BIOLOGICAL PHOSPHORUS REMOVAL FROM A PHOSPHORUS RICH DAIRY PROCESSING WASTEWATER

A thesis presented in partial fulfilment of the requirements

for the degree of

Doctor of Philosophy

in

Environmental Engineering

at

Massey University

Turitea Campus, Palmerston North,

New Zealand

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2005

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ABSTRACT

A phosphorus rich wastewater, typical of a dairy processing site producing milk powder, was biologically treated in a continuous activated sludge reactor.

A literature review indicated there was a vast amount of information on the mechanisms of the Enhanced Biological Phosphorus Removal (EBPR) process and its application to domestic wastewaters, but little successful research on its application to dairy processing wastewater.

The biodegradability of the wastewater organic fractions was assessed due to their impact on the EBPR process. Continuous anaerobic fermentation tests were used to determine the concentration of volatile fatty acids that could be generated, as these are required for successful EBPR. A fermenter hydraulic retention time of 12 hours and a temperature of 35 ^{o}C generated the highest concentration of volatile fatty acids, with an acidification rate of 65% (based on 0.45μm filtered COD).

To permit improved dissolved oxygen control and increased flexibility, a multi-zone reactor was designed. A fermentation stage was also incorporated prior to the activated sludge reactor. This reactor was operated with anaerobic, anoxic and aerobic zones at an SRT of 10 days and stable biological phosphorus removal was achieved. A maximum of 41.5 mg P/L was removed and phosphorus release and PHA storage occurred in both the anaerobic and anoxic zones. The soluble COD consumed in the unaerated zones (anaerobic + anoxic) totalled 484 mg COD/L on the day of the zone study (day 158). The aerobic sludge phosphorus concentration averaged 7.0% mg P/mg VSS after system optimisation. The anaerobic volume was doubled in order to increase the anaerobic consumption of volatile fatty acids. This change increased the amount of soluble COD consumption in the unaerated zones to 632 mg P/L after 40 days but did not result in a significant increase in biological phosphorus removal.

In the next series of trials, the concentration of nitrogen in the wastewater was decreased and the anoxic zone removed. This change did not improve the amount of biological phosphorus removal, which was 35 mg P/L at an SRT of 10 days. The effect of different sludge retention times was then investigated. Increasing the SRT to

15 days resulted in little change in phosphorus removal (34.5 mg P/L). Decreasing the SRT to 5 days resulted in the loss of EBPR.

The medium term effect on the EBPR process by removing the fermentation stage was also assessed using an AO configuration at an SRT of 10 days. The amount of phosphorus removed decreased slightly after 34 days to 34 mg P/L, but the soluble COD consumed in the anaerobic zone increased to 624 mg P/L.

It was concluded that a stable EBPR process could be established when treating a dairy processing wastewater with a continuous activated sludge reactor. The biological stability was sensitive to changes in the solids retention time and the removal of the fermentation stage.

ACKNOWLEDGEMENTS

I wish to acknowledge Professor Rao Bhamidimarri for establishing this project and encouraging me to undertake it and also his patience during this long process of completion. I also wish to acknowledge the financial support and input of the New Zealand Dairy Research Insitute (now Fonterra Reseach), especially Mike Donkin, Jim Barnett and latterly John Russell and Joanna Shepherd. Their patience has also been much appreciated.

The assistance of the technical staff of the Massey University Institute of Technology has also been much appreciated. Without the input of Don McLean in the mechanical workshop and Bruce Colins in the electrical workshop, this project would not have been possible as they made my experimental concepts a reality. Thanks very much Don and Bruce. Thanks also to John Sykes for providing analytical assistance and keeping the instruments functioning and to Anne-Marie Jackson for placing consumables orders and finding equipment. Mention must be made of the golf outings with Don and John that kept me partially sane. The companionship and knowledge of Magnus Christensson during his two years working on this difficult project was also invaluable.

The encouragement and support of Andy Shilton who completed his own thesis while continuing a busy lecturing schedule has been much appreciated. Thanks mate.

Finally thanks to my family for their support. To my wife Fiona and our daughters Sera and Amy for putting up with this whole process. As we have progressed through marriage, having a family, changing cities and employment this study has always been present, hopefully now we can look forward to more time together. Leana Hola! Thanks also to my mother for her solid support as always, this thesis is dedicated to her.

TABLE OF CONTENTS

LIST OF FIGURES

Page

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 8

Chapter 9

LIST OF TABLES

Chapter 5

Chapter 6

Chapter 7

Chapter 8

Chapter 9 No Tables

Chapter 10

ABBREVIATIONS AND NOMENCLATURE

AAO	Anaerobic-Anoxic-Oxic
AO	Anaerobic-Oxic
ASM1	Activated Sludge Model No. 1
ASM2	Activated Sludge Model No. 2
BNR	Biological Nutrient Removal
BOD	Biochemical Oxygen Demand (mg/L)
COD	Chemical Oxygen Demand (mg/L)
DO	Dissolved Oxygen (mg/L)
EBPR	Enhanced Biological Phosphorus Removal
GAO	Glycogen Accumulating Organisms
HRT	Hydraulic Retention Time (d)
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
MUCT	Modified University of Cape Town
NUR	Nitrate Uptake Rate
OUR	Oxygen Uptake Rate
P	Phosphorus
PAO	Polyphosphate Accumulating Organisms
PHA	Poly-β hydroxyalkanoates
PHB	Poly-β hydroxybutyric Acid
PHV	Poly-β hydroxyvaleric Acid
RAS	Return Activated Sludge
RBCOD	Readily Biodegradable Chemical Oxygen Demand
S_A	Fermentation Products as Acetate Equivalents (mg/L)
SBCOD	Slowly Biodegradable Chemical Oxygen Demand
SCVFA	Short Chain Volatile Fatty Acids
S_F	Fermentable Readily Biodegradable Substrates (mg/L)
S_{II}	Inert Soluble Substrate (mg/L)
SOUR	Specific Oxygen Uptake Rate
SRT	Sludge Retention Time (d)

S_{S1}	Readily Biodegradable Substrate (mg/L)
SVI	Sludge Volume Index (ml/g)
S/X	Substrate to Biomass Ratio
TKN	Total Kjehldahl Nitrogen (mg/L)
TSS	Total Suspended Solids (mg/L)
UCT	University of Cape Town
VFA	Volatile Fatty Acids
VSS	Volatile Suspended Solids (mg/L)
X_{S1}	Slowly Biodegradable Substrate (mg/L)
Y_H	Heterotrophic Yield Coefficient (mg Cell COD/mg COD consumed)
Y_{HD}	Anoxic Yield Coefficient (mg Cell COD/mg COD Consumed)
Y_{PO4}	Ratio of Phosphorus Released to COD consumed (mg P/mg COD)

CHAPTER 1
Introduction

1.1 The New Zealand Dairy Industry and Wastewater Management

The dairy industry comprises around 20% of New Zealand's exports by commodity on a revenue basis and consists of 15,000 dairy farmers and 3.2 millions cows (Statistics New Zealand, 2003). Of the annual milk production of 12 billion litres, 96% is converted into 1.6 million tonnes of product at around 25 processing sites (mainly butter, cheese, milk powder and casein), of which 90-95 % is exported (NZ Dairy Board, 2001). With a conservative ratio for the volume of wastewater produced to volume milk processed at a processing plant of 1.0 (Danalewich *et al.*, 1998; Dick, 1998; Environment Canada, 1997; Rule, 1997), there is at least 12 million m^3 of dairy processing wastewater that needs to be managed each year in New Zealand.

In New Zealand, land irrigation of dairy processing wastewater is the predominate method of disposal with varying degrees of primary and secondary treatment prior to irrigation (Leonard, 1996). Irrigation is feasible where the processing site is surrounded by suitable agricultural land and agreements can be reached with landowners to use the nutrient content of the wastewater as a fertiliser. With the move to fewer centralised dairy processing facilities there is a corresponding increase in the wastewater volume generated at each processing site, resulting in greater land areas being required for wastewater irrigation. The soil type, nutrient content of the wastewater and seasonal weather characteristics largely determine the rate of wastewater irrigation per unit area of land. However, the mass of nitrogen applied per area per annum rather than the hydraulic loading is one of the principal determinants controlling the rate of irrigation. For those processing sites where irrigation, is either not an option, or cannot manage increasing volumes of wastewater, other methods of treating the wastewater and removing nutrients need to be assessed. These other nutrient removal methods are typically chemical or biological methods.

1.2 Phosphorus and the Dairy Industry

Until recently nitrogen has been the primary nutrient of environmental concern, with limitations on the wastewater nitrogen concentrations prior to discharge to receiving

waters. The role of phosphorus as a nutrient in the eutrophication of receiving waters is now recognised in New Zealand, with the introduction of phosphorus discharge limits to sensitive receiving waters. The phosphorus content of New Zealand dairy processing wastewaters can range from 12 mg/L to over 100 mg/L depending on the process, product and cleaning procedures used (Barnett *et al.*, 1994). Internationally the phosphorus content of dairy processing effluents has occasionally been reported at over 200 mg/L (Danalewich *et al.*, 1998). This is substantially greater than domestic wastewaters (~15 mg/L). The bulk of the phosphorus in dairy processing wastewaters originates from detergents and cleaners.

1.3 Biological Phosphorus Removal and Dairy Processing Wastewaters

In New Zealand and internationally chemical phosphorus precipitation is typically the phosphorus removal method of choice from dairy processing wastewater (Papagiannis, 1996). However, several New Zealand dairy processing sites currently use biological treatment processes (anaerobic and aerobic) to remove organic matter (carbonaceous) from wastewater. For those sites that use aerobic activated sludge processes, the conversion of these facilities to full biological nutrient removal (BNR) treatment processes is a potential option to reduce both nitrogen and phosphorus discharges.

The adaptation of the activated sludge process to remove phosphorus biologically from domestic wastewater is common place internationally and is used occasionally in New Zealand. This process is known as Enhanced Biological Phosphorus Removal (EBPR). Over the last decade, a significant amount of research has been carried out into the mechanisms of biological phosphorus removal. Most of this research has investigated biological phosphorus removal from domestic wastewater or have been laboratory investigations with acetate as the primary substrate. Biological phosphorus removal from industrial wastewaters, especially dairy processing wastewater, has not been researched to the same degree.

There is little literature available on the successful application of the EBPR process to dairy processing wastewater (Comaeu *et al.*, 1996; Kolarski and Nyhuis, 1995). Some pilot and full-scale wastewater treatment plants have been constructed that can potentially remove phosphorus biologically, but have not achieved significant

biological removal. The lack of EBPR activity was in some cases due to the operational conditions or no consideration was given to the wastewater organic substrate characteristics and nitrogen concentrations and their effect on the EBPR process (Donkin and Russell, 1997; Papagiannis, 1996). It is generally accepted that short chain volatile fatty acids (SCVFA's), or readily biodegradable COD are required for biological phosphorus removal. Previous studies have shown that there is a significant amount of readily biodegradable COD present in dairy processing wastewaters, most likely as sugars (Sozen and Orhon, 1999; Leonard, 1996). There is some doubt as to whether this could be converted to SCVFA in the anaerobic zone of a BNR system or sequestered directly. Some studies using glucose as the organic substrate have lead to the deterioration of EBPR in BNR reactors by encouraging the proliferation of competing bacteria, that can sequester glucose directly in the anaerobic zone, without the subsequent storage of polyphosphate under aerobic conditions (Mino *et al.*, 1998).

1.4 Research Approach

Successful biological phosphorus removal requires an easily metabolised substrate, especially volatile fatty acids (VFA). For this reason in Chapter 4, the initial experimental work sought to determine the organic fraction biodegradability of the synthetic dairy processing wastewater used in this study. The generation of VFA by external fermentation of the wastewater or sludge is now a common part of many EBPR processes. Fermentation trials were used to evaluate the ease and degree of VFA generation from the synthetic dairy processing wastewater used.

A preliminary continuous activated sludge trial (without external fermentation) provided valuable information (Chapter 5) as to the requirements for, and the ease of, obtaining successful biological phosphorus removal. A continuous activated sludge configuration was used rather than sequencing batch systems, as in New Zealand all existing dairy processing activated sludge systems are continuous flow reactors. Biological phosphorus removal was not achieved with this preliminary reactor system.

In the next phase of the study in Chapter 6, an improved system was designed and operated consisting of ten zones. This permitted improved control, and increased

flexibility in regard to unaerated and aerated zone requirements. It also required less inter zone connections and pumps. This improved reactor system also included an external fermentation stage in order to make the conditions for EBPR as favourable as possible. This phase of the study concentrated on maximising and maintaining stable phosphorus removal with a combined phosphorus and nitrogen removal system (AAO). By extending the volume of the anaerobic zone it was found that anaerobic VFA consumption increased and the removal of phosphorus slightly improved. Batch tests used mixed liquor from the reactor with acetate as the sole carbon source, in order to provide rate and stoichiometric information that could be compared with literature values.

In order to assess biological phosphorus removal performance without the requirement for nitrogen removal, the wastewater was modified to decrease its nitrogen concentration. The effect of varying the sludge retention time was investigated and provided valuable information as to the best operational parameters. This information when operating with an AO configuration is detailed in Chapter 7.

Three factors that could influence the EBPR process when specifically treating dairy processing wastewater were investigated (Chapter 8). These were: 1) simultaneous chemical precipitation, 2) the effects of withdrawal of the fermentation stage and, 3) the requirement of metal ions during biological phosphorus removal from a dairy processing wastewater.

The performance of different reactor configurations on EBPR is discussed in Chapter 9, along with recommendations for further research and a discussion of the implications for full-scale systems.

1.5 Specific Objectives

- Assess and quantify the readily biodegradable fraction of a synthetic wastewater that was typical of a New Zealand dairy processing wastewater and compare this fraction's rate of utilisation to acetate.

- Assess the amount of VFA that could be generated from this wastewater when using a prefermentaion step.
- Determine whether biological phosphorus removal could initially be established with an AAO continuous activated sludge reactor without incorporating a prefermentation step.
- Establish biological phosphorus removal with an AAO configuration and a prefermentation step and determine the overall amount of phosphorus removal and the role of the anoxic zone in the phosphorus removal process.
- Remove the anoxic zone, while maintaining the same anaerobic zone HRT, and determine the impact on overall phosphorus removal, including anaerobic COD consumption.
- Assess the effect of varying the SRT on biological phosphorus removal.
- Perform batch tests with acetate as the standard substrate for each system that achieves biological phosphorus removal and determine COD consumption and phosphorus release rates and stoichiometric coefficients.
- Compare removal rates, uptake rates and stoichiometric coefficients between the different reactor operations and the corresponding batch tests.

CHAPTER 2
Literature Review

2.1 Phosphorus and the Environment

Growing importance has been assigned to the effects of the nutrients, phosphorus and nitrogen on the aquatic environment. The nutrients, phosphorus and nitrogen are essential elements in all-living matter and are necessary for the growth of living organisms. The discharge of these nutrients to waterways can stimulate a series of changes in the ecosystem known as eutrophication. Eutrophication is defined as the waterway becoming rich in nutrients with a subsequent acceleration in plant and plankton growth, leading to the lowering of dissolved oxygen levels (Figure 2.1). This lack of oxygen leads to the death of fish and other living organisms in the water body. Eutrophication primarily threatens poorly replenished water bodies such as natural lakes, bays and sluggish streams.

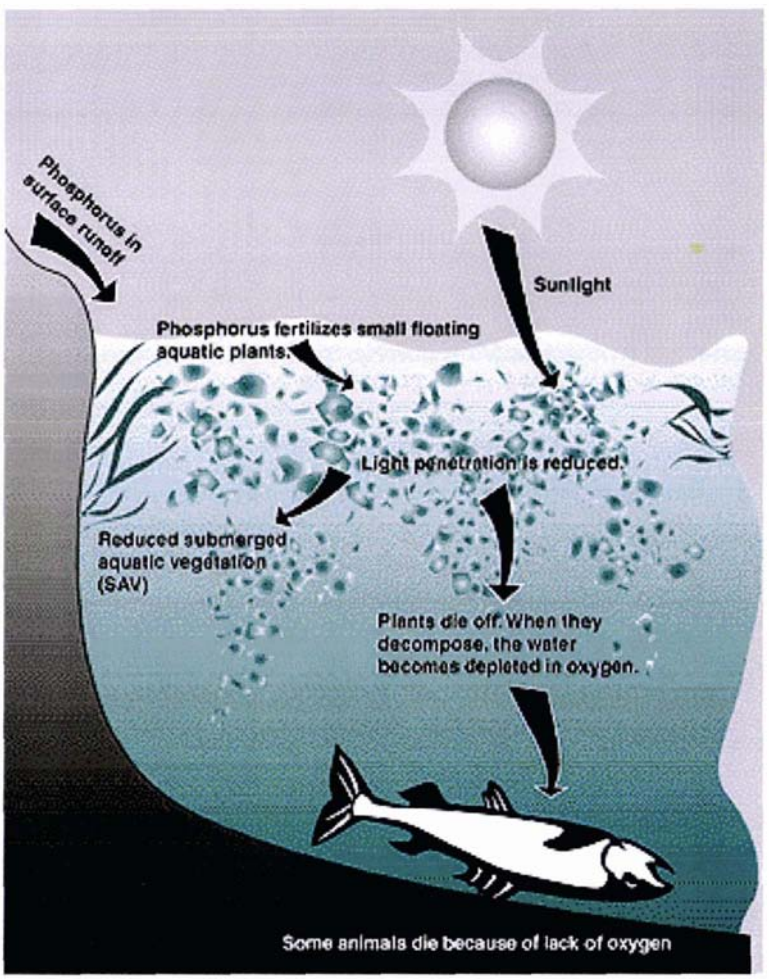


Figure 2.1: Biological effects of eutrophication (Queens University, 2003).

If the phosphorus input into the aquatic system is large enough, nitrogen becomes the limiting factor for plant growth and the growth of blue-green algae and other nitrogen-fixing bacteria is activated, bringing the system back into balance. Due to this phenomenon, the eutrophication control strategies are generally based upon the removal of phosphorus in effluent discharges to water bodies.

Phosphorus in water bodies may originate from either point (eg. domestic and industrial wastewaters) or non-point sources (eg. surface run-off from farms using nutrient rich fertilisers). Typical phosphorus concentrations in domestic wastewater range from 6 - 20 mg/l, with detergents contributing a significant proportion of the phosphorus. Nearly 70% of the total phosphorus in domestic wastewater is inorganic (orthophosphates and polyphosphates) with the remainder organically bound. Orthophosphates (PO_4^{-3},HPO_4^{-2}, $H_2PO_4^-$, and H_3PO_4) are easily assimilated by microrganisms, whereas other forms of phosphorus (polyphosphates and organic phosphorus) become available to microorganisms only after hydrolysis to orthophosphates. The phosphorus fractions in industrial wastewaters are largely dependent of the type of industry. Wastewater from food processing industries that use large amounts of detergents for cleaning process equipment will have higher phosphorus concentrations, than wastewater from industrial processes with less rigorous cleaning requirements.

2.2 Chemical and Biological Phosphorus Removal Options

2.2.1 Chemical

Traditionally phosphorus has been removed from wastewater using chemical processes. This usually involves the addition of certain chemicals to produce insoluble or low-solubility salts when combined with phosphate (Metcalf and Eddy, 2003). The principal chemicals used for chemical phosphorus removal are calcium salts usually lime (at a pH between 8 and 11), ferric chloride (pH 5.3), and aluminium sulphate (Alum) (pH 6.3). The reactions associated with these three chemicals when used for phosphorus removal are shown in Table 2.1.

Table 2.1: Precipitation reactions of phosphorus with lime, alum and iron Fe (III).

Chemical	Reactions	Chemical Species in sludge
Lime	$10Ca^{+2} + 6PO_4^{-3} + 2OH^- \leftrightarrow Ca_{10}(PO_4)_6(OH)_2$	$Ca_{10}(PO_4)_6(OH)_2$
	$Mg^{+2} + 2\ OH^- \leftrightarrow Mg(OH)_2$	$Mg(OH)_2$
	$Ca^{+2} + CO_3^{-2} \leftrightarrow CaCO_3$	$CaCO_3$
Alum	$Al^{+3} + PO_4^{-3} \leftrightarrow AlPO_4$	$AlPO_4$
	$Al^{+3} + 3\ OH^- \leftrightarrow Al(OH)_3$	$Al(OH)_3$
Iron Fe (III)	$Fe^{+3} + PO_4^{-3} \leftrightarrow FePO_4$	$FePO_4$
	$Fe^{+3} + 3\ OH^- \leftrightarrow Fe(OH)_3$	$Fe(OH)_3$

With chemical phosphorus removal it is principally the orthophosphate that is precipitated in the reaction, therefore chemical addition after secondary treatment (i.e. as a tertiary treatment step) is usually the most effective. This is because polyphosphates and organic phosphorus are transformed into orthophosphate during secondary treatment. De Hass *et al.* (2000a), identified that chemical addition may take place at one of three stages of wastewater treatment, namely:

- Primary treatment, (addition before primary sedimentation) for which the term pre-precipitation is used;
- Secondary treatment (addition before secondary sedimentation) for which the term simultaneous precipitation is used;
- Tertiary treatment (chemical flocculation followed by sedimentation) for which the term post-precipitation is used.

2.2.2 Biological

Biological phosphate removal from wastewater can be achieved in two ways: as stoichiometric coupling to microbial growth or enhanced storage in the biomass as polyphosphate (Mino *et al.*, 1998). The typical phosphorus content of conventional microbial cells is 1 to 2 percent on a dry weight basis (Bond and Rees, 1998). Wasting of excess biological solids from a conventional activated sludge process may result in 10% to 30% phosphorus removals. This is of course dependent on the biomass

production and solids retention time (SRT). The key to enhanced biological phosphorus removal (EBPR) is the activity of certain bacteria that take up phosphorus under aerobic or anoxic conditions, accumulating it as polyphosphate within the cell. The waste sludge from activated sludge plants incorporating biological phosphorus removal is typically in the range of 3% to 7%. This processes involves design or operational modifications to conventional activated sludge systems that result in the growth of a biological population that has a much higher cellular phosphorus content. For EBPR activated sludge systems treating domestic wastewater, effluent phosphorus concentrations of 1 mg P/l can be achieved. Chemical phosphorus precipitation within the activated sludge system can also occur due to the presence of calcium and magnesium ions in solution at a pH of between 7.5 and 8.5 (de Hass *et al.*, 2000a)

2.3 Enhanced Biological Phosphorus Removal (EBPR)

Enhanced Biological Phosphorus Removal (EBPR) is achieved by exposing bacteria in the activated sludge process to alternating anaerobic and aerobic conditions. These conditions favour the growth of a certain group of organisms called polyphosphate-accumulating organisms (PAO's), which are able to store more phosphorus than they need for growth. The process was first extensively investigated by Levin and Shapiro (1965) who created the expression "luxury uptake" to describe the excess phosphorus storage (as polyphosphate). The release of phosphorus under anaerobic conditions was reported by Shapiro *et al.* (1967), but the requirement of an anaerobic zone as a prerequisite for phosphorus removal was first reported by Barnard (1975, 1976) and Fuhs and Chen (1975).

The first full-scale plants with biological phosphorus removal were constructed from the middle 1970's to the early 1980's. The first full-scale plant designed for high-rate simultaneous biological nitrogen and phosphorus removal was the Meyerton plant in South Africa in 1974 (Randell *et al.*, 1992). In New Zealand the operation of full-scale biological phosphorus removal plants did not begin until the early 1990's and is still not widespread. The use of chemicals to remove phosphorus is still often favoured in New Zealand as it is seen as more reliable and less complex.

2.3.1 Principles of EBPR

When exposed to anaerobic conditions PAO's convert organic carbon to intracellular organic polymers called poly-β hydroxyalkanoates (PHA). The PHA's comprise poly-β-

hydroxbuytric acids (PHB) and poly-β-hydroxyvaleric acids (PHV). The energy for PHA formation is generated from the hydrolysis of stored polyphosphate and glycogen. The preferred forms of organic carbon are volatile fatty acids (VFA's). During anaerobic conditions, the hydrolysis of polyphosphate releases orthophosphate into solution with a subsequent rise in orthophosphate concentrations. On re-exposure to aerobic (or anoxic) conditions the stored PHA is utilised as a source of organic carbon and energy for polyphosphate storage resulting in a net uptake of phosphorus (Figure 2.2).

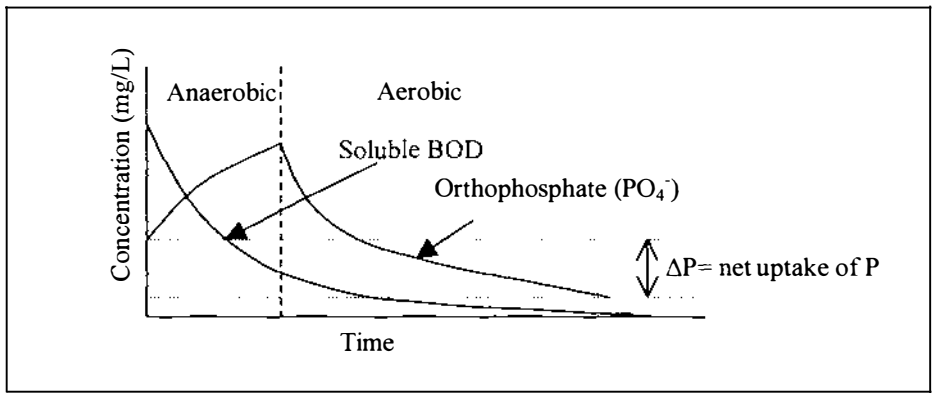


Figure 2.2: Profiles of soluble BOD and phosphorus during the EBPR process.

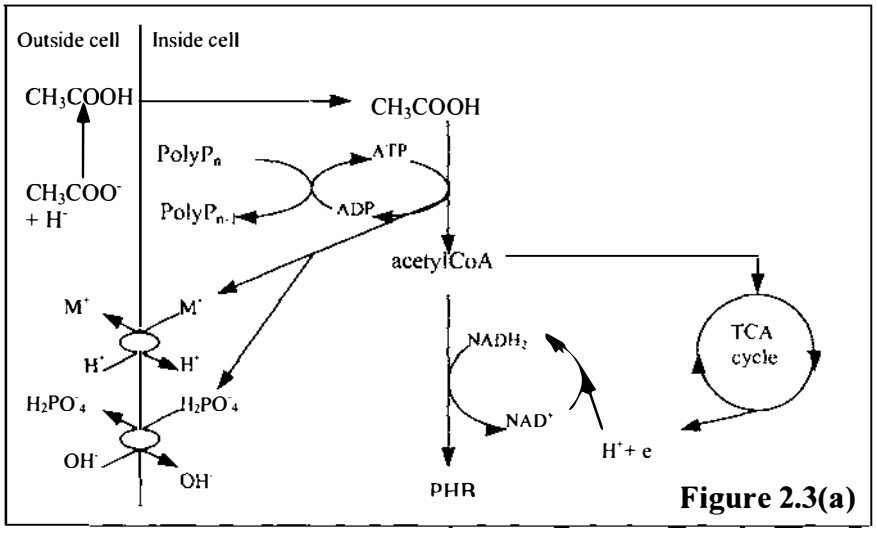
2.3.2 Biochemical Principles of EBPR

The biochemical principles of anaerobic PHA synthesis have been described in two biochemical models; Wentzel *et al.* (1991), described them as the Comeau/Wentzel model (Comeau *et al.*, 1986; Wentzel *et al.*, 1986) and the Mino model (Mino *et al.*, 1987).

In the Comeau/Wentzel model, the biochemical reactions under anaerobic and aerobic conditions are shown in Figure 2.3. The Comeau/Wentzel model proposed that under anaerobic conditions, neutral acetic acid is transported across the cell membrane with a decrease of one H^+ in the pH gradient with every acetate molecule transported (in solution, at a pH > 6.5, more than 99% of the acetate is in the ionic form). *Acinetobacter spp.* is used as the organism typifying the PAO group of organisms. Comeau *et al.* (1986), describes the energised state of the cell membrane as the proton motive force

(pmf) gradient. The intracellular acetate is activated to acetyl-CoA by coupled ATP hydrolysis. Hydrolysis of the ATP releases metal cations M^+, (usually K^+ or Mg^{2+}) and the anion $H_2PO^-_4$. The phosphate released is co-transported with the cations (M^+) out of the cell by the action of a pH-sensitive carrier. Polyphosphate is used to generate ATP from ADP by transfer of a phosphoryl group from the polyphosphate to the ADP. Two molecules of acetyl-CoA condense to form acetoacetyl-CoA, which is then reduced by $NADH_2$ to form hydroxylbutyryl-CoA, which is then polymerised to poly-β-hydroxybutyrate (PHB). Under aerobic conditions, the accumulated PHB will generate energy, by feeding the TCA cycle with acetyl-CoA, the electron transport chain will generate the PMF required for ATP formation. The ATP/ADP ratio will increase and the formation of polyphosphate from ATP will take place. Wentzel *et al.*, (1986) modified this model by describing how the ATP/ADP and NADH/NAD ratios control polyphosphate and PHB synthesis. The NADH/NAD ratio rises under anaerobic conditions because of a shortage of electron acceptors, and the repression of polyphosphate degradation leads to a decrease in ATP/ADP. This leads to an increased amount of acetyl-CoA, which controls the TCA cycle and stimulates PHB synthesis and decreases the NADH/NAD ratio and ensures that acetyl-CoA is shared between the two pathways.

In the Mino model (Mino *et al.*, 1987), the reducing power is derived from the degradation of intracellularly stored glycogen (to pyruvate then to NADH), as compared to the Comeau-Wentzel model were partial oxidation of acetyl-CoA through the TCA cycle produces the reducing power. The Mino model proposes that NADH as well as ATP is generated from the anaerobic metabolism of stored intracellular carbohydrate such as glycogen (Figure 2.4). Experimental data supports the Mino model, as levels of cell glycogen seem to fall in the anaerobic period, and are regenerated under aerobic conditions.



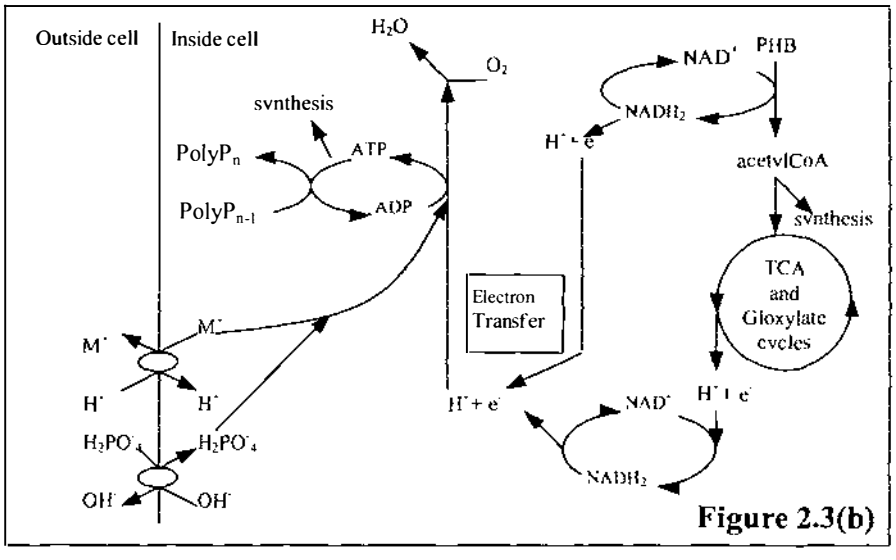


Figure 2.3: Schematic diagrams for the behaviour proposed by the Comeau/Wentzel model under anaerobic (a) and aerobic (b) conditions.

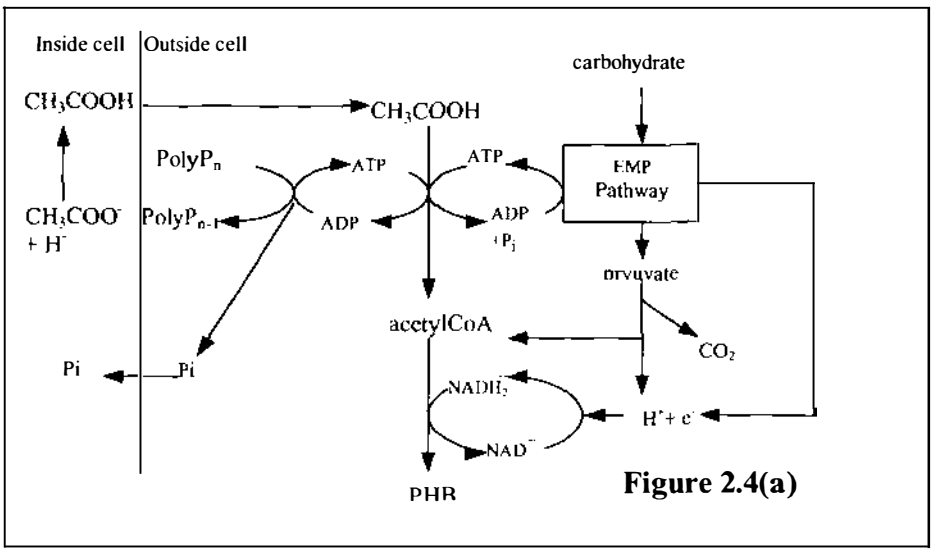


Figure 2.4(a)

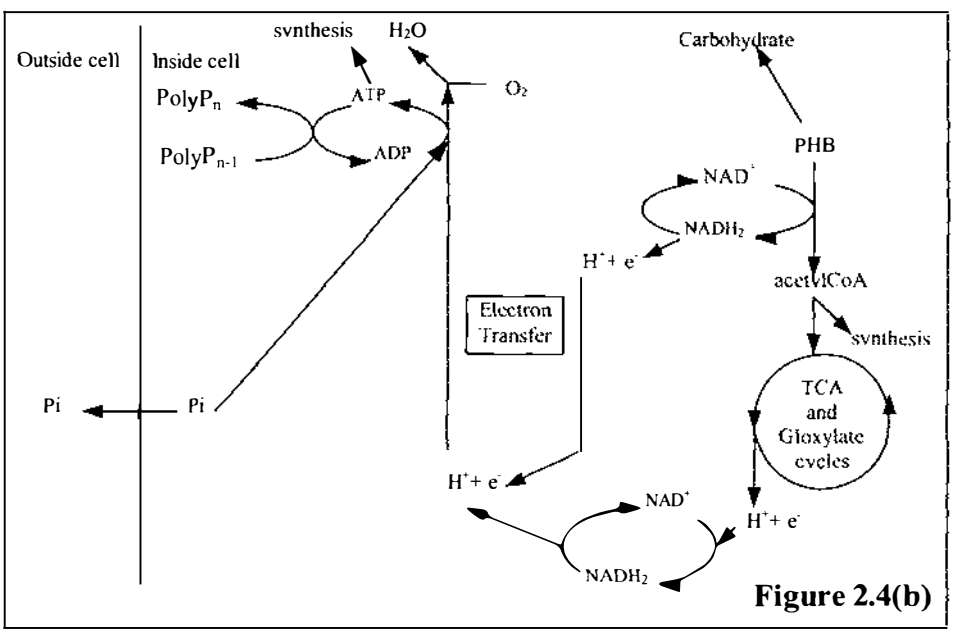


Figure 2.4(b)

Figure 2.4: Schematic diagrams of the biochemical mechanisms proposed by the Mino model under anaerobic (a) and aerobic (b) conditions.

The Comeau-Wentzel and Mino models were reviewed by Mino *et al.* (1998), in which research was summarised that strongly supported the Mino model. It was concluded that although the experimental evidence favoured the Mino model, the partial functioning of the TCA cycle could not be totally excluded. A study by Pereira *et al.* (1996), with PAO-enriched sludge under anaerobic conditions using ^{13}CNMR showed that a small

amount of the carbon in acetate was released as CO_2 supporting the partial functioning of the TCA cycle.

2.3.3 Microbiology of EBPR

The identification and isolation of the microorganisms involved in the EBPR process has been the centre of a significant amount of research. Phosphorus Accumulating Organisms (PAO's) were initially thought to be comprised principally of *Acinetobacter spp.* (Fuhs & Chen, 1975), and the early biochemical models were based on accepting *Acinetobacter spp.* to be the typifying PAO organism group (Wentzel *et al.*, 1986; Wentzel *et al.*, 1991). It is now accepted that *Acinetobacter spp.* is not primarily responsible for EBPR (Mino *et al.*, 1998). Microbiological research has shown that *Acinetobacter spp.*comprise less than 10% of the bacteria responsible for EBPR (Cloete and Steyn, 1987; Hiraishi *et al.*, 1989; Wagner *et al.*, 1994; Bond *et al.*, 1995; Kampfer *et al.*, 1996). It appears that the initial predominance of *Acinetobacter spp.* in EBPR sludges can be attributed to the culture dependent methods used, as only bacteria that are culturable on artificial media can be identified and isolated (Mino *et al.*, 1998).

It now appears that there is no single dominant group of microorganisms responsible for EBPR activity. Research using gene probes (Wagner *et al.*, 1994; Bond *et al.*, 1995; Kampfer *et al.*, 1996), denaturing gel gradient electrophoresis (DGGE) (Brdjanovic *et al.*, 1997), and restriction fragment length polymorphism (RFLP) (Liu *et al.*, 1997) indicates that there are at least three dominant groups. In a review of EBPR microbiology (Mino *et al.*, 1998) suggests that PAO's consist of several different bacterial groups and can change from time to time and place to place.

2.3.4 Substrate Influences on EBPR

The characteristics of the available wastewater organic substrates are an influential determinant in effective phosphorus removal. The beneficial effect of maximising readily biodegradable COD (RBCOD), particularly short-chain VFA's in the influent to the anaerobic stage of EBPR processes has been recognised for sometime (Barnard, 1984; Nicholls *et al.*, 1979; Wentzel *et al.*, 1985). Sometimes these VFA's may be present in the wastewater or may be created in a previous and separate fermentation stage (Cañizares, *et al.*, 1999). It is also generally accepted that VFA generation can occur in the anaerobic zone of many EBPR systems due to fermentation of readily

biodegradable substrate (Bamard, 1992). In most experimental studies of EBPR processes acetate (or acetic acid) has been added to stimulate EBPR, and in some cases used in comparative studies with reactors were unfermented domestic wastewater has been added (Tasli, *et al.*, 1999). Studies have also been performed to determine the most favourable VFA's for EBPR (Gerber *et al.*, 1986; Randall and Khouri, 1998).

Gerber *et al.* (1986), tested the response of sludge from full-scale EBPR plants treating wastewater in South Africa to various organic substrates. The study showed that for sludge from seven different plants, formic, acetic, propionic, butyric, lactic and succinic acids induced effective phosphorus release. Acetic and propionic acids were utilised the most rapidly and resulted in the most rapid phosphorus release rates. Citric acid, glucose, ethanol, methanol, 2,3 butanediol and settled sewage resulted in much lower rates of phosphorus release.

The release of phosphorus and the concurrent storage of PHB and PHV were quantified in the presence of different simple carbon substrates by Comeau *et al.* (1987). This was performed using a series of batch tests, with a 3.5 hour anaerobic phase after carbon addition followed by a 3.5 hour aerobic phase, using sludge from a pilot plant treating domestic wastewater. The various ratios of P release, P uptake and PHA storage polymers (PHB, PHV) from Comeau *et al.* (1987), are summarised in Table 2.2 for a set of experiments. In this study by Comeau *et al.*, (1987), acetic and propionic acids had uptake rates of 50 mg COD/L/h compared to mixtures of these two SCVFA's with uptake rates of 80 mg COD/L/h. Lactic, butyric and valeric acids had uptake rates of 7.0 to 3.5 mg COD/L/h, while fermented sewage had an uptake rate of 40 mg COD/L/h . It was suggested that acetic and propionic acids were stored directly as PHA whereas the larger molecular weight SCVFA's had to be metabolised by other fermentative bacteria first. Substrates with an even number of carbons (acetate, butyric) favour the storage of PHB while substrates with an odd number of carbons (propionic, lactic, valeric acids) favour the storage of PHV (Table 2.2).

Abu-ghararah and Randall (1990) investigated the effect of varying organic compounds on a continuous EBPR reactor operating as a UCT process with an SRT of 13 days. Seven different SCVFA's were tested (formic acid, acetic acid, propionic acid, butyric

Table 2.2: Summary of molar ratios during anaerobic and aerobic periods for various organic substrates (from Comeau *et al.*, 1987).

	ANAEROBIC PHASE				AEROBIC PHASE	
Substrate Added	PHB/ PHA %	$P_{released}/PHA_{stored}$ mole/mole	$P_{released}/substrate_{consumed}$ Mole/mole	$PHA_{stored}/Substrate_{consumed}$ %	$P_{uptake}/P_{released}$	$P_{uptake}/PHA_{consumed}$ mole/mole
Control	(36)	(7.7)	-	-	46	2.0
Acetic acid	77	2.5	1.5	125	101	2.7
Acetic & propionic acid (2A + 1P)	45	2.5	1.5	125	101	2.7
Acetic & propionic acid (1A + 2P)	15	2.9	1.7	111	101	3.1
Propionic acid	1	6.9	2.1	51	101	6.0
Formic acid	6	3.6	0.2	30	55	2.1
Butyric acid	52	5.8	3.7	71	101	5.3
Valeric acid	0	1.8	3.1	176	102	2.4
Lactic acid	15	3.5	1.9	88	100	3.6
Fermented primary sludge	26	2.4	0.9	75	89	2.4

acid, isobutyric acid, valeric acid and isovaleric acid) by their separate addition to the reactor influent (at a concentration of 100 mg COD/L) and the performance of the reactor measured. All the VFA's except formic acid were removed from the anaerobic stage (actual HRT = 2.1 hours). The branched chain VFA's (isobutyric and isovaleric acid) had greater phosphorus release and uptake than the straight chain forms of the same acids. The phosphorus released per mole of VFA consumed increased as the molecular weight of the VFA increased, except for acetic acid. If the release rate was recalculated as mg P released per mg COD substrate consumed (Table 2.3) then the greatest amount of phosphorus release was produced by acetic acid, with the higher acids phosphorus release increasing with an increase in molecular weight. To remove 1 mg of phosphorus from the influent, 18.8 COD mg of acetic acid was consumed; compared to 94 mg COD/mg P removed for valeric acid (Table 2.3). The authors calculated that the Comeau-Wentzel biochemical model did not accurately predict these phosphorus release and uptake rates.

Table 2.3: Ratios of phosphorus released and VFA consumed under anaerobic conditions (from Abu-ghararah and Randall, 1990).

Organic substrate	Anaerobic		Overall
	moles $P_{released}$/ moles $VFA_{consumed}$	mg $P_{realeased}$/ mg $COD_{consumed}$	mg $COD_{consumed}$/ mg $P_{removed}$
Acetic acid	0.77	0.37	18.8
Propionic acid	0.44	0.12	31.5
Butyric acid	0.78	0.15	39.0
Isobutyric acid	0.80	0.16	36.1
Valeric acid	1.72	0.19	94.0
Isovaleric acid	2.31	0.25	23.5

An SBR study using synthetic wastewater (Randall *et al.*, 1994) at an SRT of 6 days, showed that C_1-C_5 SCVFA's except propionic acid promoted phosphorus removal, and again branched chain SCVFA's were superior to their straight chain isomers. Glucose and propionic acid were detrimental to the EBPR process. An SBR feed starch as the sole carbon source displayed only marginal EBPR with a sludge phosphorus content of 3.7 % as opposed to the SBR sludge fed with the fermentation products (SCVFA's) of glucose that had a sludge phosphorus content of 6.7%. The short-term addition of alcohols (C_1-C_5) did not improve or decrease the amount of phosphorus removal.

2.3.5 Influence of Substrates on the Microbial Population.

Bacteria other than PAO's have been reported to successfully compete for substrate under anaerobic conditions and in some instances result in the failure of the EBPR process (Fukase *et al.*, 1985; Cech and Hartman, 1990, 1993; Cech *et al.*, 1993; Satoh *et al.*, 1994; Bond *et al.*, 1998). Cech and Hartman (1990) operated two SBR's under identical conditions, except that one reactor was fed acetate as the sole carbon source and the other was fed an acetate-glucose mixture. Both SBR's were started with the same EBPR capable sludge. The phosphorus removing ability of the acetate-glucose mixture fed reactor deteriorated to a stage were minimal phosphorus removal was observed, whereas the acetate only fed reactor increased in EBPR capability to a stage were the phosphorus content of the sludge was 8.4% (w/w). Both reactors displayed substrate removal during the anaerobic phase. The authors called this deterioration in

EBPR 'glucose induced breakdown' of the EBPR process. They noticed that the bacteria in the acetate-glucose fed reactor were large gram-negative spherical bacteria (~2 μm) and occurred in tetrads. The bacteria in the acetate only fed reactor were by comparison relatively small gram-negative bacterial rods. Cech and Hartman (1993), named these anaerobic substrate accumulating non-PAO organisms "G-bacteria". Satoh *et al.* (1992), proposed that some bacteria might be able to obtain the energy required for substrate uptake under anaerobic conditions by the consumption of intracellular carbohydrates rather than by polyphosphate hydrolysis. Satoh *et al.* (1994), used acetate and propionate as the substrate source. The metabolic model for energy supply without polyphosphate utilisation was proposed by Satoh *et al.* (1992 and 1994). In this model glycogen is the sole energy source under anaerobic conditions resulting in the accumulation of PHA.

Satoh *et al.* (1994), summarised possible influences for the deterioration of the EBPR systems as follows:

(a) Polyphosphate is chemically more unstable than glycogen. If the storage period (anaerobic period) of the reserve material (PHA) is long, glycogen may be preferred to polyphosphate by microorganisms because of the stability. Relatively long HRT's or SRT's may induce the competitive growth of non-PAO's.

(b) Changing influent organic carbon concentrations may induce the accumulation of non-PAO organisms. That is, occasional organic carbon starvation may induce the accumulation of glycogen if surplus carbon then exists in the system.

(c) If the wastewater is lacking in amino acids or proteins, the growth of PAO bacteria may be retarded and permit the successful competition of non-PAO's. This is explained in that the microorganisms would have to produce amino acids from inorganic nitrogen and carboxylic acids derived from the TCA cycle. The carboxylics for the TCA cycle then needs to be supplemented, and are possibly supplied from the stored PHA. When the PHA is consumed, the microorganisms have to use glycogen as the raw material.

(d) If the wastewater contains saccharides (eg. glucose) non-PAO's may be preferred. It has been shown that glucose can be taken up and accumulated as PHA in the same way as glycogen is converted to PHA in non-PAO's.

These non-PAO organisms were named glycogen-accumulating bacteria (GAB) by Liu *et al.* (1996 and 1997), but are known as GAO's (glycogen accumulating organism's) to be consistent with the PAO acronym. Liu *et al.* (1996), suppressed the growth of PAO's by feeding a low phosphorus/carbon ratio (2/100 wt/wt) with the subsequent growth of GAO's evidenced by the uptake of substrate and production of PHA under anaerobic conditions. Liu *et al.* (1997), also found that the PAO cells were generally rod or oval shaped, whereas GAO cells usually occurred in pairs or tetrads. Liu *et al.* (1997), proposed that dominance of PAO's over GAO's may be achieved by: (a) increasing the P/C feed ratio (high polyP energy pool); (b) increasing the number of anaerobic-aerobic cycles per day (low substrate/ biomass ratio or (c) increasing the sludge biomass or HRT (low substrate/biomass ratio).

2.4 Biological Phosphorus Removal Systems

Activated sludge is at present one of the most widely used biological treatment processes for both domestic and industrial wastewaters. The activated sludge process refers to a continuous or semi-continuous aerobic method for biological wastewater treatment. The process relies on a dense microbial population being mixed in suspension with the wastewater under aerobic conditions. Under appropriate conditions, a high rate of microbial growth and respiration is achieved. Activated sludge treatment removes from the wastewater the biodegradable organics as well as the unsettleable suspended solids and other constituents, which can be absorbed or entrapped by the activated sludge floc. With the addition of unaerated zones/reactors and extra recycle streams to the basic activated sludge process, nitrogen and phosphorus removal can also be accomplished.

The common feature in all EBPR systems is the inclusion of an anaerobic zone. Typical retention times in the anaerobic range from 1 to 3 hours (Cloete and Muyima, 1997). Systems may be classified as those just removing carbon and phosphorus and those

removing carbon, phosphorus and nitrogen. Most systems are a combination of phosphorus and nitrogen removal processes, comprising multiple reactors/zones.

2.4.1 EBPR Systems without Nitrogen Removal

When nitrification is not required, a staged anaerobic/oxic (AO) system as shown in Figure 2.5 is suitable. To avoid adverse affects of nitrification on phosphorus removal (the intrusion of nitrate into the anaerobic zone), a solids retention time (SRT) below the minimum SRT required for nitrification is usually selected. The size of the anaerobic zone will be dependent on the wastewater characteristics. For a high COD/P ratio or a wastewater with a high fraction of readily biodegradable COD, a smaller anaerobic zone can be employed. One potential disadvantage with this system is that unintended nitrification may occur during warmer summer months.

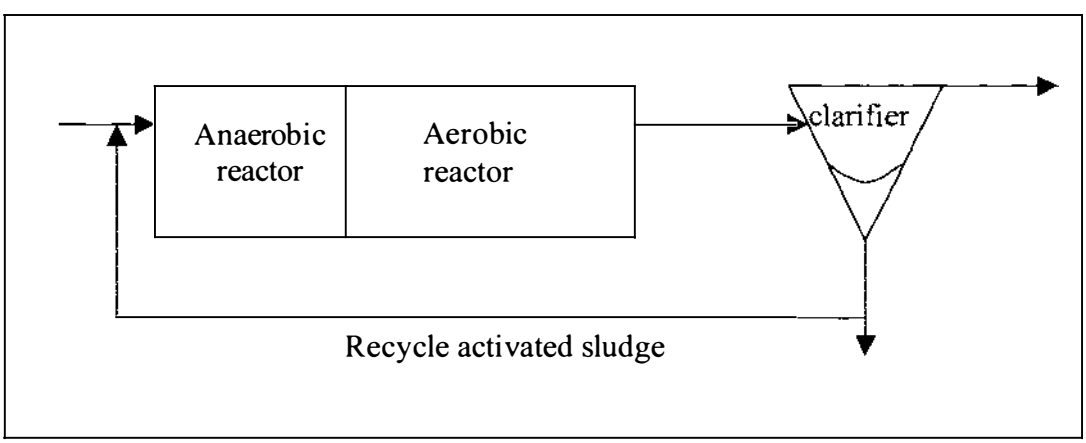


Figure 2.5: EBPR system without nitrogen removal (AO)

An alternative method to avoid the potential intrusion of nitrates into the anaerobic zone is to provide a preanoxic reactor (placed before anaerobic zone), whereby recycled nitrates are reduced by endogenous respiration. The recycle activated sludge stream would enter the preanoxic zone and the influent wastewater directly enters the anaerobic reactor.

2.4.2 EBPR with Nitrogen Removal

This necessitates employing longer SRT's to maximise nitrification. The AO process shown in Figure 2.5 can be modified to incorporate an anoxic stage between the anaerobic and aerobic stages and is called the AAO (A^2/O) process (Figure 2.6). The

AAO process is suitable for less stringent nitrogen removal requirements. For wastewater with a higher COD/TKN ratio, to achieve almost complete denitrification a secondary anoxic zone is required (Figure 2.7) (Metcalf and Eddy, 2003).

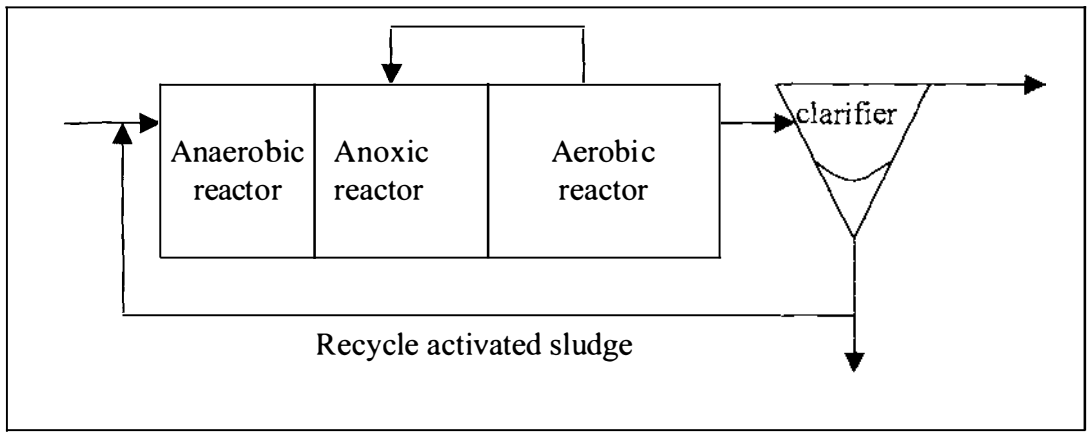


Figure 2.6: AAO system or 3-stage modified Bardenpho.

The 5-stage Bardenpho system is essentially the addition of an anaerobic zone to the 4-stage Bardenpho system used for nitrogen removal. If effluent nitrate levels are kept to a minimum, the removal of phosphorus and nitrate will be maximised.

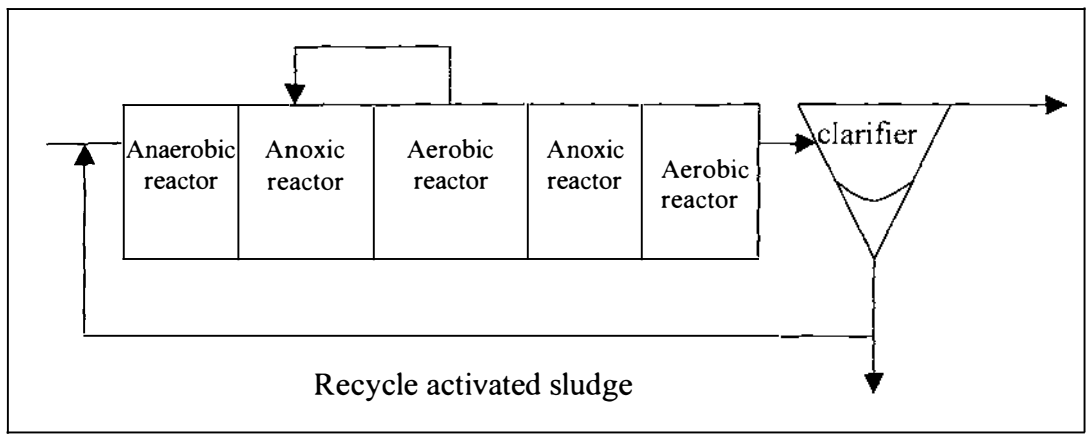


Figure 2.7: Modified (5-stage) Bardenpho, Phoredox.

A variation on the EBPR systems already discussed is the recycling of the return activated sludge to the first anoxic zone instead of the anaerobic zone. The mixed liquor from the anoxic zone is then recycled to the anaerobic zone; this system is called the UCT process (Figure 2.8) (Metcalf and Eddy, 2003). The basis for this change is that

biological phosphorus removal can be negatively affected by the presence of nitrate in the return sludge stream. For wastewater with a relatively high low BOD/TKN ratio, the nitrate concentration in the return sludge may utilise a high proportion of the wastewater soluble BOD and result in a decrease in phosphorus removal. The mixed liquor from the aerobic zone to the anoxic zone can be controlled so the denitrification potential of the anoxic zone is not exceeded; thereby assuring minimal nitrate is recycled to the anaerobic zone.

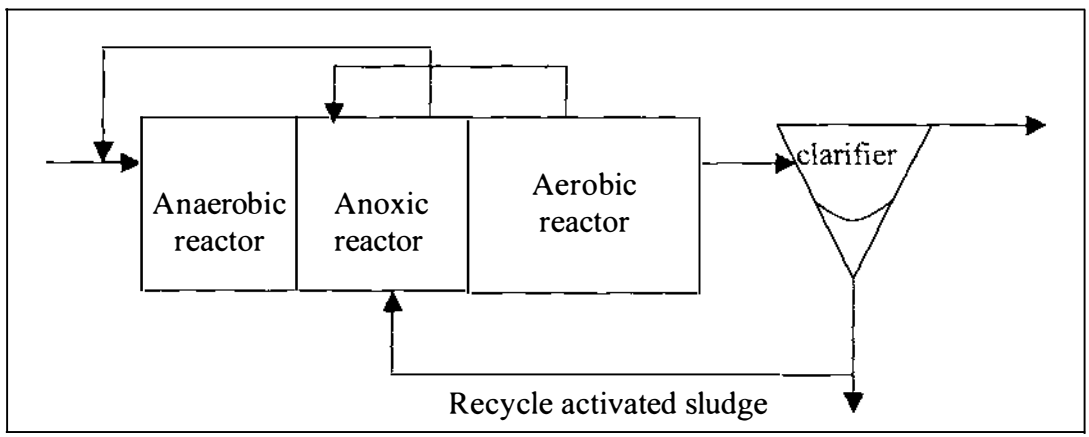


Figure 2.8: UCT Process

A modified version of the UCT process is shown in Figure 2.9. The first anoxic zone is designed to reduce only the nitrate in the return activated sludge while the second anoxic zone is designed for higher quantities of nitrate removal.

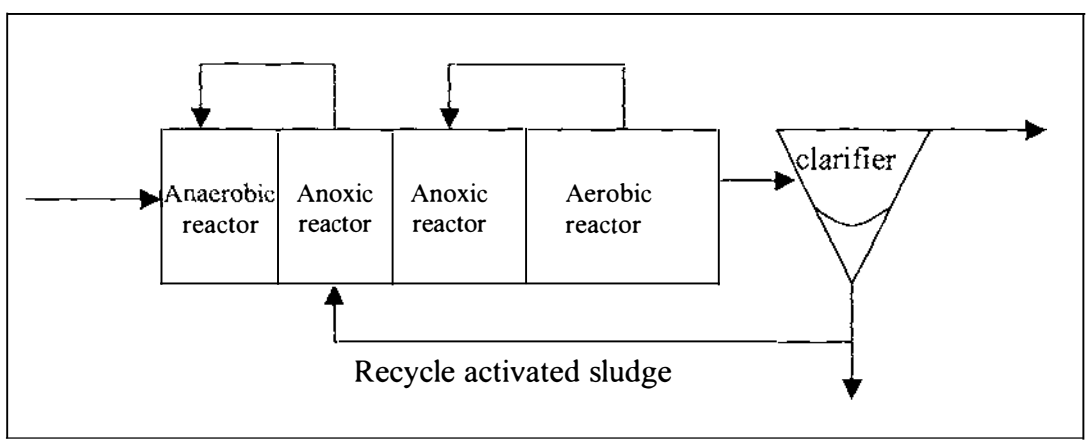


Figure 2.9: Modified UCT

The Sequencing Batch Reactor (SBR) can be readily converted to achieve EBPR by the inclusion of an anaerobic phase at the beginning of the cycle (Figure 2.10). Altering the duration of the different phases if required can readily optimise the nutrient removal performance of an SBR (Metcalf and Eddy, 2003)

Fill SBR	1st Anaerobic mix	1st Aerobic Stage	2nd Anoxic mix	2nd Aerobic Stage	Settle	Decant SBR	Sludge waste

Figure 2.10: SBR sequencing for biological phosphorus removal

2.4.3 Factors affecting EBPR System Performance

Factors affecting EBPR activity relate to wastewater characteristics, system design and operational methods. There are environmental factors such as dissolved oxygen, temperature and pH; and design parameters such as SRT and anaerobic zone retention time (Metcalf and Eddy, 2003). Wastewater characteristics like substrate availability and the presence of nitrates also affect phosphorus removal. The overall performance of the process is also affected by the effluent total suspended solids concentration (TSS). For example assuming a 4% phosphorus concentration of the mixed liquor (reactor biomass solids), then TSS concentrations of 20 mg/L would contribute an effluent particulate phosphorus concentration of 0.8 mg P/L.

Experience with the operation of full-scale biological removal systems treating domestic wastewater shows that effluent total phosphorus concentrations of less than 1 mg/L are not always achieved. This can often be due to insufficient soluble BOD being present in the form of fermentation products such as acetic acid. The addition of a fermentation reactor to generate fermentation products can also help improve the phosphorus removal capacity of the system (Barnard, 1992).

2.5 Dairy Processing Wastewater

Dairy processing plants produce a variety of products including milk, cheese, butter, milk powder, whey, protein powder, lactose, ice cream, yoghurt, and numerous specialised nutritional and baking products. The characteristics of dairy processing wastewaters vary between processing sites depending on the products manufactured,

waste minimisation practices and the cleaning methods used. The contributing sources to dairy processing effluents are principally (Marth and Steele, 2001):

1. Spills and leaks of milk, products and by-products

2. Residual milk and milk products in piping and equipment before cleaning

3. Wash solutions from equipment and floors

4. Condensate from evaporation processes

The majority of both the wastewater load and volume is generated during tanker and truck cleaning at milk reception facilities and during cleaning of piping and processing equipment after production runs. The next significant source is spills of milks, products and by-products during receiving, processing (including leakage from pipes and pumps), packaging and during equipment malfunctions. Significant wastewater volume may also be generated from evaporation condensate and cooling water discharges (Papagiannis, 1996).

2.5.1 Characteristics of Dairy Processing Wastewaters

The majority of waste components in dairy processing wastewaters are organic in nature, consisting mainly (~95%) of diluted milk and milk products. The BOD_5 of whole milk is about 100,000 mg/L with the contributions of milkfat being 17.8%, milk protein 43.3% and lactose 39% of the total BOD_5 of whole milk. Approximately 1 kg of BOD in dairy processing wastewater represents 9 kg of whole milk (Marth and Steele, 2001). The chemical characterisation of milk as summarised by Danalewich *et al.* (1998), is shown in Table 2.4.

Due to distinct production and cleaning cycles during milk processing, wastewater from dairy processing plants is characterised by a large variability in organic and hydraulic loads. This hourly and daily variability is also accentuated over a production season due to the large milk volume received during the spring flush decreasing as the season progresses. This variability makes it difficult to provide a typical chemical characterisation for dairy processing wastewater. To conduct a successful and stable laboratory study of wastewater treatment efficiencies, and to determine both kinetic and

stoichiometric constants, a wastewater with relatively constant characteristics is required. To provide these desired constant chemical characteristics a synthetic dairy-processing wastewater can be used. As dairy processing wastewaters are mainly composed of diluted milk and milk products the components required for a synthetic wastewater are readily available. The chemical characteristics of various dairy processing wasters from different studies in the 1990's are summarised in Table 2.5 in order to provide a basis for an appropriate synthetic wastewater composition. These characteristics represent both New Zealand and international studies and it should be noted that the type of processes and waste minimisation practices would vary between countries. The values shown in Table 2.5 for the Upper Midwest, USA (Danalewich *et al.*, 1998) are the average and the range of values from 15 dairy processing sites in that region while all other values shown relate to specific dairy processing plants.

Table 2.4: Chemical characterisation of whole milk (from Danalewich *et al.*, 1998).

Parameter	Whole milk concentration (mg/L)
pH	7.0
BOD_5	102,500
COD	150,000
Alkalinity (as $CaCO_3$)	200
Total Nitrogen	7,200
Total Phosphorus	1,000
Total Solids	125,000
Total Volatile Solids	117,000
Chloride	1,000
Potassium	1,500
Sodium	400
Calcium	1,200
Magnesium	500

The organic content of dairy processing wastewater varies widely between sites with an average value from Table 2.5 of about 3300 mg COD/L. In New Zealand the COD of dairy processing wastewaters at sites with good waste minimisation procedures is generally less than 4000 mg COD/L and averages about 2000 mg COD/L (Leonard, 1996; Rule, 1997), but this is very dependent on the products manufactured. Nationally the average total COD of New Zealand dairy processing wastewaters is most likely near the average value from Table 2.5 of over 3000 mg/L. The suspended solids concentration in dairy processing wastewaters will be very dependent on the type of pretreatment used and are essentially completely biodegradable (Leonard, 1996). In New Zealand, the TSS concentration of dairy processing wastewaters is generally less than 500 mg/L (Rule, 1997; Dick, 1998).

The use of cleaners and sanitizers such as nitric acid, phosphoric acid and caustic soda has a strong influence on the pH of dairy processing discharges. The pH of dairy processing wastewater mainly ranges from near neutral to strongly alkaline (Table 2.5) depending on the sampling protocol and the use of flow balancing tanks. Flow balancing tanks with retention times of greater than several hours may induce some fermentation of the wastewater resulting in pH values in the order of 5 to 6.5 (Comeau *et al.*, 1996; Jovcic, 1998).

Due to the importance of nitrogen and phosphorus in nutrient removal system, these compounds are considered separately in the following sections.

2.5.1.1 Nitrogen

The amount of nitrogen may impact on EBPR systems due to the intrusion of oxidised nitrogen (nitrate and nitrite) into the anaerobic zone/phase and its use as an electron acceptor under unaerated conditions. The occurrence of nitrification and the subsequent presence of nitrate or nitrite will determine whether a combined nitrogen/EBPR system is used or not. In dairy processing wastewater, organic nitrogen from milk proteins comprises the bulk of the nitrogen. Dick (1998), reported that over a six week period of wastewater sampling and analysis, 93% of the TKN was due to organic nitrogen with only 7% in the form of ammonia. In this same study, oxidised nitrogen concentrations averaged only 2.7 mg N/L. The occurrence of high nitrate concentrations in some wastewaters (Comeau *et al.*, 1996; Danalewich *et al.*, 1998) is related to the use of nitric acid during process equipment cleaning.

Location (Source)	Products	Volume (m^3/d)	BOD_5 (nmg/L)	COD (mg/L)	TS (mg/L)	TSS (mg/L)	TP (mg/L)	TKN (mg/L)	TN (mg/L)	NO_3-N (mg/L)	pH
Waitoa, New Zealand (Rule, 1997)	Milk powder, cheese, nutritional products	7760	1665 (max 2300)	2340 (max 3600)	363	-	125	60	-	4 (max 6)	(max 11.8)
Hautapu, New Zealand (Dick, 1998)	Cheese, Casein, Lactose, Whey protein concentrate	5050-8110	-	5485 (4130-8570)	-	389 (40-955)	96 (89-100)	114 (82-213)	117 (85-214)	2.7 (1.4-4.2)	5.5 (3.8-11.4)
Quebec, Canada (Comeau *et al.*, 1996)	Cheese, Cheese Whey	918	1755	2808	-	-	91	63	-	24	11.9
Batatais, Brazil (Filho *et al.*, 1996)	Pasturised milk, cheese, butter, milk paste.	48000	-	6318 (1548-7668)	(1210-3461)	(299-1970)	(5-32)	(76-181)	-	-	8.6 (5.8-12.3)
Turkey (Orhon *et al.*, 1993)	Pasturised milk, yoghurt, butter, cheese and ice cream	-	600	950	-	-	7.4 (soluble P)	60	-	-	6.9
California, USA (Goronszy, 1990)	Fluid Milk	-	1390	2120	-	-	32	33	-	-	-
Germany (Kolarski and Nyhuis, 1995)	-	644-1817	(680-4500)	(980-17500)	-	300	(18-162)	-	(50-380)	(20-350)	-
Upper Midwest, USA (Danalewich *et al.*, 1998)	Primarily cheese products	835 (170-2081)	1856 (565-5722)	2855 (785-7619)	4545 (1837-14205)	-	71 (29-181)	91 (14-140)	-	21 (1-80)	8.4 (6.2-11.3)

Table 2.5: Chemical characteristics of dairy processing wastewaters.
Average values of parameters are given along with either the range of values or maximum value shown in brackets ( ).

As nitrogen is a constituent of microbial cells, there is a minimal requirement for nitrogen during biological wastewater treatment. The empirical nitrogen content of microbial cells is approximately 12.5% (on a dry basis), meaning any net growth of biomass will cause some nitrogen removal by assimilation (Metcalf and Eddy, 2003). Two studies with both conventional and biological nutrient removal activated sludge systems have shown that the biomass nitrogen content averaged 9.8% (McClintock *et al.*, 1993) and 9.7% (Suwa *et al.*, 1992), lower than the empirical value. The amount of nitrogen required for balanced metabolic functioning is related to the wastewater BOD concentration, with a BOD/N ratio of 25/1 typically used in design (Eckenfelder, 1989). Ammonia is the most readily available form of nitrogen for growth while other nitrogen compounds must first be converted to ammonia. Nitrogen deficient conditions will stimulate the growth filamentous organisms resulting in sludge bulking. For a typical wastewater COD of 3000 mg/L and a BOD/COD ratio for dairy processing wastewater of 0.6 to 0.7 (Table 2.6), then approximately 72 to 84 mg N/L is required for stable biological treatment assuming a BOD/N ratio of 25. If a cellular nitrogen content of 9.8 % is used instead of 12.5%, then this decreases the metabolic nitrogen required for a typical dairy processing wastewater (COD ≈ 3000 mg/L) to a range of 56 to 66 mg N/L. Excess nitrogen beyond that required for growth will result in nitrate production (nitrification) with the possibility of nitrate intrusion into the anaerobic zone/phase. The chemical characteristics listed in Table 2.5 indicate that the Californian wastewater (Goronszy, 1990) had potential nitrogen limiting conditions. At this site a full-scale SBR was operated for biological wastewater treatment and filamentous bulking was observed which was attributed to occasional deficiencies in nitrogen. The COD/TKN ratio's in Table 2.5 range from 16 (Orhon *et al.*, 1993) to 64 (Goronszy, 1990). If these two extremes are not considered then the average COD/N ratio is 42 for these dairy-processing wastewaters.

2.5.1.2 Phosphorus

The phosphorus content of New Zealand dairy processing wastewaters can range from 12 mg P/L to over 100 mg P/L depending on the process, product and cleaning procedures used (Barnett *et al.*, 1994; Rule, 1997; Dick, 1998). Internationally the phosphorus content of dairy processing effluents has occasionally been reported at over 200 mg P/L (Danalewich *et al.*, 1998). A significant amount of the phosphorus originates from wasted detergents and cleaners, which may contain significant amounts

of phosphorus (Danalewich et al., 1998; Papagiannis, 1996; Rule, 1997; Dick, 1998), while one percent of milk in a wastewater produces about 12 mg P/L (Goronszy, 1990). An important component in alkaline cleaners is polyphosphates which are used for emulsification, dispersion, and protein peptising while phosphoric acid is also frequently used as an acid cleaner to clean high temperature equipment (Danalewich *et al.*, 1998).

There is not much information available in published literature on the sources of and relative quantities of phosphorus in dairy processing wastewaters. Danalewich *et al.* (1998), however identified in their analysis of composite samples from 15 dairy processing plants that orthophosphate (PO_4^{-2}) concentrations in these samples only averaged 27% of the total phosphorus, with the remaining phosphorus in the organic and polyphosphate form. This indicated that the majority of the phosphorus in these wastewaters from the American Midwest originated from milk, alkaline cleaners and emulsifiers. This is in contrast to the analysis by Rule (1997), of a New Zealand dairy processing wastewater where an average of 90% of the total phosphorus was present as orthophosphate indicating high phosphoric acid use.

Successful EBPR treatment resulting in effluent phosphorus levels of less than 1 mg P/L requires a BOD/TP ratio greater than 20 (Randall *et al*, 1992). In Table 2.5 these ratios range from 11 to 43 (where BOD value are provided) indicating that for some wastewaters complete phosphorus removal using EBPR systems will potentially be achievable, whereas for others it is likely that chemical phosphorus removal will also be required. If as mentioned previously the COD for a typical dairy processing wastewater is about 3000 mg COD/L, and the BOD/COD ratio ranges from 0.6 to 0.7, then about 90 mg P/L might potentially be removed using the EBPR process. This is however a very simplistic approach. The actual potential for phosphorus removal however contains more complexities such as the type of organic substrate, nitrogen concentration and the biological community present in the reactor.

2.5.2 Synthetic Dairy Processing Wastewater

To successfully research the degree of EBPR possible from dairy processing wastewater and the mechanisms involved, a wastewater with constant properties is required.

Papagiannis (1996), summarised the recipes and resulting characteristics of some synthetic dairy wastewaters used in previous studies as a basis for creating a synthetic wastewater for a nutrient removal study. Previous studies had principally used diluted low fat and whole milk and been concerned only in the total COD rather than other constituents. One synthetic wastewater used a more complex combination of dry non-fat milk, dried sweet whey, salted butter and water. Papagiannis's own study used cottage cheese and evaporated milk to add the milk derived components and acetic acid as a VFA source. Extra phosphorus, nitrate, chloride, magnesium and calcium were added separately in the form of various chemicals. Cottage cheese was later removed from the synthetic wastewater in the study and the proportion of evaporated milk increased due to the high influent suspended solids interfering with the reactor operation.

New Zealand based studies by Leonard (1996), and Donkin and Russell (1997) have used synthetic wastewaters that have tried to mimic a wastewater typical of a butter/milkpowder processing plant. This is because butter and milkpowder are commonly produced at New Zealand dairy processing plants. The composition of the synthetic wastewater used by Leonard (1996), (Table 2.6) comprised the readily available milk products of skim milk powder, whole milk powder and full cream salted butter. The pH was increased to 11.0 using NaOH, a pH typical of wastewater discharged during cleaning (Leonard, 1996).

2.6 Biological Phosphorus Removal From Dairy Processing Wastewater

The removal of organic matter from dairy processing wastewater by biological treatment is well established with the current use of both anaerobic and aerobic treatment systems in the New Zealand dairy processing industry. The use of biological nutrient removal systems in the dairy processing industry is less common. Some research has been conducted on aerobic treatment of dairy processing wastewater with systems containing unaerated selectors, in order to prevent bulking and explore the possibility of nutrient removal (Donkin *et al.*, 1995; Leonard, 1996; Donkin and Russell, 1997). Other research has used SBR's with anaerobic/anoxic phases to treat dairy processing wastewater and remove nitrogen and phosphorus (Goronszy, 1990; Kolarski and Nyhuis, 1995; Comeau *et al.*, 1996; Papagiannis, 1996; Dick, 1998).

Table 2.6: Synthetic wastewater composition and characteristics as used by Leonard (1996).

Ingredient or Characteristic	Concentration (mg/L)
Whole milk powder	650
Skim milk powder	700
Butter	150
Total COD	2200
Soluble COD	1650
Total P	14
Total N	89
TSS	150

Donkin and Russell (1997), used a laboratory scale continuous reactor fed synthetic dairy processing wastewater with an AAO configuration to alleviate sludge bulking and encourage nutrient removal. The system had an overall hydraulic retention time (HRT) of 7 days and a solids retention time (SRT) of 20 days, the anaerobic selector had an HRT of 1 hour and the anoxic selector an HRT of 0.75 hours. Based on an influent TKN and TP of 49 mg N/L and 10.4 mg P/L respectively 68% of the nitrogen was removed and 63 % of the phosphorus (based on effluent soluble P) was removed. This amounted to 6.6mg P/L of phosphorus removal from a wastewater with a COD of 2050 mg COD/L, not representative of good EBPR. Lack of EBPR was partially due to an effluent oxidised nitrogen concentration of 11 to 14 mg N/L. Leonard (1996), used unaerated selectors to prevent bulking in a laboratory scale continuous activated sludge system (HRT= 1 day, SRT = 10 days). The size of the selectors was varied during the trial with the selector HRT ranging between 33 and 111 minutes. The phosphorus content of the biomass ranged from 1.5 to 2.6 % g P/ g VSS indicating minimal EBPR. Again, effluent nitrate concentrations resulted in predominately anoxic conditions as opposed to anaerobic conditions in the selector.

Kolarski and Nyhuis (1995), reported the performance of two full-scale SBR systems with an anoxic/anaerobic fill phase treating dairy processing wastewater. The SBR at the Muller Milch dairy processing plant was designed to treat 1500 m^3/d of wastewater at an influent BOD_5 of 1200 mg/L with an SRT of 25 days and an F/M ratio of 0.06 d^{-1}. The operating cycle was 8 hours duration consisting of 2 hours anoxic/anaerobic shock fill and mix, four hours of aerated react, one hour of settling and one hour of decanting. Over a 3 year period this SBR had an influent total phosphorus ranging from 18 to 162 mg P/L while the effluent total phosphorus ranged from 0.5 to 3.8 mg P/L. Due to the large fluctuations in the influent phosphorus levels, chemical phosphorus precipitation was also installed. It is not stated explicitly but it appears from the data that the effluent phosphorus concentration of 0.5 to 3.8 mg P/L is inclusive of chemical phosphorus removal. This makes it difficult to make an assessment of the degree of phosphorus removal by EBPR within the SBR. The other full-scale SBR at the Westmilch dairy processing facility was operated with the same cycle duration and structure as at Muller. EBPR was observed after four weeks and the influent total phosphorus concentration of 15 - 30 mg/L was consistently reduced to less than 0.5 mg P/L without the requirement for chemical precipitation. No data was provided as to the phosphorus content of the SBR sludge for either case.

Papagiannis (1996), operated laboratory-scale SBR's treating synthetic dairy processing wastewater with COD contributions from both milk components/products and from acetic acid (to promote EBPR) and an average total phosphorus concentration of 56 mg P/L. An SBR was also operated in parallel as a control with a conventional fully aerated cycle treating the same influent. The conventional SBR removed 30 % of the phosphorus as opposed to only 16 and 20% for the two SBR's configured for nutrient removal. It was noted that phosphorus removal was however very inconsistent. Operational difficulties were continually encountered, especially poor sludge settling, resulting in very poor SRT control, with would be detrimental to EBPR establishment. It is possible that the control SBR had a greater amount of phosphorus precipitation as it was fully aerated and most likely operated at a higher pH due to carbon dioxide stripping.

Dick (1998), operated a pilot-scale SBR with a volume of 13 m^3, at a New Zealand dairy processing plant. This SBR was operated with both a 24 and 12 hour cycle duration using at different times, both aerated and unaerated fill phases. The influent

had an average COD, TKN and TP of 5485 mg/L, 114 mg N/L and 96 mg P/L respectively, suggesting that adequate organic matter was present for nutrient removal. The removal efficiency of nitrogen averaged 90% and phosphorus removal averaged 24% during a three-week period of stable operation. However in this study consistently stable operation again proved difficult to achieve. No operational SRT control value was mentioned but the SBR mixed liquor concentration (TSS) was 6000 mg/L or greater suggesting a relatively high SRT. With this high sludge concentration, the majority of the phosphorus was most likely removed by assimilation, although inadequate data is presented to confirm either the presence or lack of EBPR.

Of the studies published, that reported by Comeau *et al.* (1996), achieved the best EBPR, when treating wastewater from a cheese factory in Quebec, Canada with a 15 L bench-scale SBR. Successful EBPR was established when the SBR treated effluent from a wastewater equalisation tank at the cheese factory. The equalisation tank had an HRT of about 20 hours, long enough for volatile fatty acid production. The VFA concentration in the influent averaged 1130 mg COD/L, the orthophosphate concentration of the influent averaged 60 mg P/L. The SBR was operated with a 12 hour cycle, 3 hour initial anaerobic phase, a 2 day HRT and at an SRT of 15 days. A sludge phosphorus content of up to 7.6 % g P/g MLSS was observed with 2.1 % g P/g MLSS of that estimated to be precipitated absorbed phosphorus, indicating good EBPR performance. It was claimed that over 50 mg P/L was removed.

2.7 Fermentation of Dairy Processing Wastewater

As previously mentioned in this review, short chain VFA are the preferable carbon source for the EBPR mechanism. This can be achieved by simultaneous fermentation in the reactor zone/phase or by fermentation in a separate acidogenic anaerobic reactor. Fermentation of primary sludge in order to supplement VFA for a subsequent EPBR process is now relatively commonplace at domestic wastewater treatment plants. Due to the readily biodegradable nature of the organic matter in milk processing wastewater fermentation should not be difficult as evidenced by the amount of VFA generated in the equalisation tank of the Comeau *et al.* (1996) study.

Kisaalita *et al.* (1987a; 1987b; 1989), investigated the acidogenic degradation of lactose and whey, both potential components of dairy processing wastewater. Complete degradation of lactose was achieved at 35°C and at reactor HRT's greater than 2.5 hours, with maximum acetic acid and minimum lactic acid produced at HRT's greater than 6.7 hours. The potentially optimum pH levels for lactose acidogenesis were identified to be 4.5 and 6.0. When whey protein was added to the lactose mixture between 65 and 70% of the protein was acidified without any negative effect on lactose acidification.

Fang and Yu (2001), showed that optimum lactose acidification (~80%) was achieved at a lactose COD of 2000 to 5000 mg COD/L in a reactor with a 0.5 day HRT, a pH of 5.5 and at a temperature of 35°C. The degree of lactose acidification did not increase substantially once the HRT increased above 12 hours. Lactose acidification decreased sharply below a pH of 5 with a peak at a pH of 5.5 but there was not a large effect on lactose acidification (75 - 81%) between a pH of 5 and 6.5. The effect of temperature on lactose acidification was also assessed and the optimum temperature was 55°C with an acidification of 86%. At 35°C the acidification decreased only slightly to 82% with a greater decrease observed at temperatures lower than 35°C.

Kasapgil *et al.* (1995), determined the optimum operating conditions for an acidogenic reactor when treating dairy processing wastewaters with COD's ranging from 2000 to 6000 mg/L. They found that VFA production was affected by changes in temperature more than pH. They found that a pH range of 5.7 to 5.8 and a temperature of 34-36 °C were the best combination of operating conditions at an HRT of 0.5 days resulting in a maximum acidification rate ($VFA_{COD}/COD_{soluble}$) of 71%. The major VFA's produced were acetic, propionic, n-butyric and n-valeric acid.

Yu and Fang (2001), investigated the acidification of dairy processing wastewaters with a COD ranging from 2000 to 30,000 mg/L. It was shown that acidification of protein began after carbohydrate was depleted at a pH of 5.5 and a temperature of 37 °C. At an HRT of 0.5 days acidification decreased with the increase in COD, from 57.1% at 2000 mg COD/L to 28.8% at 30,000 mg COD/L. By measuring the individual components of the milk processing wastes they determined that 92-99% of carbohydrates, 59-85% of protein and 12-42% of lipids were acidified under continuous conditions.

2.8 Summary

Previous studies have established the mechanisms involved in EBPR and the design and operational protocols when EBPR is applied to domestic wastewater treatment (or synthetic wastewaters of comparative strength). The use of EBPR for treating dairy processing wastewater is however not well established, especially when using continuous reactors. The majority of previous attempts to biologically remove phosphorus from dairy processing wastewater have proved unsuccessful with operational problems also occurring. Only one study (Comeau *et al.*, 1998) has clearly demonstrated the establishment of the EBPR mechanism when treating fermented dairy processing wastewater with an SBR over the period of their study. As fermented wastewater is essentially a mixture of short chain VFA, this can be considered pre-fermented due to the degradation of the milk based components and their conversion to organic acids. As the presence of VFA (or conversion of organic substrates to VFA) is an essential requirement of EBPR the success with the fermented wastewater could be expected. Yet information on the EBPR process with high VFA wastewaters is also limited.

As yet no published studies with continuous activated sludge BNR reactors have established stable EBPR operation with significant biological phosphorus removal from either fermented or unfermented dairy processing wastewaters. In New Zealand due to a number of dairy processing sites having conventional non-BNR continuous activated sludge treatment systems the retrofitting and conversion of these systems for EBPR is a possibility.

This study will attempt to try and fill some of these knowledge gaps in the application of the EBPR to dairy processing wastewater especially for continuous activated sludge BNR systems. A laboratory scale continuous activated sludge configured for biological phosphorus removal will be used to generate information. A continuous EBPR process configured for both combined nitrogen and phosphorus removal and for phosphorus removal only will be tested when treating dairy processing wastewater. Both the ease of fermentation of the synthetic wastewater used, and the requirement of a fermented wastewater for EBPR will be explored.

CHAPTER 3
Analytical and Experimental Methodology

3.1 Introduction

The methods used for the quantitative measurement of the relevant analytical parameters and for the experimental reactor systems performance evaluations are described in this chapter. All analytical methods where possible were performed as described in *Standard Methods* (APHA, 1998), otherwise they were based on established techniques presented in literature and used by other researchers.

3.2 Analytical Methodology

The analytical parameters measured are those that are required to describe the performance of biological nutrient removal reactors and the chemical characteristics of the wastewater. Measurement of the intracellular storage compounds glycogen, poly-ß-hydroxybutyrate and poly-ß-hydroxyvalerate describe the metabolic storage processes occurring during biological phosphorus removal.

3.2.1 Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS)

The TSS and VSS concentrations provide measurements of both the suspended solids concentrations present in the wastewater, and the biomass concentration present in the biological reactor. The VSS measures the amount of organic matter present in the suspended solids. A high VSS/TSS ratio for the suspended matter present in a wastewater indicates that the suspended solids consist of mostly organic matter and are therefore most likely biodegradable. As dairy wastewaters are comprised of mostly dilutions of milk and milk products the suspended organic matter, would consist mainly of milk fat and milk proteins resulting in a high VSS/TSS ratio (Leonard, 1996; Danalewich *et al.*, 1998). The estimation of the microbial concentration using the VSS measurement is widespread in wastewater treatment. The reactor biomass concentration when expressed as the VSS concentration is only an estimate of the viable biomass as it may also include inactive (inert) cellular matter and non-biodegradable solids (small for dairy processing wastewater).

Both the total suspended solids (TSS) and the volatile suspended solids (VSS) were determined according to sections 2540 D and 2540 E of *Standard Methods* (APHA, 1998) as follows:

1. Glass fibre filters (Whatman GF/C and Schleider & Schnell GF52 filters were used, both of 90 mm diameter circles) were prepared by placing in an NEY M525 Series II muffle furnace at 550 °C(± 50), for 15 minutes. The filters were then cooled to room temperature in a desiccator that contained silica gel.

2. Filters were then weighed (Mettler AE 200 balance) in grams to four decimal places. Filters were placed wrinkled side up in the filtration apparatus and lightly wet with distilled water. A volume of a well-mixed sample (usually 20 ml) was then pipetted on to the filter paper.

3. Filters were dried in a Contherm Series Five oven at 103°C usually overnight. After drying fi ters were cooled in a desiccator and reweighed.

4. For VSS determination, filters were then ignited in the Muffle furnace for 30 minutes before subsequent drying and weighing.

5. The values for TSS and VSS were calculated as follows:

$$\text{TSS} = \frac{(A - B) \times 10^{6}}{\text{sample volume (mL)}} \qquad (3.1)$$

$$\text{TSS} = \frac{(A - C) \times 10^{6}}{\text{sample volume (mL)}} \qquad (3.2)$$

where:

A= weight of filter plus residue dried at 103°C (g)

B= weight of filter (g)

C= weight of filter plus residue after ignition at 550°C (g)

The TSS method was verified by filtering a standard of a known TSS concentration. A 20 ml volume of a 500 mg/L TSS standard of 20 μm cellulose particles (Sigma Cell Cellulose type-20) was filtered and then dried at 103°C in parallel with samples for

the first two months of the project. In every instance, the TSS recovery was 95% to 105% of the standard.

3.2.2 Chemical Oxygen Demand (COD)

The organic carbon content of the wastewater was measured as the chemical oxygen demand using the COD test. The COD method is used in most nutrient removal activated sludge models to describe the wastewater characteristics due to the fact that it is a relatively rapid method of quantification. The closed reflux, colorimetric method as described in section 5220 D of *Standard Methods* (APHA, 1998) was used. Total (COD_{total}) and soluble COD ($COD_{soluble}$) measurements were determined, with soluble COD defined as the fraction of COD that passed through GFC filter paper (<1.2μm). Occasionally the COD of the filtrate from 0.45μm membrane filtration was determined and is shown in the data as $COD_{<0.45\mu m}$.

The COD test was performed as follows:

1. An acidified (pH<2) 900 mg COD/L standard solution of potassium hydrogen phthalate was used to create a calibration curve with appropriate dilutions to give a range of calibration points. Samples with a COD greater than 900 mg/L were diluted before analysis.

2. A 2.5 mL volume of either sample, standard or distilled water (zero COD blank) was added to HACH COD tubes, followed by 1.5 mL of digestion solution and 3.5 mL of catalyst solution. The tubes were closed with Teflon lined caps. All samples, standards and blank were performed in triplicate.

3. Samples were heated at 150°C for 120 minutes in a 25 tube capacity HACH COD digestion block and then cooled.

4. The absorbance of each sample and standard were measured at 600nm on a UV/VIS Spectrophotometer (Shimadzu, UV-1201), using a cuvette with a 1 cm path length. The absorbance of the digested blank was used as the zero point for the spectrophotometer.

5. The sample COD was calculated as follows:

$$\text{COD (mg / L)} = \frac{A \times D}{\text{Slope}} \qquad (3.3)$$

where:

A = sample absorbance at 600nm

D=dilution factor

Slope= gradient of calibration plot (absorbance versus COD)

3.2.3 Total Phosphorus (TP)

The total phosphorus content of wastewater is important to determine the degree of phosphorus removal by the biological reactor. The phosphorus content of the biomass in the reactor is also important in order to perform a mass balance on the reactor and to calculate the phosphorus content of the biomass. The determination of total phosphorus is a two-stage process consisting of sample digestion and then phosphate measurement. The Sulphuric Acid-Nitric Acid digestion method outlined in section 4500-P B of *Standard Methods* (APHA, 1998) was used, followed by phosphorus measurement using the Ascorbic Acid Method in section 4500-P E of *Standard Methods* (APHA, 1998).

The TP of samples was analysed as follows:

1. To generate a calibration curve, phosphorus standards as well as samples were firstly digested in 300 mL digestion tubes (Tecator Digestion System 6, 1007 Digester). Due to the high phosphorus levels in the samples and the resulting small sample size, 20 mL of deionised water was firstly added to each digestion tube to prevent the sample boiling dry. Standard phosphorus amounts of 0, 0.0125, 0.025 and 0.05 mg P were added to digestion tubes. This equated to 0, 0.25, 0.5 and 1.0 mL of a 50 mg/L phosphorus standard. Sample volumes of 0.20 mL of were pipetted into the digestion tubes. To each tube 1 mL of concentrated acid and 5mL of concentrated nitric acid were added followed by heating at 350°C in the electric heating block (Tecator) until the solution became colourless. After cooling 10 mL of deionised water was added to each tube. One drop of phenolphthalein indicator was added to

each tube and the solution neutralised to a faint pink colour with a 12.5 M NaOH solution.

2. The contents of the digestion tubes were transferred to 100 mL capacity volumetric flasks with repeated rinsing of tubes until about 50 to 70 mL of solution remained in the volumetric flasks.

3. Ascorbic acid reagent (16 mL) was added to each 100 mL flask and the volume made up 100 mL using deionised water and the flask inverted several times to ensure complete mixing of sample and reagent. After 20 minutes (but no more than 30 minutes), the absorbance of each sample was measured at 880 nm in a spectrophotometer using 1cm pathlength cuvette. The blank (0 mg P) was used to zero the spectrophotometer.

4. The TP of the sample were calculated as follows:

$$TP\ (mg\ /\ L) = \frac{A}{V\ x\ slope} \qquad (3.4)$$

where:

A = absorbance at 880 nm

V= sample volume (L)

Slope = gradient of calibration plot (absorbance versus mg P)

3.2.4 Soluble anions (PO^{-3}_4, NO^{-}_2 and NO^{-}_3)

The soluble anions were measured by ion chromatography (IC) according to section 4110 C of *Standard Methods* (APHA, 1998). All samples were filtered prior to analysis with 0.45μm membrane filters before injection in the IC (Dionex, DX-100). A Dionex IonPac column (AS 9-HC 4-mm) was used with 9 mM Na_2CO_3 used as the eluent at a flowrate of 1.0 ml/min and with a sample injection volume of 25 μL. Five point external calibration plots were generated (using mixed anion standards) and stored in the IC computer prior to analysis, with the sample concentrations generated from peak areas. The maximum standard concentration for each anion was as follows; nitrite (41.5mg/L), nitrate (79.5mg/L), phosphate (201.8mg/L).

3.2.5 Total Kjeldahl Nitrogen (TKN)

The TKN was determined using a digestion procedure similar to that of section 4500-N_{org} B, while the resulting ammonia concentration was determined by distillation and auto-titration as described in sections 4500-NH_3 B and 4500-NH_3 C of *Standard Methods* (APHA, 1998).

The digestion stage used a BÜCHI 435 digestion system and tubes. An appropriate sample volume (~50 mL), 13 mL concentrated H_2SO_4, and two 5g Kjeltabs (THOMPSON & CAPPER LTD, 97.5% Na_2SO_4, 1.5% $CuSO_4.5H_2O$, 1% Se) were digested at 350°C until the sample became clear and white fumes were emitted.

Each tube was then attached to an BÜCHI 323 distillation unit in which 45% w/w NaOH was automatically added and the ammonia distilled off and collected in 4% boric acid (H_3BO_3) that contained screened methyl red indicator. In the final stage ammonia was measured by auto-titration (METTLER DL125 auto titrator) with 0.1N HCl.

3.2.6 Ammonia

Ammonia was analysed using a TECHNICON Autoanalyser continuous flow analyser using a modification of the automated phenate method (section 4500-NH_3 G *Standard Methods* (APHA, 1998)). The calibration curve was constructed using standards up to 3 mg/L ammonia with the peak height used to determine the relative concentrations.

3.2.7 Glycogen

The glycogen content of lyophilised sludge (VIRTIS Model 10-020 freeze dryer) was measured as glucose residues by heating at 100 °C with 3 mL of 0.6 M HCL for two hours. The glucose was then measured using a YSI 2700 Select Biochemistry Analyzer fitted with an enzyme membrane. A 2.5 g/L glucose standard was used for calibration of the YSI Analyzer. To check glycogen and glucose recoveries, standards of both glycogen (BDH, oyster glycogen $(C_6H_{10}O_5)_x$) and glucose (BDH, Analar) were taken through the same heating, hydrolysis and measurement process as the samples.

3.2.8 Poly-β-hydroxyalkanoate (PHA)

PHA's were measured as poly-β-hydroxybutyric acid (PHB) and poly-β-hydroxyvaleric acid (PHV) similar to the method developed by Braunegg *et al.* (1978), and used for activated sludge PHA analysis by Comeau *et al.* (1988).

- Approximately 30 mL mixed liquor samples were collected and immediately centrifuged at 4°C (JOUAN MR1812 refrigerated centrifuge) and the pellets then frozen until analysed. Later the samples were lyophilised (VIRITIS Model 10-020 freeze dryer). PHB and PHV data are shown for samples that were lyophilised prior.

- A weighed amount (about 20 mg) of lyophilised sludge was combined with 2 mL of acidified methanol (3% H_2SO_4) containing benzoic acid (300 mg/L) as the internal standard and 2 mL of chloroform in a 15 mL HACH test tube with tight fitting Teflon lined caps. The acidified methanol solution was made by adding 150 mg benzoic acid and 15 mL concentrated H_2SO_4 to 400 mL methanol and then making up to 500 mL with methanol. All samples were analysed in duplicate.

- External standards were prepared using a commercial preparation of PHB and PHV (ALDRICH CHEMICALS) consisting of Poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid) of natural origin with a composition of 88 % w/w PHB and 12% w/w PHV as follows:
    - 25 mg PHB/PHV standard was weighed and dissolved in 25 mL of chloroform (880 mg/L PHB and 120 mg/L PHV).
    - Three different standards were made in duplicate in 15mL HACH test tubes:
        1. Add 1.17 mL of chloroform, 0.29 mL of PHB/PHV stock solution and 2 mL of methanol solution (with internal standard) to a test tube (127.6 mg/L PHB and 17.4 mg/L PHV).
        2. Add 1.15 mL of chloroform, 0.85 mL of PHB/PHV stock solution and 2 mL of methanol solution (with

internal standard) to a test tube (374 mg/L PHB and 51 mg/L PHV).

3. Add 0.6 mL of chloroform, 1.4 mL of PHB/PHV stock solution and 2 mL of methanol solution (with internal standard) to a test tube (616 mg/L PHB and 84 mg/L PHV).

- Samples and standards were heated for 3.5 hours at 100 C in boiling water.

- After cooling to room temperature 1 mL of distilled H_2O was added to each tube and the tubes were shaken vigorously in a GRIFFIN flask shaker for 15 minutes.

- The chloroform phase was then settled to the bottom of the tube and then drawn off using a Pasteur pipette and transferred to a vial along with three pellets of an ALLTECH 1/8" molecular sieve as a drying agent. The vials were stored at $-18^{o}C$ until analysed.

- The PHB and PHV extracts (1μL of sample was injected) were analysed by gas chromatography (GC 6000 VEGA SERIES 2 with FID detector, helium carrier gas at 30 kpa, 1:5 split, SUPELCO NUKOL capillary column 15 m length x 0.53 mm diameter and 0.5 μm film thickness). The GC was operated using a temperature program (80 ^{o}C for 1 minute then increased at a rate of 6 ^{o}C/min to 155 ^{o}C and maintained at 155 ^{o}C for 1 minute). The injection and detector ports were both operated at 230 ^{o}C.

An example of a GC chromatogram for PHB and PHV analysis is shown in Figure 3.1. With the temperature program used, good separation of the PHB, PHV and the benzoic acid internal (IS) standard was achieved, with a retention time of 4 minutes for PHB, 5 minutes for PHV and 6 minutes for benzoic acid.

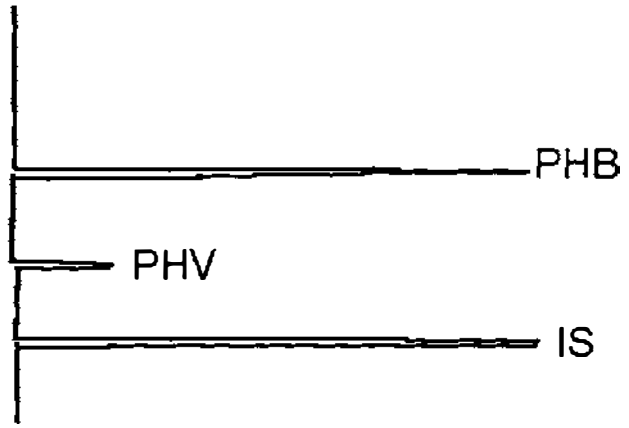


Figure 3.1: Example of GC chromatogram for PHB and PHV analysis.

3.2.9 Volatile Fatty Acids (VFA)

The volatile acids measured were acetic acid, propionic acid, n-butyric acid, iso-butyric acid, n-valeric acid, iso-valeric acid and caproic acid. These VFA's were analysed by gas chromatography (GC 6000 VEGA SERIES 2 with FID detector) using a SUPELCO NUKOL capillary column (15 m length x 0.53 mm diameter and 0.5μm film thickness), with helium as the carrier gas (30 kpa). The oven temperature program used was 10 seconds at 100°C, the temperature was increased at a rate of 10°C/min to 180°C and then held constant at 180°C for 1 minute. The injector and detector ports were both operated at 220°C. The sample volume injected was 1μL.

3.2.10 Dissolved Oxygen (DO) and Oxygen Uptake Rate (OUR and SOUR)

A dissolved oxygen (DO) meter and probe (YSI 57 oxygen meter, YSI 5750 probe) was used to measure both the dissolved oxygen and the oxygen uptake rate. The DO meter was connected to a SECKONIC chart recorder (Model SS-250F). The recorder speed was set at 30mm/minute, with 1 mg/L of DO corresponding to 30.3 mm of distance on the y-axis of the chart. The oxygen uptake was measured by filling a 20 mL conical flask with mixed liquor, adding a small stirring bar and placing on a magnetic stirrer. The DO probe was then inserted into flask until it formed a seal at the top of the flask to prevent oxygen intrusion. The decrease in DO with time could then be calculated from the slope of the recorder line. The OUR set up is shown

schematically in Figure 3.2. The OUR and specific oxygen uptake rate (SOUR) were calculated as shown in Equation 3.5 and 3.6 respectively.

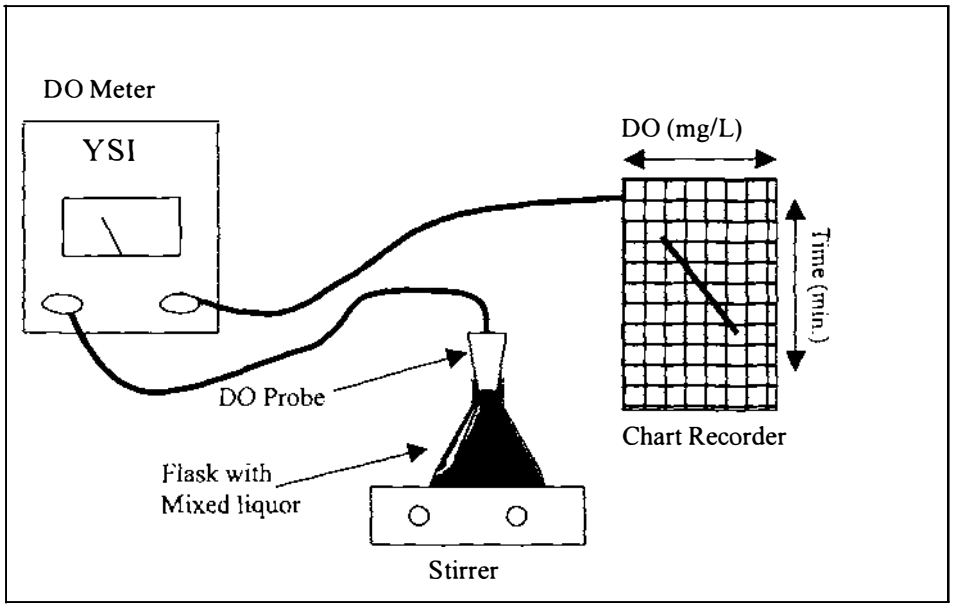


Figure 3.2: Schematic of OUR measurement technique.

$$\mathbf{OUR\ (mgO_2 / L / min)} = \frac{\mathbf{\Delta DO(mgO_2 / L)}}{\mathbf{\Delta time(min)}} \tag{3.5}$$

$$\mathbf{SOUR\ (mgO_2 / mgVSS / min)} = \frac{\mathbf{OUR}}{\mathbf{VSS(mg / L)}} \tag{3.6}$$

For wastewater characterisation studies using aerobic batch tests (Chapter 4) nitrification was inhibited with allythiourea (BDH Chemicals) at a concentration of 1 mg/L. OUR measurements during reactor zone testing and phosphorus release batch tests were not nitrification inhibited.

3.2.11 Sludge Volume Index

The sludge volume index (SVI) is used to measure the settling characteristic of the reactor mixed liquor and is the volume in millimetres occupied by 1 g of sludge after 30 minutes settling. The SVI was measured according to section 2710 D of *Standard Methods* (APHA, 1998). Mixed liquor (1000 mL) was collected from the final aerated section (or zone) in a 1000 mL measuring cylinder and inverted several times. The contents were then left to settle for 30 minutes and the volume occupied by the sludge was recorded. The SVI was then calculated as in Equation 3.7:

$$SVI = \frac{\text{settled sludge volume (ml / L) x 1000}}{\text{TSS(mg / L)}} \quad (3.7)$$

3.2.12 pH Measurement

The pH of samples and reactors was measured using a Orion Model 230A pH meter. The pH meter was calibrated before use with a two point calibration procedure using pH 7 and pH 4 or pH 10 colour key buffer solutions (BDH Chemicals).

3.2 Laboratory-Scale Reactors

In the biological nutrient removal (BNR) studies an activated sludge reactor configuration was required that contained both unaerated, aerated zones, and a clarifier. The BNR reactor was also required to be robust and reliable so that steady state operation could be achieved with a minimum of interruptions from equipment failure and tube blockages.

Initially a BNR system comprising readily available components was constructed to help determine the required operating parameters, important reliability issues and whether biological phosphorus removal could be achieved with a relatively simple system and without fermentation (Chapter 5). The sludge from this system was also used in the wastewater characterisation studies (Chapter 4). It was decided during this preliminary study that a much more reliable and flexible system was needed, and an improved reactor system was designed. This improved design was the reactor system that was used for the remainder of the study due to its ease of operation, reliability and flexibility (Chapter 6, 7 and 8).

3.3.1 Preliminary AAO Reactor System

Previous research using a similar wastewater composition for laboratory scale biological wastewater treatment investigations showed that nitrification occurred and nitrate and nitrite were present in the treated effluent (Leonard, 1996; Donkin and Russell, 1997). On this basis, an activated sludge configuration capable of removing both nitrogen and phosphorus was required. As seen in the review in Chapter 2 (Section 2.4.2) a number of activated sludge configurations are available that can be used for biological phosphorus and nitrogen removal. Of these, the AAO system is one of the least complicated and was initially tested. In New Zealand activated sludge

systems treating dairy processing wastewater generally have extended hydraulic retention times in the order of 5 to 7 days. The laboratory scale AAO system used by Donkin *et al.* (1995), also had an extended HRT of 7 days. In the preliminary reactor studies (Chapter 5), a relatively long HRT of 3.6 days (AAO) and 3.2 days (MUCT) was used. A longer HRT was not used due to the difficulty of achieving accurate low pumping rates and the fact that long aeration times have been reported as detrimental to the EBPR process (Satoh *et al.*, 1994; Brdjanovic *et al.*, 1998). A total reactor volume of 18 litres was used with an influent flow rate of 5 litres/day, to give an overall retention time of 3.6 days. The actual retention time (including recycle streams) in the anaerobic zone of 7 hours (Table 3.1) was greater then those typically encountered in BNR systems of 1 to 3 hours (Cloete and Muyima, 1997) so as to promote *insitu* substrate fermentation. The sludge recycle ratio was kept constant at 1.74 times the influent flow rate (1.74Q) to prevent sludge accumulation in the clarifier and the anoxic recycle was operated at 2Q. A schematic of the reactor configuration is shown in Figure 3.3. This AAO configuration was operated for a total of 50 days with a sludge retention time (SRT) of 15 days.

Table 3.1. Retention times in respective zones of laboratory AAO system.

	Anaerobic zone	Anoxic zone	Aerobic zone
Volume (litres)	4	2	12
Actual HRT (hours)	7	2	12
Nominal HRT (hours)	19.2	9.6	57.6

Initially the reactor was seeded with sludge from the Rotorua municipal wastewater treatment plant, which has a 5 stage Bardenpho configuration for both biological nitrogen and phosphorus removal. The mixed liquor was transferred between the reactor sections under gravity. The outlets in the anaerobic and anoxic stages were fixed at a certain level to maintain the desired volume (Figure 3.3). The anaerobic and anoxic zones were both completely mixed using magnetic stirrers at a low rotation to minimise oxygen intrusion into the mixed liquor. The aerated reactor consisted of a New Brunswick Scientific Co., laboratory fermenter with independent mixing, aeration and temperature control. The aerated reactor temperature was maintained at 25°C. The volume of the aerated reactor was kept constant by positioning the base of the outlet tube to the clarifier at the required depth in the reactor. The dissolved

oxygen in this reactor was controlled at 3.0 mg/L using a YSI dissolved oxygen probe connected to a feed back controller attached to a solenoid valve in the air supply line. The SRT was controlled by continuously wasting sludge directly from the aerated reactor. A photo of the system is shown in Figure 3.4.

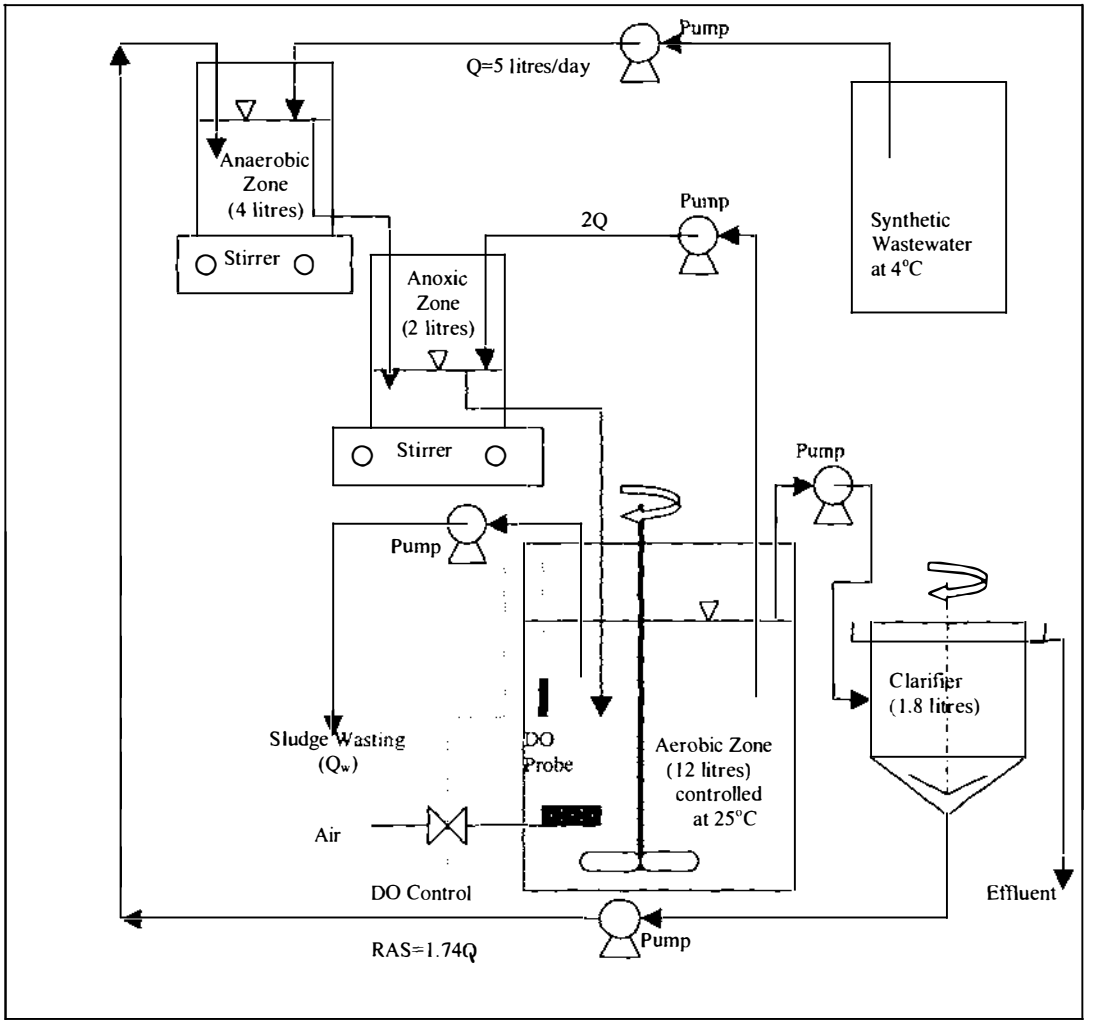


Figure 3.3: Schematic of AAO laboratory activated sludge system.

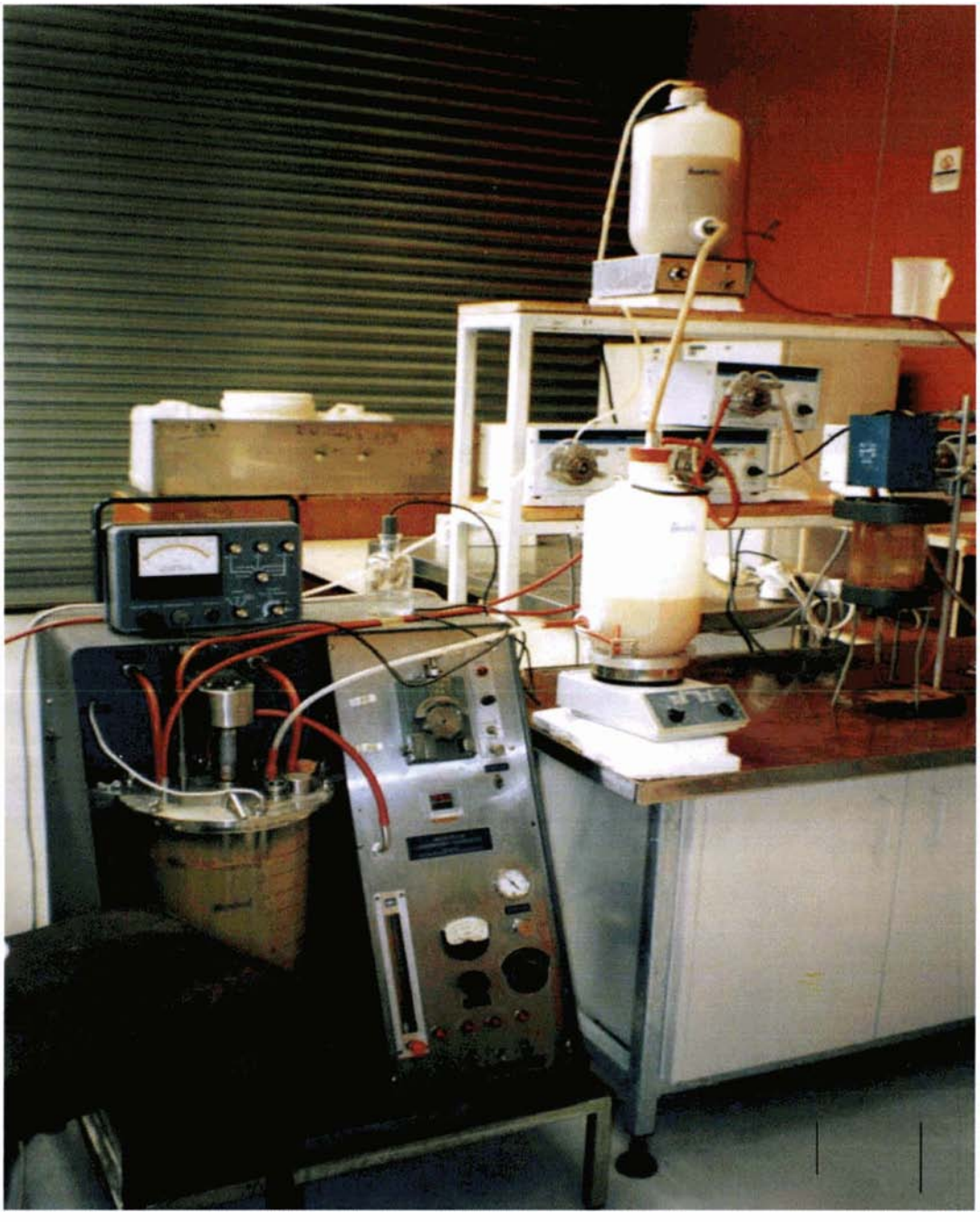


Figure 3.4: Picture of initial AAO laboratory activated sludge system.

3.3.2 Preliminary MUCT Reactor System

In the next phase of the study the AAO configuration was changed to a MUCT process by the addition of one extra anoxic zone (Figure 3.5). The recycle from the first anoxic zone to the anaerobic zone had a recycle ratio of 1Q. The volume of both anoxic zones was 2 litres and the aerobic-anoxic recycle ratio was 2Q. The volume of the anaerobic zone was decreased to 2 litres to maintain a unaerated volume of 6 litres. The aerated zone was decreased slightly to 10 litres in order to decrease the retention to 3.2 days and the influent flowrate (Q) remained at 5 L/day. There was a possibility that the lower HRT might be beneficial to phosphorus removal (Satoh *et al.*, 1994; Brdjanovic *et al.*, 1998). The respective retention times and volumes of each zone are shown in Table 3.2.

Table 3.2: Hydraulic retention times in respective zones of laboratory MUCT system.

	Anaerobic zone	1st Anoxic zone	2nd Anoxic zone	Aerobic zone
Volume (litres)	2	2	2	10
Actual HRT (hours)	4.8	3.2	2.4	12
Nominal HRT (hours)	9.6	9.6	9.6	48

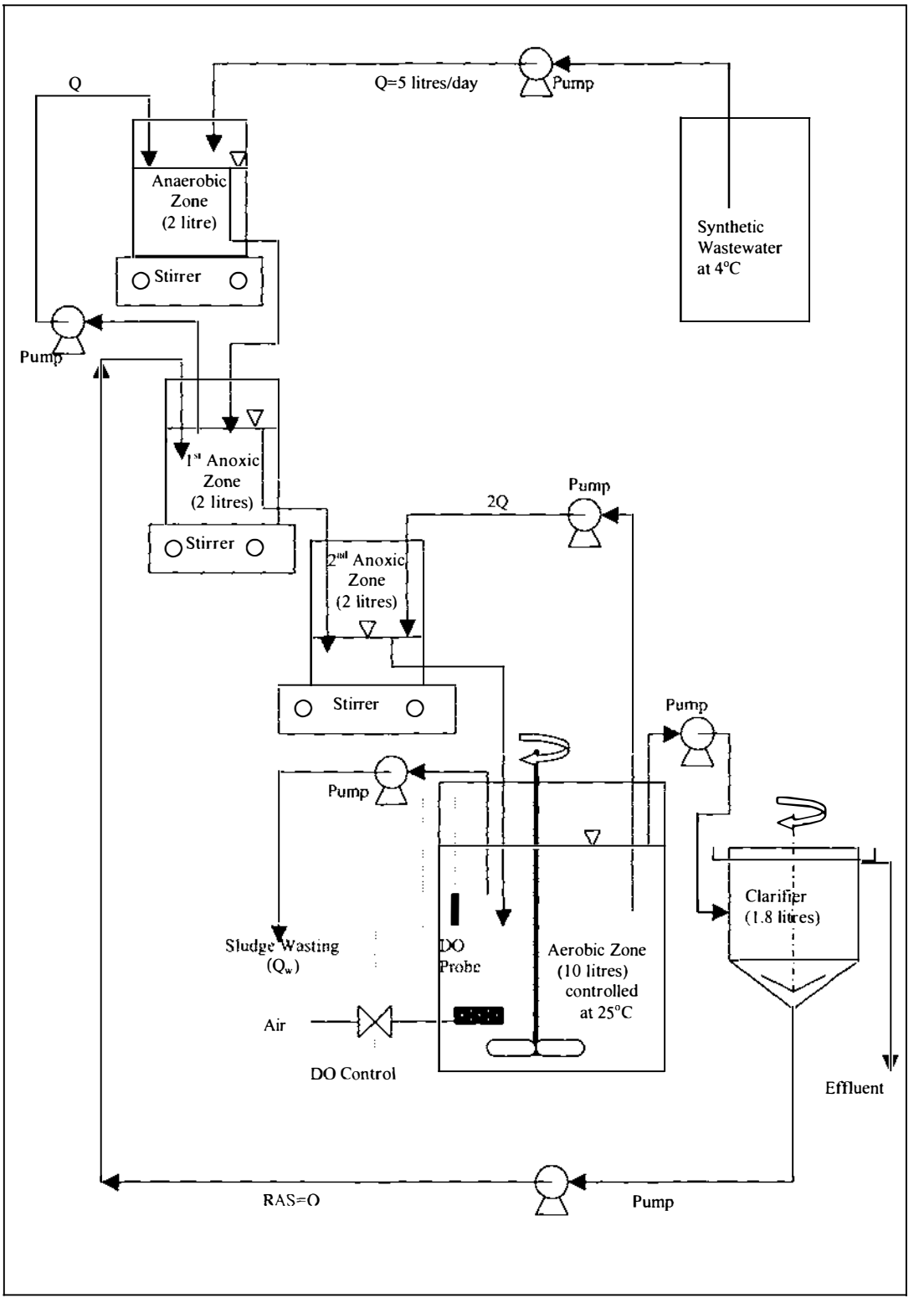


Figure 3.5: Schematic of MUCT laboratory activated sludge system.

3.3.3 Zoned Activated Sludge Reactor System.

During the preliminary reactor study a new activated sludge reactor system was designed and constructed to improve reliability, stability and flexibility. The improved reactor consisted of 10 individually mixed and aerated zones in series. The aeration the each zone could be turned off or on to each of the ten zones allowing for unaerated and aerated zones in any part of the system. Zone mixing was achieved using magnetic mixers. Each mixer consisted of former computer hard drives fitted with magnets and was connected to a variable speed drive. Each zone had aeration connections, in which the compressed air-lines were connected to ball valves on the outside of each zone and fitted with an aquarium air diffuser inside each zone. When either an anaerobic or anoxic zone was required, the ball valve was simply turned off. Each zone was 100mm by 100mm wide and 250mm high giving a total volume of 2.5 litres. The volume of mixed liquor in each zone was limited to 2.0 litres, (providing a freeboard in each zone of 50mm) giving a total reactor liquid volume of 20 litres. A water jacket (25mm wide) enclosed the wall and base of the reactor to allow the circulation of water at 20 ^{o}C, providing a constant temperature environment for the reactor contents. A temperature probe connected to a temperature control unit was positioned in the mixed liquor contents of the final zone providing feedback control to maintain a constant temperature. A baffled hole of 10mm diameter in each zone separation wall, 25 mm from the base of the zones allowed the movement of the reactor contents in the flow direction shown in Figures 3.6 and 3.7. A circular clarifier of (volume 2 litres) positioned at an appropriate height controlled the liquid level and hence the reactor volume. A scraper/stirrer with rubber edges was operated at a speed of 3 rpm and prevented sludge accumulation in the clarifier. This scraper was connected to a timer that controlled an intermittent cycle in which the stirrer speed suddenly increased for 4 seconds. In this study, the interval between these stirrer cycles was set at 20 minutes. This periodic increase in scrapper speed prevented the accumulation of sludge in the clarifier by periodically breaking up clumps of sludge. The accumulation of clarifier sludge in clumps had been an operational problem in the preliminary reactor system.

Aeration was controlled using the same DO control unit used previously, with the DO probe positioned in the second aerated zone and the DO controlled at 2 mg/L in this zone. This did not control the DO concentration in each zone and resulted in higher

DO levels in subsequent zones. This insured that sufficient aeration was provided to the initial zones. The D.O. concentration in the final zone was controlled at about 0.5 mg/L by closing the air input ball valve. This prevented oxygen intrusion into the anaerobic zone through excessive DO levels in the return activated sludge (RAS) line.

Sampling ports with ball valves were positioned on the side of each zone at a height of 100 mm from the base to permit either sub-surface sampling or stream recirculation entry/exit points. This reactor system minimised the amount of tubing and pumps required, resulting in a much more reliable process.

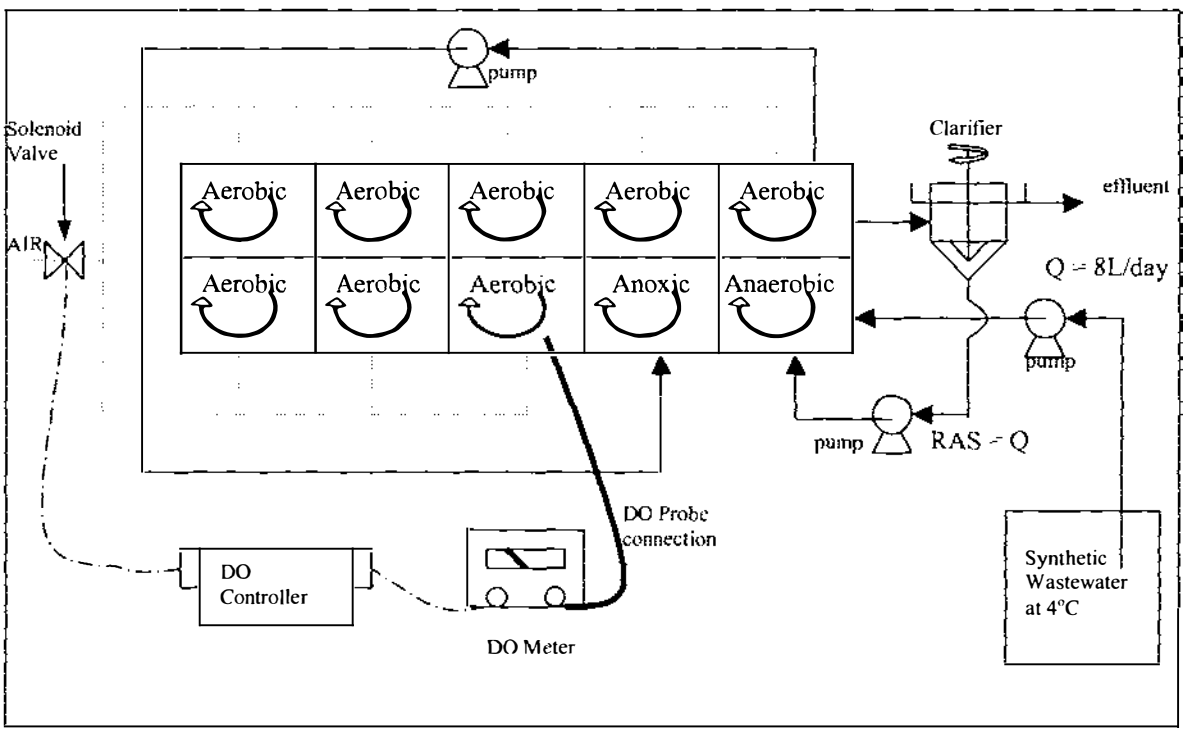


Figure 3.6: Schematic of improved laboratory zoned reactor system.

The wastewater was fermented using a continuous fermenter as described in the next section and then pumped to the AAO configured activated sludge zoned reactor. The last part of the study involved operating the reactor with an a AO configuration by converting the anoxic zone into an aerobic zone (zone 2) by simply opening the air supply valve (Chapter 7).

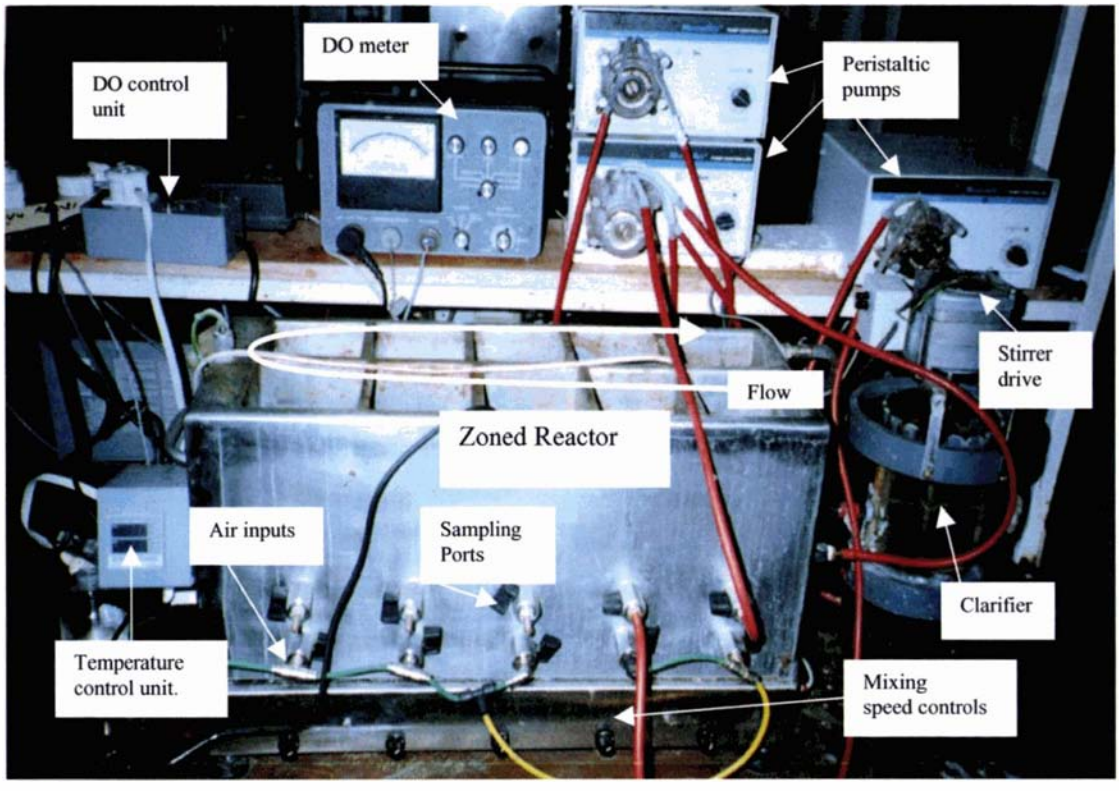


Figure 3.7: Zoned reactor system showing clarifier and stirrer, DO control system and temperature control unit.

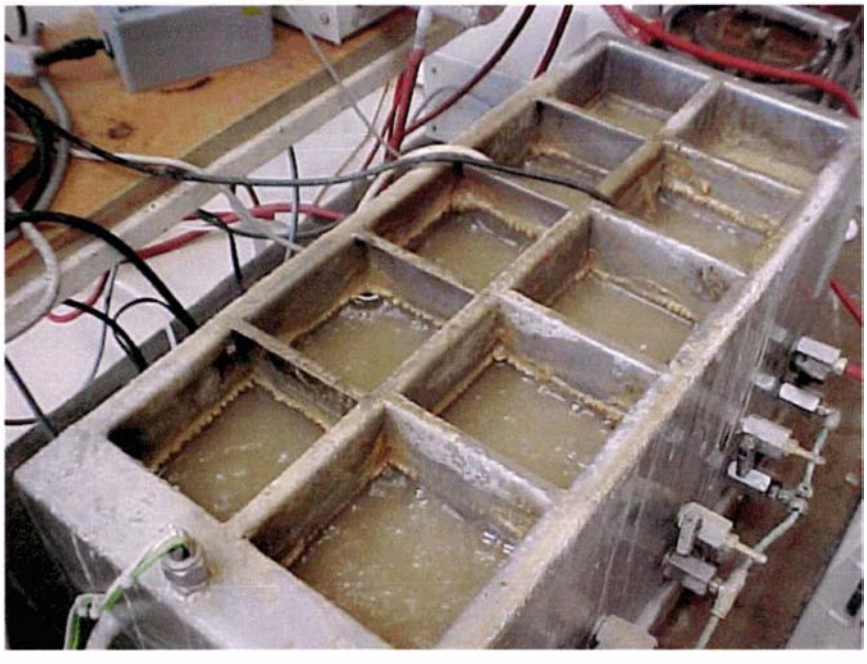


Figure 3.8: Mixed liquor contents of zoned reactor system.

3.3.4 Fermentation Reactor

To provide a consistent source of VFA's to the reactor, a fermenter was added before the activated sludge reactor. The fermenter consisted of a 15 litre New Brunswick Scientific Company (Model MF-14) fermenter with temperature control, pH control and an internal mixer (Figures 3.9 and 3.10). In the initial fermentation trial, the fermenter was opearted at HRT's of 12, 18 and 24 hours, temperatures of 30 and 35^{O}C and pH settings of 6.0 and 6.5 (Chapter 4). The fermenter was operated at an HRT of 12 hours, a constant temperature of 35^{0}C and a constant pH of 6.5 during the EBPR reactor studies. Synthetic wastewater refrigerated at 4^{o}C, was pumped to the fermenter, converted to volatile fatty acids, and then pumped to another holding refrigerated plastic container at 4^{o}C before pumping to the subsequent activated sludge system. The volume in the fermenter was controlled using a level control probe attached to a pump. The pH was adjusted by adding 6N NaOH via a pump connected to a pH controller (Horizon Model 5997-20). The headspace in the fermenter was purged with nitrogen gas before start up or whenever the top of the fermenter was removed. The operating volume of was maintained at 10 litres, which produced 20 litres of fermented dairy processing wastewater per day.

3.3.5 Laboratory Pumps

All the pumps used in reactor systems were peristaltic pumps. The activated sludge reactors used Masterflex (Cole-Parmer Instrument Co, Models 7554-60 and 7554-30) pumps fitted with 7016 pump heads and Norprene Masterflex tubing (6404-16). The fermenter influent and effluent lines were connected to a double head pump (ISCO Co., Model 1612) with Norprene Masterflex tubing (6404-15). All connections between pumps, reactors, influent, and effluent storage containers consisted of red rubber tubing with a 5 mm inside diameter.

3.3.6 Reactor System Steady State

Before complete sampling and analysis of the reactor systems they were operated for a period of at least three solids retention times (SRT's) in order to reach 'pseudo steady state' after which changes were made in system parameters (Leonard, 1996). For the biological systems tested, the so-called steady state was only an indication of the system response to changes in operating conditions.

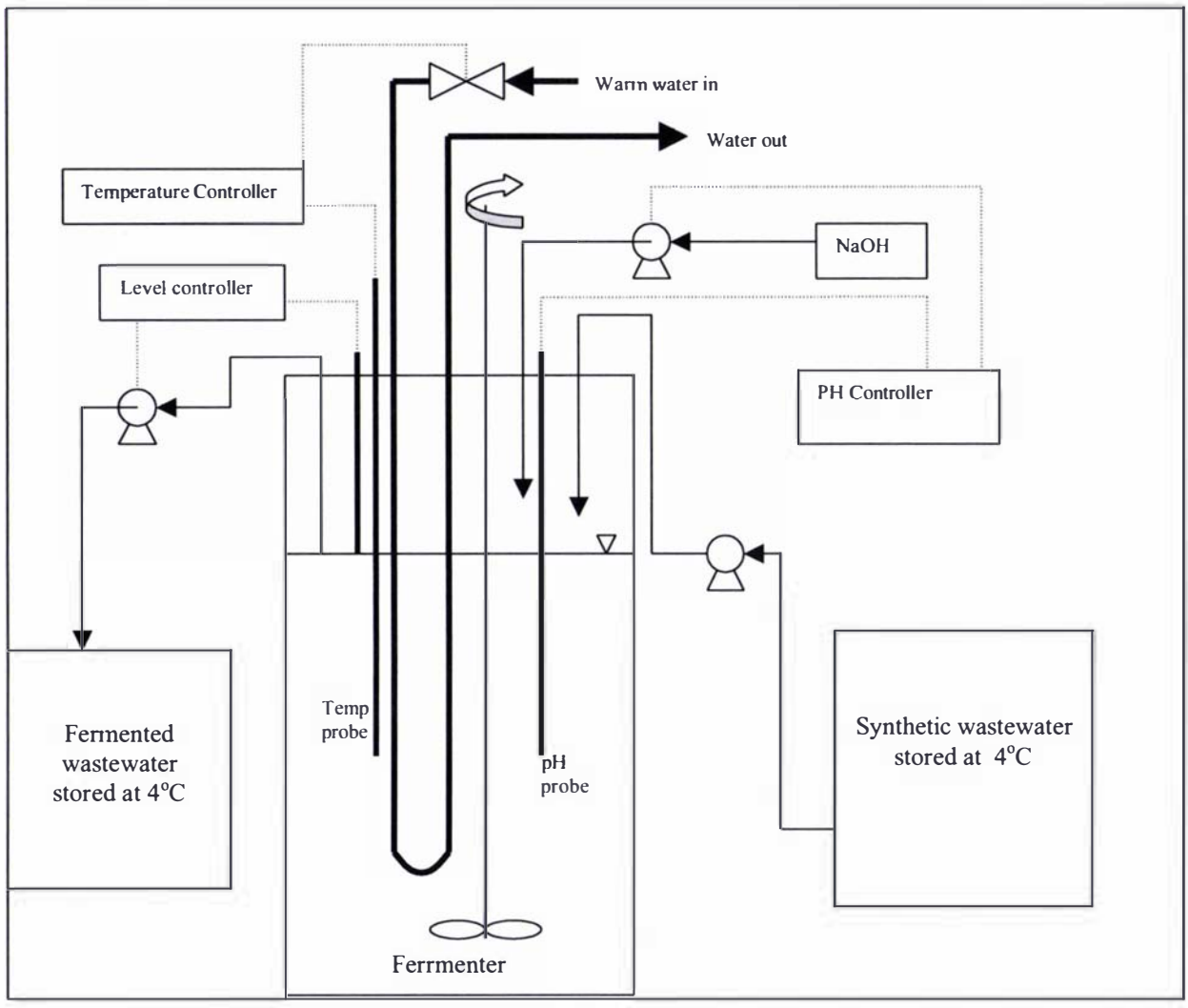


Figure 3.9: Schematic of fermenter process.

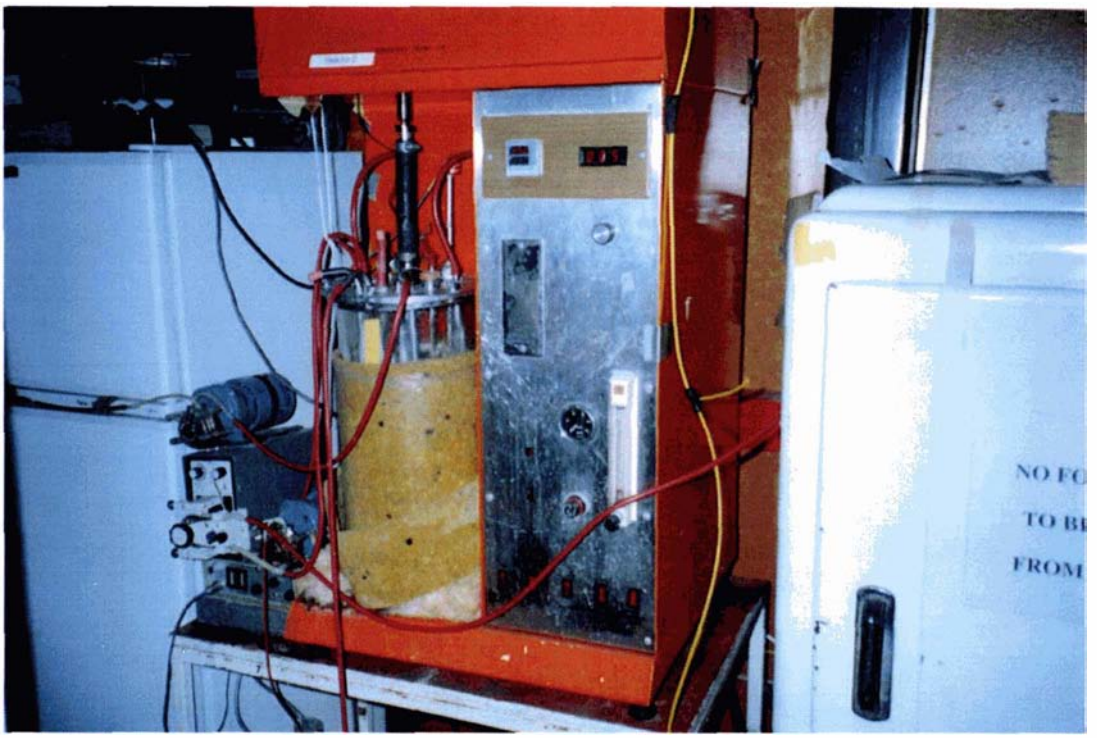


Figure 3.10: Photo of fermenter system showing 4°C synthetic wastewater storage refrigerator on the right and fermented wastewater refrigerator on the left.

3.4 Synthetic Wastewater Composition

The synthetic wastewater was initially composed of whole milk powder, skim milk powder and butter with additional phosphorus added as KH_2PO_4 (Table 3.3). This recipe was similar to that used by Leonard (1996), with the individual components increased in proportion to create a COD of approximately 3000 mg/L. This wastewater had a COD/TKN ratio of 28. This wastewater was used in Chapters 4, 5 and 6. The wastewater initially had a total phosphorus concentration of 105 mg P/L for the experimental work in Chapters 4 and 5. For Chapter 6, the KH_2PO_4 concentration was decreased to 3.74 g/10 L, decreasing the total phosphorus concentration to 98 mg P/L. In the later studies (Chapter 6,7 and 8) the butter was removed from the recipe, as under refrigeration it tended to separate from the mixture and float on the surface of the wastewater.

The synthetic wastewater was subsequently altered to increase the COD/TKN ratio to 32 in order to minimise the occurrence of nitrification and the resulting nitrate

production (Chapter 7). The recipe for this modified wastewater is listed in Table 3.4. Adding lactose made up the COD that was removed, by decreasing the amount of whole and skim milk powder, as lactose does not contain nitrogen. This wastewater had a total phosphorus concentration of 95 mg P/L

Table 3.3: Synthetic wastewater recipe with COD/TKN ratio of 28.

Components	Amount to make 10 litres
Whole milk powder	10 g
Skim milk powder	10.8 g
Butter	2.3 g (Chapter 4 and 5)
	0 g (Chapter 6)
KH_2PO_4	4.1 g (Chapter 4 and 5)
	3.74 (Chapter 6)
NaOH (45% w/w)	20 ml to adjust pH to 11

Table 3.4: Recipe for synthetic wastewater with COD/TKN ratio of 32.

Components	Amount to make 10 litres
Whole milk powder	7.2 g
Skim milk powder	7.8 g
Lactose	6.0 g
KH_2PO_4	3.75 g
NaOH (45% w/w)	20 mL to adjust pH to 11

The milk powder used (both skim and whole milk) was manufactured by Anchor Milk Products New Zealand. The characteristics of whole and skim milk powder shown in Table 3.5 were calculated from information supplied on the packaging showing the composition per 100 ml of reconstituted milk. The lactose was analytical grade and supplied by BDH (Poole, England).

Table 3.5: Milk powder characteristics according to the manufacturer (Anchor Milk Products Ltd, N.Z.).

	Whole milk powder per g powder	Skim milk powder per g powder
Milk fat (g)	0.2595	0.0054
Protein (g)	0.2595	0.2054
Carbohydrate (g)	0.3511	0.2595
Calcium (mg)	9.1603	6.4865
Phosphorus (mg)	6.8702	5.4054
Energy (KJ)	20	7.945

Based on the above information there is 12.7 mg P/L of phosphorus that was due to the milk powder in the first synthetic wastewater (COD/TKN=28) and 9.2 mg P/L in the second synthetic wastewater (COD/TKN=32).

CHAPTER 4
Wastewater Characterisation and Fermentation Studies

4.1 Introduction

The success of the Enhanced Biological Phosphorus Removal (EBPR) process is largely dependent on the characteristics of the organic carbon present in the wastewater. The COD/TKN content of the wastewater will also determine whether a combined nitrogen and phosphorus removal EBPR system is required.

In this study the conventional gross analytical properties of the "high nitrogen" synthetic dairy processing wastewater (COD/TKN=28) were determined along with greater specification of the readily biodegradable COD (RBCOD) component. In order to increase the RBCOD component and generate volatile fatty acids (VFA) for the EBPR process, fermentation (acidification) of this dairy processing wastewater was also assessed.

4.2 Synthetic Dairy Processing Wastewater

Dairy processing wastewater is comprised mainly of diluted milk, milk products and cleaning solutions. The characteristics of dairy processing wastewater at any time will be dependent on both the products produced at the site at that time and whether process equipment is being cleaned. To meet the requirement of operating a biological reactor under controlled conditions, a wastewater without widely variable characteristics was required. In this study, a synthetic dairy processing wastewater with properties typical of an actual wastewater was used. The composition of the synthetic wastewater used in this characterisation study was based on the recipe used by Leonard (1996), comprising a mixture water, milk powder and initially butter (Table 4.1). The soluble phosphorus concentration was increased by 92 mg P/L in order to create a phosphorus rich dairy processing effluent. The organic content (COD) of the wastewater was increased by 50% over that used by Leonard (1996), to give a COD of 3200 mg/L, slightly more than the typical dairy wastewater COD of about 3000 mg/L (Table 2.5). This COD is also typical of a New Zealand phosphorus rich dairy processing wastewater (Barnett *et al.*, 1994). The synthetic wastewater recipe is shown in Table 4.1.

Table 4.1: Synthetic dairy processing wastewater recipe.

Component	Amount to make 10 litres
Whole milk powder	10.0 g
Skim milk powder	10.8 g
Butter	2.3 g
KH_2PO_4	4.1 g
NaOH (45% w/w)	20 mL

The chemical and physical characteristics of the synthetic wastewater are shown in Table 4.2. The total COD of the wastewater in the refrigerated storage container varied depending on when the sample was collected. If the sample was collected soon after the wastewater was mixed the total COD was about 3200 mg COD/L. When the wastewater was sampled after more than 1 day after mixing (under refrigeration), then some solids could settle and the total COD would approach 3500 mg COD/L if the sample was collected from the lower part of the container. The wastewater was usually replaced daily with a fresh mixture when possible.

4.3 COD Fractionation of Wastewater

If substrate is to be available for conversion and storage as PHA's in the anaerobic zone of the EBPR process, the substrate must firstly be converted to VFA or be present as VFA. Substrate that is part of the readily biodegradable COD (RBCOD) fraction but not present as VFA needs to firstly be converted to VFA within the anaerobic zone before conversion to PHA. It is likely that with the relatively small actual HRT of the anaerobic zone that only the RBCOD organic fraction will be converted to SCVFA. The overall RBCOD requirement for biological phosphorus removal has been estimated as 10 g RBCOD/g P removed (Clayton *et al.*, 1991).

In Activated Sludge Model No. 1 (ASM1) (Henze *et al.*, 1986), the influent wastewater total COD comprises four fractions: readily biodegradable COD (S_{S1}), slowly biodegradable COD (X_{S1}), inert particulate COD (X_{I1}), and inert soluble COD (S_{I1}).

Orhon *et al.* (1993), showed experimentally that S_{I1} was insignificant for diary processing wastewater. Leonard (1996), suggested that the inert particulate COD fraction was negligible for this synthetic dairy processing wastewater as the VSS/TSS ratio was measured as 1, indicating zero ash content. This was also the case in this study were the VSS/TSS ratio of the wastewater was always measured as being equal to one.

Table 4.2: Synthetic dairy processing wastewater chemical and physical characteristics.

Characteristic	Concentration/Value
COD_{total}	3200 mg COD/L
$COD_{GFC\ filtered\ (<1.2\ \mu m)}$	2150 mg COD/L
$COD_{filtered\ (<0.45\ \mu m)}$	1950 mg COD/L
TKN	115 mg N/L
Total Phosphorus	105 mg P/L
Soluble Phosphorus	95 mg P/L
TSS	470 mg/L
pH	11
Lactose	880 mg/L

Activated Sludge Model No. 2 (ASM2) (Henze *et al*, 1995), which is an extension of ASM1 incorporating phosphorus removal, further subdivides some COD fractions. In ASM2 the readily biodegradable substrate comprises two fractions, firstly the fermentation end products (S_A), which are considered to be acetate, and the fermentable readily biodegradable organic substrates (S_F). In effect the S_{S1} (RBCOD) fraction of ASM1, is replaced by $S_A + S_F$ in ASM2. ASM2 assumes that the S_F fraction is directly available as a substrate for fermentation by heterotrophic organisms. In ASM2 the soluble COD comprises S_A and S_F, although analytically the soluble COD component also comprises slowly biodegradable COD (X_S). With the synthetic dairy wastewater used in this study, no VFA was initially present so the S_F fraction is equivalent to the S_{S1} fraction given in ASM1.

Due to the reliance of the EBPR process on the S_A and S_F COD fractions they will be considered further. As previously stated S_A in this synthetic wastewater is insignificant, therefore the remaining readily biodegradable COD (RBCOD) needs to be quantified. The RBCOD fraction of the synthetic dairy processing wastewater used in this study was experimentally determined using aerobic batch tests by Leonard (1996) as 42.5% of the total COD and 60% of the soluble COD. If these RBCOD values are recalculated using the same data it is evident that they have been over estimated. This is due to the incorporation of the baseline due to endogenous respiration, instead of including the baseline due to substrate hydrolysis in the oxygen consumption calculations. The corrected RBCOD fraction (S_{S1}) would be approximately 20% of the total COD. This is much more comparable to that obtained by Sozen and Orhon (1999) of 22.8% for an actual dairy processing wastewater. The RBCOD fraction of any dairy processing wastewater will vary however depending on the products being manufactured and the waste minimisation procedures that are used at a particular processing site.

To confirm the RBCOD values obtained by Leonard (1996) both aerobic batch tests and anoxic batch tests were performed. It was anticipated that the anoxic batch test method would clarify the position of the baselines due to substrate hydrolysis and confirm the RBCOD calculations from aerobic batch tests. Both aerobic and anoxic tests also simultaneously permit the calculation of the heterotrophic yield coefficient Y_H (Solfrank and Gujer, 1991; Kujawa and Klapwijk, 1999).

4.3.1 Aerobic Batch Tests

The RBCOD fraction describes the organic carbon fraction with a low molecular weight, which sustains a high rate of bacterial respiration. The RBCOD fraction is determined by measuring the rate of consumption of the electron acceptor under either aerobic conditions (O_2) or anoxic conditions (NO_3-N and NO_2-N), when sludge is exposed to the wastewater.

The aerobic batch test method for evaluation of S_{S1} as described by Ekama *et al.* (1986), and Solfrank and Gujer (1991), measures the oxygen uptake rate (OUR) after a preselected volume of wastewater of known COD is mixed with a known volume of mixed liquor. It is desirable that the initial high OUR plateau due to RBCOD

consumption is long enough to be accurately measured, but short enough to also minimise heterotrophic growth and hydrolysed substrate. Kappeler and Gujer (1992), recommend an elevated OUR duration due to RBCOD of 30 minutes. A suitable substrate to biomass ratio (S/X) needs to be determined for a particular wastewater to obtain this duration. Once the RBCOD is depleted, the OUR drops to a second plateau, which is the rate associated with the utilisation of COD generated by hydrolysis. The RBCOD fraction is proportional to the area between the initial high OUR and the OUR due to hydrolysis (ΔO) (Figure 4.1). The RBCOD concentration is then calculated as follows:

$$S_{SI} = \frac{\Delta O}{(1 - Y_H)} \frac{V_{ml} + V_{ww}}{V_{ww}} \qquad (4.1)$$

Where Y_H is the heterotrophic yield coefficient (mg cell COD/ mg COD consumed) and V_{ml} is the volume of mixed liquor used and V_{ww} is the volume of wastewater used. The requirement of Y_H necessitates either the presence of a suitable literature value or the separate calculation of Y_H. Fortunately Y_H can be simultaneously estimated during these aerobic batch tests by relating the total oxygen consumed (due to added COD) to the COD degraded as shown in equation 4.2 (Solfrank and Gujer, 1991).

$$Y_H = 1 - \frac{\text{Oxygen consumed}}{\text{COD consumed}} \qquad (4.2)$$

Figure 4.1 shows an example of the oxygen uptake rate (OUR) curve for the initial aerobic batch test, using mixed liquor from a reactor system with anaerobic, anoxic and aerobic zones and operated at an SRT of 15 days (Chapter 5). The batch test consisted of 1 litre of mixed liquor (MLVSS=1635mg VSS/L) and 4 mls of concentrated GFC filtered synthetic dairy processing wastewater (10 times strength, the ingredients for 10 L mixed in only 1 L) resulting in an initial soluble COD of 81 mg/L.

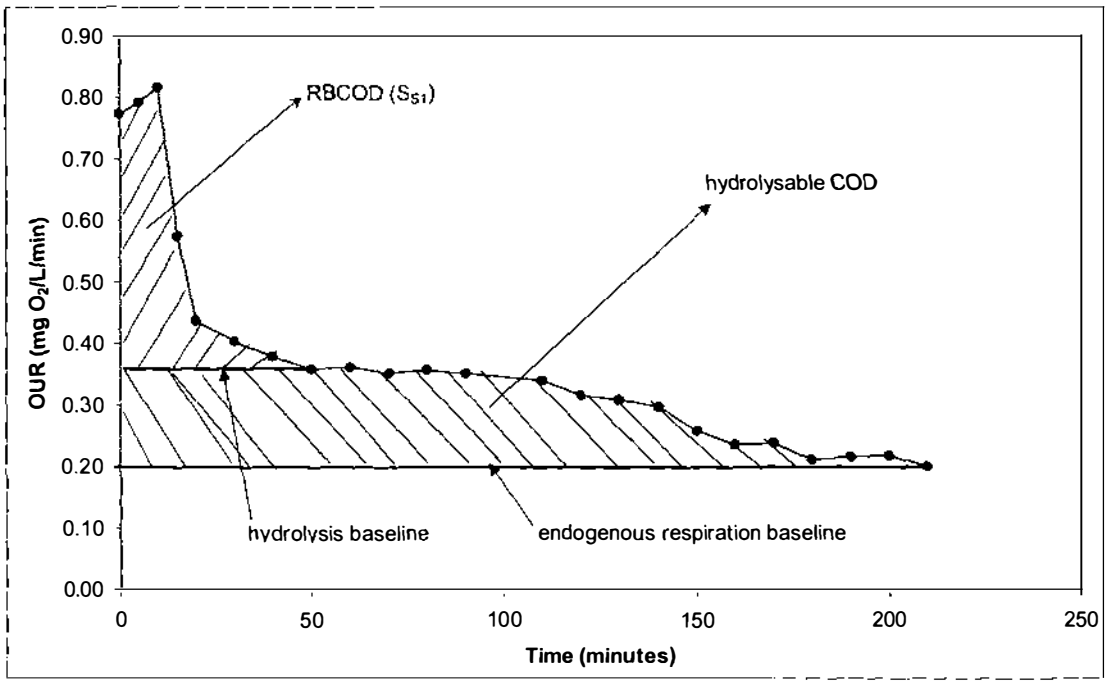


Figure 4.1: RBCOD determination using aerobic batch test method for synthetic dairy processing wastewater (S/X= 0.05).

The total oxygen consumption due to added substrate (area above endogenous baseline) is 29.5 mg O_2/L giving a value of Y_H for this batch test of 0.64 mg cell COD/mg COD consumed (1-(29.5/81)). The oxygen consumption due to RBCOD is 8.5 mg O_2/L resulting in an S_{S1} value of 21 mg COD/L for this batch test. The RBCOD fraction of the soluble COD is calculated as 29 % of soluble the COD (18% of the total wastewater COD). The hydrolysable component comprised the remainder of the GFC filtered COD at 72%.

The aerobic batch test was repeated at a higher S/X ratio (0.12) by increasing the amount of filtered concentrate added to 10 mL, (202 mg COD/L) to increase the duration of the initial OUR plateau. The total oxygen consumption of 68.6 mg O_2/L resulted in a Y_H value of 0.66 mg cell COD/mg COD consumed. The RBCOD fraction was calculated as 22% of the total COD and 35% of the soluble COD. The hydrolysable COD was 65% of the soluble COD.

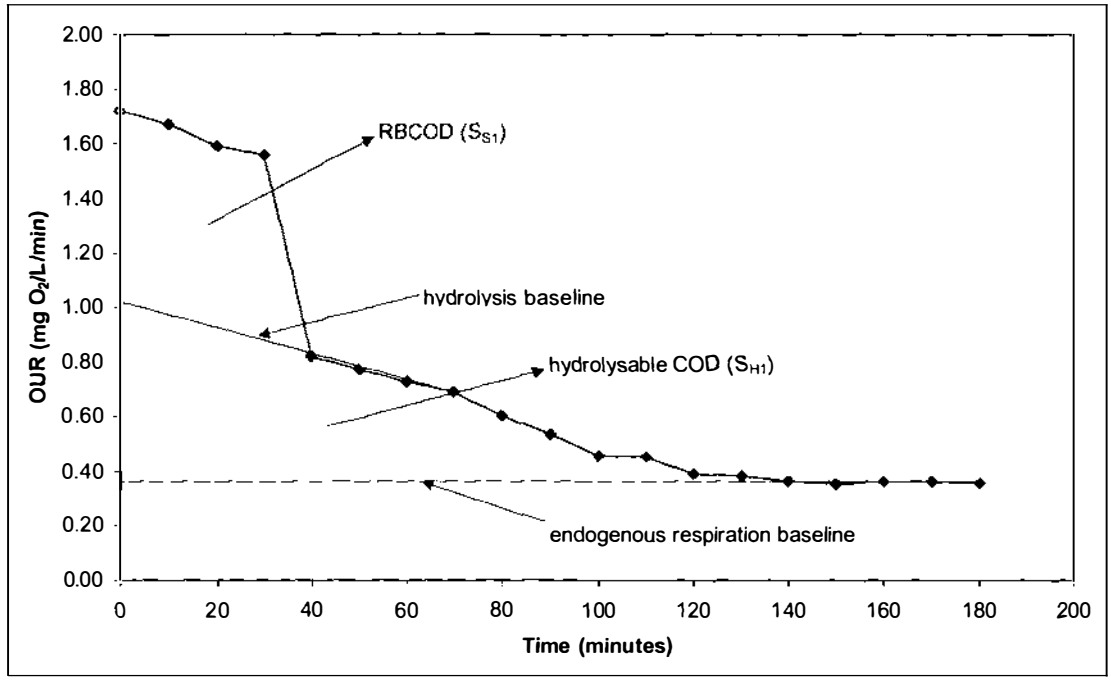


Figure 4.2: RBCOD determination using aerobic batch test method for synthetic dairy processing wastewater (S/X= 0.12).

The RBCOD fraction obtained in these two aerobic tests of 18 and 22% are similar to the corrected values obtained by Leonard (1996) and the values for dairy processing wastewater obtained by Sozen and Orhon (1999).

4.3.2 Anoxic Batch Tests

In order to check the reliability of the aerobic batch test results, anoxic batch tests were performed. Anoxic tests have the advantage that they can more precisely define the changes in the utilisation rate of the electron acceptor due to changes in the COD fractions. A high nitrate uptake rate (NUR) due to RBCOD of at least 30 minutes is recommended for the anoxic batch test (Kujawa and Klapwijk, 1999). The same data as for the aerobic batch test is required except that the nitrate and nitrite concentration is measured with time instead of the oxygen concentration. The RBCOD concentration is proportional to the amount of nitrate utilised (ΔNO_3) during the initial high NUR rate (equation 4.4).

$$S_{SI} = \frac{2.86\Delta NO_3}{(1-Y_H)} \frac{(V_{ww}+V_{ml})}{V_{ww}} \quad (4.4)$$

If nitrite is present then it is included in the nitrate total by multiplying by 0.6 and adding to the nitrate, as nitrite has 0.6 of the electron acceptor value of nitrate (Kujawa and Klapwijk, 1999). This sum has been denoted as the total oxidised nitrogen (NO_X-N) in the NUR graphs presented in this study. The yield coefficient used in RBCOD calculations for anoxic batch tests can be either previously determined values from aerobic tests, literature values, or experimentally determined. Orhon *et al.* (1994), derived a theoretical value for Y_H under aerobic conditions, using the energetics of aerobic growth on lactate, of 0.65 as a theoretical value for dairy processing wastewater. They suggested that an anoxic yield coefficient (Y_{HD}) should be used in anoxic data calculations with the value suggested for dairy processing wastewater of 0.52. There are however no experimentally derived values for anoxic yield coefficients for activated sludge processes treating actual industrial wastewater. Cokgor *et al.* (1998), determined the RBCOD component of domestic and industrial wastewaters using both aerobic and anoxic batch tests using the aerobic yield (Y_H) in the calculations. This is despite suggesting that Y_{HD} should theoretically be used.

In this study the anoxic heterotrophic yield coefficient used in the calculations was determined for each experiment. The value of Y_{HD} was determined by addition of known quantities of either mixed VFA or acetate as a readily biodegradable organic substrate (S_{SI}) in the anoxic batch tests, and then was calculating Y_{HD} by rearranging Equation 4.4 and solving for Y_{HD}.

Initially three anoxic batch tests were performed in parallel, with varying substrates. As with the aerobic batch tests the mixed liquor used was obtained from a reactor system with anaerobic, anoxic and aerobic zones that was operated at an SRT of 15 days (Chapter 5). The substrates used were filtered synthetic dairy wastewater, filtered fermented dairy processing wastewater (obtained from the fermentation study in Section 4.4), and one reactor with no added substrate (endogenous). The dairy processing wastewater was not concentrated for these NUR tests. The soluble portion of the fermented wastewater consisted of a known concentration of VFA as the RBCOD

source. This was used to both calculate Y_{HD} and provide a reference NUR comparison for the RBCOD components. The initial substrate and biomass (VSS) concentrations for the four batch tests are shown in Table 4.3 for each batch test.

Table 4.3: NUR batch test parameters.

Substrate	Added Soluble COD (mg/L)	Reactor VSS (mg/L)	S/X (based on Soluble COD)	Liquid Volume (litres)
Dairy Processing WW	85	3065	0.03	2.00
Fermented WW	85	2975	0.03	1.84
Endogenous	0	3170	0	1.86

The mixed liquor was mixed aerated for 4 hours before the tests to consume any residual substrate and the dissolved oxygen concentration was decreased to zero before substrate addition. To obtain an initial nitrate concentration of between 33 and 40 mg NO_3-N/L, 60 mls of a 1000 mg NO_3-N/L solution was added to each reactor. The results from the three substrates are shown in Figure 4.3.

The NUR gradient(s) for each substrate can be defined as K_1, K_2, and K_3 were the subscript 1 defines an initial high NUR gradient due to RBCOD, 2 denotes that due to hydrolysed COD and 3 is the NUR gradient due to endogenous respiration (Table 4.4).

The initial high NUR gradient K_1 for the fermented wastewater was due to readily biodegradable substrate (VFA). If the initial rate for the raw wastewater is comparable then this can also be classified as K_1 (RBCOD substrate). NUR gradients less than or equal to the endogenous substrate rate (batch test without added substrate) can also be classified as endogenous (K_3), while rates in between K_1 and K_3 are classified as the rate due to substrate hydrolysis (K_2). The values of K_1, K_2 and K_3 are shown in Table 4.5 for each batch test.

The anoxic yield coefficient (Y_{HD}) was calculated as 0.62 from the known VFA concentration of the fermented dairy wastewater. VFA comprised 100% of the soluble COD (85 mg/L) for the fermented wastewater. Using this value for Y_{HD} the RBCOD

fraction of the dairy processing wastewater was calculated as being 22% of the total COD (33% of the soluble COD). Within the 240 minutes duration of the synthetic wastewater batch test no equivalent endogenous rate was obtained. The endogenous NUR gradient (K_3) of the fermented wastewater was 40% of the endogenous rate of the reactor without an additional carbon source. Some of the difference was due to the slightly lower VSS concentration in the fermented wastewater batch test.

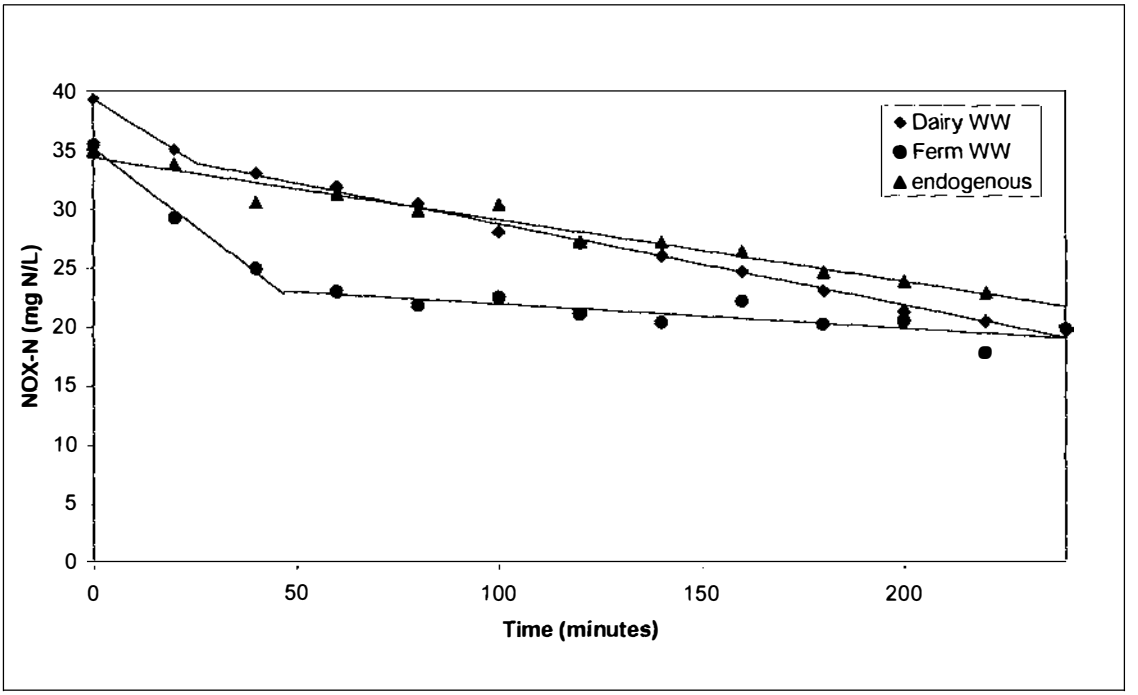


Figure 4.3: Anoxic batch test NUR graph for four different substrates.

Table 4.5: NUR gradients for each substrate used in the anoxic batch test.

Substrate	NUR gradient (mg N/L/minute)		
	K_1	K_2	K_3
Fermented WW	0.263		0.020
Dairy WW	0.214	0.069	
Endogenous			0.052

Another anoxic batch test was performed as only two data points confirmed the initial NUR gradient for the dairy processing wastewater in the first set of anoxic batch tests. This time acetate (sodium acetate) was used as the RBCOD substrate to measure Y_H at a starting concentration of 130 mg/L. The S/X ratio for the dairy processing wastewater reactor was 0.08 (volume of wastewater =300 mL, initial soluble COD = 325 mg/L, VSS= 3850). The volume of the batch test was 2.0 litres for both the acetate and the dairy processing wastewater. In the wastewater batch test the mixed liquor used was allowed to settle and 300mL of supernatant decanted, and at the start of the test, 300 mL of filtered wastewater was added. The same procedure as the previous NUR tests was followed. Samples were collected every 15 minutes for 240 minutes and immediately filtered with 0.45 μm filter paper. The results are plotted in Figure 4.4.

The anoxic yield coefficient was calculated as 0.68 mg COD/mg COD (ΔNO_X-N=14.5 mg N/L). The initial NUR gradient of the wastewater test is comparable to that of acetate indicating again that this initial rate is due to the RBCOD component of the wastewater (Table 4.6). The RBCOD fraction of the dairy processing wastewater (570 mg/L) was calculated as 18 % of the total COD (ΔNO_X-N=9.6 mg N/L). It would be expected that K_3 for the acetate reactor would reflect an endogenous rate, yet it is in between the K_2 and K_3 values for the wastewater results. Care needs to be taken when assigning descriptions to these rates, as it is likely that part of the acetic acid (or other RBCOD substrates) may be converted to internal storage compounds such as PHA that could then be utilised after RBCOD expiry. This would explain the reason why K_3 for acetic acid is similar the hydrolysis rate (K_2) for the wastewater. It has also previously been shown that internal storage compounds may influence the outcome of aerobic batch tests (Dircks *et al.*, 1999; Carcucci *et al.*, 2001).

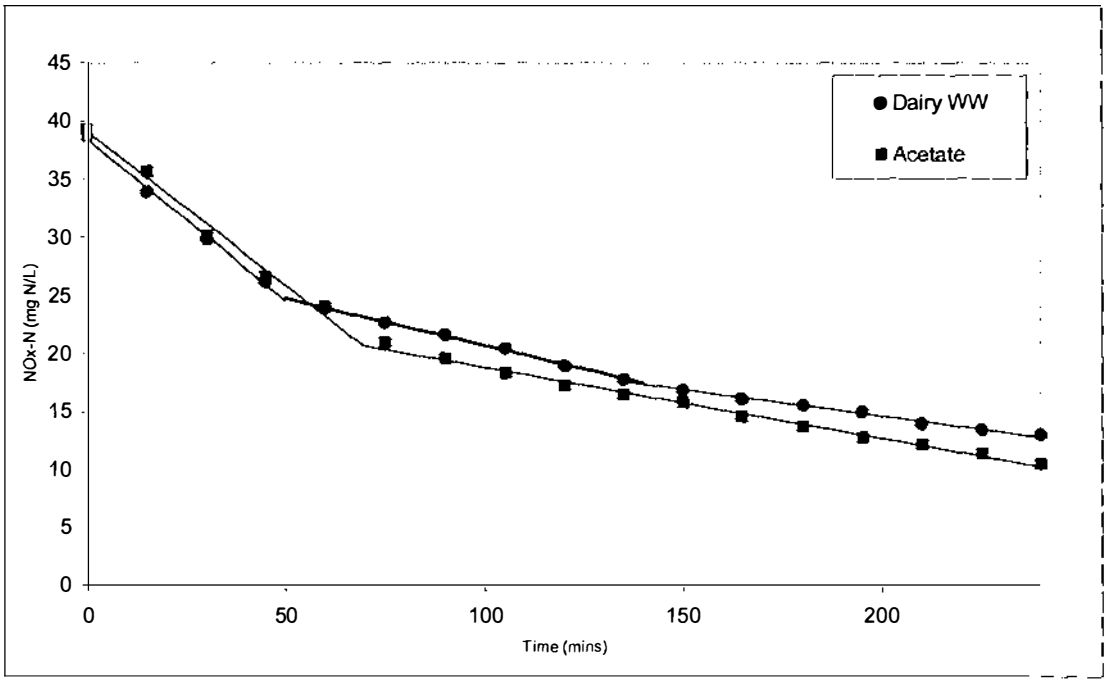


Figure 4.4: Anoxic batch test comparison for acetate and synthetic dairy processing wastewater.

Table 4.6: NUR gradients for each substrate used in second anoxic batch test.

Substrate	NUR gradient (mg N/L/minute)		
	K_1	K_2	K_3
Acetic acid	0.264	-	0.061
Dairy WW	0.277	0.081	0.045

The values of RBCOD and the yield coefficients (Y_H) and (Y_{HD}) determined are summarised in Table 4.7 below and compared to previous values for dairy processing wastewater from literature. The values for RBCOD determined in this study averaged 20%, which is very close to the values of Leonard (1996), and Sozen and Orhon (1999). It most likely that the RBCOD compounds are sugars present in the milk. Table 3.6 gives the carbohydrate content of each of the milk powders, which when calculated is 631 mg/L for this synthetic wastewater. The packaging states that carbohydrates are present as sugars. If it is assumed that the majority of the carbohydrate is lactose, then

this equates to a COD of 705 mg COD/L or 22% of the total wastewater COD. Based on this it appears reasonable to assume that the RBCOD is due to this carbohydrate component.

Table 4.7: RBCOD fraction and yield coefficients from this study and literature.

	RBCOD fraction (S_{S1})	Y_H (mg Cell COD/mg COD consumed)
Aerobic tests (this study)	18 %, 22%	0.64, 0.66**
Anoxic tests (this study)	22%, 18%	0.62, 0.68***
Leonard (1996)	20%	0.62*, 0.70**
Sozen & Orhon (1999)	23%	
Orhon *et al.* (1993)	-	0.6, 0.6*
Orhon *et al.* (1994)	-	0.65[1], 0.52[2]
Huang (1984)	-	0.69*

*Aerobic batch growth tests. ** Aerobic batch respirometric tests

***Anoxic NUR test using VFA.

1 Theoretical aerobic yield based on energetics for lactate

2 Theoretical anoxic yield based on energetics for lactate

4.4 Fermentation of Dairy Processing Wastewater

The supply of VFA to the anaerobic zone of EBPR is typically achieved in two ways. Firstly the wastewater substrate may be fermented to VFA within the anaerobic zone/phase and simultaneously converted to storage polymers as PHA. Secondly, the wastewater may already contain VFA that is directly available for conversion to PHA. If VFA is not already present in the wastewater, as with this synthetic dairy processing wastewater, a fermentation process can be used before the EBPR reactor to generate the required fermentation products. Comeau *et al.* (1996), investigated the EBPR process using an SBR when treating a cheese factory wastewater from a balance tank (HRT~20 hours). The balance tank had no temperature or pH control. The effluent from the balance tank generally had a pH of 6.2 and a VFA COD concentration averaging 1130 mg/L, showing that dairy processing wastewater is readily fermentable. Other researchers have studied the acidogenic fermentation of dairy wastewaters and lactose as a means to optimise anaerobic treatment processes (Kisaalita *et al.*, 1987a, 1987b,

1989; Kasapgil *et al.*, 1995; Fang and Yu, 2001; Yu and Fang 2001). Yu and Fang (2001), demonstrated that carbohydrate was preferentially acidified as compared to protein and lipids. At a pH of 5.5 and an HRT of 12 hours, wastewater with a COD of 2000 mg/L had the highest degree of acidification of 57.1%. The degree of acidification is quantified using the percentage of the initial soluble substrate concentration converted to VFA and other fermentation products. Fang and Yu (2001) investigated the acidification of lactose and found that while the degree of acidification was greatest at an HRT of 24 hours, there was not a lot of difference between 12 and 24 hours. Also between a pH of 4 and 6.5, the optimum pH was 5.5, but the variation in acidification was only ±6% between a pH of 5 and 6.5.

Kisaalita *et al.* (1987a), found that the optimum pH range for acidogenesis of lactose was found to be 6.0 - 6.5 using an HRT of 20 hours, with up to 70% of the soluble COD converted to VFA. At fermenter HRT's ranging from 10 to 20 hours there was good VFA production with minimal lactic acid formed, whereas at lower HRT's, VFA production decreased and lactic acid increased. Wastewater from a milk and cream bottling plant with a COD ranging from 2000-6000 mg/L had optimum fermentation conditions of a pH of 5.8, HRT of 12 hours and a temperature of 35°C. Decreasing the temperature had the largest negative impact on VFA production.

Some preliminary fermentation trials were performed to confirm that the synthetic dairy processing wastewater used in this study was readily fermentable. The anaerobic fermenter (operating volume 10 litres) was initially seeded using screened anaerobic biomass from an anaerobic sewage sludge digester (Palmerston North City Council, Wastewater Treatment Plant). The analytical parameters of importance to the EBPR process such as total and soluble COD and the VFA type and quantity were measured. The operating parameters chosen were based on the results from the previous studies mentioned. A pH of either 6.0 or 6.5 was used as it was anticipated that this would not disrupt the maintenance of a reasonable pH within the anaerobic zone of any subsequent EBPR process. These pH values are also typical of effluent from balance tanks without pH control at dairy processing sites (Comeau *et al.*, 1996; Jovcic, 1998).

Typical flow balancing tanks have HRT's of around 24 hours, however during periods

of high flows this may decrease. As previously mentioned successful acidification of dairy wastewater and lactose was achieved at an HRT of 12 days, but a decrease in acidification was experienced at HRT's less than 12 hours (Fang and Yu, 2001). For these reasons acidification of the synthetic dairy processing wastewater was investigated at HRT's between 12 and 24 hours.

4.4.1 Fermentation Studies

The background in the previous section shows that there is a reasonable amount of published and successful dairy wastewater fermentation information. For this reason, this study was limited to only four variations in operating conditions. This was to determine the most suitable fermentation parameters for this wastewater based on information from previous studies, and conditions typical of balance tanks. The fermentation reactor and equipment are described in detail in Section 3.3.5. In this study, four trials were performed using a 10 litre New Brunswick (Model MF-14) fermenter with variations in the HRT, pH and COD. The pH was adjusted using a pH controller and only required adjusting upwards using 6N NaOH (due to organic acid generation). The fermenter was operated for 7 days before sampling. The operating parameters were:

1) HRT=24, pH=6.0, Temperature=30^{O}C, COD=3200 mg/L;
2) HRT=12, pH=6.5, Temperature=35^{O}C, COD=3200 mg/L;
3) HRT=12, pH=6.5, Temperature=35^{O}C, COD=6400 mg/L;
4) HRT=18, pH=6.0, Temperature=30^{O}C, COD=3200 mg/L.

The results from each of the 4 trials are summarised in Table 4.8. The trials using an HRT of 12 hours have a similar degree of acidification ($VFA_{produced}$/ COD_{in}) that is independent of the COD of the raw wastewater (and COD loading of the fermenter). These values are comparable to a study by Ince (1998), who achieved acidification rates (based on soluble influent COD) of 50 to 70% for a range of pH values. The pH of 6 or 6.5 was used as this had been identified as the optimum pH range for VFA generation (Kisaalita *et al.*, 1987a) and is typical of the pH in dairy processing wastewater balancing tanks (Comeau *et al.*, 1996; Jovcic, 1998). The COD of the 0.45 μm filtered fraction is the 'true' soluble COD of the incoming wastewater and the % acidification is

based on this value. The 24 and 18 hour HRT trials, which were also at a lower temperature, produced about 80% of the VFA's that were produced with a 12 hour HRT and a temperature of 35°C. This is most likely due to the conversion of some VFA to methane gas (methanogenesis) at the longer HRT, and the lower growth rate at 30°C. Acetic acid was produced at a higher concentration then other VFA's, with propionic acid and n-butyric acid the next most common VFA. Based on the milkpowder specifications the carbohydrate total COD amounts to approximately 705 mg/L. Yu and Fang (2001), demonstrated that carbohydrate was preferentially converted to VFA. Assuming that all the carbohydrate is converted to VFA, then it accounts for between 57 and 75% of the VFA COD, which suggests some other milk components, such as protein, are also converted to VFA.

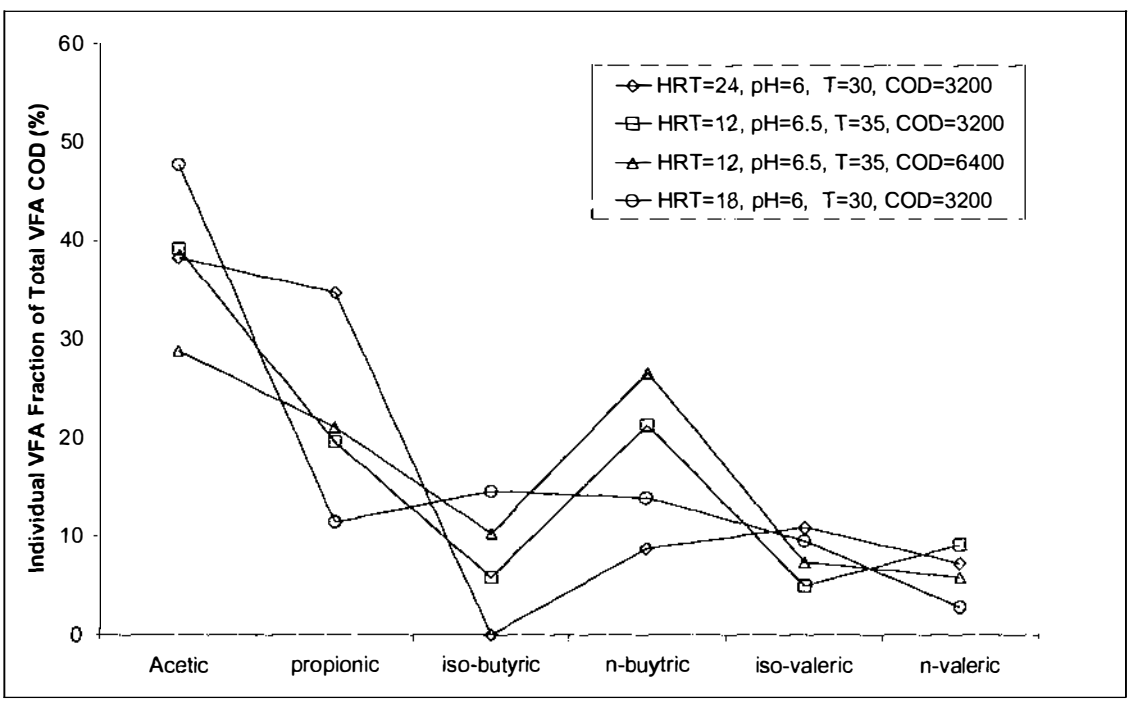


Figure 4.5: The percentage of each VFA as a fraction of the total VFA COD.

Table 4.8: Operating parameters and analytical data from the four different fermenter operations.

Parameter	Value			
HRT (hours)	24	12	12	18
PH	6.0	6.5	6.5	6.0
Temperature (^{o}C)	30	35	35	30
Total COD of wastewater	3200	3200	6400	3200
GFC filtered (1.2 μm) COD (mg/l) of wastewater	2150	2150	4300	2150
0.45 μm filtered COD of wastewater	1950	1950	3900	1950
0.45 μm filtered COD from fermenter	1130	1300	2650	1010
VFA COD produced (mg/L)	935	1200	2470	995
% Acidification based on total influent COD	29	38	39	31
% Acidification based on 0.45 μm filtered CODin	48	62	62	51
% Acidification based on GF/C filtered CODin	43	56	57	46
Fermenter TSS (mg/L)	730	710	625	710
Acetic acid (mg COD/L)	358	470	712	475
Propionic acid (mg COD/L)	325	235	521	114
Iso-Butyric acid (mg COD/L)	0	70	254	145
n- Butyric acid (mg COD/L)	82	255	656	138
Iso-Valeric acid (mg COD/L)	102	60	182	95
n-Valeric acid (mg COD/L)	68	110	145	28

An HRT of 12 hours, a pH of 6.5 and a temperature of 35°C were used as the fermenter operating parameters in subsequent EBPR studies (Chapters 6, 7, and 8). A pH of 6.5 would insure that a near neutral wastewater was pumped to the activated sludge reactor, avoiding potential pH complications in the EBPR system. An HRT of 12 hours and a temperature of 35°C appeared to optimise VFA production based on literature data and this fermentation study. At an HRT of 12 hours, the 10 litre fermenter would generate 20 litres of fermented wastewater per day, which would easily meet the requirements for EBPR activated sludge studies.

4.5 Discussion

The fraction of RBCOD in the wastewater (20%) is similar to that for domestic wastewaters, however the magnitude of RBCOD is much greater (~640 mg COD/L). The rest of the soluble COD according to ASM1 and ASM2 (Henze *et al.*, 1986 and 1995) belongs to the slowly biodegradable (X_S) fraction. ASM2 describes S_F as the readily biodegradable substrate that is composed of small molecules that can be metabolised directly, or quickly fermented/hydrolysed before being metabolised. These compounds can be soluble proteins, carbohydrates and other easily degradable compounds. The RBCOD component of this synthetic wastewater appears to be primarily carbohydrates (i.e. sugars) as the carbohydrate content COD of 22% is very close to the RBCOD fraction determined. The RBCOD does not necessarily describe the only substrate available for successful EBPR functioning, by way of PHA formation under anaerobic conditions. It may be that some of the X_S COD fraction can also be converted to VFA within the anaerobic zone of an EBPR process.

The two anoxic yield coefficients found in this study are comparable to the aerobic yields and are greater than the theoretical anoxic value proposed by Orhon *et al.* (1994). The anoxic yields were measured using VFA as the substrate compared to the aerobic yield value that was calculated from the wastewater soluble substrate added to the batch reactor. Dircks *et al.* (1999), showed that the same sludge sources had different Y_H values with various substrates. The value for Y_H determined under anoxic conditions using VFA should be a reasonable estimation of that for the RBCOD fraction of the dairy processing wastewater as the values of K_I for both VFA's and the RBCOD component of the wastewater had very similar values.

Fermentation trials demonstrated that similar acidification rates (60%) to other published studies could be achieved for this wastewater and up to 1200 mg COD/L generated form the synthetic wastewater with a COD of 3200 mg/L. Doubling the total COD of the influent wastewater resulted in a similar acidification rate. Comeau *et al.* (1996), successfully removed about 45 to 50 mg/L of phosphorus from a cheese processing wastewater with a influent VFA concentration of 1200 mg COD/L. For future EBPR trials, fermentation operating parameters of an HRT =12 hours, pH=6.5 and a temperature of 35 ^{o}C were selected.

4.6 Conclusions

The readily biodegradable fraction (RBCOD) of the synthetic wastewater (COD/TKN=28) used in this characterisation study, was 20% (18 to 22%) or 640 mg/L. It appeared that the RBCOD consisted mostly of carbohydrates (sugars) as the carbohydrates made up about 22% of the total COD of this wastewater.

Fermentation of this wastewater generated a maximum VFA concentration of 1200 mg COD/L at an HRT of 12 hours, a controlled temperature of 35^{O}C and a controlled pH of 6.5. The total VFA generation was greater than the RBCOD fraction, suggesting that compounds other than sugars were converted to VFA. The acidification rate of 60% at these operating conditions was comparable to other dairy wastewater fermentation studies.

CHAPTER 5
Preliminary EBPR Reactor Studies

5.1 Introduction

Most design and research information available for the EBPR process is directly related to wastewater of comparable organic strength and phosphorus content to domestic wastewater. Some studies have achieved biological phosphorus removal from dairy processing wastewater using laboratory scale SBR systems (Comeau *et al.*, 1996) or full scale SBR's (Goronszy, 1990; Kolarski and Nyhuis, 1995), however little detailed analytical information was available. The approach used in this study was to study biological phosphorus removal from dairy processing wastewater using a laboratory scale continuous flow BNR activated sludge system. The rational for the use of a continuous BNR system was that the biological treatment systems used in the New Zealand dairy industry are generally continuous flow.

In Chapter 4 it was shown that about 20% of the total COD (640 mg/L) is present as readily biodegradable COD. The first reactor system in this chapter used a long anaerobic HRT in an attempt to achieve in situ fermentation. Previous continuous reactor laboratory scale studies, using similar synthetic dairy processing wastewater, have shown that nitrate and nitrite were present in the recycle activated sludge stream (Donkin and Russell, 1997; Leonard, 1996) and that an anoxic zone for denitrification would be required. The first configuration tested in this preliminary study used an AAO configuration with an extended anaerobic HRT and the second system was configured as a MUCT system. Neither of these systems used external fermentation.

5.2 AAO Configuration

Typical anaerobic zone hydraulic retention times range from 1 to 3 hours (Henze *et al.*, 2002). As this dairy processing effluent has a relatively high concentration of soluble COD, and a significant RBCOD component, it was decided to start with an actual anaerobic zone HRT of 7 hours. The anaerobic zone was 22.2% of the total reactor volume and the anaerobic retention time was 22.2% of the total reaction time, similar to the 25% used by Comeau *et al.* (1996), for their SBR operation. An AAO

configuration (Figure 5.1) was used, as it is one of the least complicated. The reactor had a total volume of 18 litres and an overall retention time of 3.6 days. The actual retention times of the different zones (including recycle streams) are shown in Table 5.1. The return sludge recycle ratio was kept constant at 1.74 times the influent flow (1.74Q) and the anoxic recycle was operated at 2Q. The system was operated for a total of 50 days, at an SRT of 15 days. The reactor was initially seeded with sludge from a full scale 5 stage Bardenpho treating domestic wastewater (Rotorua District Council).

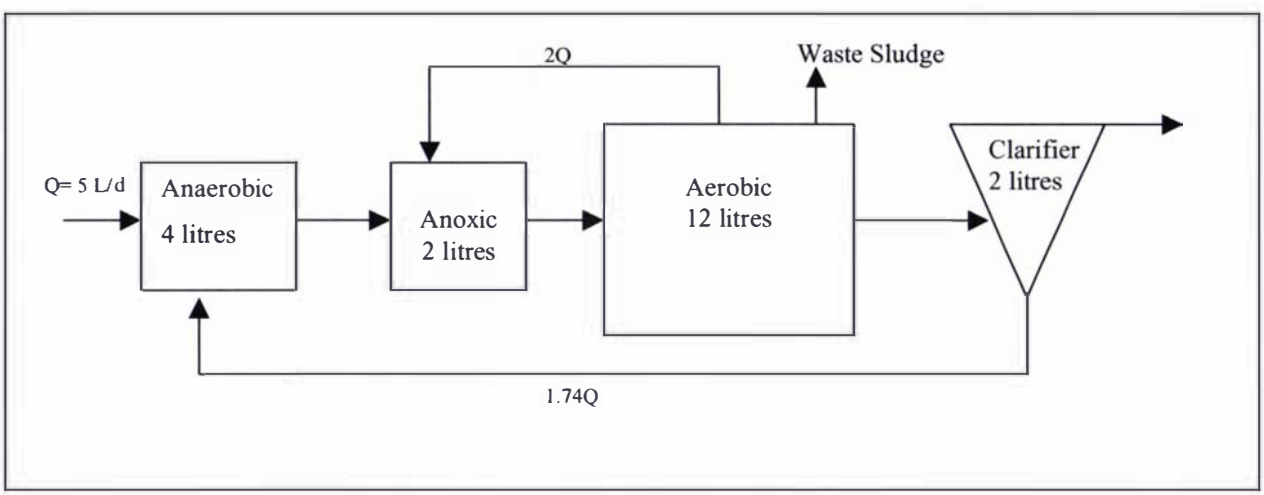


Figure 5.1: AAO activated sludge preliminary lab-scale system.

The organic loading rate of the system averaged 0.39 kg COD/kg VSS/d, similar to that used by Comeau *et al.* (1996), of 0.37 to 0.49 kg COD/VSS.d. The volumetric loading of 0.92 kg COD/m^3.d was lower that that used by Comeau *et al.* (1996), of 1.47 kg COD/m^3.d.

Table 5.1: Retention times in respective zones of laboratory AAO system

	Anaerobic zone	Anoxic zone	Aerobic zone
Volume (litres)	4	2	12
Actual HRT (hours)	7	2	12.2
Nominal HRT (hours)	19.2	9.6	57.6

5.2.1 Reactor Operation

The TSS concentration was measured in each zone during the period of operation and is shown in Figure 5.2. Initially the suspended solids concentrations in each zone were similar, although the TSS concentration in the anaerobic zone was greater than the other zones when measured on days 14 and 19 due to blockages in the anaerobic zone inlet. Most of the variation in solids concentrations between zones is explained by the operational difficulties in maintaining a steady operation due to tube blockages. The TSS concentration generally ranged between 2000 and 3000 mg/L in each zone. The VSS/TSS ratio provides an indication of the amount of non-volatile solids present in the biomass and decreases with an increase in cellular polyphosphate content. For an EBPR process there should be a noticeable decrease in the VSS/TSS ratio from the anaerobic zone (due to P-release) to the aerobic zone (due to P-uptake). The VSS/TSS ratio's for the anaerobic zone during the period reactor of operation (Figure 5.3) were nearly always greater than the aerobic zone. During the final 10 days of operation, the difference between that anaerobic and aerobic VSS/TSS ratio's decreased. This suggests that the phosphorus removal also decreased in the final ten days of operation.

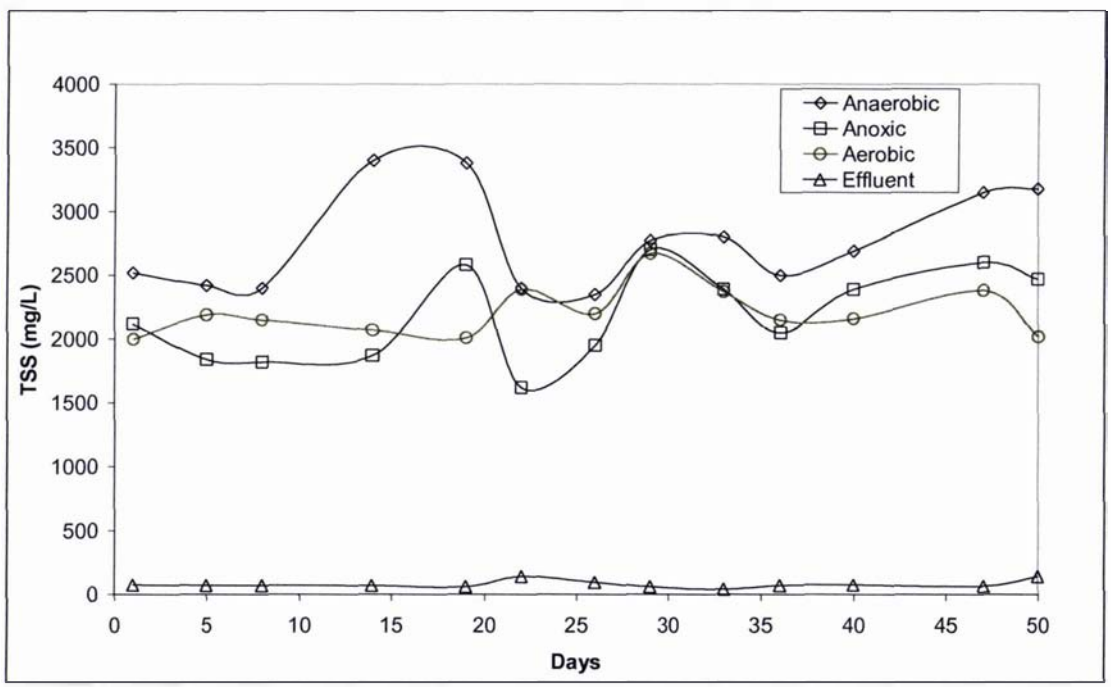


Figure 5.2: TSS concentration in each zone.

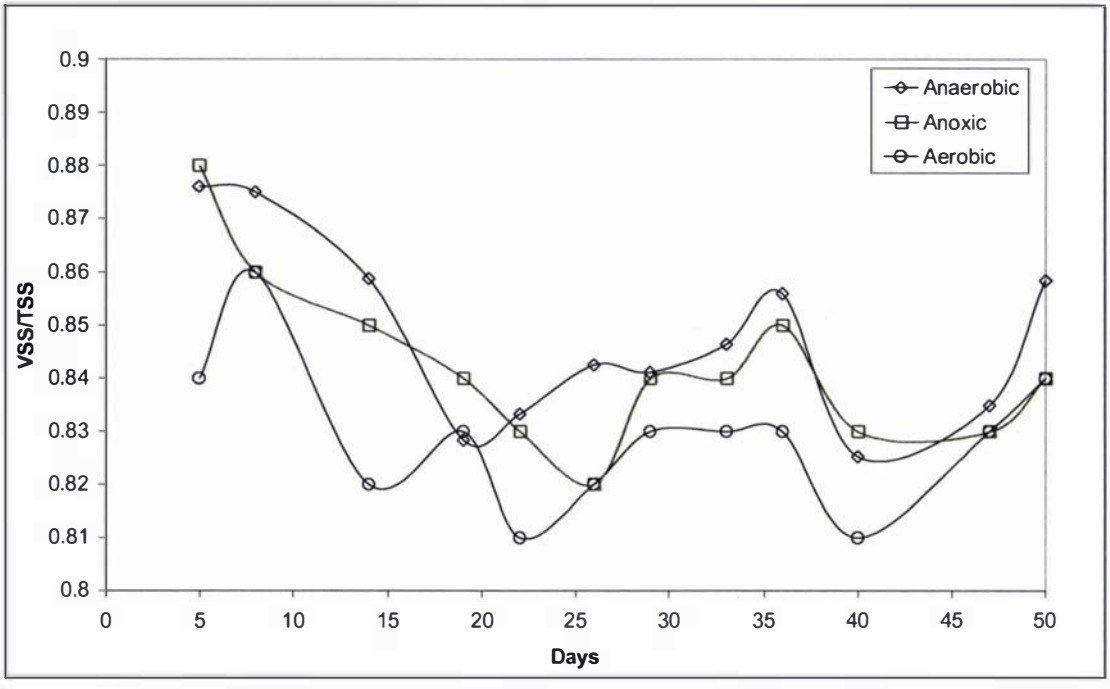


Figure 5.3: Zone VSS/TSS ratio's during AAO system operation.

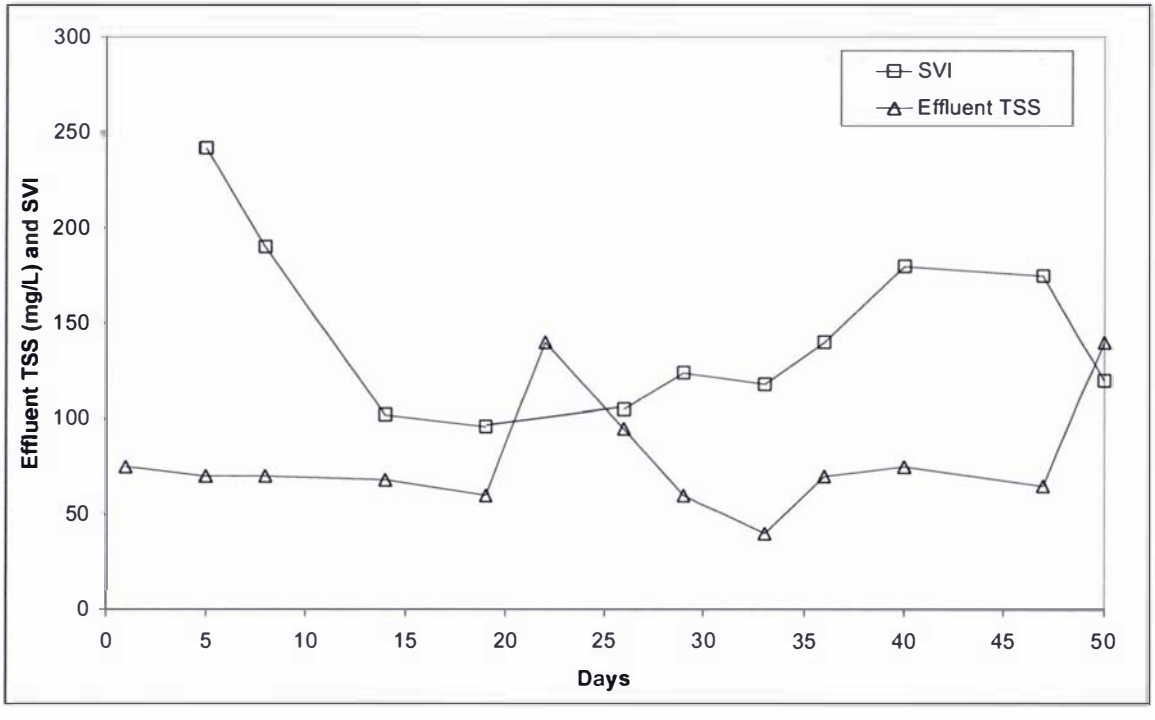


Figure 5.4: Variation in the SVI and the effluent TSS during AAO system operation.

The stability of activated sludge reactors is largely dependent on the settling properties of the biomass in the clarifier measured as the sludge volume index (SVI). After seeding, the SVI decreased steadily to an SVI of 102 after 14 days and remained between 100 and 120 until day 40 when it increased to 180 (Figure 5.4). The effluent TSS averaged 79 mg/L and peaked at 140 mg/L on two occasions. The SVI values averaged 145 which is comparable to the lower range of values obtained by Leonard (1996) in a study on the effects of selectors on bulking when treating dairy processing wastewater. The SVI values in this study were much lower than the values obtained by Donkin and Russell (1997), with a laboratory AAO system where the SVI values were always greater than 150 and occasionally as high as 600. The effluent TSS concentrations were higher than expected from the SVI values obtained, suggesting that an improvement in the clarifier design or operation is required.

The removal of COD was quantified by measuring the soluble COD in each zone. The soluble COD profiles shown in Figure 5.5 show that there was a progressive decrease in COD through the three zones. Dilution due to recycle streams will account for some of this decrease. COD uptake in the anaerobic zone is usually due to either denitrification or PHA storage by polyphosphate accumulating organisms (PAO's) or glycogen accumulating organisms (GAO's). In this trial, an average of 790 mg/L of the total influent COD and 430 mg/L of the soluble COD was removed in the anaerobic zone. These calculations assume that for the total COD removed, all the particulate COD would be converted to soluble COD within the anaerobic zone, and for the soluble COD removed no particulate COD is converted to soluble COD. In reality, the average amount of COD consumed in the anaerobic zone would be between 790 mg/L and 430 mg/L COD as some particulate COD would be hydrolysed. Of the soluble COD entering the anoxic zone from the previous anaerobic zone an average of 68 mg/L was consumed, most likely due to denitrification. Overall 99% of the influent COD was removed through the reactor (based on effluent soluble COD).

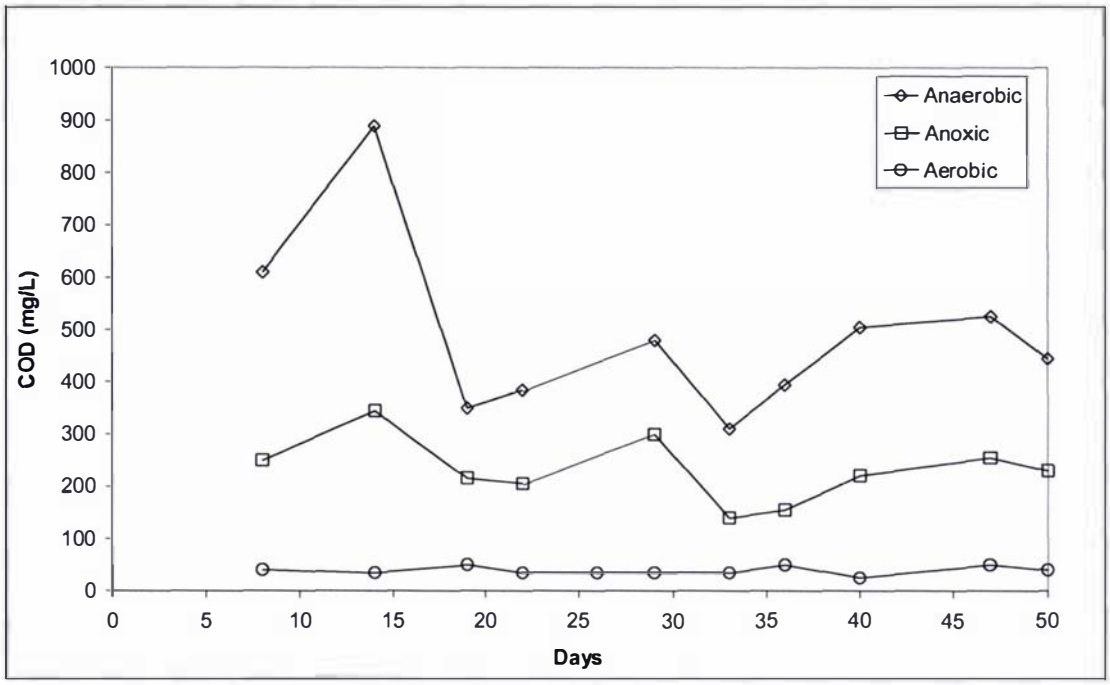


Figure 5.5: Soluble COD concentrations in each zone.

5.2.2 Nitrogen Removal

The principle importance of nitrogen removal to success of the EBPR process is to minimise the amount of nitrate or nitrite that enters the anaerobic zone in subsequent recycle streams. After day 15, samples were periodically analysed for nitrate and ammonia (Figures 5.6 and 5.7). Almost complete nitrification occurred in the system with an average ammonia concentration in the aerobic zone of just 0.5 mg N/L compared to 10.6 mg N/L in the anaerobic zone. Aerobic and effluent nitrate concentrations averaged 8.2 and 7.4 mg N/L respectively. This effluent nitrate concentration, while moderate, was not enough to account for the average 430 mg/L of soluble COD consumed in the anaerobic zone. Almost complete denitrification was achieved in the anoxic zone were the nitrate concentration averaged 0.3 mg N/L, which suggests that the 2Q anoxic recycle rate was close to optimum.

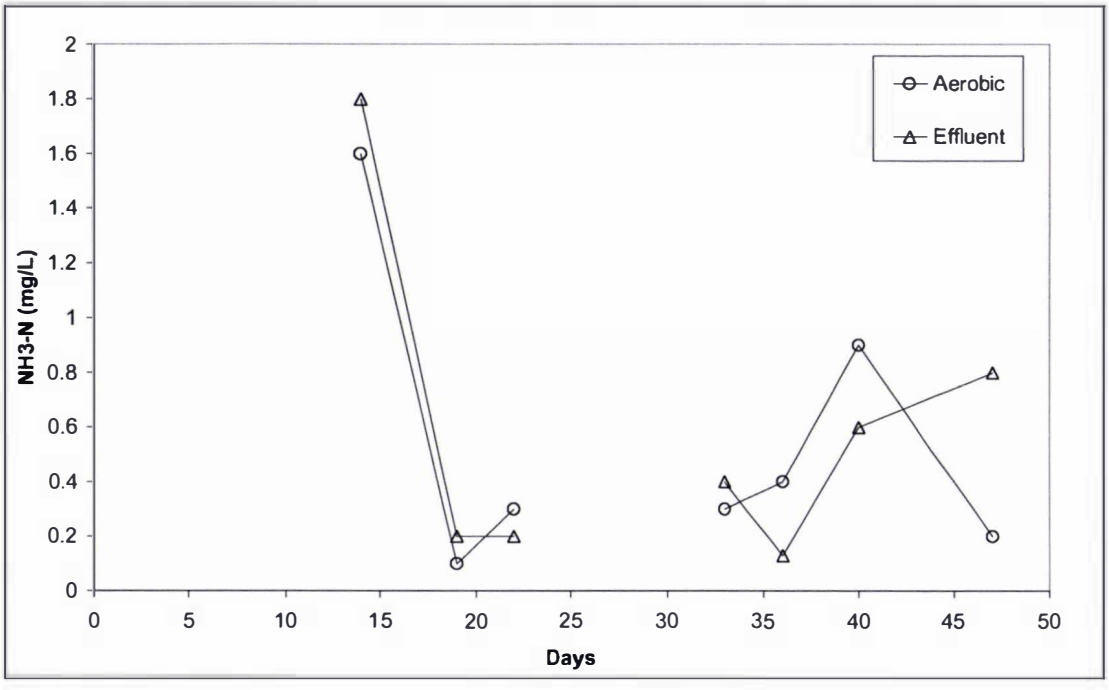


Figure 5.6: Ammonia concentrations in the aerobic zone and effluent.

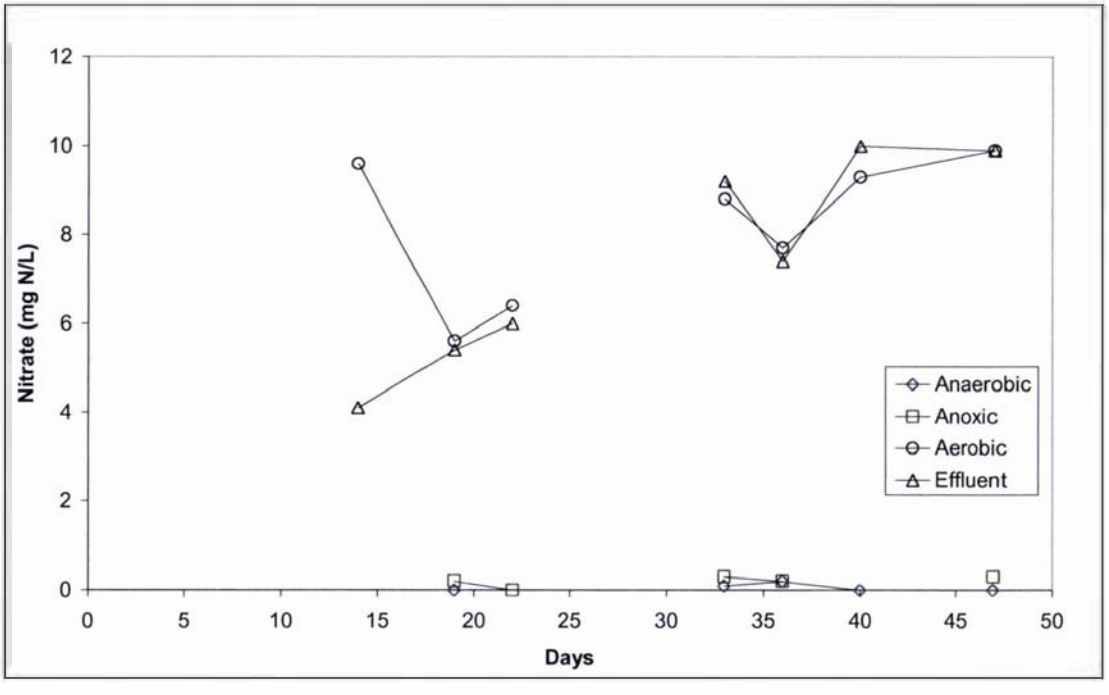


Figure 5.7: Nitrate concentrations in each zone.

5.2.3 Phosphorus Removal

The amount of phosphorus removed was calculated from measurements of the soluble phosphate concentration (PO_4^{-3}) and the phosphorus content of the activated sludge. These values are shown in Figure 5.8. The amount of phosphorus released in the anaerobic and anoxic zones is shown in Figure 5.9, with the P release values in the anaerobic zone calculated based on both the influent total and soluble phosphorus concentrations. An average of 20 mg/L of phosphorus was removed after day 5. The aerobic phosphorus concentration steadily increased over two SRT's (30 days). The decrease in phosphorus removal is reflected in Figure 5.9, where the amount of phosphorus released in the anaerobic zone decreased to 5 or less mg/L (based on influent total phosphorus). In the anoxic zone, there was mostly phosphorus uptake, as expected in the presence of an electron acceptor. The phosphorus content of the sludge averaged 3.0 % mg P/mg VSS after 8 days of operation. The majority of the phosphorus removed was due to that required for microbial growth, with very little removed by other mechanisms. This compares to values of about 7 % mg P/mg VSS for systems with significant EBPR (Comeau *et al.*, 1996) and 3.7% with a starch fed BNR system that had exhibited minimal EBPR (Randall *et al.*, 1994).

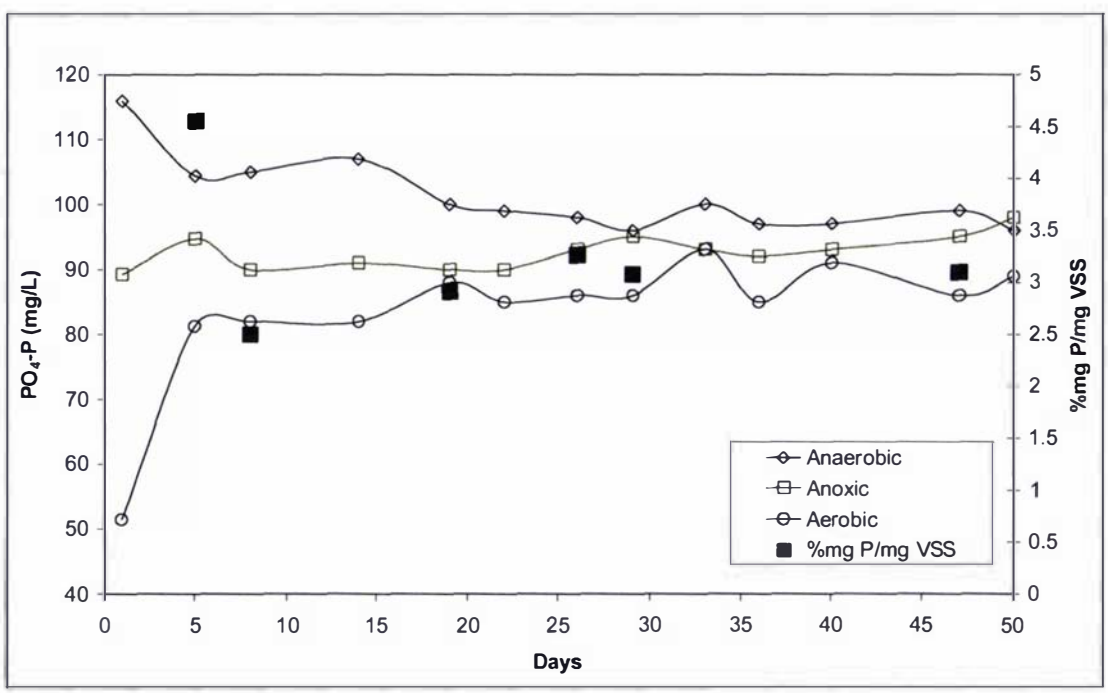


Figure 5.8: Soluble phosphorus (PO_4-P) concentration in each zone.

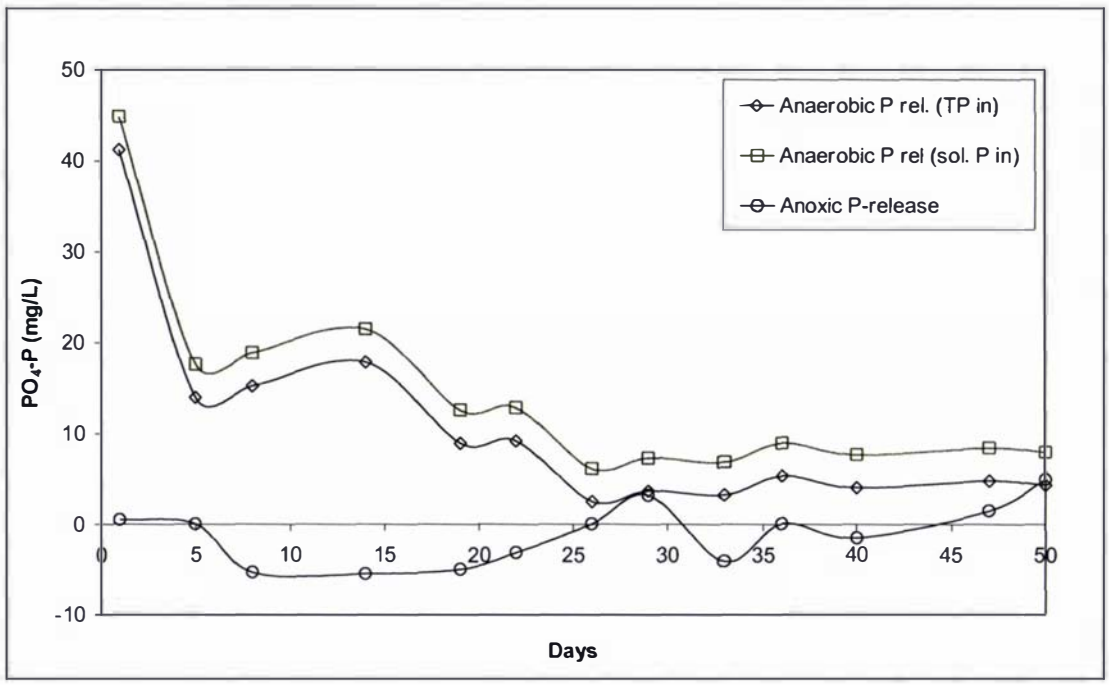


Figure 5.9: P release in anaerobic and anoxic zones based on both total influent phosphorus and the influent soluble phosphorus concentrations.

5.2.4 Discussion

For this preliminary AAO system trial, the intention was to determine if the system was robust and reliable, and also to assess whether biological phosphorus could occur without a pre-fermentation stage but instead with an extended anaerobic HRT. Reliable reactor operation at times was difficult with sludge accumulation occurring in both the anaerobic and anoxic zones due to blockages of the zone outlets. Consistent pump flowrates were also difficult to obtain due to the low influent flowrate used (5 L/d). Although an internal stirrer was used in the clarifier, sludge accumulation still occurred periodically, which meant an improved method of clarifier operation was required. The dissolved oxygen (DO) concentration in the aerated zone was controlled at 3 mg/L O_2 that resulted in measurable DO levels (generally <2 mg/L) in the clarifier, possibly resulting in some oxygen intrusion in to the anaerobic zone via the RAS line. Overall, the system used in this trial, required modification and the addition of more complex control.

The decrease of anaerobic phosphorus release with time shows that the sludge

composition changed with time, with the initial PAO population in the seed sludge decreasing with time. The amount of anaerobic phosphorus release on day 50 at the end of the AAO system operation was calculated at only 4 mg P/L. The anaerobic phosphorus released to soluble COD consumed after 50 days was only 0.01mg P/mg COD much less than the value of 0.22 mg P/mg COD used in a model for the SBR treatment of dairy processing wastewater by Ky *et al.* (2001). This low ratio and the continued consumption of anaerobic soluble COD suggests organisms other than PAO's competing for soluble COD under anaerobic conditions had increased (possibly GAO's) over the 50 days of reactor operation (Oehman *et al.*, 2000). Besides the operational difficulties, influences on the changing microbial population may have been the long overall HRT (3.6 days), the long anaerobic retention time (7 hours) and the presence of sugars in the wastewater (Satoh *et al.*, 1994).

A high degree of overall COD removal and almost complete nitrification were achieved. To minimise nitrate intrusion into the anaerobic zone via the RAS line the denitrification capacity needs to be increased although a high amount of denitrification was still achieved. The phosphorus content of the sludge (3%) was not much more than that removed by assimilation through growth (2%). To accurately assess the capacity for biological phosphorus removal on a laboratory scale without interruptions to steady operation an improved system was required. The average analytical parameters for each zone are shown in Table 5.2.

During the later part of this study, a new modified system was constructed with individual aeration and mixing and with minimal pumping requirements as the flow between zones was via slots in the zone separators. The problem of sludge retention in the clarifier was minimised by incorporating a period of rapid mixing in the clarifier for 4 seconds every 20 minutes in order to break up sludge clumps so that the sludge flowed through the clarifier outlet rather than bridging the outlet. It was intended to control the DO in the final aerobic zone to 0.5 mg/L in order to prevent oxygen intrusion into the anaerobic zone via the RAS recycle. This would also minimise oxygen intrusion into the anoxic zone via the internal recycle line.

Table 5.2: Average analytical parameters for each zone during operation of AAO lab-scale configuration.

	Zone			
Parameter	Anaerobic	Anoxic	Aerated	Effluent
TSS (mg/L)	2765	2185	2210	80
$P_{soluble}$ (mg/L)	102	93	84	83
P removed (mg/L)				20
P released (mg/L)	10	-1		
% mg P/mg VSS			3.0	
pH	6.8	7.2	8.1	
COD (mg/L)	490	230	40	145*
Nitrate (mg N/L)	0.2	0.3	8.2	7.4
Ammonia (mg N/L)	10.6	7.3	0.5	0.6
SVI			145	

*Effluent COD value is expressed as total COD

5.3 MUCT Configuration

The preliminary reactor system was modified to a MUCT configuration by the addition of an extra zone and recycle stream. This configuration prevented the intrusion of both nitrate and oxygen into the anaerobic zone with only a small modification, although it did not solve the issues related to operational reliability. The MUCT configuration had a total reaction volume of 16 litres, an influent flow rate of 5 litres/day, and an HRT of 3.2 days (Figure 5.10). The system was operated with a sludge recycle (RAS) rate of 5 L/d (Q), an anoxic recycle of 2Q and an anaerobic recycle of 1Q. The retention times in each zone are shown in Table 5.3. The actual anaerobic HRT was decreased from the 7 hours in the previous AAO system to 4.8 hours in this MUCT configuration. This shorter anaerobic HRT was closer to the typical domestic wastewater values of up to 3 hours.

Table 5.3: Hydraulic retention times in respective zones of laboratory MUCT system.

	Anaerobic zone	1st Anoxic zone	2nd Anoxic zone	Aerobic zone
Volume (litres)	2	2	2	10
Actual HRT (hours)	4.8	3.2	2.4	12
Nominal HRT (hours)	9.6	9.6	9.6	48

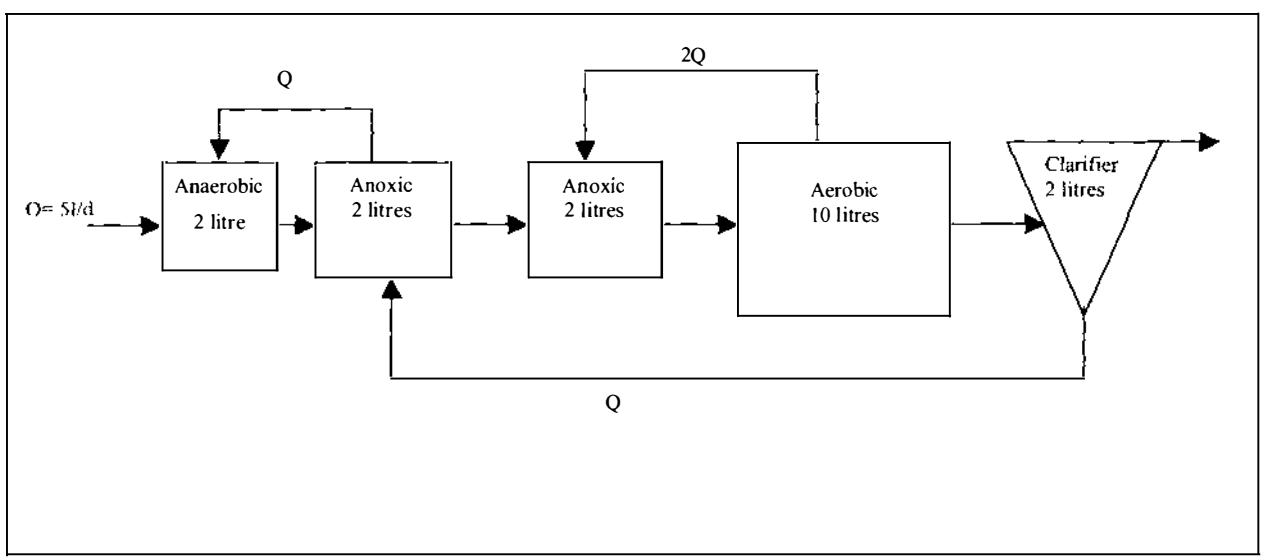


Figure 5.10: Modified UCT activated sludge lab-scale system for treatment of synthetic dairy processing wastewater.

5.3.1 Reactor Operation

This system was operated for a period of 38 days at an SRT of 15 days. The period of operation was less than 3 SRT's duration as the reactor operation was ceased due to a lack of significant EBPR and the completion of the improved zoned reactor system. With this system there was some difficulty maintaining a similar TSS concentration in the anaerobic zone compared to the rest of the reactor (Figure 5.11). The TSS concentration in the anaerobic zone averaged 1525 mg/L compared to over 2000 mg/L in the other zones during the 38 days of operation. A higher anaerobic recycle rate may have increased the TSS concentration in the anaerobic zone. The average

value of the SVI (256) was greater then with the AAO configuration, but did progressively decrease during the period of operation to a value of 178 after 38 days.

The COD profile in each zone is shown in Figure 5.12. The soluble COD in the anaerobic zone averaged 890 mg/L, which was higher than for the previous AAO system. The soluble COD consumed within the anaerobic zone averaged 495 mg/L (Figure 5.13). Leonard (1996) found that from 50 to 84% of the available soluble COD for dairy processing wastewater was consumed in unaerated selectors with HRT's of 33 to 131 minutes. In this trial only 38% of the soluble COD was consumed. The reason for this difference may be due to the solubilisation of influent particulate COD because of the long HRT of the anaerobic zone in this study (4.8 hours) compared to a maximum unaerated selector HRT of 2.2 hours employed by Leonard (1996).

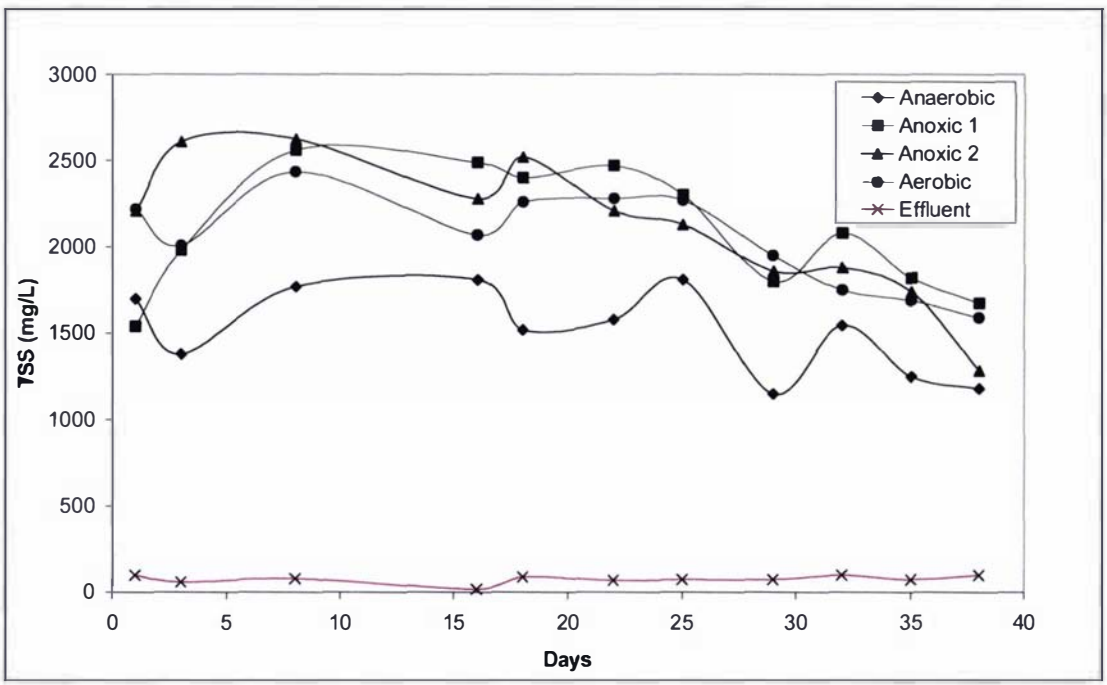


Figure 5.11: TSS concentration in each zone for the MUCT system.

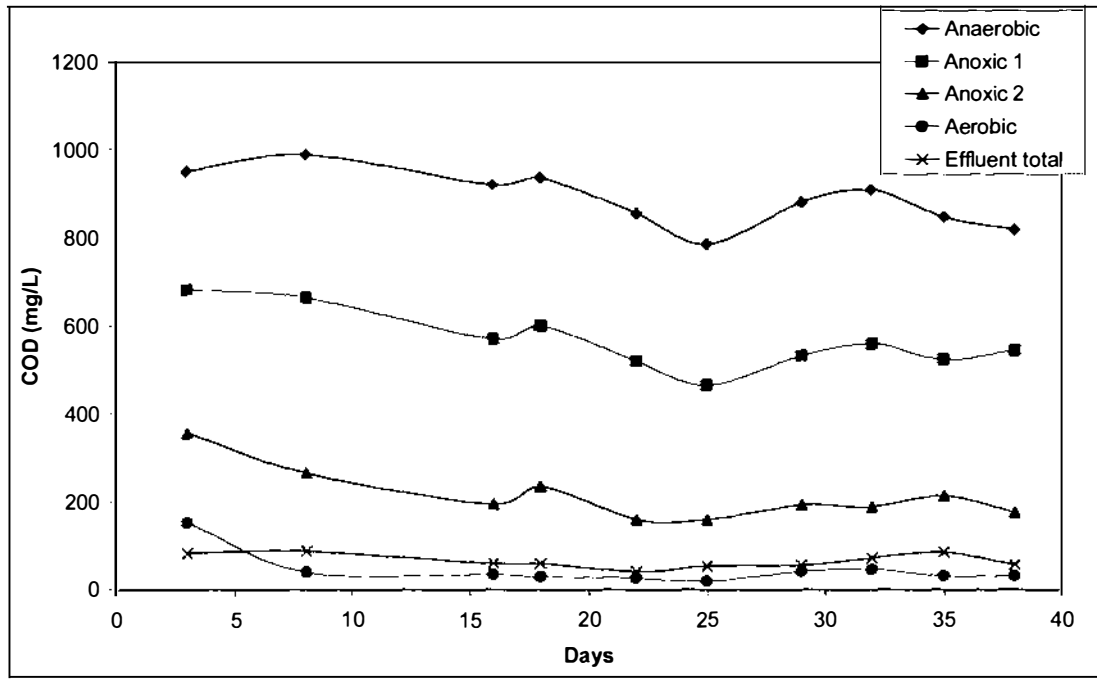


Figure 5.12: COD concentration in each zone for the MUCT system.

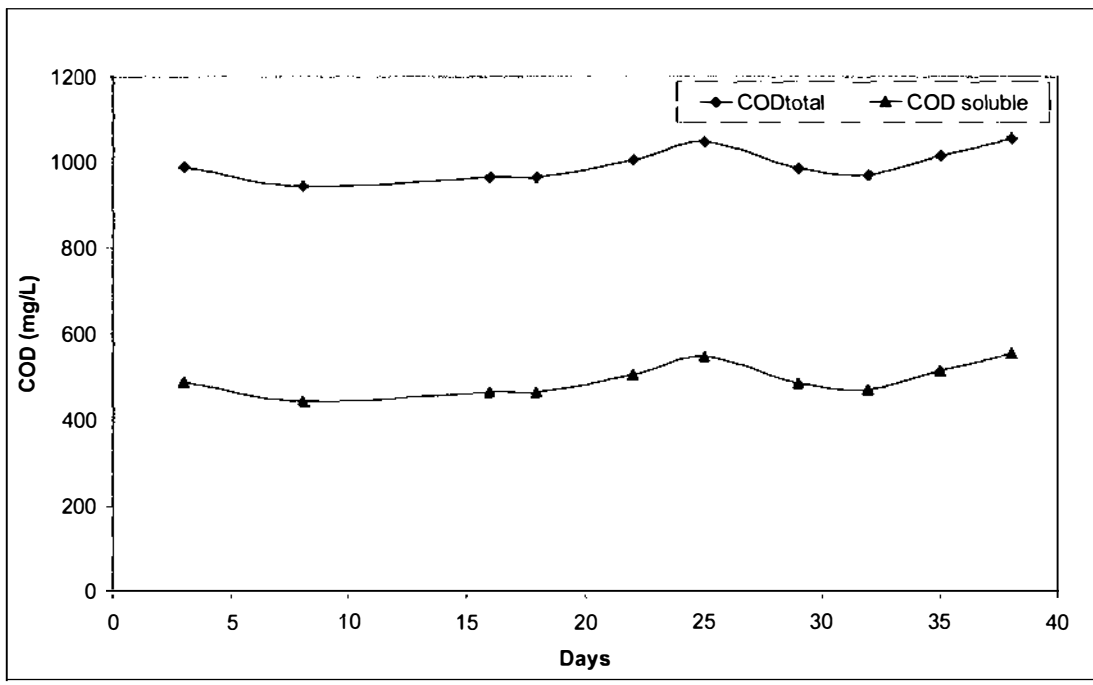


Figure 5.13: Anaerobic zone total and soluble COD consumption.

The ammonia concentration was measured in each zone (Figure 5.14). The ammonia was almost completely nitrified. This is reflected in the NO_3-N concentrations in the aerobic zone and effluent of 1.3 to 9.0 mg N/L (Figure 5.15). The nitrate concentration in the anaerobic zone was undetectable. After day 3, the nitrate concentration in anoxic zones 1 and 2 was also undetectable. This showed that this system could potentially provide conditions that are suitable for EBPR in a continuous activated sludge system due to the prevention of nitrate intrusion into the anaerobic zone. This system could be further optimised for nitrogen removal by changing internal recycle rates and anoxic volumes, but this particular system still had the operational difficulties of tube blockages and non-consistent flows between zones of the AAO system. Increasing the anoxic recycle from 2Q to 4Q would have most likely decreased the effluent and aerobic nitrate concentrations, however this was not required due to the fact that no nitrate entered the anaerobic zone.

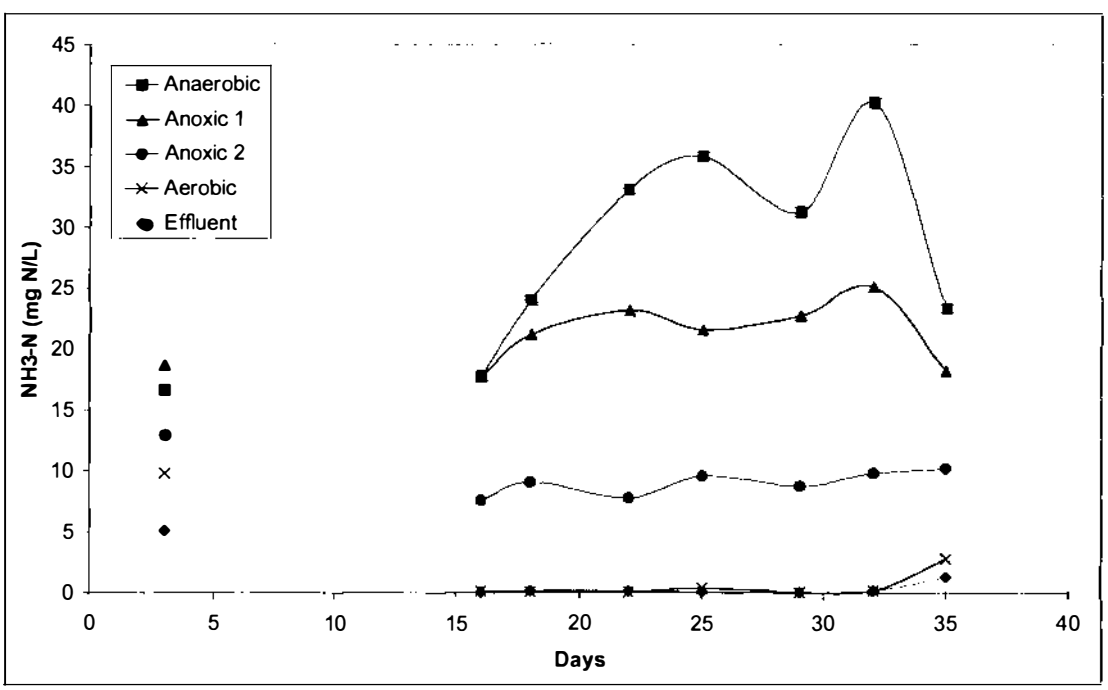


Figure 5.14: Ammonia concentration in each zone for the MUCT system.

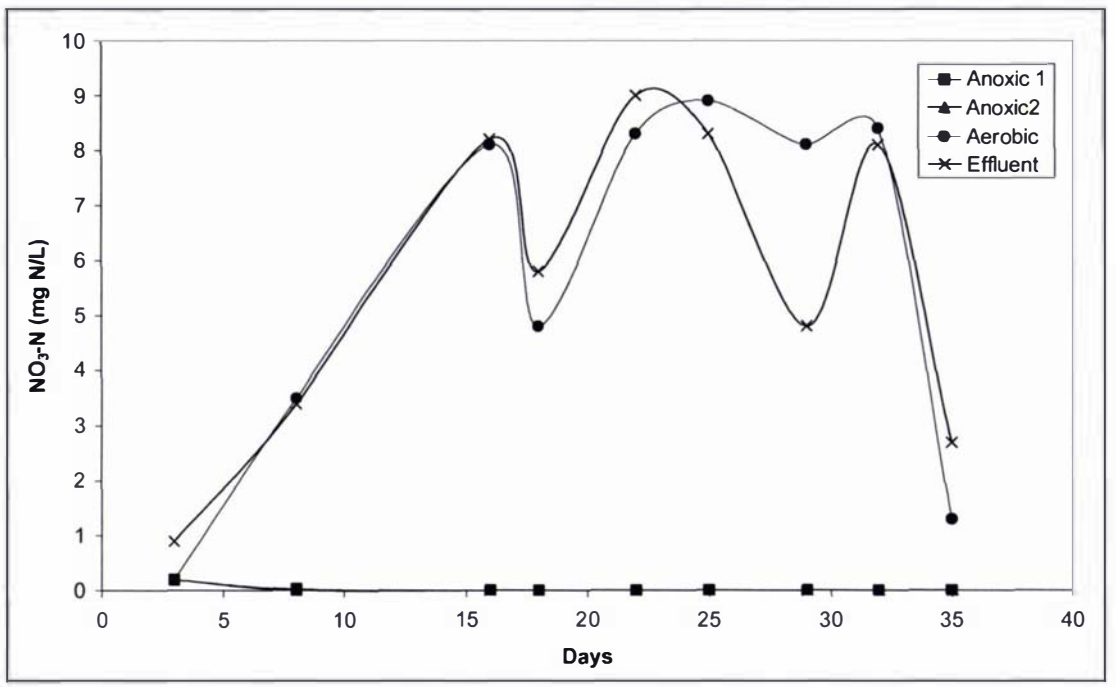


Figure 5.15: Nitrate concentrations in each zone for the MUCT system.

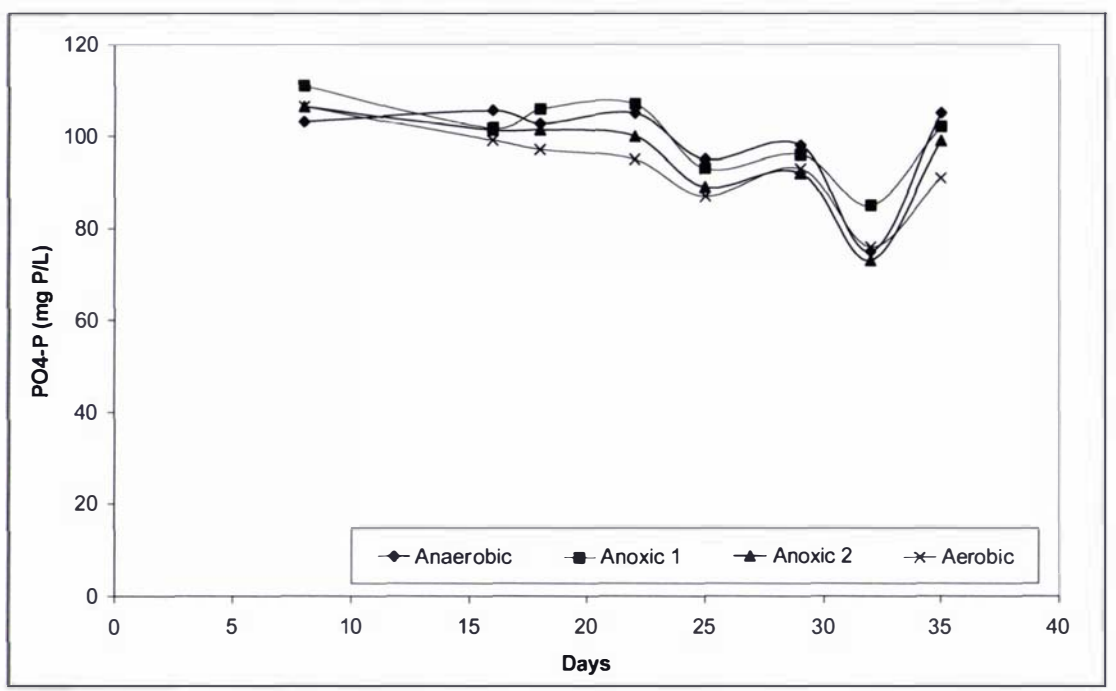


Figure 5.16: Orthophosphate (PO_4-P) concentrations in each zone for the MUCT system.

Phosphorus removal within the reactor was not significant, as evidenced by the lack of noticeable phosphorus release and uptake in the anaerobic and aerobic zones respectively (Figure 5.16). The decrease in PO_4-P on day 32 was due to a decrease in the phosphorus feed concentration, which was tracked to an error in the composition of the wastewater during mixing of the synthetic wastewater. It is evident that although anaerobic conditions were maintained by the prevention of nitrate intrusion into this zone that phosphorus release did not improve. While the sludge in this MUCT configuration originated from the preliminary AAO configuration, there was no noticeable improvement in the amount of phosphorus removed. The amount of phosphorus removed averaged only 15 mg P/L. The phosphorus content of the aerated sludge averaged only 2.5 %mg P/mg VSS.

5.3.2 Discussion

The average analytical parameters are shown in Table 5.4. The average value of the settling volume index (SVI) is greater than with the previous AAO configuration. With the MUCT configuration there was difficulty in maintaining a similar TSS concentration in the anaerobic zone compared to rest of the reactor. The amount of phosphorus removed (15 mg P/L) was also less then with the AAO (20 mg P/L). The phosphorus content of the sludge in the aerobic stage also decreased to 2.5 % mg P/mg VSS.

Table 5.4: Average analytical parameters for each zone during operation of MUCT lab-scale configuration.

	Section				
Parameter	Anaerobic	1st Anoxic	2nd Anoxic	Aerated	Effluent
TSS (mg/L)	1525	2195	2090	2040	70
DRP (mg P/L)	98	99	94	91	89
pH	6.7	7.0	7.6	8.0	8.1
COD (mg/L)	875	540	190	35	60*
Nitrate (mg N/L)	<0.1	<0.1	<0.1	6.8	6.7
Ammonia (mg N/L)	29.4	21.4	9.0	0.6	0.3
SVI				250	

*Effluent COD value is expressed as total COD

To confirm whether precipitated phosphorus was contributing to phosphorus removal, the separate phosphorus components of a sludge sample were determined using the method given by Aspegren (1995). In this test the total phosphorus content of the biomass is determined, the polyphosphate content determined from the phosphorus release in an anaerobic batch test with excess acetate (500 mg COD/L) and the precipitated phosphorus determined from extraction in cold acid (HCl). The organic phosphorus content of the cell can then be calculated by subtracting the polyphosphate and the precipitated phosphorus concentrations from the total phosphorus concentration. The phosphate release curve after 250 minutes of anaerobic conditions is shown in Figure 5.17, where negligible P-release occurred. The results of this simple fractionation are shown in Table 5.5. The polyphosphate content was negligible as minimal phosphorus was released under anaerobic conditions. Although the pH in the MUCT acrobic zone was 8.1, precipitated phosphorus was minimal in the system.

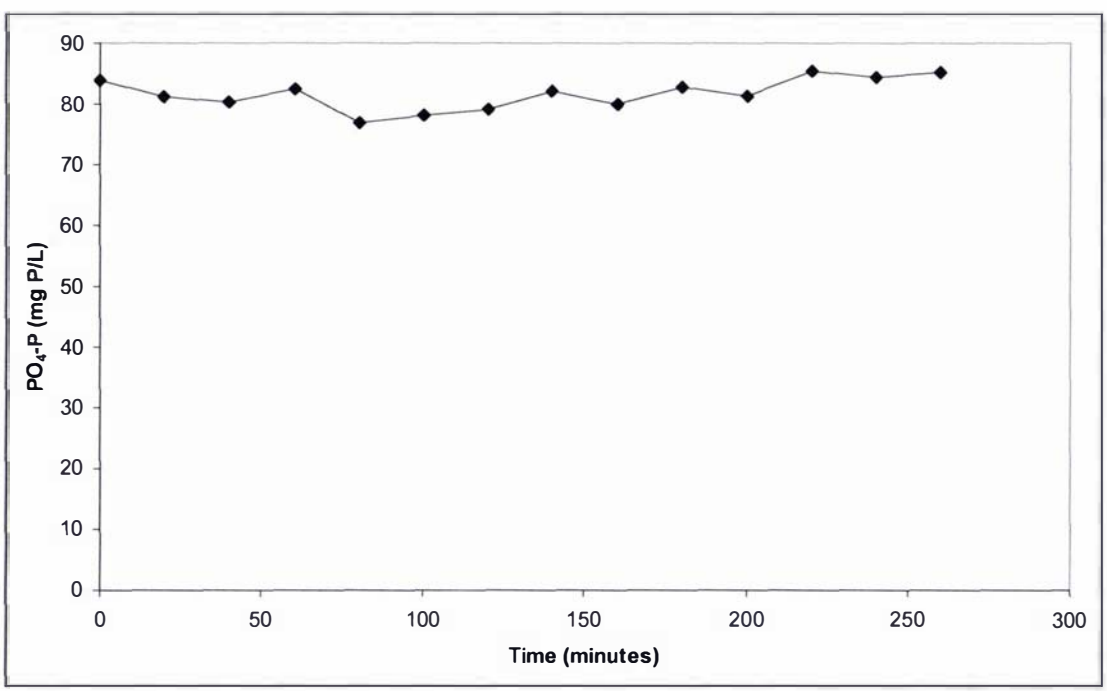


Figure 5.17: Phosphorus concentration in anaerobic batch test.

Table 5.5: Phosphorus fractionation of aerobic sludge sample (Day 35).

Phosphorus fraction	% P of sludge (wt/wt)	Fraction % of total P content
Total P of aerobic sludge	2.20% mg P/mg VSS	
Precipitated P	0.18% mg P/mg VSS	8.1% of total solids P content
Poly P content	Negligible	Negligible
Organic P	2.02% mg P/mg VSS	91.9 % of total solids P content

5.4 Conclusions

This preliminary study used unfermented wastewater and extended anaerobic retention times. Both the AAO and MUCT configurations removed 20 and 15 mg P/L respectively, much less than the 45 to 50 mg P/L achieved by Comeau *et al.* (1996) in their study of the SBR treatment of a fermented cheese processing wastewater. Phosphorus removal with the AAO and MUCT reactors was an improvement on the continuous reactor studies by Donkin and Russell (1997) of 3 to 5 mg P/L, and that achieved by Leonard (1996) with unaerated selectors of 6.5 to 10.8 mg P/L. A small amount of biological phosphorus removal possibly occurred in the AAO system, as the sludge phosphorus content was 3.0 mg P/mg VSS. After initially seeding the AAO reactor with active EBPR sludge there was a progressive decrease in anaerobic phosphorus release during the 50 days of operation. Continuing anaerobic COD consumption along with decreasing phosphorus removal indicated that the PAO population was decreasing and that the competing organism population was increasing.

Modifying the AAO system to an MUCT configuration, without reseeding the sludge, did not improve the amount of phosphorus removed over a period of 38 days. At the end of the study, the sludge phosphorus content was 2.2 % mg P/mg VSS, with 0.18 % mg P/mg VSS due to chemical phosphorus precipitation.

Operational difficulties due to tube blockages and the low pumping rates, were considered in designing a zoned system that was subsequently used in Chapters 6, 7 and 8.

CHAPTER 6
Combined Nitrogen and Phosphorus Removal -AAO Zoned Reactor Studies

6.1 Introduction

The reactor systems used in Chapter 5 did not exhibit significant EBPR thought to be due to the wastewater characteristics, operational instability, the long anaerobic zone HRT and the long overall HRT, or a combination of these. In this chapter an improved reactor system was used that allowed greater system stability through the incorporation of multiple zones. The mixed liquor flow between the zones was controlled by the height of the clarifier weir and therefore did not rely on pumps and tubing. Pumps were only used for the influent, recycle activated sludge (RAS) and anoxic recycle streams (Section 3.34). Each zone had separate aeration and mixing if required, which permitted any of the ten zones to be either unaerated or aerated. The possibility that the wastewater characteristics were a factor in the previous systems lack of EBPR, was addressed by including a separate fermentation step to convert soluble COD to VFA (Figure 6.1). Detailed information on the EBPR and fermentation reactor specifications is provided in Chapter 3.

6.2 Reactor System

The synthetic wastewater composition used is listed in Table 3.3. The characteristics of this wastewater (COD/TKN = 27.8) were the same as those shown in Table 4.2 except that the total phosphorus concentration was decreased slightly to 98 mg P/L. The RAS recycle rate was 1Q and the anoxic recycle rate was 2Q. The synthetic wastewater (stored at 4° C) was firstly fermented in a 10 litre reactor at 35°C with an HRT of 12 hours and a set pH of 6.5. The fermented wastewater was collected and stored in a refrigerator at 4°C and then pumped at a rate of 8 litres/day to the zoned EBPR reactor which was operated at a temperature of 20°C, and an HRT of 2.5 days. One zone was anaerobic, one zone anoxic and 8 zones aerobic, resulting in an unaerated fraction of 20% (Table 6.2). The actual retention time in the anaerobic zone was 3 hours, which is a typical maximum for systems treating domestic wastewater (Cloete and Muyima, 1997). The reactor was operated at an SRT of 10 days.

A new batch of wastewater was made daily, the storage containers cleaned to prevent wastewater deterioration during storage, and all pump tubing was cleaned weekly. The schematic of the overall process is shown in Figure 6.1. Photo's of the zoned reactor and fermenter are shown in Figures 6.2 and 6.3 respectively.

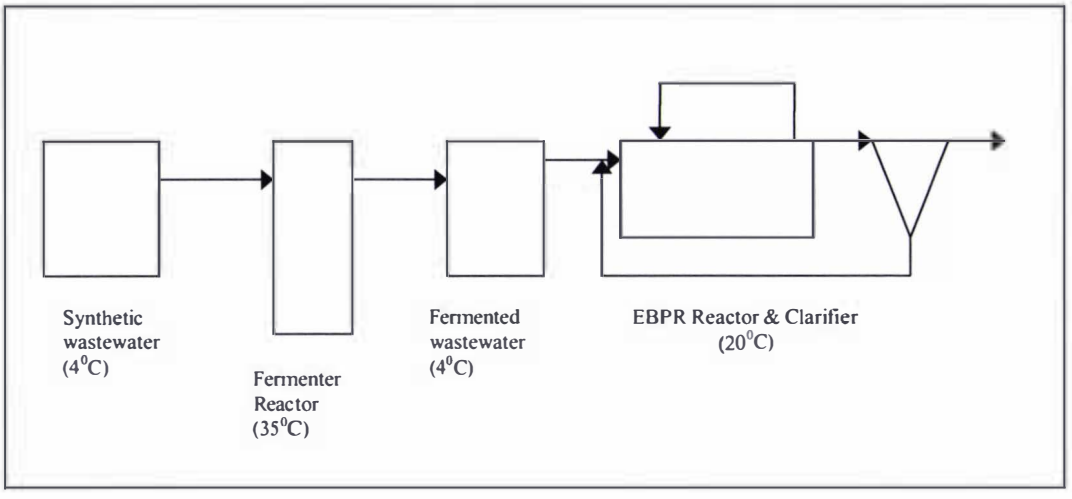


Figure 6.1: Schematic of laboratory treatment system.

Table 6.1: Reactor system operational parameters.

	Influent rate(L/d)	Temperature (^{0}C)	RAS rate (L/d)	Anoxic Recycle (L/d)	pH
Fermenter	10	35	-	-	6.5
EBPR Reactor	8	20	8	16	-

Table 6.2: Volume, number of individual zones and the HRT in the anaerobic, anoxic and aerobic steps along with the overall HRT.

	Anaerobic	Anoxic	Aerobic	Overall
Volume (litres)	2	2	16	20
Number of zones	1	1	8	10
Actual HRT (hours)	3	1.5	12	16.5
Nominal HRT (hours)	6	6	48	60

The system was operated for a total of 158 days. On day 95, improved dissolved oxygen control was installed in the reactor. The improvement included, finer valve control on the aeration input into the final aerated zone to prevent oxygen intrusion into anaerobic zone via the RAS recycle steam. The DO in the final aerated zone was maintained at 0.5 mg/L, while the first aerobic zone DO was controlled at 1.5 mg O_2 /L. The other aerobic zones did not have DO control. A complete analytical study of each zone was performed on day 158 after it was felt that this particular reactor configuration was optimised as much as possible.

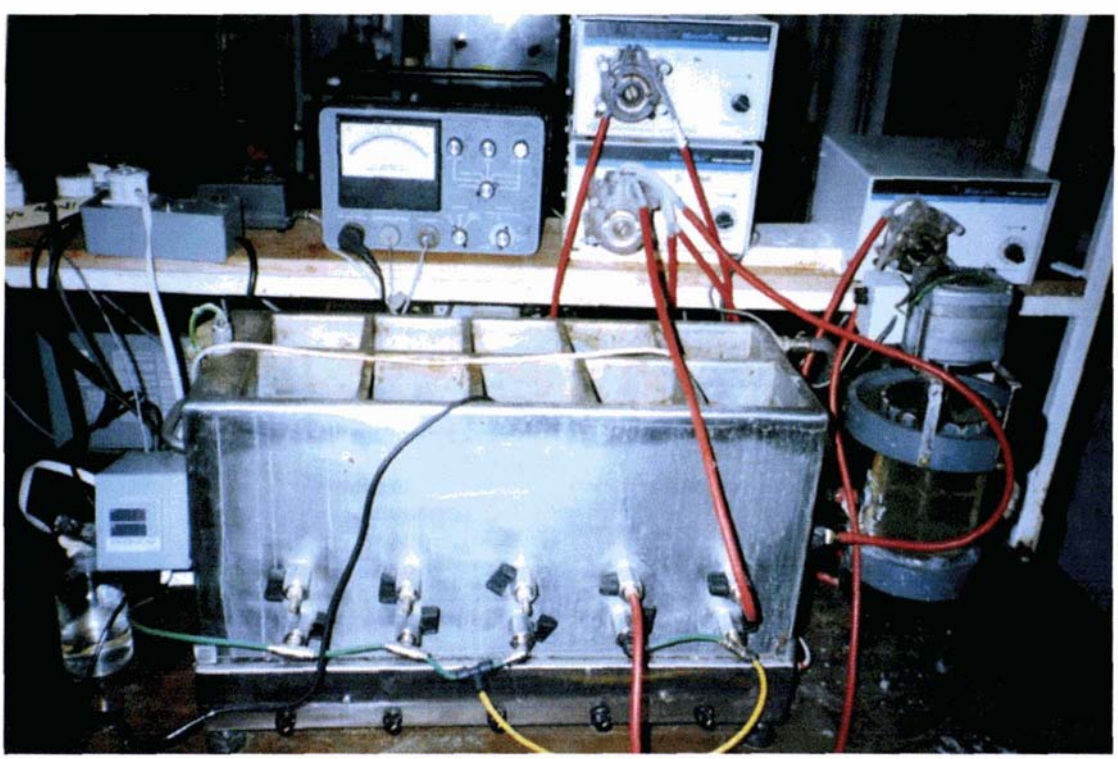


Figure 6.2: Zoned laboratory-scale activated sludge EBPR AAO reactor system.

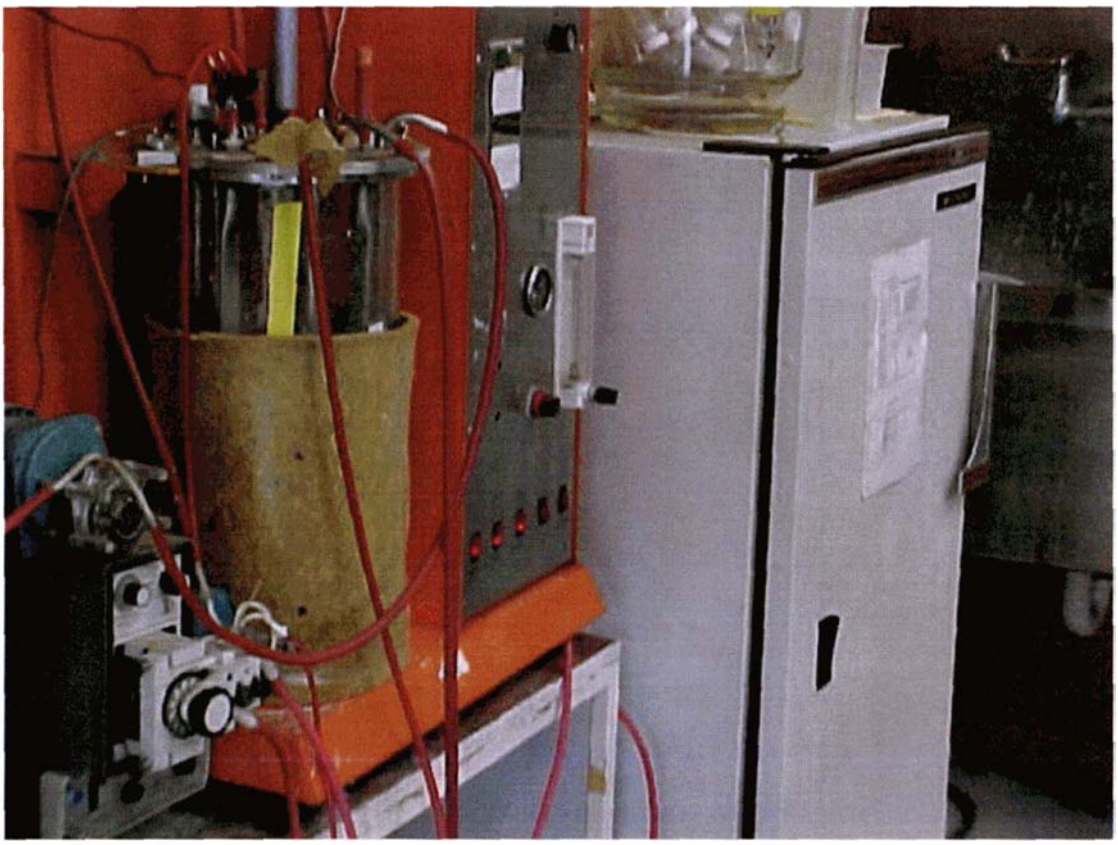


Figure 6.3: Influent fermentation system

6.3 Fermenter Operation

During the fermenter operation, the suspended solids concentration of the effluent from the fermenter varied due to the settling of solids in the storage containers. After 80 days of operation, the fermenter effluent total COD concentration was stabilised by withdrawing settled solids daily from the fermenter effluent collection container. The soluble COD of the effluent remained relatively constant during the reactor operation and is shown in Figure 6.4. The individual and total VFA COD's are shown in Figure 6.5, with the average values summarised in Table 6.3. Acetic acid was the principal VFA generated, averaging 40.2% of the average total VFA COD of 1155 mg/L. The composition of the fermenter effluent soluble COD (<0.45μm) was on average 85.7% VFA COD. The percent acidification of the synthetic wastewater averaged 51% based on the synthetic wastewater GFC filtered (<1.2μm) soluble COD (Kasapgil et al, 1995). This is slightly less than the value of 56% (based on GF/C influent soluble COD) in the short-term fermentation study with the same operating conditions in Section 4.4.1 (Table 4.6). Fang and Yu (2001), obtained a value of 57.1% for a dairy processing wastewater with a COD of 2000 mg/L, and a reactor HRT of 12 hours.

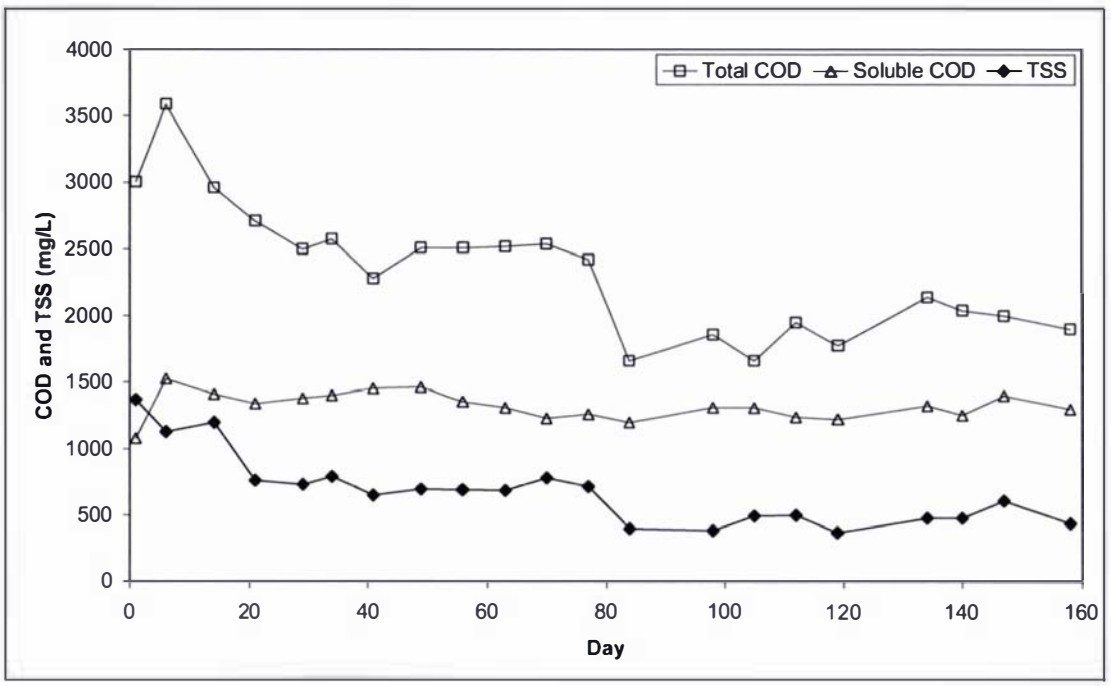


Figure 6.4: TSS and COD profile during reactor operation of fermenter effluent.

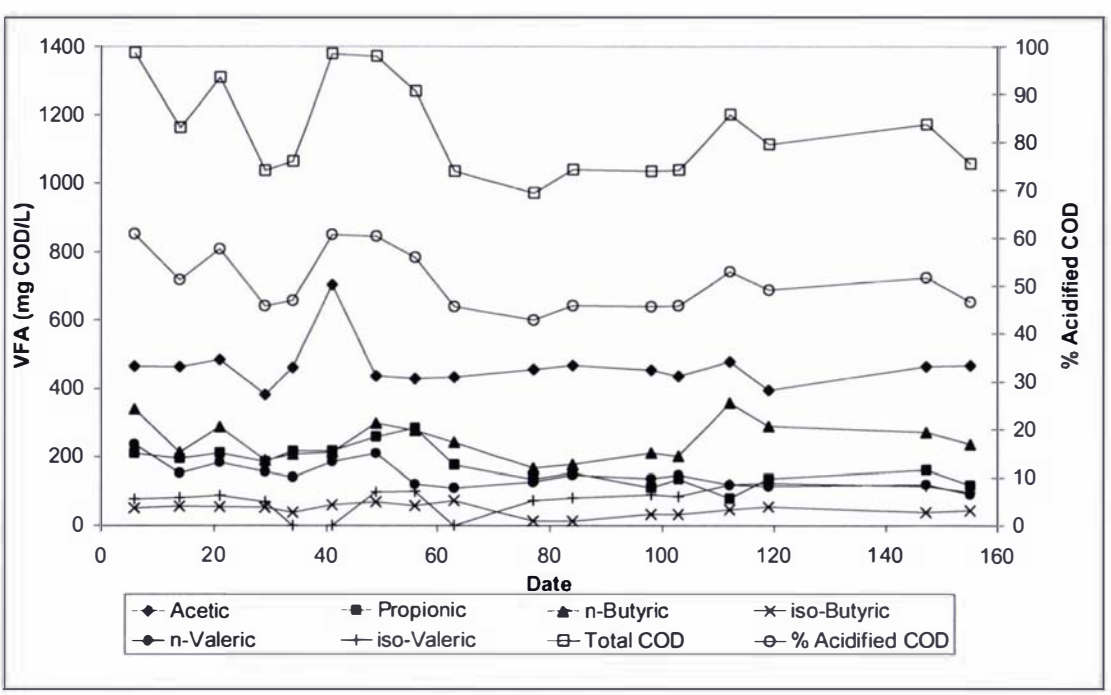


Figure 6.5: VFA COD fractionation, total VFA COD and % acidification of fermenter effluent.

Table 6.3: Fermented wastewater VFA COD concentrations and proportions of total VFA and soluble $COD_{<0.45\mu m}$.

	COD (mg/L)	% Total VFA COD	% Fermenter Effluent COD (<0.45μm)
Acetic acid	464	40.2	34.4
Propionic acid	176	15.2	13.0
n-Butyric	246	21.3	18.3
iso-Butyric	47	4.0	3.5
n-Valeric	147	12.7	10.9
iso-Valeric	76	6.6	5.6
Total VFA COD	1155	-	85.7
Fermeneter Effluent	1345 (<0.45μm)	-	-

6.4 Zoned BNR Reactor Operation

The 158 day operation of the reactor are described in this section. The D.O. control improvement with the installation of the different aeration valve on zone 10, started from day 95. The anaerobic, anoxic and aerobic zones all had similar MLSS concentrations during most of the reactor operation (Figure 6.6). After the addition of improved D.O. control the reactor MLSS decreased from about 3500 mg/L to 2500 mg/L, most likely due to the TSS concentration of the fermented effluent decreasing as previously shown in the TSS and total fermented effluent COD trend in Figure 6.4. The F/M ratio averaged 0.36 g COD/g VSS based on the fermented wastewater total COD and 0.21 g COD/g VSS based on the fermented wastewater soluble COD.

The COD profile for each zone during the reactor operation is shown in Figure 6.7. The soluble COD in the anaerobic zone varied between 320 and 55 mg/L and averaged 175 mg/L. An average of 520 mg/L of soluble COD was consumed in the anaerobic zone. If an amount of soluble COD equal to the soluble final effluent COD is approximated as the inert soluble fraction, then an average of 80% of the biodegradable soluble COD was consumed in the anaerobic zone. The soluble COD consumed in the anoxic zone based on a daily COD mass balance averaged 20 mg/L COD and ranged from –11.5 to 55.5 mg COD/L. The negative value suggests that

soluble COD has been formed during further hydrolysis in this zone. This increase in soluble COD within the anoxic zone/phase was also noticed by Thayalakumaran (2003) during the treatment of meat processing wastewater in a combined nitrogen and phosphorus SBR system and was attributed to the hydrolysis of residual particulate material.

The SVI (Figure 6.8) decreased to 73 after 29 days and then ranged between 130 and 54. There were no settling problems experienced during the reactor operation. The SVI averaged 108 during the total 158 days of reactor operation, but averaged only 78 after day 29. The SVI achieved in this section was much lower than obtained in previous continuous activated laboratory studies with a similar wastewater (Donkin and Russell, 1997; Leonard , 1996).

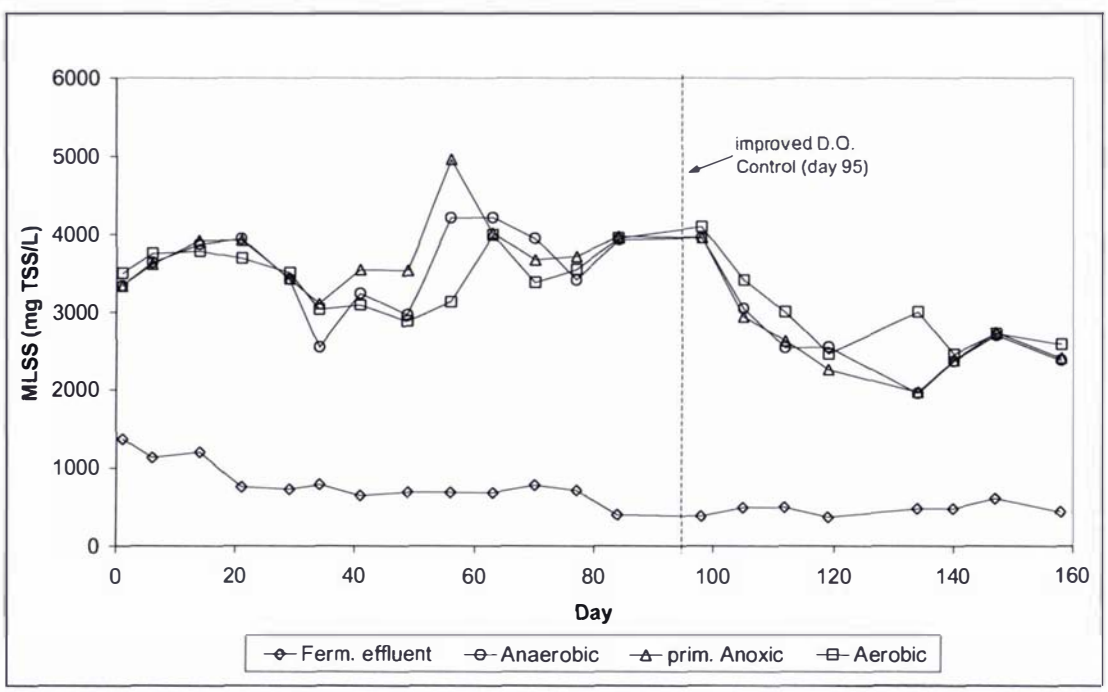


Figure 6.6: TSS profile during zoned AAO reactor operation

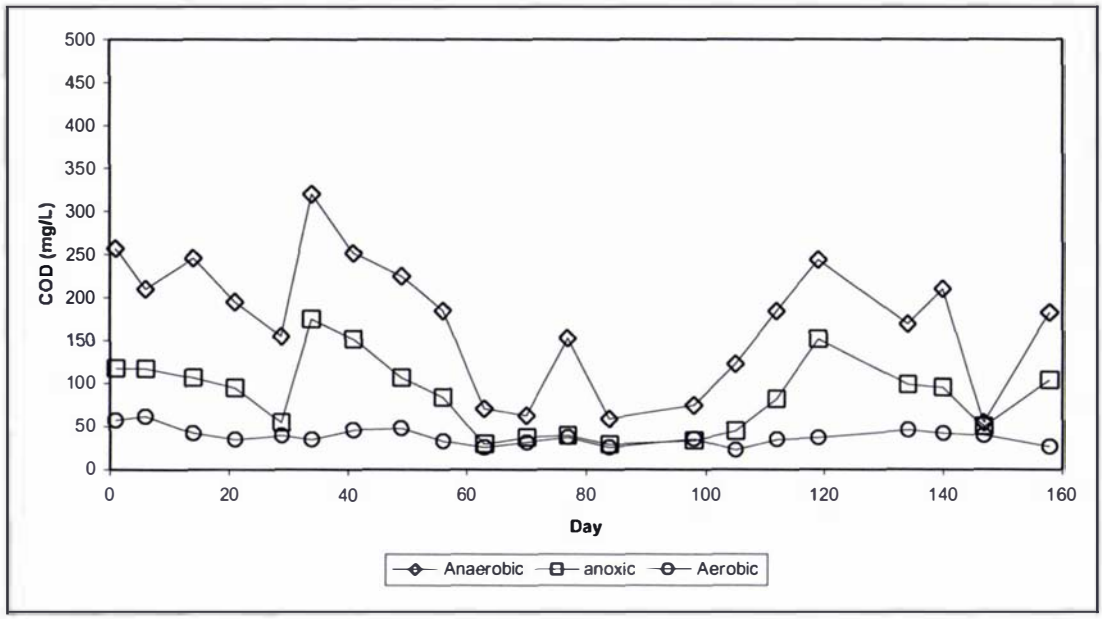


Figure 6.7: COD profile during reactor operation in the anaerobic, anoxic and last aerobic zone.

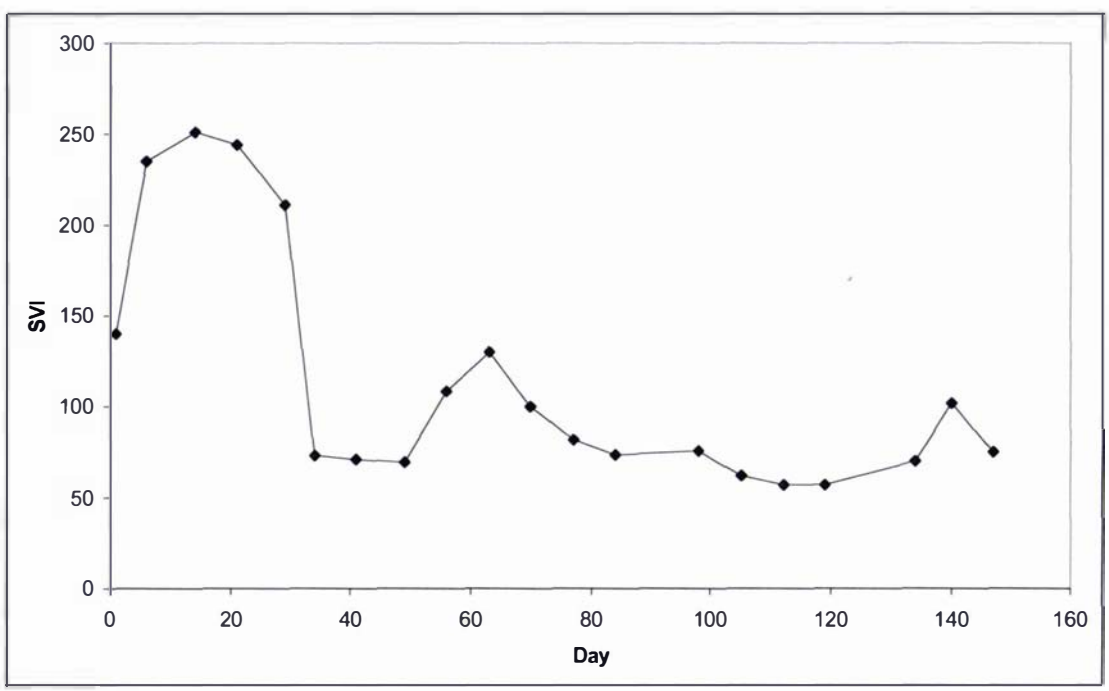


Figure 6.8: SVI variation during reactor operation.

The effluent nitrate concentrations varied between 0 and 19.3 mg NO_3-N/L and averaged 6.5 NO_3-N/L (Figure 6.9), which was similar to effluent concentration in the preliminary AAO study in Chapter 5. The concentration peaked at day 85 and then steadily decreased after the installation of improved D.O. control on day 95. Nitrate concentrations in the anaerobic and anoxic zones were undetectable. The effluent nitrate concentrations did not appear to significantly inhibit the biological phosphorus removal process as shown in the following results on phosphorus removal.

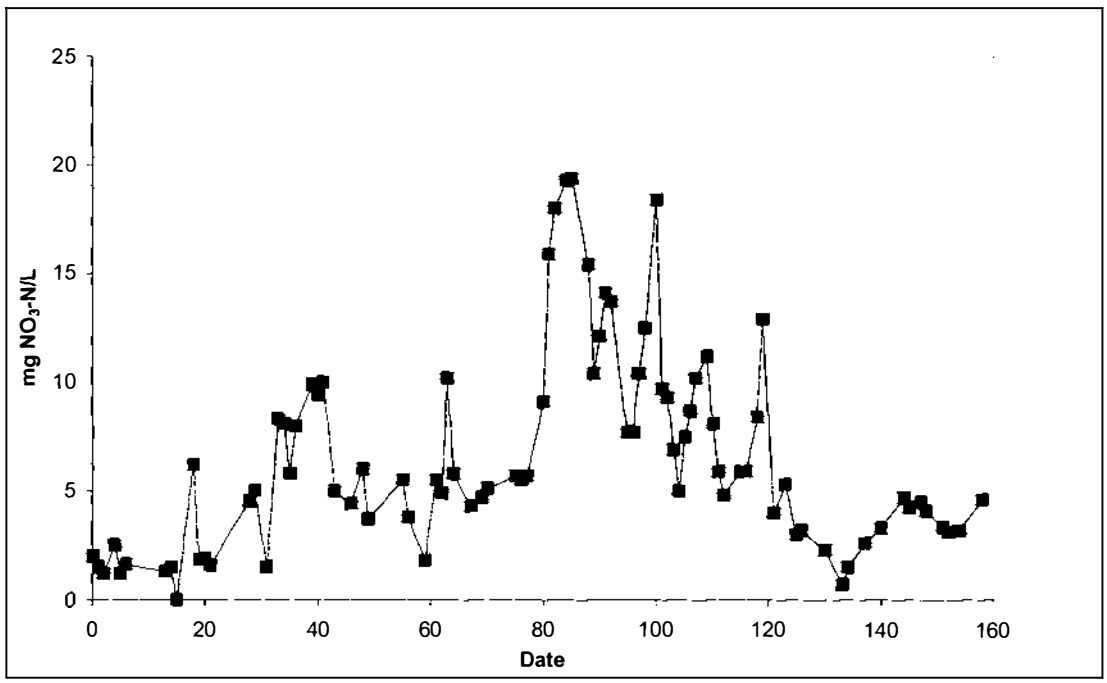


Figure 6.9: Effluent nitrate concentrations during the reactor operation.

The amount of phosphorus removal was initially moderate and then improved after the improved D.O. control system was added on day 95 (Figure 6.10). The high soluble phosphorus concentration in the anoxic zone from days 105 to 119 (Figure 6.10) occurred after the D.O. concentration in zone 10 was stabilised at 0.5 mg/L which resulted in less oxygen being transferred to the anoxic recycle stream, which stimulated phosphorus release (Figure 6.7). After the initial seeding of the reactor the phosphorus content of the aerobic sludge decreased from 7 %mg P/mg VSS to 3 %mg P/mg VSS, and after day 95 the phosphorus content increased markedly (Figure 6.11). After day 56 when increased anaerobic phosphorus release started, an average of 34 mg P/L of phosphorus was removed (based on the fermented effluent) with a maximum of 41.5 mg P/L removed on day 105 (Figure 6.12). After day 95, the

reactor removed an average of 36.3 mg P/L. The ratio of anaerobic phosphorus release to COD consumed averaged 0.04 during the reactor operation.

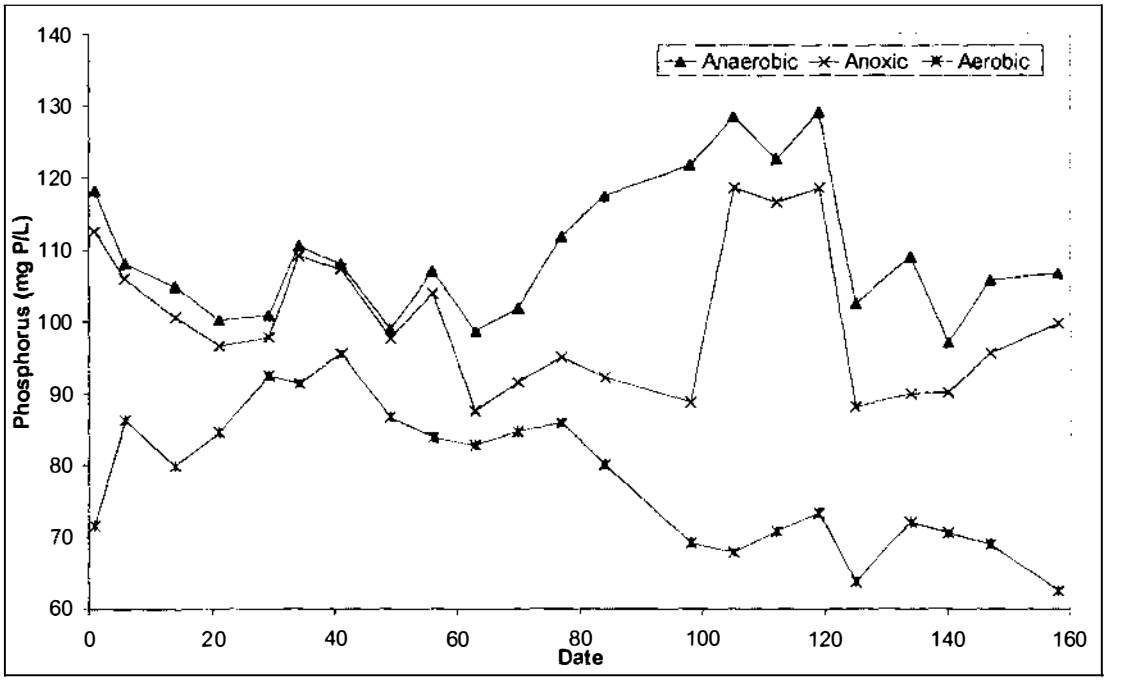


Figure 6.10: Phosphorus concentrations during the reactor operation.

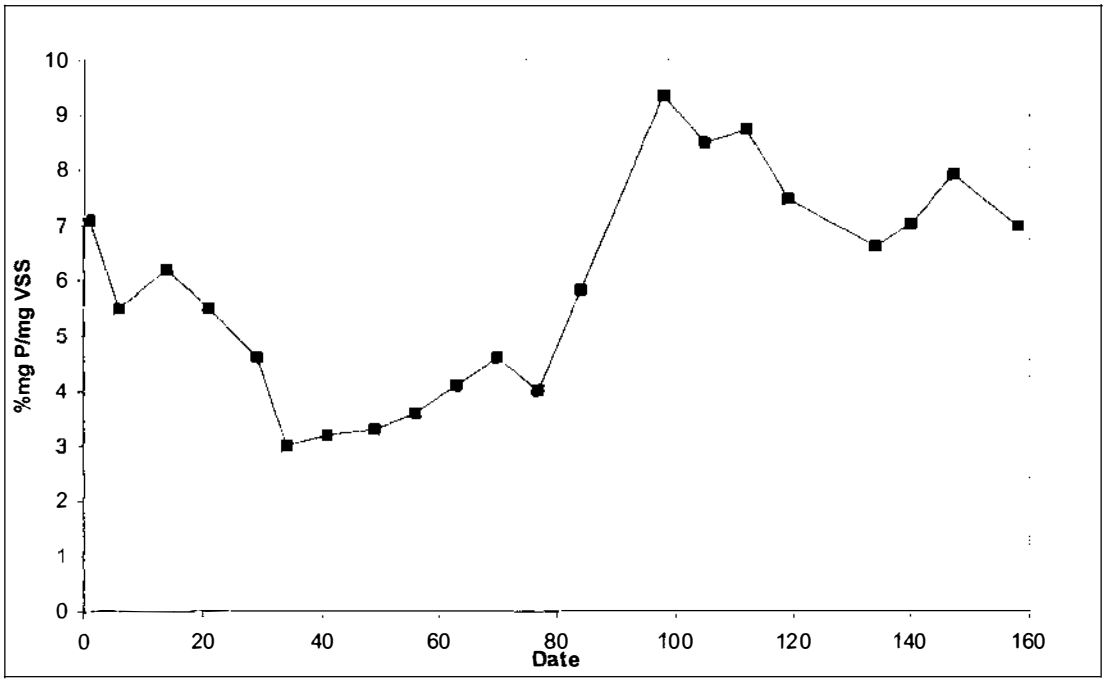


Figure 6.11: Sludge phosphorus concentration in the final aerobic zone during the reactor operation.

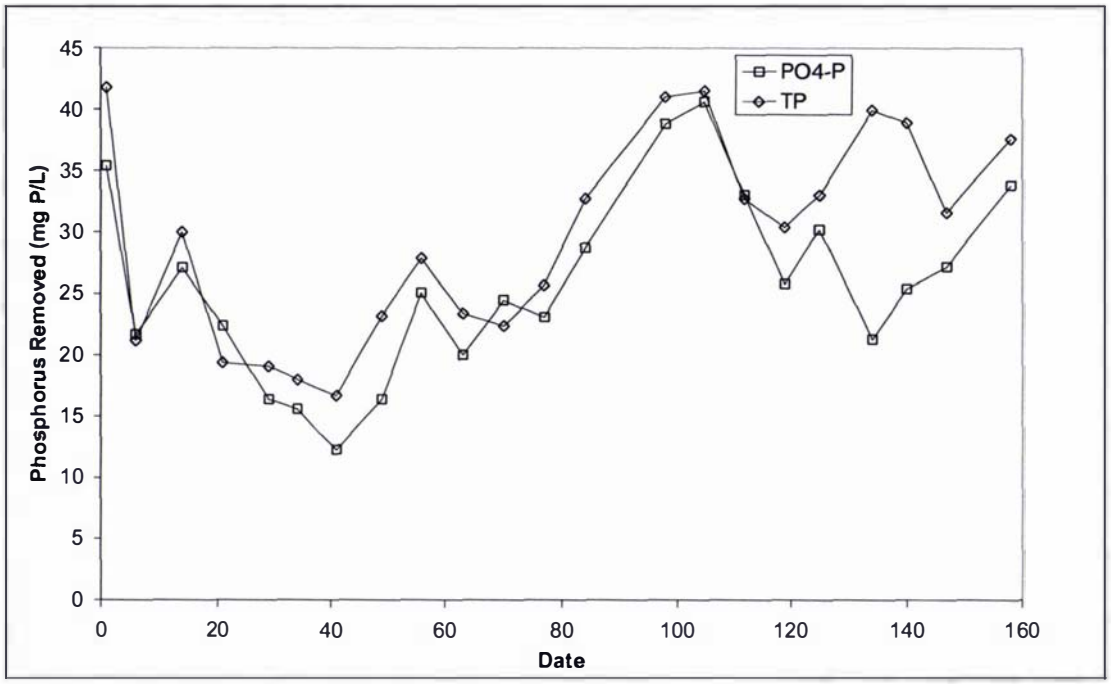


Figure 6.12: Phosphorus removal during the reactor operation, based on both the fermented effluent total phosphorus (TP) and soluble phosphate (PO_4-P).

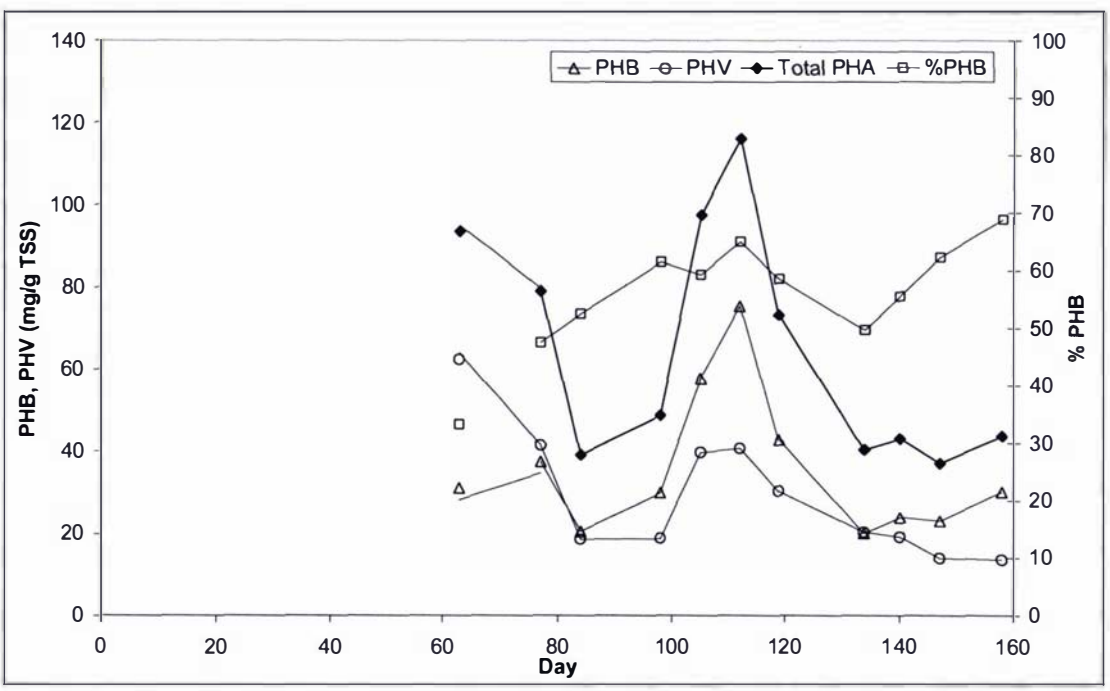


Figure 6.13: Anaerobic zone PHA, PHB and PHV concentration during the reactor operation.

The amount of intracellular PHA stored during in the anaerobic zone reached a maximum on day 112 (coinciding with the increased phosphorus release). Both PHB and PHV were present due to the mixed VFA content of the influent. PHB was the principal PHA compound (56%). Satoh *et al.* (1992) operated a continuous reactor fed with a mixture of acetate and propionate and found that in batch tests with this sludge that acetate formed predominately PHB while propionate formed mostly PHV under anaerobic conditions.

6.4.1 Zone Analysis

On day 158 a detailed analysis of each zone was undertaken, as the system was well established. This provided an instantaneous picture of each zone and the sequential EBPR process through the reactor. The TSS concentration was relatively constant across the reactor (Figure 6.14), and ranged from 2380 mg/L in zone 1 to 2585 mg/L in zone 10. The VSS/TSS ratio in the first zone was 0.828 and in the final zone was 0.768. The decrease in VSS/TSS reflected the increase in the cellular polyphosphate content due to phosphorus uptake.

The soluble COD consumed in the anaerobic zone was 483 mg COD/L at a rate of 82 mg COD/g VSS/h, and the soluble COD was almost completely utilised by zone 3 (Figure 6.15). Soluble COD consumption in the anoxic zone amounted to only 1 mg COD/L. Hydrolysis of particulate material may have occurred in this zone affecting the soluble COD mass balance. A mass balance calculation of the VFA consumption within these two zones shows that 450 mg COD/L and 19 mg COD/L of VFA were consumed within the anaerobic and anoxic zones respectively. The difference between the soluble COD and VFA consumption in the anaerobic zone (483-450) was 33 mg COD/L. This difference is most likely due to a combination of: 1) the hydrolysis of particulate material; 2) consumption of non-short chain VFA soluble compounds; 3) mass balance errors; and the production of microbial inert soluble COD. The difference of minus 18 mg COD/L ($COD_{soluble}$-VFA COD) within the anoxic zone (1-19) is most likely due to both the hydrolysis of particulate substrate to soluble non-VFA COD and experimental error in the mass balance. This emphasises the potential difficulty in obtaining accurate COD mass balances in the anaerobic and anoxic zones, when treating a mixed substrate wastewater containing particulate material.

Soluble phosphorus concentrations decreased until zone 9, with the greatest rate of phosphorus uptake in zones 3 and 4 (Figure 6.15), of 2.2 and 4.8 mg P/g VSS/h respectively. Figure 6.16 shows the net phosphorus uptake in each zone. Zones 1 and 2 exhibited net phosphorus release (negative uptake) of 24.2 and 15.1 mg P/L respectively, which equates to a phosphorus release rate of –4.1 and –5.1 mg P/g VSS/h respectively. The ratio of phosphorus released to both soluble COD and VFA COD consumed in the anaerobic zone was 0.04 mg P/g COD. This is much lower than the value of 0.22 mg P/mg COD used in the EBPR model of SBR treatment of a fermented cheese processing effluent by Ky *et al.* (2001). The phosphorus content of the biomass reached a maximum of 7.4 % mg P/mg VSS by zone 5 (Figure 6.17) and then levelled off, which correlated with the VSS/TSS ratio levelling off by zone 5 (Figure 6.14). The final aerobic zone sludge concentration was 6.99 %mg P/mg VSS. The sludge phosphorus content of the anaerobic and anoxic zones was 3.65 and 4.34 % mg P/mg VSS respectively. These two values suggest that there was potential for more phosphorus release in both the anaerobic and anoxic zones on the day of this analysis. A total of 37.6 mg P/L was removed at the time of the zone study.

Nitrification was complete by zone 5 when nitrate reached a maximum concentration of 4.5 mg NO_3-N/L (Figure 6.18), with an average nitrate concentration of 1.5 mg NO_3-N/L in zones 3, 4 and 5. Nitrite as an intermediate product of nitrification reached a maximum of 1.1 mg NO_2-N/L in zone 4 after which it decreased to zero in zone 6 and remained at zero in subsequent zones. Neither nitrate nor nitrite was detected in the anaerobic or anoxic zones. The aerobic nitrate concentration in this study was 53% of the average nitrate concentration in the preliminary unfermented AAO system in Chapter 5.

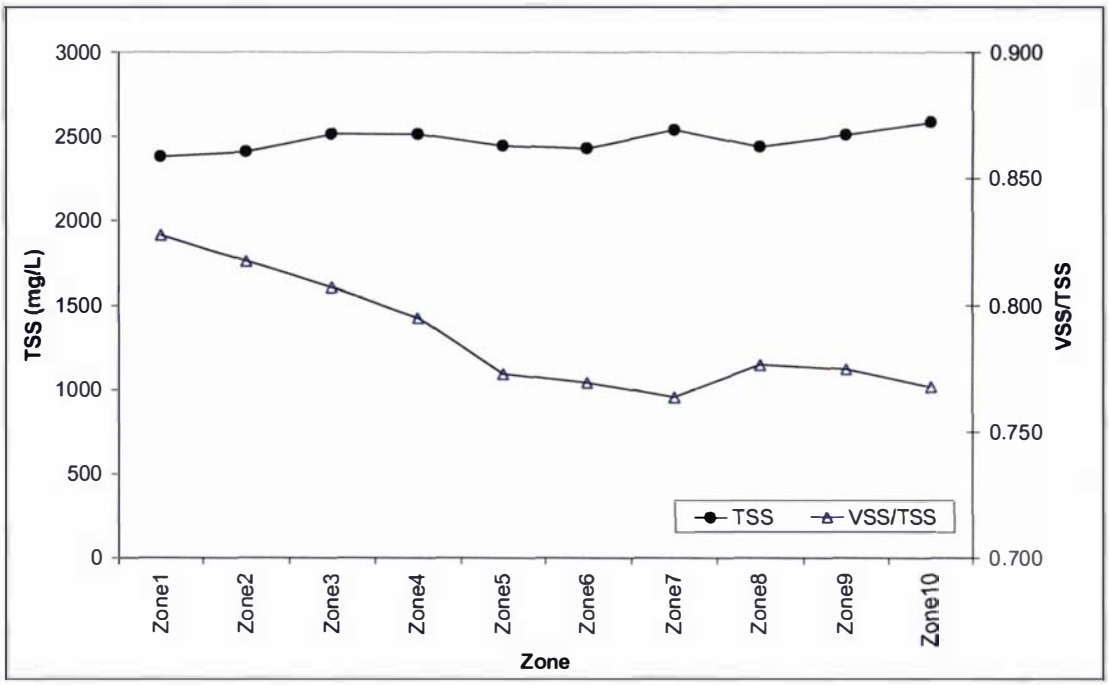


Figure 6.14: TSS concentration and VSS/TSS ratio for each zone on day 158.

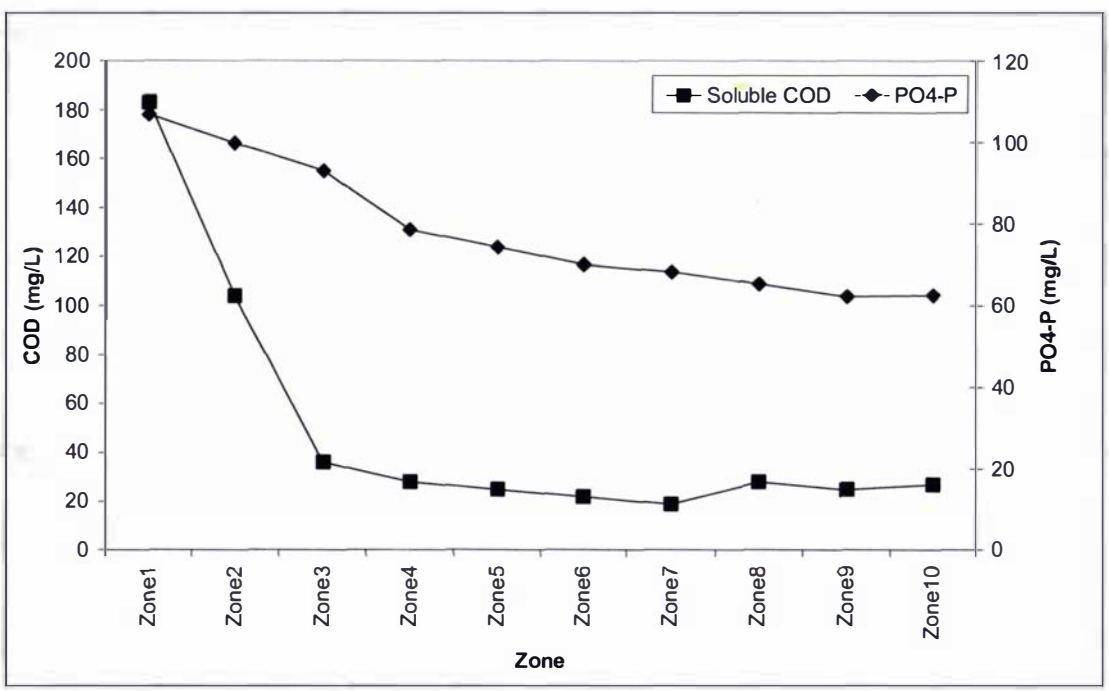


Figure 6.15: Soluble COD and PO_4-P decrease through each zone on day 158.

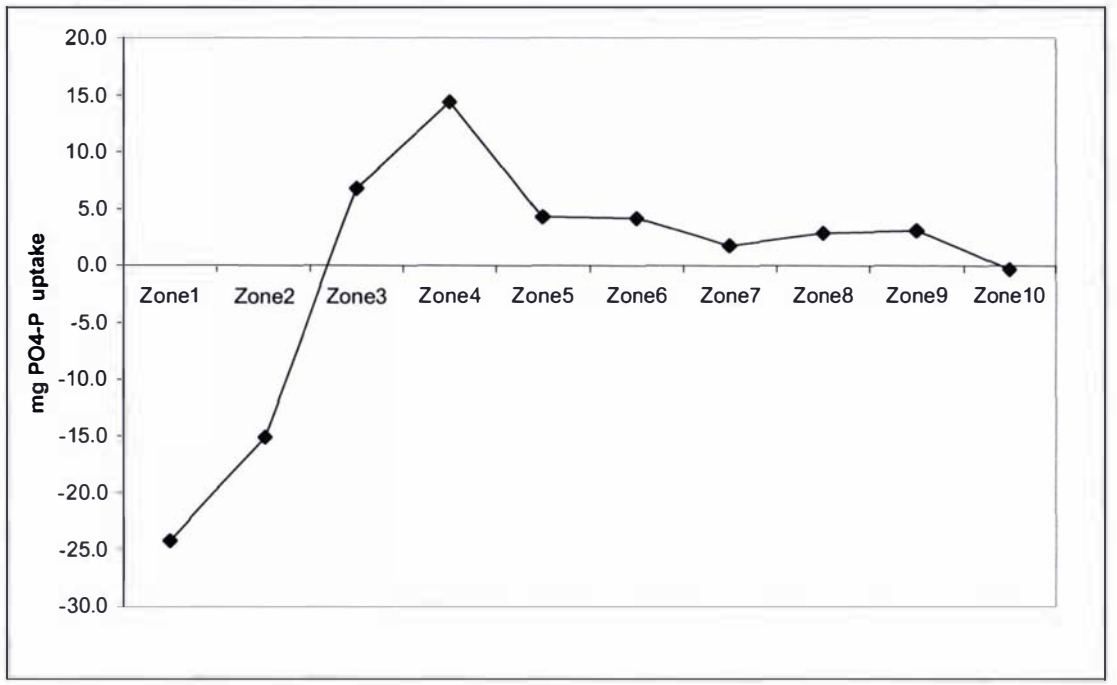


Figure 6.16: Net phosphorus uptake in each zone (negative uptake means phosphorus release).

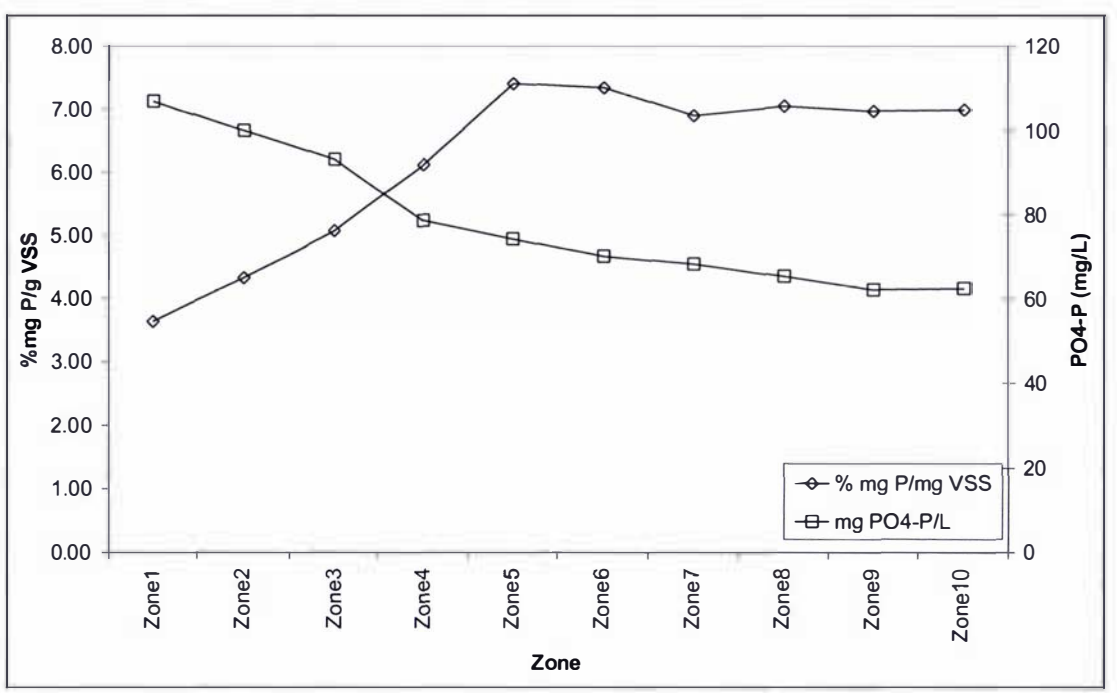


Figure 6.17: Biomass phosphorus content relative to the soluble PO_4-P concentration in each zone.

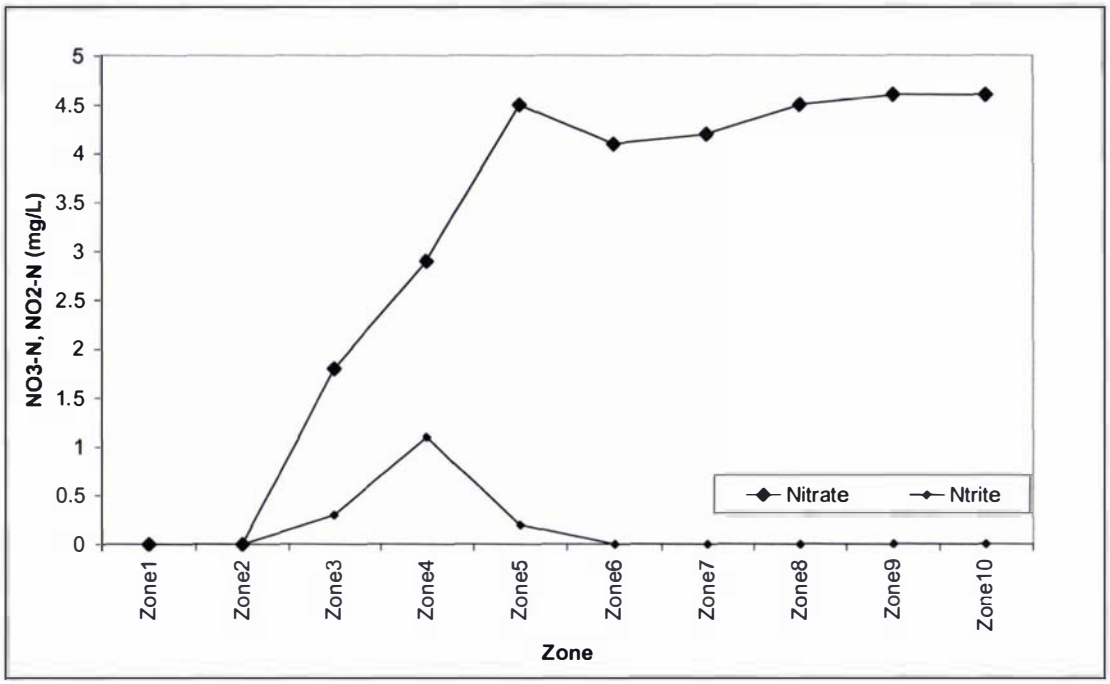


Figure 6.18: Nitrate and nitrite zone concentrations for each zone on day 158.

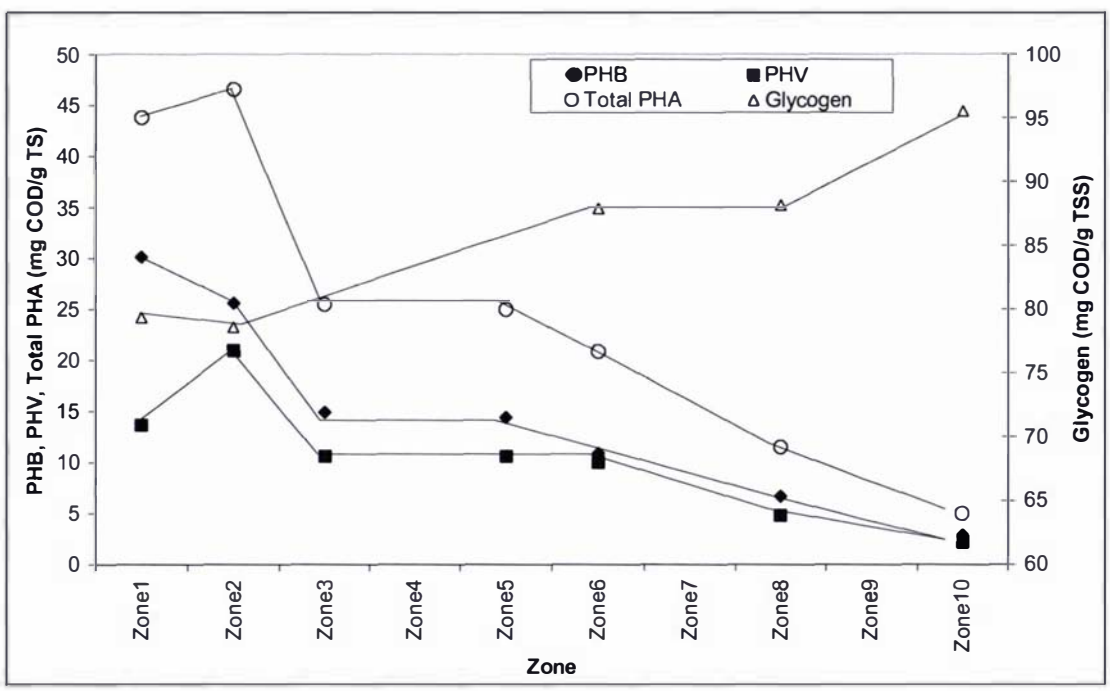


Figure 6.19: Total PHA, PHB, PHV and glycogen concentrations in the sludge.

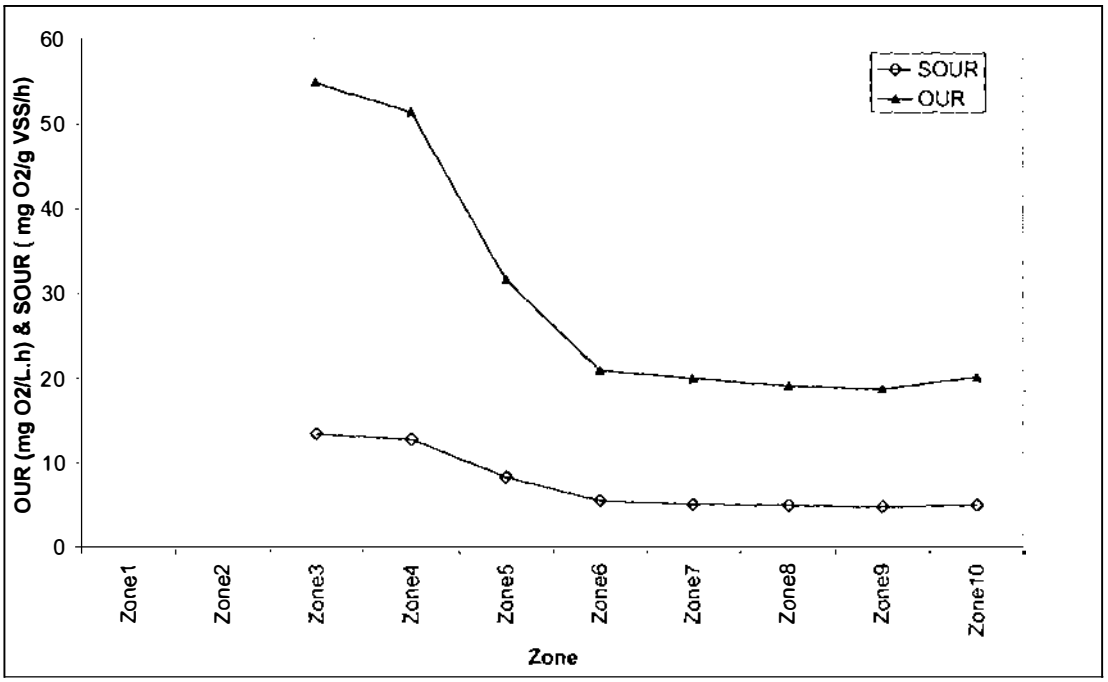


Figure 6.20: Our uptake rates (OUR) and specific oxygen uptake rates (SOUR) in each aerobic zone.

The intracellular PHA storage concentration reached a maximum in the anaerobic zone (zone 1) of 43.8 mg COD/g TSS and the anoxic zone (zone 2) of 46.5 mg COD/g TS (Figure 6.18). The storage of PHA in the anoxic zone supported the previous phosphorus calculations that showed that phosphorus release occurred in the anoxic zone, and that this zone essentially acted as a second anaerobic zone. The PHA production rate in zone 1 was 15.1 mg COD/g VSS/h and 35 mg COD/g VSS/h in zone 2. The fraction of total PHA present as PHB was 68.9% in zone 1 and 55% in zone 2 then ranged between 52.1% and 58.5% in the subsequent zones. The significant fraction of PHA present as PHV is a result of the multiple VFA components in the fermented influent (Satoh *et al.*, 1992), as acetate was only 36 % of the total VFA COD. The ratio of anaerobic PHA stored to VFA COD consumed was 0.2 mg COD/mg COD. The total PHA concentration decreased to 5 mg COD/g TS by zone 10. Glycogen was measured in zones 1, 2, 6, 8 and 10, was 79 mg COD/g TS in zones 1 and 2, and increased to 95 mg COD/g TS in zone 10. The amount of glycogen used in the anaerobic and anoxic zone was 100.6 mg COD/L and 28.5 mg COD/L

respectively. The amount of anaerobic glycogen consumed to VFA COD consumed was 0.22 mg COD/mg COD.

Respirometry was used to measure the oxygen uptake in each aerobic zone (3 to 10) and the results are shown in Figure 6.20. A sharp drop in the OUR and SOUR (Figure 6.20) occurred when the amount of phosphorus uptake decreased in zone 5. At this stage oxygen is used mainly for glycogen synthesis (Figure 6.19), growth and maintenance (Smolders *et al.*, 1994). The main individual zone parameters for each zone are shown in Table 6.4.

6.4.2 Batch Test

A phosphorus release and uptake batch test of the mixed liquor was done by adding 200 mg COD/L of sodium acetate to 2 litres of mixed liquor taken from the final zone of continuous reactor (once the dissolved oxygen was equal to zero). The mixed liquor was stirred and purged with nitrogen gas during the 240 minute anaerobic phase followed by a 240 minutes aerobic phase. Samples were collected at 30 minute intervals and filtered using 0.45 μm filter paper. Total and volatile solids were measured at 30 minute intervals. The initial VSS concentration was 2175 mg/L with an initial sludge phosphorus concentration of 6.6 %mg P/mg VSS. There were three different rates of COD utilisation of 32.8, 10.2 and 6.2 mg COD/g VSS/h respectively (Figure 6.21). The initial nitrate concentration was 5.7 mg NO_3-N/L, which contributed to some of the initial COD utilisation. If the initial high rate of COD utilisation is corrected for the COD utilised by denitrification (4 mg COD/mg NO_3-N), then the initial rate of COD consumption decreases to 22.4 mg COD/g VSS/h. The initial and second phosphorus release rates were 4.5 mg P/g VSS/h and 2.3 mg P/g VSS/h respectively (Figure 6.21). The COD consumed to phosphorus released ratio was 0.34 mg P/mg COD. The initial phosphorus content of the sludge was 6.57 % mg P/mg VSS and decreased to 3.7% at the end of the anaerobic zone. This value of 3.7 % was very close to the zone analysis anaerobic zone value of 3.65%. The initial VSS/TSS ratio was 0.759 and increased to 0.834, a decrease of 7.5% in the ash content of the sludge.

In the aerobic phase, there were three different rates of phosphorus uptake of 6.2, 4.5 and 1.2 mg P/g VSS/h. The sludge phosphorus content at the end of the aerobic phase was 6.88 % mg P/g VSS, slightly greater than the initial value at time zero of 6.57 %. At the end of the aerobic phase, the VSS/TSS ratio was 0.76, similar to the initial value of 0.76. This overall net increase in the sludge phosphorus content and the ash content was reflected in the overall net decrease in the soluble phosphorus concentration of 1.9 mg P/L.

Due to a loss of frozen samples for PHA analysis because of refrigeration failure, only glycogen was able to be determined up to 420 minutes. There was a net glycogen decrease in the anaerobic phase of 21.6 mg COD/g TS and then an increase through the aerobic phase of 40 mg COD/g TS up to 420 minutes. This was an overall glycogen increase of 18.4 mg COD/g TS. The specific oxygen uptake rate (SOUR) was initially high due to a small amount of remaining acetate and then gradually decreased along with the phosphorus uptake rate and glycogen synthesis. The values of the batch test parameters are shown in Table 6.5 for the start of the anaerobic phase (t =0), end of the anaerobic phase (t=240) and end of the aerobic stage (t=660).

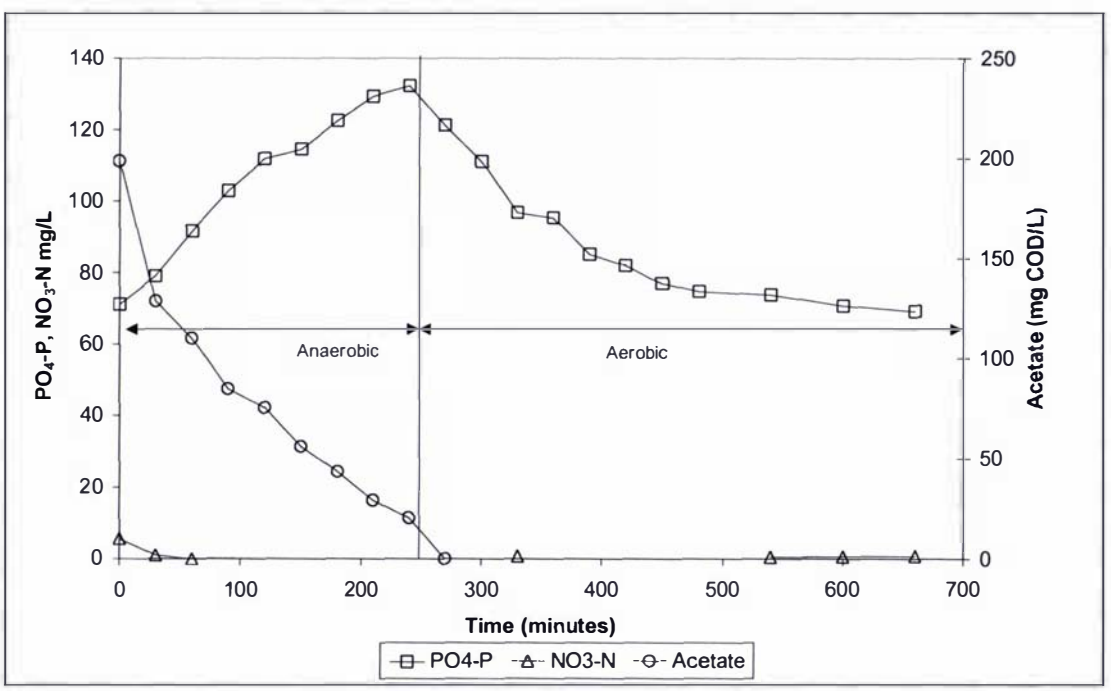


Figure 6.21: Phosphorus release/uptake and acetate uptake during the batch test with acetate as sole carbon source.

Table 6.4: Individual zone parameters for AAO configuration.

	Zone									
Parameter	1 Anaerobic	2 Anoxic	3 Aerobic	4 Aerobic	5 Aerobic	6 Aerobic	7 Aerobic	8 Aerobic	9 Aerobic	10 Aerobic
$COD_{soluble}$ (mg/L)	183	104	36	28	25	22	19	28	25	27
PO_4-P (mg/L)	106.8	99.8	93	78.6	74.3	70.1	68.3	65.4	62.3	62.6
%mg P/g VSS	3.65	4.34	5.08	6.12	7.40	7.34	6.90	7.04	6.97	6.99
NO_3-N (mg/L)	0	0	1.8	2.9	4.5	4.1	4.2	4.5	4.6	4.6
SOUR mgO_2/mg VSS/h			13.5	12.9	8.4	5.6	5.2	5.0	4.8	5.1
Total PHA mg COD/g TSS	25.1	26.2	14.4		14.1	11.7		6.5		2.8
Glycogen mg COD/g TSS	79.4	78.6				87.9		88.2		95.5
TSS (mg/L)	2380	2410	2515	2515	2445	2430	2540	2440	2510	2585
VSS/TSS ratio	0.83	0.82	0.81	0.80	0.77	0.77	0.76	0.78	0.78	0.77

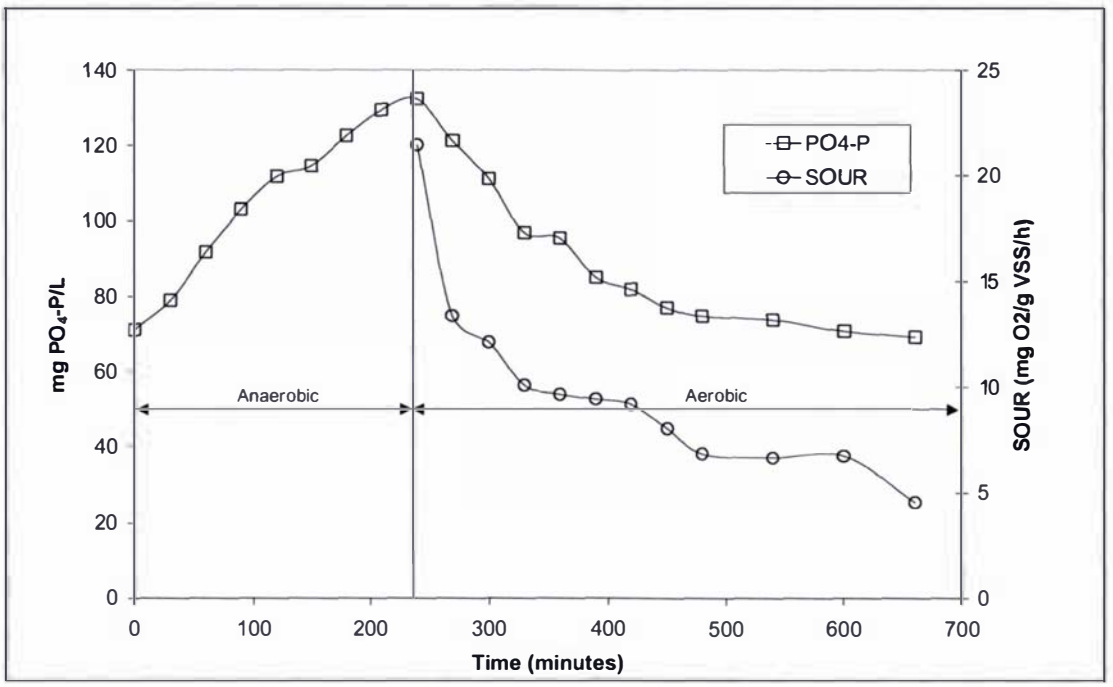


Figure 6.22: Specific oxygen uptake rate (SOUR) and phosphorus release/uptake during batch test.

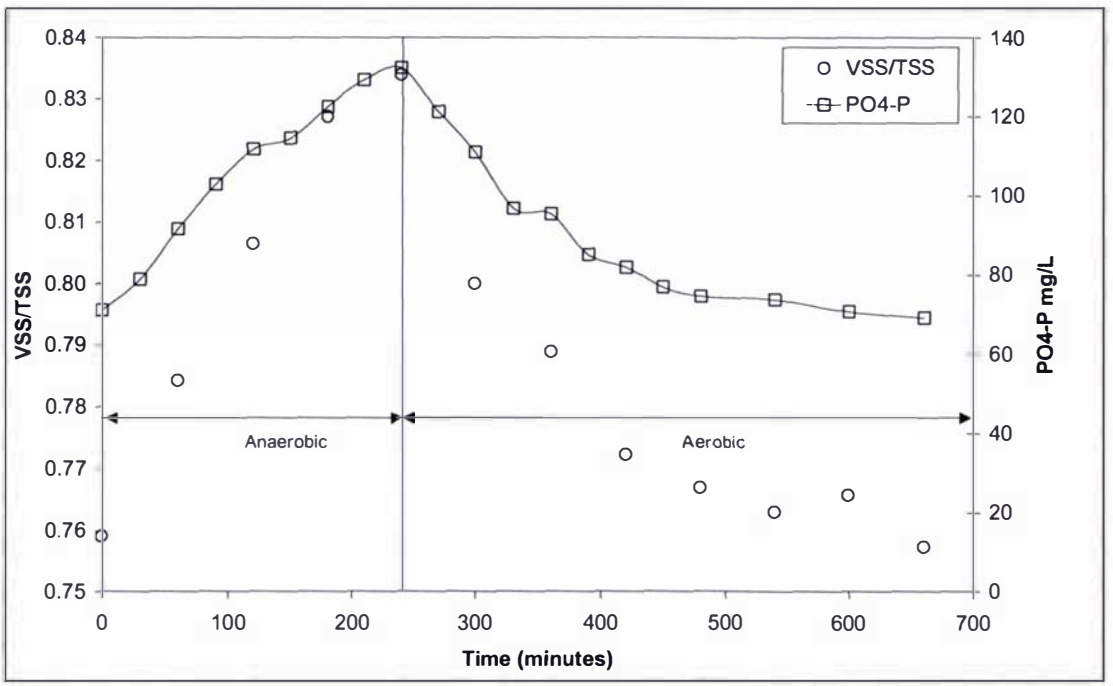


Figure 6.23: VSS/TSS ratio and phosphorus release/uptake during batch test.

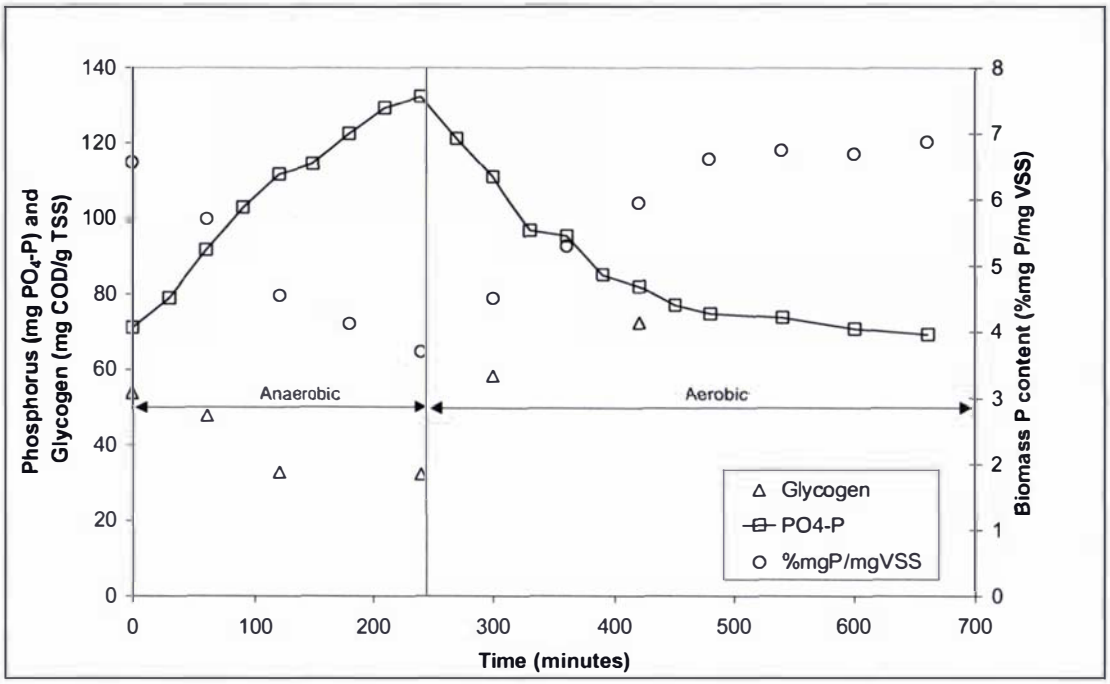


Figure 6.24: Glycogen, sludge phosphorus content and soluble phosphorus profiles during batch test.

Table 6.5: Analytical parameters at time 0, 180, and 660 minutes for the batch test for 10 day SRT AAO system. Glycogen concentration is given for 420 minutes instead of 600 minutes.

	Time		
Parameter	0 minutes	240 minutes	660 minutes
PO_4-P (mg P/L)	71.1	132.3	69.2
Acetate COD (mg/L)	198.7	20.5	0
VSS (mg/L)	2175	2210	2105
VSS/TSS	0.759	0.834	0.760
%mg P/mg VSS	6.57	3.70	6.74
Glycogen (mg COD/g TS)	54.0	32.4	72.4 (420 minutes)
OUR(mg O_2/L/h)	-	47.4	9.6
SOUR mg O_2/g VSS/h)	-	21.5	4.6

6.5 Extended Anaerobic Retention Time Test

6.5.1 Extended Anaerobic HRT AAO (EAAO) Reactor Zone Study

The reactor configuration tested in Section 6.3 was altered slightly by making the first two zones anaerobic and the third anoxic, which resulted in the number of aerobic zones decreasing from 8 to 7. This was the only change that was made to the reactor. The reason for increasing the anaerobic volume fraction from 10% to 20% was to determine if the increased anaerobic retention time would result in significantly greater anaerobic VFA uptake and phosphorus removal. In the previous study VFA were detected in both the anaerobic and anoxic zones, and the anoxic zone displayed phosphorus release and essentially acted as a second anaerobic zone. The extended anaerobic zone system was operated for a total of 40 days at an SRT of 10 days (4 SRT's duration) before a full zone study was performed on the reactor. As this system was operated with an AAO configuration but with an extended anaerobic HRT, it is called an EAAO system in this thesis. Based on literature information there was also a possibility that extending the anaerobic zone HRT could lead to phosphorus removal deterioration. Satoh *et al.* (1994), had previously identified that GAO organisms may be preferred under both extended anaerobic and aerobic conditions as polyphosphate is chemically more unstable than glycogen.

The TSS concentrations varied between 3270 and 3635 mg/L through the zones (Figure 6.25). There was a slight decrease from the first anaerobic zone to the second anaerobic zone of 365 mg/L and then a slight increase in the last three aerobic zones. The VSS/TSS ratio increased from zone 1 (0.86) to zone 2 (0.88), reflecting a further decrease in the cellular polyphosphate content as continued anaerobic EBPR activity occurred. As the cellular polyphosphate content increased during aeration the VSS/TSS concentration decreased to 0.81 in zone 10. The VSS/TSS ratio increased in zone 8, due to a small decrease in the sludge phosphorus concentration in this zone. The main change from the previous single anaerobic zone reactor was that the ash content of the sludge decreased by 7.5% through the reactor as opposed to 6.0% with the previous configuration.

The soluble COD in zone 1 was 210 mg/L and decreased to 60 mg/L in zone 2. The amount of soluble COD consumed was 465 mg/L in zone 1 and 150 mg/L in zone 2,

which implies that most of the COD consumption occurred in zone 1 in the first three hours. The VFA consumed in zone 1 of 429 mg/L was slightly less than the soluble COD consumed (465 mg/L). This difference of 36 mg/L between soluble COD and VFA consumption as with the previous system, could be due to further fermentation, consumption of non-VFA compounds or mass balance errors. The VFA consumed in zone 2 of 150 mg/L matched the soluble COD consumption. The COD consumption rates were 24.7 and 8.7 mg COD/g VSS/h in the anaerobic zones 1and 2 respectively. In the anoxic zone, there was only a small amount of COD consumption (17 mg/L), which was almost equal to the VFA consumed (19 mg/L).

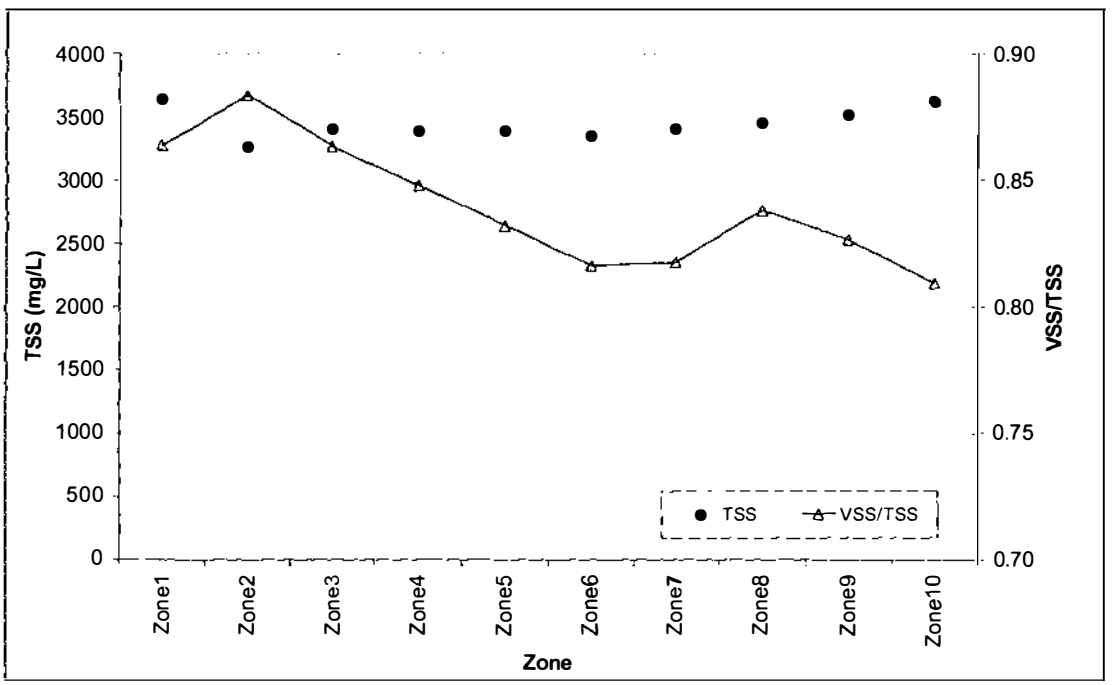


Figure 6.25: EAAO system TSS concentrations and VSS/TSS ratio profiles.

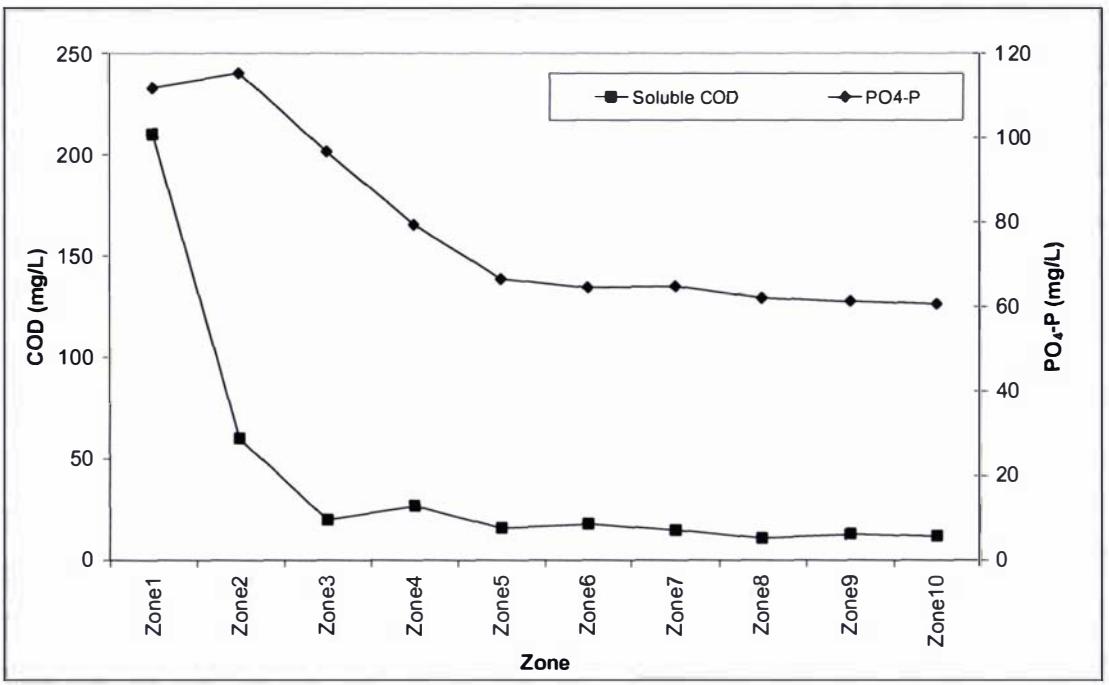


Figure 6.26: EAAO system soluble COD and soluble phosphate zone concentrations.

The soluble phosphorus profile (Figure 6.26) varies from the previous system in that phosphorus release occurred in three zones. The greatest amount of release occurred in zone 1 (-29.9 mg/L), with a small amount occurring in zone 2 (-3.5 mg/L) and zone 3 (-8.3 mg/L) (Figure 6.27). This amount of release equated to rates of 3.2, 0.4 and 1.9 mg P/g VSS/h in zones 1, 2 and 3 respectively. Phosphorus uptake was greatest in zone 4 the first aerobic zone (17.3 mg/L) and was essentially complete by zone 6. The specific rate of phosphorus uptake in zone 4 was 3.9 mg P/g VSS/h and in zone 5 was 3.0 mg P/g VSS/h. The sludge phosphorus content is shown in Figure 6.28, where the maximum value was 5.61 %mg P/mg VSS in zone 10. Based on the amount of phosphorus removed each day and the daily amount of VSS wasted, a mass balance gives the theoretical sludge phosphorus content value for zone 10 calculated as 5.45 % mg P/mg VSS, close to the analytical value. Near maximum phosphorus release appears to have occurred by zone 2 as the sludge phosphorus content of 2.31 %mg P/mg VSS is typical of a non-EBPR sludge while the value of 2.43 %mg P/mg VSS in zone 1 was slightly greater. The sludge phosphorus content showed an increase in the final two zones but did not result in further soluble phosphorus removal in these two zones. A total of 41.3 mg P/L of phosphorus was removed at the time of the zone

study from a fermented wastewater concentration at the time of the study of 102 mg P/L.

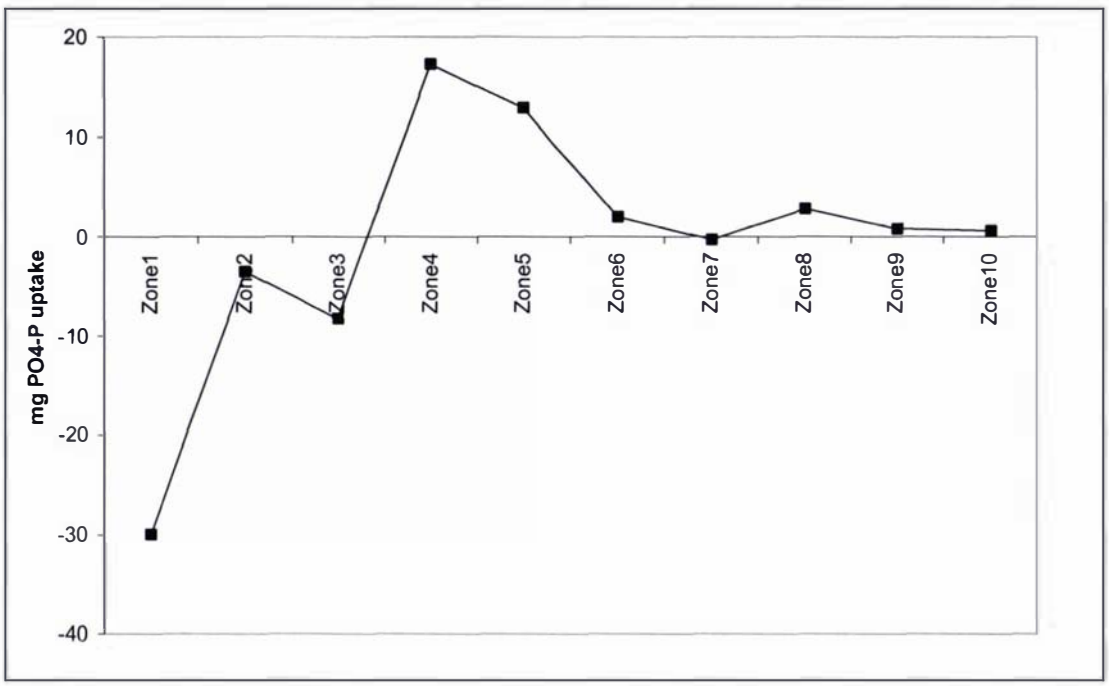


Figure 6.27: EAAO system net phosphate uptake profiles (negative uptake denotes phosphate release).

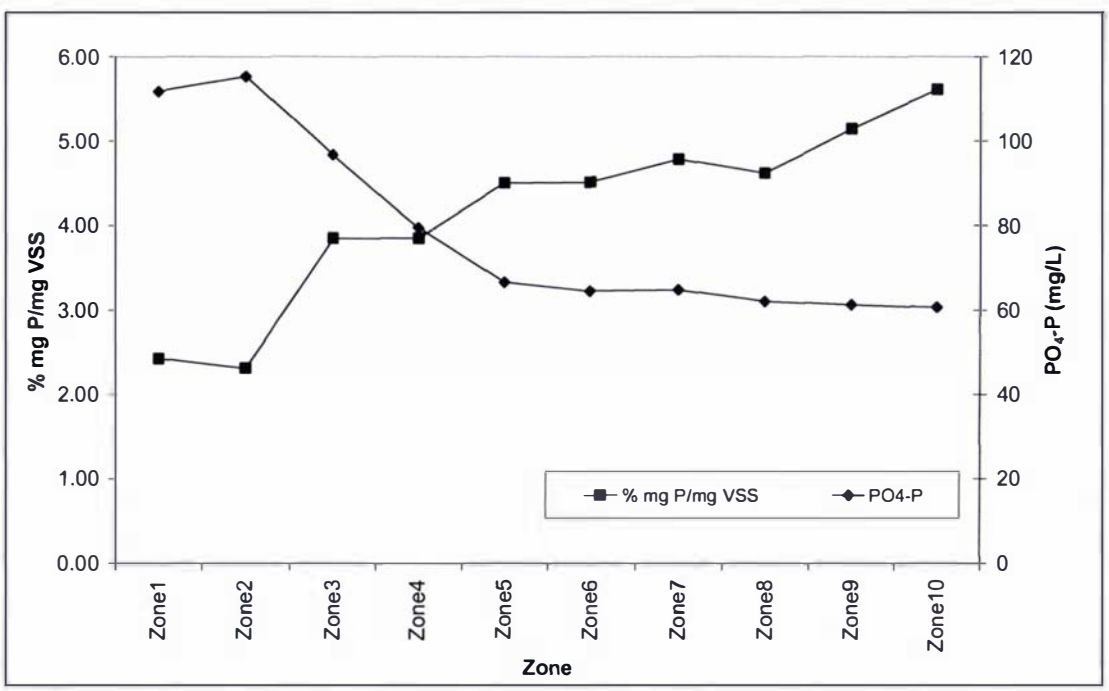


Figure 6.28: EAAO system biomass phosphorus content relative to soluble phosphate.

Complete denitrification occurred in zone 3 as the nitrate and nitrite concentration was zero in the anoxic zone. Although the non-aerated fraction of the reactor was increased by 10 %, nitrification was still active (Figure 6.29), with both the nitrate and nitrite concentrations in the final aerobic zone slightly higher than for the previous system (6.2 mg NO_3-N/L and 1.9 mg NO_2-N in zone 10). This aerobic zone nitrate concentration was only 2.4 mg N/L less than for the unsuccessful AAO study in Chapter 5, suggesting that the effluent nitrate concentration was not a factor in the lack of EBPR for that system.

The aerobic respiration rates are shown in Figure 6.30. The first aerobic zone (zone 4) was 28.2 mg O_2/g VSS/h compared to the previous system with the smaller anaerobic fraction where it was 13.5 mg O_2/mg VSS/h. This is unusual as the soluble COD flowing through from the previous zone (zone 3) was 20 mg/L in this extended anaerobic zone system and 104 mg/L in the previous system. The reason is most likely due to extra storage of PHA (not measured due to sample loss) from increased COD consumption. The amount of phosphorus removal was 41.3 mg P/L for this EAAO system compared to 37.6 mg P/L with the previous AAO configuration at the time of the zone study.

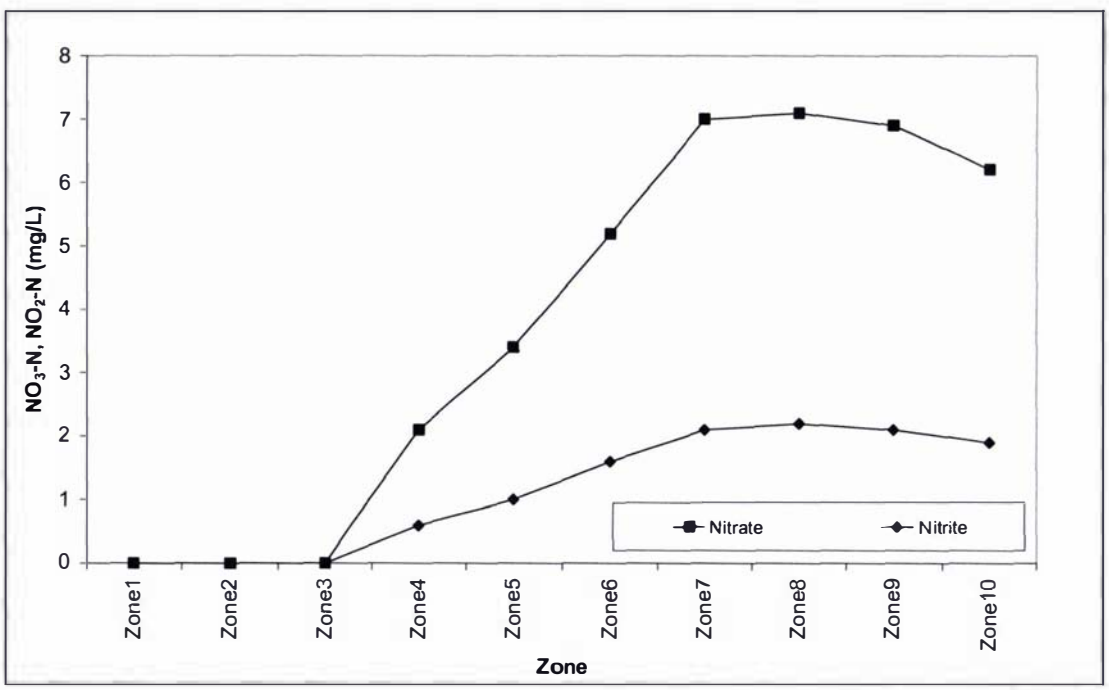


Figure 6.29: EAAO system nitrate and nitrite concentrations.

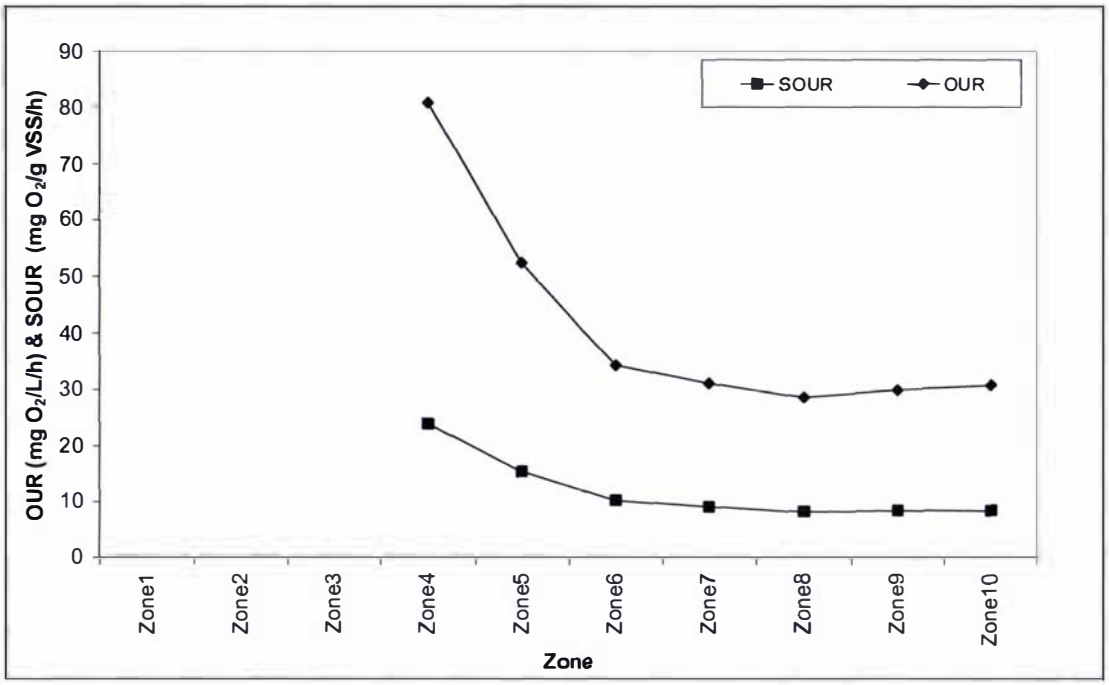


Figure 6.30: EAAO system nitrate and nitrite concentrations.

6.5.2 EAAO Anaerobic Batch Test

After the EAAO zone study, an anaerobic only batch test was performed to determine the rates and duration of phosphorus release with acetate used as the substrate. The amount of sodium acetate added was 500 mg/L, with a COD equivalence of 533 mg COD/L. The mixed liquor was continuously mixed and purged with nitrogen during the batch test, with the acetate added when the dissolved oxygen was measured as zero. The duration of the batch test was 480 minutes (8 hours), two hours longer than the combined 360 minute retention time of both anaerobic zones in the continuous reactor system. Samples (20 mL) were collected at 60 minute intervals and immediately filtered with 0.45 μm filter paper. The initial TSS concentration was 1815 mg/L, which was about half what was present in the reactor. This concentration of TSS was obtained by withdrawing 1 litre of mixed liquor from the final aerobic zone and diluting it with 1 litre of clarifier effluent. It was decided that withdrawing 1 litre of mixed liquor would not have too much impact on the continued reactor operation. The concentration of phosphorus, nitrate, and acetate were measured during the batch test and are shown in Figure 6.31 (nitrite was undetectable).

Table 6.6: Individual zone parameters for EAAO configuration.

	Zone									
Parameter	1 Anaerobic	2 Anoxic	3 Aerobic	4 Aerobic	5 Aerobic	6 Aerobic	7 Aerobic	8 Aerobic	9 Aerobic	10 Aerobic
$COD_{soluble}$ (mg/L)	210	60	20	27	16	18	15	11	13	12
PO_4-P (mg/L)	111.8	115.3	96.8	79.5	66.6	64.6	64.9	62.1	61.3	60.7
%mg P/g VSS	2.43	2.31	3.58	3.85	4.50	4.51	4.79	4.62	5.14	5.61
NO_3-N (mg/L)	0	0	0	2.1	3.4	5.2	7.0	7.1	6.9	6.2
SOUR mgO_2/mg VSS/h	-	-	-	28.2	18.5	12.6	11.2	9.9	10.3	10.5
Phosphorus Uptake (mg/L)	-29.9	-3.5	-17.2	17.3	12.9	2.0	-0.3	2.8	0.8	0.6
TSS (mg/L)	3635	3270	3405	3385	3400	3350	3400	3460	3520	3620
VSS (mg/L)	3140	2890	2940	2870	2830	2735	2780	2900	2910	2930
VSS/TSS ratio	0.86	0.88	0.86	0.85	0.83	0.82	0.82	0.84	0.83	0.81

A total of 226 mg/L of acetate COD was consumed, at two rates of 25.2 and 8.4 mg COD/g VSS/h, which are very similar to the rates for the single anaerobic zone (AAO) batch test. The COD consumption rate in the anaerobic zone of the continuous reactor was calculated as 24.7 mg COD/g VSS/h, which is also similar to the first high rate calculated in this batch test. The second slower COD consumption rate of 8.4 mg COD/g VSS/h is also similar to the continuous reactor second anaerobic zone rate of 8.7 mg COD/g VSS/h.

For an influent VFA concentration of 1270 mg COD/L, a consumption rate of 22.8 mg COD/g VSS/h and a VSS concentration of 3140 mg/L a theoretical retention of 4.4 hours would be required for complete VFA uptake in the zoned reactor. As seen from the data although the 2-zone retention time was greater than 4.4 hours, there was still some residual VFA in zone 2 as the COD consumption rate in this zone was much slower. This shows that although the fermenter effluent, on the day of the zone study, consisted of only 37% acetic acid, the COD consumption rate was similar to the batch test which used only acetate over the six hour period. The majority of the available COD was consumed in the first 3 hours for both the batch test (62%) and the continuous reactor (67%).

A total of 44.7 mg/L of phosphorus was released at two rates of 3.9 and 1.0 mg P/g VSS/h. The COD consumed to phosphorus released ratio was calculated as 0.20 mg P/mg COD, less than that for the previous AAO batch test of 0.34 mg P/mg COD. Phosphorus release in this batch test was complete after 360 minutes, the same as the anaerobic time available in one anaerobic zone in the continuous reactor. When considering only the two continuous reactor anaerobic zones, 89% of phosphorus release occurred in the first anaerobic zone, compared to 73% of the phosphorus release occurring in the first three hours of this batch test.

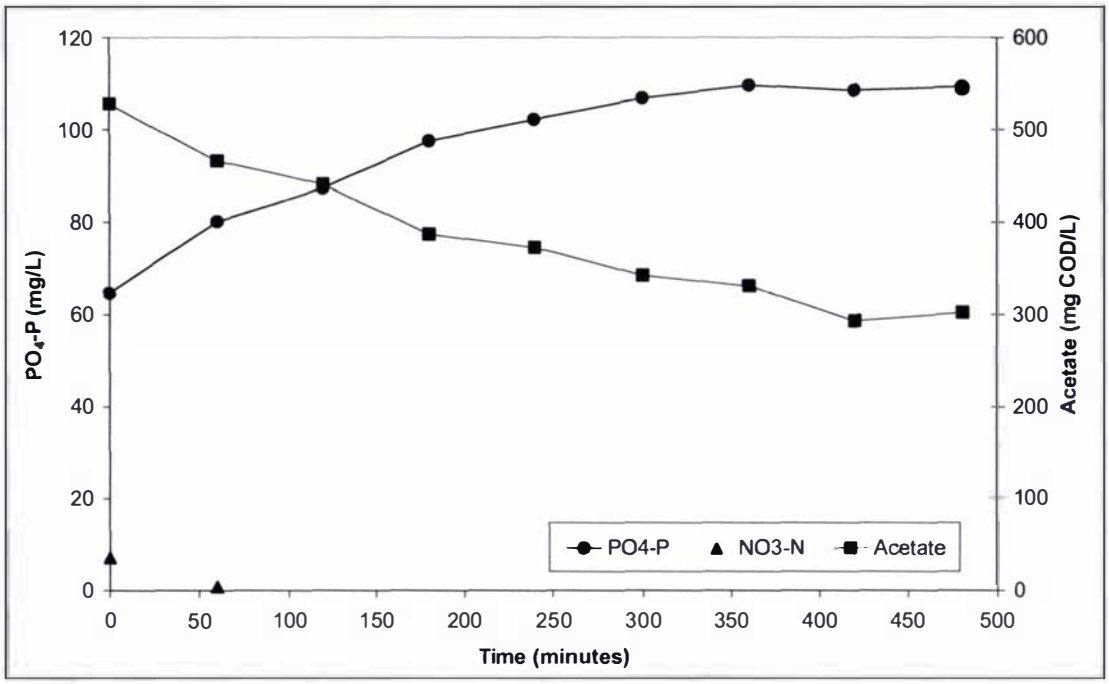


Figure 6.31: Anaerobic batch test using sludge from EAAO.

6.6 Discussion

The reactor performance in terms of achieving phosphorus removal was optimised by careful reactor design and operation. The reactor design both maximised the operational stability and minimised dissolved oxygen entry into the anaerobic and anoxic zones. The suitability of the synthetic dairy wastewater was also improved by the addition of a fermenter to produce VFA prior to the EBPR reactor. A maximum of 44.6 mg P/L of phosphorus was removed, showing that a system could be designed to remove a large amount of phosphorus from this synthetic dairy processing wastewater. Figure 6.32 shows the theoretical amount of phosphorus that could be removed based on mass balance calculations for different sludge phosphorus concentrations, different mixed liquor concentrations at an SRT of 10 days. The lines on Figure 6.32 for a mixed liquor concentration of 2000 mg VSS/L and a sludge phosphorus concentration of 7.0 mg P/VSS equate to 35 mg P/L of phosphorus removal. This value is very close to the value of 37 mg P/L removed on day 159 of the AAO system when the zone study was performed, at this time the MLVSS was 1985 mg VSS/L and the sludge phosphorus concentration was 6.99 %mg P/mg VSS. A sludge phosphorus concentration of 7 %mg P/mg is typical of an EBPR process with good phosphorus removal performance. Unless a greater sludge phosphorous

content could be achieved on a consistent basis then it would be very difficult to remove more phosphorus than achieved with the initial single anaerobic zone system. When the dissolved oxygen control was improved in the final zone (day 96) and the reactor stabilised then the final aerobic zone VSS concentration varied between 1830 and 2365 mg VSS/L. For a sludge phosphorus concentration of 7 % mg P/mg VSS this equates to between 32 and 41 mg P/L of phosphorus removal. It is unlikely that a higher concentration of VFA n the fermenter effluent would further improve the amount of phosphorus removal, as excess soluble COD was available in the anaerobic zone. In fact on day 159, when the zone analysis was performed, 159 mg COD/L of VFA was detected in the anaerobic zone and 53 mg COD/L of VFA was detected in the anoxic zone. When Comeau *et al.* (1996), operated a pilot scale SBR at a 20 day SRT treating fermented cheese-processing wastewater containing 1310 mg COD/L as VFA's, about 40 to 50 mg P/L of phosphorus was claimed to be removed. The exact level of phosphorus removal was difficult to accurately calculate due to the variable influent characteristics. On day 191 when Comeau *et al.* (1996), performed their cycle analysis, the MLVSS was 2870 mg VSS/L and the sludge phosphorus content was 7.6 % mg P/mg VSS. About 45 mg P/L of phosphorus was removed by the SBR on this day.

For the extended anaerobic zone system after 40 days of operation, at an SRT of 10 days, the VSS concentration averaged 2890 mg/L in the reactor with a final aerobic zone sludge phosphorus content of 5.61 % mg P/mg VSS. While this value of 5.61 is less than the single anaerobic zone system of 6.99 % mg P/mg VSS, the mixed liquor VSS concentration in the final aerobic zone (2930 mg/L) was 1.48 times greater. The amount of phosphorus removal for the EAAO system increased to 41 mg P/L compared to 37 mg P/L for the single anaerobic zone system on the days of the respective zone studies. The theoretical amount of phosphorus removed with an aerobic sludge of 5.6 % mg P/mg VSS and 2930 mg VSS/L is 41 mg/L (Figure 6.30). It is difficult to quantify why the VSS concentrations in the extended anaerobic zone system were greater than the single zone system. The reason is most likely related to the fact the specific oxygen uptake rate (SOUR) in the final zone for the extended anaerobic zone system was 10.5 mg O_2/g VSS/h as opposed to 5.1 mg O_2/g VSS/hr for the single anaerobic zone system. Generally the respiration rate for each aerobic

zone of the extended anaerobic zone system was twice that of the single anaerobic zone system, which means that the rate of cell lysis would be less for the extended system resulting in a higher biomass concentration. This suggests that there may be some merit in decreasing the aerobic HRT, although conversely increasing the anaerobic HRT had only minimal benefit in the medium term (40 days operation). It is fair to say that long term operation of the EAAO system may have generated different results. The high amount of anaerobic zone 2 COD consumption but the low amount of phosphorus release (Y_{PO4}=0.02) possibly indicates a potentially slow but progressive deterioration in EBPR due to the growth of organisms able to compete for COD under anaerobic conditions such as GAO's. Satoh *et al.* (1994,) found that during long anaerobic storage periods of the reserve material (PHA) glycogen may be preferred to polyphosphate by certain microorganisms because glycogen is more stable, resulting in the growth of competing GAO's.

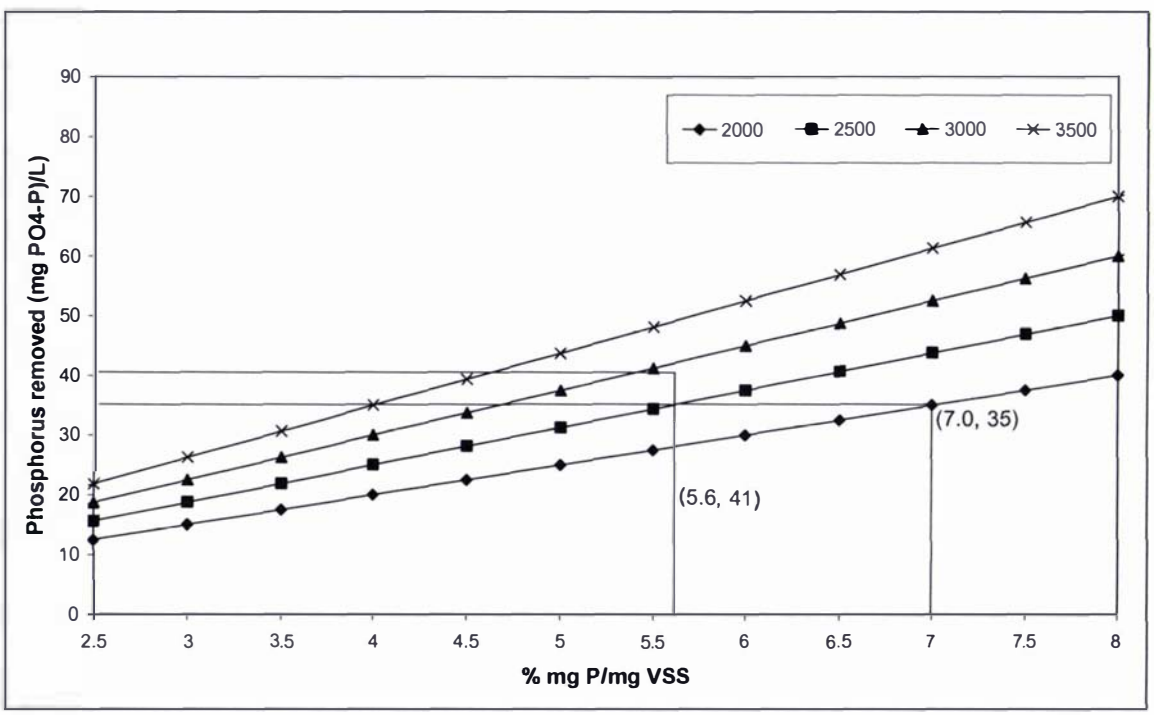


Figure 6.32: Theoretical phosphorus removed as a function of sludge phosphorus concentration for individual mixed liquor VSS concentrations for a reactor at an SRT of 10 days.

Table 6.7: Anaerobic and anoxic COD and VFA consumption and phosphorus release values for the single anaerobic zone AAO system (AAO) and for the extended anaerobic zone AAO system (EAAO).

	COD consumed (mg/L)		VFA consumed (mg/L)		Phosphorus released (mg/L)	
	AAO	EAAO	AAO	EAAO	AAO	EAAO
Anaerobic zone 1	483	465	450	429	24.2	29.9
Anaerobic zone 2	-	150	-	150	-	3.5
Anoxic zone	1	17	19	19	15.1	8.3
Total	484	632	469	598	39.3	41.3

The amount of COD consumed and phosphorus released in each unaerated zone is shown in Table 6.7. The COD consumed and phosphorus released in the first anaerobic zone was similar for each system, with slightly more phosphorus release in the EAAO system. The most significant variation was in the anoxic zone of the AAO system, which exhibited more phosphorus release, yet consumed the same amount of VFA COD. There was phosphorus release in both anoxic zones, which suggests that maintaining an anoxic zone is beneficial with this particular reactor configuration, as it results in further VFA consumption and PHA storage. The extra phosphorus release in the anoxic zone was most likely due to polyphosphate release from 'fresh' biomass recycled via the aerobic-anoxic recycle stream. The unusual aspect of the extended zone system was that while a relatively small amount of phosphorus was released in the second anaerobic zone a relatively large amount of COD was consumed, with a Y_{PO4} value of only 0.02. The stoichiometric relationships between phosphorus release, VFA consumed, PHA and Glycogen are shown in Table 6.8, for zones 1 and 2 of the continuous AAO reactor on the day of the zone study. Only phosphorus release and COD consumed ratio's are shown for the EAAO reactor and for both batch tests. The values for the batch test provide stoichiometric values with a single substrate carbon source (acetate) that can more easily be compared to other single substrate literature values.

The theoretical stoichiometric relationship between phosphorus release and COD uptake under anaerobic conditions has different theoretical values depending on the model used. Wentzel *et al.* (1991), predicted a theoretical value of 0.48mg P/mg COD (1.0 mol P/mol acetate), while the ASM2 model (Henze *et al.*, 1995) gives a typical value of 0.4 mg P/mg COD. The value will also vary depending of the VFA used or the mixture of VFA's used. Abu-ghararah and Randall (1990), reported values ranging from 0.37 mg P/mg COD for acetic acid to 0.12 mg P/mg COD for propionic acid, with other short chain VFA's with ratios in between this range. Based on VFA COD consumption only, low values of 0.06 and 0.07 mg P/mg VFA COD were obtained for zone 1 of both the AAO and EAAO continuous reactors for the mixed VFA influent used. As mentioned the value of Y_{PO4} obtained for the second anaerobic zone of the EAAO reactor was even lower at 0.02. In the second anaerobic zone the phosphorus release rate was much lower than for first zone (0.4 compared to 3.2 mg P/g VSS/h).

This was also seen in the EAAO batch test, were the two rates of phosphorus release were 3.9 and 1.0 mg P/g VSS/h respectively. In the EAAO batch test two stage Y_{PO4} values were obtained of 0.23 and 0.12 mg P/mg COD respectively with an overall value of 0.19 mg P/mg COD. The batch test with the single anaerobic zone AAO sludge had a single Y_{PO4} value of 0.34 mg P/mg COD, as the anaerobic phase was only 180 minutes duration with just a single phosphorus release rate. The ratio for this batch test was much closer to the previously mentioned literature values. By using the data obtained from the SBR pilot scale trial with fermented cheese processing wastewater by Comeau *et al.* (1996), a phosphorus release to soluble COD consumed ratio of about 0.07 was obtained. Possible reasons for the low Y_{PO4} ratio in the continuous reactor studies in this chapter are; the mixed VFA source, further fermentation occurring within the anaerobic zone, the partial consumption of VFA during denitrification, and the possible presence of organisms such as GAO's that can competitively sequester substrate under anaerobic conditions. Ky *et al.* (2001), in calibrating a model for the EBPR process from a fermented cheese whey effluent determined that a value of 0.22 mg P/mg acetate COD, was required for successful model calibration. This was attributed to the mixed VFA source and the presence of GAO's. Cech and Hartman (1993), observed that GAO's can take up organic

substrates without using polyphosphate and that the $P_{released}/COD_{consumed}$ ratio decreases as the relative dominance of GAO bacteria increases. Tasli *et al.* (1997), established EBPR using an SBR with an influent of 80% Tryptone Soya Broth (TSB) and 20% acetate, that resulted in an mixed liquor phosphorus content of 15% mg P/mg VSS and a $P_{released}/COD_{consumed}$ ratio of 0.26. When the acetate was replaced with glucose, a substrate that can favour the growth of GAO's, the $P_{released}/COD_{consumed}$ ratio decreased to 0.15. A value of 0.70 mg P/mg COD was also obtained from a batch test using mixed liquor with a sludge phosphorus content of 14.2 %mg P/mg VSS from one SBR trial in which the actual SBR operating ratio was 0.22. The amount of glucose was further increased to 50% (the TSB was decreased to 50%), and the $P_{released}/COD_{consumed}$ ratio decreased to 0.095. There was no significant variation or decrease in anaerobic COD utilisation during these changes in influent composition, which was attributed to the fact that the proportion of GAO's had increased when acetate was replaced with glucose.

Oehmen *et al.* (2001), performed batch tests with acetate as the sole carbon source using mixed liquor from six full scale Australian EBPR treatment plants and also performed microscopic identification of bacteria. The $P_{released}/COD_{consumed}$ ratio's varied from 0.05 to 0.23 mg COD/mg P, which equated to between <1% to 12% of bacteria that were identified as a type of GAO called *Competibacter*. The value of 0.34 mg P/mg COD obtained from the batch test of the AAO system in this study, with acetate as the sole carbon source, is greater than the highest value obtained by Oehmen *et al.* (2001), of 0.23. Oehmen *et al.* (2001), identified only a small amount of GAO's as being present at the treatment plant with Y_{PO4} value of 0.23. The ratio of 0.34 obtained from the AAO batch test is close to the value of 0.37 calculated by Abu-ghararah and Randall (1991), when using acetic acid. This suggests that the presence of GAO's were not significant in the single anaerobic zone AAO system study, but more were more significant in the EAAO were the value of Y_{PO4} generated from the batch test was 0.19.

Smolders *et al.* (1994), also attributed a variation in the ratio of anaerobic phosphorus release to VFA uptake ratio to varying pH values, with higher ratio's obtained at higher pH values. The pH in zone 1 for the AAO and EAAO continuous reactors was

7.3 and 7.2 respectively. The pH in both the batch test anaerobic phases was 7.2. Using the empirical relationship between pH and Y_{PO4} calculated by Smolders *et al.*, (1994), the theoretical ratio at a pH of 7.2 would be 0.25 mg P/mg COD. This value is close to the value of 0.23 mg P/mg COD calculated for the initial phosphorus release stage of the EAAO batch test but less than the value from the AAO batch test (0.34).

Table 6.8: Anaerobic/anoxic phase stoichiometric constants from AAO and EAAO zone studies and for both batch test of mixed liquor.

Stoichiometric Constant	Anaerobic zone			Anoxic zone		Batch Test	
	AAO	EAAO		AAO	EAAO	AAO	EAAO**
Zone number	1	1	2	2	3	-	-
$P_{release}$/ COD $_{consumed}$ (mg P/mg COD)	0.05	0.06	0.02	15.1	0.99	0.34	0.19
$P_{release}$/ VFA COD $_{consumed}$ (mg P/mg COD)	0.05	0.07	0.02	0.78	0.89	0.34	0.19
$P_{release}/PHA_{stored}$ (mg P/mg COD)	0.27			0.28			
$PHA_{stored}/VFA_{consumed}$ (mg COD/mg COD)	0.2			2.76			
$Glycogen_{consumed}/VFA_{consumed}$ (mg COD/mg COD)	0.22			1.96		0.39	

** EAAO batch test had two separate Y_{PO4} values of 0.23 and 0.12 mg P/mg COD.

The ratio of phosphorus released to PHA stored for the continuous AAO reactor in this study was 0.27 mg P/mg PHA COD in the anaerobic zone and 0.28 mg P/mg PHA COD in the anoxic zone. Both of these values are unaffected by the further production of short chain VFA's in the anaerobic and anoxic zones as was potentially the case for the phosphorus to acetate ratio. Due to the loss of samples for PHA analysis from the AAO batch test, EEAO reactor and EEAO batch test, due to refrigeration failure, a ratio for these tests was not calculated. The AAO anaerobic and anoxic zone values are both slightly less than the value of 0.32 calculated from the

data of Smoulders *et al.* (1994). The value by Smoulders *et al.* (1994), was calculated with acetate as the sole carbon source, while the fermented effluent VFA fraction fed to the AAO system anaerobic zone contained only 34% acetate. The anoxic zone received VFA from the anaerobic zone of which 53% was acetate. If a significant proportion of competing organisms such as GAO's were present in the sludge then this ratio would be expected to be low due to the anaerobic storage of PHA's by both PAO's and GAO's, but phosphorus release by PAOs only.

The mixed nature of the short chain volatile acids in the anaerobic zone resulted in a low anaerobic zone ratio of PHA produced to VFA COD consumed. The anaerobic zone value of 0.2 mg PHA COD/mg VFA COD is much less than the value of 1.5 found in the biochemical model of Smolders *et al.* (1994), and the value of 1.24 calculated by Christensson (1997), when using ethanol as a carbon source for EBPR. By using the models proposed by Comeau *et al.* (1986), and Wentzel *et al.* (1986), Abu-Ghararah and Randall (1991), calculated theoretical ratios for acetic acid and butyric acid of 0.67 mg PHB/mg COD used. The ratio for valeric acid was calculated as 0.36 mg PHB/mg COD (no actual values were experimentally determined for this ratio during their study).

The glycogen consumed to the VFA consumed ratio in the anaerobic zone for the continuous AAO reactor of 0.22 mg glycogen COD/mg VFA COD was also less than the value of 0.5 calculated by Smolders *et al.* (1994). The value calculated from the AAO batch test of 0.39 mg glycogen COD/mg VFA COD was slightly greater that the AAO continuous reactor, but still less than this literature values. Liu *et al.* (1997), varied the P/COD ratio in order to influence competition between between PAO's and GAO's and found that the lower the glycogen consumed to the acetate consumed ratio the higher the phosphorus content of the sludge and the lower the dominance of GAO's. This was because as GAO's became more dominant, the anaerobic degradation of glycogen became more important. This again suggests that GAOs were not a significant influence in the AAO system, and other factors contributed to the low Y_{PO4} values in the AAO reactor such as the mixture of VFA and continued fermentation in the unaerated zones.

6.6 Conclusion

A combined nitrogen and phosphorus removal system with a 3 hour actual anaerobic retention time was able to remove a maximum of 41.5 mg P/L, and an average of 36.3 mg P/L. Effluent nitrate concentrations of up to 19.3 mg NO_3-N/L and 4.6 mg NO_3-N/L did not adversely affect phosphorus removal. An average of 74% of the fermenter soluble COD was consumed in the anaerobic zone at the time of the zone study. After the reactor was optimised as much as possible, phosphorus release and PHA storage occurred in both the anaerobic and anoxic zones. On the day of zone study, 24.2 and 15.1 mg P/L of phosphorus release occurred in the anaerobic and anoxic zone respectively. The final aerobic sludge phosphorus concentration averaged 7.0 % mg P/mg VSS after system optimisation.

By slightly modifying the previous reactor system and doubling the actual anaerobic retention time to 6 hours, 41.3 mg P/L of phosphorus was removed at the time of the zone study after 40 days operation. The final aerated zone sludge had a phosphorus concentration of 5.61 %mg P/mg VSS, lower than the previous system, as the VSS concentration of the EAAO system was 47% greater than the AAO system. After 40 days operation this system had effluent nitrate concentrations of 6.2 mg NO_3-N/L. In the first anaerobic zone 69% of the fermenter soluble COD was consumed while in the second anaerobic zone 71% of the available soluble COD was consumed in the second anaerobic zone. Phosphorus release occurred in both anaerobic zones (29.9 and 3.5 mg P/L) and the anoxic zone (8.3 mg P/L). The time required for maximum phosphorous release to occur, was confirmed by an anaerobic batch test were maximum phosphorus release took 6 hours. The increased anaerobic HRT in the AAO system appeared to have only a relatively small benefit in the medium term (40 days operation).

Due to the mixed short chain VFA composition of the fermented effluent, and possible further fermentation in the anaerobic zone, low anaerobic zone phosphorus release to VFA COD consumed ratios were observed in the continuous reactor. Batch tests with acetate as the sole carbon source showed that the ratio's obtained from the batch tests were closer to the literature values used in previous models of the EBPR process. This lead to the conclusion that GAO bacteria were not a significant factor in

the competition for carbon sources under anaerobic conditions in these initial AAO and EAAO systems.

CHAPTER 7
Phosphorus Removal -AO Zoned Reactor Studies

7.1 Introduction

In this chapter, the zoned AAO reactor system used in Chapter 6 was reconfigured as an AO process with one anaerobic zone only, and the other 9 zones were aerobic. The anaerobic zone retention time was maintained at 3 hours. It was decided that the extended anaerobic zone (6 hour HRT) system would not be used as there appeared to be only a small amount of benefit and the potential long term negative effects through the growth of competing organisms were not known. As the system was no longer configured for nitrogen removal, the nitrogen content of the synthetic wastewater was decreased. This was to minimise the potential inhibition of the EBPR process by nitrate (Table 7.1). The proportion of whole and skim milk powder added in the AAO study (Chapter 6) was by decreased by 28% in this AO study to decrease the COD/TKN ratio from 28 to 32. The soluble COD (GFC filtered) was maintained close to that of the previous wastewater (~2200 mg/L) by adding COD in the form of lactose. The average wastewater characteristics of this modified synthetic wastewater are listed in Table 7.2.

By operating the zoned reactor with an AO configuration, it was intended to determine the maximum amount of phosphorus removal possible with this type of system and how this compared with the previous AAO configuration. The influence of the anoxic zone role on the phosphorus removal process with the previous configuration could also be assessed by its removal in this study. In some instances in New Zealand, dairy processing wastewaters have COD/TKN ratio's greater than the value of 32 used in this Chapter. In Rules (1997) study of a full-scale aerated activated sludge process (non-BNR) study at a New Zealand dairy-manufacturing site, an average COD/TKN ratio of 39 was calculated during the period of the study. The fermented cheese processing wastewater used in the SBR study by Comeau *et al.* (1996), also had a COD/TKN ratio of 32, with only a minimal amount of nitrate formed during the reactor operation (0.6 mg NO_3-N/L).

Table 7.1: Synthetic dairy processing wastewater used in AO zoned reactor studies.

Component	Amount to make 10 litres
Whole milk powder	7.2 g
Skim milk powder	7.8 g
Lactose	6.0 g
Butter	1.5 g
KH_2PO_4	3.75 g
NaOH (45% w/w)	20 ml

Table 7.2: Chemical and physical characteristics of the modified low nitrogen synthetic dairy processing wastewater.

Characteristic	Concentration/Value
COD_{total}	2600 mg COD/L
$COD_{GFC\ filtered\ (<1.2\ \mu m)}$	2280 mg COD/L
$COD_{filtered\ (<0.45\ \mu m)}$	2050 mg COD/L
TKN	82 mg N/L
Total Phosphorus	95 mg P/L
Soluble Phosphorus	85 mg P/L
TSS	280 mg/L
pH	11

7.2 Reactor System

As with the AAO configuration, the AO reactor was operated with an HRT of 2.5 days and a feed rate of 8 litres/day of fermented wastewater. The schematic of the overall process is shown in Figure 7.1, which is identical that used in the Chapter 6 except that the anoxic zone and the internal recycle have been eliminated. Figure 7.2 shows a photo of the zoned reactor in an AO configuration. The synthetic wastewater

(stored at 4°C) was again first fermented in a 10 litre reactor at 35°C with an HRT of 12 hours and a set pH of 6.5. The zoned reactor SRT was maintained at 10 days, without reseeding. During the initial 10 day SRT operation, another zoned reactor was constructed, enabling two zoned activated sludge reactors to be operated simultaneously in the next part of the study. The 20 litres of fermented wastewater produced per day was sufficient to provide the required 16 L/d for two reactors (8 L/d each). The fermented wastewater (refrigerated at 4°C) was pumped to the zoned EBPR reactors, which were operated at a temperature of 20°C. The reactor temperatures were controlled by circulating water from a 20°C temperature controlled water bath through water jackets surrounding the perimeter of the reactors. A temperature probe in the mixed liquor of reactor measured the temperature, which was displayed on a digital display attached to side of each reactor.

As previously mentioned the initial AO reactor was initially operated at an SRT of 10 days for 40 days (4 SRT's duration) before a full zone study was performed. During the 40 days of operation at an SRT of 10 days, the stability of the reactor was monitored by periodically measuring soluble phosphorus concentrations in the effluent. After the initial 40 days operation at an SRT of 10 days a full study was performed. In the next phase of the AO system study, two reactors were operated each at a different SRT. One was operated at a 15 day SRT for 79 days before performing a full zone study and one was operated at a 5 day sludge age for 56 days before a full zone study was performed. Each reactor was identical and each operated in an AO configuration receiving the same wastewater. The objective of operating at a range of SRT's was to determine if there were either beneficial or negative effects on the EBPR process at different SRT's with this type of wastewater. The operational parameters for the AO reactors are shown in Table 7.3.

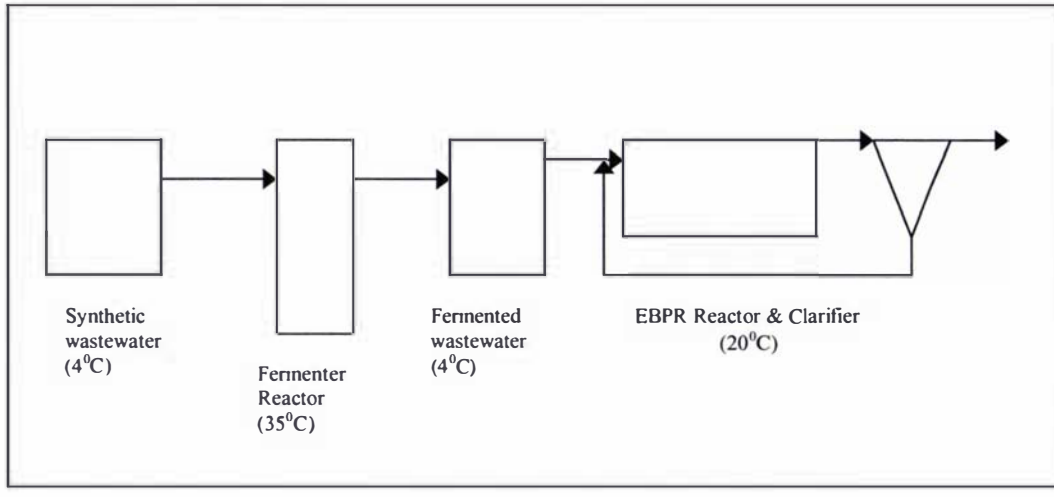


Figure 7.1: Schematic of laboratory treatment system.

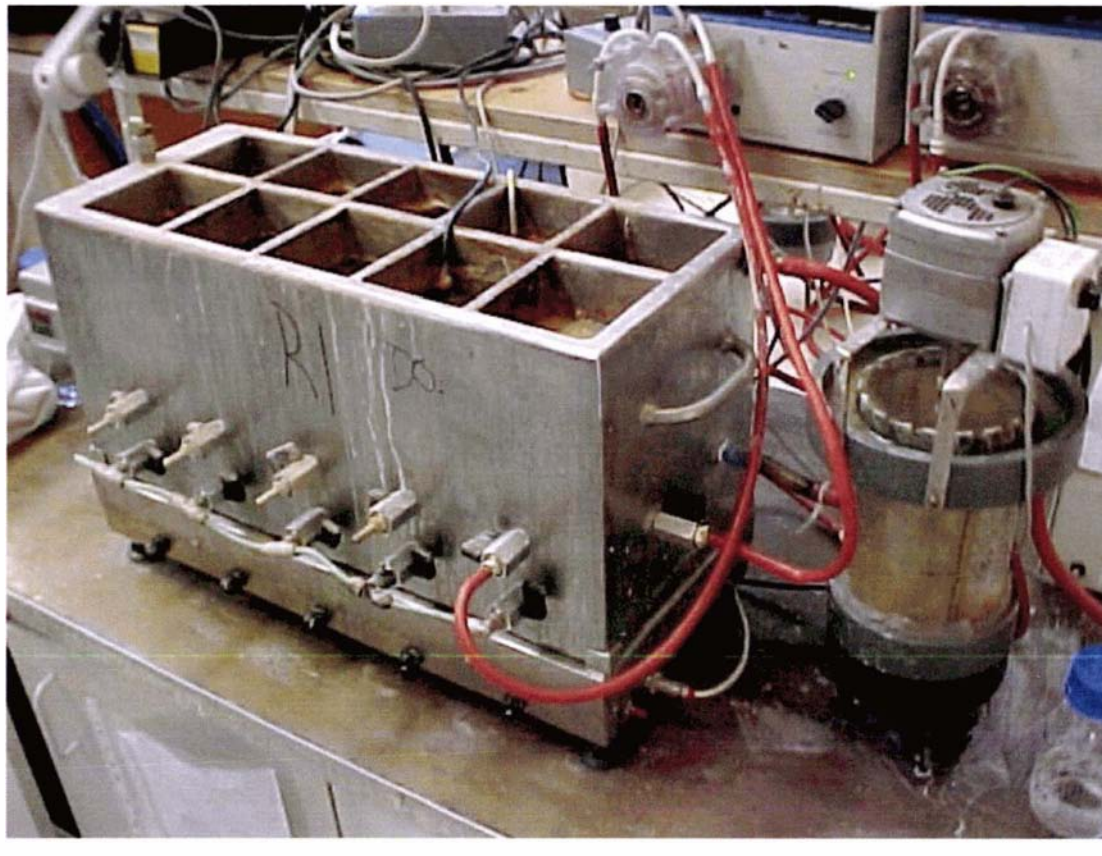


Figure 7.2: Zoned laboratory-scale AO activated sludge EBPR reactor system.

Table 7.3: AO reactor system operational parameters.

	Influent flowrate(L/d)	Temperature (^{0}C)	RAS flowrate (L/d)	pH set-point
Fermenter	20 L/d	35	-	6.5
EBPR Reactor	8	20	8	-

7.3 Fermenter Operation

The fermenter operation was periodically monitored during a 79 day period of operation by monitoring the COD and short chain volatile acids. This provided additional fermentation data for the modified low nitrogen wastewater. The total and soluble (GFC filtered) COD profiles for the fermenter effluent are shown in Figure 7.3. The fermenter effluent soluble COD averaged 1570 mg/L compared to the previous study (Chapter 6) with the high nitrogen wastewater where the soluble COD was 1320 mg/L. This increase in soluble COD was most likely due to the increased lactose concentration in the synthetic wastewater and the fact that it is easily fermented. The soluble COD of the fermented wastewater was 69% of the soluble COD of the initial synthetic wastewater. The individual VFA are plotted in Figure 7.4 and summarised in Table 7.4. Acetic acid was again the main VFA produced (39.9%) and n-butyric acid was the next most common VFA (21.8%). The total short chain VFA concentration averaged 1028 mg/L, which was lower than the average VFA concentration for the high nitrogen fermented wastewater (1345 mg/L). The fraction of each individual VFA was similar between both wastewater's except for n-valeric acid which was only 6.7% of the total VFA COD compared to 12.7% for the high nitrogen wastewater. The main difference with the low nitrogen wastewater was that the total VFA COD accounted for only 67.8% of the soluble COD (<0.45μm) as opposed to 85.7% for the high nitrogen wastewater. This difference was most likely due to the production of intermediate products such as lactic acid, that are not measured as short chain VFA. The percent acidification (based on synthetic wastewater GF/C soluble COD) of the low nitrogen wastewater averaged 45% compared to 51% for the previous high nitrogen wastewater.

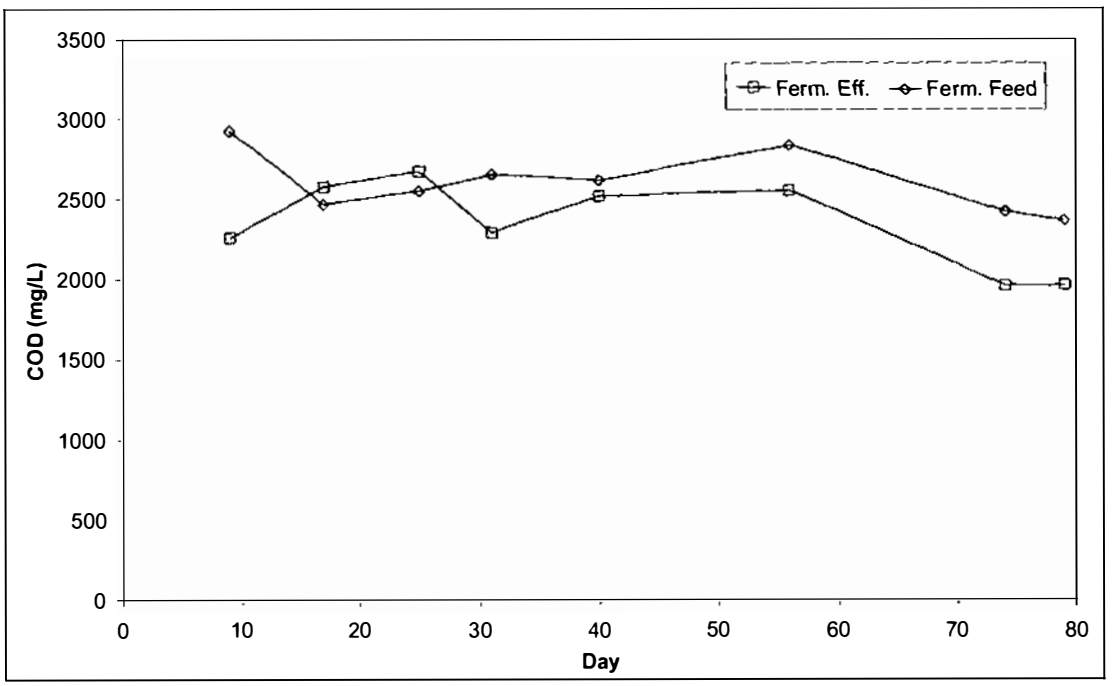


Figure 7.3: Fermenter feed and effluent COD profile during reactor operation.

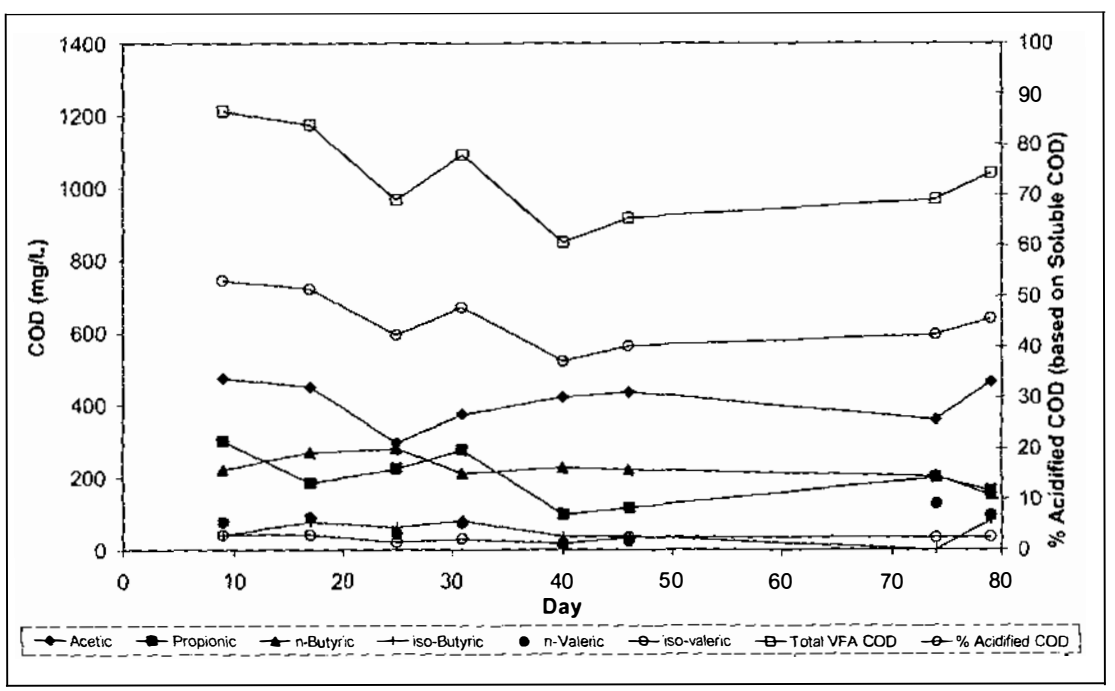


Figure 7.4: VFA COD fractionation, total VFA COD and % acidification of fermenter effluent.

Table 7.4: Average fermented wastewater individual and total VFA COD concentrations and fractions and total soluble $COD_{0.45\mu m}$.

	COD (mg/L)	% Total VFA COD	% Fermenter Effluent COD (<0.45μm)
Acetic acid	410	39.9	27.0
Propionic acid	196	19.1	12.9
n-Butyric	224	21.8	14.8
Iso-Butyric	53	5.2	3.5
n-Valeric	69	6.7	4.5
Iso-Valeric	65	6.3	4.3
Total VFA COD	1028	-	67.8
Fermeneter Effluent	1517 (<0.45μm)	-	-

7.4 10 Day SRT AO Reactor Zone Study

A full zone analysis of the zoned reactor was performed after 40 days operation at an SRT of 10 days. The zones mixed liquor TSS averaged 2825 mg/L throughout the reactor with an anaerobic zone VSS/TSS ratio of 0.857 (ash content of 14.3%) and a final aerobic zone VSS/TSS ratio of 0.793 (ash content of 20.7%) (Figure 7.5). The increase in ash content of 6.4% from zone 1 to zone 10 was due to the sludge intracellular storage of phosphorus which resulted in a phosphorus removal of 34.9 mg PO_4-P /L (total phosphorus of fermenter effluent was 104.2 at time of zone analysis). The soluble phosphorus concentration decreased from zone 1 (128.5 mg PO_4-P/L) to zone 6 (67.7 mg PO_4-P/L) and then remained relatively constant for the remaining aerobic zones (Figure 7.6). The soluble COD concentration in the anaerobic zone was 358 mg COD/L with the VFA COD component in this zone measured as 335 mg COD/L. The amount of VFA consumed within the anaerobic zone was 225 mg COD/L or 40% of the available VFA. This compared to anaerobic zone soluble COD consumption of 390 mg COD/L or 52% of the available soluble COD, assuming that no particulate COD was hydrolysed. The rate of anaerobic COD consumption was 33 mg COD/g VSS/h. The quantities and of each individual short chain VFA are shown in Table 7.5

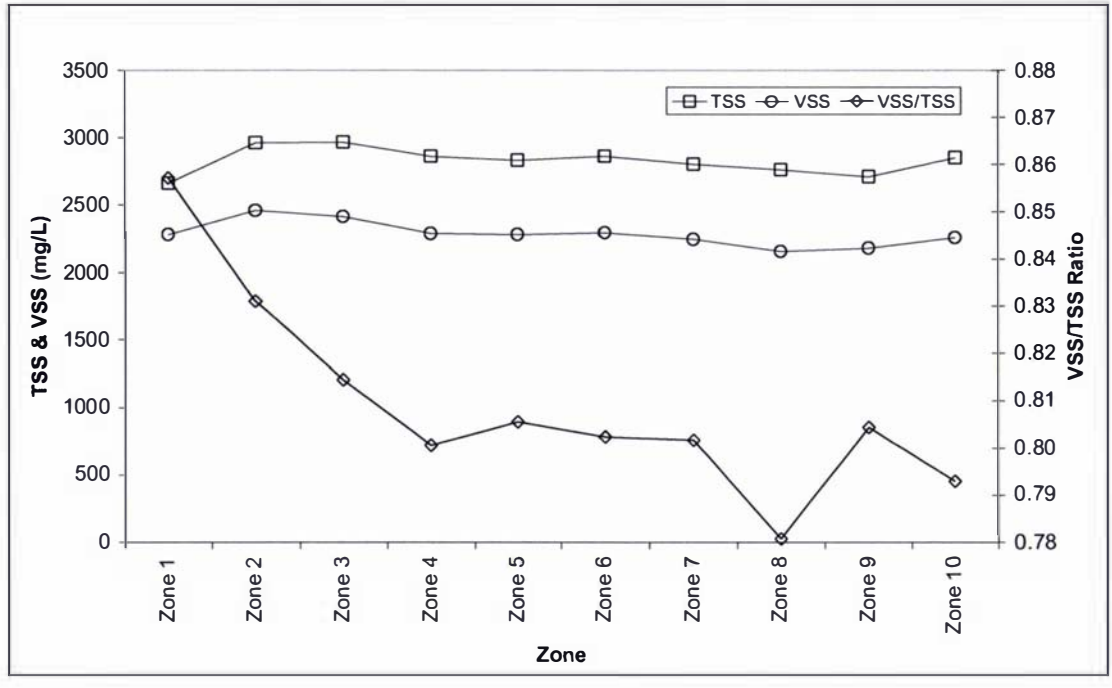


Figure 7.5: Zone profiles of TSS, VSS and the VSS/TSS ratio for 10 day SRT AO zoned reactor.

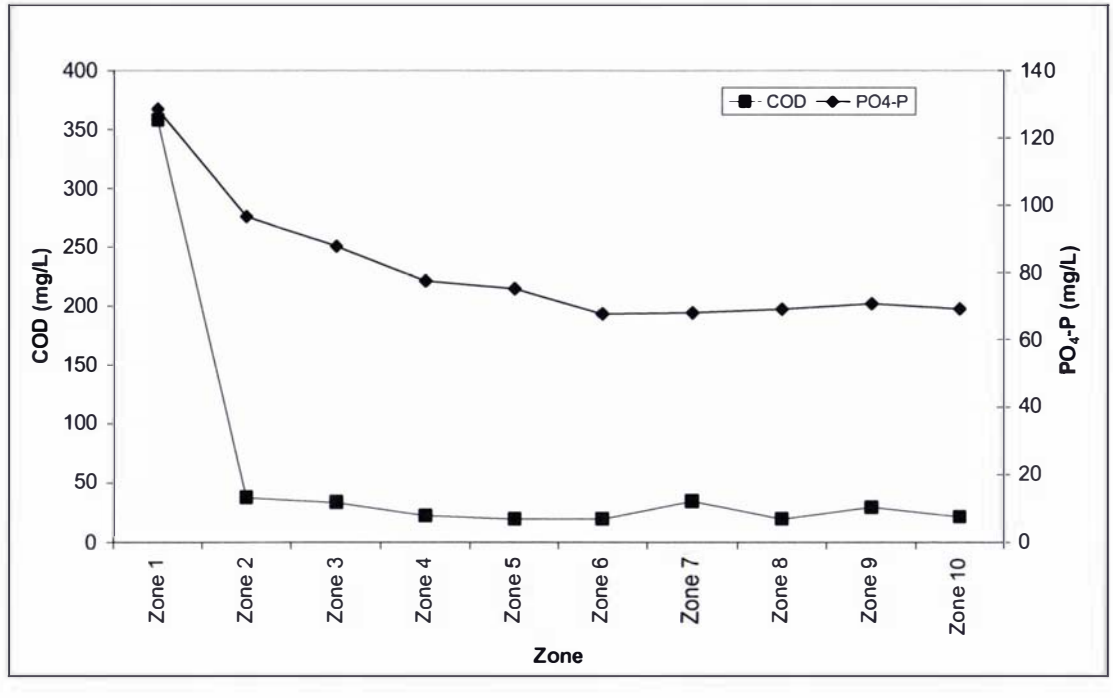


Figure 7.6: Zone profiles of soluble COD and PO_4-P for the 10 day SRT AO zoned reactor.

Table 7.5: Fermenter effluent and anaerobic zone (zone 1) individual short chain VFA COD concentrations, the % of total VFA COD and the % consumption of anaerobic zone individual VFA based on fermenter effluent VFA concentrations.

VFA	Concentration (mg COD/L)		% Total VFA COD		Consumption of VFA COD (%)
	Fermenter Effluent	Anaerobic Zone	Fermenter Effluent	Anaerobic Zone	Anaerobic Zone
Acetic acid	352.8	138.6	31.0	41.4	20.2
Propionic acid	202.0	39.6	17.8	11.9	60.2
n-Butyric acid	283.1	66.8	24.9	19.9	52.0
Iso-Butyric acid	57.0	8.5	5.0	2.5	69.6
n-valeric acid	162.1	48.0	14.2	14.3	40.0
Iso-Valeric acid	63.5	26.9	5.6	8.0	14.0
n-Caproic acid	16.8	6.6	1.5	2.0	19.8
Total VFA	1137.3	335	-	-	40.2
Soluble COD	1515	358	-	-	-

The acetic acid concentration in the anaerobic zone comprised 41.4% compared to 31.0% for the fermenter effluent. This increase in acetic acid proportion mostly likely reflected a conversion of some of the larger molecular weight VFA to acetic acid before consumption. In Table 7.5 the percent consumption of the anaerobic zone VFA COD is based on the fermenter effluent VFA composition and does not include possible inter-VFA conversion. The value of only 20.2% consumption for acetic acid, as opposed to the value of 69.6% for iso-butyric acid, also reflects conversion of other VFA to acetic acid. The difference between the consumption of soluble COD (390 mg COD/L) and short chain VFA (225 mg COD/L) was 165 mg COD/L. While some of this difference may reflect the direct consumption of non-VFA COD, most of the difference is likely to be due to the conversion of non-VFA soluble COD to VFA. This makes it very difficult to accurately quantify routinely the amount of VFA consumption with the anaerobic zone.

The amount of phosphorus uptake in each zone is shown in Figure 7.7. The amount of phosphorus release in the anaerobic zone was 41.6 mg/L at a rate of 6.9 mg P/g VSS/h. Aerobic phosphorus uptake was greatest in the first aerobic zone (31.8 mg/L in zone 2) with decreased uptake in zones 3 to zones 6, with very little uptake or release occurring in the final four aerobic zones. The ratio of P-release to soluble COD consumed in the anaerobic zone was 0.11 mg P/mg COD while the ratio of P-release to VFA COD consumed was 0.18 mg P/mg VFA COD. The ratio of 0.18 mg P/mg VFA COD is greater than what the actual value would be which would be due to additional fermentation occurring within the anaerobic zone. If there was hydrolysis of any particulate matter within the anaerobic zone than the value of 0.11 mg P/mg COD could also be slighter greater than the actual ratio. This Y_{PO4} value is greater than the values obtained in Chapter 6 for the AAO and EAAO configurations of 0.05 and 0.06 mg P/mg COD consumed.

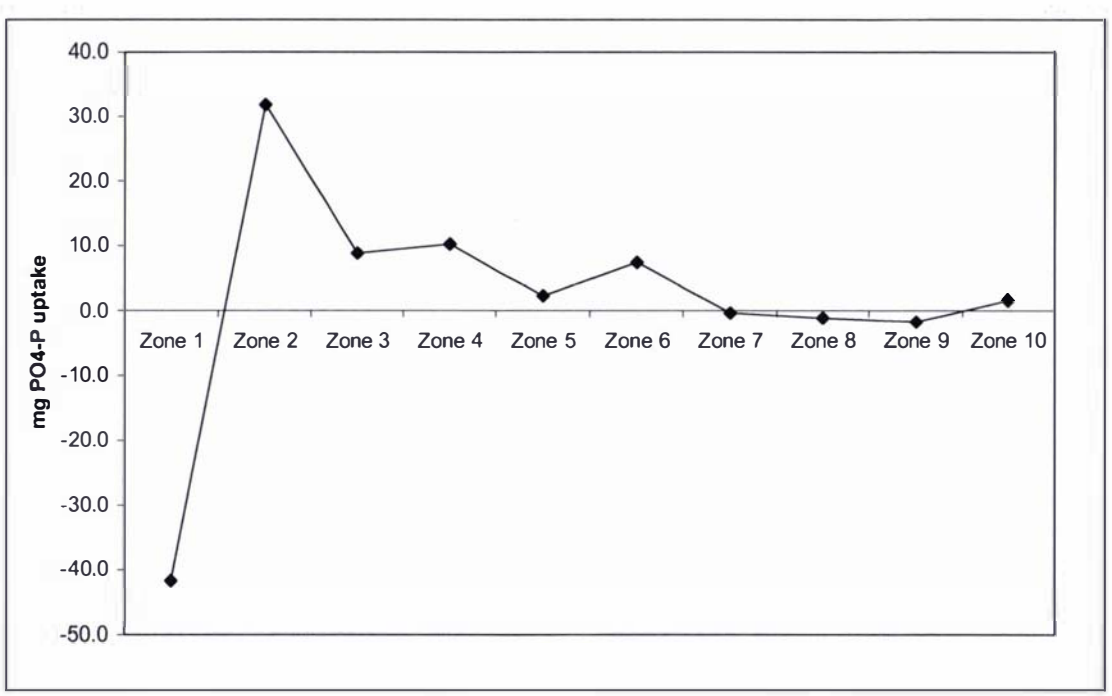


Figure 7.7: PO_4-P uptake for each zone for the 10 day SRT AO zoned reactor.

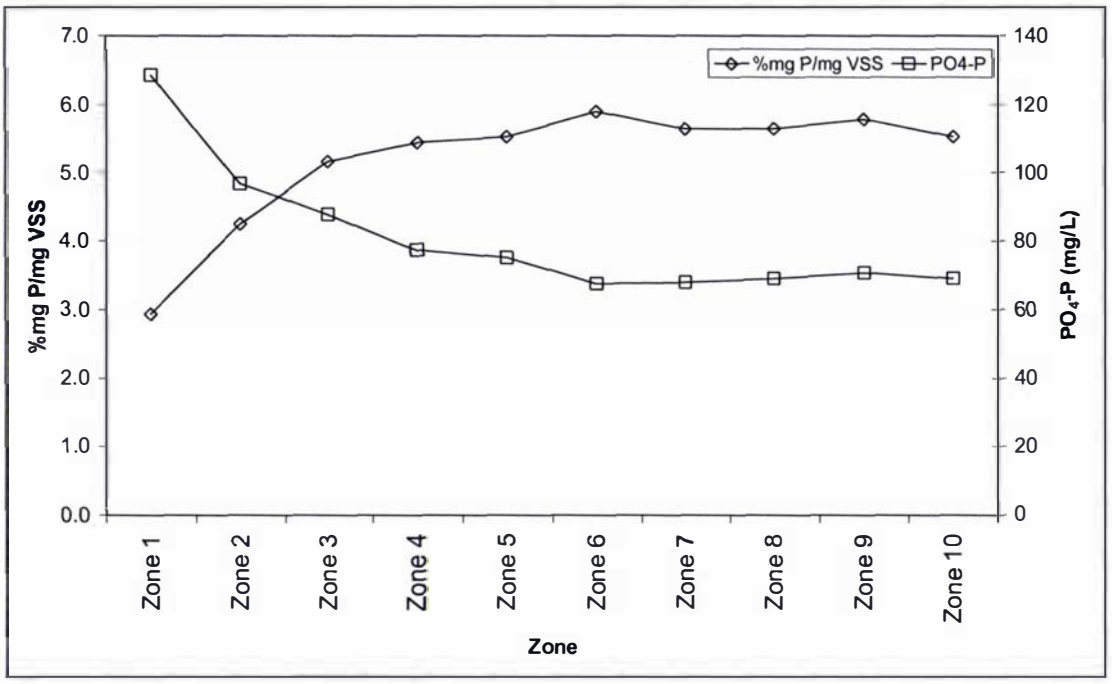


Figure 7.8: Soluble PO_4-P and sludge phosphorus profiles for each zone for the 10 day SRT A O reactor.

The phosphorus content of the sludge varied from 2.94 % mg P/mg VSS in the anaerobic zone to 5.53 % mg P/mg VSS in the final aerobic zone (Figure 7.8), with a maximum value of 5.89 in zone 6. A total of 34.9 mg P/L was removed by the reactor. This resulted in 279.2 mg P removed per day in the 2 litres of waste activated sludge volatile solids both pumped from the final aerobic zone and lost in the effluent (4520+520 =5040 mg VSS/day). This is equivalent to a theoretical waste activated sludge phosphorus concentration of 5.56 % mg P/mg VSS in the final aerobic zone, which is almost the same as the measured value. The sludge phosphorus content of 2.94 % mg P/mg VSS in the anaerobic zone suggests there may possibly be more phosphorus that could be released in this zone. Especially when compared to the value of 2.3 % mg P/mg VSS in the second zone of the EAAO system in Chapter 6.

The zone nitrate concentrations shown in Figure 7.9 means that there was slight surplus of nitrogen in the wastewater to that required for metabolic requirements by the activated sludge system. The nitrate reached a maximum of 1.4 mg NO_3-N/L in zone 9 and decreased to 1.0 mg NO_3-N/L in zone 10 were the dissolved oxygen was

0.5 mg O_2/L. This low nitrate concentration would not have interfered with the EBPR process.

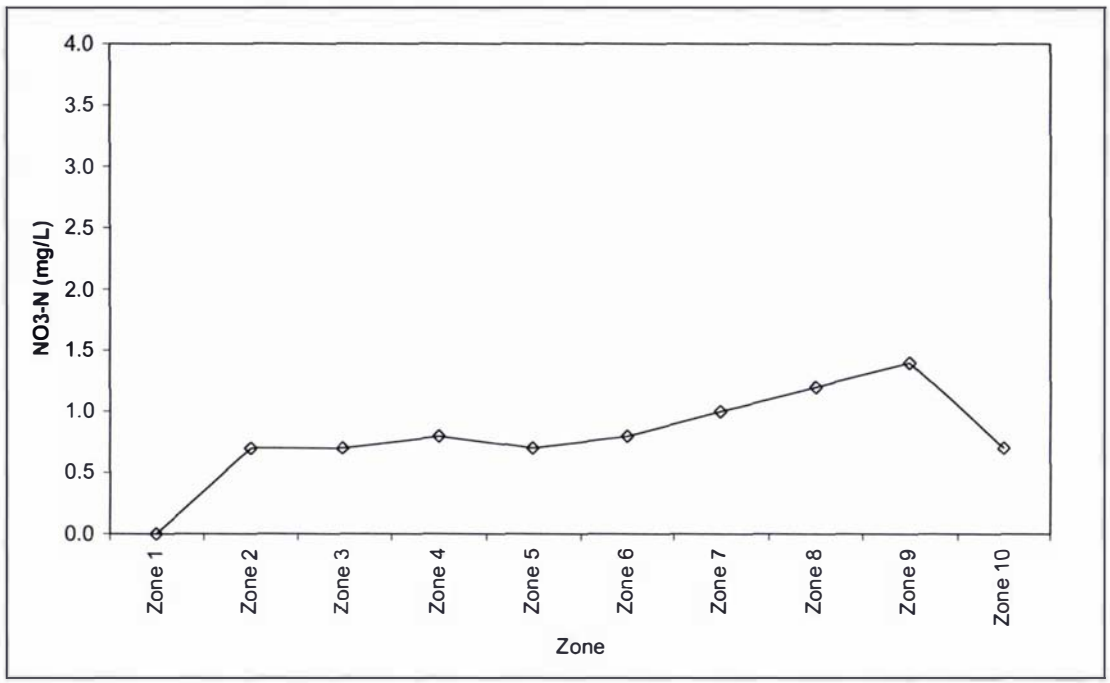


Figure 7.9: Zone nitrate concentrations for each zone for the 10 day SRT AO zoned reactor.

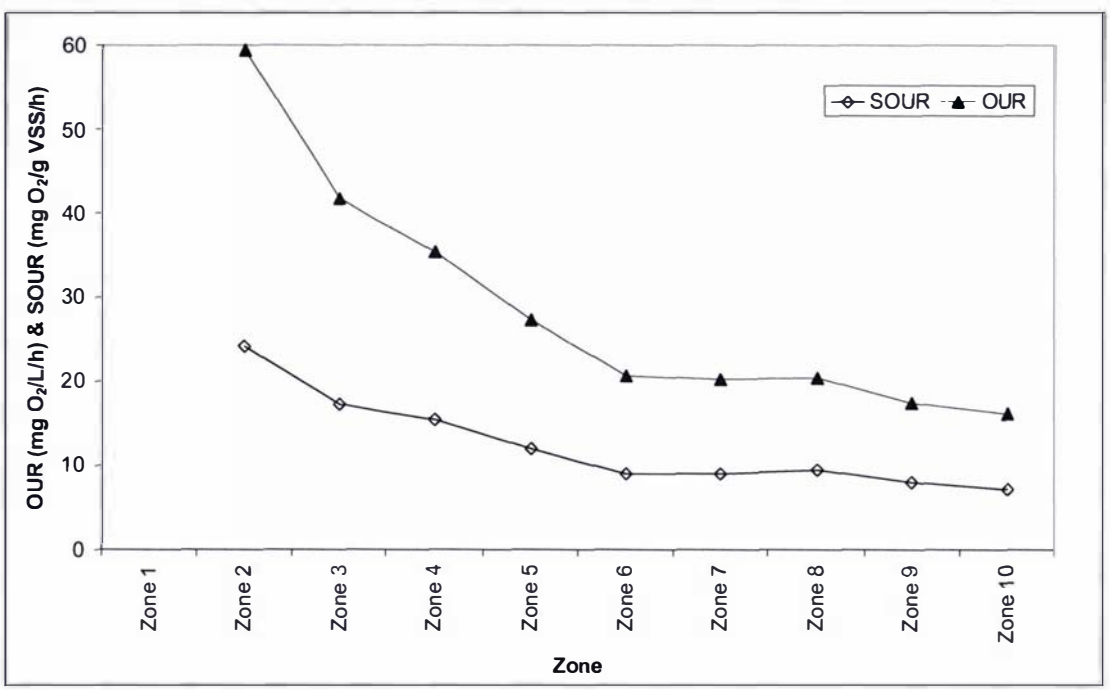


Figure 7.10: Specific oxygen uptake rates (SOUR) and oxygen uptake rates (OUR) for each zone for the 10 day SRT AO reactor.

Table 7.6: Individual zone parameters for AO configuration at an SRT of 10 days.

	Zone									
Parameter	1 Anaerobic	2 Aerobic	3 Aerobic	4 Aerobic	5 Aerobic	6 Aerobic	7 Aerobic	8 Aerobic	9 Aerobic	10 Aerobic
$COD_{soluble}$ (mg/L)	358	38	34	23	20	20	35	20	30	22
PO_4-P (mg/L)	128.5	96.7	87.8	77.5	75.2	67.7	68.0	69.1	70.8	69.2
%mg P/g VSS	2.94	4.25	5.16	5.44	5.52	5.89	5.64	5.64	5.78	5.53
NO_3-N (mg/L)	0	0.7	0.7	0.8	0.70	0.80	1.0	1.2	1.4	1.0
SOUR mgO_2/mg VSS/h		24.2	17.3	15.5	12.0	9.0	9.0	9.5	8.0	7.2
Phosphorus Uptake (mg P/L)	-41.6	31.8	8.9	10.3	2.3	7.5	-0.3	-1.1	-1.7	1.6
TSS (mg/L)	2660	2960	2965	2860	2830	2860	2800	2760	2710	2850
VSS (mg/L)	2280	2460	2415	2290	2280	2295	2245	2155	2180	2260
VSS/TSS ratio	0.86	0.83	0.82	0.80	0.81	0.80	0.80	0.78	0.80	0.79

The biological activity was measured using oxygen uptake rates (OUR) (Figure 7.10) which reached a maximum in the first aerobic zone (Zone 2) with an OUR value of 59.4 mg O_2/L/h and a specific OUR (SOUR) value of 20.1 mg O_2/g VSS/h. The OUR values levelled off by zone 6, the same zone that the phosphorus decrease levelled off (Figure 7.6). The SOUR value of 7.2 mg O_2/g VSS/h in the final zone is between that of the previous AAO and EAAO systems. The analytical parameters for each zone are shown in Table 7.6.

7.4.1 Batch Test

A batch test was performed with the mixed liquor from the 10 day SRT AO reactor. Two litres of mixed liquor was used for the test with an anaerobic phase of 300 minutes and an aerobic phase of 300 minutes. The long anaerobic phase was used to ensure that the maximum amount of phosphorus release occurred. Sodium acetate (391 mg/L) was added to give an initial starting COD of 320 mg/L. The initial VSS concentration was 2330 mg VSS/L and the initial sludge phosphorus concentration was 5.4 % mg P/mg VSS. The pH was controlled at 7.0 throughout the anaerobic phase and aerobic phases.

Figure 7.11 shows the COD consumption and phosphorous release trend. There was an initial high COD consumption rate of 21.6 mg COD/g VSS/h which then decreased to 7.9 mg COD/g VSS/h. The initial high rate of phosphorus release of 4.6 mg P/g VSS/h decreased to 2.9 mg P/g VSS/h after 150 minutes. The phosphorus released to COD consumed ratio was 0.45 mg P/mg COD, which is close to the theoretical value of 0.48 mg P/mg COD in the biochemical model by Wentzel *et al.* (1991). The fact that consumption ceased after 180 minutes suggests that the design of the continuous reactor with an anaerobic retention time of 180 minutes was close to optimal.

After the anaerobic phase, the phosphorus content of the sludge decreased to 2.5 % mg P/mg VSS from an initial value of 5.4 % and increased to 5.0% after the aerobic phase (Figure 7.12). The VSS/TSS ratio initial value was 0.75 and increased to 0.80 after the anaerobic phase, a decrease in the ash content of 4.9%. After aerobic conditions the ratio decreased to 0.76. Both the net change in the sludge phosphorus content and the change in the ash content, reflect the fact that there was a net increase

in the soluble phosphorus concentration over the duration of the batch test of 5.5 mg P/L. This net decrease was most likely due to the aerobic consumption of the remaining acetate decreasing the rate of PHA consumption and the uptake of phosphorus. Due to this residual acetate a longer aerobic phase was required than the 300 minutes used.

The very high oxygen uptake rate in Figure 7.13 at the beginning of the aerobic phase is due to unconsumed COD of 173 mg COD/L. After the initial high oxygen uptake rate, three separate phases are evident, 330 to 390 minutes, 420 to 510 minutes and 540 to 600 minutes. Each of these phases correspond to the changing rates of phosphorus uptake over the same three time intervals (Figure 7.11) of 19.0, 10.4 and 5.1 mg P/L/h. The analytical parameters at the beginning of the anaerobic (t=0) and aerobic (t=300) phases and the end of the batch test (t=600) are presented in Table 7.7.

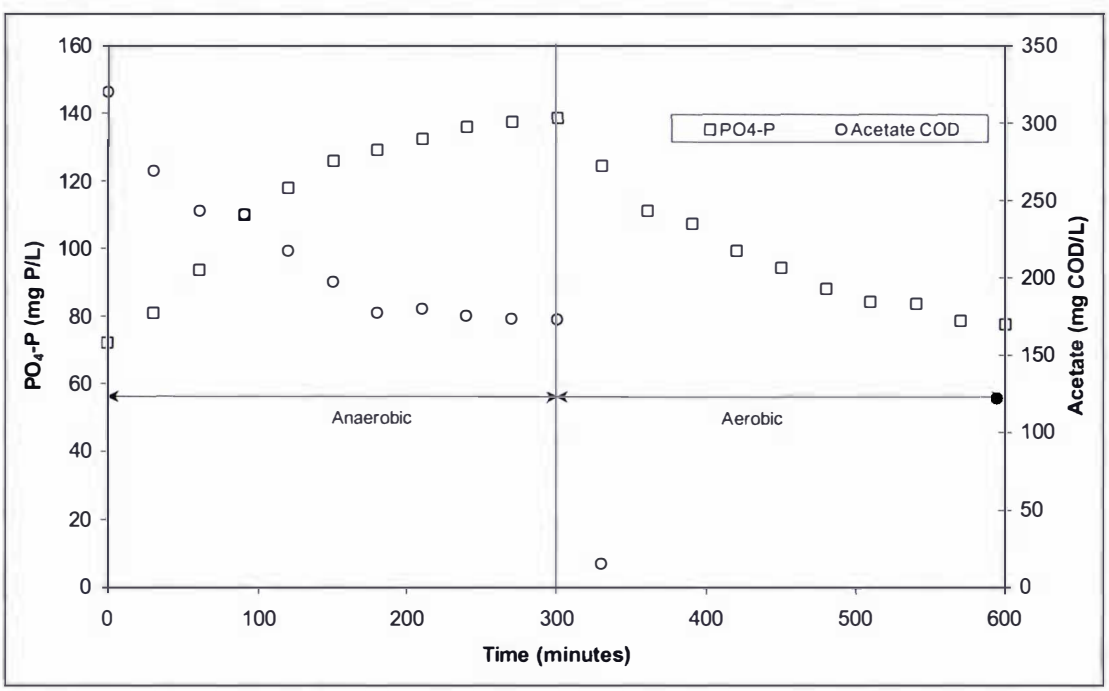


Figure 7.11: Acetate COD and PO_4-P profiles during the batch test for the 10 day SRT AO zoned reactor.

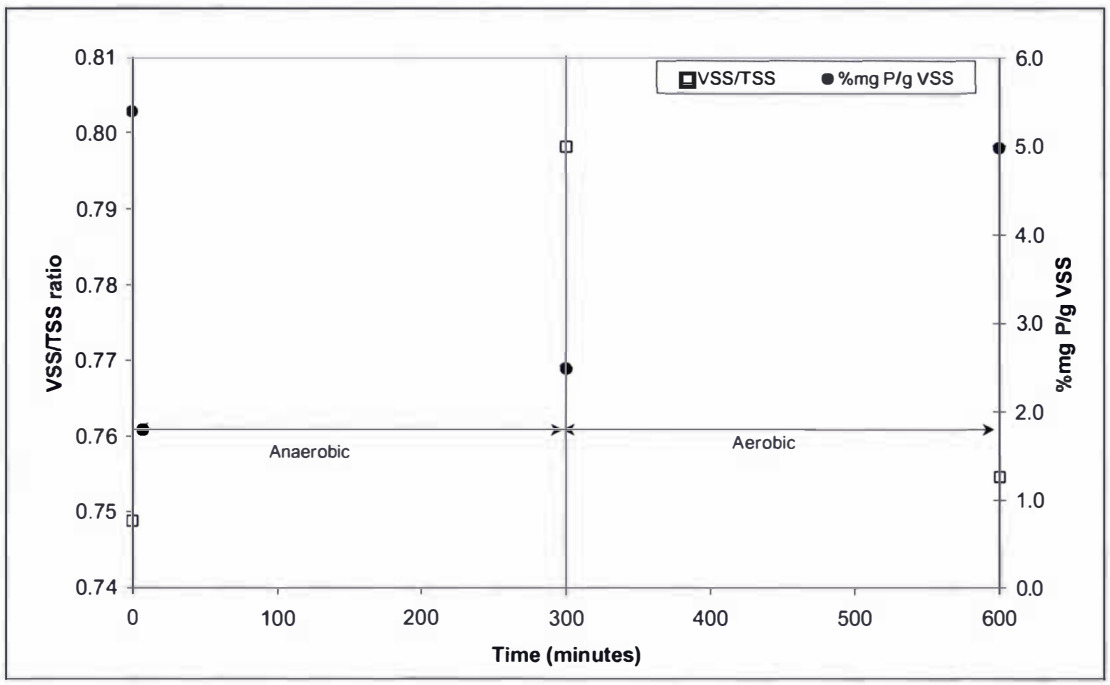


Figure 7.12: Sludge phosphorus content and VSS/TSS variation during the batch test at 0, 300 and 600 minutes.

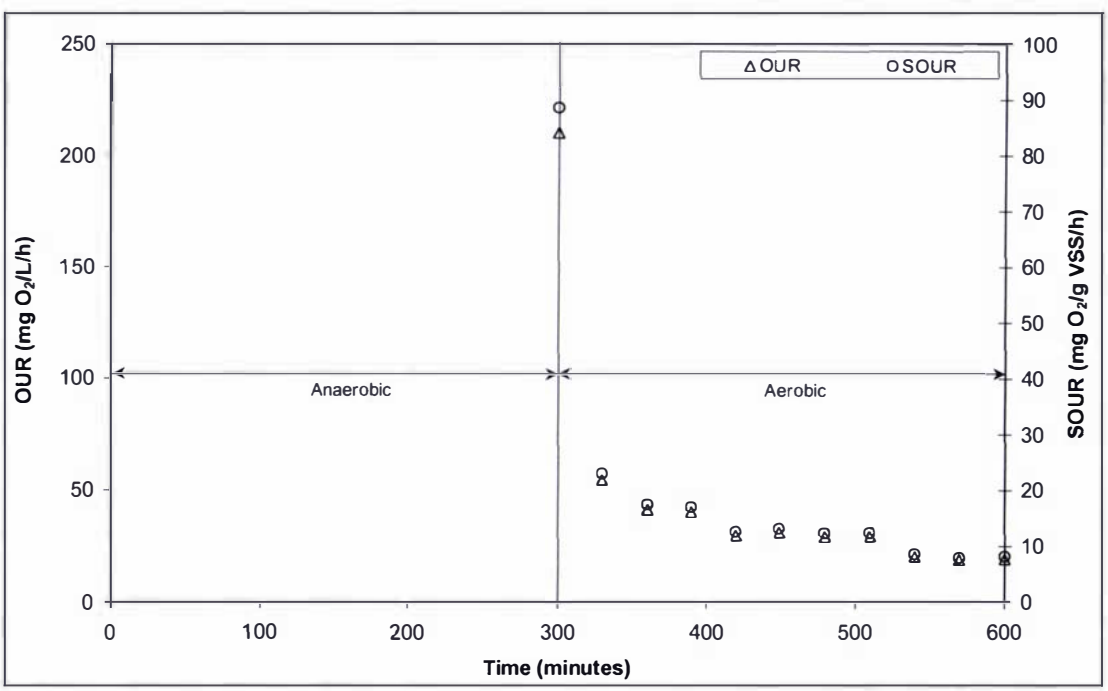


Figure 7.13: SOUR and OUR profiles during the batch test aerobic phase.

Table 7.7: Analytical parameters at time 0, 300 and 600 minutes for batch test using mixed liquor of AO system operated at an SRT of 10 days.

	Time		
Parameter	0 minutes	300 minutes	600 minutes
PO4-P (mg P/L)	72.1	138.5	238.1
Acetate COD (mg/L)	320	162	0
VSS (mg/L)	2330	2373	2410
VSS/TSS	0.749	0.798	0.755
%mg P/mg VSS	5.38	2.5	4.97
OUR (mg O_2/L/h)		210	19.5
SOUR (mg O_2/g VSS/h)		88.6	8.2

7.5 AO Reactor Operation at an SRT of 15 days

The activated sludge reactor used for the study at an SRT of 10 days was altered to an SRT of 15 days and operated for 79 days with the parameters measured during the operation shown in Section 7.5.1 and the zone study is detailed in Section 7.5.2. An additional activated sludge reactor that had been constructed was seeded using waste activated sludge from the SRT 15 reactor, and was operated simultaneously at an SRT of 5 days and is described in Section 7.6.

7.5.1 Reactor Operation

The total suspended solids concentrations of the anaerobic zone, final aerobic zone, the fermented effluent and the VSS/TSS ratios for the anaerobic and aerobic zones are shown in Figure 7.14. After 30 days the anaerobic and final aerobic zone TSS became similar and remained similar for the duration of the reactor operation with the TSS varying between 3810 g/m^3 and 4535 g/m^3 for these zones between 30 and 79 days.

The VSS/TSS ratio was stable after 30 days (Figure 7.14) and varied between 0.84 and 0.86 for the anaerobic zone and 0.79 and 0.81 for the aerobic zone. After 79 days, the difference between the ash content of the biomass of the anaerobic and aerobic zone was 6.5%.

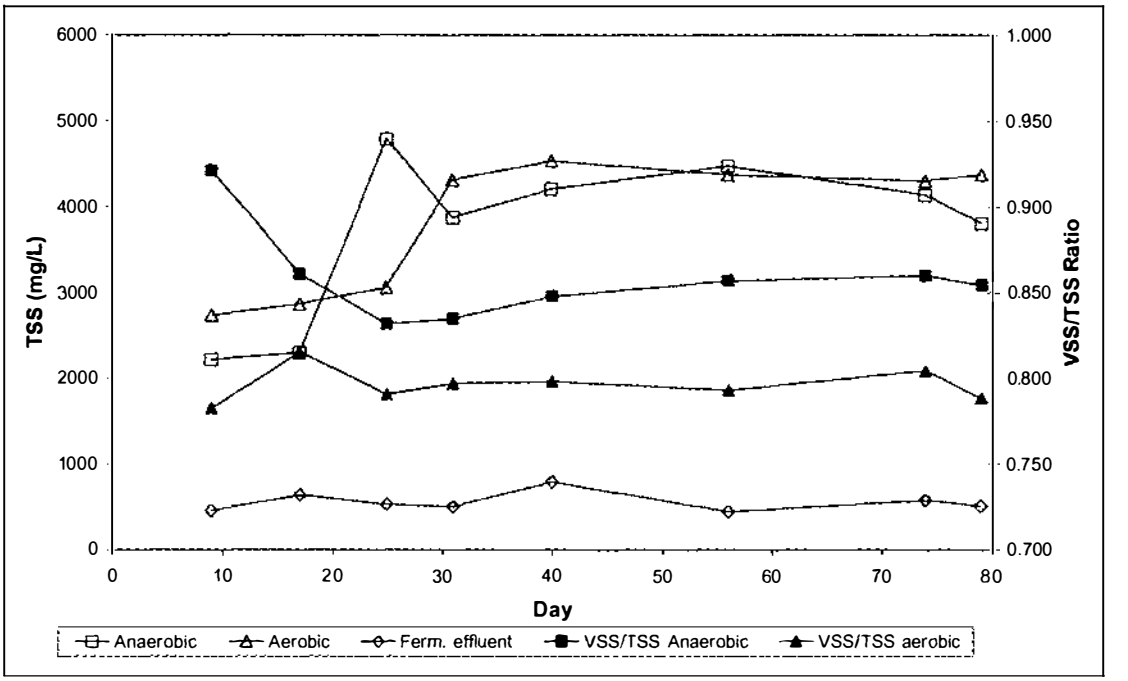


Figure 7.14: TSS and VSS/TSS profiles during 15 day reactor operation.

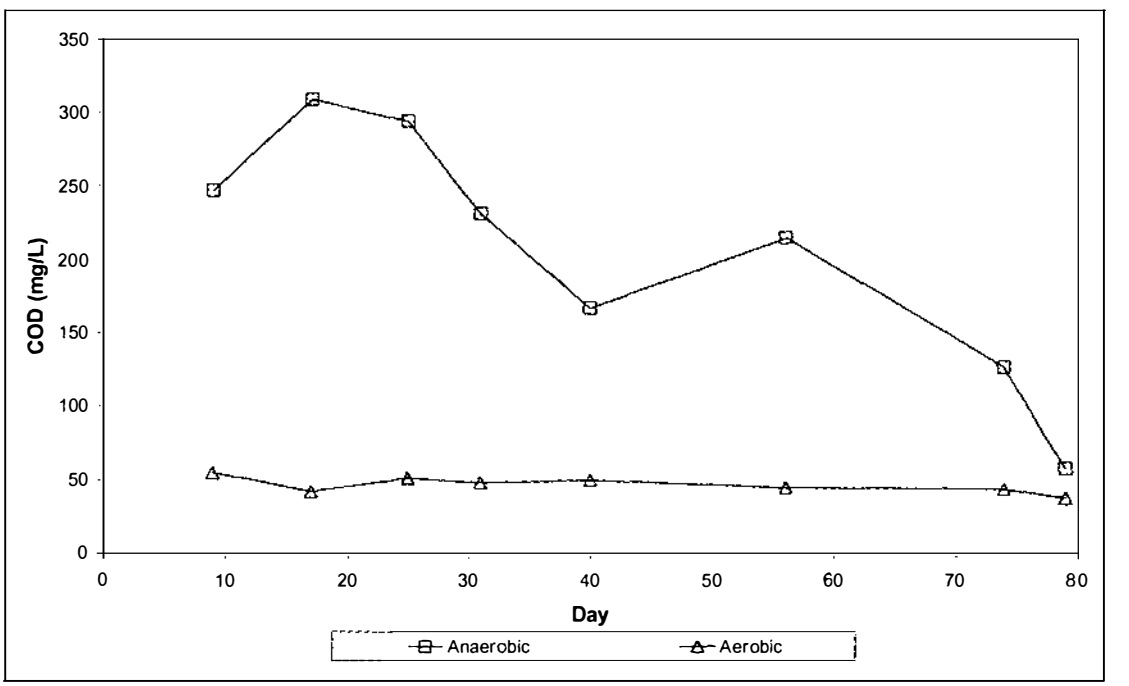


Figure 7.15: Soluble COD profiles of anaerobic zone and final aerobic zones for 15 days SRT reactor.

The soluble COD in the final aerobic zone (38 to 50 mg/L) was relatively constant during the reactor operation (Figure 7.15) while the anaerobic zone soluble COD varied between 230 mg/L at 30 days and 60 mg/L at 79 days in the anaerobic zone. This variation was largely due to changes in the fermenter VFA COD (Figure 7.4). The soluble COD consumed in the anaerobic zone displayed relatively less variation (523 to 720 mg COD/L) then the VFA COD consumed (257 to 485 mg COD/L). This suggests that there was some conversion to VFA of non-VFA substrate. After 79 days 93 % of both the available soluble COD and VFA COD was consumed.

The SVI of the reactor mixed liquor was variable, with a low of 79, a high of 237 and an average of 148 (Figure 7.17). The operability of the reactor was not affected by these SVI variations. The effluent nitrate concentrations shown in Figure 7.18 were generally small, but increased during the reactor operation to 3.7 mg NO_3-N/L by 79 days of operation. These nitrate concentrations (Figure 7.19) did not have a negative impact on the phosphorus removal process as detailed below.

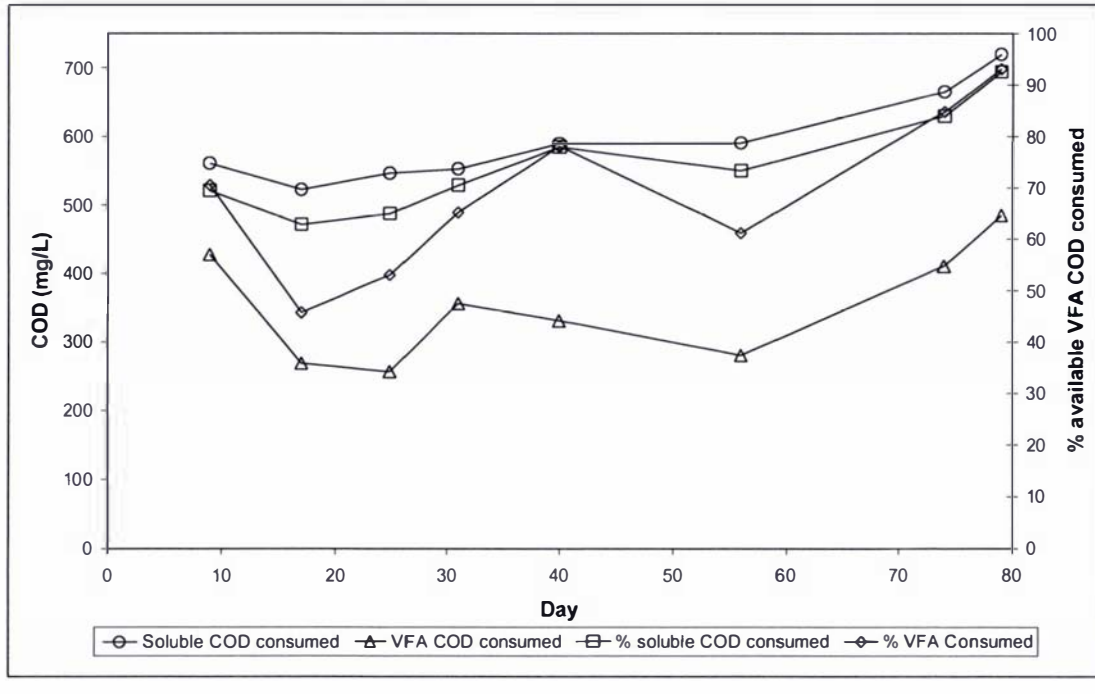


Figure 7.16: The amount of soluble and VFA COD consumed in the anaerobic zone and the % of the available COD and VFA consumed within the anaerobic zone for the 15 day SRT AO reactor.

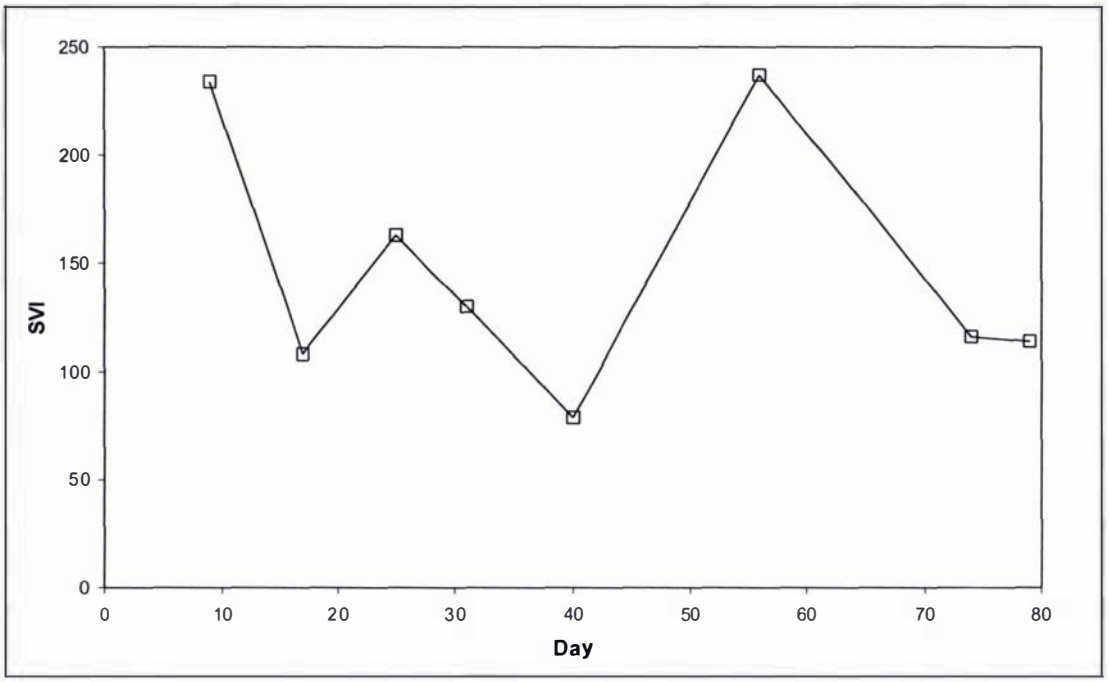


Figure 7.17: SVI variations during the 15 SRT AO reactor operation.

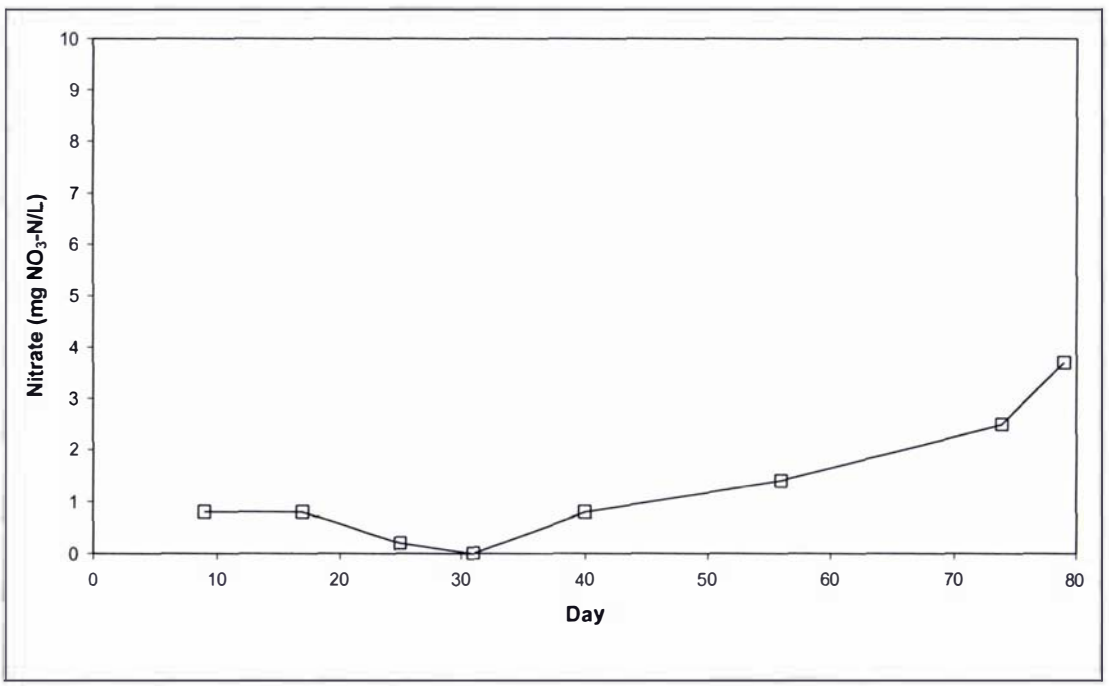


Figure 7.18: Effluent nitrate concentration during reactor operation at an SRT of 15 days.

The final aerobic zone phosphorus concentration increased to 76.7 mg P/L 17 days after changing the SRT to 15 days and then gradually decreased a low value of 64.5 mg/L on day 74 (Figure 7.20). The anaerobic zone increase in soluble phosphorus over the period of operation reflected the increasing anaerobic phosphorus release of 6.5 mg P/L after 9 days to 53.1 mg P/L after 74 days. The sludge phosphorus concentration reached a maximum of 5.15 % mg P/mg VSS after 40 days and was 5.12 mg P/mg VSS after 79 days (Figure 7.1). The ratio of phosphorus release to COD consumed (Y_{PO4}) was 0.08 after 40 days, 0.11 after 74 days and 0.08 after 79 days, which was similar to the ratio of 0.11 at a 10 day SRT. The value for this ratio is only slightly greater than the value of Y_{PO4} for the AAO and EAAO systems.

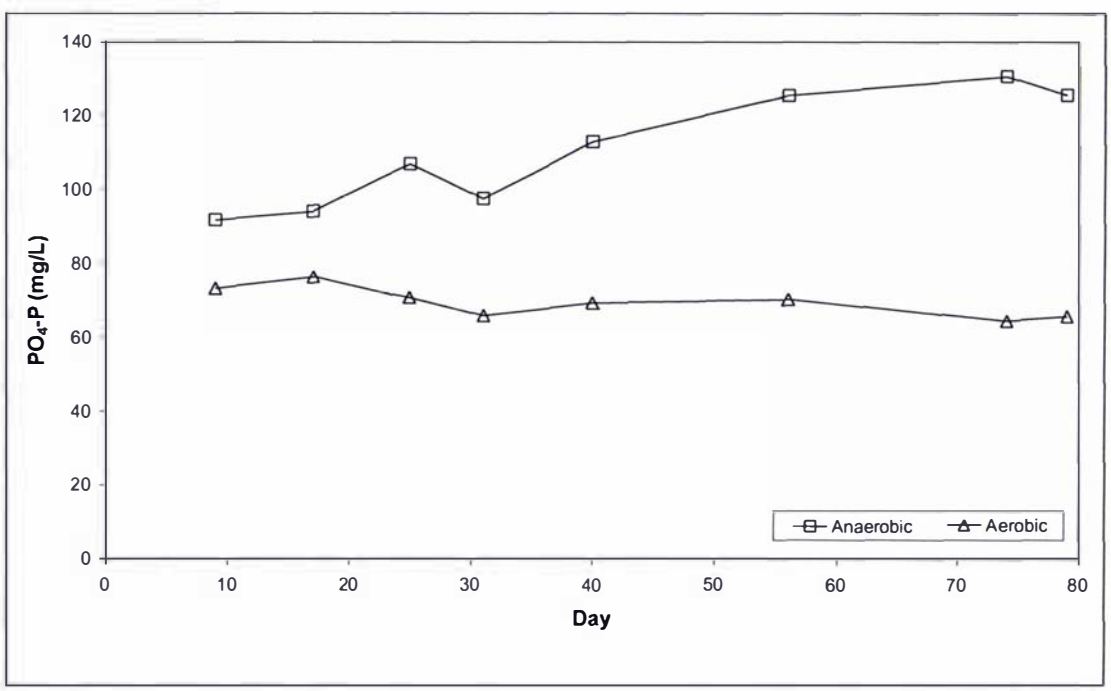


Figure 7.19: Soluble phosphorus concentrations in the anaerobic zone and final aerobic zone during 15 day SRT reactor operation.

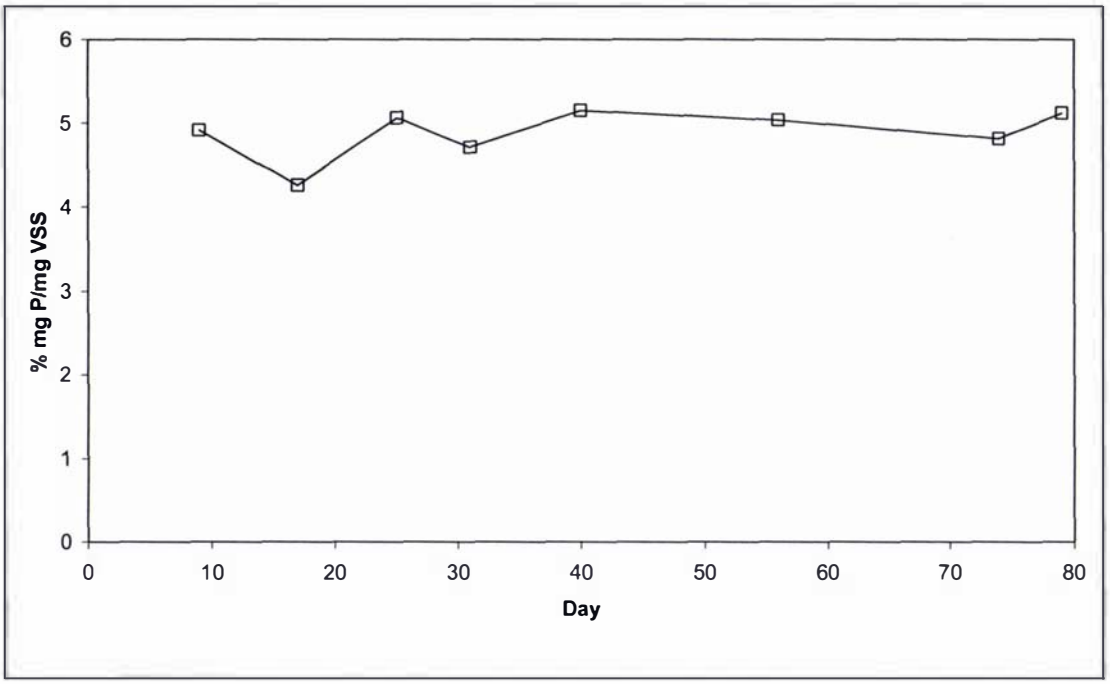


Figure 7.20: Sludge phosphorus concentration in the final aerobic zone.

7.5.2 Zone Analysis

After 79 days a full zone analysis was performed on the reactor. The VSS concentration varied only slightly across the reactor (3255 mg/L in zone 1 and 3460 in zone 10), while the TSS concentration increased across the reactor (3810 mg/L in zone 1 and 4375 mg/L in zone 10) as shown in Figure 7.21. The VSS/TSS ratio was 0.85 (14.6% ash) in the anaerobic zone (Zone 1) and 0.79 (20.9% ash) in the final aerobic zone (Zone 10).

The phosphorus profile for each zone is shown in Figure 7.22 where the soluble phosphorus concentrations in the anaerobic zone was 125.7 mg P/L, and 65.7 mg P/L in the final aerobic zone. A total of 34.5 mg P/L was removed by the reactor. The total phosphorus concentration of the fermented effluent was 100.2 mg P/L at the time of the zone study. A large amount of COD removal occurred in the anaerobic zone where the soluble COD concentration was only 58 mg COD/L (Figure 7.22). The amount of anaerobic COD consumption was 709 mg/L, with 472 mg COD/L of VFA consumed (assuming no insitu fermentation). The utilisation rate of soluble COD was 38.2 mg COD/g VSS/h and the VFA utilisation rate was 25.4 mg COD/g VSS/h. The increase in soluble COD in zones 8 to 10 are in fact only slight, as the scale used in

Figure 7.22 is small. The amount of phosphorus release in the anaerobic zone was 42.2 mg P/L at a rate of 4.6 mg P/g VSS/h. Maximum phosphorus uptake occured in zone 2 of 20.9 mg P/L and at a rate of 2.3 mg P/g VSS/h. Phosphorus uptake was close to 0 in zones 7 to 10 (Figure 7.24). The required retention time until phosphorus uptake was complete was between 14.2 to 17 hours (zones 5 and 6), based on an actual feed rate of 8.2 litres/day and a RAS rate of 8.6 litres/day.

The final aerobic zone sludge phosphorus concentration was 5.12 % mg P/mg VSS (Figure 7.24). Using mass balance calculations based on the amount of phosphorus removed, the theoretical sludge phosphorus concentration for the final zone is 5.6 %mg P/mg VSS. The analytical values showed a maximum sludge phosphorus concentration of 5.94 mg P/mg VSS in zone 7. The reason for the subsequent decrease in the sludge phosphorus concentration after zone 7 may be due to a slight increase in the VSS concentration in these zones (Figure 7.21). The anaerobic zone sludge concentration was 3.16 % mg P/mg, suggesting incomplete phosphorus release. The nitrate concentration increased rapidly between zones 3 and 5 and was 4 mg NO_3-N/L in the final aerobic zone, showing that there was a slight nitrogen excess over that required for metabolic requirements.

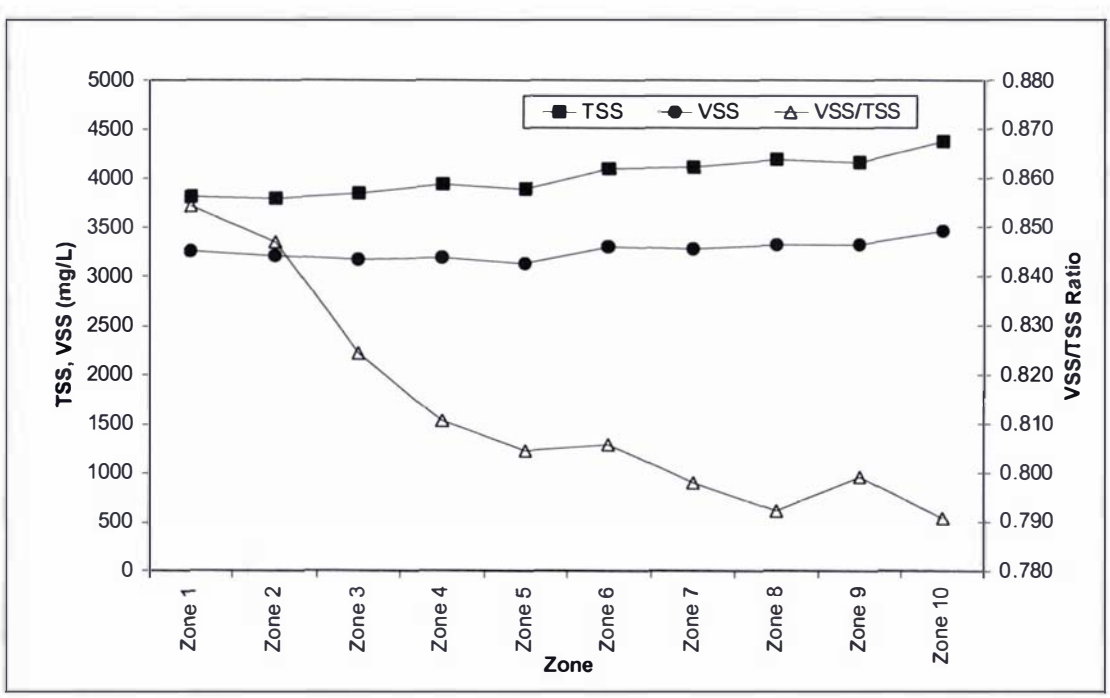


Figure 7.21: TSS, VSS and VSS/TSS ratio's for each zone for 15 day SRT AO reactor.

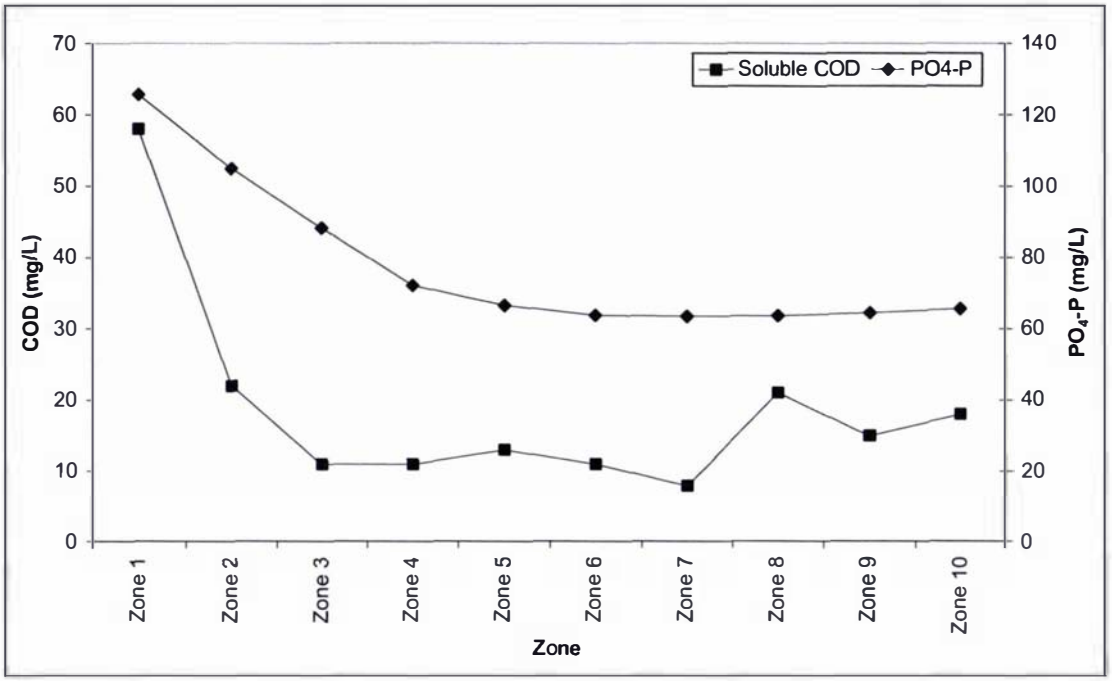


Figure 7.22: Soluble COD and PO_4-P profiles for each zone for 15 day SRT AO reactor.

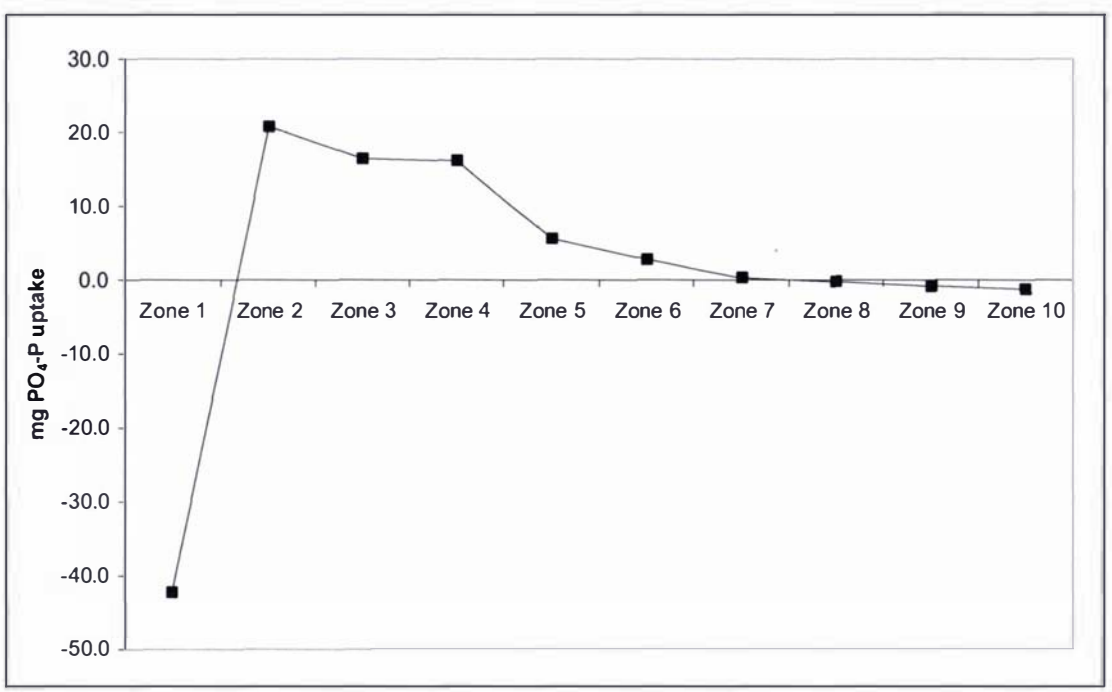


Figure 7.23: Phosphorus uptake in each zone for 15 day SRT AO reactor.

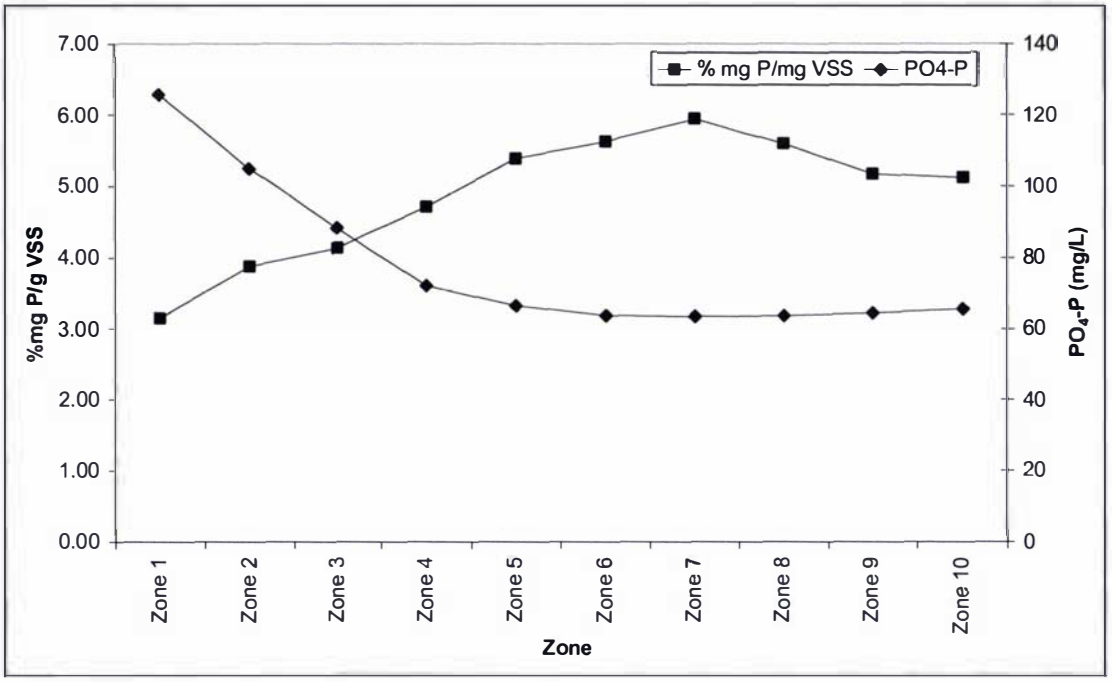


Figure 7.24: Sludge phosphorus content and PO_4-P concentrations for each zone for the 15 day SRT AO reactor.

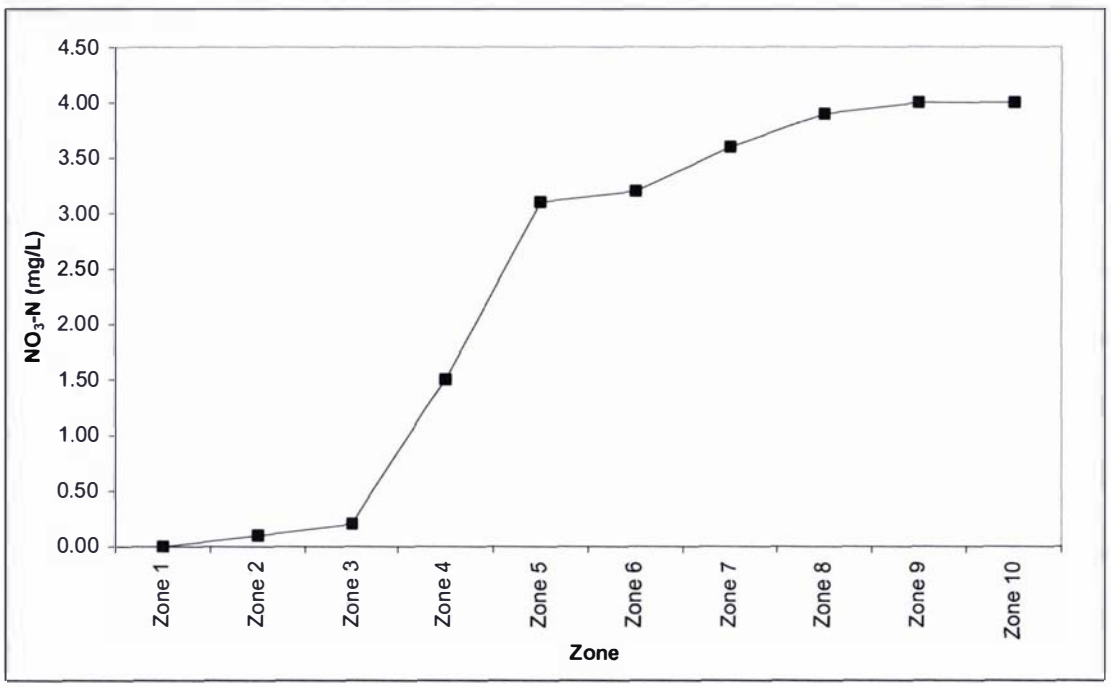


Figure 7.25: Nitrate concentrations in each zone for the 15 day SRT AO reactor.

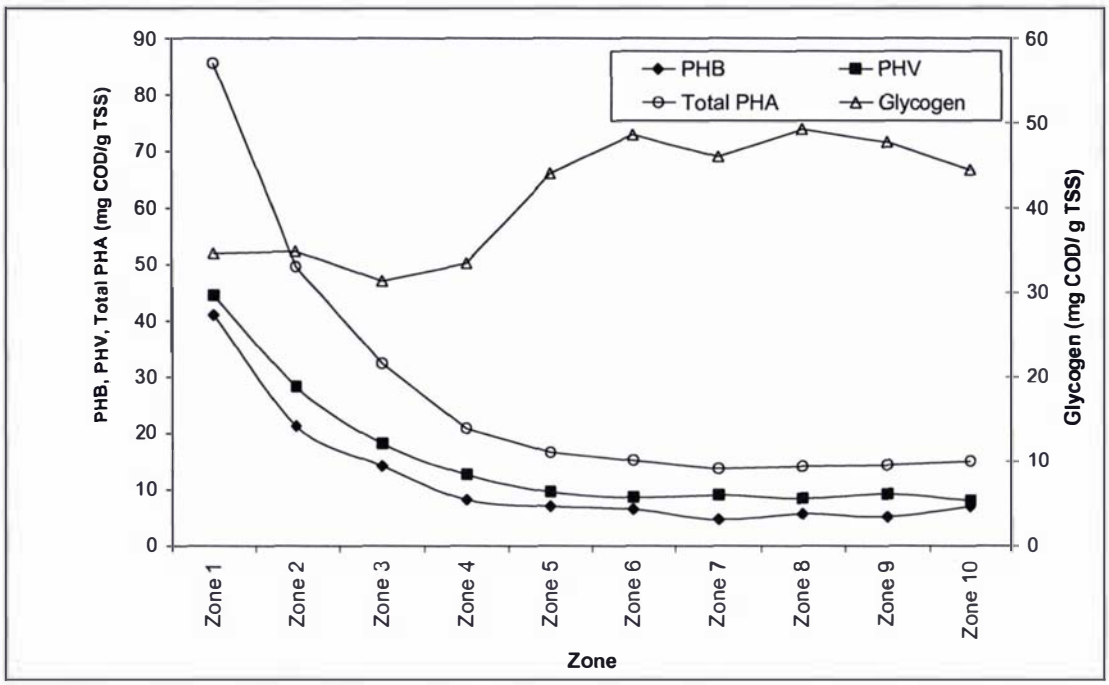


Figure 7.26: PHB, PHV, Total PHA and glycogen concentrations for each zone for the 15 day SRT AO reactor.

The profiles of the intracellular storage compounds are shown Figure 7.26. The relative PHB and PHV concentrations were similar through the reactor zones. The total PHA concentration reached a maximum of 85.6 mg COD/g TSS in zone 1 and decreased until zone 7. The PHA concentration was 15.1 mg COD/g TS in zone 10. The anaerobic zone PHA concentration was substantially greater than the AAO reactor were it was 44 mg COD/g TS. The fraction of total PHA present as PHB was 48% in zone 1 (compared to 69% in AAO system) and then ranged between 34.2% and 46.6% in the subsequent zones. The specific rate of PHA production in the anaerobic zone was 63.3 mg COD/ g VSS/hr. The glycogen concentration was 34.7 mg COD/g TSS in zone 1 and remained relativcly constant until zone 4 and was 44.4 mg COD/g TSS in zone 10.

The biological activity (OUR & SOUR) reached a maximum OUR of 75.8 mg O_2/L/h in the first aerobic zone (zone 2) as expected due to the high PHA concentration in this aerobic zone (Figure 7.27). Both the OUR and SOUR reached a plateau at zone 6, which was the same zone that the PO_4-P, PHA and glycogen concentrations levelled

off. The SOUR had a value of 5.2 mg O_2/g VSS/h in zone 10, the same value as for the AAO zone study.

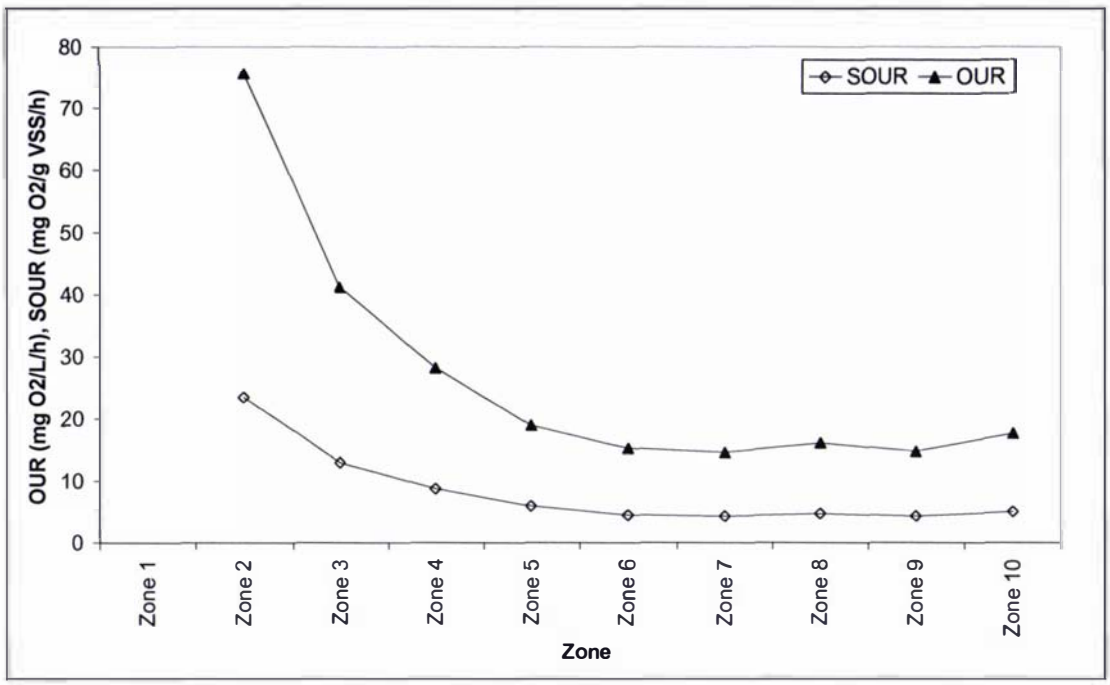


Figure 7.27: SOUR and OUR rates for each zone for the 15 day SRT AO reactor.

Table 7.8: Individual zone parameters for AO configuration at an SRT of 15 days.

	Zone									
Parameter	1 Anaerobic	2 Aerobic	3 Aerobic	4 Aerobic	5 Aerobic	6 Aerobic	7 Aerobic	8 Aerobic	9 Aerobic	10 Aerobic
$COD_{soluble}$ (mg/L)	58	22	11	11	13	11	8	21	15	18
PO_4-P (mg/L)	125.7	104.9	88.4	72.2	66.6	63.8	63.5	63.7	64.5	65.7
%mg P/g VSS	3.16	3.88	4.14	4.71	5.38	5.62	5.94	5.59	5.17	5.12
NO_3-N (mg/L)	0	0.1	0.2	1.5	3.1	3.2	3.6	3.9	4.0	4.0
SOUR mgO_2/mg VSS/h		23.6	13.0	8.9	6.1	4.7	4.5	4.9	4.5	5.2
Total PHA mg COD/g TS	85.6	49.7	32.5	20.9	13.6	16.8	11.8	14.1	14.4	15.1
Glycogen mg COD/g TS	34.7	34.9	31.4	33.5	44.1	48.6	46.0	49.3	57.3	44.4
TSS (mg/L)	3810	3790	3845	3935	3890	4095	4110	4190	4160	4375
VSS/TSS ratio	0.85	0.85	0.82	0.81	0.81	0.81	0.80	0.79	0.80	0.79

7.5.3 Batch Test

The batch test used two litres of the mixed liquor from the SRT 15 AO reactor and consisted of a 180 minute anaerobic phase (same as continuous reactor) and a 240 minute aerobic phase. Sodium acetate was added to give an initial starting COD concentration of 300 mg/L. The initial VSS concentration was 3870 mg/L with a sludge phosphorus concentration of 4.40 %mg P/mg VSS. Figure 7.28 shows that the acetate COD was 23 mg/L after 90 minutes and 0 at 120 minutes, with full consumption occurring at about 100 minutes. Two distinct COD utilisation rates are evident with the initial high rate of 80.5 mg COD/g VSS/h (initial nitrate and nitrite concentrations were zero) and a second rate of 31.4 mg COD/g VSS/h. There were also two distinct phosphorus release rates of 12.6 mg P/g VSS/h and 3.9 mg P/g VSS/h. As expected, phosphorus release ceased after acetate consumption. The COD consumed to phosphorus released during the anaerobic phase was 0.2 mg COD/mg P. In the subsequent aerobic phase there was an initial higher phosphorus uptake rate of 6.4 mg P/g VSS/h followed by a decreased uptake rate of 2.1 mg P/g VSS/h. The phosphorus uptake rate decreased to a very low value of 0.4 mg P/g VSS/h after in the last hour of the batch test.

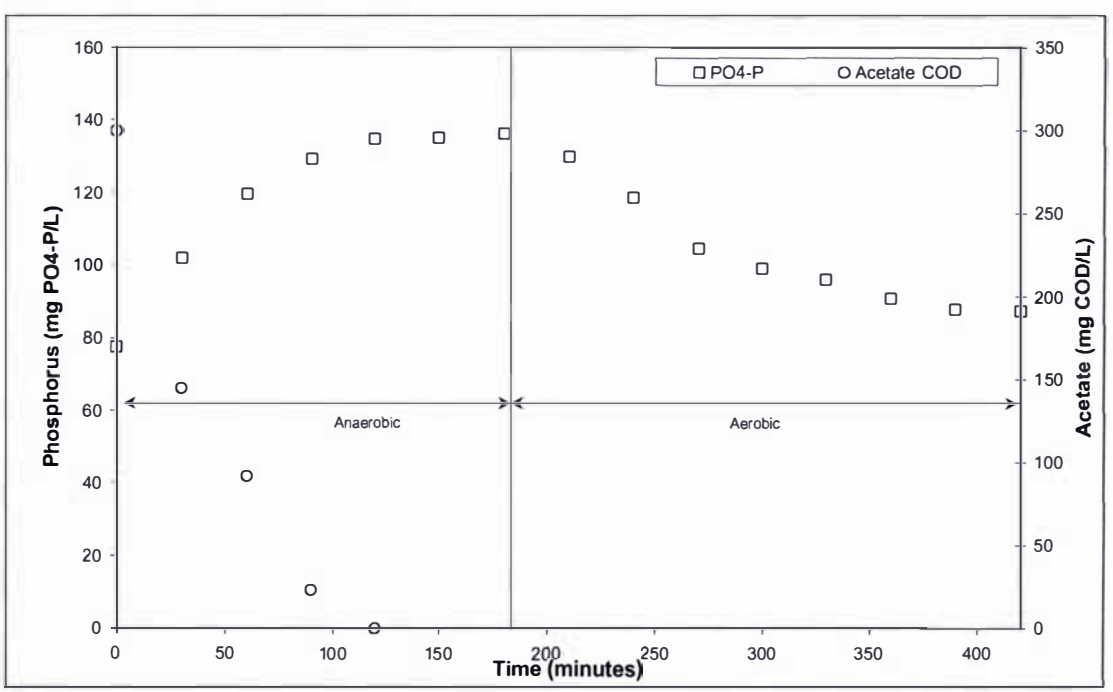


Figure 7.28: Soluble COD and PO_4-P profiles during batch test for the 15 day SRT AO reactor.

The phosphorus content of the sludge at the end of the anaerobic phase decreased to 2.8 % mg P/mg VSS from an initial value of 4.40 % and increased to 4.1% after the aerobic phase (Figure 7.29). The initial VSS/TSS ratio was 0.790 and increased to 0.825 after the anaerobic phase, a decrease in the biomass ash content of 3.5%. At the end of the batch test the VSS/TSS ratio decreased to 0.796, an increase during the aerobic phase of 2.9%. This was a net overall decrease in the ash content of the sludge of 0.6% (2.9%-3.5%= -0.6%). The net decrease in the sludge phosphorus content and ash content is reflected in a net change in the soluble phosphorus concentration during the batch test of -9.9 mg P/L. This suggests that a longer aerobic phase was required to obtain complete phosphorus uptake.

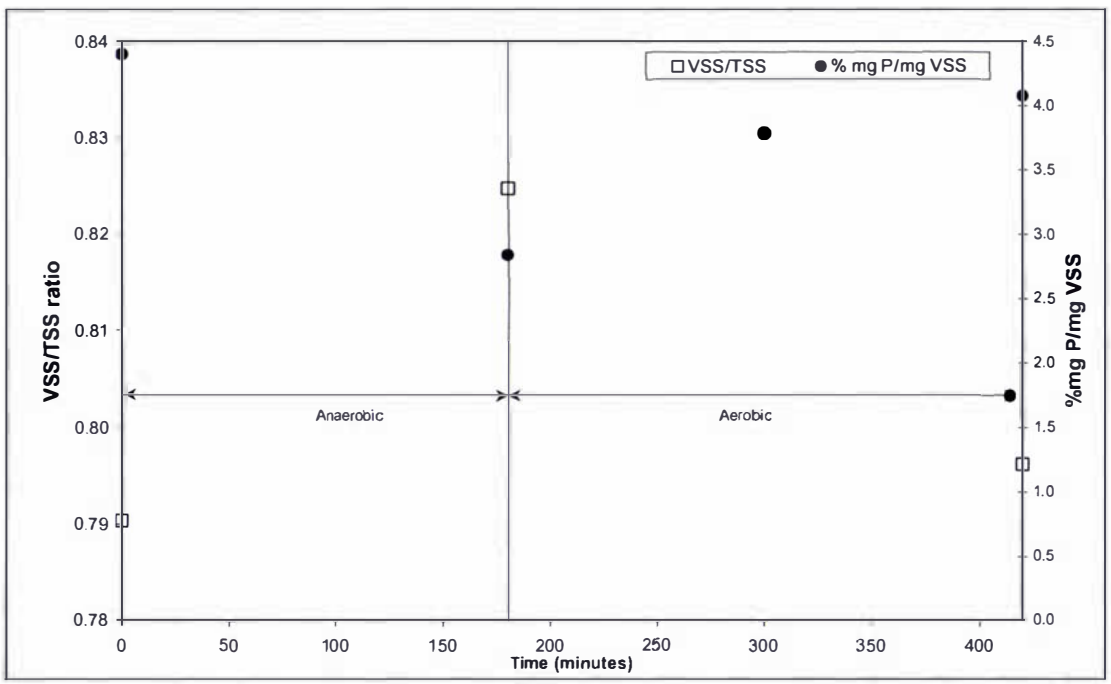


Figure 7.29: Sludge phosphorus content and VSS/TSS variations during SRT 15 mixed liquor batch test.

The changes in the intracellular storage compounds PHB, PHV, PHA and glycogen along with the specific oxygen uptake rate (SOUR) are shown in Figure 7.30. There was an initial rapid rate of PHA production (75% as PHB) at a rate of 36.1 mg COD/g TS/h which then decreased at 60 minutes to a slower rate of PHA production (6.3 mg COD/g TS/h) corresponding to the decreased rate of phosphorus release. A slight decrease in the PHA concentration occurred after the acetate was depleted. The

glycogen had a net decrease through the anaerobic phase (-10.2 mg COD/g TS) as expected and then increased during the aerobic phase (+20.7 mg COD/g TS) resulting in an overall net increase during the batch test of 10.2 mg COD/g TS. The specific oxygen uptake rate (SOUR) remained high for the first 60 minutes of the aerobic phase and then decreased as the PHA was utilised. The SOUR changed in four separate phases, up to 240 minutes it remained high and then decreased rapidly from 240 to 300 minutes and then was constant from 300 to 330 minutes and again decreased from 330 to 420 minutes. Each of these phases generally matches the changes in PHA utilisation.

The analytical parameters at the start and end of the anaerobic zone (t= 0 and t= 180 minutes) and at the end of the aerobic phase (t=420 minutes) are shown in Table 7.6.

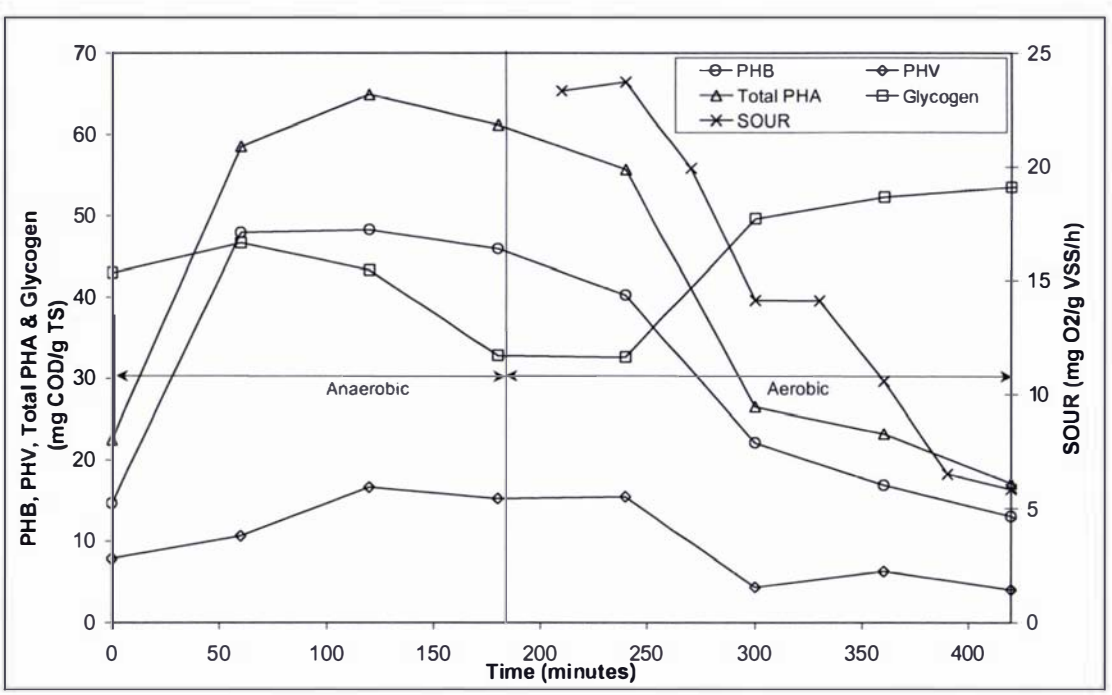


Figure 7.30: PHB, PHV, total PHA, Glycogen and SOUR profiles during batch test for the 15 day SRT AO reactor.

Table 7.9: Analytical parameters at time 0, 180 and 420 minutes for batch test using mixed liquor of AO system operated at an SRT of 15 days. Except OUR and SOUR are given for 210 minutes instead of 180 minutes.

	Time		
Parameter	0 minutes	180 minutes	420 minutes
PO4-P (mg P/L)	77.6	136.2	87.5
Acetate COD (mg/L)	300	0	0
VSS (mg/L)	3870	3940	4310
VSS/TSS	0.790	0.825	0.796
%mg P/mg VSS	4.40	2.8	4.08
PHB (mg COD/g TS)	14.6	45.9	13.0
PHV (mg COD/g TS)	7.9	15.2	4.1
PHA (mg COD/g TS)	22.5	61.2	17.1
Glycogen (mg COD/g TS)	43.0	32.8	53.5
OUR (mg O_2/L/h)		91.9 (210 minutes)	23.0
SOUR (mg O_2/g VSS/h)		23.3 (210 minutes)	5.8

7.6 AO Reactor Operation at an SRT of 5 days

7.6.1 Reactor Operation

This reactor was operated for period of 56 days at an SRT of 5 days to further minimisc nitrification and to assess the stability of phosphorus removal at a relatively low SRT. The changes in TSS concentration and the VSS/TSS ratio are shown in Figure 2.31. The final aerobic zone concentration varied between 1350 mg/L and 2860 mg/L compared to SRT's of 10 days and 15 days in which the TSS concentrations stabilised at about 2800 mg/L and 4300 mg/L respectively. The drop in the mixed liquor solids concentration on day 17 was due to the RAS pump tubing blocking before sampling and a large sludge blanket developing in the clarifier. The reactor mixed liquor concentration varied more during this operation than for the other SRT operations. This variation was principally due to poorer settling in the clarifier as evidenced by progressively higher SVI values during the operation, which increased from an SVI of 157 at day 1 to 405 after day 56.

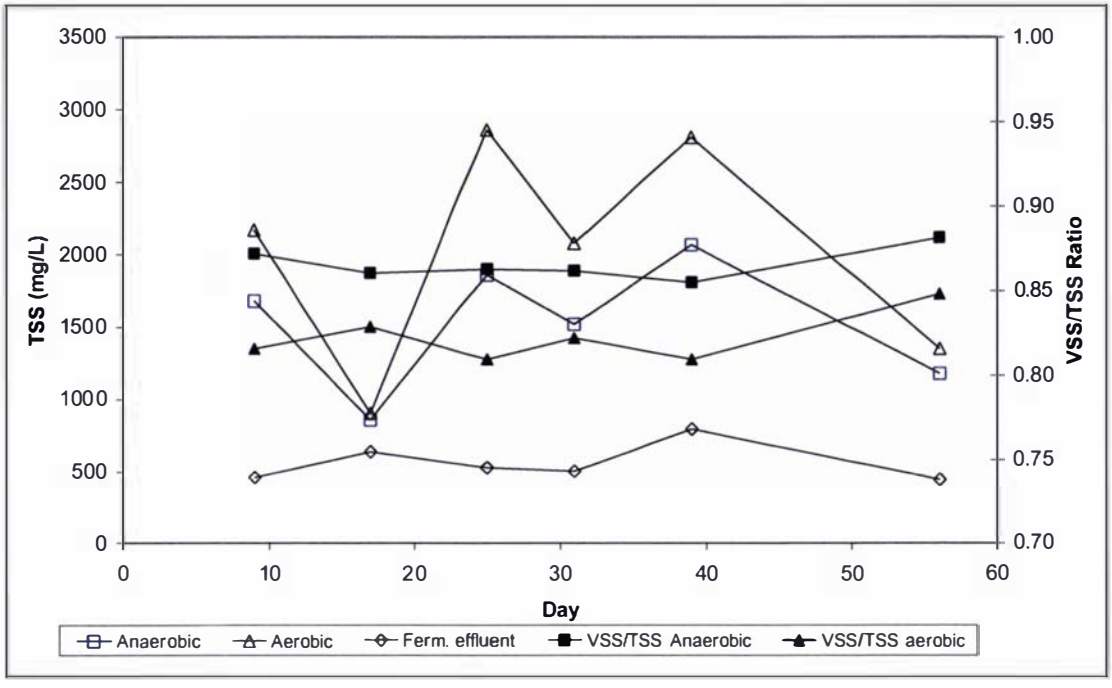


Figure 7.31: TSS, VSS and VSS/TSS ratio's for the anaerobic zone and the final aerobic zone during the 5 day SRT AO reactor operation.

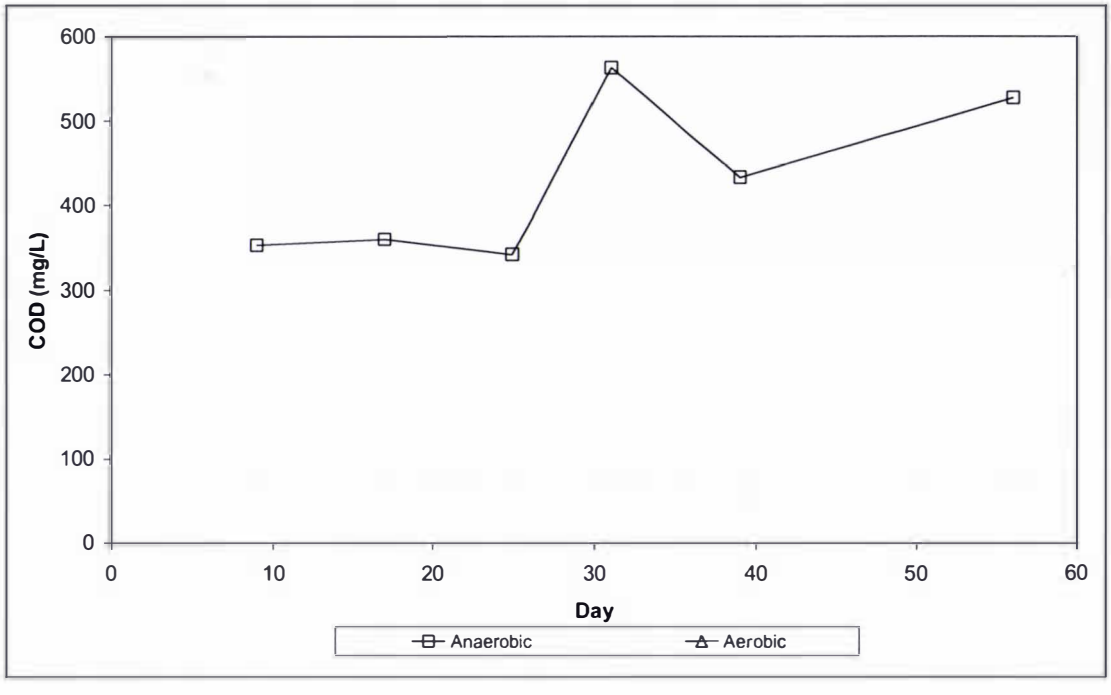


Figure 7.32: Soluble COD for the anaerobic zone and the final aerobic during the 5 day SRT AO reactor operation.

The soluble COD in both the anaerobic and final aerobic zones are shown in Figure 7.32 while the anaerobic VFA concentration and the amount of VFA consumed are shown in Figure 7.33 throughout the 56 days of operation. Initially 55 % of the available VFA COD was consumed within the anaerobic zone and then progressively decreased to 0% after 56 days of operation. The decrease in anaerobic VFA consumption was also matched by a decrease in the amount of phosphorous release in the anaerobic zone, which was initially 8.7 mg P/L, decreased to zero on day 39, and was then negative (uptake) until day 56. A small amount of phosphorus release (negative uptake) was measured on day 39 of 5.6 mg P/L, otherwise no anaerobic release was measured on other days that sampling occurred. Phosphorus removal was poor with the effluent soluble phosphorus concentration reaching a low of 73.7 mg P/L after 25 days and then increasing to 80.5 mg P/L on day 39 (Figure 7.35). The final aerobic sludge phosphorus content was initially 5.1 % mg P/mg VSS and decreased to 2.6 % mg P/mg VSS after 59 days of operation, reflecting the decrease in phosphorus removal over the period of operation. Nitrate inhibition of phosphorus removal was not a significant factor as the maximum nitrate concentration in the final aerobic zone reached only 2.1 g NO_3-N /m^3 (Figure 7.37).

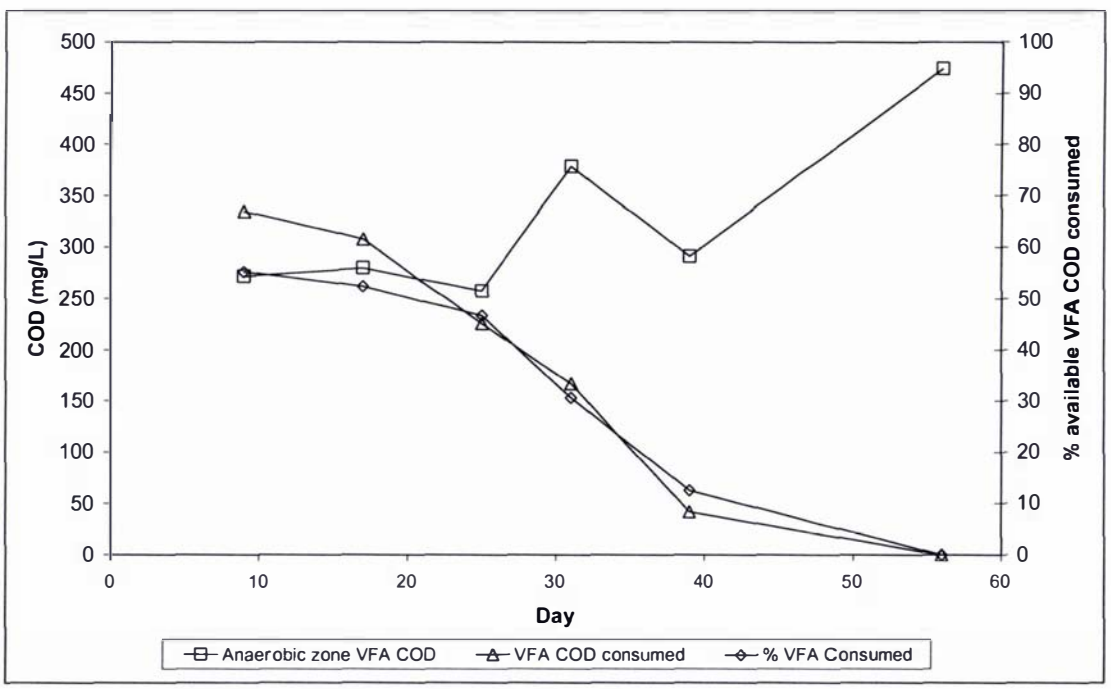


Figure 7.33: Total VFA COD concentration in the anaerobic zone, the amount of VFA COD consumed in the anaerobic zone and the % of the available VFA consumed within the anaerobic zone for 5 day SRT AO reactor.

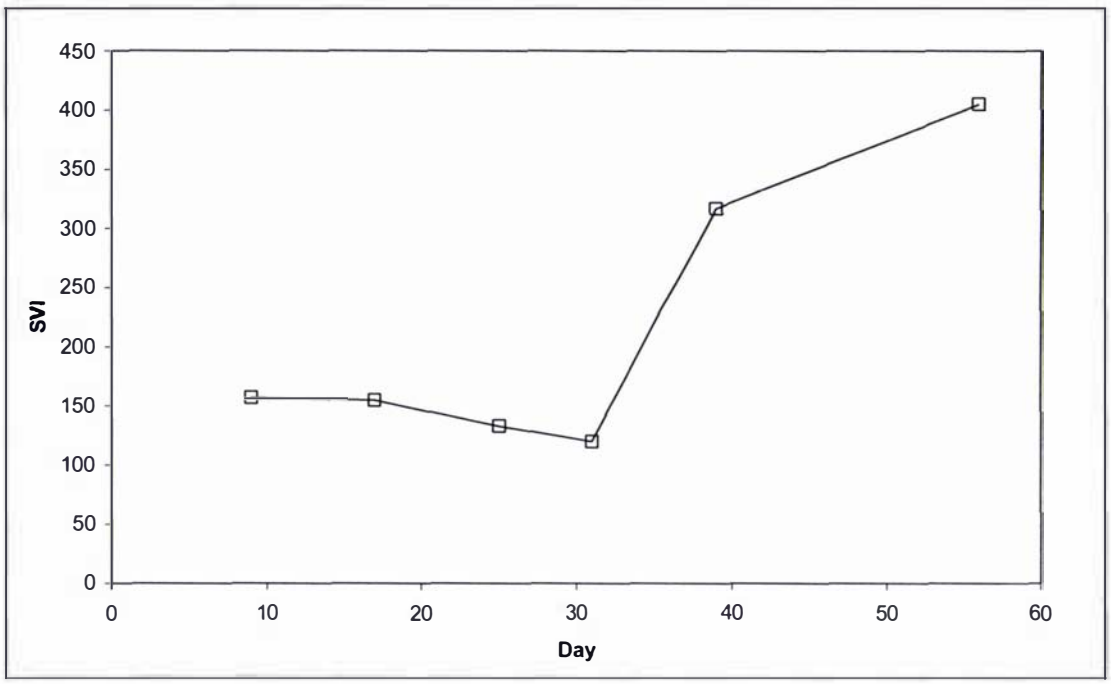


Figure 7.34: SVI variation during the operation of the 5 day SRT AO reactor.

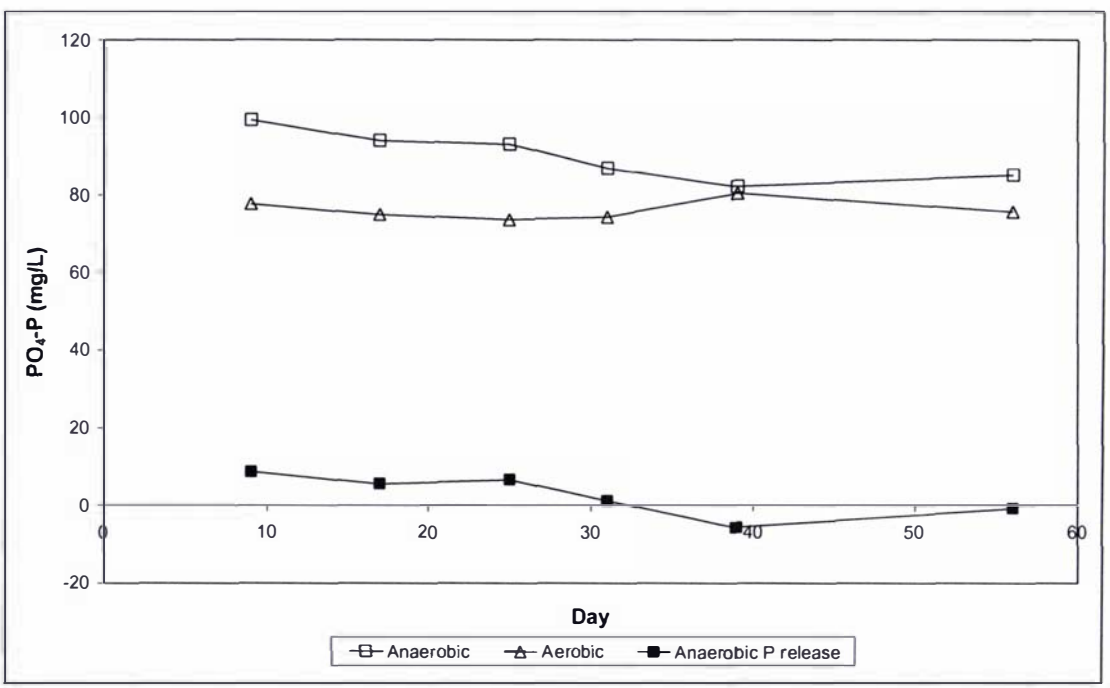


Figure 7.35: Anaerobic and aerobic PO_4-P concentrations and the amount of anaerobic zone P-release during the operation of the 5 day SRT AO reactor.

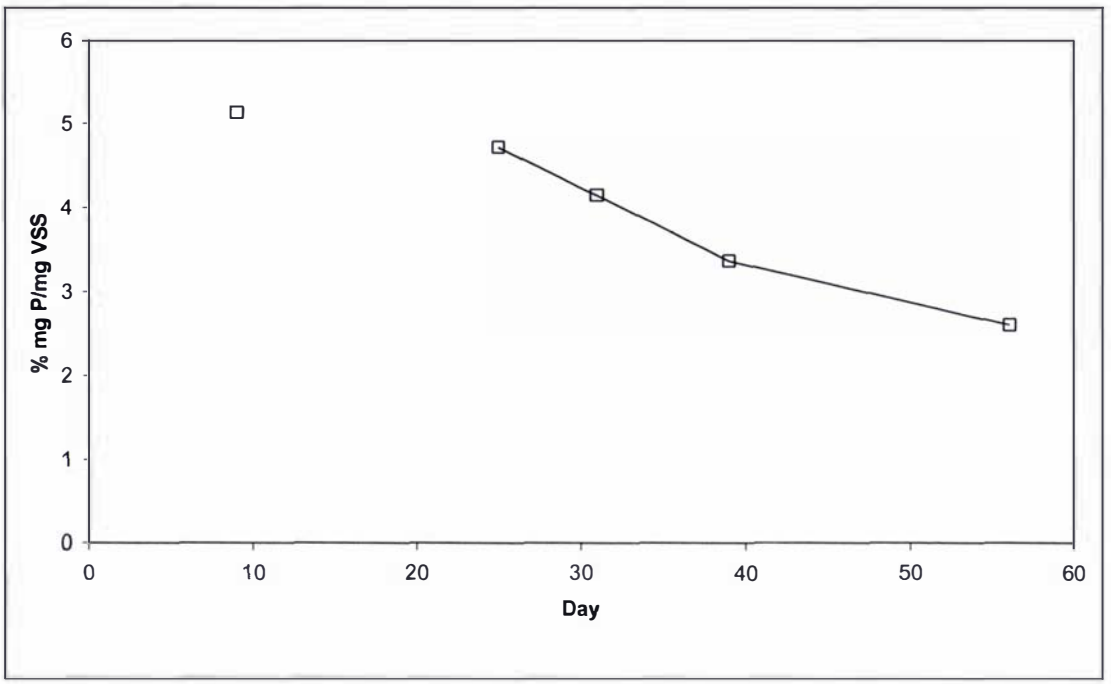


Figure 7.36: Final aerobic zone sludge phosphorus content during the operation of the 5 day SRT AO reactor.

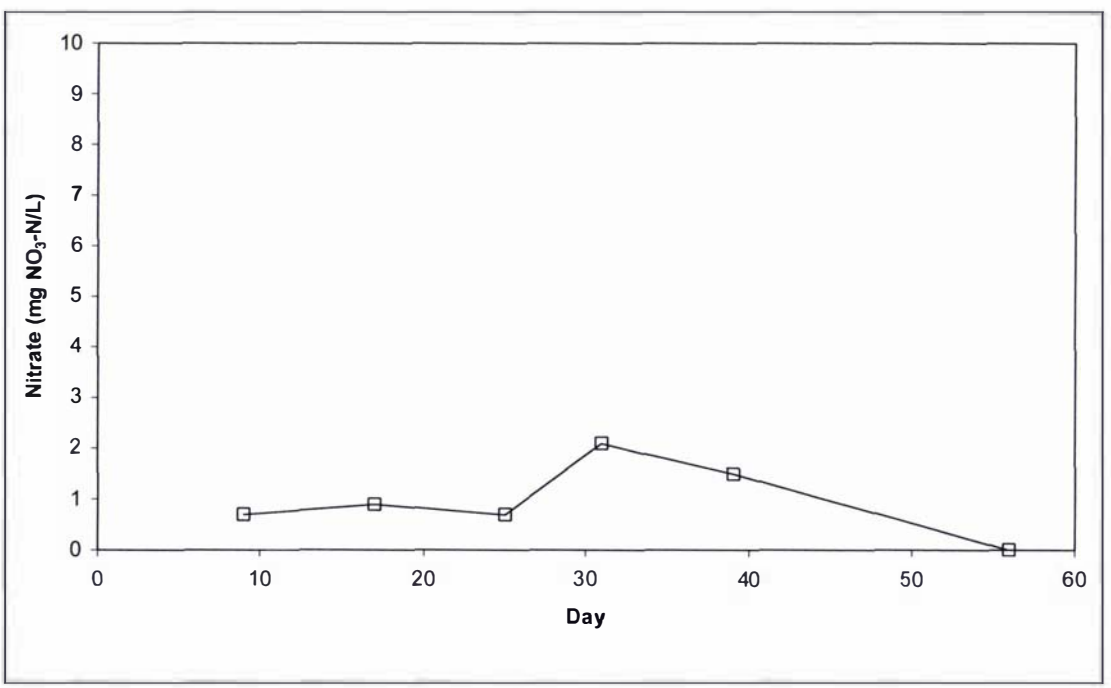


Figure 7.37: Final aerobic zone nitrate concentration during the operation of the 5 day SRT AO reactor.

7.6.2 Zone Study

After 56 days of operation a zone study was performed on the reactor, all the main parameters were analysed except for the storage compounds PHB, PHV and glycogen, as the phosphorus removal was minimal. As previously discussed the stability of the reactor was poor during the trial especially when the settleability of the biomass deteriorated (SVI=405). The internal operational stability of the reactor was also difficult to control, as the solids mixed liquor concentration was uneven, especially in the first three zones were the TSS concentration ranged from 1180 g/m^3 to 1780 g/m^3 (Figure 7.38). The VSS/TSS ratio (0.88 in zone 1 and 0.85 in zone 10) did not display the decreasing trend typical of an EBPR process but was variable through the reactor. The value of 0.85 in zone 10 compares to the ratios of 0.79 for both the 10 and 15 day SRT operations.

The anaerobic zone (zone 1) soluble COD was 525 g/m^3 with 90% of this present as VFA compounds, principally acetic and n-butyric acid. A total of 277 mg/L of the influent soluble COD was consumed in the anaerobic zone, this compares to the VFA consumption which was calculated as –11 mg/L of VFA. The negative value implies VFA production through further fermentation. Full consumption of biodegradable soluble COD did not occur until zone 3 (Figure 7.39). The small increase in the soluble COD from zones 3 to zone 10 was probably due to the production of a small amount of microbiologically produced inert soluble COD. The phosphorus profile throughout the reactor was relatively constant (Figure 7.39), with no phosphorus release occurring in the anaerobic zone. The amount of phosphorus removal was 18.2 mg P/L on the day of this zone study compared to 34 mg P/L for the 15 day SRT zone study. The total phosphorus concentration in the fermented wastewater on the day of the study was 95 mg P/L. The actual amount of phosphorus uptake in each zone is shown in Figure 7.40. There was no phosphorus release in zone 1 but rather a small amount of uptake of 0.7 mg/L calculated. The sludge phosphorus content of the final aerobic sludge was 2.6 % mg P/mg VSS (Figure 7.41), similar to systems without significant biological phosphorus removal. A phosphorus mass balance generated a theoretical waste activated sludge phosphorus concentration of 2.85 % mg P/mg VSS. There was no effect of nitrate on phosphorus removal as nitrate effluent concentrations were very low (Figure 7.42) with nitrate only being detected in zone 9.

The fact that there was no anaerobic phosphorus release but still a significant amount of soluble COD consumption shows that there was a microbial population present that was capable of anaerobic COD consumption without phosphorus release.

The oxygen uptake rates (Figure 7.43) for each aerobic zone is related to COD removal rather than the combination of COD removal and phosphorus uptake (due to the lack of EBPR). The trend generally follows the expected pattern apart from the decrease in zone 5. The SOUR value in zone 10 was 7.7 mg O_2/g VSS/h. Due to the lack of phosphorus removal a batch test was not performed with this sludge.

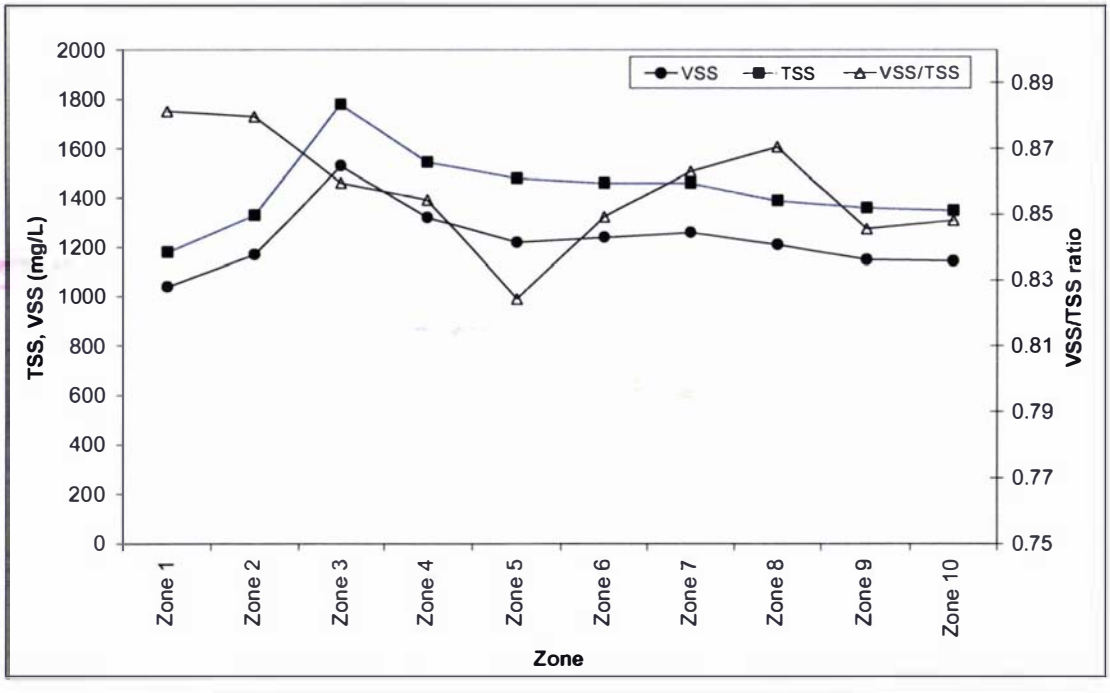


Figure 7.38: TSS, VSS and VSS/TSS ratio in each zone for the reactor operated at an SRT of 5 days.

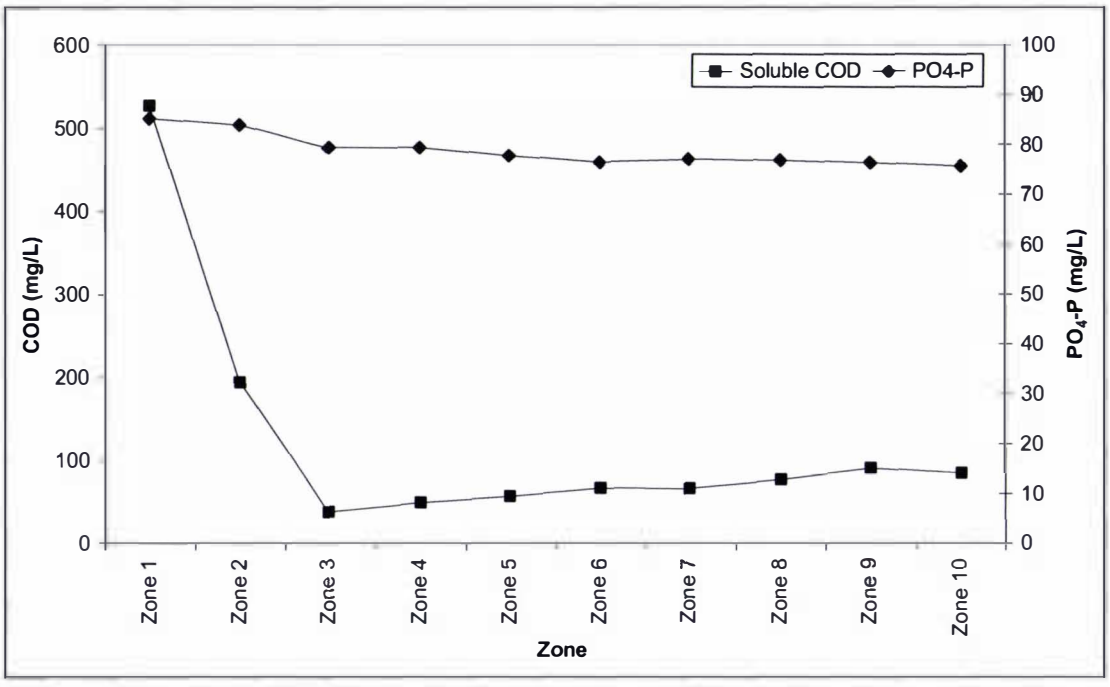


Figure 7.39: Soluble COD and PO_4-P concentration in each zone for AO zoned reactor operated at an SRT of 5 days.

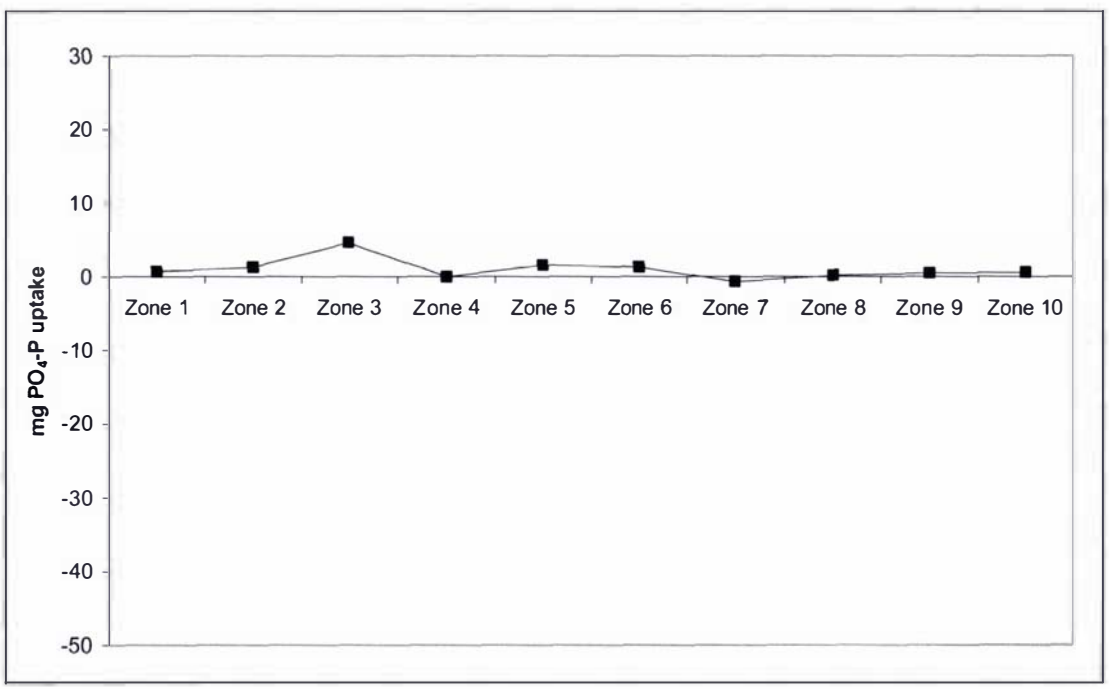


Figure 7.40: Anaerobic zone phosphorus uptake in each zone for AO zoned reactor operated at an SRT of 5 days.

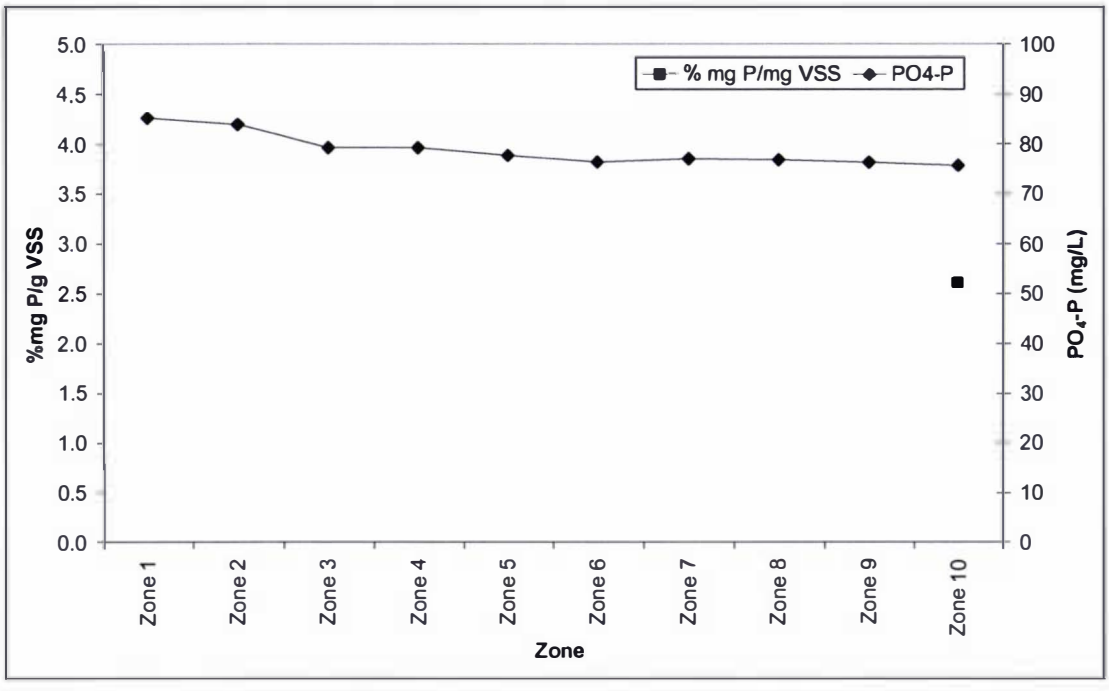


Figure 7.41: Final aerobic zone sludge phosphorus content and PO_4-P for zones of AO reactor operated at an SRT of 5 days.

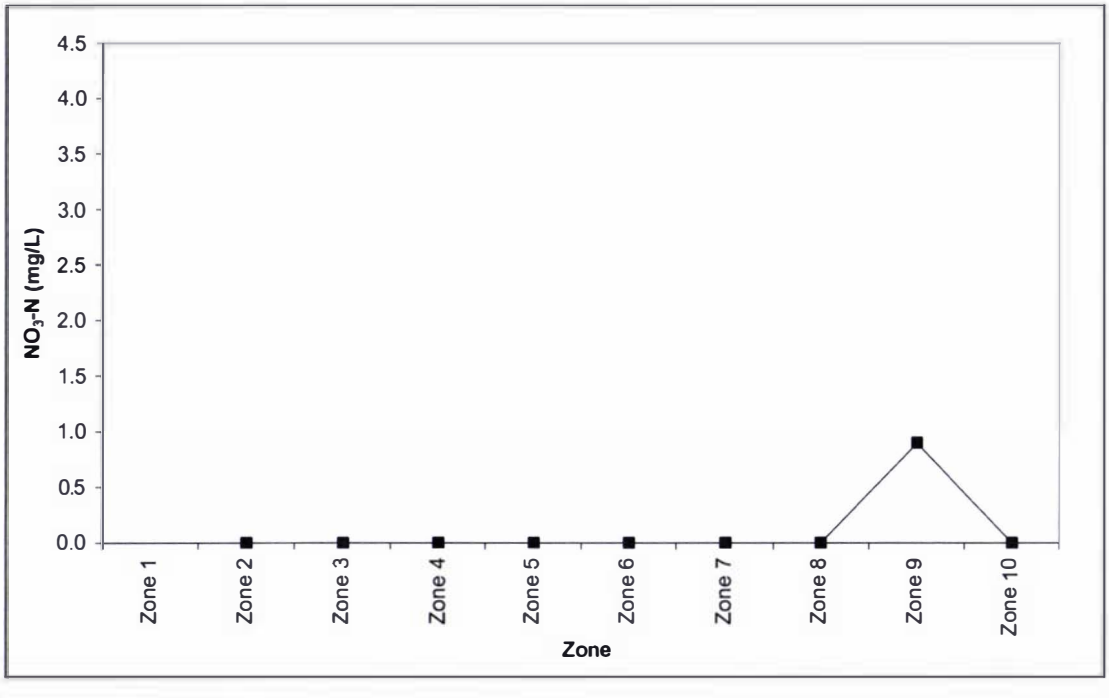


Figure 7.42: Nitrate in each zone of AO reactor operated at an SRT of 5 days.

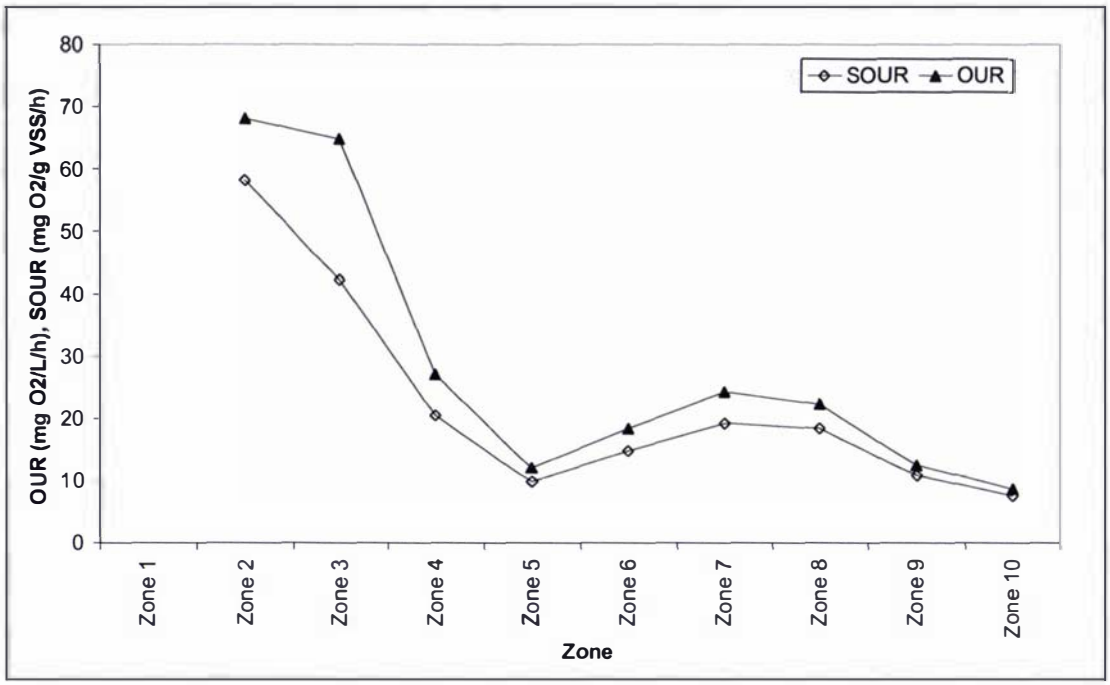


Figure 7.43: SOUR and OUR zone profiles for AO reactor operated at an SRT of 5 days.

7.7 Discussion

An AO configured activated sludge reactor exhibited sustainable biological phosphorus removal at SRT's of 10 and 15 days but not at an SRT of 5 days. After 79 days 34.5 mg P/L was removed at an SRT of 15, and at an SRT of 10 days, 34.9 mg P/L of phosphorus was removed at the time of the zone studies. At an SRT of 5 days, only 18.2 mg P/L of phosphorus was removed with a sludge phosphorus concentration of 2.6% mg P/mg VSS. This compares to the combined nitrogen and phosphorus removal systems, were at the time of the zone studies, the AAO system removed 37.6 mg P/L and the EAAO system removed 41.3 mg P/L. The removal of the anoxic zone had a slight negative effect on phosphorus removal. The important anaerobic zone parameters influencing the EBPR process for AO and AAO systems are shown in Table 7.11. All parameters are for the anaerobic zone except the following two values: the value for phosphorus removal is calculated from the difference between the fermenter effluent total phosphorus concentration and the activated sludge reactor zone 10 soluble phosphorus concentration and the sludge phosphorus concentration was for the final aerobic zone sludge. The anaerobic stoichiometric ratios are

summarised in Table7.12 for both the AO continuous reactor and AO sludge batch tests.

Table 7.10: Individual zone parameters for AO configuration at an SRT of 5 days.

	Zone									
Parameter	1 Anaerobic	2 Aerobic	3 Aerobic	4 Aerobic	5 Aerobic	6 Aerobic	7 Aerobic	8 Aerobic	9 Aerobic	10 Aerobic
$COD_{soluble}$ (mg/L)	527	194	38	49	57	67	66	77	91	85
PO_4-P (mg/L)	85.2	83.9	79.3	79.3	77.7	76.7	77.0	76.8	76.3	75.7
%mg P/g VSS	-	-	-	-	-	-	-	-	-	2.61
NO_3-N (mg/L)	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0
SOUR mgO_2/mg VSS/h	-	58.3	42.4	20.7	10.4	15.0	19.4	18.6	11.0	7.7
Phosphorus Uptake (mg P/L)	0.8	1.3	4.6	0.0	1.6	1.3	-0.6	0.2	0.5	0.6
TSS (mg/L)	1180	1330	1780	1545	1480	1460	1460	1390	1360	1350
VSS (mg/L)	1040	1170	1530	1320	1220	1240	2260	1210	1150	1145
VSS/TSS ratio	0.88	0.88	0.86	0.85	0.82	0.85	0.86	0.87	0.85	0.85

Table 7.11: Summary of anaerobic zone parameters during zone studies, except for P removed and % mg P/mg VSS which relate to final aerobic zone (zone 10).

System	AO	AO	AO	AAO	EAAO (zone 1)
SRT(days)	15	10	5	10	10
TSS (mg/L)	3810	2660	1180	2380	3635
VSS (mg/L)	3255	2280	1040	1970	3140
COD (mg/L)	58	358	527	183	210
COD consumed (mg COD/L)	709	399	278	483	465
COD consumption rate (mg COD/g VSS/h)	38.2	33.0	48.4	41	24.7
VFA (mg COD/L)	36	335	474	146	208
VFA consumed (mg COD/L)	472	225	-11	450	429
VFA consumption rate (mg COD/g VSS/h)	25.4	18.6	-2	38	22.8
PO4-P (mg/L)	125.7	128.5	85.2	106.8	111.8
P release (mg P/L)	42.1	41.6	-0.7	24.2	29.9
P release rate (mg P/g VSS/h)	4.5	6.9	-0.5	4.1	1.9
P removed overall (mg P/L)	34.5	34.9	18.2	37.6	41.3
(%mgP/mg VSS) -Zone 10	5.12	5.53	2.6	6.99	5.6
PHA (mg COD/ g TS)	85.6	-	-	43.8	
Glycogen (mg COD/g TS)	34.7	-	-	79.4	

Table 7.12: Stoichiometric constants from each zone study and batch test for the AO systems.

Stoichiometric Constant	Anaerobic zone (zone 1)			Batch Test	
SRT	15	10	5	15	10
$P_{release}$/ COD $_{consumed}$ (mg P/mg COD)	0.06	0.10	0	0.19	0.45
$P_{release}$/ VFA COD $_{consumed}$ (mg P/mg COD)	0.09	0.19	0	0.19	0.45
$P_{release}/PHA_{stored}$ (mg P/mg COD)	0.17	-	-	0.31	
$PHA_{stored}/VFA_{consumed}$ (mg COD/mg COD)	0.54	-	-	0.61	
$Glycogen_{consumed}/VFA_{consumed}$ (mg COD/mg COD)	0.17	-	-	0.18	

The 15 day SRT AO reactor had a similar TSS and VSS concentration to the EAAO system but a much greater soluble COD consumption value (709 mg/L). The 15 day reactor also had slightly more zone 1 anaerobic VFA consumption (472 mg COD/L), than the AAO system (450mg COD/L) and the EAAO system (429 mg COD/L). Further fermentation in the anaerobic zone of the 15 day SRT system might have converted non-VFA soluble COD to VFA COD before consumption and conversion to PHA. This is unlikely to account for all of the difference between the COD and VFA consumption of 237 mg COD/L. During the period of the 15 day SRT reactor operation the anaerobic zone soluble COD progressively decreased (Figure 7.15), by 251 mg/L between days 17 and 79. Judging by the batch test value of Y_{PO4} for this reactor of 0.19 mg P/mg COD, compared to the 10 day SRT batch test value of 0.45 mg P/mg COD, it appears that there may have been growth of organisms that can successfully compete with PAO's (such as GAO's) during the reactor operation. This difference is also seen in the continuous reactor Y_{PO4} values which were 0.06 for the 15 day SRT reactor yet and 0.1 for the 10 day SRT. As mentioned previously (Chapter 6) Smolders *et al.* (1994), determined that higher ratio's occurred at higher

pH values, yet the pH in the anaerobic zone of the 15 day and 10 day SRT systems were 7.48 and 7.25 respectively. The pH for the 15 day batch test was controlled at 7.0, while the pH of the 10 SRT AO batch ranged from 7.05 to 7.24 for the anaerobic phase, not a significant pH difference. While the presence of competing organisms in the 15 day SRT system appeared to be more significant than that at the 10 day SRT, the amount of phosphorus removal was similar for both the 10 day and the 15 day SRT systems. The system operated at a 5 day SRT deteriorated to the stage that, at the time of the zone study, zero phosphorus was released under anaerobic conditions. It appeared that the growth of the PAO's could not be sustained at the 5 day SRT and they were gradually washed from the system during the reactor operation. Henze *et al.* (2002), also found a low SRT value of 4 days gave unstable biomass settling characteristics and a more variable biomass.

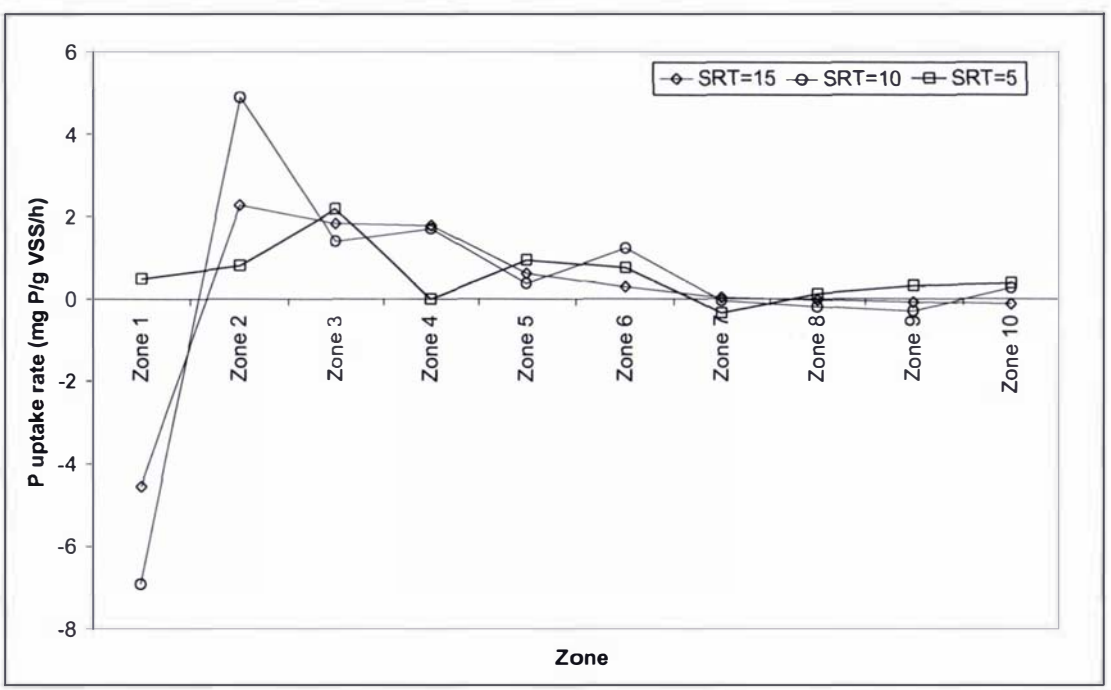


Figure 7.44: Phosphorus release (negative) and uptake (positive) rates for each AO system zone study.

In Figure 7.44 it can be seen that the specific phosphorus release and uptake rates are higher for the 10 day SRT system. The rates in zones 1 and 2 are –6.9 and 4.9 mg P/g VSS/h respectively compared to the 15 day SRT system which were –4.5 and 2.3 mg

P/g VSS/h respectively. This difference reflects that a higher fraction of the VSS concentration in the 15 day system were non-PAO organisms (eg. GAO's). Sludge that is GAO enriched takes up acetate slower than sludges that are dominated by PAO's. (Mino *et al.*, 1998).

The storage of PHA under anaerobic conditions was measured in the 15 day SRT AO system only. This reactor stored a higher amount of anaerobic PHA (85.6 mg COD/g TS) than the AAO 10 day SRT system (43.8 mg COD/g TS). This is possibly due to the greater anaerobic zone COD consumption of the 15 day SRT AO system and the fact that both GAO's and PAO's store PHA under anaerobic conditions. GAO's generally produce more PHV than PAO's under anaerobic conditions (Oehman *et al.*, 2000) and with the 15 day SRT AO system, 52% was present as PHV while with the AAO 10 day SRT system, 31% was present as PHV. The 15 day SRT anaerobic phosphorus released to PHA stored ratio of 0.17 and 0.31 for the batch test compares to a value of 0.27 for the AAO continuous reactor and a value of 0.34 found by Smolders *et al.*, (1994). The ratio of PHA stored to VFA consumed of 0.54 for the continuous reactor and 0.61 for the batch test is greater than the value of 0.2 for the AAO continuous system. The increased PHA storage without a corresponding increase in phosphorus removal is also a characteristic of a system with greater proportions of competing organisms. It is possible that while the 15 day SRT reactor was successfully operated for a total of 79 days, the increasing anaerobic soluble COD consumption, without a corresponding increase in phosphorus removal could have lead in the long term to a gradual deterioration of EBPR due to the growth and subsequent domination of competing organisms such as GAO's. It appears that long term, operating the AO reactor at a 10 day SRT may be more favourable than operating at a 15 day SRT. Operating at a 5 day SRT was not sustainable even in the short term.

7.8 Conclusions

Operating a biological phosphorus removal only configuration (AO) removed less phosphorus than for a combined phosphorus and nitrogen removal reactor (AAO). About 35 mg P/L was removed by both the 10 day and 15 day SRT operation, with sludge phosphorus concentrations of 5.53 and 5.12 %mg P/mg VSS respectively. The

previous AAO and EAAO systems removed 37.6 and 41.3 respectively, which suggests that the inclusion of an anoxic zone was beneficial to biological phosphorus removal.

There was no improvement in phosphorus removal by increasing the SRT to 15 days although there was almost complete VFA consumption in the anaerobic zone at the higher SRT. It appeared that increasing the SRT permitted competing organisms increase in the reactor, which while it increased the anaerobic COD consumption did not results in improved phosphorus removal. This was confirmed by the anaerobic batch tests which produced a Y_{PO4} value of 0.45 for the 10 day SRT system and but a much lower value of 0.19 for the 15 day SRT system.

Decreasing the SRT to 5 days lead to a progressive deterioration in the EBPR process to a stage, that eventually there was no anaerobic phosphorus release or anaerobic VFA consumption. The sludge phosphorus concentration decreased to only 2.6 %mg P/mg VSS and resulted in only 18.2 mg/L of phosphorus removal. The principal reason for the poor performance at a 5 day SRT, was due to reactor instability resulting from poor biomass settleability. This resulted in a progressive washout of PAO organisms.

CHAPTER 8
Influences on Phosphorus Removal

8.1 Introduction

The influence of phosphorus precipitation, partially fermented or non-fermented wastewater and the metal cations of magnesium, potassium and calcium, on dairy processing wastewater EBPR systems are investigated in this Chapter. Phosphorus precipitation could potentially be influenced by the calcium concentration in milk processing wastewater. Comeau *et al.* (1996), had previously reported a significant amount of chemical precipitation from a pilot plant treating fermented cheese processing wastewater. Non-fermented or partially fermented dairy processing wastewater could also potentially lead to the deterioration of the EBPR process as milk products contain carbohydrates such as lactose. Glucose has previously been identified as a possible cause for GAO proliferation and subsequent EBPR deterioration (Cech and Hartman, 1990, 1993). The metal cations magnesium, calcium and potassium are important counter-ions for the EBPR process to satisfy electroneutrality (Pattarkine and Randell, 1999). Danalwich *et al.* (1998), identified magnesium and potassium as potentially limiting cations in some dairy processing wastewaters and Ky *et al.* (2001), found low concentrations of magnesium in a cheese processing wastewater. In this study, potassium is present in excess due to its addition as part of phosphorus supplementation.

8.2 Phosphorus Precipitation

If significant phosphorus precipitation occurs it will affect the phosphorus release and uptake rates and stoichiometric constants that are attributed to biological phosphorus removal. Also if phosphorus precipitation occurs then it is desirable and should be maintained or increased if possible. In the De Haas *et al.* (2000a), literature review of simultaneous phosphorus precipitation and biological phosphorus removal, it was stated that iron salts were most commonly used for the combined phosphorus removal. However, whole milk has a high concentration of calcium (Table 2.5), which would be the most likely pathway for phosphorus precipitation in this study. In whole milk the calcium concentration is about 1200 mg/L and in the synthetic wastewater

used in the AO (COD/TKN=32) study it was measured at 15.3 mg/L in the fermented wastewater. The ratio of calcium in the low nitrogen synthetic wastewater to that typical of whole milk (Table 2.4) is 0.0128. This ratio is also close to the ratio of the low nitrogen synthetic wastewater COD to whole milk COD of 0.0129, which makes the concentration of calcium measured close to what is theoretically expected for a wastewater with a COD of 2600 mg/L that is derived from diluted milk components. Chemical phosphorus removal with calcium salts is usually in the pH range of 8 to 11. De Haas *et al.* (2000a), states that precipitation with calcium occurs around a pH of 9, which is outside the pH operating range of most biological treatment systems. Carlsson *et al.* (1997), studied calcium phosphate precipitation in biological phosphorus removal systems and found that at a pH of 7 the phosphorus concentration must be at least 50 mg/L for precipitation to occur at a calcium concentration of 100 mg/L. The phosphorus must also be about 100 mg P/L at a calcium concentration of 50 mg/L for precipitation to occur at a pH of 7. However at a pH of about 8.5 and a calcium concentration of 50 mg/L the phosphorus concentration should be at least 8 mg/L for precipitation to occur. The pH in the final aerobic zone was generally about 8 and the calcium concentration in the synthetic dairy processing wastewater used here is much less than 50 mg/L. The phosphorus concentrations in the aerobic zones of 104.9 to 63.5 mg P/L, which are much higher than 8 mg/L may possibly permit some phosphorus precipitation at the pH values measured in the aerobic zones (Figure 8.1).

Comeau *et al.* (1996), in their pilot scale study on nitrogen and phosphorus removal from a fermented cheese processing wastewater performed a phosphorus fractionation on the waste sludge. The sludge had a phosphorus content of 7.6 % mg P/mg VSS, with 2.1% mg P/mg VSS attributed to the precipitated and adsorbed fraction. Therefore, this precipitation accounted for 27.6% of the total phosphorus removal. Subtracting the precipitated/adsorbed fraction from the total gives 5.5 % mg P/mg VSS, this amount can be attributed to both phosphorus assimilation and luxury uptake. This value of 5.5 % mg P/mg VSS is closer to the values obtained in Chapter 7 for the AO reactors. Comeau *et al.* (1996), did not state what the pH value was during the aerobic or sludge wasting phase or the wastewater calcium concentration. It was therefore difficult to attribute the precipitated/adsorbed phosphorus to calcium or

another ion or some other factor, but as previously stated is most likely to be from calcium precipitation. In Chapter 5 the results were presented for phosphorus fractionation of the sludge when the pH was 8. The phosphorus content of the sludge at the time of the fractionation study (Day 35) was 2.2 %mg P/mg VSS, without enhanced phosphorus removal occurring. The precipitated phosphorus in this instance was only 0.18% mg P/mg VSS or 8.2% of the sludge phosphorus content. The fermented cheese processing wastewater used in the Comeau *et al.* (1996), study may have had a higher calcium concentration than the synthetic wastewater used here, making phosphorus precipitation a more significant factor.

In the continuous reactor studies discussed previously in Chapters 6 and 7, the pH in the final aerobic zones was usually above 8 (Figure 8.1). The highest pH values were 8.51 in zones 7 and 8 of the 10 day SRT AO system and the pH in zone 10 ranged between 7.9 for the AAO 10 days SRT system and 8.3 for the 15 day SRT AO system. This pH increase in the aerobic zones was due to carbon dioxide stripping. For the 15 day SRT AO system, the soluble calcium concentration was measured for three zones and is shown in Figure 8.2. The total calcium concentration in the fermented wastewater was 15.3 mg/L. This information can provide an estimate of the phosphorus precipitated from the calcium decrease. The influent flow rate was measured at 8.21 L/day (influent calcium concentration of 15.3 mg/L) and the RAS rate measured at 8.64 L/day (zone 10 calcium concentration of 11.7 mg/L). A mass balance calculation assuming no calcium uptake or release gives a calcium concentration in the first zone of 13.5 mg/L. The actual measured soluble value in zone 1 (anaerobic zone) was 18.7 mg/L, which suggests there was some redissolution of the calcium precipitate from the recycle sludge in this zone (due to the lower pH of 7.48). The calcium concentration decreased to 12.7 mg/L in zone 5 and then to 11.7 mg/L in zone 10. As only a small amount of calcium is be used as a counter ion in the EBPR biochemical mechanism (Comeau *et al.*, 1986), it will be assumed that the decrease in calcium is due to precipitation only. Carlsson *et al.* (1997), stated at a pH of about 8.5 a compound resembling $CaHPO_4$ (dicalcium phosphate) is formed during precipitation in an activated sludge process. As the pH in zones 5 to 10 ranges from 8.18 to 8.4, it will be assumed that the principle precipitate is $CaHPO_4$. The Ca:P molar ratio for this is 1:1 or 1.3:1 on a weight basis. A calcium mass balance through

the reactor shows there is an overall calcium decrease through the reactor of 1.8 mg/L, which equates to 1.4 mg/L of phosphorus, removed as a calcium precipitate. That is, 11.5 mg P/day (1.4 mg/L x 8.2 L/day) is removed in 4498 mg VSS/day of volatile solids (1.3 L x 3460 mg VSS/L) at an SRT of 15 days. This equates to approximately 0.26 % mg P/mg VSS of the waste sludge being chemically precipitated phosphorus for the 15 day SRT reactor. This is only 5.1 % of the sludge phosphorus content of 5.12 %mg P/mg VSS being due to precipitated phosphorus. The value of 0.26 %mg P/mg VSS is reasonable close to the value of 0.18 %mg P/mg VSS determined for the preliminary MUCT reactor in Chapter 5. Based on this information chemical phosphorus precipitation is not a significant factor with the wastewater and reactor systems used in this study.

If it assumed that with the zoned reactor system, about 0.25 % mg P/mg VSS results from precipitated phosphorus, than, how will this affect the stoichiometric ratios that are related to phosphorus release in the anaerobic zone? The pH in the anaerobic zones of each of the four zone studies ranged from 6.98 to 7.48, which means that precipitation will most likely be minimal, but with the high anaerobic phosphorus concentrations due to phosphorus release some precipitation, may be possible. The fact that redissolution of calcium was evident in the 15 day SRT system anaerobic zone shows that the opposite occurred. The amount of phosphorus release in the anaerobic zone was a combination of, release due to PAO's and redissolution of the phosphate precipitate. This would affect the value of Y_{PO4} by making it slightly greater than if no redissolution occurred. The amount of redissolution of calcium was 5.2 mg/L at the 15 day SRT which equates to approximately 4 mg/L of phosphorus released (based on a Ca:P ratio of 1.3:1) due to redissolution or 9.5 % of the total phosphorus released. If the value of Y_{PO4} were adjusted to account for the redissolution of precipitated phosphorus, it would change from only 0.09 to 0.08 mg P/mg VFA COD.

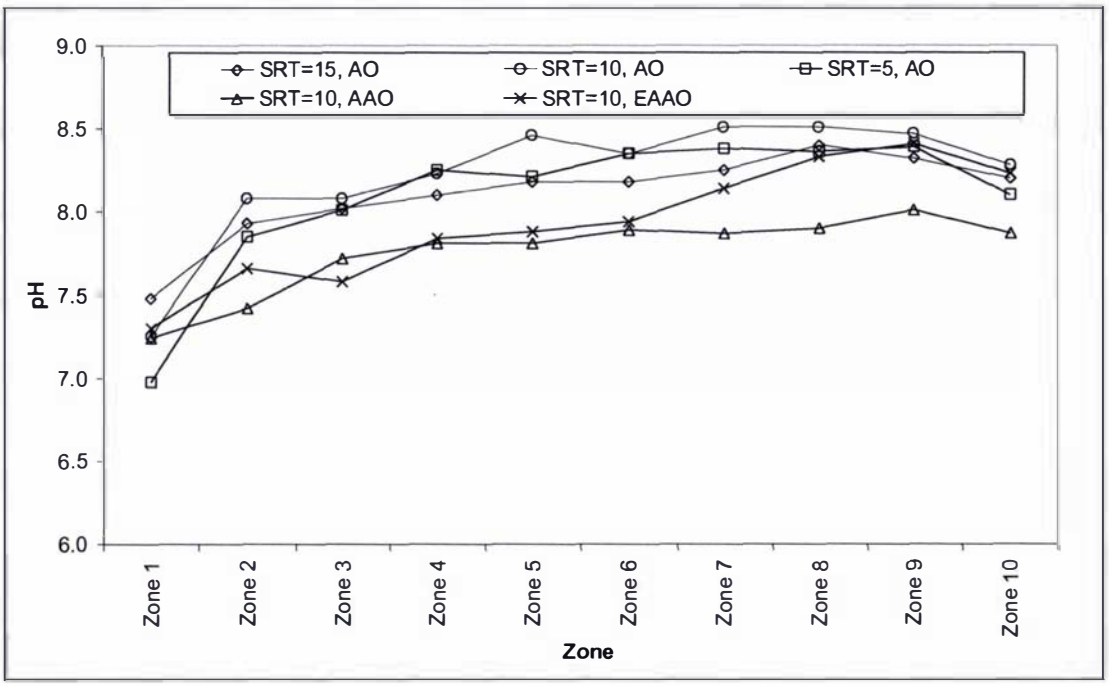


Figure 8.1: Zone pH values for each zone study.

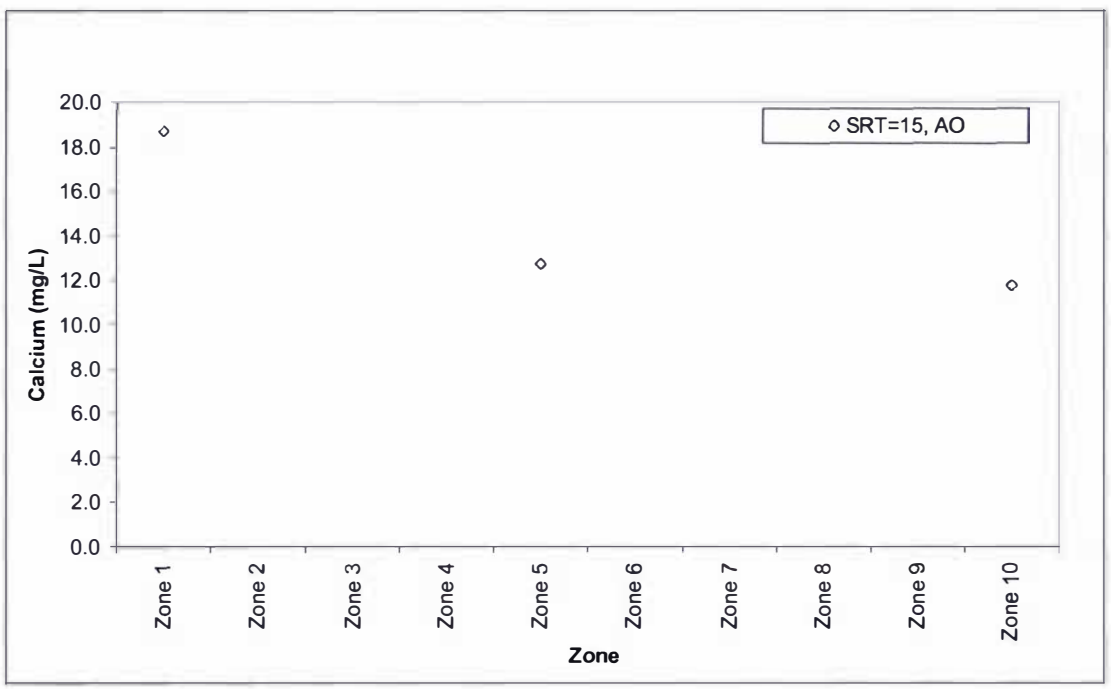


Figure 8.2: Soluble calcium values for zones 1 ,5 and 10 for the 15 day SRT AO system The total calcium concentration value in the influent is 15.3 mg/L.

8.3 Cessation of External Fermentation - Medium Term Effects

It is possible, that occasionally the degree of wastewater acidification during fermentation will be low or absent due to process control failure or process overloads. It is therefore valuable to assess whether, in such instances the viability of the EBPR process will remain. After completion of the 15 day SRT AO, the reactor was altered to a 10 day SRT (to minimise potential EBPR deterioration due to the growth of competing organisms) and the raw wastewater was pumped to directly to the zoned activated sludge reactor. The reactor effluent soluble phosphorus concentration was periodically monitored for a 34 days and then a full zone study performed. It was initially expected that a noticeable deterioration would occur within the first week, however after 34 days (slightly more than three SRT's), phosphorus removal had not deteriorated (Figure 8.2) and a zone study was performed.

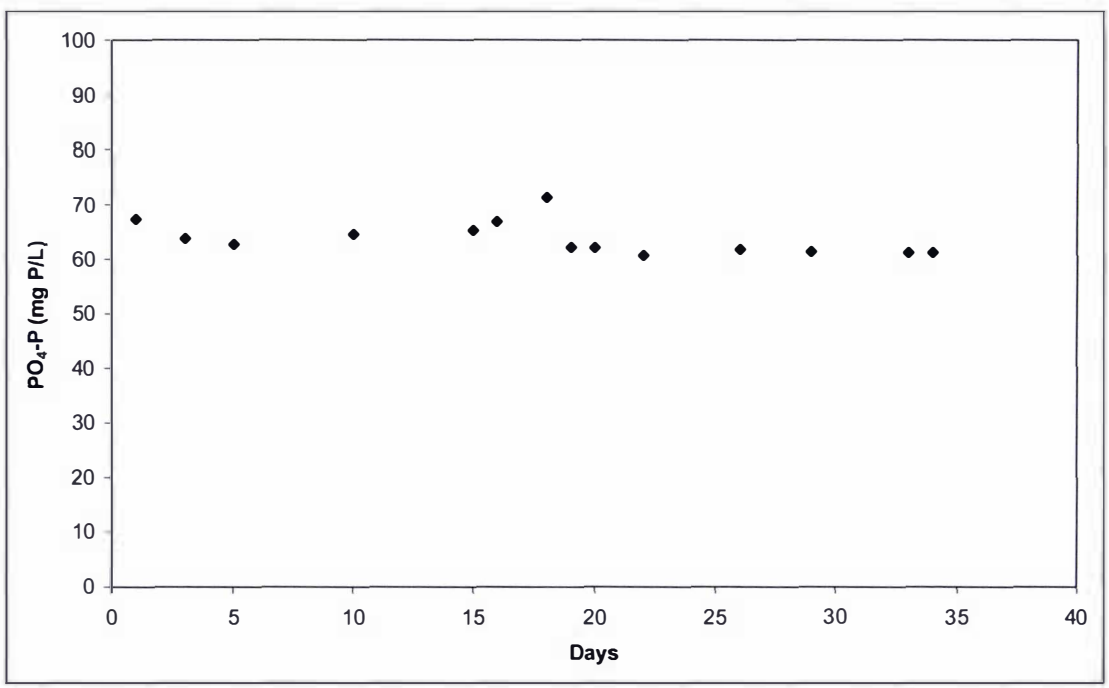


Figure 8.3: Soluble effluent phosphorus concentration over a 34 day period for a AO configured reactor fed unfermented wastewater.

On day 34, the synthetic wastewater was analysed by disconnecting the feed tube from the reactor and allowing the wastewater to pump into a 50 mL sample container. The COD and VFA analysis results are shown in Table 8.1. The VFA analysis showed that some acetic acid (88 mg COD/L) and iso-valeric acid (16 mg COD/L)

was present in the synthetic wastewater (Table 8.2). It is most likely that some fermentation most likely occurred within the length of rubber tubing (~3.5m length) that conveyed the wastewater to the peristaltic pump and than to the reactor, rather than in the refrigerator. The rubber tubes were cleaned of solids accumulation every two days. At the time of this study, it was summer and while the reactor was temperature controlled (20°C), and the wastewater refrigerated (4°C), the reactor tubing was at room temperature in a room that was not air-conditioned. Essentially then, this study simulated a situation were the amount of wastewater acidification was low (5% of 0.45μm filtered COD or 4.5% of GF/C filtered COD), with the rest the soluble COD fraction belonging to the readily biodegradable fraction and the slowly biodegradable fraction. There may also have been some conversion from the slowly biodegradable fraction to the readily biodegradable fraction due to hydrolysis during the pumping process.

Table 8.1: Characteristics of synthetic wastewater immediately before entry to zoned reactor.

Parameter (all units mg/L)	Value
Total COD	2510
GF/C (1.2μm) Filtered COD	2155
0.45 μm Filtered COD	1955
VFA COD	105
TSS	325
Total Phosphorus	95
Soluble Phosphorus	74

On the day of the study, there was a slight uneven profile of the sludge concentration through the reactor as seen in Figure 8.3. The increase in zone 10 was due to a partial blockage in the tube between the final aerobic zone and the clarifier. The average TSS and VSS concentrations were 2730 and 2300 mg/L respectively, close to the values of 2825 and 2285 obtained from the 10 day SRT AO study (Chapter 7). The VSS/TSS ratio in the anaerobic zone was 0.88 and in the final aerobic zone was 0.82, with equated to an increase in the ash content of the sludge from zone 1 to zone 10 of 5.3%

(compared to 6.4% change in 10 day SRT AO system). The increase in ash content was due to the typical EBPR phosphorus release and uptake profile as seen Figure 8.5.

The soluble COD concentration in the anaerobic zone was 455 mg/L of which 156 mg/L of COD or 34% was due to short chain VFA compounds. The concentrations of the individual VFA are shown in Table 8.2 for each zone that VFA were detected. From mass balance calculations, 624 mg/L of soluble COD was consumed in the anaerobic zone (58% of available soluble COD), and a net amount of 105 mg/L of VFA COD was produced in the anaerobic zone. The amount of COD consumption was larger than all the other zoned systems, except the 15 day SRT system. The COD consumption rate was 45 mg COD/g VSS/h. The production of VFA compounds within the anaerobic zon,e showed that fermentation within the anaerobic zone is possible as has been previously proposed (Wentzel *et al.*, 1985; Barnard, 1994). The DO concentration in the first aerobic zone (zone 2) was relatively low at about 0.5 mg/L, which contributed to the high soluble COD concentration (215 mg/L) in this zone, of which 92 mg/L was due to acetic acid.

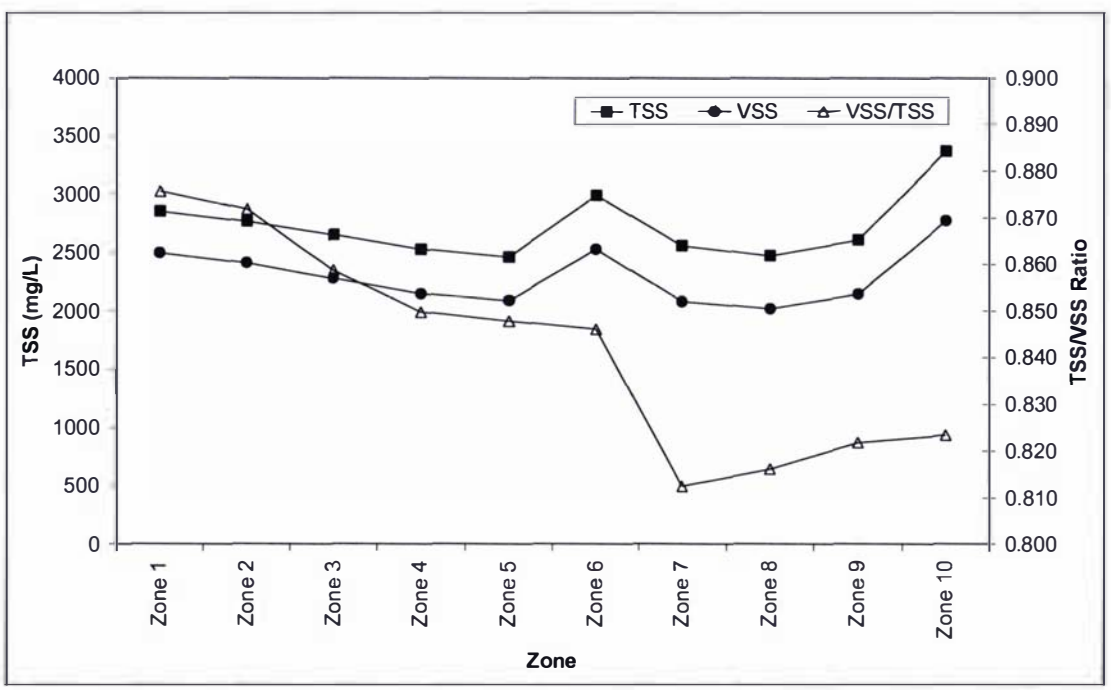


Figure 8.4: TSS, VSS and VSS/TSS zone profiles for AO system treating unfermented wastewater.

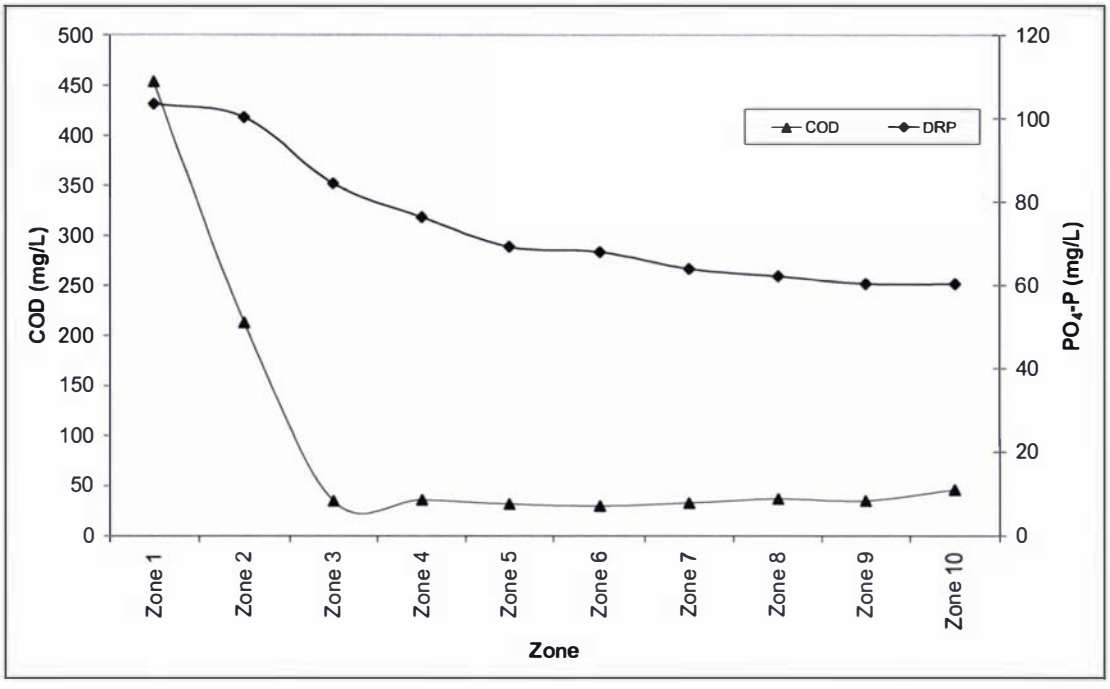


Figure 8.5: Soluble COD and phosphorus profiles for AO system treating unfermented wastewater.

Table 8.2: Individual and total VFA concentrations.

	Wastewater (after pumping) (mg COD/L)	Zone 1 (Anaerobic) (mg COD/L)	Zone 2 (Aerobic 1) (mg COD/L)
Acetic acid	88	116	92
Propionic acid	0	20	0
n-Butyric acid	0	0	0
iso-Butyric acid	0	10	0
n-Valeric acid	0	6	0
iso-Valeric acid	16	0	0
n-Caproic acid	0	5	0
Total VFA COD	105	156	92

The amount of anaerobic phosphorus release of 25.8 mg/L, was similar to the AAO and EAAO reactors (24.2 and 29.9 mg/L) but less than the previous AO 10 day and 15 day SRT studies (42.1 and 41.6 mg/L). The rate of anaerobic phosphorus release was 1.9 mg P/g VSS/h, similar to the EAAO system. This system had the lowest ratio of phosphorus released to soluble COD consumed of 0.04 mg P/mg COD. Full phosphorus release appears to not have occurred in the anaerobic zone as the sludge phosphorus content was 3.18 % mg P/mg VSS (Figure 8.7). Most of the phosphorus uptake occurred in zone 3 (15.9 mg P/L) when the soluble COD concentration had reached a low value. The phosphorus content of the sludge in the final zone reached a concentration of 4.42 % mg P/mg VSS, which is slightly lower than the previous systems exhibiting EBPR. A maximum value of 4.94 % mg P/mg VSS was reached in zone 6. A total of 33.8 mg/L of phosphorus was removed at the time of the zone study. A mass balance based on the phosphorus removed and the amount of sludge wasted each day results in a theoretical waste sludge concentration of 4.81 % mg P/mg VSS. As with the other zoned reactor studies there were some variations in the sludge phosphorus content profiles that were not matched by comparative variations in the soluble phosphorus concentrations. The nitrate concentration was 1.5 mg NO_3-N in the final zone, which was less than for the fermented 10 day SRT AO reactor and again did not appear to have any negative effect on phosphorus removal.

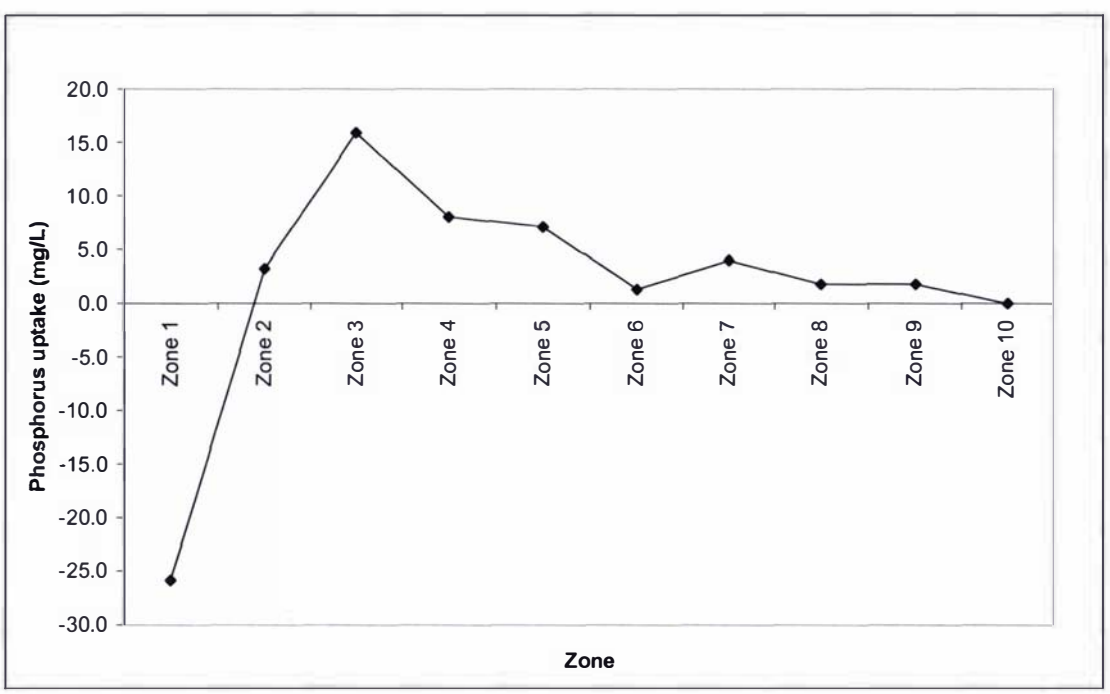


Figure 8.6: Anaerobic zone phosphorus uptake for non-fermented system.

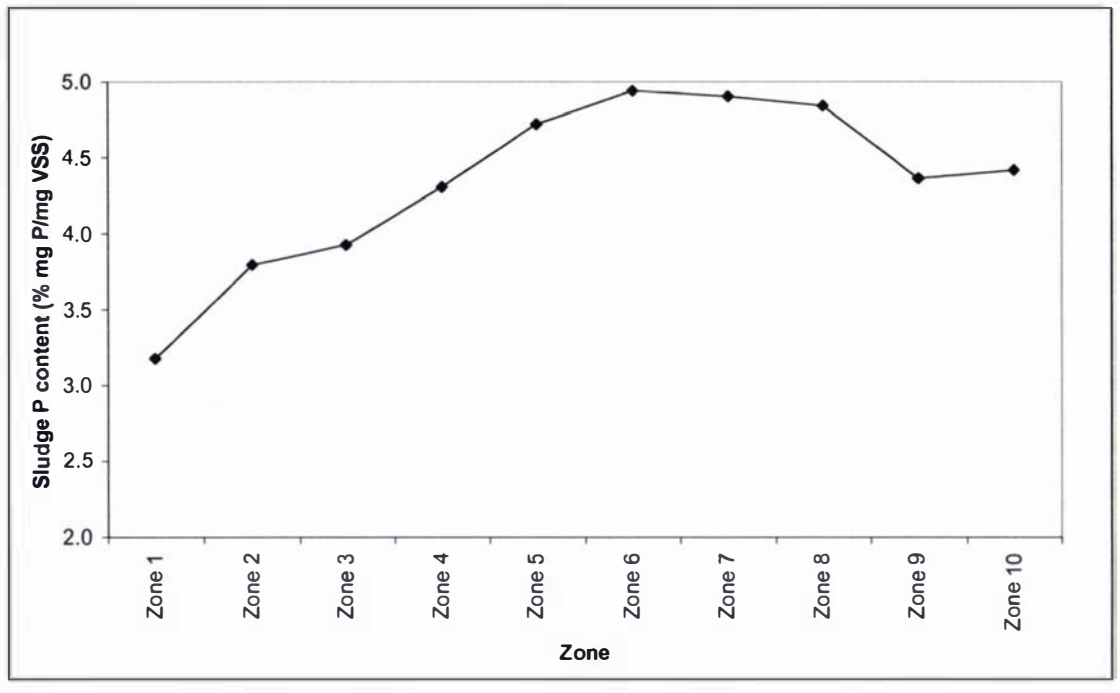


Figure 8.7: Sludge phosphorus content for each zone.

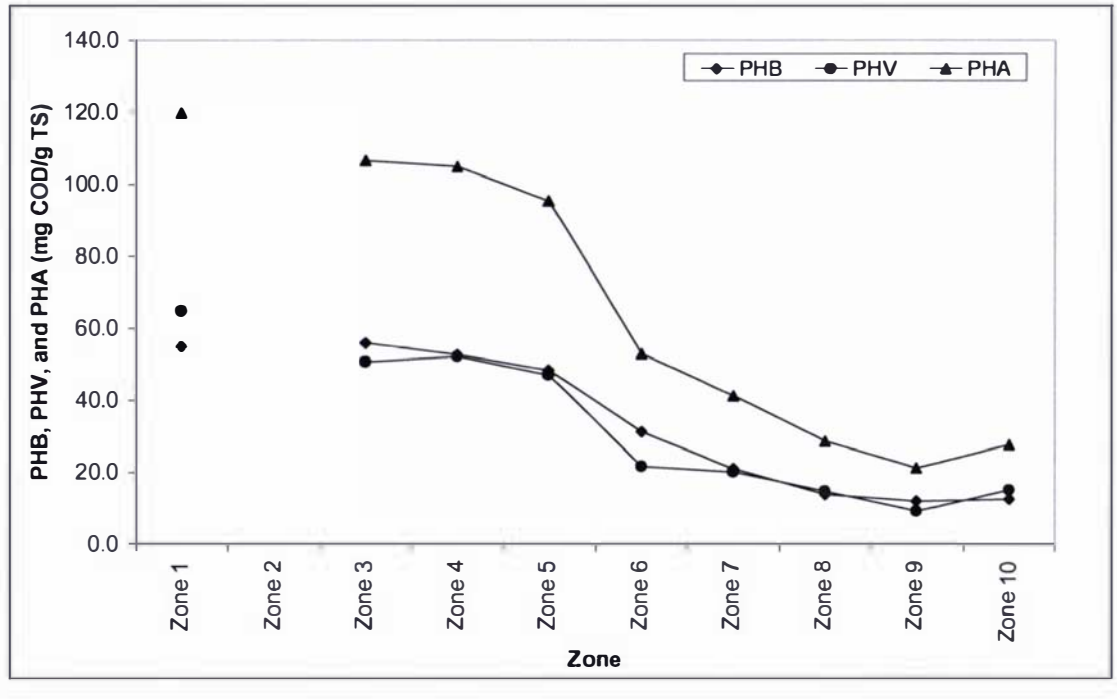


Figure 8.8: Zone PHB, PHV and PHA concentrations.

The storage compounds PHB and PHV were analysed for each zone (Figure 8.8), except zone 2 as the sample for this zone lost during vacuum drying. The amount of PHB was slightly greater in zone 1 and zone 6 than the amount of PHV, but was not substantially different throughout the reactor. The total PHA concentration in zone 1 was 120 mg COD/g TS, much greater than the 10 day SRT AAO system (43.8 mg COD/g TS) and 40% greater than the 15 day SRT AO system (85.6 mg COD/ TS). The high PHA concentration is most likely attributable to the presence of competing organisms such as GAO's as is the low value of Y_{PO4} (0.04). The ratio of phosphorus released to PHA stored was 0.07, much lower than both the 15 day SRT AO system (0.17) and the 10 day SRT AAO system (0.31), an indication of anaerobic PHA synthesis by both GAO's and PAO's but phosphorus release by PAO's only.

The respiration rate in zone 2, the first aerobic zone, was high as expected due to the high soluble COD concentration (Figure 8.8), otherwise the respiration rates were similar to the other systems. Table 8.3 summarises the analytical parameters for each zone.

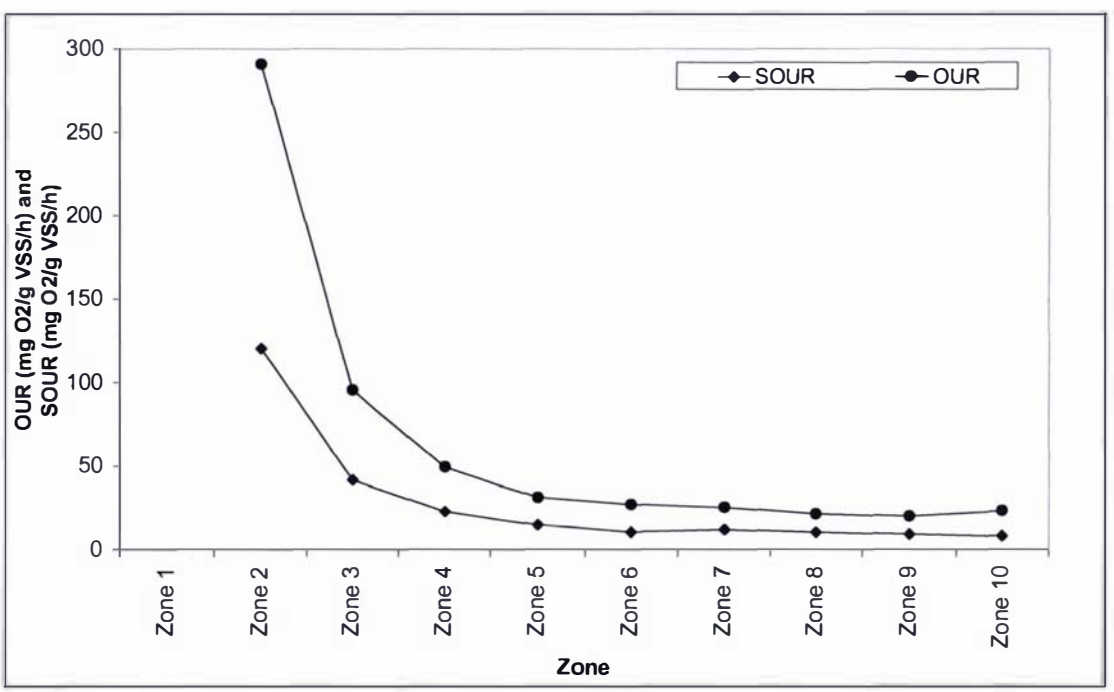


Figure 8.9: SOUR and OUR respiration rates for each zone.

8.3.1 Batch Test

This batch test used two litres of mixed liquor from the 10 day SRT AO reactor fed unfermented wastewater and had a 240 minute anaerobic phase and a 240 minute aerobic phase. At time zero 500 mg/L of acetate (as sodium acetate) was added to give a starting COD concentration of 533 mg/L. The initial TSS concentration was 3880 mg/L and the VSS concentration was 3165 mg/L. Also at the start of the batch test the pH was adjusted to 7.0. There was a single rate of COD consumption of 25.4 mg COD/g VSS/h. The phosphorus released at a relatively constant but slow rate of 1.6 mg P/g VSS/h until 180 minutes and then there was a small soluble phosphorus decrease for the last hour of the anaerobic phase. The phosphorus released to COD consumed ratio was 0.04 mg COD/mg P. This value was much lower than any of the previous batch tests that exhibited EBPR behaviour. This reactor was likely to be in a state of slow EBPR deterioration due to the gradually dominance of competing organisms that can sequester substrate under anaerobic conditions. The analytical parameters and the start and end of the anaerobic phase and the end of the aerobic phase are shown in Table 8.4 below.

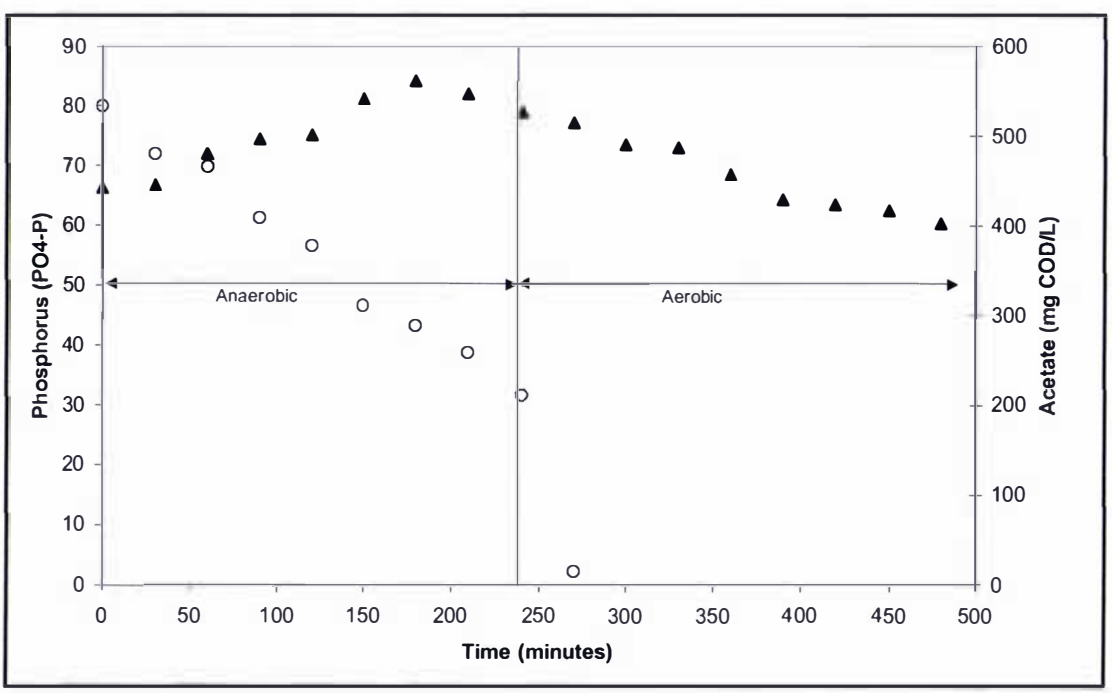


Figure 8.10: Soluble COD and PO_4-P profiles during batch test for non-fermented system.

Table 8.3: Individual zone parameters for non-fermented wastewater AO configuration at an SRT of 10 days.

	Zone									
Parameter	1 Anaerobic	2 Aerobic	3 Aerobic	4 Aerobic	5 Aerobic	6 Aerobic	7 Aerobic	8 Aerobic	9 Aerobic	10 Aerobic
$COD_{soluble}$ (mg/L)	455	213	35	36	32	30	33	37	35	46
PO_4-P (mg/L)	103.6	100.4	84.5	76.4	69.3	68.0	64.0	62.2	60.4	60.4
%mg P/g VSS	3.18	3.80	3.93	4.31	4.72	4.94	4.90	4.84	4.36	4.42
NO_3-N (mg/L)	0	0.0	0.0	0.0	0.3	0.2	0.7	1.2	1.5	1.0
SOUR mgO_2/mg VSS/h	-	120.5	41.9	23.0	15.0	10.8	12.2	10.6	9.4	8.4
Total PHA (mg COD/g TS)	119.6	-	106.6	105.0	95.4	53.0	41.3	28.7	21.3	27.7
TSS (mg/L)	2855	2770	2655	2530	2465	2990	2560	2475	2610	3370
VSS (mg/L)	2500	2415	2280	2150	2090	2530	2080	2020	2145	2775
VSS/TSS ratio	0.88	0.87	0.86	0.85	0.85	0.85	0.81	0.82	0.82	0.82

Table 8.4: Analytical parameters at time 0, 240 and 480 minutes for the batch of the unfermented 10 day SRT AO system.

	Time		
Parameter	0 minutes	240 minutes	480 minutes
PO_4-P (mg P/L)	65.0	79.0	60.4
Acetate COD (mg/L)	533	211	0
VSS (mg/L)	3165	3275	3285
VSS/TSS	0.816	0.845	0.809
OUR (mg O_2/L/h)	-	480	53
SOUR (mg O_2/ g VSS/h)	-	147	16

8.4 Nutrient Requirements

The metal ions potassium and magnesium are used as counter ions during phosphate release and uptake to satisfy electroneutrality (Pattarkine and Randall, 1999). Smolders *et al.* (1994), estimated the composition of polyphosphate as $Mg_{1/3}K_{1/3}PO_3$ based on the release of phosphorus, magnesium and potassium under anaerobic conditions. The molar ratios of potassium and magnesium co-transported with phosphorus are typically 0.20 to 0.40 K^+/P and 0.24 to 0.30 Mg^{2+}/P (Comeau *et al.*, 1986; Christensson, 1997) or 0.25 to 0.51 K^+/P and 0.19 to 0.24 Mg^{2+}/P on a weight basis. Calcium has also been proposed as metallic ion that is also co-transported with phosphorus and may be important for stabilisation of the EBPR process (Schonborn *et al.*, 2001). The molar ratio of calcium co-transported with phosphorus has been reported to range from 0.0 to 0.12 Ca^{2+}/P (Comeau *et al.*, 1986) or 0.0 to 0.16 on a weight basis. In some instances, the calcium concentration remains constant under both anaerobic and aerobic conditions. Schonborn *et al.* (2001), used X-ray spectroscopy to show that Ca, Mg and K were the principal metal components of polyphosphate granules and that the ratios of Ca, Mg and K varied, depending on the influent concentration of these metals. They reported that with an influent concentration of 16.4 mg/L (similar to the wastewater in this study) of calcium the polyphosphate granules contained no calcium but when the influent calcium was increased to 164 mg/L then calcium rich granules were found.

Ky *et al.* (2001), created a model for biological phosphorus removal from a fermented cheese factory effluent using an SBR and added a Monod-type saturation function for magnesium to account for magnesium limitations. The half saturation constant for magnesium was estimated as 10 mg/L and the stoichiometric ratio between magnesium and phosphate was experimentally determined as 0.157 g Mg/g P.

On the day of the zone study for the AO system operated at an SRT of 15 day, magnesium, potassium and calcium were measured in three zones (1,5 and 10). Figure 8.9 shows the concentrations measured of these three compounds. There was a progressive decrease in all three cations through the reactor. The concentration of magnesium, calcium and potassium in the fermented wastewater were 4.2, 15.3 and 158 mg/L respectively. The potassium concentration was high due to the synthetic recipe containing additional phosphorus as KH_2PO_4 (3.75 g). This contributed an extra 108 mg/L of potassium than what was present in the diluted milk compounds. The amount of magnesium in the fermented wastewater (4.2 mg/L) was lower than the half saturation value of 10 mg/L determined by Ky *et al.* (2001), below which the phosphorus uptake rate decreases. The magnesium concentration in the fermented cheese processing wastewater in the study by Ky *et al.* (2001), was 7.8 mg/L also below the value at which decreases in the phosphorus uptake rate occur. By using the ratio of this synthetic wastewater COD (without including the contribution of the added lactose) to the whole milk COD, of 0.0129, and multiplying this ratio by the magnesium concentration in whole milk of 500 mg/L a magnesium concentration 6.45 mg/L is obtained. This shows that for this type of wastewater that consists of diluted milk powders or diluted milk, relatively low magnesium concentrations are likely. In their characterisation of dairy processing wastewaters, Danalewich *et al.* (1998), found that magnesium concentrations ranged from 6.5 to 46.3 mg/L.

There was a progressive decrease in the concentration of all three cations through the reactor (Figure 8.11). As expected magnesium release (7.2 mg/L) occurred in the anaerobic zone, with the ratio of magnesium release to phosphorus release of 0.17 mg Mg/mg P and a ratio of 0.16 mg Mg/mg P for aerobic magnesium uptake to phosphorus uptake (Table 8.4). A total of, 2.0 mg/L of magnesium was removed by the biomass. This stoichiometric relationship is close to the value of 0.157 mg Mg/mg

P experimentally determined by Ky *et al.* (2001), but slightly below the range of literature values mentioned previously. It appears that the low magnesium concentration, in the AO study at an SRT of 15 days (Chapter 7), did not adversely affect phosphorus removal, as phosphorus uptake was complete by zone 6. It must be noted that this reactor operated for a period of 79 days before the zone study, and EBPR stability was maintained throughout.

There was a net anaerobic zone calcium release of 5.2 mg/L and then a subsequent decrease through the aerobic zones. The stoichiometric ratios calculated in this study (15 day SRT AO system) of 0.12 mg Ca/mg P for both anaerobic release and aerobic uptake are at the upper end of the typical range mentioned earlier. As mentioned in the previous section on phosphorus precipitation, the amount of calcium removed through the reactor was 1.8 mg/L. Pattarkine and Randell, (1999), when determining the requirement of metal cations for the EBPR process with batch tests, also found that calcium decreased steadily throughout the aerobic phase, but not by more than was released in the anaerobic phase. In this study with the 15 day SRT system the net uptake of 1.8 mg/L has been associated with phosphorus precipitation.

Potassium had a very high release value in the anaerobic zone (26.8 mg/L) and a net overall release of 8.8 mg/L through the reactor. The ratio of 049 mg K/mg P for anaerobic release (Table 8.5), is in the upper part of the typical range of 0.25 to 0.51 mg K/mg P. The value of 0.33 mg K/mg P for the aerobic uptake is 33% less than the anaerobic release ratio. Some of this variation may be due to slight analytical errors, as the potassium required a 1:50 dilution before instrumental analysis whereas magnesium required no dilution and calcium only had a dilution of 1:2. The cations K^+, Mg^{2+} and Ca^{2+} are co-transported with phosphate in a total molar charge ratio of 1.0 irrespective of the direction (Comeau *et al.*, 1986) and were calculated for this system as 1.01 during phosphorus release and 0.84 during phosphorus uptake. The variation in the total charge ratio for release and uptake was again most likely due to the multiplication of analytical errors in the potassium analysis.

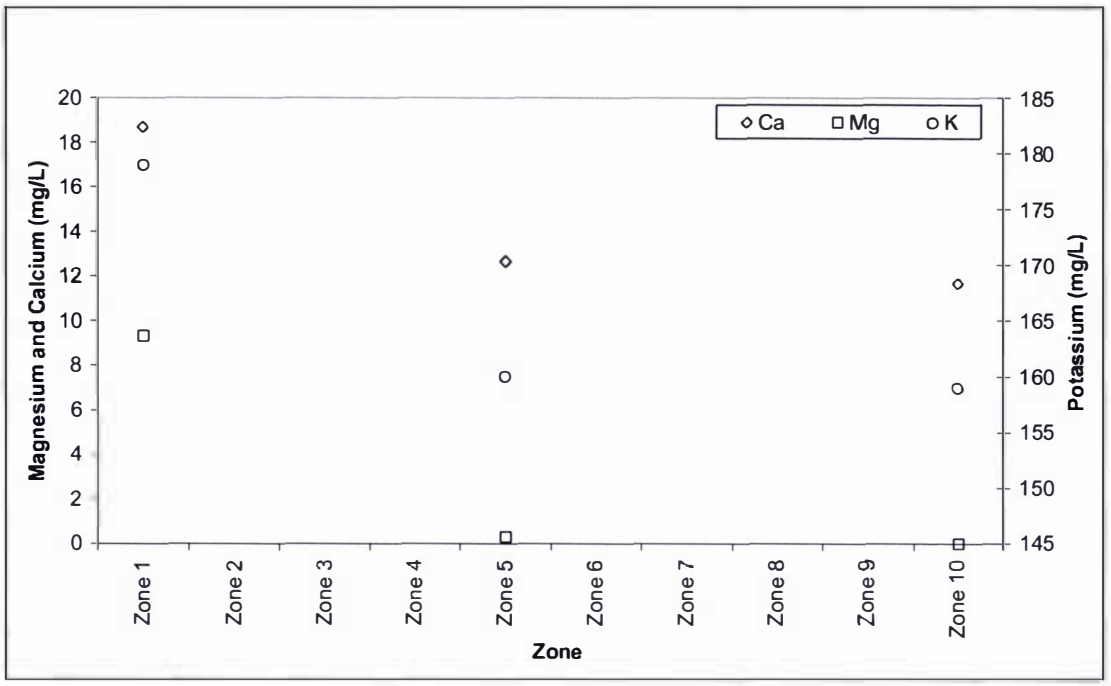


Figure 8.11 Concentrations of magnesium, calcium and potassium for zones 1, 5 and 10 at the time of the 15 day SRT AO system zone study.

Table 8.5: Amount of magnesium, potassium and calcium release in the anaerobic zone and the ratio of each cation to both phosphorus release and uptake (Also shown is the sum of the charges on a molar basis).

	Anaerobic Zone Release (mg/L)	M^+/P ratio (release) (mg M^+/mg P)	M^+/P ratio (uptake) (mg M^+/mg P)
Magnesium	7.2	0.17	0.16
Potassium	20.5	0.49	0.33
Calcium	5.2	0.12	0.12
Sum of Charges (molar basis)		1.01	0.84

8.5 Discussion

The influence of phosphorus precipitation, partially or non-fermented wastewater and the metal cations of magnesium, potassium and calcium have been investigated. Comeau *et al.* (1996), and Ky *et al.* (2001), have previously stated that a significant amount of chemical precipitation took place when applying biological phosphorus removal to a cheese processing wastewater (2.1 % mg P/mg VSS). A previous phosphorus fractionation test (Chapter 5) had found that only 0.2 % mg P/mg VSS was attributable to chemical precipitation at an aerobic zone pH of 8. A mass balance with the SRT 15 day AO system showed that the reactor removed a net value of 1.8 mg/L of calcium. Assuming that the pathway for chemical precipitation would be via precipitation with calcium, and all the calcium removed was due to chemical precipitation, 0.26 %mg P/mg VSS was calculated as the amount of precipitated phosphorus. The Ca:P ratio of the precipitate was assumed to be 1.3:1 (Carlsson *et al.*, 1997). The pH during the zone studies was measured up to 8.4 in zone 9 of the 15 day SRT AO system, less than the value of about 9 that de Haas *et al.* (2000a), state is required for significant phosphorus precipitation with calcium. The data supplied by Ky *et al.* (2001), in their model calibration is based on the experimental data determined by Comeau *et al.* (1996). Ky *et al.* (2001), stated a final aerobic phase pH of 7.7 and an influent calcium concentration of 61 mg/L. As the pH is less than the values in this study, yet a higher fraction of precipitated phosphorus was obtained, the difference may well be related to the higher calcium influent concentration of 61 mg/L compared to 15 mg/L in this study (Carlsson et al, 1997). There is little published information of the range of calcium concentrations in New Zealand dairy processing wastewaters, although Danalewich *et al.* (1998), found ranges of 1.4 to 58.5 mg/L for 15 processing plants in the USA. The combined influence of pH and the calcium concentration on phosphorus precipitation with dairy processing wastewater could be more intensively explored in a future study, with the aim of maximising both simultaneous biological and chemical phosphorus removal, when treating dairy processing wastewater.

Withdrawing the fermentation step and pumping the synthetic wastewater directly to the EBPR reactor, resulted in a partially fermented wastewater that had a VFA concentration of 105 mg/L COD compared to the previous concentration of about

1200 mg/L. This small amount of VFA was most likely generated by *insitu* fermentation within the pumping tubes. Cech and Hartman (1990, 1993), and Satoh *et al.* (1994), had previously attributed the proliferation of GAO's and the deterioration of EBPR to the presence of glucose as a substrate. Occasionally good EBPR can be achieved when glucose as used as the major carbon source (Mino *et al.*, 1998). With a substantial portion of the synthetic wastewater soluble COD present as lactose and possibly other sugars, the effect of removing the fermentation step could be detrimental to the EBPR process. Liu *et al.* (1996), developed a glycogen enriched system and found that while acetate and glucose were consumed under anaerobic conditions and stimulated some phosphorus release, lactose was only consumed in trace amounts and resulted in no phosphorus release, but did result in some PHA storage. It should be noted though that the activated sludge biomass was cultivated using acetate and a low P/COD feed ratio, different than the conditions used in this study. After 34 days without the fermentation stage the reactor continued to remove phosphorus (33 mg/L). The amount of soluble COD consumed in the anaerobic zone (624 mg/L) was greater than all the other previous zone systems except the 15 day SRT AO system. The consumed COD was also slightly greater than the combined COD consumption (615 mg/L) of the extended anaerobic zone AAO (EAAO) system. A net amount of 105 mg/L VFA COD (acetic acid and iso-valeric acid) was generated in the anaerobic zone. The amount of anaerobic phosphorus release (25.8 mg/L) was similar to the amount of anaerobic phosphorus release in the AAO and EAAO systems but less than the release in the AO systems. This showed that simultaneous fermentation, COD consumption and phosphorus release can occur in the anaerobic zone. The value of Y_{PO4} (0.04mg P/mg COD) for the continuous reactor was less than the other reactor systems with active EBPR supporting the possibility that the fraction of competing organisms in the sludge had increased due to the change in wastewater characteristics (increased carbohydrates). The most noticeable change was the value of Y_{PO4} for the acetate batch test using mixed liquor from the reactor of only 0.04 mg P/mg COD. The high PHA concentration in the anaerobic zone of 120 mg COD/g TS also supported the possibility that the fraction competing organisms had increased.

In this study, the viability of the EBPR process was maintained for 34 days after the cessation of separate wastewater fermentation, but as stated there were indications

that the biomass characteristics had changed. It can be concluded that failure of the fermentation stage would not lead to the immediate collapse of the EBPR process.

The importance and requirement of counter ions to the EBPR has been well documented (Comeau *et al.*, 1986; Pattarkine and Randall, 1999; Wentzel *et al.*, 1991). More recently, Danalewich *et al.* (1998), and Ky *et al.* (2001), have identified a potential limitation of magnesium in some dairy processing wastewaters. A profile of the magnesium, calcium and potassium concentrations for the 15 day SRT AO system showed that all three metal ions were released under anaerobic conditions with subsequent uptake occurring under aeration. Magnesium was measured at only 4.2 mg/L in the fermented wastewater, below the suggested limiting concentration of 10 mg/L (*Ky et al.*, 2001). By zone 5 the magnesium concentration had decreased to only 0.3 mg/L. The stoichiometric ratio of both magnesium release to phosphorus release and magnesium uptake to phosphorus uptake were 0.17 and 0.16 mg Mg/mg P respectively. This was similar to the experimental value determined by Ky *et al.* (2001), of 0.157 mg Mg /mg P, and slightly less than the typical range suggested by Comeau *et al.* (1986), and Christensson (1997). While low magnesium concentrations lead to a decrease in the phosphorus uptake rate, there was no negative effect on the stability of the EBPR process. Calcium and potassium can also function as counter ions. The total sum of the charges under both anaerobic and aerobic conditions (molar basis) should be equal to about 1 (Comeau *et al.*, 1986). This was the case under anaerobic conditions with a sum of 1.01, but under aerobic conditions, the sum was only 0.84. The lower aerobic total charge is likely due to analytical errors in potassium determination due to the high dilution required (1:50) and also the depletion of magnesium under aeration.

8.6 Conclusion

Chemical phosphorus precipitation in the study in Chapter 5 was only 0.18 % mg P/mg VSS. It was assumed that the precipitation of phosphorus as calcium phosphates would be the most likely form of chemical phosphorus removal with dairy processing wastewater. A mass balance of the calcium concentration for the 15 day SRT AO system showed that a maximum of 0.26 %mg P/mg VSS could be due to precipitation with calcium. Chemical phosphorus was not a significant factor in this study.

Removal of the fermentation stage from a well functioning EBPR system did not lead to an immediate deterioration of the EBPR process. After 34 days a large amount of biological phosphorus removal was still occurring. By analysing the stoichiometric ratio's that are used to characterise the EBPR process, it appeared that the fraction of competing organismss had increased. By this stage, the value of Y_{PO4} had decreased to 0.04 mg P/mg VSS for both the continuous reactor and the acetate batch test. This is typically an indication of a higher proportion of competing organisms in the sludge, as this lowers the value of Y_{PO4}.

Anaerobic release and aerobic uptake was observed for the counter ions magnesium, potassium and calcium with the 15 day SRT AO system. The low magnesium influent concentration of 4.2 mg/L resulted in a near zero final aerobic zone magnesium concentration. The stoichiometric magnesium to phosphorus release and uptake ratios were not substantially different to literature values. The low influent magnesium concentration in this study did not have a detrimental effect on the EBPR stability in this system, which was operated for 79 days. Both calcium and phosphorus were present in excess to their metabolic requirements.

CHAPTER 9
Final Discussion and Conclusions

9.1 Introduction

This project was undertaken to study the feasibility of enhanced biological phosphorus removal from a phosphorus rich dairy processing wastewater. The synthetic wastewater used was similar to New Zealand milk powder manufacturing sites. The objective was to determine the operating parameters and the configuration of a continuous activated sludge system that would achieve a high and stable level of biological phosphorus removal.

The literature review provided very little information on the use of continuous EBPR reactor technology for successful biological removal from dairy processing wastewater. Information on successful biological phosphorus removal had only been reported with sequencing batch reactor (SBR) technology (Comeau *et al.*, 1996; Ky *et al.*, 2001). It was noted that there was a vast amount of information on the biochemical mechanisms and microbial characteristics of the EBPR process as well as its application to domestic wastewater treatment. Information on the application of the EBPR process to the treatment of industrial wastewaters is scarce, especially full-scale processes.

The activated sludge reactor trials conducted during this study initially concentrated on developing a system that would achieve stable EBPR. Because dairy processing wastewater can have highly variable nutrient concentrations, it was desirable to investigate nutrient removal systems that could remove both nitrogen and phosphorus or phosphorus only. As full anaerobic VFA consumption was not achieved, an extended anaerobic zone HRT system was also tested. Using information from literature, the influences of certain wastewater characteristics on the EBPR process were studied. Some of these characteristics are unique to dairy processing wastewater, such as the high carbohydrate content and the concentration of certain metal cations.

9.2 Phosphorus Removal

The wastewater phosphorus concentration of between 95 mg P/L and 105 mg P/L used in this study could be reduced generally by between 35 and 40 mg P/L. This compared to the 40 to 50 mg P/L of phosphorus removal claimed by Comeau *et al.* (1996). The fermented wastewater treated in that study was similar to the fermented wastewater treated in these trials. The principle difference was that the Comeau *et al.* (1996), SBR sludge contained 2.1 %mg P/mg VSS of precipitated/adsorbed phosphorus. In this study, the precipitated fraction amounted to approximately 0.25% mg P/mg VSS. The fermented cheese processing wastewater used in the Comeau *et al.* (1996) study, had a calcium concentration of 61 mg/L compared to 15.3 mg/L in this study. An extra 2.0 % mg P/mg of sludge phosphorus due to precipitation with calcium amounts to about an extra 10 mg/L of phosphorus removal. If this extra amount of precipitation was achieved in this study then the amount of phosphorus removed would increase to between 45 and 50 mg/L, similar to the Comeau *et al.* (1996) study.

The intention of this study was to maximise biological phosphorus removal, when treating a typical milk power manufacturing wastewater, as opposed to maximising phosphorus precipitation by the addition of extra calcium. Other studies have tried to maximise the role of chemical phosphorus precipitation with high phosphorus containing wastewaters. Manga *et al.* (2000), studied an SBR system with what they called a high phosphorus concentration of 30 mg/L and an acetate COD of only 230 mg/L and they attributed 5 mg P/L of phosphorus removal to precipitation at an SRT of 17 days. Filauro *et al.* (1991), used a phostrip pilot plant that achieved 99% phosphorus removal from a combined municipal and pig wastewater with an influent phosphorus concentration of 100 mg/L. The COD of the wastewater was 2800 mg/L. In this system phosphorus is anaerobically released from the RAS stream using COD (VFA) from part of the influent stream and then chemically removed through precipitation. While no information was provided on the specifics of any EBPR details, chemical phosphorus removal appeared to be the main removal mechanism.

9.3 Reactor System Comparisons

In this section the reactor systems that exhibited phosphorus removal with fermented wastewater are compared. Combined nitrogen and phosphorus removal systems generally exhibit phosphorus release in the anaerobic stage and phosphorus uptake in the anoxic and aerobic stages. The AAO and EAAO systems in Chapter 6 exhibited both phosphorus release and COD consumption in the anoxic zone, which was beneficial to phosphorus removal. The AAO and EAAO systems at 10 day SRT's removed an extra 2.7 to 6.8 mg P/L over the AO systems at 10 and 15 day SRT's (Figure 9.1).

The total amount of phosphorus release in the unaerated zones at the time of the zone study for each system in Chapters 6 and 7 that exhibited EBPR, is shown in Figure 9.2. It is interesting that despite the AAO and EAAO systems having a greater unaerated HRT (4.5 and 7.5 hours respectively), the total amount of phosphorus release was not substantially different for the AAO, EAAO and AO systems. The main difference was either a low anaerobic phosphorus release rate (EAAO system) or a combination of a low release rate and a lower VSS concentration (AAO system). The lower VSS concentration in the AAO system was due to cell lysis from the low aerobic respiration rate from zones 6 to 10 of 5.6 to 4.8 mg O_2/g VSS/h. The low phosphorus release rate for the EAAO reactor was attributed to an increase in the population of competing organisms over the 40 days of operation. These indications of an increase in this microbial population was not unexpected as Satoh *et al.* (1994), had previously identified long anaerobic retention times as a stimulus for GAO growth.

The consumption of COD in the anaerobic/unaerated zones of EBPR systems is not necessarily related to the efficiency of phosphorus removal in sludges that have a reasonable proportion of competing organisms (Oehmen *et al.*, 2000). The factors that are thought to stimulate the growth of competing organisms are discussed in Chapter 2, and have been summarised by Satoh *et al.* (1994), and Mino *et al.* (1998). Figure 9.3 shows the total amount of COD consumption in the unaerated zones and the amount of phosphorus removed relative to the unaerated retention time for the four systems that exhibited EBPR when treating fermented wastewater. The slope for the

COD consumption data is 51.4 compared to only 1.4 for the phosphorus removal data. While this figure indicates that increasing the unaerated fraction results in a high amount of COD consumption, it results in only a relatively small increase in phosphorus removal. This divergence shows that there is an increasing proportion of microorganisms, that can remove COD without contributing to the EBPR process with larger unaerated reactor fractions. Once aspect that was not investigated here was the long term effect of doubling the anaerobic retention time as with the EAAO system. The AAO reactor was operated for 158 days compared to 40 days for the EAAO reactor. The assumption stated in Chapter 3 that operating a reactor for at least three SRT's lead to "psuedo steady state' may not necessarily be true for these EBPR configurations. Long term operation of the EAAO reactor may risk the further dominance of competing organisms and a resulting deterioration in biological phosphorus removal. Whether a system such as the EAAO configuration can maintain stable, long term high phosphorus is a factor that is worthy of further research.

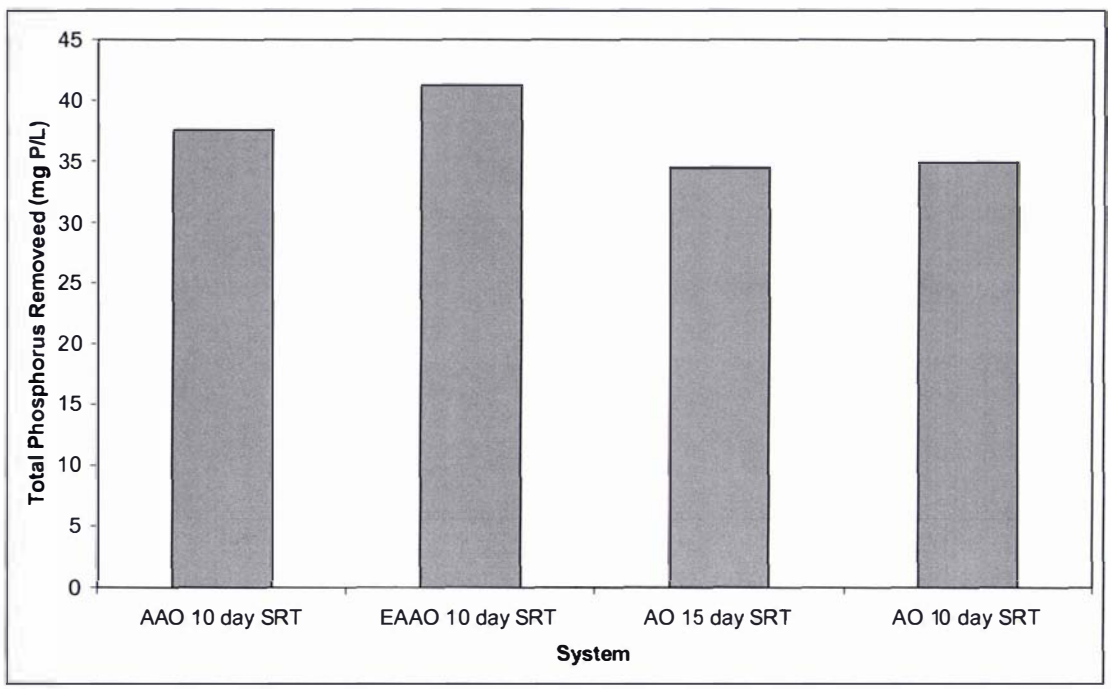


Figure 9.1: The total phosphorus removed in each system at the time of the zone studies.

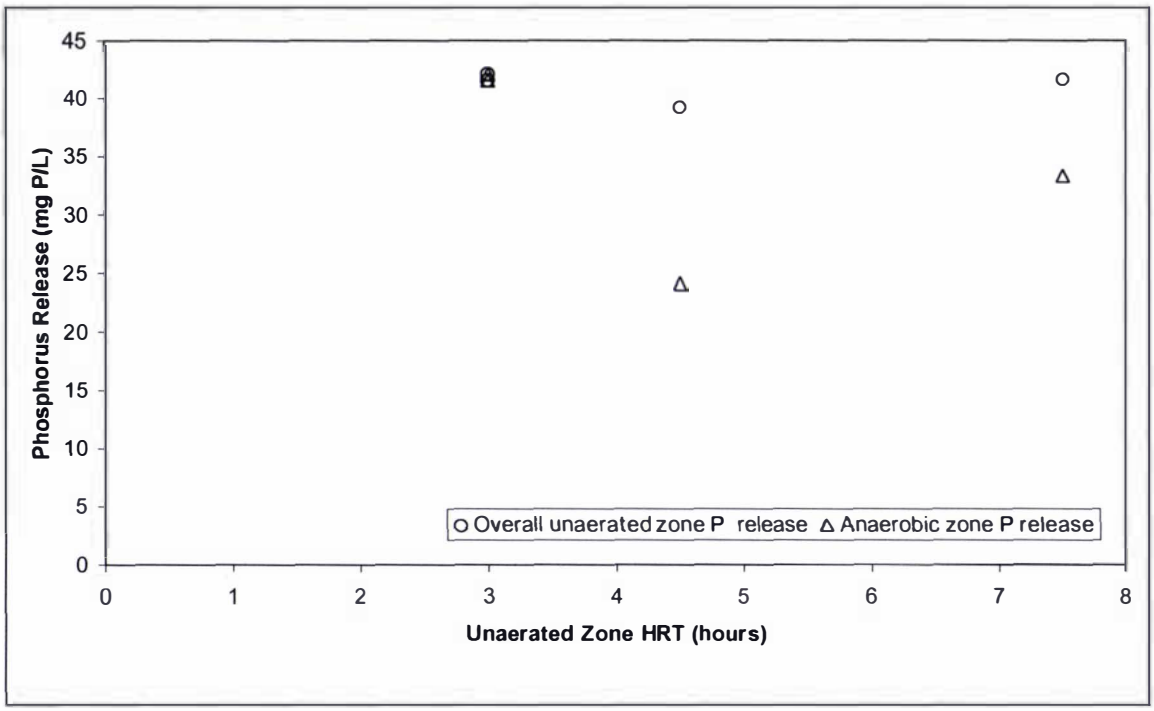


Figure 9.2: Overall unaerated zone phosphorus release and anaerobic zone only phosphorus release relative to the unaerated zone(s) HRT.

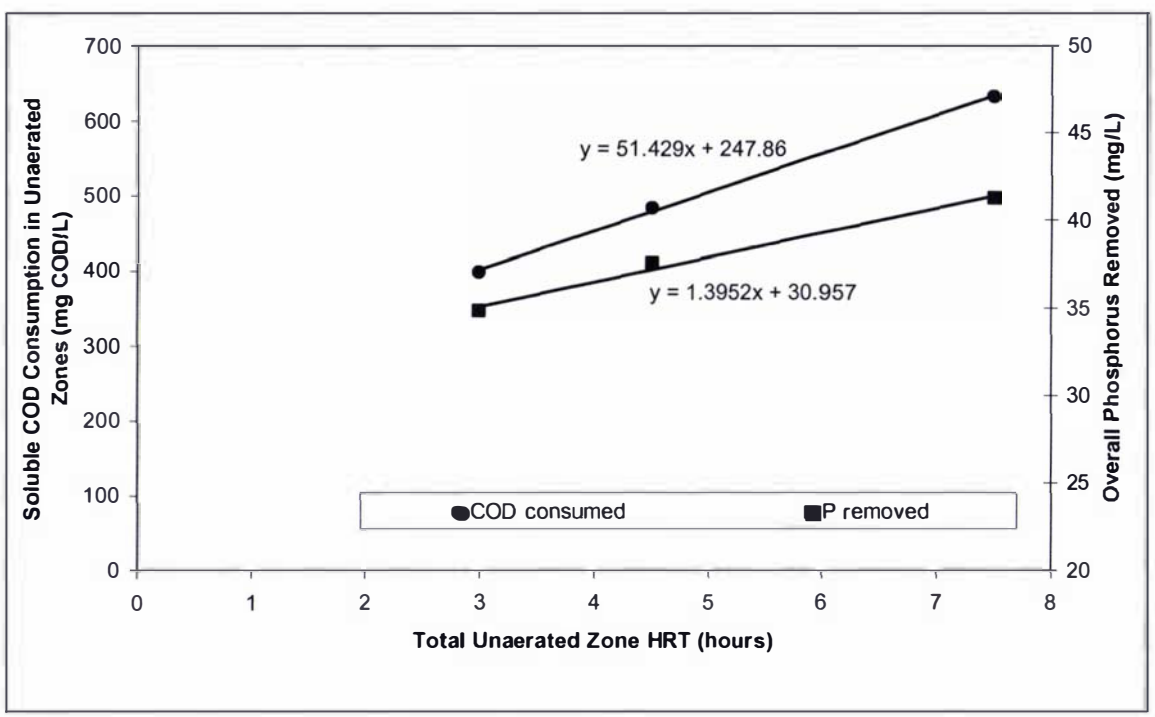


Figure 9.3: The relationship of the soluble COD consumption in the unaerated zones and overall phosphorus removed relative to the unaerated fraction HRT for 10 day SRT reactors.

The AO reactor when operated at an SRT of 15 days had a high amount of COD consumption (709 mg/L), yet only removed 34.5 mg/L of phosphorus after 79 days of operation. The amount of phosphorus release for this reactor was very similar to the 10 day SRT system, yet consumed 310 mg/L more soluble COD. It was noted that over the period of operation there was a decreasing anaerobic zone COD concentration to the stage that at 70 days it had reduced to 58 mg/L. The conditions at an SRT of 15 days appeared to be favourable for the gradual growth of competing organisms.. Comeau *et al.* (1996), operated an SBR at an SRT of 20 days in their EBPR study using fermented cheese processing wastewater for two periods of about 80 days without any obvious deterioration in phosphorus removal. As mentioned for the EAAO system, long term operation for a period of about 6 months (~180 days) or longer at the same configuration and parameters, would be necessary to investigate the sensitivity of these EBPR process to EBPR deterioration at longer SRT's and anaerobic HRT's.

A decreasing ratio of anaerobic phosphorus release to COD consumed (Y_{PO4}) is also used as an indication of GAO activity (Oehmen e*t al.*, 2000). Figure 9.4 shows the change in the batch test Y_{PO4} value with increasing anaerobic/anoxic COD consumption. The values for Y_{PO4} were determined from batch tests with acetate as the sole carbon source. The decrease in Y_{PO4} is a strong indication that increasing unaerated COD consumption may result in an increase in the proportion of competing organisms.. The value of Y_{PO4} used in Figure 9.4 from the EAAO batch test, was calculated from the initial high rate of phosphorus release in the first 180 minutes.

The medium term influence of partial fermentation on an existing EBPR reactor was investigated as it has been reported that sugars, especially glucose can in some instances result in EBPR deterioration (Cech and Hartman, 1990, 1993). After 34 days of operation, a reactor that was feed a partially fermented dairy processing wastewater (VFA= 156 mg COD/L) did result in a small decrease in the overall amount of phosphorus removal (33.8 mg P/L). The most notable change was that while the Y_{PO4} value had decreased to 0.04 mg P/mg COD for the continuous reactor the value calculated from the acetate batch test was only 0.04 mg P/mg COD as well. The rate of phosphorus removal decreased to 1.9 mg P/g VSS/h almost the same as for the

EAAO system (1.6 mg P/g VSS/h). From the perspective of phosphorus removal only, the medium term effect of removing the fermentation step was only minor although the sludge metabolic characteristics had changed significantly. This supports the earlier comment that the changing of biomass towards a sludge dominated by competing organisms appears to be a very gradual process.

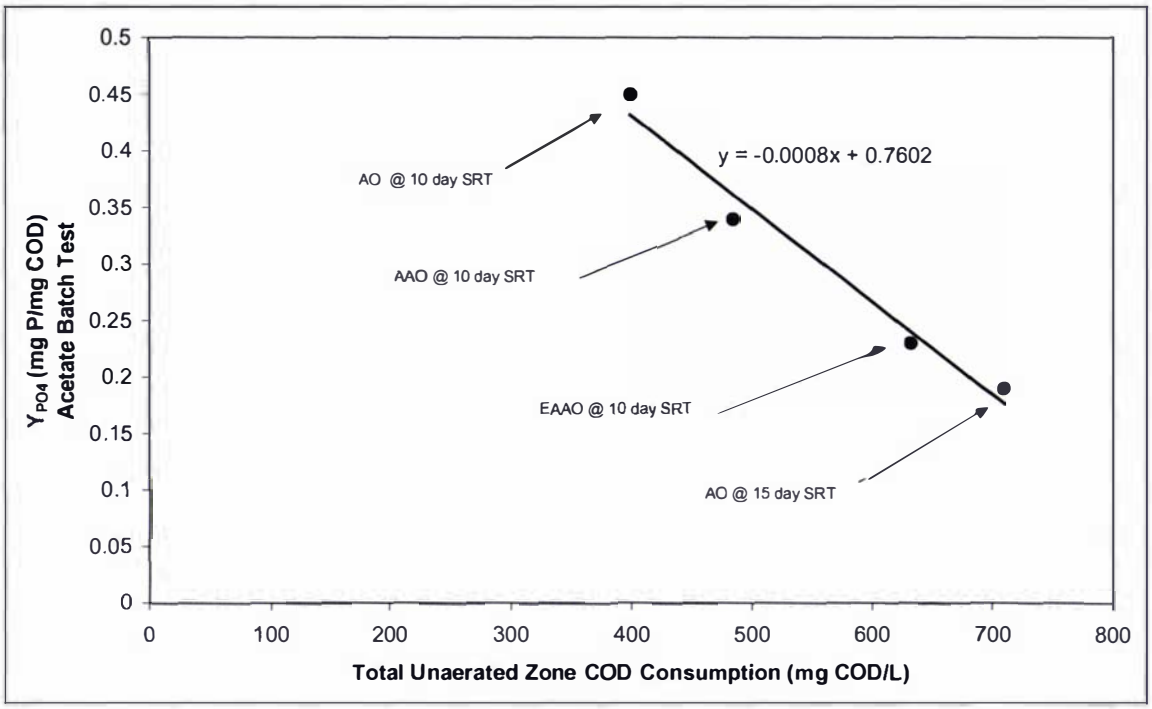


Figure 9.4: The change in Y_{PO4} determined from acetate batch tests relative to the continuous reactor total soluble COD consumption in the unaerated zones.

9.4 Full-Scale Implications

Based on: 1)the lack of EBPR in Chapter 5 with the preliminary systems, 2) the changing activated sludge biomass when the fermenter was removed, and 3) previous literature information, the inclusion of a separate pre-fermentation step is a fundamental requirement. A fermentation reactor with pH and temperature control may not be required. Comeau *et al.* (1996), and Jovic (1998), have shown that simple flow balancing tanks with an HRT between 12 and 24 hours can generate high concentrations of VFA's. This also has the advantage of evening out variations in the wastewater characteristics that result from in-plant alternate production and cleaning cycles. This study has shown that while insitu anaerobic fermentation can take place there is also a high risk of milk carbohydrates resulting in a gradual change in the

sludge characteristics that may eventually lead to EBPR deterioration. It appears that the occasional 'slug' of unfermented wastewater, if the balance tank is overloaded or bypassed for maintenance purposes, should not result in the rapid deterioration of the EBPR mechanism.

Operating at an SRT of 10 days and an anaerobic retention time of 3 hours resulted in a stable system in this study. These parameters can be easily achieved in full-scale design, but care should be taken to maintain an accurate SRT. Failure to do this may result in a gradual deterioration in phosphorus removal. The use of a flow- balancing fermentation stage, should also assist in maintaining a consistent anaerobic retention time. The flexibility to readily alter the anaerobic HRT (volume) is recommended, as this could be useful for process optimisation.

In this study chemical phosphorus precipitation was minimal, but where possible simultaneous phosphorus precipitation should be encouraged, by maintaining an elevated aerobic phase pH. It should be noted that the amount of chemical precipitation is highly dependent on the wastewater characteristics especially the calcium concentration (Carlsson *et al.*, 1997). This will vary not only between manufacturing sites, but also potentially within a site. It is possible that changing production schedules will result in changing wastewater calcium concentrations.

Magnesium has been identified as a potentially limiting nutrient with dairy wastewaters. While this study showed that a relatively low wastewater magnesium concentration of 4.2 mg/L did not inhibit the EBPR process, routine wastewater magnesium measurements would be recommended in any full-scale application of the EBPR process.

Due to the fact that approximately 40 mg/L of phosphorus is the maximum that could be expected to be removed without significant simultaneous chemical precipitation, it is recommended that the provision for chemical dosing (eg. Fe Cl_3) be included for wastewaters that have phosphorus concentrations above about 35 mg/L. For wastewaters with phosphorus concentrations above 100 mg/L, chemical phosphorus

removal is probably the technology of choice unless waste minimisation practices can reduce the influent phosphorus concentration.

The influence of temperature on the reactor operation was not quantitatively investigated in this study as all reactors were operated at constant temperature. The EBPR process is not as sensitive to decreasing temperatures as nitrification and high levels of phosphorus removal have been observed down to 6 °C (Brdjanovic *et al.*, 1997). As nitrification is the limiting factor at low temperatures, a full-scale system would be designed to permit the required degree of nitrification at the lowest anticipated operating temperature. Dairy processing wastewaters can be discharged above 40 °C (Danalewich *et al.*, 1998), due to the requirement to clean process equipment with hot water. The relatively close distances from the manufacturing plant to the treatment process would result in only small decreases in the wastewater temperature prior to treatment, even in cooler seasons. If the fermentation/balance tank stage lacked temperature control the amount of acidification would decrease during low temperatures and the presence of carbohydrates in the fermenter/balance tank effluent could result in EBPR deterioration due to a changing biological population and the dominance of competing organisms. In cooler regions it would be wise to have supplementary heating for the fermentation stage.

9.5 Future Research

While this study has provided answers and established the basis for the application of continuous reactor EBPR technology to the treatment of dairy processing wastewater it has also raised some questions. This thesis provides the basis for future research, because little published information is available that investigates the application of EBPR to dairy processing wastewaters. Recommendations for future research are as follows:

- Establish the optimum anaerobic retention times for specific VFA concentrations and investigate the effect of operating with an extended anaerobic HRT over the long term (eg. 6 months).

- Determine the effect on the EBPR process of different reactor HRT's. In this study an HRT of 2.5 days was used, but the effect of decreasing or increasing this HRT was not investigated. Retrofitting an existing fully aerated long HRT aerobic activated sludge plant may not be successful. Literature suggests that excessive aeration can lead to the deterioration of EBPR systems (Brdjanovic *et al.*, 1998).

- Undertake a comprehensive characterisation of New Zealand dairy processing wastewaters, especially in regard to magnesium, calcium and potassium concentrations. Currently this information is very limited.

- Investigate the possibility of increasing simultaneous phosphorus precipitation by artificially increasing the wastewater calcium concentration.

- Different reactor operational parameters should be assessed for long term trends, as it appears that the negative influence of competing organisms is a gradual process.

- During activated sludge reactor trials, the concurrent characterisation of the microbial population is also recommended if possible.

9.6 Recommendations

- A fermentation stage is critical to both generate a high concentration of VFA and to prevent carbohydrate intrusion into the activated sludge anaerobic zone. While insitu fermentation can take place in the anaerobic zone there is also a risk that carbohydrates present in the wastewater will result in the deterioration of phosphorus removal due to a changing population favouring competing organisms.

- Operate the EBPR process at an SRT of 10 days. Failure to accurately control the SRT at 10 days could also cause phosphorus removal to deteriorate due to the proliferation of competing organisms.

- An activated sludge reactor HRT of 2.5 days is recommended as this resulted in stable and sustainable biological phosphorus removal at an SRT of 10 days.

- Size the anaerobic zone for an actual retention time of 3 hours, as this will both maximise the utilisation of VFA COD and maximise anaerobic zone phosphorus release. Longer anaerobic retention times resulted in moderate increases in VFA consumption, but only a marginal increase in phosphorus removal.

- Carefully characterise the dairy processing wastewater to determine whether nitrogen removal is required or not. The EBPR process is marginally more effective with anoxic zones but is still stable and capable of removing significant amounts of phosphorus without anoxic zones.

- Routine wastewater characterisation will also identify whether it there is a limitation of magnesium or potassium as these are important counter ions in the EBPR process and are occasionally present at low concentrations in dairy processing wastewaters.

- As about 40 mg P/L is the maximum amount of phosphorus that can be removed biologically, greater phosphorus removal would require chemical precipitation or phosphorus minimisation at source.

CHAPTER 10
Appendix

Chapter 4 - Data

Table 10.1: Aerobic Readily Biodegradable Test Data, S/X ratio of 0.05

Concentrate	COD filtered=	20217 mg/L
Substrate COD	COD total =	32411 mg/L

Volume of concentrated feed added (filtered)=	4ml	TSS=	1925 mg/l
Volume of mixed liquor (aerated)= 1 litre		VSS=	1635 mg/l
Mixed liquor aerated for 1.5 hours prior to test		COD added=	80.8667355 mg/l

Chart recorder settings		
1mg/l DO=	30.4	mm
chart speed=	30	mm/min

Time	mm time	mm DO	Time (minutes)	delta DO (mg/L)	OUR mg/l/min
0	81	63.5	2.70	2.09	0.77
5	61	49	2.03	1.61	0.79
10	93	77	3.10	2.53	0.82
15	36	21	1.20	0.69	0.58
20	98	43.5	3.27	1.43	0.44
30	78	32	2.60	1.05	0.40
40	55	25	1.83	0.82	0.38
50	107	39	3.57	1.28	0.36
60	77.5	28.5	2.58	0.94	0.36
70	100.5	36	3.35	1.18	0.35
80	69	25	2.30	0.82	0.36
90	77	27.5	2.57	0.90	0.35
110	104	36	3.47	1.18	0.34
120	70	22.5	2.33	0.74	0.32
130	70	22	2.33	0.72	0.31
140	76	23	2.53	0.76	0.30
150	78	20.5	2.60	0.67	0.26
160	87.5	21	2.92	0.69	0.24
170	64	15.5	2.13	0.51	0.24
180	100	21.5	3.33	0.71	0.21
190	75	16.5	2.50	0.54	0.22
200	49.5	11	1.65	0.36	0.22
210	73.5	15	2.45	0.49	0.20

Table 10.2: Aerobic Readily Biodegradable Test Data, S/X ratio of 0.12

Concentrate	COD filtered=	20217	mg/L
Substrate COD	COD total =	32411	mg/L

Volume concentrated feed added (filtered)=	10 ml	TSS=	2050 mg/l
Volume mixed liquor (aerated)= 1 litre		VSS=	1750 mg/l
Mixed liquor aerated for 1.5 hours prior to test		COD added=	202 mg/l

Chart recorder settings	
1mg/l DO=	30.4 mm
chart speed=	30 mm/min

Real Time	Time	mm time	mm DO	time (min)	delta DO	OUR	OUR
						mg/l/min	mg/l/hr
21:20	0	27.5	48	0.92	1.58	1.72	103.35
21:30	10	36	61	1.20	2.01	1.67	100.33
21:40	20	31	50	1.03	1.64	1.59	95.50
21:50	30	31	49	1.03	1.61	1.56	93.59
22:00	40	18	15	0.60	0.49	0.82	49.34
22:10	50	51	40	1.70	1.32	0.77	46.44
22:20	60	46	34	1.53	1.12	0.73	43.76
22:30	70	45	31.5	1.50	1.04	0.69	41.45
22:40	80	36	22	1.20	0.72	0.60	36.18
22:50	90	46	25	1.53	0.82	0.54	32.18
23:00	100	41	19	1.37	0.63	0.46	27.44
23:10	110	50	23	1.67	0.76	0.45	27.24
23:20	120	48	19	1.60	0.63	0.39	23.44
23:30	130	54	21	1.80	0.69	0.38	23.03
23:40	140	23	9.5	0.77	0.29	0.36	21.88
23:50	150	49	17.5	1.63	0.58	0.35	21.15
0:00	160	49	18	1.63	0.59	0.36	21.75
0:10	170	49	18	1.63	0.59	0.36	21.75
0:20	180	36	13	1.20	0.43	0.36	21.38

Table 10.3: Anoxic Readily Biodegradable Test (S/X ratio of 0.03)

Substrate	Added Soluble COD (mg/L)	Reactor VSS (mg/L)	S/X (based on Soluble COD)	Liquid Volume (litres)
Dairy Processing WW	85	3065	0.03	2.00
Fermented WW	85	2975	0.03	1.84
Endogenous	0	3170	0	1.86

	Fermented Dairy Processing Wastewater			Raw Dairy Processing Wastewater			No Substrate Addition (Endogenous)		
Time (mins)	NO2-N	NO3-N	NOx-N	NO2-N	NO3-N	NOx-N	NO2-N	NO3-N	NOx-N
0	0.5	35.1	35.4	0.8	38.8	39.3	0.8	34.4	34.9
20	0.8	28.7	29.2	0.0	35.0	35.0	0.8	33.3	33.8
40	0.4	24.7	24.9	1.8	32.0	33.1	0.8	30.1	30.6
60	0.0	23.0	23.0	2.4	30.4	31.9	0.9	30.7	31.2
80	0.0	21.8	21.8	3.0	28.6	30.5	0.8	29.4	29.8
100	0.0	22.5	22.5	3.6	25.9	28.1	0.9	29.8	30.4
120	0.0	21.1	21.1	4.1	24.6	27.0	0.9	26.6	27.2
140	0.0	20.4	20.4	4.8	23.1	26.0	1.0	26.6	27.2
160	0.0	22.2	22.2	5.0	21.7	24.7	1.1	25.7	26.4
180	0.0	20.2	20.2	5.1	19.9	23.0	1.1	24.0	24.6
200	0.4	20.3	20.5	5.1	18.2	21.3	1.2	23.1	23.8
220	0.5	17.4	17.7	5.6	17.1	20.5	1.2	22.1	22.9
240	0.0	19.8	19.8	6.3	16.1	19.9	1.5	23.4	

Table 10.4: Anoxic Readily Biodegradable Test (S/X ratio of 0.08)

Substrate	Added Soluble COD (mg/L)	Reactor VSS (mg/L)	S/X (based on Soluble COD)	Liquid Volume (litres)
Dairy Processing WW	325	3850	0.08	2.00
Acetate	130	4445	0.03	2.00

	Acetate					Dairy Processing Wastewater				
Time	Nitrate	NO_3-N	Nitrite	NO_2-N	Nox-N	Nitrate	NO_3-N	Nitrite	NO_2-N	NOx-N
0	173.4	39.2	0.6	0.2	39.3	170.7	38.6	0.6	0.2	38.7
15	140.3	31.7	21.8	6.6	35.7	126.6	28.6	29.1	8.8	33.9
30	100.6	22.7	40.7	12.4	30.2	91.3	20.6	50.7	15.4	29.9
45	66.7	15.1	63.3	19.3	26.6	61.5	13.9	67.5	20.5	26.2
60	35.2	8.0	88.2	26.8	24.0	40.7	9.2	80.8	24.5	23.9
75	6.3	1.4	107.0	32.5	20.9	28.7	6.5	88.8	27.0	22.7
90	0.2	0.0	107.2	32.6	19.6	20.2	4.6	93.4	28.4	21.6
105	0.0	0.0	100.4	30.5	18.3	13.1	3.0	95.9	29.1	20.5
120	0.0	0.0	94.5	28.7	17.2	5.2	1.2	97.4	29.6	18.9
135	0.0	0.0	90.3	27.5	16.5	0.6	0.1	96.7	29.4	17.8
150	0.8	0.2	85.7	26.1	15.8	0.0	0.0	92.3	28.1	16.8
165	0.1	0.0	79.6	24.2	14.6	0.0	0.0	88.2	26.8	16.1
180	0.0	0.0	75.3	22.9	13.7	0.0	0.0	85.3	25.9	15.6
195	0.0	0.0	70.1	21.3	12.8	0.0	0.0	82.2	25.0	15.0
210	0.0	0.0	66.9	20.3	12.2	0.5	0.1	76.0	23.1	14.0
225	0.1	0.0	62.5	19.0	11.4	0.4	0.1	73.4	22.3	13.5
240	0.0	0.0	57.5	17.5	10.5	0.5	0.1	70.6	21.5	13.0

- Fermentation Tests - Refer to Table 4.8 in Chapter 4 for analytical data.

Chapter 5 - Data

Table 10.5: AAO Continuous Reactor Data – Unfermented Wastewater

	TSS (mg/L)				VSS(mg/L)			VSS/TSS		
Day	Anaerobic	Anoxic	Aerobic	Effluent	Anaerobic	Anoxic	Aerobic	Anaerobic	Anoxic	Aerobic
1	2520	2120	2000	75						
5	2420	1840	2190	70	2120	1550	1840	0.88	0.88	0.84
8	2400	1820	2150	70	2100	1570	1840	0.88	0.86	0.86
14	3400	1870	2070	68	2920	1580	1700	0.86	0.85	0.82
19	3380	2580	2010	60	2800	2140	1850	0.83	0.84	0.83
22	2400	1620	2390	140	2000	1340	1930	0.83	0.83	0.81
26	2350	1950	2200	95	1980	1590	1810	0.84	0.82	0.82
29	2770	2700	2670	60	2330	2270	2210	0.84	0.84	0.83
33	2800	2390	2370	40	2370	2000	1960	0.85	0.84	0.83
36	2500	2050	2150	70	2140	1740	1790	0.86	0.85	0.83
40	2690	2390	2160	75	2220	1990	1740	0.83	0.83	0.81
47	3150	2600	2380	65	2630	2160	1970	0.83	0.83	0.83
50	3180	2470	2020	140	2730	2070	1700	0.86	0.84	0.84

	pH			Dissolved Reactive phosphorus (mg/L)				P removed	%P removed
Day	Anaerobic	Anoxic	Aerobic	Anaerobic	Anoxic	Aerobic	Effluent	(mg/L)	
1	6	6.5	7.7	116	89.3	51.5	57.4	47.6	45.3
5	6.2	6.6	7.9	104.4	94.7	81.3	82	23	21.9
8	6.5	7.2	7.9	105	90	82	81	24	22.9
14	6.5	7.1	8.1	107	91	82	80	25	23.8
19	7	7.3	8.1	100	90	88	83	22	21.0
22	7	7.2	8.2	99	90	85	81	24	22.9
26	7.1	7.5	8.1	98	93	86	90	15	14.3
29	6.9	7.2	8.3	96	95	86	85	20	19.0
33	7.05	7.55	8.18	100	93	93	92	13	12.4
36	7.1	7.56	8.27	97	92	85	84	21	20.0
40	6.9	7.5	8.3	97	93	91	86	19	18.1
47	6.9	7.3	8.2	99	95	86	86	17	16.2
50	7.07	7.35	8.18	96	98	89	84	21	20.0

	Total Phophorus(mg/L)				Anoxic P-release	Anaerobic P-release (mg/L)		%mg P/mg VSS		
Day	Anaerobic	Anoxic	Aerobic	Effluent	(mg/L)	baedon TPin	based on DRPin	Anaerobic	Anoxic	Aerobic
1					0.52	41.23	44.88			
5	168	131	185		0.05	14.01	17.66	2.63	1.97	4.55
8	147	134	128		-5.30	15.24	18.89	1.75	2.42	2.50
14					-5.45	17.88	21.53			
19	188	155	136	87	-4.94	8.97	12.62	2.60	2.52	2.91
22					-3.09	9.24	12.89			
26	214	169	145	85	0.06	2.53	6.18	4.94	3.90	3.26
29	276	257	154		3.22	3.70	7.35	6.50	6.00	3.08
33					-4.05	3.26	6.91			
36					0.06	5.34	8.99			
40					-1.47	4.07	7.72			
47	189	166	147	91	1.49	4.80	8.45	2.86	2.73	3.10
50					4.95	4.34	7.99			

	SVI	COD(mg/L)								
Day		Anaerobic	CODsol removed	CODtot removed	Anoxic	COD removed	Aerobic	% COD removed	Effluent total	Effluent sol
1										
5	242									
8	190	610	218.3	583.3	250	119.5	40	98.8		60
14	102	890			345	184.2	35	98.9	280	50
19	96	350	484.7	849.6	216	7.4	50	98.4	180	45
22		385	440.1	805.1	206	31.3	35	98.9	115	40
26	105		825.1	1190.1			35	98.9	83	45
29	124	480	345.1	710.1	300	-7.8	35	98.9		50
33	118	311	514.1	879.1	140	54.5	35	98.9		30
36	140	395	439.7	804.8	155	94.4	50	96.4	100	70
40	180	505	313.8	678.6	221	81.5	25	99.2		25
47	175	525	309.7	674.6	255	69.6	50	98.4		30
50	120	445	383.3	748.3	230	44.1	40	98.8	100	

	Nitrate (mg N/L)				NH3-N (mg N/L)			
Day	Anaerobic	Anoxic	Aerobic	Effluent	Anaerobic	Anoxic	Aerobic	Effluent
1								
5								
8								
14	0	0	9.6	4.1			1.6	1.8
19	0	0.2	5.6	5.4	2.76	5.6	0.1	0.2
22	0	0	6.4	6		-	0.3	0.2
26								
29								
33	0.1	0.3	8.8	9.2	11.4	5.6	0.3	0.4
36	0.2	0.2	7.7	7.4	13.9	7.6	0.4	0.13
40	0	0	9.3	10	9.9		0.9	0.6
47	0	0.3	9.9	9.9	15	10.4	0.2	0.8
50								

Table 10.6: MUCT Continuous Reactor Data – Unfermented Wastewater

	TSS (mg/L)					VSS (mg/L)				VSS/TSS			
Day	Anaerobic	Anoxic 1	Anoxic 2	Aerobic	Effluent	Anaerobic	Anoxic 1	Anoxic 2	Aerobic	Anaerobic	Anoxic 1	Anoxic 2	Aerobic
1	1700	1540	2210	2220	100	1565	1400	1970	1950	0.92	0.91	0.89	0.88
3	1380	1980	2610	2010	60	1295	1840	2420	1865	0.94	0.93	0.93	0.93
8	1770	2560	2625	2435	80	1650	2360	2380	2190	0.93	0.92	0.91	0.90
16	1810	2490	2280	2070	20	1700	2290	2100	1890	0.94	0.92	0.92	0.91
18	1520	2400	2520	2260	90	1410	2180	2250	2020	0.93	0.91	0.9	0.89
22	1580	2470	2210	2260	70	1460	2240	2010	2070	0.93	0.91	0.91	0.91
25	1810	2300	2130	2270	75	1680	2080	1920	2020	0.92	0.9	0.9	0.89
29	1150	1800	1860	1950	75	1040	1610	1660	1700	0.90	0.89	0.89	0.87
32	1545	2080	1680	1750	100	1380	1810	1610	1510	0.88	0.87	0.68	0.86
35	1250	1820	1740	1690	75	1140	1630	1570	1510	0.91	0.90	0.90	0.89
38	1180	1675	1285	1590	100	1070	1460	1100	1375	0.91	0.87	0.86	0.86

	pH					Dissolved Reactive Phosphorus (mg/L)				
Day	Anaerobic	Anoxic 1	Anoxic 2	Aerobic	Effluent	Feed	Anaerobic	Anoxic 1	Anoxic 2	Aerobic	Effluent
1											
3	5.31	6.1	6.74	7.2							
8	6.15	6.5	6.91	7.45	7.42	92.3	103.2	110.9	106.5	106.5	92.7
16	6.6	6.9	7.5	8	8.1	102.3	105.6	101.7	101.3	99.1	97.6
18	6.6	6.9	7.6	8	8.2	94.5	102.7	105.9	101.4	97.1	98.8
22	6.5	6.9	7.7	8.2	8.3	99	105	107	100	95	96
25	6.6	7.1	7.5	7.9	8		95	93	89	87	92
29	6.8	7.3	7.7	7.9	8.2	96	96	96	92	93	93
32	7	7.2	7.6	8.1	8.2	72	75	85	73	76	54
35	6.6	6.9	7.4	8.1	8	99	105	102	99	91	94
38	6.6	6.95	7.6	8.1	8.3						

	Total Phosphorus (mg/L)						% mg P/mg VSS				SVI
Day	Feed	Anaerobic	Anoxic 1	Anoxic 2	Aerobic	Effluent	Anaerobic	Anoxic 1	Anoxic 2	Aerobic	
1											
3	128.3	125.9	139.1	147.6	141.3	90.9					
8	116	128	138	139	130	92	1.48	1.14	1.36	1.42	
16	115	137	151	141	144	104	1.85	2.15	1.89	2.38	333
18	150	163	168	161	154	157	4.28	2.85	2.65	2.82	319
22	140	134	144	132	173	110	1.99	1.65	1.59	3.77	338
25	-	134	144	135	136	99	2.35	2.45	2.40	2.43	
29	111	114	121	123	124	92	1.54	1.55	1.87	1.82	185
32	148	151	152	141	141	141	5.59	3.70	4.22	2.20	184
35	122	126	136	129	132	97	1.84	2.09	1.91	2.72	178
38											

	COD (mg/L)								COD consumed	
Day	Anaerobic	Anoxic 1	Anoxic 2	Aerobic	Effluent total	Effluent sol	feed total	feedsoluble	Anaerobic (CODt)	Anaerobic COD sol
1										
3	951	682	357	153	84	63			990	490
8	988	664	266	40	88	48	3308	2774	944	444
16	920	570	195	35	60	35	3495	2880	965	465
18	935	600	235	30	60	35	3290	2240	965	465
22	855	520	160	27	42	36	3020	2440	1005	505
25	785	465	160	20	54	25			1048	548
29	880	532	193	42	56	23	3527	2145	986	486
32	908	559	189	47	73	26	3268	2229	972	472
35	847	523	214	32	88	31	3187	2369	1015	515
38	818	544	176	32	57	25	3427	2210	1054	554

	NO_x-N (mg/L)					NO_3-N (mg/L)				
Day	Anaerobic	Anoxic 1	Anoxic2	Aerobic	Effluent	Anaerobic	Anoxic 1	Anoxic 2	Aerobic	Effluent
1										
3						0.1	0.2	0.2	0.2	0.9
8	0	0.04	0.1	5.6	5.2	0	0.0	0.0	3.5	3.4
16	0	0.2	0.1	2.8	3.7	0	0	0	6.1	8.2
18	0	0.1	0.2	1.2	2.7	0	0	0	4.8	5.8
22	0	0.1	0.1	4.1	3.8	0	0	0	8.3	9
25						0	0	0	8.9	8.3
29	0	0.2	0.1	3.7	3.6	0	0	0	8.1	4.8
32	0	0.2	0.1	3.7	4.3	0	0	0	8.4	8.1
35	0	0.1	0.1	1.4	1.2	0	0	0	1.3	2.7
38										

	NH_3-N (mg/L)				
Day	Anaerobic	Anoxic 1	Anoxic 2	Aerobic	Effluent
1					
3	16.6	18.7	12.9	9.8	5.1
8					
16	17.8	17.8	7.6	0.2	0.1
18	24	21.2	9.1	0.1	0.2
22	33.1	23.2	7.8	0.1	0.2
25	35.8	21.6	9.6	0.4	0.1
29	31.3	22.8	8.8	0.1	0.1
32	40.2	25.1	9.8	0.2	0.2
35	23.3	18.2	10.2	2.8	1.3
38					

Chapter 6 - Data

Table 10.7: Zoned AAO System – Fermented Wastewater

	Total Suspended Solids (TSS mg/l)					
Day	Ferm. feed	Ferm. effluent	Anaerobic	prim. Anoxic	Aerobic	Effluent
1	305	1370	3340	3340	3500	70
6	450	1130	3635	3615	3750	100
14	265	1200	3870	3920	3780	405
21	445	760	3940	3920	3690	50
29	330	730	3420	3430	3505	80
34	295	790	2545	3105	3030	90
41	330	650	3240	3545	3090	90
49	325	695	2965	3535	2880	95
56	345	690	4210	4960	3130	75
63	355	685	4215	4015	3990	70
70	300	780	3945	3670	3380	75
77	775	715	3415	3715	3550	55
84	295	400	3930	3970	3950	60
98	525	385	3955	3960	4095	60
105	305	495	3045	2940	3410	45
112	450	500	2540	2630	3000	60
119	590	370	2550	2260	2460	70
134	360	480	1955	1970	3000	50
140	470	480	2370	2380	2455	80
147	355	610	2700	2735	2720	60
158	290	440	2380	2410	2585	75

	Volatile Suspended Solids (VSS mg/l)				VSS/TSS Ratio			
Day	Anaerobic	Anoxic	Aerobic	Ferm. Eff	Anaerobic	prim. Anoxic	Aerobic	Ferm. Eff
1	2670	2670	2665		0.800	0.800	0.761	
6	2940	2890	2910		0.808	0.799	0.776	
14	3110	3165	2930		0.803	0.807	0.775	
21	3190	3173	2910		0.810	0.810	0.790	
29	2775	2770	2740		0.811	0.808	0.782	
34	2170	2610	2470		0.850	0.840	0.820	
41	2800	3035	2593		0.864	0.856	0.839	
49	2530	3000	2410	610	0.853	0.849	0.837	0.878
56	3585	4160	2580	620	0.852	0.839	0.824	0.899
63	3440	3360	3190	660	0.816	0.837	0.800	0.964
70	3325	3090	2745	725	0.843	0.842	0.812	0.929
77	2920	3070	2870	675	0.855	0.826	0.808	0.944
84	3270	3230	2970	385	0.832	0.814	0.752	0.963
98	2975	2820	2835	355	0.752	0.712	0.692	0.928
105	2330	2250	2290	305	0.765	0.765	0.672	0.961
112	2035	2055	2100	480	0.801	0.781	0.700	0.960
119	2025	1795	1830	-	0.794	0.794	0.744	
134	1635	1620	2365	475	0.836	0.821	0.788	0.990
140	1925	1905	1880	455	0.812	0.800	0.766	0.948
147	2180	2130	2035	570	0.807	0.778	0.748	0.934
158	1970	1970	1985	435	0.828	0.817	0.768	0.989

	COD (mg/l)							
Day	Fermenter Effluent	Fermenter Effluent Sol.	Anaerobic	Anaerobic COD_{sol} con.	Anoxic	Anoxic COD_{sol} con.	Aerobic	Effluent total
1	3005	1080	257		118	39.0	57	70
6	3588	1526	210	584	117	19.0	62	89
14	2960	1409	246	480	107	37.0	42	404
21	2710	1340	195	493	95	20.0	35	50
29	2500	1380	155	555	55	42.5	40	80
34	2575	1400	320	398	175	2.5	35	77
41	2280	1455	252	499	152	-3.0	46	63
49	2509	1465	225	532	107	29.5	48	80
56	2510	1355	185	509	84	25.0	33	40
63	2520	1310	71	597	30	18.5	26	46
70	2540	1230	63	568	38	9.5	32	45
77	2422	1262	153	497	40	55.5	38	48
84	1660	1200	59	554	29	13.5	26	34
98	1858	1312	75	599	34	21.5	36	49
105	1660	1310	123	544	45	28.0	23	35
112	1950	1240	184	454	82	27.5	35	60
119	1776	1225	244	387	152	-11.5	37	55
134	2140	1325	169	517	99	8.5	46	58
140	2040	1255	210	439	96	30.0	42	63
147	2000	1400	55	665	50	-2.5	40	47
158	1900	1300	183	481	104	1.0	27	59

Table 10.7: Continued…

	Reactor F/M		Dissolved Reactive Phosphprus (DRP mg/l)					
Day	g CODsol/g VSS/d	g CODtot/g VSS/d	Ferm.Feed	Ferm. Eff.	Anaerobic	Anoxic	Aerobic	Effluent
1	0.16	0.45	108.9	107	118.2	112.6	71.6	68.2
6	0.21	0.49		107	108.1	106	86.3	87.8
14	0.18	0.39	113.9	108	104.9	100.6	79.9	78
21	0.17	0.35	80.6	107	100.2	96.6	84.6	86.6
29	0.20	0.36	101.6	108.9	100.9	97.8	92.5	90.9
34	0.23	0.43	97.1	107	110.6	109.2	91.4	91.5
41	0.21	0.32	94.2	107.8	108	107.3	95.5	93.3
49	0.22	0.38	96.6	103.1	98.9	97.7	86.7	85.8
56	0.16	0.29	102.6	109	107.1	104	83.9	81.8
63	0.16	0.30	98.9	102.9	98.7	87.6	82.9	83
70	0.16	0.33	96.6	109.3	101.9	91.6	84.8	84.3
77	0.17	0.33	98.4	114.1	111.9	95	85.9	85.3
84	0.15	0.21	101	108.8	117.5	92.3	80.1	78.3
98	0.18	0.26	101	112.8	121.8	88.8	69.2	69
105	0.23	0.29	94.2	112.1	128.5	118.6	67.9	67.5
112	0.24	0.38	107.6	103.9	122.6	116.6	70.9	74.3
119	0.26	0.38	94.4	99.2	129.2	118.6	73.4	70.1
134	0.28	0.46		83.4	143.5	126.6	82.1	63.1
140	0.26	0.43	89.8	96	97.1	90.2	70.6	63.1
147	0.26	0.38	91.3	96.2	105.8	95.6	69	69.4
158	0.26	0.38	89.7	96.4	106.8	99.8	62.6	65.1

	P removed (mg/L)	P removed (mg/L)	%P	P-release (mg/L)	Prel/CODcon	Total Phosphorus (TP mg/l)		
Day	based on TP	based on DRP	removed	anaerobic	(mg/mg)	Ferm. Eff.	Aerobic	%mgP/mgVSS
1	40.4	38.8	36.1	28.1		112	262.8	7.1
6	30.3	19.2	26.0	5.9	0.01	116.6	245.4	5.5
14	35.1	30	30.5	8.4	0.02	115	263	6.2
21	21.4	20.4	20.2	3.9	0.01	106	243.5	5.5
29	26.5	18	22.3	-4.05	-0.01	119	267.7	4.6
34	18.1	15.5	16.5	10.1	0.03	109.5	165.2	3
41	5.5	14.5	5.4	10.85	0.02	101	179.7	3.2
49	17.5	17.3	16.8	3.9	0.01	104.2	165.8	3.3
56	25.8	27.2	23.5	11.35	0.02	109.7	175.8	3.6
63	23.5	19.9	22.1	4	0.01	106.4	213.5	4.1
70	21.9	25	20.5	6.4	0.01	106.7	211.7	4.6
77	27.4	28.8	24.2	12.6	0.03	113.3	201.2	4
84	23.1	30.5	22.4	26.75	0.05	103.2	253.2	5.83
98	43.6	43.8	38.7	30.9	0.05	112.8	334.1	9.35
105	44.2	44.6	39.4	38.7	0.07	112.1		8.5
112	41.6	29.6	37.0	29.2	0.06	112.5	254.3	8.74
119	27.1	29.1	27.0	43.9	0.11	100.5	210.6	7.49
134	21.0	20.2	20.4	60.3	0.12	103.1	238.7	6.62
140	34.5	32.9	32.8	13	0.03	105.1	202.6	7.03
147	39.2	26.8	36.2	17	0.03	108.2	230.1	7.92
158	37.6	31.3	37.5	24.2	0.05	100.2	201.4	6.99

	SVI	PHB and PHV Anaerobic(mg/g TSS)			PHB and PHV Anaerobic(mg COD/g TSS)			
Day		PHB	PHV	Total	PHB	PHV	Total	%PHB
1	140							
6	235							
14	251							
21	244							
29	211							
34	73							
41	71							
49	69.4							
56	108.5							
63	130	18.60	32.50	51.10	31.14	62.40	93.54	33.29
70	100							
77	81.7	22.40	21.60	44.00	37.50	41.47	78.97	47.48
84	73.4	12.30	9.70	22.00	20.59	18.62	39.21	52.51
98	75.7	17.90	9.80	27.70	29.96	18.82	48.78	61.43
105	62	34.50	20.70	55.20	57.75	39.74	97.50	59.24
112	57	45.00	21.20	66.20	75.33	40.70	116.03	64.92
119	57	25.60	15.80	41.40	42.85	30.34	73.19	58.55
134	70	12.00	10.60	22.60	20.09	20.35	40.44	49.67
140	101.8	14.30	10.00	24.30	23.94	19.20	43.14	55.49
147	75	13.8	7.3	21.10	23.10	14.02	37.12	62.24
158		18.00	7.10	25.10	30.13	13.63	43.76	68.85

Table 10.7: Continued...

	Nitrate (NO3-N mg/l)		Nitrite (NO2-N mg/l)		pH			
Day	Aerobic	Effluent	Aerobic	Effluent	Anaerobic	Anoxic	Aerobic	Effluent
1	2	1.5			7.58	7.82	8.25	8.14
6	1.2	1.6	0.4	0.3	7.35	7.53	7.9	7.93
14	3.3	1.5	0.5	0.8	7.31	7.64	8.34	8.27
21	1.6	1.6		0.4	7.78	7.98	8.3	8.06
29	5.2	5		0.7	7.65	7.95	8.18	8.12
34	6.7	5.8			7.31	7.55	8.17	8.05
41	10.6	10		0.3	7.58	7.82	8.24	8.1
49	3.7	3.7	0.7	0.8	7.52	7.75	8.23	8.1
56	4.9	3.8	0.7	0.9	7.88	8.02	8.41	8.25
63	6.1	10.2			8.18	8.31	8.79	8.7
70	4.4	5.1			8.15	8.25	8.68	8.61
77	6.1	5.7			7.83	8.16	8.56	8.53
84	19.8	19.3	1	0.9	8	8.18	8.52	8.38
98	13.2	12.5			7.69	7.9	7.98	7.95
105	8.4	7.5			7.52	7.65	8.09	8
112	6.1	4.8			7.59	7.77	8.04	7.98
119					7.49	7.72	8.1	8.01
134	2.5	1.5						
140	4	3.3			7.37	7.59	7.88	7.93
147					7.33	7.5	7.88	7.86
158					7.24	7.42	7.87	7.83

Table 10.8: AAO Reactor Fermented Wastewater VFA Concentrations

Day	Acetic (mg COD/L)	Propionic (mg COD/L)	n-Butyric (mg COD/L)	iso-Butyric (mg COD/L)	n-Valeric (mg COD/L)	iso-Valeric (mg COD/L)	Total (mg COD/L)
6	466	210	340	51	237	78	1381
14	464	195	213	57	153	82	1163
21	485	211	288	55	184	88	1310
29	383	186	189	53	157	69	1037
34	462	216	207	38	141	0	1064
41	702	217	213	60	186	0	1379
49	437	258	298	69	210	98	1371
56	430	284	277	58	120	100	1269
63	434	177	242	73	110	0	1036
77	456	135	167	13	127	73	971
84	468	154	177	13	147	81	1040
98	454	111	211	33	137	90	1036
103	436	136	202	32	147	85	1039
112	479	79	357	47	118	120	1201
119	395	136	290	55	115	123	1114
147	465	162	271	39	120	115	1172
155	468	118	237	45	92	98	1058

- AAO Zone Study Parameters - Refer to Table 6.4 in Chapter 6 for analytical data.

- AAO Batch Test Parameters - Refer to Table 6.4 in Chapter 6 for analytical data.

- Extended AAO (EAAO) Zone Study parameters - Refer to Table 6.6 in Chapter 6 for analytical data.

Chapter 7 - Data

Table 10.9: Fermenter Operation during AO System Operation (15 day SRT)

	Acetic acid	Propionic	iso-Butyric	n-Butyric	iso-Valeric	n-Valeric	n-Caproic	Total VFA COD
Day	mg COD/L	mg COD/L	mg COD/L	mg COD/L	mg COD/L	mg COD/L	mg COD/L	mg COD/L
9	474	302	41	222	87	78	10	1214
17	451	186	78	271	87	89	15	1177
25	296	225	63	281	43	46	13	967
31	374	277	82	213	62	75	10	1093
40	421	98	36	228	33	15	18	849
46	436	117	38	223	71	25	8	918
74	359	200	0	204	68	127	10	968
79	464	165	82	153	73	95	11	1043

- AO Zone Study parameters at an SRT of 10 days (Chapter 7) - Refer to Table 7.6 in Chapter 7 for analytical data.

- AO Batch Test Parameters at an SRT of 10 days - Refer to Table 7.7 in Chapter 7 for analytical data.

Table 10.10: 15 Day SRT AO Reactor System

	TSS (mg/L)					VSS mg/L	
Day	Feed	Fermenter	Anaerobic	Aerobic	Effluent	Anaerobic	Aerobic
9	225	460	2220	2740	110	2045	2145
17	270	640	2300	2870	105	1980	2340
25	235	530	4790	3060	330	3985	2420
31	185	505	3875	4315	105	3235	3440
40	260	795	4210	4535	175	3570	3620
56	260	445	4475	4375	160	3835	3470
74	220	580	4140	4300	95	3560	3460
79	580	515	3810	4375	80	3255	3450

	COD (mg/L)					VFA COD mg/L	
Day	Fermenter total	Fermenter Soluble	Anaerobic	Aerobic	Effluent total	Fermenter	Anaerobic zone
9	2261	1615	247	55	86	1213	179
17	2581	1663	309	42	98	1176	319
25	2675	1680	294	51	258	967	227
31	2294	1567	231	48	88	1092	190
40	2520	1512	167	50	83	848	93
56	2555	1610	215	45	91	918	178
74	1962	1584	127	44	75	969	74
79	1965	1555	58	38	50	1042	36

	% VFA COD		VFA Utilisation Anaerobic zone		Anaerobic COD consumption	
Day	Fermenter (%0.45um Sol)	Anaerobic zone	mg COD/L	% utilisation	mg COD/L	% utilisation
9	76.3	72.4	427.8	70.5	560.5	69.4
17	73.1	100.0	268.9	45.7	522.5	62.8
25	59.3	77.1	256.7	53.1	546.0	65.0
31	70.8	82.2	356.2	65.2	552.5	70.5
40	68.9	55.8	331.0	78.0	589.0	77.9
56	59.6	82.8	280.8	61.2	590.0	73.3
74	64.0	58.1	410.7	84.8	665.0	84.0
79	70.4	61.8	485.0	93.1	719.5	92.5

Table 10.10: Continued...

	Soluble Phosphorus (mg P/l)				
Day	Feed	Fermenter	Anaerobic zone	Aerobic Zone	Effluent
9	90.4	92.2	91.8	73.3	78.3
17	79.9	95.3	94.1	76.4	72
25	80	94.3	106.9	70.7	66.8
31	84.8	84.9	97.5	65.8	62.5
40	82.8	82.4	113	69.3	67.9
56	83	86.6	125.5	70.2	72.3
74	78.7	91.2	130.8	64.5	64.2
79	75.7	93.3	125.7	65.7	67

	Total Phosphorus (mg P/L)			% mg P/mg VSS	Ypo4 -anaerobic	Anaerobic release
Day	Fermenter	Aerobic zone	Premoval	Aerobic	mg P/mg COD	(mg P/l)
9	99.9	178.7	26.6	4.91	0.01	2.70
17	102.5	176	26.1	4.26	0.03	6.85
25	97.7	193.2	27	5.06	0.10	24.65
31	107.4	227.9	41.6	4.71	0.04	12.55
40	107.4	255.7	38.1	5.15	0.08	25.35
56	95	245	24.8	5.04	0.15	41.85
74	104.3	231	39.8	4.81	0.11	46.55
79	101.1	236.6	35.4	5.12	0.09	41.65

	pH		Nitrate (mg N/L)		Nitrite (mg N/L)		DSVI
Day	Anaerobic	Aerobic	Aerobic	Effluent	Aerobic	Effluent	aerobic
9	7.4	8.4	0.8	0.8	0	0.3	234
17	7.47	8.26	0.7	0.8	0	0	108
25	7.07	8.08	0.2	0.2	0	0	163
31	7.41	8.38	1.1	0	0.5	0	130
40	7.64	8.02	0	0.8	0	0	79
56	6.85	7.5	2.8	1.4	1	0.5	237
74	7.19	8.42	2.3	2.5	0.3	0.3	116
79	7.48	8.2	4	3.7	0.1	0	114

- AO Zone Study Parameters at an SRT of 15 days.- Refer to Table 7.8 in Chapter 7 for analytical data.

- AO Batch Test Parameters at an SRT of 15 days - Refer to Table 7.9 in Chapter 7 for analytical data.

Table 10.11: 5 Day SRT AO Reactor System

	Fermented Wastewater							
	Acetic acid	Propionic	iso-Butyric	n-Butyric	iso-Valeric	n-Valeric	n-Caproic	Total VFA
Day	mg COD/L	mg COD/L	mg COD/L	mg COD/L	mg COD/L	mg COD/L	mg COD/L	mg COD/L
9	474	302	41	222	87	78	10	1213
17	451	186	78	271	87	89	15	1176
25	296	225	63	281	43	46	13	967
31	374	277	82	213	62	75	10	1092
39	421	98	36	46	33	15	18	667
56	436	117	38	223	71	25	8	918

	TSS (mg/L)					VSS (mg/L)	
Day	Feed	Fermenter	Anaerobic	Aerobic	Effluent	Anaerobic	Aerobic
9	225	460	1680	2170	[illegible]	1465	1770
17	270	640	860	905	90	1640	1650
25	235	530	1860	2860	130	1605	2315
31	185	505	1520	2080	95	1310	1710
39	260	795	2070	2810	145	1770	2275
56	260	445	1180	1350	415	1040	1145

	COD (mg/L)				
Day	Fermenter total	Fermenter Soluble Anaerobic		Aerobic	Effluent total
9	2261	1615	353	68	88
17	2581	1663	360	70	89
25	2675	1971	342	75	110
31	2294	1567	563	79	65
39	2520	1512	433	64	64
56	2555	2118	527	85	421

Table 10.11: Continued...

	VFA COD (mg/L)		% VFA COD		VFA Utilisation Anaerobic zone	
Day	Fermenter	Anaerobic zone	Fermenter (%0.4	Anaerobic zone	(mg COD/L)	% utilisation
9	1213.1	271.7	76.3	77.0	334.9	55.2
17	1175.8	280.0	73.1	77.8	307.9	52.4
25	967.1	257.6	59.3	75.3	226.0	46.7
31	1092.2	378.9	70.8	67.3	167.2	30.6
39	666.9	291.4	54.1	67.3	42.0	12.6
56	917.6	473.9	44.0	89.9	0.0	0.0

	Soluble Phosphorus (mg P/L)					Total Phosphorus (mg P/L)		
Day	Feed	Fermenter	Anaerobic zone	Aerobic Zone	Effluent	Feed	Fermenter	Aerobic zone
9	90.4	92.2	99.3	77.7	81.3	107	99.9	168.6
17	79.9	95.3	94	75	77.8	103	99	135.3
25	80	94.3	93.1	73.7	75.2	106	97.7	183.2
31	84.8	84.9	86.9	74.4	73.5	107	97.8	145.5
39	82.8	82.4	82.2	80.5	83.7	96	92	157.2
56	78	81.3	85.2	75.7	76.8	98	95	105.6

	% mg P/mg VSS	Anaerobic release			Nitrate (mg N/L)		Nitrite (mg N/L)		SVI
Day	Aerobic	(mgP/L)	Anaerobic	Aerobic	Aerobic	Effluent	Aerobic	Effluent	
9	5.14	8.70	7.22	8.36	0	0.7	0	0	157
17	3.65	5.60	7.01	8.48	0.2	0.9	0	0	155
25	4.73	6.65	7.11	7.96	0	0.7	0	0	133
31	4.16	1.25	7.15	8.41	2.1	2.1	0.5	0.6	120
39	3.37	0.00	7.48	8.38	0	1.5	0	0.5	317
56	2.61	0.00	6.98	8.1	0	0	2.1	0	405

- AO Zone Study Parameters at an SRT of 5 days - Refer to Table 7.10 in Chapter 7 for analytical data.

Chapter 8 - Data

- AO Zone Study Parameters using Non-Fermented Wastewater at an SRT of 10 days – Refer to Table 8.3 in Chapter 8 for analytical data.

- AO Batch Test Parameters using Non-Fermented Wastewater at an SRT of 10 days - Refer to Table 8.4 in Chapter 8 for analytical data.

CHAPTER 11
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