left in the effluent. It also had the highest COD and ammonia removal rates.

The batch test in day 276 was designed to be similar to the day 256 reactor cycle times as shown by Table 4.7 as it was successful in removing the nitrate formed in the reactor. This time the first aerobic period was increased to 7 hours in order to remove all of the ammonia as oppose to 81.1% in the day 256 batch test. After 7 hours 84.3% of the ammonia disappeared, however the nitrate concentration increased to 76.0 mg N/l. Although the subsequent 4 hour anoxic cycle was able to remove around 20 mg N/l of

nitrate in day 276 and day 256, but there was still a 54.8 mg N/l of nitrate as opposed to 11.0 mg N/l in day 256. There was still some 28.0 mg N/l at the start of the second aerobic period and there was a build up of nitrate levels in subsequent aerobic periods. The shortened anoxic period in the middle of the cycle was shorter and removed very little nitrate. In the end, full nitrification was achieved but the effluent contained 111.4 mg N/l.

Time (hrs)	256	276
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45		
46		
47		
48	Settling	Settling

Table 4.7 Reactor Cycle Test for Days 256 and 276.

The shaded area represents anoxic periods and clear area represent aerobic periods .

Therefore the results in day 160, 166 and 232 show that the rate of ammonia removal during the first aerobic period was important in determining nitrate removal. This was because the faster the ammonia removal, in a short initial aerobic period the more COD for denitrification reducing the concentration of nitrate in the effluent.

However in the day 256 test, more nitrate was removed than day 276 despite the reactor cycle times being very similar. This could be due to a higher concentration of ammonia in day 276 of 193.8 mg N/l whereas day 256 had only 131.2 mg N/l. There was still ammonia left in the second aerobic period to increase nitrate levels to 130.7 mg N/l. At the same time, there was less readily degradable COD as subsequent anoxic periods only slightly reduced the nitrate concentration.

4.11 Yield Coefficient Test

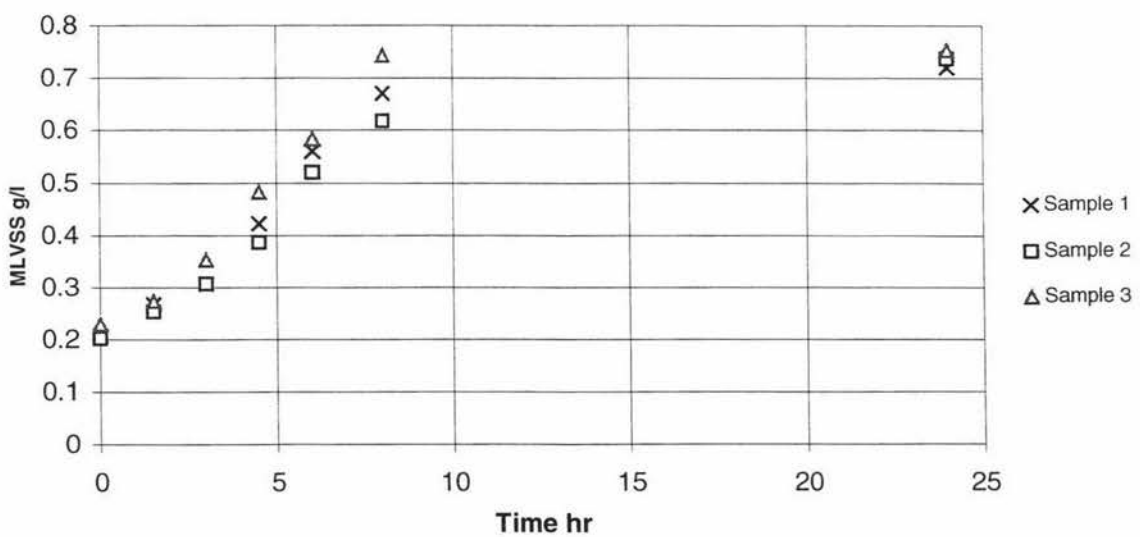
Shaker tests were used to evaluate observed yield of the heterotrophic population in the SBR. Results from Table 4.8 show that the average observed yield was 0.47 g VSS/ g COD and average specific growth rate (μ) was 0.15 hr^{-1}. As they all started with similar concentrations of dissolved COD and similar concentrations of initial biomass, the values of yield and μ are comparable.

Table 4.8 Yield Coefficient of Shaker Test

Sample	Yield coefficient	μ hr-1
1	0.48	0.1524
2	0.44	0.144
3	0.49	0.154

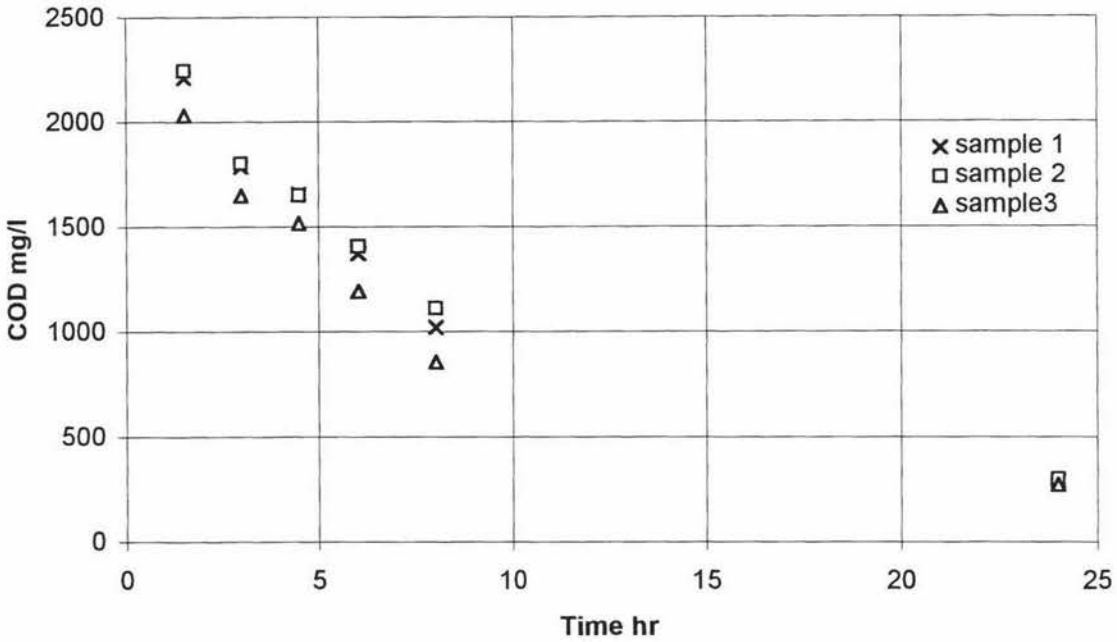
Figures 4.14 and 4.15 show the Mixed Liquor Volatile Suspended Solids (MLVSS) and COD during the shaker test.

Figure 4.14 Shaker Tests MLVSS



The change in MLVSS was plotted for the three samples and the μ was determined by the gradient of the graph when MLVSS was directly proportional to time.

Figure 4.15 Shaker Tests COD



4.12 Half Saturation Constant (K_s) and maximum specific growth Rate (μ_{max})

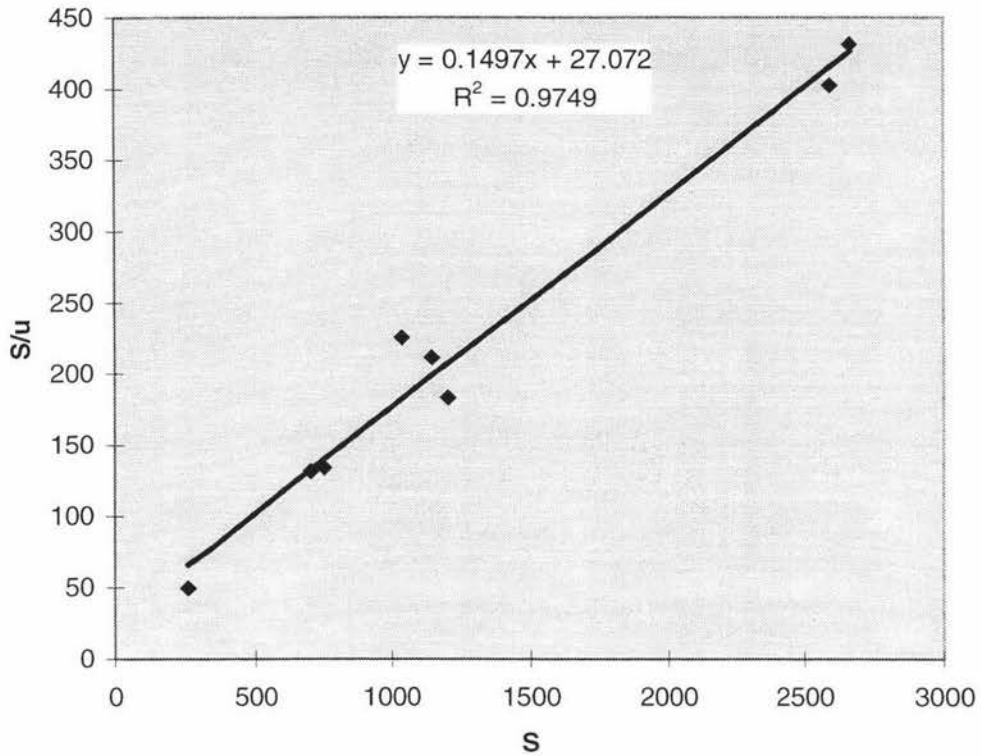
A growth rate study at different substrate concentrations indicated that the μ_{max} was 6.84 d^{-1} and the K_s = 293.6 mg/l. This was done by analysing different substrate concentrations. Seven flasks with different dilutions were monitored. The results are summarised in Table 4.8 and μ_{max} and K_s were calculated by plotting S/μ versus S as shown in Fig 4.16.

Table 4.9 Results from Batch Growth Kinetic Test

Sample	So mg/l	μ d^{-1}	Yo
1	2,596	6.43	0.37
2	2,668	6.19	0.28
3	1,206	6.59	0.55
4	1,149	5.43	0.49
5	754	5.62	0.59
6	706	5.36	0.64
7	260	5.23	0.49

So = Initial Substrate Concentration Yo = Observed Yield

Figure 4.16 Hanes-Woolf Plot for Growth Kinetic Test



Graphical method used for the determination of K_s and μ_{max} .
The slope = $1/\mu_{max}$ and when S =0 then $S/\mu = K_s/\mu_{max}$

Gupta & Sharma (1996) investigated heterotrophic kinetics during oxidation of high strength nitrogenous wastewater from the manufacture of fertilisers. Their heterotrophic yield coefficient measured at 0.498 (COD basis) was very similar to the average yield coefficient in this study (0.49). The half saturation constants K_s recorded in their study was 56.3 mg/l which was much lower than the K_s found in this study, but the heterotrophic substrates are expected to be very different between piggery waste and fertiliser wastewater.

4.13 Nitrification kinetics

The results of nitrification rates presented in this project assume no inhibition or limitation of nitrification by environmental factors such as dissolved oxygen and pH. This was because dissolved oxygen, pH and temperature were controlled at optimum values as suggested by Barnes & Bliss (1983).

In the batch tests, MLVSS was measured, but results showed no significant increase or decrease in biomass during the nitrification process. It was difficult to assess the biomass in the large reactor as there were fibrous material that were not readily biodegradable but affected the MLVSS measurement. Thus the MLVSS does not show the true amount of biomass present in the reactor.

Nitrification rates were calculated from the gradient of nitrite and nitrate formation when their concentration increased in direct proportion with time. As there were more than one nitrate forming episodes during the alternating aerobic and anoxic periods, all periods with significant nitrite, nitrate formation and ammonia oxidation were recorded in Table 4.10.

Table 4.10 Nitrification Rates from Ammonia Oxidation and Nitrite and Nitrate Formation during Reactor Cycle Tests in SBR.

Batch Test Day	Aerobic period measured	Initial Ammonia Conc. mg N/l	Nitrite Formation mg N/l.h	Nitrate Formation mg N/l.h	Ammonia Oxidation mg N/l.h
160	First	112.6		1.8	6.9
166	First	82.2	7.1	3.1	6.7
232	Second	189.2	11.5	4.7	14.6
	Third	56.6	9.7	9.7	
256	First	131.2	10.3	9.4	19.2
	Second	4.0		8.1	
276	First	193.8	11.5	12.0	24.6
	Second	28.0		15.5	
	Third	70.0		7.1	

Table 4.11 Batch tests: Average nitrification rates from ammonia oxidation and nitrite and nitrate formation.

Batch Test Day	Aerobic period measured	Initial Ammonia Conc. mg N/l	Nitrite Formation mg N/l.h	Nitrate Formation mg N/l.h	Ammonia Oxidation mg N/l.h
270	First	82.2	6.5	7.3	9.8
282	First	118.2	8.9	7.2	11.0

The ammonia oxidation rates were always greater than oxidised nitrogen formation rates for all batch tests. Ammonia oxidation rates shown in Table 4.10 increased from 6.8 mg N/l.h to 24.6 mg N/l.h from day 160 to 276 in the batch tests. Most of them measured in the first aerobic period as most of the ammonia was removed in this period. The increasing rate was not due to initial ammonia concentration as day 256 had a higher ammonia oxidation rate of 19.2 mg N/l.h than day 232 where it was 14.6 mg N/l.h, but had a lower initial concentration of 131.2 mg N/l compared to 189.2 mg N/l in day 232. The ammonia oxidation rates continued to increase with each batch test despite the changes in length of the first aerobic period. The results suggests that the SBR became more effective in removing ammonia with time.

Similarly nitrate formation in the first aerobic period increased in the SBR through time from 1.8 to 12.0 mg N/l h. At the same time the ammonia and COD removal increased in the reactor to 100% and 88 % respectively which was indicated in Table 4.7. Nitrate formation rates were higher in the second aerobic period indicated by day 232 and 276. This was because most of the ammonia formed nitrite by that stage and higher nitrite concentrations would pose as a substrate for forming nitrate.

The nitrite and nitrate forming rates are similar in the last three reactor cycle tests. While the nitrate formation rate increases through time, nitrite formation increases from 7.1 mg N/l h at day 166 to 11.5 mg N/l.h by day 232 and seemed to have peaked at this point.

In the SBR batch tests experiments, the nitrification rate according to nitrite formation or nitrate formation may not be constant even within the one batch test. It was observed that at the start of each aerobic period, there were different ammonia and nitrite concentrations. For example, nitrite formation rates decreased in day 232 from 11.5 to 9.7 mg N/l.h as the ammonia concentration at the start of the aerobic periods were 189.2 and 56.6 mg N/l. Similarly nitrate formation rates increased on day 232 and 276 during the second period as nitrite concentrations were higher. Therefore there was some indication that these affected the nitrite and nitrate formation rates during these batch tests.

The most nitrate removed in the waste was in the day 256 test. However in day 276 where higher rates of nitrite, nitrate and ammonia oxidation were observed, there was build up of nitrate. Thus indicating that other factors other than reaction rate affected the overall removal of nitrate. This will be discussed in later sections.

The nitrate formation rate increased from 1.7 mg N/l.h to an average of 11.6 mg N/l.h during the running of the SBR. These nitrification rate results were comparable to Mahne *et al.* (1996) who showed different nitrification rates at different substrate concentrations. They indicated a combined nitrite and nitrate formation rate of 177 mg N/l.day to 218 mg N/l.day with a substrate concentration between 500 to 2000 mg NH_4^+-N/l. The highest nitrate formation rate in the large reactor on day 276 ranged from 170 to 372 mg N/l.d with an initial ammonia concentration of 193.8 mg N/l. The rates in this project were generally higher than those shown in the study above but with a lower concentration of substrate. They used a high strength synthetic waste which was formulated to be similar to piggery wastewater.

Although the nitrite formation rates were very consistent in the large reactor, however in the small reactor where filtered feed was used there were more consistent nitrate formation rates. Similar to the large reactor was that the ammonia oxidation rate also increased with time. The nitritisation reaction were both slower than the nitratisation reaction. The nitrification rate using nitrate formation was 208.8 mg N/l.h with an initial

ammonia concentration of 118.2 mg N/l. The rate was very similar to those found in Mahne *et al.* (1996) but initial ammonia concentrations were much lower than that study.

The ammonia oxidation, nitrite and nitrate formation can be very different rates, whereas studies such as Bortone *et al.* (1992) used ammonia oxidation as an indication of nitrification rates. From the results shown here they may have over estimated the nitrification rate as the ammonia oxidation rates were much higher than nitrate and nitrite formation rates. This removal of ammonia is due to nitrification and assimilation of cells (Sozen *et al.* 1996). Barnes & Bliss (1983) stated that up to 1/3 of the ammonia would be used for cell synthesis. Therefore ammonia oxidation rates are higher than nitrite and nitrate formation. Nitrite and nitrate formation rates can be different thus combining the formation rate of these two compounds as done by Mahne *et al.* (1996) can be misleading especially in the situation where nitrate formation is inhibited and nitrite builds up.

4.14 Denitrification Tests

Batch tests were performed in 1 litre vessels with subcultured biomass and filtered feed to determine the denitrification rate. The denitrification rate was also monitored on day 230 in the large SBR.

The denitrification test conducted in the large reactor started with an initial nitrate concentrations of 19.3 mg N/l which decreased to 0.1 mg N/l over a 10 hour period. The denitrification rate measured was 2.18 mg N/l.hour based on the removal of nitrate. During the small SBR batch tests using filtered feed, the denitrification rate was at least six times that found in the big reactor as shown in Table 4.13. The nitrate removal rate increased from 13.6 to 22.1 mg N/l.h measured from the batch tests performed on day 270 and day 275 respectively.

Table 4.12 Denitrification Rate from Large Reactor and Batch Tests.

Test Date	Denitrification Rate mg N/l.h	mg N/g MLVSS
230	2.2	
270	13.6	5.4
275	15.1	7.1
282	22.1	16.2

shaded: test conducted in large reactor, other tests were carried out with subcultered biomass and filtered feed.

Bortone *et al.* (1994) who used industrial wastewater found a slightly higher denitrification rate of 36.5 mg/l . h compared to the denitrification rates found in this study. Their batch tests used pure nitrogen gas to maintain anoxic conditions while this study used a close reactor which might not have ensured fully anoxic conditions.

4.15 COD: Ammonia Ratio

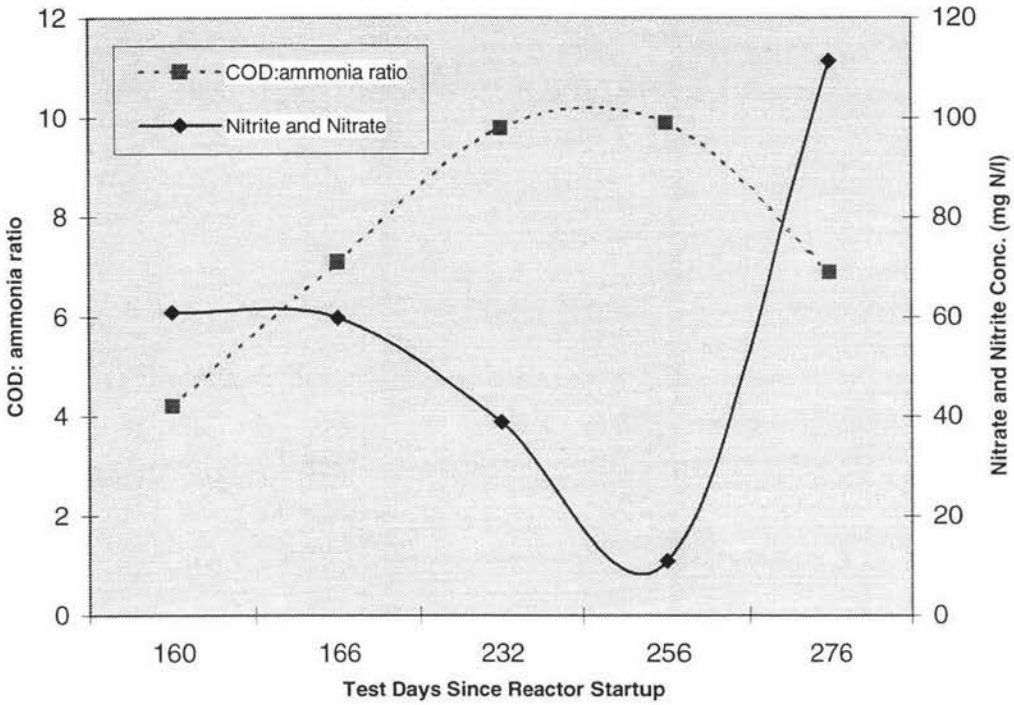
Observations of the running of the SBR showed that when the ammonia was quickly removed in the initial aerobic period, there was enough COD left for denitrification later on. Therefore the COD: ammonia ratio was an important determinant in how the SBR treats incoming waste. The COD: ammonia ratios were calculated in Table 4.13.

Table 4.13 COD: Ammonia Ratios in Batch Tests

Test Day	COD: Ammonia Ratio
160	4.2
166	7.1
232	9.8
256	9.9
276	6.9

Table 4.13 and Fig. 4.14 shows that the COD: ammonia ratio was highest during day 232 and day 256 batch tests. These tests had lower nitrate levels in the effluent. A high enough COD: ammonia ratio indicates that there was enough COD remaining after nitrification in order for denitrification to occur. Although the day 276 test had higher nitrification rates, the COD: ammonia ratio was much lower (6.9) than that of the day 256 test (9.9). Yet nitrate accumulated to 112 mg/l in the effluent of the day 276 test. The results suggests that there was not enough COD left for denitrification which in turn produced a high nitrate concentration.

Figure 4.17 COD :Ammonia Ratio and Effluent Oxidised Nitrogen Concentrations in Batch Tests



* Results show that as the COD:ammonia ratio increases, the nitrite and nitrate concentration in the effluent decreased. Higher COD: ammonia ratios mean that more COD remains after the first aerobic period when most of the ammonia was removed, therefore allowing dentrification to occur.

Chapter 5

Conclusion

The project achieved the objective of acclimating a culture of micro organisms to piggery wastewater in a SBR and biologically treated the effluent for COD and ammonia removal from the piggery effluent. The COD of the raw piggery effluent in Stage 1 used in this study were on average 3686 mg/l and 12679 mg/l for filtered and non filtered feed respectively. Ammonia concentrations averaged at 681 mg N/l for non filtered feed. The average TKN values were 1103 mg N/l for non filtered feed. In Stage 2 of the experiments, the raw feed was diluted in order to create a more consistent feed input into the reactor. The filtered and non filtered COD feed averaged to 614 and 8842 mg/l respectively. The non filtered feed ammonia concentration dropped to 478 mg N/l and non filtered feed TKN was averaged at 641 mg N/l.

During stage 1, there was no nitrification activity which was ideal for determining heterotrophic kinetics. The heterotrophic kinetic constants measured during this period were the yield coefficient which was measured as 0.49, the maximum specific growth rate (μ_{max}) as 6.84 d^{-1} and half saturation constant (K_s) as 293.6 mg/l. The reactor cycle time was then increased to 2 days (Stage 2) at day 87. Full nitrification was achieved when the SRT was increased to 30 days and HRT 6.7 days.

Nitrite accumulated in the effluent when the cycle included an initial 18 hour aerobic period followed by 4 hour anoxic period. Nitrate formation was inhibited as little was formed throughout the 48 hour cycle. A 4 hour anoxic period was then added at the beginning of the reactor cycle time. This denitrified the accumulated nitrite from the previous reactor cycle and the nitrification was completed with the formation of nitrate.

The highest average weekly COD removal rate for non filtered feed was 92.7% in week 29 and the corresponding highest weekly ammonia removal was 95.8% in week 30. Batch tests indicated that ammonia needs to be removed in the first 10 hours so there would be enough COD for denitrification. The reactor cycle tests on day 256 achieved full

nitrification and denitrification and was able to reduce the nitrate levels in the effluent to 11.0 mg N/l.

The ammonia oxidation and nitrate formation rates increased over time to 24.6 mg N/l.h and 11.5 mg N/l.h respectively. Nitrite formation rates peaked at 11.5 mg N/l.h . Overall the SBR has become more effective in removing ammonia and promoting the nitrification process over time.

Ammonia oxidation rates were much higher than nitrite or nitrate formation rates. This was attributed to lost of ammonia to cell synthesis. Therefore determining nitrification rate with ammonia oxidation can over estimate the rate. By measuring nitrite and nitrate formation rates, a more detail picture of nitrification rates was shown rather than oxidised nitrogen formation rates which are misleading especially if nitrate formation was inhibited and there was a build up of nitrite.

The SBR biomass population was able to remove nitrate efficiently as batch tests over 4 to 10 hours showed that denitrification rates could reach 22.1 mg N/l.h.

The COD: ammonia concentration ratio were important in determining the nitrification and denitrification activity in the SBR. This was shown by the nitrate levels in the effluent. Higher COD: ammonia concentration ratio had lower nitrate levels in the effluent. Thus there was not enough COD needed for the denitrification of the proportionally higher ammonia concentration and in turn nitrate concentration.

To summarise the study was able to determine heterotrophic kinetics and nitrification rates. Denitrification rates were also assessed with batch tests. Finally over time it was shown that a series of aerobic and anoxic periods were the best way in providing for COD removal, nitrification and denitrification of piggery effluent.

5.1 Further Investigations

The project determined nitrification rates using the product nitrite and nitrate formation rates. In order to support these data, nitrification rates can be determined by other methods such as using respirometrically. Heterotrophic and autotrophic biomass kinetics constants can also be determined by respirometric methods and batch analysis. Thus one can compare these results to ones collected during this study. The ammonia oxidation rates and nitrate formation rates were still increasing by the end of the study. Higher values may have been obtained if the SBR was allowed to continue.

Chapter 6

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Appendix 1.1 Average Weekly COD concentrations (mg/l) used in SBR

Week	COD non filtered effluent	COD non filtered feed	COD filtered effluent	COD filtered feed
5		21524	2361	6442
6	2020	9657	1993	4490
7	1485	12185	1081	3126
8	1137	5825	881	3242
9	3135	10866	2292	6216
10	3650	11620	3308	8965
11	1851	11949	843	5061
12	2928	17339	2957	7504
13	2318	17811	1537	5315
14	1251	8273	507	2921
15	890	6479	390	3527
16	814	5813	497	3514
17	775	7582	576	3639
18	1272	8583	822	5437
19	1032	9272	544	5488
20	1566	9025	691	4175
21	663	8019	470	3525
22	619	10108	293	4247
23	699	11416	215	5389
24	357	7471	305	3303
25	251	8253	266	4325
26			306	3715
27	198	7058	244	4289
28	384	7104	187	3314
29	163	6839	119	3298
30	161	8096	127	3499

shaded area denotes Stage 1, unshaded was Stage 2

Appendix 1.2 Weekly Ammonia Concentrations in SBR (mg/l) in 1998

Week	Ammonia Filtered Effluent	Ammonia Filtered Feed	Ammonia Non filtered Effluent	Ammonia Filtered Feed
1	258	414	257	430
2	522	611	513	881
3	576	750	521	752
4	551	876	657	985
5	1029	681	1015	735
6	353	426	418	388
7	273	336	268	306
8	290	379	217	539
9	517	1010	404	796
10	1032	1358	773	965
11	549	589	589	631
12	792	1101	1530	732
13	585	587		
14	365	341	408	186
15	239	438	234	300
16	218	423	249	576
17	14	465	22	443
18	14	844	17	856
19	14	784	10	774
20	9	581	5	546
21	14	315	10	313
22	8	283	8	293
23	29	356	32	366
24	9	366	10	360
25	2	380	5	404
26			3	457
27			7	413
28	2		5	377
29	5	331	5	303
30	4	295	3	291

shaded area denotes Stage 1, unshaded was Stage 2

Appendix 1.3 Percentage COD Removal from SBR

Week	COD (Non Filtered)	COD (Filtered)
5		37.9
6	66.8	36.4
7	70.5	29.1
8	69.5	45.3
9	67.6	48.4
10	64.6	34.6
11	47.5	40.4
12	44.6	43.4
13	56.5	15.9
14	48.2	41.9
15	48.1	56.8
16	52.9	49.9
17	66.6	66.0
18	45.7	49.1
19	56.9	66.4
20	71.8	70.2
21	72.7	55.2
22	80.3	76.8
23	78.4	86.5
24	84.3	68.4
25	89.8	79.1
26		74.3
27	91.1	81.0
28	83.1	80.8
29	92.3	87.8
30	93.2	87.9

Appendix 1.4 Percentage Ammonia Removal in SBR

Week	Ammonia (Non Filtered)	Ammonia (Filtered)
17	77.5	89.1
18	96.7	95.5
19	95.6	94.8
20	96.8	94.5
21	89.5	85.8
22	90.8	90.4
23	67.7	72.7
24	95.0	92.2
25	96.0	98.4
26	97.8	
27	94.7	
28	95.2	
29	96.7	97.0
30	97.5	96.5

Appendix 2.1 Batch Test Results for 1 day Reactor Cycle (Stage 1)

Time hrs	COD mg/l	Nitrate mg N/l	Ammonia mg N/l
0.5	895	0.64	269
2	781	0.58	299
4	510	0.61	291
6	398	0.79	265
8	345	0.58	269
16	300	0.7	231
17	287	1.18	250
18	345	1.12	261
19	457	0.61	250
19.6	330	0.64	257
20	171	0.82	227
21	260	0.64	231
21.5	330	0.64	250
22	323	0.61	246
23	334	0.58	205

Shaded are denotes anoxic periods
unshaded denotes aerobic periods

Appendix 2.2 Batch Test Results of 2 Day Reactor Cycle (Stage 2)

Time (hrs)	Ammonia mg N/l	Nitrite mg N/l	Nitrate mg N/l	Phosphorus mg/l
0.0	251	188.0	14.4	46.6
2.0	271	200.8	12.4	53.9
6.5	213	212.7	13.3	73.0
16.0	56	265.0	16.8	100.1
17.5	79	256.6	31.9	130.4
20.5	55	261.7	14.5	123.0
21.5	70	280.1	12.9	123.4
23.0	42	269.5	10.7	104.0
24.0	44	291.3	11.8	121.2
25.0		251.7	13.9	108.4
30.5		330.3	23.4	140.8
31.5		323.5	12.2	109.8
42.5		295.2	12.7	103.2
44.0		357.8	13.6	108.0
45.0		286.5	10.1	75.8
46.0		284.4	9.0	79.6
47.0	12	300.8	11.5	59.7

Shaded are denotes anoxic periods
unshaded denotes aerobic periods

Appendix 3.1 Nitirification Batch Test day 160 (11/10/1998)

Time (hr)	Nitrate mg N/l	Nitrite mg N/l	Phosphate mg/l	Ammonia mg N/l	COD mg/l
0	2.9	0.0	50.3		654
1.8	2.8	0.0	29.5	125.0	784
3.8	3.0	0.0	30.5	103.1	864
6.4	0.0	0.0	30.0	112.6	855
7.2	0.9	0.1	19.1	111.9	910
8	0.6	0.0	18.6	110.8	484
18.5	28.7	35.6	46.0	8.8	249
19.5	27.3	29.5	47.4	23.8	180
21	24.7	18.1	43.9	9.7	156
22	30.0	17.0	58.2	8.9	160
23	65.8	23.6	73.2		154
25	36.4	5.4	45.8		139
27	36.1	0.0	49.5		139
30.2	43.2	0.9	40.6		141
31	41.8	0.6	44.5	5.4	134
43.5	58.1	0.4	44.6		127
44.5	60.1	0.6	75.2		117
46.2	55.8	0.6	51.1		125
47	60.2	0.7	48.8	6.8	121

First Aerobic Period

Nitrate formation
y = 1.751x - 9.4572
R2 = 0.938

Ammonia Oxidation
y = -6.9119x +148.02
R2 = 0.8724

Appendix 3.2 Nitrification Batch Test Day 166 (17/10/1998)

Time (hr)	Nitrate mg N/l	Nitrite mg N/l	Phosphate mg/l	Ammonia mg N/l	COD mg/l
0	47.7	0.0	129.2	117.3	834
1.7	15.0	0.1	79.8		672
4	16.5	0.5	89.9	117.6	886
6	5.7	15.7	80.8	82.2	201
7	9.2	21.7	72.2	73.8	198
8	11.7	28.7	76.1	65.5	207
18.7	45.0	29.7	67.2	7.2	183
23.5	0.0	10.3	62.5	6.4	198
24	54.0	6.7	79.5		237
28	51.9	1.5	79.4		142
33	56.8	0.0	71.9		148
40.7	62.1	0.0	67.4	7.8	133
40.7	51.3	0.0	74.2		539
41.5	57.9	0.0	66.0		524
42.4	58.2	0.2	257.6		491
44	57.7	0.0	68.0		485
46	61.6	0.0	79.6		355
47	59.9	0.0	82.2		428

st aerobic period

Nitrite formation
y = 7.0139x - 27.184
R2 = 0.9979

Nitrate formation
y = 3.0898x - 12.773
R2 = 0.9999

Ammonia Oxidation
y = -6.6925x + 127.75
R2 = 0.9292

Appendix 3.3 Nitrification Batch Test day 232 (22/12/98)

Time hr	Nitrite mg N/l	Nitrate mg N/l	Phosphate mg/l	Ammonia mg N/l	COD mg/l
0	6.2	17.8	280.6	233.8	2293
2	0.0	0.2	273.2	217.6	1041
4	0.0	0.2	259.2	218.5	2112
5	0.0	0.4	202.4	212.8	1836
6	0.0	0.2	171.5	191.6	1509
7.3	0.0	0.1	151.7	173.0	1190
8	0.0	0.7	151.7	187.4	1043
9	0.0	0.4	141.1	177.0	888
10	0.0	0.1	29.6	171.9	479
14	0.0	0.1	36.7	189.2	550
15	6.1	0.2	129.8	169.5	443
16	20.3	2.0	113.4	148.9	419
17	35.7	7.4	115.4	122.5	429
18	43.3	12.2	110.6	102.5	514
19	55.0	18.5	114.1	80.4	419
22	0.0	0.0	89.8	70.3	379
23	12.1	3.4	88.3	54.4	383
28	0.0	0.0	101.5	56.6	353
29	14.9	4.9	81.6	40.1	334
30	23.9	12.4	86.6	26.6	362
31	29.4	22.3	89.8	9.6	350
32	25.6	32.5	86.5	2.5	345
33	17.1	45.1	92.3	1.2	326
34	9.9	57.1	87.7	0.0	336
38	0.1	39.3	96.1	20.4	319
39	0.0	40.4	83.9		300
40	0.0	46.5	97.9	0.0	334
41	0.0	43.3	80.7		295
42	0.0	45.8	82.5		284
43	0.0	47.3	82.4		303
44	0.0	42.8	80.6	1.7	298
45	0.0	31.1	68.6		284
46	0.0	36.0	84.3	21.5	281
47	0.0	38.9	79.8		293

First Aerobic Period

Nitrite formation
y = 11.487x - 162.8
R2 = 0.9884

Nitrate formation
y = 4.6919x - 71.701
R2 = 0.973

Ammonia Oxidation
y = -14.611x + 380.23
R2 = 0.9223

Second Aerobic Period

Nitrite formation
y = 9.7355x - 270.16
R2 = 0.9561

Nitrate formation
y = 9.7085x - 276.07
R2 = 0.9822

Appendix 3.4 Nitrification Batch Test Day 256 (15/01/1999)

Time (hr)	Nitrite mg N/l	Nitrate mg N/l	Phosphate mg/l	Ammonia mg N/l	COD mg/l
0			168.4	135.4	1345
2		0.1	183.3	135.4	1138
4			178.5	131.2	1069
5		0.1	122.6	119.5	397
6	12.5	4.2	130.2	99.9	379
7	24.4	12.4	126.9	73.9	255
8	35.9	22.4	119.4	50.8	262
9	42.9	34.1	125.0	35.1	266
10	42.5	45.8	137.3	24.7	250
12	26.0	28.5	124.2		234
15	9.8	11.0	113.6	4.0	205
16	9.3	20.6	106.8	0.0	203
17	3.1	30.3	101.2		193
18		33.9	93.1		190
19		33.8	95.0	0.0	179
20		30.7	94.5		179
21		28.2	99.8		183
22		24.2	102.5	0.0	176
23		24.1	90.1		173
24		23.7	107.0		173
25		21.6	96.0		176
26		18.6	97.8		162
27		16.9	91.2		176
28		15.2	95.5		172
29		12.2	93.4		171
30		10.0	96.7	0.0	174
31		11.7	79.8		162
32		12.4	126.2		172
33		12.7	78.7		171
34		15.8	76.1		167
39	0.1	6.7	85.4		165
41		10.3	71.4		179
43		12.2	71.3		160
44		11.7	77.7		155
45		10.7	75.3		155
46		9.6	79.5		172
47		11.0	75.8	0.0	162

First Aerobic Period

Nitrite formation
y = 10.251x - 47.95
R2 = 0.9867

Nitrate formation
y = 9.379x - 50.508
R2 = 0.978

Ammonia Oxidation
y = -19.195x + 210.81
R2 = 0.9867

Second Aerobic Period

Nitrate formation
y = 8.0719x - 109.44
R2 = 0.96

Appendix 3.5 Nitrification Batch Test day 276 (04/02/1999)

Time	Nitrite mg N/l	Nitrate mg N/l	Phosphate mg/l	Ammonia mg N/l	COD mg/l
0	0.00	0.07	108.44	166.33	1147
2	0.00	0.19	115.06		962
4	0.00	0.00	112.77	193.82	860
5	8.45	1.61	84.53	138.67	313
6	20.54	7.34	79.45	107.87	229
7	32.85	15.34	77.23	92.29	237
8	45.64	26.34	79.86		250
9	55.64	39.30	88.59	53.88	226
10	54.63	51.92	91.75	31.87	235
11	54.47	75.99	116.83	30.42	224
15	35.54	54.78	107.90	28.01	215
16	33.91	68.29	105.66	19.86	209
17	27.41	85.43	123.12	3.87	196
18	17.70	99.82	132.42		183
19	9.40	117.88	147.31		165
20	2.63	130.67	160.06		157
23	0.00	118.81	155.07		159
24	0.00	110.04	150.51		165
25	0.00	101.37	144.53		157
26	0.00	99.32	145.76		59
27	0.00	95.24	147.80		185
28	0.00	90.92	143.98		161
30	0.00	85.07	139.52		165
31	1.14	90.82	133.88		163
32	0.02	98.10	138.89		166
33	0.00	106.91	137.63		155
34	0.00	112.41	144.47		152
39	15.24	120.01	174.30		152
43	0.00	105.92	135.47		146
47	0.00	111.36	146.28		140

First Aerobic Period

Nitrite formation
$y = 11.488x - 47.486$
$R2 = 0.997$

Nitrate Formation
$y = 12.009x - 64.955$
$R2 = 0.9567$

Ammonia Oxidation
$y = -24.605x + 271.2$
$R2 = 0.9483$

Second Aerobic Period

Nitrate Formation
$y = 15.503x - 178.49$
$R2 = 0.9984$

Third Aerobic Period

Nitrate Formation
$y = 7.0776x - 127.82$
$R2 = 0.9945$

Appendix 3.6 Nitrification 1 litre Batch Test Day 270 (29/1/99)

Time (hr)	Nitrite mg N/l	Nitrate mg N/l	Phosphate mg/l	Ammonia mg N/l	COD mg/l
0	0.0	95.3	203.2	118.0	758
2	0.0	42.5	81.5		
4	0.4	34.8	71.8		
4.5	3.7	30.2	78.9		1044
5	7.8	30.8	69.2	82.2	175
5.5	10.7	30.6	75.3		
6	13.2	30.7	66.2	83.5	195
7	23.3	40.0	67.1	74.6	186
8	29.5	43.4	65.7	62.1	189
9	34.4	48.7	69.9	50.5	193
10	40.0	56.5	77.3	40.3	203
11	44.5	70.6	98.1	38.1	202
17.5	35.6	73.4	122.1		195
18.25	36.6	79.6	116.8		187
19	32.4	81.7	113.8		202
20	26.6	82.7	120.2		195
21	24.7	98.1	140.5		202
22	18.1	93.8	127.6		177
24	10.4	102.5	135.1		
25	0.0	129.3	130.1		

29/01/1999

Nitrite
y = 6.4906x - 24.674
R2 = 0.9913

Nitrate
y = 7.2622x - 13.398
R2 = 0.9556

Ammonia Oxidation
y = -9.7513x + 141.07
R2 = 0.9755

Appendix 3.7 Nitrification Batch Test day 282 (10/2/99)

Time hr	Nitrite mg N/l	Nitrate mg N/l	Phosphate mg/l	Ammonia mg N/l	COD mg/l
0	0.0	105.6	171.2		701
1	0.0	77.9	132.3		425
2	0.0	41.9	67.1		246
3	0.0	29.5	63.3		193
4	0.0	19.2	64.8	118.2	175
5	9.9	20.7	54.2	92.3	167
6	20.1	23.2	52.8	88.5	183
7	33.4	29.1	59.7	82.0	181
8	39.6	33.8	59.8	65.3	185
9	46.7	39.9	57.3	51.4	185
10	51.6	46.9	72.9	43.0	192
11	53.4	55.2	99.5	35.8	203
12	54.7	67.2	119.5	26.4	201
13	51.0	72.7	128.9	28.4	201
20.2	47.7	85.7	161.4		219
21	42.5	86.1	154.1	26.8	181
22	36.2	90.6	160.4		183
23	32.9	109.9	177.9		201
24	23.4	111.1	156.4		181
25	14.4	120.9	155.5		153
26	0.0	138.4	170.8		140
27	0.0	142.8	162.8		146
28	0.0	144.6	167.9		135
29	0.0	141.2	162.2		158

First Aerobic Period

Nitrite
$y = 8.8567x - 33.234$
$R2 = 0.98$

Nitrate
$y = 7.2374x - 22.742$
$R2 = 0.9833$

Ammonia Oxidation
$y = -10.971x + 154.76$
$R2 = 0.9771$

Appendix 3.8 Reactor Cycle Times for Nitrification Batch Tests in Large SBR

Test day	160	166	232	256	276
Time (hrs)					
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
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42					
43					
44					
45					
46					
47					
48	Settling	Settling	Settling	Settling	Settling

Shaded Region Denotes Anoxic Period
Unshaded Region Denotes Aerobic Period

Settling denotes settling time

Appendix 4.1 Yield Coefficient Batch Test in Stage 1

Parameter Time (hrs)	mlvss g/l Sample 1	cod mg/l Sample 1	mlvss g/l Sample 2	cod mg/l Sample 2	mlvss g/l Sample 3	cod mg/l Sample 3
0	0.207		0.203		0.229	
1.5	0.267	2210	0.253	2244	0.273	2032
3	0.307	1784	0.307	1800	0.353	1647
4.5	0.423	1651	0.387	1647	0.483	1515
6	0.560	1373	0.520	1406	0.583	1193
8	0.670	1019	0.617	1109	0.743	853
24	0.720	291	0.737	300	0.753	271

	u hr^{-1} Gradient of Graph
Sample 1	y = 0.1524x - 1.5704 R2 = 0.9864
Sample 2	y = 0.144x - 1.5915 R2 = 0.9914
Sample 3	y = 0.1536x - 1.4851 R2 = 0.9913

Appendix 4.2 Batch Growth Kinetic Test to Determine Ks and umax

MLVSS of Batch Test in g/l

Sample Time (Hrs)	1	2	3	4	5	6	7	8
0	0.156	0.160	0.162	0.156	0.160	0.162	0.160	0.156
1.5	0.393	0.314	0.357	0.473	0.347	0.350	0.307	0.413
3	0.587	0.620	0.500	0.523	0.453	0.453	0.307	0.530
4.5			0.570		0.470	0.453	0.333	
6	0.877	0.773	0.620	0.740	0.380	0.373	0.307	0.577
8	0.733	0.737	0.620	0.577	0.423	0.430	0.277	0.550
15	0.925	0.910	0.645	0.640	0.415	0.440	0.330	0.570
24	1.245	1.000	0.805	0.750	0.495	0.550	0.370	0.625

(ln MLVSS) of Batch Test used to Determine Specific Growth Rate (u)

Sample Time (Hrs)	1	2	3	4	5	6	7	8
0	0	0	0	0	0	0	0	0
1.5	0.924	0.677	0.791	1.109	0.776	0.772	0.654	0.973
3	1.323	1.358	1.129	1.209	1.044	1.031	0.654	1.222
4.5			1.260		1.081	1.031	0.737	
6	1.725	1.579	1.344	1.556	0.868	0.837	0.654	1.306
8	1.547	1.530	1.344	1.306	0.976	0.978	0.551	1.259
15	1.779	1.741	1.384	1.410	0.956	1.001	0.727	1.295
24	2.076	1.836	1.605	1.569	1.132	1.224	0.841	1.387

COD of Batch Test in mg/l

Sample / Time (Hrs)	1	2	3	4	5	6	7	8
0	2596	2668	1206	1149	754	706	260	1038
1.5	1833	1672	915	982	486	424	186	672
3	1494	1468	682	631	257	253	116	268
4.5	944	877	462	362	241	207	132	234
6	672	862	255	350	247	267	149	217
8	832	808	270	310	235	178	123	215
15	284	287	172	163	156	103	92	108
24	462	531	258	272	230	184	138	106

Parameter / Sample	Specific growth rate u	Initial Substrate conc. S	S/u	Observed Yield yo
1	0.268	2596	403.306	0.37
2	0.258	2668	431.213	0.28
3	0.275	1206	183.060	0.58
4	0.226	1149	211.555	0.49
5	0.234	754	134.259	0.59
6	0.224	706	131.618	0.64
7	0.218	260	49.717	
8	0.192	1038	225.731	0.49

$$\text{Observed Yield} = \frac{S_0 - S1}{MLVSS_1 - MLVSS_0}$$

Plot from, S/u vs S

y = 0.1497x + 27.072
R2 = 0.9749

1/ umax = 0.1497
umax = 6.68 hr-1

When S = 0 then S/u = Ks/umax
Ks = 180.84 mg/l

Appedix 5.1 Denitrification Test in Large Reactor on day 230 (20/12/98)

Parameter Time (hrs)	Nitrate mg N/l	COD mg/l	MLVSS g/l
0	19.37	1750	2.560
1	13.69	2129	3.245
2	13.90	1517	2.728
3	10.40	2388	2.646
4	7.60	2250	2.650
5	6.27	1845	2.663
6	4.42	1991	2.654
7	2.57	1983	3.252
8	0.72	1683	2.755
9	0.10	1641	2.617
10	0.05	1859	2.720

Denitrification Rate = gradient of graph

y = -2.1845x + 17.511
R2 = 0.9692

Appedix 5.2 Denitrification Test in Large Reactor on day 270 (29/1/99)

Parameter Time (hrs)	Nitrate mg N/l	COD mg/l	MLVSS g/l
0	95.28	758	2.290
2	42.50		2.471
4	34.83		2.861
4.5	30.16	1044	2.510

Denitrification Rate = gradient of graph

y = -13.576x + 86.327
R2 = 0.8569

Appedix 5.3 Denitrification Test in 1 litre Reactor on day 282 (10/2/99)

Parameter Time (hrs)	Nitrate mg N/l	COD mg/l	MLVSS g/l
0	123.19	701	2.418
0.5	107.12	425	2.553
1	80.44	246	2.535
1.5	64.42	193	2.431
2	43.64	175	2.500

Denitrification Rate = gradient of graph

$y = -22.114x + 99.043$
$R2 = 0.9432$

Appedix 5.4 Denitrification Test in 1 litre Reactor on day 275 (3/2/99)

Parameter Time (hrs)	Nitrate mg N/l	COD mg/l	MLVSS g/l
0	44.98	1767	1.990
0.5	41.12	1786	2.048
1	33.14	1674	2.110
1.5	25.74	1665	2.180
2	14.88	1471	2.250
2.5	17.93	1360	2.350
3	0.00	1332	2.267

Denitrification Rate = gradient of graph

$y = -15.118x + 47.09$
$R2 = 0.9761$