THE USE OF A SEQUENCING BATCH REACTOR (SBR) FOR THE
REMOVAL OF ORGANICS AND NUTRIENTS WHEN SUBJECTED TO
INTERMITTENT LOADING

by

Kenneth Allen Haggerty P.Eng.

A thesis submitted in conformity with the requirements
for the degree of Master of Applied Science
Graduate Department of Civil Engineering
University of Toronto

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ABSTRACT

A synthetic waste water has been treated with lab scale sequencing batch reactors (SBRs) operating in a mode designed to remove organics and nutrients (nitrogen and phosphorus). These SBRs were subjected to starvation under differing operational conditions for between 58 and 117 days. The ability of an SBR to maintain its capability to remove organics and nutrients after periods of inactivity was determined. By not decanting the SBR at the end of the cycle preceding starvation, and allowing the SBR to remain quiescent during the starvation period, the retention of the treatment capability was substantial after 58 days at 22° C. Survival of treatment capability is further enhanced by operating the SBR prior to starvation in a mode with an extended anoxic period (3.5 hr) during and after fill. Conversely the process survival is significantly reduced if the SBR is decanted after the last cycle preceding starvation, and is further reduced by mixing, and or aerating the remaining mixed liquor. Higher temperatures appear to reduce the process survivability.
ACKNOWLEDGMENTS and DEDICATION

I would like to express my gratitude to my supervisor Professor David M. Bagley for his insightful guidance throughout this work, and his encouragement throughout my studies.

I thank my wife Eeva: without who's forbearance, encouragement, and understanding, I would never have been able to maintain the resolve necessary to complete this project.

I dedicate this work to my brother Gordon, whose untimely death caused me to examine and repriorize my objectives.
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<td>acradine orange direct count</td>
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<td>artificial wastewater</td>
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<td>BPR</td>
<td>biological phosphorus removal</td>
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<td>COD</td>
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<td>dissolved oxygen</td>
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<td>direct viable count</td>
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<td>mixed liquor</td>
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<td>MLVSS</td>
<td>mixed liquor volatile suspended solids</td>
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<td>ORP</td>
<td>oxidation reduction potential</td>
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<td>viable but non cultureable</td>
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<td>VFA</td>
<td>volatile fatty acid</td>
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<td>wastewater treatment plant</td>
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CHAPTER 1
INTRODUCTION

1.1 The Wastewater Treatment Problem

Soluble reactive phosphorus (SRP), or orthophosphate ($\text{PO}_4^{3-}$, P) is generally the limiting nutrient in the oligotrophic lakes in Precambrian regions, and eutrophication is often attendant its addition [Hutchinson, 1973]. Figure 1 (reprinted with permission) clearly shows this close relationship between eutrophic status and nutrient availability [Nurnberg, 1996].

![Figure 1 Chlorophyll versus Nutrient Concentrations](image)

**Figure 1 Chlorophyll versus Nutrient Concentrations**

The present practice of using septic systems to treat domestic sewage from cottages introduces SRP into the soil where, due to the varying geochemical factors involved, the time required for phosphorus to migrate from the tile bed into the nearby
surface waters will vary considerably. However it must be assumed that all of the septic SRP will eventually reach surface waters [Dillon and Mollot, 1996]. In order to prevent this with certainty it is necessary to remove the SRP prior to releasing the wastewater into the soil.

Phosphorus can be removed from wastewater by employing either chemical, [Brandes, 1976; Ptacek et al., 1994] or biological processes [Wentzel et al., 1985; Ketchum et al., 1987]. However biological processes offer the capability of removing organics and ammonia concurrent with the removal of SRP.

Sequencing batch reactors (SBRs) have been successfully used to treat wastewater generated by small communities [Melcer et al., 1987; Rusten and Eliassen, 1993] and, due to their flexibility, may be an appropriate technology for treating domestic wastewater from individual residences. However, no references to this possibility were found in the literature.

The treatment problem presented by wastewater generated at cottages is markedly different from that of a permanent residence due to the highly intermittent flow. In addition to the usual diurnal fluctuations, extreme weekly and seasonal fluctuations are expected, wherein flows will vary from nil to several times that normally expected for a single family residence. High flow events can be dealt with hydraulically using a combination of equalization capacity and shorter hydraulic retention times (HRTs) in the SBR. The situation is quite different for periods of no
flow which occur when the cottage is unoccupied. During these periods the microorganisms which normally facilitate the conversion of wastes will be starved.

Recent studies of bacteria under starvation conditions have established that many microbes are capable of withstanding long periods of starvation [Kjelleberg, 1993], and this is considered to be the normal situation in nature [Morita, 1982]. However these studies have involved either marine isolates, or pure cultures, and as such do not reflect either the environment, or the ecosystem diversity present in wastewater treatment systems. These findings will not likely be predictive of the bacterial starvation-survival in an SBR. Chudoba et al. (1991) subjected return activated sludge to 8 hours of anoxia and starvation, and Ford and Eckenfelder (1967) studied the effects of up to 72 hours anoxic starvation on chemical oxygen demand (COD) uptake and oxygen uptake rate (OUR) for the purpose of observing the effects on mixed liquor floc formation and settling. These studies demonstrated that the mixed liquor from aerobic treatment systems was capable of surviving relatively short periods of starvation and anoxia. However, while confirmatory of the ability to survive short term starvation, they do not provide any findings from which the recovery response of an aerobic treatment system starved for several weeks or months can be predicted.

1.2 The Effects of Inactivity on The Operating Performance of an SBR

Sewage treatment systems employing aerobic biological processes for the removal of organics, nitrogen and phosphorus, and other contaminants rely on a
relatively continuous albeit fluctuating flow-stream containing these constituents. Virtually all studies concerning the mechanisms, dynamics, and kinetics of microbial behavior relating to sewage treatment have implicitly assumed these conditions. To successfully operate a waste treatment system serving a single residence, and employing biological nutrient removal technology, it is necessary to understand the behavior of the microbes in conditions when the supply of organics and nutrients varies from being relatively continuous (residence occupied) to being completely absent (residence unoccupied). A search of the literature does not reveal any study of the behavior of any sewage treatment systems under conditions of discontinuous loading.

1.3 Objectives

The objectives of this research are:

1) To determine variations in effectiveness of organics and nutrient removal when an SBR is operated at different hydraulic loadings.

2) To determine the recovery responses of the treatment processes after the supply of organics and nutrients has been discontinued for various periods of time (i.e. SBR subjected to starvation conditions).

3) To use the results from (2) above to propose possible operating and restart strategies for the SBR in order to achieve removal of organics and nutrients after extended starvation periods.
CHAPTER 2
LITERATURE SURVEY

2.1 Some Aspects of Bacterial Growth and Starvation Responses

2.1.1 Introduction

Starvation appears to be the most predominant condition faced by microbes in nature, [Morita, 1982; Novitsky and Morita, 1977] and is also a prevalent condition in the bacterial cultures existing in reactors and wastewater treatment plants with observations revealing only 40-50% of the cells in a slowly growing chemostat culture being plateable [Kaprelyants and Kell, 1992].

During growth, cells have the ability to respond rapidly to challenges by turning on expressions of sets of genes in response to stress. In a starved cell such a rapid response is not likely to be possible. Therefore cells must prepare in advance to deal with the possibility of eventual environmental challenges such as heat, osmotic or oxidative stress, which may occur when they are in a starved state. Reeve et al., (1984) and subsequently Givsov (1994) and others showed that protein synthesis during the initial phase of multiple-nutrient starvation is essential for conferring starvation resistance. Starved cells lack the energy reserves required to respond quickly to changes in the environment, and it appears that they have evolved a means of protecting themselves in this event by modifying the cell envelope [Givskov et al., 1994], and making global changes to cell physiology, such as transcript modification
and stability, for both long term persistence during starvation as well as the efficient recovery of the cells [Kolter et al., 1993, Kjelleberg et al., 1993]. The starvation response includes expression of a catalase to protect against oxidative damage.

Another response of cells to starvation stress is to increase their spontaneous mutation rate [Siegele and Kolter, 1992]. In an experiment to prove mutation under stationary conditions, ten day old cells (stationary phase) were added to a culture of young (growing) cells. The stationary phase cells actually increased in number at the expense of the young cells indicating that they had mutated to increase survival [Kolter et al., 1993].

One other survival response to starvation appears to be the ability of some bacteria to enter a state termed viable but non cultureable (VBNC). In this condition the microorganisms require a stimulus other than simply the presence of nutrients in order to reactivate. Oliver (1991) and Whitesides and Oliver (1997) observed that the starvation response appears to be different than the noncultureable response, and may repress the latter. They observed that cells starved before a temperature downshift from 37 °C to 5 °C do not become nonplateable to the same extent as non-starved cells subjected to the same temperature drop, with a difference of a 2-log reduction in cultureability versus a 6-log reduction.

2.1.2 The Cell Cycle

The cell cycle is conventionally divided into four phases, i) the lag phase, ii) the exponential growth phase, iii) the stationary phase, and iv) the endogenous growth
phase. It would appear that the cell cycle is viewed in this order for reasons solely related to the set-up and conduct of most growth-related experiments. An organization of the cycle in a way which more accurately depicts the cycle as generally experienced by microorganisms would be: starvation, lag phase, exponential growth phase, stationary phase, and starvation phase, with the understanding that the stationary phase actually represents nutrient deprivation, and indicates the onset of starvation.

Stationary-phase does not represent a homogeneous physiological state, but is only a descriptive term, describing cultures in which the number of bacterial cells ceases to increase. Starvation-induced differentiation appears to be gradual: the slower the growth rate of the culture, the more growing cells resemble starved cells. It does not appear to be an all-or-none process involving an irreversible commitment to a program [Kolter et al., 1993].

Rapidly growing bacteria may contain several non-segregated nuclei or cell origins. This feature of growth accommodates the problem presented by the fact that the time required for a round of DNA initiation often takes longer to complete than is available within a single cell cycle [Muray and Hunt, 1993; James et al., 1995; Kolter et al., 1993; Neidhardt et al., 1990]. When deprived of nutrients, bacteria immediately begin a process of reductive division which results in an increase in the number of viable cells and a decrease in their size. This is often accompanied by morphological changes (e.g. rod shapes to coccibacilli) [Torella and Morita, 1981].
2.1.3 The Starvation Regulon

The requirement for the synthesis of survival-related proteins was first noted by Reeve in 1984. It is now known that the starvation response is governed by an alternate sigma transcription factor protein named $\sigma^*$ (also referred to as katF, nur, appR, csi-2, abrD and rpoS). This protein controls a regulon of 30 or more genes expressed in response to starvation and during the transition to stationary phase. There is evidence for a multilayered, cascade-regulation mechanism within the $\sigma^*$ regulon. However many factors besides $\sigma^*$ are involved in the regulation of stationary phase-induced genes, with different genes being under the control of different combinations of regulators. The interrelationships of these many factors is not well understood and many of the genes have not yet been identified [Loewen and Henge-Arronis, 1994].

2.1.4 Viability

The study of the starvation responses of microbes has suffered from a long held misunderstanding due to an unfortunately assumed relationship between cultureability and viability. Cultureability has long been held to be a proxy for viable or living cells, and the reciprocal non-cultureable state has been associated with dead cells. This assumption held sway in the published works of microbiologists and engineers until the 1980s [Amy and Morita, 1983; Moyer and Morita, 1989; Postgate and Hunter, 1962], and continues to be an accepted concept in environmental engineering literature. Cultureability tests are still used by public health officials to
determine the presence of coliforms and other bacterial contaminants [Standard Methods, 1989], and its suitability in this regard is still being studied and debated [McFeters, 1990, Craun et al., 1997].

There are significant variations amongst species in responses to starvation as measured by cultureability, with some marine isolates showing a decline in cultureability of 3 to 4 orders of magnitude after 8 weeks starvation, and others showing a decline of about 1 order of magnitude after 28 weeks starvation, [Amy and Morita, 1983]. The assumed relationship between living cells and cultureability has been definitively shown to not always be correct in several studies which employed acrdine orange direct count (AODC), and direct viable count (DVC) techniques, the INT assay, or flow cytometry, [Kjelleberg et al., 1987; Rollins and Colwell, 1986; Rozak et al., 1984; Tabor et al., 1981]. There is furthermore a large variation between cultureability on agar, and cultureability in liquid medium, [MacDonell and Hood, 1982].

Cultureability is not necessarily an appropriate exclusionary measure of viability. For example, loss of cultureability and decrease in cell volumes of starved ANT-300 cells varied directly and significantly with pre-starvation growth rates (i.e., higher growth rates resulted in a more gradual decrease in cultureability and less rapid reduction in cell volumes). On the other hand total cell counts as determined by AODC remained relatively unchanged throughout 175 days of starvation [Moyer and Morita, 1989; Nelson and Parkinson, 1978].
2.1.5 General Starvation Responses

Cell response to multiple nutrient starvation (starvation for glucose, amino acids, ammonium, and phosphate simultaneously) is a 3-phase process [Kjelleberg et al., 1993; Malmconna-Friberg et al., 1986; Nystrom et al., 1990; Marden et al., 1985].

1.0 Stringent control phase. This first phase encompasses accumulation of guanosine 5'-diphosphate 3'diphosphate (ppGpp) and decreases in RNA [Postgate and Hunter, 1962] and protein and peptidoglycan synthesis during the first 40 minutes of starvation [Kaprelyants and Kell, 1993]. The rate of proteolysis increases 16 fold [Siegel and Kolter, 1992] to 25 fold [Kjelleberg et al., 1993]. For cells receiving succinate alone the ATP/ADP ratios for growing and starved cultures dropped precipitously from 9 to 0.2 immediately upon entering the stationary phase [Jensen et al., 1995]. When the same experiment was conducted using glucose as the substrate the drop was precipitous but not as extreme (from 9 to 1). The researchers postulated that the small amount of acetate secreted during growth on glucose allows the cells to continue ATP synthesis after depletion of the glucose.

2.0 Reorganizational events 0.5-6 hr. During this phase a temporary increase in RNA and protein synthesis has been observed to occur between 40 and 180 minutes after the onset of starvation with a parallel decrease in the ppGpp pool [Malcrona-Fiberg, 1986]. This coincided with degradation of poly-a-hydroxybutyrate, which was rapidly utilized within the first hour and could no longer be detected after 3 hr starvation. In
another study Marden (1985) observed poly-hydroxy-butyrate, (PHB) granules in S14 cells starved for 1 hr, but the PHB granules were not visible after 27 hr starvation. Respiration rates observed by Marden were seen to increase for up to 1 hr after the onset of starvation and then decline. This corresponded with observations by Malcorna-Friberg of a transient decrease in the total amount of fatty acids. After 3 hr starvation Marden reported a peak in the substrate uptake ability which corresponded with an increase in the ratio of mono-unsaturated to saturated fatty acids and increased amounts of short chain fatty acids. This corresponded with a transient increase in the permeability of the membrane after 3 hr of starvation.

3.0 Gradual decline in metabolic activities. During this phase (after 3 hr starvation) a gradual decline in macromolecular synthesis and a gradual decrease in endogenous metabolism to low, or undetectable levels has been observed. It was found that amino acid starvation, or CdCl₂ addition which induced the most stringent response, were the most effective in conferring enhanced survival during starvation. In the case of Ant 300 the endogenous respiration dropped to less than 1% after 7 days [Moriya, 1982; Novitsky and Morita, 1977]. The ability to quickly metabolize substrates was maintained, but declined over time [Amy and Morita, 1983]. Using cultureability as a criterion for viability, Amy and Morita observed that marine isolates survived for 9 months without appreciable loss of cultureability in 75% of the isolates studied. The rate of respiration as shown by ¹⁴C declined with the length of the starvation period.
2.1.6 Resuscitation

After exposing E. coli to nutrient limitation for 20 hrs, Jacobsen and Gillespie (1968) observed that the optical density had dropped to one half the initial, however upon addition of substrate there was no observed lag period in the commencement of cell growth as observed by cell mass or optical density. The lag period for Vibrio S14 cells starved for 50-60 minutes was the same as that for cells shifted from rich medium to minimal medium [Albertson et al., 1990]. Cells starved for longer than 2 hours showed progressively longer lag periods with a relationship for commencement of increases in cell numbers of: \[ \text{Lag Time (min)} = 60 + 2.65 \times T_s \] (Ts = starvation time, hr.) applying for Ts up to 200 hrs. DNA synthesis followed the same relationship except that the initial 60 minute lag did not exist. This presumably reflected the time needed for cells to reach a critical size prior to division. The phenomenon of lag period being proportional to starvation period has been reported by Amy and Morita (1983) and Kaprelyants and Kell (1992) as well.

Kaprelyants and Kell (1993) found that 50% of 75-day-old cells were dormant and could be resuscitated after 16-18 hr. as measured by increases in total counts, viable counts and percent of cells taking up Rhodamine123. After 20 hr the viable count increased.

MacDonell and Hood (1982) found that of 27 marine isolates, 89% were initially incapable of growth in a full strength nutrient rich broth. When tested in dilute concentrations growth was observed with maximum initial growth being at 1/8 full
strength broth (approximately 400-500 mg/l trypticase soy broth). Agars were found to be inhibitory.

Nilsson, Oliver and Kjelleberg (1991) were able to resuscitate cells of *Vibrio vulnificus* which had shown a gradual decline in cultureability and become noncultureable after 27 days at 5°C by placing them at room temperature. The cells became cultureable after 3 days and were detectable at the original numbers. Two more cycles of noncultureability and resuscitation were examined without changes in the total cell count.

2.1.7 Cell Death

Davis *et al.*, (1986) listed irreversible damage to a vital element of the genome, irreversible damage to the cell membrane, and complete loss of a certain species of macromolecules as three classes of mechanisms which could be responsible for cell death. Of these they concluded that the excessive loss of ribosomes was the most likely cause of cell death during the studies of phosphate, carbon and nitrogen deprivation. Their findings confirm those of Jacobson and Gillespie (1968) who found that 95% of detectable ribosomes had disappeared after 20 hr of starvation. They also agree with some of the observations of Postgate and Hunter (1962) that starved cells maintained an intact osmotic barrier when viability count (cultureability) had dropped by 80%, and of Moyer and Morita, (1989) who observed minimal lysis in Ant300 cells starved for 175 days.
2.1.8 Nutrient uptake systems

The induction of nutrient scavenging systems such as those belonging to the global regulatory networks, e.g. cAMP/CAP and phosphate starvation responses, may allow for the utilization of additional or alternative substrates if such are available so that the adaptation towards the non-growing state is delayed or even reversed, [Kjelleberg, 1993].

Matin et al. (1979) concluded that bacteria generally increase the quantity of enzymes involved in the capture of the nutrient concerned, synthesize alternate proteins having a higher affinity for the nutrient, and/or acquire new capacities to obtain the scarce nutrient from a range of different substrates in response to deprivation of a specific nutrient. Kolter et al. (1993) and Amy and Morita (1983) observed that starved bacteria also are observed to produce fimbrae-like structures which may be involved in aggregation and scavenging.

2.1.9 Intracellular Inclusions, PHA and Polyphosphates

PHA is accumulated within cells in a wide variety of bacteria under unbalanced growth conditions when cells become limited for an essential nutrient, such as nitrogen, oxygen, phosphorus, sulfur, or magnesium, but are exposed to an excess of carbon [Doi, 1989]. PHB is degraded under anaerobic or aerobic conditions and serves as a major source of endogenous substrate with anaerobic degradation occurring at a much faster rate than aerobic. Doi (1989) subjected cells with 53% PHB/cell mass wt/wt to carbon, phosphorus and nitrogen starvation and found that
under aerobic conditions PHB content declined to 44% and 38% after 48 and 72 hours respectively, whereas under anaerobic conditions PHB declined to 20% and 8% in the same time frames. Jones and Rhodes-Roberts (1981) observed that PHB prolonged cultureability, but did not appear to fully account for overall survival time variations amongst the species tested. Matin et al., (1979) found that after 30 hr starvation the starvation-survival rate increased with increasing dilution rate for Pseudomonas sp., but for Spirillum the survival rate correlated with the PHB content of the culture at the onset of starvation and was not directly correlated with dilution rate. PHB content was a parabolic function of dilution rate for Spirillum. The rate of RNA breakdown increased with increasing dilution values and was not related to PHB content.

Bock (1976) found that Nitrobacter accumulated PHB, polyphosphates, and glycogen-like storage granules when grown under chemooorganotrophic conditions. After showing a first order decline in activity for the first 6 days of starvation the microorganisms then survived for at least 3 months before a decrease in viability commenced.

A widespread property of bacteria is that glycogen reserves can be enormously increased when growth is restricted by other than carbon and energy depletion. Under these conditions the cell converts available substrate into large quantities of intracellular glycogen to be readily available when growth is again possible. These glycogen granules are of uneven appearance 20-100 nm in diameter, and serve as
storage deposits. These granules disappear during carbon starvation as the glycogen is used up. Other bacteria store carbon as poly-α-hydroxyalkanoate granules and some have the capacity to store carbon in either of these forms [Neidhart et al., 1990].

2.1.10 Extra-Cellular Products

Many organisms release exoprotease in response to starvation, [Brock et al., 1994]. Albertson et al., (1990b) observed exoprotease release commencing 4 hr after onset of starvation conditions, and reaching a maximum after 24 hr. Release was coincident with the release of an exopolysaccharide produced in response to starvation. Chudoba (1985) found that microbial waste products which were intentionally increased in an SBR had the following effects.

(1) Dispersed growth changed from 0.3% to 30% of the bacterial population.
(2) The specific rate of COD removal fell from 100 to 55 mg·g⁻¹·hr⁻¹.
(3) Nitrification ability was lost, as was the ability to accumulate phosphate.

2.1.11 Starvation Related Experimental Observations in Wastewater Treatment

In a batch experiment Chudoba (1991) subjected sludge from a continuous system to an 8 hr anaerobic period and measured ATP, redox potential, and O₂. The ATP concentration decreased quickly during the first hour from 210 x 10⁻⁷ mol/L to approximately 70 x 10⁻⁷ mol/L. This decrease continued until stabilizing at 20 x 10⁻⁷ mol/L after 4 hours. Redox potential showed a similar decline from approximately 80 millivolts (mv) at the beginning to -380 mv after 8 hours. O₂ concentration decreased from 6 mg/L to almost 0 within 1 hour and reached 0 by the end of the second hour.
No solids concentration was given, however other experiments reported in the same article indicated biomass in the order of 30 to 220 mg/L. Chudoba et al., (1991) theorized that the manufacture of ATP and rebuilding of energy reserves would be at the expense of growth. The timing for the loss of ATP reported coincides with the timing for the release of $\text{PO}_4^{3-}$ - P reported by Manning and Irvine (1985).

Ford and Eckenfelder (1967) subjected sludges from continuous units to anaerobic periods of 0, 6, 12, 23, 48, and 72 hours and observed COD removal efficiency and OUR after both 15 minutes and 6 hours. Their observations are summarized as follows:

The OUR 15 minutes after end of starvation was substantially unchanged until after 48 hrs. After 72hrs starvation the OUR was 37 % below the initial level. The OUR 6 hours after the end of starvation was unchanged for all starvation periods studied, and the same percentage of COD was removed after the end of starvation for all of the periods studied. Improved settling characteristics accompanied increased anaerobic exposure.

In work with an SBR in the biological phosphorus removal (bioP) mode Smolders, (1994) subjected sludges to starvation up to 25 hours in a respirometer in order to determine maintenance coefficients. From the start of the aerobic phase he found that it took approximately one hour to remove all the phosphate from the medium, after which there was a marked drop in the oxygen consumption rate. This was followed by a further drop after the cells had used up their PHB stores (a first
order reaction extending about 8 additional hours). After 9 hours of starvation the oxygen consumption rate was constant at 0.25 mmol O₂/L·hr. The maintenance per C-mol biomass \( m_{\text{ATP}} \) was calculated as 0.019 mol ATP/C-mol·h.

### 2.1.12 Observations

The aforementioned studies make little mention of the effects of starvation on any nutrient removal capability. However it seems reasonable to anticipate that there will be no degradation in capability until sometime after 3 days of shutdown. Based on the findings of several researchers it also seems reasonable to expect that there will be significant survival after over 100 days of starvation. Coupling the findings of Postgate (1962) that Mg²⁺ ions delayed the onset of non-cultureability ("death" in the parlance of the time) with more recent observations by Comeau (1987) that Mg²⁺ ions are released in conjunction with P, it can be postulated that the presence of polyphosphate accumulating organisms (PAO) may extend the survivability of all the organisms present which are capable of accumulating polyphosphate.

### 2.2 Nutrient Removal Mechanisms

#### 2.2.1 Ammonia and Nitrate Removal

**2.2.1.1 Introduction** Unionized ammonia (NH₃) is toxic, and can be fatal to some species of fish at concentrations greater than 0.2 \( \mu \text{gNH}_3/\text{L} \), whereas the ammonium ion is not considered to be toxic [Sawyer, McCarty, and Parkin, 1994]. The unionized fraction of ammonia present in water can be found by the formula:
\[ f = \frac{1}{(10^{pK_a-pH} + 1)}. \]  \hspace{1cm} (1)

where:

\[ pK_a = 0.09018 + \frac{2729.92}{T}. \] \hspace{1cm} (2)

\( T \) = ambient water temperature in Kelvin (\( K = ^{\circ}C + 273.6 \)) [Provincial Water Quality Objectives (Ontario), 1994].

The Provincial water quality objective (PWQO) for NH\(_3\) in Ontario is 20 \( \mu g \) NH\(_3\)/L. This translates to a total concentration of ionized and unionized ammonia of 5 mg NH\(_3\)/L at pH 7 and 20\(^\circ\)C (3.9 mg/L as NH\(_4^+\) - N). The Ontario regulations require secondary treatment as the norm for all sewage treatment plants and may require further treatment as determined by the Ministry of the Environment on a case by case basis. Typically, effluent from a conventional activated sludge plant with secondary treatment and nitrification will contain 3 mg NH\(_4^+\) - N/L [Guidelines for the design of sewage treatment works (Ontario), 1984].

Unionized ammonia at concentrations in excess of 8.2 mg NH\(_4^+\) - N/L (equivalent to approximately 4300 mg/L total ammonia at 10 \( ^{\circ}C \), pH 7) is reported to be inhibitory to *Nitrosomonas* and therefore nitrification [Anthonisen et. al, 1976]. These levels are far outside the range which will be encountered in a wastewater treatment plant (WWTP) treating residential wastes where total ammonia will be approximately 30 mg/L. Mahn (1996) successfully operated a nitrification test on piggery wastes at a total ammonia concentration of 3000 mg NH\(_4^+\) - N/L.
2.2.1.2 Ammonia Removal. In a biological treatment system with adequate solids retention time (SRT) processes mediated by microorganisms will be responsible for virtually all observed ammonia removal. The effects of air stripping will be insignificant in any municipal WWTP operating at a pH near 7 as virtually all of the ammonia will be ionized (e.g. @ 10°C and pH 7, $f = 0.0019$, as determined by equation (1) above.)

A small portion of the ammonia removed will be as a result of the growth and subsequent removal of microorganisms. Taking the composition of bacteria as $C_3H_7O_2N$ it follows that there is 1 mole N per mole biomass, or 12.4% of the biomass weight [Sawyer, McCarty and Parkin, 1994]. Battley (1987) indicates that the measured nitrogen component of *E. Coli* K12 is 14.7% which is in close agreement with the empirical composition noted.

It was believed until approximately a decade ago that ammonia was also removed biologically only by conversion to nitrate as the result of a two step autotrophic process, described in section 2.2.1.3. However in the last decade several different biological pathways which facilitate ammonia removal have been discovered. Kuenen and Roberston (1994) suggest a treatment process where heterotrophic nitrifiers are the predominant species, but none are presently known to have been developed into fully functional systems. It is possible that the processes are functional to varying degrees in municipal WWTPs, and may need to be considered when examining the ammonia removal phenomenon.
A process termed Anamox [Mulder et al., 1995; van de Graff 1995] relies on a newly recognized mechanism whereby ammonia is converted to N₂ (gas) using NH₄⁺ as the electron donor and NO₃⁻ as the electron acceptor. This process operates anaerobically and it is not known to what extent, if any, it contributes to the removal of ammonia in a conventional WWTP.

It had long been held that Nitrosomonas europaea, the principle species involved in nitrification was a strictly autotrophic bacterium until Bock (1976), Poth and Focht (1985), and Stuven et al. (1992) demonstrated that at low oxygen levels N. europaea was able to reduce nitrite and cause nitrogen loss (changed from 14% of N lost at 0.4 mg O₂/L to 60 % of N lost at 0.2 mg O₂/L) using acetate, pyruvate and various other electron donors. A number of bacteria, some of which are also heterotrophic have been shown to be capable of simultaneously respiring on oxygen and nitrate [Robertson et al., 1995; Mulder et al., 1995]. The significance of these capabilities in the conventional WWTP is not known. Most wastewater treatment technology for ammonia removal is based on the assumption that the two biologically mediated processes of nitrification and denitrification occur sequentially.

2.2.1.3 Nitrification. The major biological process whereby ammonia is removed from wastewater is termed nitrification, and involves a sequential oxidation produced by two classes of bacteria, namely, ammonia oxidizers (NH₄⁺ → NO₂⁻), and nitrite oxidizers (NO₂⁻ → NO₃⁻).
The ammonia oxidizers are in genera prefixed *Nitroso-*, and most of the studies relating to wastewater relate to the single species, *Nitrosomonas europaea* which is one of the five *Nitroso* genera [Wood, 1988]. Nitrite oxidizers in genera prefixed *Nitro-* consist of strains which are obligate autotrophs and strains which can grow heterotrophically in the absence of nitrite [Bock, 1976; Wood, 1988]. The predominant species in wastewater is *Nitrobacter agilis* which is mixotrophic [Bock 1976; Wood, 1988].

Both of these nitrification steps result in the consumption of bicarbonate (reduced alkalinity), and use oxygen as the electron acceptor. Consider the overall oxidation equation.

\[
\text{NH}_4^+ + 2\text{O}_2 \rightarrow \text{NO}_3^- + 2\text{H}^+ + \text{H}_2\text{O} \quad \text{(not accounting for growth.)}
\]  

\[
\Delta G^o = -349 \text{ kJ/mole}
\]

Consequently, nitrification of 14 mg NH\(_4^\) - N/L produces 2 milli-equivalents (meq), or 100 mg CaCO\(_3\)/L of acidity, and nitrification of 1 mg NH\(_4^\) - N/L removes 0.14 meq or 7.14 mg CaCO\(_3\)/L of alkalinity. By calculation from the above oxidation equation 4.57 mg O\(_2\)/L is required for each mg NH\(_4^\) - N/L nitrified. When growth is accounted for the O\(_2\) requirement is 4.25 mg/L of O\(_2\) per mg/L of NH\(_4^\) - N nitrified [Henze et al., 1995].

Due to the relatively low energy yield resulting from ammonia oxidation the nitrifying bacteria exhibit a slow growth rate. This results in the solids retention time (SRT) being a limiting factor in nitrification.
Painter and Loveless (1983) arrived at a relationship for growth rate by studying nitrification with several different sludges. The relationship: 

$$k = a \cdot e^{b \cdot (T-15)}$$  \hspace{1cm} (4)$$

where: $$T = ^\circ\text{C}$$ and a and b are constants specific to a given wastewater was arrived at. They proposed values for (a) and (b) of 0.18 and 0.0729 respectively as a reasonable average, although (a) varied between 0.064 and 0.462 and (b) varied between 0.031 and 0.096.

The threshold inhibition pH level below which nitrification ceased was approximately 6.2 [Painter and Loveless, 1983]. In addition to temperature and pH, the nitrification process is affected by the presence of inhibitors. Copper, chromium, and organic substances such as thiourea, peptone, and certain amino acids are suggested as possible inhibitors [Painter and Loveless, 1983].

An expression relating growth rate to pH and temperature has been developed by Antoniou (1990). The maximum net growth rate expression is:

$$\hat{\mu}_A - b_A = \frac{mc^{-10T}}{1 + \frac{1}{10^{\text{pH} - 10^{-\text{pH}}}}$$  \hspace{1cm} (5)$$

and the minimum SRT to avoid washout is the inverse of this term. Normally this is about 12 days. (Ruston, 1993)

$$\hat{\mu}_A = \text{the maximum specific growth rate d}^{-1}$$

$$b_A = \text{the decay rate (varies between 0.05 and 0.15 d}^{-1})$$

$$m = \text{coefficient which is wastewater specific (} 4.7 \times 10^{14} \text{)}$$

$$a = \text{coefficient which is wastewater specific (} 9.98 \times 10^{3} \text{)}$$

$$b = \text{coefficient which is wastewater specific (} 2.05 \times 10^{9} \text{)}$$

$$c = \text{coefficient which is wastewater specific (} 1.66 \times 10^{-7} \text{)}$$
\[ T = \text{temperature K} \]

This expression predicts an optimal pH = \(-\log_{10}\sqrt{bc}\) (in the neighborhood of 8 for the sludge studied), and maximum net growth rate increases with temperature up to about 33°C.

2.2.1.4 Denitrification. Nitrate is reduced to \( N_2 \) in an anoxic heterotrophic reaction involving denitrifying bacteria from any of several genera. The nitrate is reduced to nitrite which in turn is reduced to \( N_2 \) and nitric and nitrous oxides [Wood, 1988]. This step produces bicarbonate and results in an increase in alkalinity.

Energetically, nitrate is a less preferable electron acceptor than is \( O_2 \). The half-reactions of interest are:

\[
0.25 \text{O}_2 + \text{H}^+ + e^- \rightarrow 0.5 \text{H}_2\text{O}, \quad \Delta G^\circ (W) = -78.14 \text{kJ/e} (6)
\]

\[
0.2 \text{NO}_3^- + 1.2 \text{H}^+ + e^- \rightarrow 0.1\text{N}_2 + 0.6 \text{H}_2\text{O}, \quad \Delta G^\circ (W) = -71.67 \text{kJ/e} (7)
\]

Accordingly oxygen is the preferred electron acceptor until its concentration reaches a low level, at which point nitrate will be used if available. This point often occurs within bacterial flocs, so that overall both nitrification and denitrification occur concurrently.

The carbon source (electron donor) for the denitrification reaction can be exogenous (either methanol, or sewage which is less expensive) or endogenous, [Alleman and Irvine, 1980; Comeau et al., 1987; Silverstein and Schroeder, 1983].

Combining the half-reaction equations for wastewater organic matter \( C_{18}H_{19}O_{9}N \) [Henze et al., 1995], and nitrate gives:

\[
\frac{1}{70} C_{18}H_{19}O_{9}N + \frac{1}{5} \text{NO}_3^- + \frac{1}{5} \text{H}^+ \rightarrow \frac{1}{10} \text{N}_2 + \frac{17}{70} \text{CO}_2 + \frac{1}{70} \text{HCO}_3^- + \frac{1}{70} \text{NH}_4^+ + \frac{1}{5} \text{H}_2\text{O}. \quad (8)
\]
One of the results of this reaction is that the consumption of 14 mg NO₃⁻ - N/L removes 1 meq of acidity, and at the same time produces 1/14 meq of bicarbonate. The net effect on alkalinity of the nitrification/denitrification process is the removal of 2 meq of alkalinity during nitrification and the addition of 15/14 meq during denitrification for a net loss of 13/14 meq for each millimole of NH₄⁺ processed.

The net alkalinity effect of nitrification/denitrification is the loss of 3.32 mg CaCO₃ per mg NH₃ - N removed.

The COD required for the denitrification reaction can be determined as follows [Henze et al., 1995].

\[
C_{18}H_{19}O_9N + 17.5O_2 + H^+ \rightarrow 18CO_2 + 8H_2O + NH_4^+
\]  \hspace{1cm} (9)

and 1 mg organic mater = 1.42 mg COD

From equation 8: denitrification of 1 mg NO₃⁻ - N requires 2 mg organic matter which may be expressed as 2.84 mg COD.

2.2.2 Biological Phosphorus Removal

2.2.2.1 Introduction. Phosphorus constitutes 2.8% of the microbial biomass in E.coli K12 [Battley 1987], and accordingly any biomass removed will contain approximately this amount of cellular phosphorus. In addition some bacteria possess the capability to take up phosphorus in amounts significantly in excess of that required for cellular structure. Removal of these bacteria from the wastewater can thus facilitate the extraction of significant amounts of phosphorus. This is referred to as biological phosphorus removal (BPR).
In BPR the soluble phosphorus (PO$_4^{3-}$ P) is removed from solution by a combination of intracellular storage and surface adsorption on the bacterial flocs. A portion of the sludge is then withdrawn from the reactor at a point when its phosphorus content is maximal and phosphorus is thus removed from the system. Recent work indicates that in a well operated BPR process, surface adsorption will not be an important mechanism [Appeldoorne et al., 1992; Smolders et al., 1994].

It is suspected that several genera of bacteria contribute to the process (Bark et al., 1992; Bond et al., 1995; Manz et al., 1994; Beacham et al., 1990; Brodish et al., 1983). Several researchers state that the process has not been demonstrated in pure cultures although Deinema et al. (1985) report to have done so with Acinetobacter.

2.2.2.2 Prescription. Prior to a solid theoretical foundation for the operation of the BPR process, a largely prescriptive regimen governed [Wentzel et al., 1985; Kortsee et al., 1994; Mamais and Jenkins, 1992; Pittman et al., 1992]. The main tenets of this regimen are as follows.

(1) Exposure of the system to anaerobic and aerobic periods. Phosphorus (P) release under anaerobic conditions must be observed in order to give rise to P uptake subsequently under aerobic or anoxic conditions, which results in a net P removal from the system by way of sludge wasting. As an alternative to using oxygen as the electron acceptor Kuba et al. (1993) operated a bioP process excluding oxygen entirely and using only nitrate as the electron acceptor.
(2) The presence of easily degradable organic compounds. For any fixed process configuration the magnitude of the P removal is linked directly to the magnitude of the readily biodegradable COD (RBCOD) concentration. The slowly biodegradable influent appears to have little direct influence on excess P removal. Wentzel et al (1988) suggested that since no short chain fatty acids were found in wastewater samples tested, only the RBCOD in the wastewater was being converted to short chain fatty acids, and that this first order reaction was actually the rate-limiting reaction in the BPR process chain.

(3) Polyphosphate accumulating organisms (PAOs) must be present in the inoculum. Up to a point, the greater the fraction of total sludge mass in the anaerobic reactor, the greater the net P removal. Appledoorn et al. (1992) demonstrated the necessity for PAOs to be present by operating an SBR in the BPR with sludge obtained from lakebed sediments and from a reactor treating potato flour wastes. In both instances he was unable to obtain P uptake after 42 days of operation, whereas when using sludge from a conventional WWTP, BPR was achieved within this time frame.

(4) The presence of electron acceptors (nitrate or oxygen) in the anaerobic phase has an adverse effect on P removal; the greater the mass of nitrate recycled, the lower the P removal possible. Tracking studies of Comeau et al. (1986) shows that denitrification occurred preferentially to substrate uptake and P release by the PAOs.

2.2.2.3 Description. Continuing research over the last 20 years has resulted in an understanding of many of the fundamentals involved in the BioP process.
The first of the two distinct environmental states which must exist for BPR to be operative is an anaerobic period during which RBCOD, which is comprised of short chain fatty acids is available. During this period the RBCOD is taken up by the bacteria and stored as polyhydroxyalkanoates (mostly PHB, e.g. C\textsubscript{3}H\textsubscript{5}O). There is some division as to the source of all of the energy to drive this reaction. Most lately Smolders et al. (1993) propose that the energy is provided by the degradation of some of the cellular stores of polyphosphate, e.g. Mg\textsubscript{1/3}K\textsubscript{1/3}(PO\textsubscript{4}) and glycogen (C\textsubscript{6}H\textsubscript{10}O\textsubscript{5}), resulting in the release of phosphate and Mg\textsuperscript{2+} and K\textsuperscript{+} ions. In the proposed model [Smolders et al., 1993] glycogen acts as a source of reduction equivalents and ATP, the remainder of the ATP is provided by polyphosphate degradation. At higher pH an increased electrical potential difference between the cell interior and the exterior exists. The cell is thus required to do more work when taking up negatively charged ions (such as acetate and other volatile fatty acids (VFAs)) against the negative potential of the cell. Smolders (1993) observed the following relationship for phosphate release between pH 5.5 and pH 8.2 using acetate

\[ Y_{\text{P/phosphate}} = 0.19\text{pH} - 0.85 \quad (\text{P-mol released/C-mol acetate uptake}) \quad (9) \]

The second environmental state must be a period following the anaerobic stage where electron acceptors (oxygen or nitrates) are present. During this period the PAOs utilize the electron acceptor and oxidize PHB to obtain energy which they use for growth and maintenance and to convert the unoxidized PHB to glycogen,
transport metal (\(\text{Mg}^{++}\), \(\text{Ca}^{++}\), and \(\text{K}^{-}\)) and phosphate ions into the cells and store them as polyphosphate [Smolders et al., 1994; van Veen et al., 1994].

2.3 Sequencing Batch Reactors:

An SBR consists of a single vessel which contains the biomass in the form of mixed liquor (ML), and into which the wastewater to be treated is introduced. The batch treatment process occurs during the following cycle:

**Fill** - The SBR is filled with wastewater to be treated.

**React** - Biological treatment takes place. This generally involves a mixed anaerobic or anoxic period followed by an aerated period. Many variations are possible.

**Settle** - A quiescent state is maintained and the biomass settles.

**Decant** - The treated supernatant is withdrawn from the SBR, leaving the settled mixed liquor as a source of biomass for the next cycle.

The process is controlled by adjusting the following environment factors within the SBR:

1.0 The quantity and hence age of the biomass.

2.0 The quantity of ML in the SBR prior to the introduction of the effluent.

3.0 Rate of filling and any subsequent addition of wastewater after the start of a cycle.

4.0 The rate and extent of mixing.

5.0 The timing and duration of aeration, and the volume of air supplied.

6.0 The length of the settle period.
The use of SBRs to treat wastewater and remove nutrients has been developed in the last 25 years [Alleman and Irvine, 1980; Ketchum et al., 1987; Manning and Irvine, 1985; Nakajima and Kaneko, 1987; Palis and Irvine, 1985; Sheker et al., 1994]. State of the art waste treatment installations using SBRs are capable of producing effluent with the following general characteristics: BOD$_3$ < 8 mg/L, SS < 8 mg/L, TKN < 8 mg/L, tot-P < 0.5 mg/L, for installations serving small communities treating the combined waste from several homes [Imura et al., 1994, Ruston and Eliassen, 1993, Marklund, 1994, Yuyama et al., 1994].
CHAPTER 3
MATERIALS AND METHODS

3.1 Overview

Six SBRs were used to treat an artificial wastewater (AWW). Each SBR consisted of a 2 - L Phipps and Bird™ jar -test vessel (VWR Canlab), outfitted with: AWW fill port, supernatant withdrawal port, paddle-type mixer with motor, aeration supply line, level control, and port for monitoring equipment. Each of two temperature controlled water baths contained three SBRs. The SBR operational cycles of fill, draw, mix, and aerate were each controlled by a seven day digital timer (# N1507, Noma Industries, Toronto).

3.2 SBR Experimental Setup

Three SBRs were contained within each of two covered aquarium tanks measuring approximately 30 cm wide x 35 cm high x 91 cm long (Battery 1 and Battery 2). The tanks were insulated with styrofoam SM™ insulation of approximately 4 cm thickness, and were each equipped with a removable styrofoam SM™ cover. Each battery was filled with water to a depth of approximately 20 cm and was maintained within a narrow temperature range (27-28 °C for Battery 1, and 20.5-24 °C for Battery 2) by a 100 watt submersible electric aquarium tank heater. Each aquarium tank was outfitted with two longitudinal aluminum bars measuring 2 cm x 2 cm which
were placed 20 cm above the tank bottom, spaced 12.5 cm apart, and fastened with L brackets to plywood plates bonded with silicone to each end of the tanks. The SBRs slid between these tubes and were supported by them on brackets.

For each SBR the operational cycles of fill, draw, mix, and aerate were individually controlled by a dedicated seven day digital timer. The arrangement was as shown in Figure 2.

![Figure 2 Overview of SBR Arrangement](image)

3.3 SBR Construction

Each SBR consisted of a 2 - L acrylic Phipps and Bird™ jar -test vessel which was modified to accommodate 12.7 mm (1/2") threaded supernatant withdrawal ports at levels which left a retained volume of 0.5 L, 0.75 L, 1.0 L, 1.25 L, and 1.5 L in the
SBR. All of these ports were closed with a threaded brass bung except for one, which was fitted with a threaded barbed connector to which was attached a 1/4" flexible hose for decanting the SBR.

Great difficulty was encountered in sourcing a mixing motor which was durable, of a small enough physical size, and capable of operating at a speed of approximately 30 rpm. In all, three different motors were used during the experiments. It was initially postulated that a two speed mixing setup was required, with a high initial mixing speed (80 rpm) to be used immediately after the AWW was introduced, and a slow speed (30 rpm) used thereafter. Therefore a small 1.5 volt motor turning at 10,000 rpm was reduced by a combination of helical gears and electronic circuitry to operate at both 30 and 80 rpm. After approximately 4 weeks of operation the brushes in all of these motors burned out, and could not be serviced. Subsequently an inexpensive motor and gear assembly used as a drive for toy vehicles was located (Active Surplus, Toronto), and modified to operate the paddle mixer. Although the minimum mixing speed at which this setup would operate was in the order of 90 rpm to 120 rpm, it was hoped that by employing intermittent mixing cycles it could serve the purpose until a suitable substitute was found. The operation of the SBRs was not satisfactory when using these motors, and after approximately three months they were removed.

The third motor examined was of a type normally used to stir the color containers in paint blending machines (DS Imports, Unit 1, 640 Hardwick Road,
Bolton, Ontario). The SBRs were retrofitted to accommodate the new 110 volt motor which was geared to turn its shaft at 28 rpm. The jar-test vessels were modified to accept a 1-cm thick Lexan\textsuperscript{TM} cover which supported the mixing motor and paddle mixer, AWW fill port, aeration line, level sensor, and was fitted with a port with removable cover for monitoring equipment. Figure 3 shows the completed SBR. Figure 4 shows SBR 2 equipped with the final revised motor and cover (left), a spare Jar-test vessel (center), and SBR 3 with the second motor installed and in operation prior to being replaced (right).
Although the fill cycle was controlled by a timer, a level control was employed to shut the fill pump off when the volume reached the 2-L level. Two types were employed: a circuit continuity type (Figure 5) designed by Dr. Mathew Malone, Department of Engineering Science, University of Toronto and shown installed in Figures 3 and 4, and installed on 5 SBRs, and a commercial capacitance activated device by Cole-Parmer (LC41-1001 and LP10-1303) installed on SBR-1. The circuit continuity device was susceptible to occasional premature shut-off due to moisture bridging.
The AWW was prepared in 100-L lots, stored at 4 °C and continuously mixed with a small aquarium circulating pump. A peristaltic fill pump (Masterflex™ 77000-30 by Cole-Parmer Instrument Company) for each SBR was controlled by a timer and pumped the AWW from the refrigerated storage to the SBR. In order to avoid heat shock to the mixed liquor the AWW was passed through a heat exchanger prior to entering the SBR (Figure 6).

Figure 5 Level Control Circuitry

Figure 6 Substrate Flow Diagram
Figure 7 shows the entire experimental setup in its operating form.

![Experimental Setup](image)

Figure 7 Experimental Setup

3.4 Artificial Wastewater

Artificial wastewater was used instead of actual sewage in order to eliminate the variables of changing concentration of organics and nutrients, and to avoid the presence of bacterial growth inhibitors. These often affect the treatability of real wastewater.
The AWW consisted of tap water to which the following constituents were added: Ensure™ (Abbott Laboratories, Montreal) - 0.77 ml/L, NH₄Cl - 114.6 mg/L, K₂HPO₄ - 22.5 mg/L, KH₂PO₄ - 17.6 mg/L, Na₂CO₃ - 100 mg/L, NaHCO₃ - 100 mg/L, trace mineral medium [Appledoome, 1992] - 2 ml/L. The trace mineral solution was prepared in 1 litre batches and stored at 4°C in opaque bottles. It consisted of 0.06 g Na₂MoO₄·2H₂O, 0.12 g ZnSO₄·7H₂O, 0.15 g CoCl₂·6H₂O, 1.5 g FeCl₂·6H₂O, 0.15 g H₃BO₃, 0.03g CuSO₄·5H₂O, 0.03g KI, 0.12 g MnCl₂·4H₂O dissolved in 1 litre of deionized water. In order to prevent growth in the lines and the storage tank, the AWW storage tank and the fill lines were chlorinated on a weekly basis for 10 to 20 minutes with a Chloriclean™ (Wine Art, Toronto) solution at 60 °C, and subsequently rinsed with clean tapwater.

3.5 SBR Operation

The SBRs were seeded with sludge taken from the reacter section of the old wastewater treatment plant in Newcastle, Ontario. This plant receives a mixture of residential and industrial waste and did not add any chemicals for phosphorus removal at the time. All SBRs were operated with the same 8 hour overall cycle time. The SBRs were operated as shown in Tables 1 and 2.

SBRs 1 and 4 employed a cycle strategy which was essentially the same as that found to be successful by others [Manning and Irvine, 1985, Ketchum et al., 1987; Okada and Sudo, 1986; and Imura et al., 1994]. The operating strategy for SBRs 2, 3, 5, and 6 initially only varied from this protocol with respect to HRT which was varied
by changing the quantity of supernatant withdrawn and subsequently the quantity of AWW added during fill. The purpose of these variations was to determine the effect of the HRT on the reactor performance.

**Table 1. Battery 1 SBR Operation**

<table>
<thead>
<tr>
<th>SBR Function</th>
<th>SBR 1&lt;sup&gt;1&lt;/sup&gt;</th>
<th>SBR 2</th>
<th>SBR 3&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fill and Anoxic Mix (min)</td>
<td>120</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Aerate and Mix (min)</td>
<td>180</td>
<td>180</td>
<td>270</td>
</tr>
<tr>
<td>Anoxic Mix (min)</td>
<td>105</td>
<td>105</td>
<td>0</td>
</tr>
<tr>
<td>Settle (min)</td>
<td>45</td>
<td>45</td>
<td>60</td>
</tr>
<tr>
<td>Decant and Idle (min)</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>27 - 28</td>
<td>27 - 28</td>
<td>27 - 28</td>
</tr>
<tr>
<td>Full Volume (L)</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>After Decant Volume (L)</td>
<td>1</td>
<td>0.75</td>
<td>1</td>
</tr>
<tr>
<td>HRT (hr)</td>
<td>16</td>
<td>12.8</td>
<td>16</td>
</tr>
</tbody>
</table>

(1) Initial anoxic mix was for first 60 minutes only. Mix during aerate was for 90 min. commencing 30 min. after start. Mix during anoxic period after aerate was for 30 minutes commencing 30 min. after end of aeration.

(2) The SBR operated on this cycle for only 2 weeks prior to onset of starvation.

The different HRTs were expected to have different P uptake and release characteristics primarily due to the difference in the quantity of NOx-N present which would act as an electron acceptor. The NOx-N would be removed before the P release and associated substrate uptake commenced. All SBRs were expected to perform BPR, as well as nitrification and denitrification. The lengths of the anoxic periods in
SBRs 5 and 6 were extended to 210 min after observing that P release was still ongoing at the end of the original 2 hr period.

<table>
<thead>
<tr>
<th>SBR Function</th>
<th>SBR 4</th>
<th>SBR 5</th>
<th>SBR 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fill and Anoxic Mix (min)</td>
<td>120</td>
<td>210</td>
<td>210</td>
</tr>
<tr>
<td>Aerate and Mix (min)</td>
<td>270</td>
<td>180</td>
<td>180</td>
</tr>
<tr>
<td>Anoxic Mix (min)</td>
<td>0</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Settle (min)</td>
<td>60</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>Decant and Idle (min)</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>20.5 - 24</td>
<td>20.5 - 24</td>
<td>20.5 - 24</td>
</tr>
<tr>
<td>Full Volume (L)</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>After Decant Volume (L)</td>
<td>1</td>
<td>1.5</td>
<td>0.5</td>
</tr>
<tr>
<td>HRT (hr)</td>
<td>16</td>
<td>32</td>
<td>10.67</td>
</tr>
</tbody>
</table>

The sludge age employed by different researchers varied between 6 and 20 days with MLSS values reported in the range of 1500 to 4500 mg/L (Ketchum et al., 1987; Manning and Irvine, 1985; Shin, 1992). One of the objectives of this research was to attempt to limit sludge wasting to that necessary for phosphate removal. However the time spent getting the SBRs to actually perform phosphate uptake and release was so long that an effective sludge wasting program was never fully employed. Sludge wasting was incorporated into the cycle for SBRs 3 and 4 by
operating the decant pump for 2 minutes (at 84 ml/min) immediately before the commencement of the settle cycle. This method was adopted after testing other methods which proved to be unsatisfactory for the experimental setup employed.

3.6 Starvation

The supply of AWW was stopped and the bacterium in the SBRs were considered to be starved. Table 3 summarizes the SBR starvation conditions examined.

<table>
<thead>
<tr>
<th>SBR</th>
<th>Decanted</th>
<th>Aerated</th>
<th>Stirred</th>
<th>Duration, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>117</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>96</td>
</tr>
<tr>
<td>3</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>72</td>
</tr>
<tr>
<td>4</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>58</td>
</tr>
<tr>
<td>5</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>78</td>
</tr>
<tr>
<td>6</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>84</td>
</tr>
</tbody>
</table>

3.7 Restart After Starvation

The SBRs were restarted on schedules as shown in Table 4, and resupplied with AWW.
Table 4 SBR Operating Schedules for Restart

<table>
<thead>
<tr>
<th>SBR Function</th>
<th>SBR 1</th>
<th>SBRs 2 and 3</th>
<th>SBRs 4, 5 and 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fill and Anoxic Mix (min)</td>
<td>120</td>
<td>210</td>
<td>210</td>
</tr>
<tr>
<td>Aerate and Mix (min)</td>
<td>240</td>
<td>180</td>
<td>180</td>
</tr>
<tr>
<td>Anoxic Mix (min)</td>
<td>45</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Settle (min)</td>
<td>45</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Decant and Idle (min)</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>27 - 28</td>
<td>27 - 28</td>
<td>20.5 - 24</td>
</tr>
</tbody>
</table>

3.8 Sampling and Analytical Methods

During the course of the studies the SBRs were individually monitored using a YSI™ 600XL sonde (Ontario Hydrometric, Toronto) connected to a Mitsubishi MP286L laptop computer with datalogging software supplied by YSI™. The sonde was equipped to monitor dissolved oxygen (DO), conductivity, temperature, pH, and oxidation-reduction potential (ORP).

Effluent samples were collected approximately 3 times per week, and analyzed for suspended solids (SS), volatile suspended solids (VSS), total chemical oxygen demand (CODt), soluble COD (CODf), turbidity, ammonia (NH₄⁺ - N), nitrate (NO₃⁻ - N), occasionally nitrite (NO₂⁻) and both PO₄³⁻ - P and total phosphorus (P₇). Solids were determined according to Standard Methods (APHA, 1992, method 2540D & 2540E), as was COD using the colorimetric method 8000; turbidity was measured with a Hach 2100P turbidimeter; and ammonia, nitrate and phosphorus were
determined with a Hach DR2000 spectrophotometer using methods and materials prescribed by Hach (Fryston Canada Inc, Mississauga, Ont.). On occasion an Orion™ ion selective electrode (from VWR Canlab Inc, Mississauga, Ont Model No. 34105-120) was used to determine ammonia concentration. Mixed liquor was analyzed approximately 3 times per week for solids (mixed liquor suspended solids, MLSS, and mixed liquor volatile solids, MLVSS), and occasionally characterized for NH$_3$ - N, NO$_3$ - N, and PO$_4$$^{3-}$ - P, and P$_T$.

Tracking studies were conducted on all SBRs during the operating period preceding starvation, and subsequently when the SBRs were recovering from starvation. During starvation, samples of the mixed liquor (ML), or supernatant in the case of non-stirred SBRs, were occasionally taken and characterized.
CHAPTER 4
RESULTS AND DISCUSSION

4.1 Pre-Starvation Results and Discussion

The performance characteristics of the SBRs were monitored for a period of 6 months prior to the commencement of starvation studies. Approximately 4 months of this period were spent modifying the experimental apparatus and its operation in order to achieve the conditions necessary for biological nutrient removal.

4.1.1 SBR Start-Up Problems

Start up required the resolution of several operating problems caused by: incomplete knowledge as to the character of the AWW; the inability to locate a satisfactory stirring motor when the SBRs were initially constructed; and a lack of appreciation as to the physical arrangements required in order that the ML achieve the prescribed anoxic state. These were corrected one at a time as follows:

(1) The pH was found to have dropped from 6.85 measured in the substrate to an average of 5.6 in the effluent. This was below the 6.2 limit for nitrification inhibition reported by Painter and Loveless (1983). It was theorized that this was the result of alkalinity consumption during nitrification, and after adding 200 mg/L of NaHCO₃, (later changed to 100 mg/L NaHCO₃ and 100 mg/L Na₂CO₃) the SBR pH stabilized at an average of 6.95. Subsequent tests indicated the tapwater alkalinity to be approximately 90 mg CaCO₃/L or 1.8 meq/L. Alkalinity requirements for
nitrification in SBR-1 for example, where approximately 15 mg NH₄-N/L was nitrified, would consume 2.14 meq of alkalinity (see equation 3), thus causing the observed pH drop.

(2) Initially it was assumed that since Ensure™ is sometimes used as a total food source for invalids, the AWW would contain all of the minerals necessary to support microbial growth. However MLVSS was below expectations, and after receiving assurances from the manufacturer that the Ensure™ did not contain any bacterial inhibitors, a supplemental mineral medium as described in Section 3.4 was added to the AWW. Thereafter higher levels of MLVSS were achieved.

(3) Prior to the sourcing of a suitable 28 rpm stir motor assembly, tracking studies revealed that PO₄³⁻ was not being released and subsequently taken up during the respective anoxic and aerated periods of the treatment cycle. This seemed to confirm that the DO present during the anoxic period (approximately 1.5 mg/L) was too high for the mechanism of polyphosphate degradation to be operative. In order to eliminate the capture of DO arising from the high stirring speed, the cycle for SBR-1 was adjusted to incorporate only 3 stir periods of 2 minutes each during the 2 hour anoxic period. Tracking studies conducted after 1 week operation (Figure 8), and 2 weeks operation (not shown) revealed that there was still no significant observed PO₄³⁻ release during the anoxic period, nor was all of the NO₃⁻ removed by denitrification.

(4) The CODf uptake rate during the anoxic mix period was of interest since it was considered to be indicative of the RBCOD present, and was expected to bear a
relationship to $\text{PO}_4^{3-}$ - $\text{P}$ release (sections 2.2.2.2 and 2.2.2.3). Tracking studies (Figure 9) indicated that CODf was substantially removed by the end of the fill cycle (15mg/L measured vs 128 mg/L calculated if no uptake during fill).

![Figure 8 SBR 1 Tracking Study Aug 13, 1996](image)

![Figure 9 SBR 1 Tracking Study Aug 13, 1996](image)
Two experiments were conducted in order to observe the CODf uptake phenomenon in greater detail. In the first experiment the AWW for SBR 1 (1L) was collected from the fill pump and then dumped into the SBR containing the ML (1L) and mixed. In order to gain a greater appreciation of the effect of CODf concentration on the uptake phenomenon, 100 ml of ML was taken from SBR 1 and 1 L of AWW was collected from the fill pump and dumped into the ML and mixed. Readings of DO and CODf were taken for these two experiments and plotted (Figure 10 ).

![Figure 10 CODf and DO Uptake Study for SBR 1](image_url)
Specific Uptake Rate (U) and OUR parameters were calculated and are shown in Table 5. No studies were attempted to determine the proportions at which CODf removal and storage was cellular vs extracellular and therefore U must only be interpreted as a capture parameter.

**Table 5 Parameters During Uptake**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Substrate:ML 1:1</th>
<th>Substrate:ML 10:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate CODf, mg/L</td>
<td>214</td>
<td>198</td>
</tr>
<tr>
<td>MLVSS after dump fill, mg/L</td>
<td>1530</td>
<td>367</td>
</tr>
<tr>
<td>Loading, mg CODf/mg MLVSS</td>
<td>0.07</td>
<td>0.49</td>
</tr>
<tr>
<td>OUR, mg O₂/L-min</td>
<td>0.66 (0.999)</td>
<td>0.48 (0.999)</td>
</tr>
<tr>
<td>Specific OUR, mg O₂/mgMLVSS/min</td>
<td>0.03</td>
<td>0.08</td>
</tr>
<tr>
<td>U, mg CODf/L-min</td>
<td>5.82 (0.841)</td>
<td>3.62 (0.939)</td>
</tr>
<tr>
<td>Specific U, mg CODf/mg MLVSS/min</td>
<td>0.23</td>
<td>0.59</td>
</tr>
<tr>
<td>U/OUR, mg CODf/mg O₂</td>
<td>7.66</td>
<td>7.37</td>
</tr>
</tbody>
</table>

(1) During first 20 min

It is notable that relationship of U/OUR was essentially unchanged for both experiments (only 4% lower in 10:1 than in 1:1) even though the specific loading rate in the 10:1 experiment was 7 times that in the 1:1 experiment. This strongly suggests that the CODf removal was dependent on the availability of O₂ as an electron acceptor. Denitrification was negligible during this study.
During the 10:1 Substrate/ML test the rate of substrate uptake dropped sharply to 23% of its previous rate after the DO dropped below 1 mg/L. Although it is possible that a maximum CODf storage capacity was reached simultaneously with the low DO level, it is more likely that this is confirmatory of the view that respiration was driving the uptake rate. The specific OUR and specific U increased by a factor of 2.7 and 2.6 respectively when the specific loading increased by a factor of 7 indicating that 0.08 mg O2/mg MLVSS min is very near the maximum OUR for this system.

Although not considered at the time of these experiments, it has been subsequently realized that the observed phenomenon of CODf uptake without PO4-3 - P release may to some extent be an experimental artifact which resulted from the high DO in the AWW. If real wastewater were being used the DO would be expected to be significantly below the 8 mg/L observed in these experiments. It is possible that under these conditions the system, which had selected for microorganisms which were capable of removing the CODf to the low levels measured before the DO was below 1 mg/L, would have allowed the PAOs to be effective, and an intermittent mix strategy during the anoxic period would have resulted in PO4-3 - P release. This will be discussed further in section 4.1.2.2.

In addition to the aforementioned these experiments allow for the following observations and conclusions.
1.0 The CODf values obtained show a high degree of linearity over the short period observed (20 min), and are indicative that the CODf fluctuations observed during the later tracking studies represent real changes and not experimental artifacts.

2.0 CODf removal is a capture phenomenon as is shown by the relationship between U/OUR (Table 5). The respective values of 7.66 and 7.37 mg CODf removed/mg \(O_2\)\(\text{min}^{-1}\) are clear evidence that the substrate is not being metabolized, but is instead being stored. Given that there was no P release observed at the time this study was conducted it is safe to assume that the CODf storage was not related to the sought for PAO activity.

Shortly after these studies were completed, a slow speed stir motor assembly was located and all of the SBRs were refitted with the new stir motor mounted on a cover which was designed to significantly reduce the quantity of fresh air available to the surface of the mixed liquor (see Figures 2, 3, and 4). Two days after the changeover the DO concentration at the end of the anoxic period was observed to be between 0.5 and 0.2 mg/L. The \(PO_4^{3-}\) - P proportion of the decanted ML was between 3.0% and 4.1% on a mass basis. Subsequently uptake and release of \(PO_4^{3-}\) was observed, as was complete denitrification during the anoxic fill period. This will be discussed in Section 4.1.2.4.

4.1.2 Pre-Starvation SBR Operation

Tracking studies were conducted on individual SBRs just prior to the onset of starvation. The results of these studies were analyzed with respect to CODf removal,
PO$_4^{3-}$ - P release and uptake, nitrification, and denitrification. The data for CODf, PO$_4^{3-}$ - P, NH$_4$ - N, and NO$_3^-$ - N from these tracking studies is plotted in Figures 10 to 15 in order to provide a sense of the processes occurring. A review of the performance of each with respect to CODf, P, and N follows in Sections 4.1.2.1 to 4.1.2.4 inclusive.

The following events influence the tracking study results.

SBR - 1 One week prior to the tracking study approximately 1/2 of the ML was inadvertently wasted. At the time of the tracking study the solids had returned to near the previous level.

SBR - 3 The operating cycle was only in effect for 2 weeks prior to the tracking study. For the previous 6 weeks a series of varied aeration and fill schedules were employed.

SBR - 4 A great deal of gray to black wall growth indicative of anoxic conditions which may have arisen due to inadequate mixing was present in the corners of the reactor vessel and chunks occasionally broke loose.
Figure 11 SBR 1 Tracking Study October 8, 1996

Figure 12 SBR 2 Tracking Study October 24, 1996
Figure 13 SBR 3 Tracking Study November 12, 1996

Figure 14 SBR 4 Tracking Study November 7, 1996
Figure 15 SBR 5 Tracking Study November 12, 1996

Figure 16 SBR 6 Tracking Study November 12, 1996
4.1.2.1 Organics removal. The CODf concentrations for the effluent and in the SBRs during the anoxic period are summarized in Table 6. It appears that the reduction of the air supply which resulted from the change to a covered reactor coupled with a slower mixing speed (28 rpm) markedly changed the organics removal profile during the anoxic stage. Although there was significant variation amongst the SBRs, the overall trend is similar, in that at the end of fill the average CODf removal was 76% prior to the aforementioned changeover, and 57% after (see Table 6, note 4).

Similarly the CODf at the end of the anoxic period was also higher in all cases except for SBR 4. The CODf in the effluent before the changeover averaged 93% removal whereas after the changeover the average CODf removed was 86% (excluding SBR3).

As will be discussed in the next section (4.1.2.2) the change to the 28 rpm mix coupled with a cover for the SBR allowed the effect of the PAOs to become evident, and \( \text{PO}_4^{3-} \)-P release was observed. However an examination of the profile of the \( \text{PO}_4^{3-} \)-P release, which shows no immediate consequential increase during fill, leads to the conclusion that a significant portion of the initial CODf removal (57%) resulted from the previously mentioned mechanism which is unrelated to \( \text{PO}_4^{3-} \)-P release.

The CODf which was removed during anoxic fill by denitrification is shown in Table 10, and its contribution to the overall CODf removal during anoxic fill is shown in Table 7.
### Table 6 CODf in Anoxic Period and in Effluent

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SBR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Calculated CODf (mg/L) if no Uptake (CODf&lt;sub&gt;substr&lt;/sub&gt;) X Vol + CODf&lt;sub&gt;ML&lt;/sub&gt; X Vol/2</td>
<td>142</td>
</tr>
<tr>
<td>End of Fill&lt;sup&gt;(1)&lt;/sup&gt;</td>
<td>(Prior to 28rpm Mix) CODf, mg/L</td>
</tr>
<tr>
<td></td>
<td>% CODf Removed</td>
</tr>
<tr>
<td></td>
<td>(28 rpm Mix) CODf, mg/L</td>
</tr>
<tr>
<td></td>
<td>% CODf Removed</td>
</tr>
<tr>
<td>End of Anoxic&lt;sup&gt;(1)&lt;/sup&gt;</td>
<td>(Prior to 28rpm Mix) CODf, mg/L</td>
</tr>
<tr>
<td></td>
<td>(28 rpm Mix) CODf, mg/L</td>
</tr>
<tr>
<td>Effluent CODf&lt;sup&gt;(2)&lt;/sup&gt;</td>
<td>(Prior to 28rpm Mix), mg/L</td>
</tr>
<tr>
<td></td>
<td>Standard Deviation, mg/L</td>
</tr>
<tr>
<td></td>
<td>% CODf removed</td>
</tr>
<tr>
<td>Effluent CODf&lt;sup&gt;(3)&lt;/sup&gt;</td>
<td>(28 rpm Mix), mg/L</td>
</tr>
<tr>
<td></td>
<td>Standard Deviation, mg/L</td>
</tr>
<tr>
<td></td>
<td>% CODf removed</td>
</tr>
<tr>
<td>HRT, hr.</td>
<td>16</td>
</tr>
</tbody>
</table>

<sup>(1)</sup> Data is from 1 tracking study for the SBR.

<sup>(2)</sup> CODf is the average of the last 5 readings taken over a 2 week period.

<sup>(3)</sup> CODf is the average of the last 5 readings taken over a 3 week period before starvation.

<sup>(4)</sup> SBR 3 was operated on several different cycles for 6 weeks. Then operated on cycle shown for 2 weeks prior to tracking study and onset of starvation SBR 3 data for this period was not used when calculating averages. 1 measurement, 16hr HRT.
Table 7 CODf in Anoxic Period (28 rpm Mix)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SBR 1</th>
<th>SBR 2</th>
<th>SBR 3</th>
<th>SBR 4</th>
<th>SBR 5</th>
<th>SBR 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CODf removed During Fill, mg/L</td>
<td>113</td>
<td>95</td>
<td>81</td>
<td>49</td>
<td>47</td>
<td>82</td>
</tr>
<tr>
<td>CODf removed During all Anoxic, mg/L</td>
<td>113</td>
<td>117</td>
<td>88</td>
<td>114</td>
<td>33</td>
<td>108</td>
</tr>
<tr>
<td>COD Removed by Denitrification, mg/L &lt;sup&gt;(A)&lt;/sup&gt;</td>
<td>6</td>
<td>4</td>
<td>7</td>
<td>14&lt;sup&gt;(c)&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CODf Removed During Fill by Other Mechanisms, mg/L &lt;sup&gt;(B)&lt;/sup&gt;</td>
<td>108</td>
<td>92</td>
<td>74</td>
<td>43</td>
<td>47</td>
<td>82</td>
</tr>
<tr>
<td>(A) as a Percent of all CODf Removed During Anoxic Fill</td>
<td>5</td>
<td>4</td>
<td>9</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(B) as a Percent of all CODf Removed During all Anoxic</td>
<td>95</td>
<td>79</td>
<td>84</td>
<td>36</td>
<td>143</td>
<td>76</td>
</tr>
</tbody>
</table>

<sup>(1)</sup> Values from Table 10. All values are for during fill except for SBR 4 which is calculated to have removed 5.4 mg/L during fill.

During the course of tracking studies (Figures 11-16) there was considerable fluctuation within the CODf readings, 47 mg/L for SBR 1 for example. Some of these fluctuations are no doubt due to the accuracy of the test method. However the COD curve was calibrated several times during the course of the study and always achieved an r<sup>2</sup> of 0.99 or better. Additionally CODf measurements during uptake experiments (Section 4.1.1) produced linear data with an r<sup>2</sup> of 0.84 and 0.94, indicative of testing accuracy. It is postulated that the CODf fluctuations are produced by a biologically mediated process, and represent release and recapture of the CODf. This process could involve the production of extracellular products as well as the release of substrate which was captured but not utilized by a particular strain of microorganism. These products, which would show up as CODf could then be all or partly recaptured.
by a different species of microorganism. In any event the phenomenon of varying
CODf is real. Similar but smaller variations were reported by Orhon et al. (1986) for
tracking studies of an SBR.

Within the range studied the HRT did not appear to be a factor which
influenced the CODf removal.

4.1.2.2 PO₄³⁻ - P Release. For all six SBRs, the concentration of PO₄³⁻ - P at the end
of the anoxic cycle (Table 8) is substantially less than the 50 - 60 mg/L levels reported
by others [Manning, 1985; Shin, 1992], and expected in these experiments. As
outlined in section 2.2.2.2 it is necessary to achieve PO₄³⁻ - P release in the anoxic
cycle in order for uptake and removal to be possible. PO₄³⁻ - P release is known to be
related to the uptake of VFAs during the anoxic period of the cycle (Section 2.2), and
it was expected that this would be expressed as a change in the CODf during the
anoxic cycle. The monitoring results are summarized in Table 8 and show no
consistent expression of this relationship.

Others have observed a wide range of PO₄³⁻ - P release yields, as is evident in
Table 9 which lists the Phosphate/acetate ratios from the literature as summarized by
Smolders et al. (1993). In the case of these experiments which used a complex
substrate, the yield relationship was further complicated by the presence of an aerobic
CODf uptake element which was discussed in the previous sections. It was not
possible to quantify what effect if any that this had on PO₄³⁻ - P yield, but it was
probably significant, given that an average of 57% of the CODf was taken up by the
end of fill, and only 7% of this average could be attributed to denitrification as is shown in Table 7. It is suspected that the short chain VFAs, which constitute RBCOD and are most readily taken up by the PAOs, were already sequestered during fill by way of the mechanisms which were put forward in Section 4.1.2.1.

Table 8 $\text{PO}_4^{3-} - \text{P Yield}$

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum $\text{PO}_4^{3-} - \text{P}$ at End of Anoxic Cycle, mg/L</td>
<td>12.3</td>
</tr>
<tr>
<td>$\text{PO}_4^{3-} - \text{P}$ Released During Anoxic Cycle, mg/L</td>
<td>5.4</td>
</tr>
<tr>
<td>CODf Removed During Anoxic, mg/L$^0$</td>
<td>107</td>
</tr>
<tr>
<td>$Y_{\text{PO}_4^{3-},P_r}$, mg $\text{PO}_4^{3-} - \text{P}$ Released/gCODf Removed During Anoxic</td>
<td>48</td>
</tr>
<tr>
<td>CODf Removed After End of Fill (Anoxic), mg/L</td>
<td>0</td>
</tr>
<tr>
<td>$Y_{\text{PO}_4^{3-},P_r}$, mg $\text{PO}_4^{3-} - \text{P}$ Released/gCODf (after fill, anoxic)</td>
<td>$\infty$</td>
</tr>
<tr>
<td>F/M, mg CODf/mg MLVSS.d</td>
<td>0.35</td>
</tr>
<tr>
<td>$Y_{\text{PO}_4^{3-},P}$, mg $\text{PO}_4^{3-} - \text{P}$/gCODf Applied</td>
<td>48.9</td>
</tr>
<tr>
<td>HRT</td>
<td>16</td>
</tr>
</tbody>
</table>

(1) Calculated losses due to denitrification during anoxic period have been deducted.

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The PO$_4^{3-}$ - P concentrations in the ML at the start of the study were in every case higher than the concentration in the effluent at the end of the tracking study. NO$_3^-$ - N concentrations in the ML were reduced from that in the effluent, but only SBRs 5 and 6 were 0. This is somewhat different from results reported by Shin et al. (1992), who only reported PO$_4^{3-}$ - P release after all of the NO$_3^-$ - N was removed. Examination of the tracking studies indicates that all NO$_3^-$ - N was removed by the end of fill in all SBRs except for SBR 4, which took 1 hr for complete denitrification to occur. The relationship between PO$_4^{3-}$ - P yield and overall NO$_3^-$ - N losses is quite strong indicating that the COD used for denitrification was related to the PO$_4^{3-}$ - P energetics. The maximum PO$_4^{3-}$ - P released during the anoxic period varied inversely with the NO$_3^-$ - N denitrified during the anoxic fill period, and can be characterized by the relationship $\text{mg} \text{PO}_4^{3-} \text{ - P released /L} = 9.23 - 2.34 \times \text{(mg} \text{NO}_3^- \text{ - N denitrified)}$. 

Table 9 Phosphate/Acetate Release Ratios from the Literature

<table>
<thead>
<tr>
<th>AUTHOR</th>
<th>YEAR</th>
<th>Release Ratio (P-mol/C-mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wentzel</td>
<td>1986</td>
<td>0.24</td>
</tr>
<tr>
<td>Arun</td>
<td>1988</td>
<td>0.21 - 0.39</td>
</tr>
<tr>
<td>Mino</td>
<td>1987</td>
<td>0.39</td>
</tr>
<tr>
<td>Wentzel</td>
<td>1988</td>
<td>0.52 - 0.57</td>
</tr>
<tr>
<td>Arvin</td>
<td>1985</td>
<td>0.62 - 0.74</td>
</tr>
<tr>
<td>Comeau</td>
<td>1987</td>
<td>0.7 - 0.75</td>
</tr>
<tr>
<td>Smolders, Y$_{pHAC}$ = 0.19pH - 0.85</td>
<td>1993</td>
<td>0.48 @ pH = 7</td>
</tr>
</tbody>
</table>
(r² = 0.92). This is shown in Figure 17, it is indicative of an RBCOD shortage, and can only be considered as specific to the systems used in these experiments. It is clear from the PO₄³⁻ - P release observed for SBRs 5 and 6 in which significant denitrification occurred prior to fill, that endogenous carbon stores, which are thought to have been consumed during denitrification, were not limiting to the PO₄³⁻ - P release.

![Figure 17 PO₄ P Release vs NO₃ N Denitrified](image)

There was no observed relationship between PO₄³⁻ - P yield or concentration and HRT.

4.1.2.3 PO₄³⁻ - P Uptake.

An examination of the tracking study results plotted in Figures 11 to 16 indicates that the PO₄³⁻ - P uptake rate had essentially reached 0 for SBRs 1, 2, 4, and
6 by the end of the aeration cycle. SBR 3 was still exhibiting $\text{PO}_4^{3-} - \text{P}$ uptake after 4.5 hours aeration. This SBR however, had only been operating in a $\text{PO}_4^{3-} - \text{P}$ removal cycle for 2 weeks prior to this study. SBR 5 was receiving aeration for 3 hours, and would have probably benefited from additional aeration time, or a higher rate of aeration. Within the overall cycle this could have been accommodated as this SBR exhibited the highest release rate of any in the study as it did not require all of the anoxic cycle for complete P release.

4.1.2.4 $\text{NH}_4^+ - \text{N}$ and $\text{NO}_3^- - \text{N}$.

The concentration of $\text{NO}_3^- - \text{N}$ in the effluent was expected to vary with HRT, being higher for shorter HRTs as the initial $\text{NH}_4^+ - \text{N}$ concentration would be higher due to the lower dilution by the ML remaining from the previous cycle. This was not the case. The effluent concentration averaged 8.4 mg/L ± 0.7 mg/L. For all SBRs an average of 70% of the $\text{NH}_4^+ - \text{N}$ applied was removed (standard deviation 2%).

In every case, except for SBR 4, $\text{NO}_3^- - \text{N}$ concentrations in the effluent were higher than in the ML at the start of the cycle (Table 10), indicating that the $\text{NO}_3^-$ was acting as an electron acceptor and denitrification was occurring. Concurrently the $\text{NH}_4^+ - \text{N}$ in the ML was higher than in the effluent. This was of special note in SBR 5 where over 3 different tracking studies the $\text{NH}_4^+ - \text{N}$ increased from undetectable (less than 0.1 mg/L) in the effluent to a level of 5 mg/L in the ML before fill. In spite of the COD required for denitrification, CODf was always higher in the ML at the start of the cycle (before fill) than in the effluent. Endogenous processes were no doubt
responsible for this effect, but the actual mechanisms were not determined. As previously mentioned all \( \text{NO}_3^- - N \) was removed by the end of fill in all SBRs except for SBR 4, which took 1 hr for complete denitrification to occur. The effluent \( \text{NH}_4^+ - N \) was uniformly near 0. Table 10 summarizes the \( \text{NO}_3^- - N \) removed during fill by denitrification and the contribution of this process to COD removal during fill.

### Table 10 COD Requirements for Denitrification During Fill

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SBR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>( \text{NO}_3^- - N ) in effluent, mg/L</td>
<td>8.3</td>
</tr>
<tr>
<td>( \text{NO}_3^- - N ) in ML before fill, mg/L</td>
<td>3.6</td>
</tr>
<tr>
<td>COD Required for denitrification, mg/L(^{(1)})</td>
<td>5.8</td>
</tr>
</tbody>
</table>

(1) Calculated at 2.84 mgCOD/mg \( \text{NO}_3^- - N \) denitrified (Section 2.2.1.4)

In order to be assured of the extent of biological \( \text{NH}_4^+ - N \) removal, a study was conducted to determine whether air stripping was a factor. Using the same type of aerator devices and airstones as were used in the SBRs, air was bubbled into a jar test vessel containing 2 L of tapwater with 9.4 mg/L \( \text{NH}_4^+ - N \) at 20 °C and at pH 7. Over a 25 hour study period \( \text{NH}_4^+ - N \) was found to be lost at a rate of 0.068 mg/L·h (12 samples, \( r^2 = 0.87 \)). Over a 4 hour aeration period this rate projects a loss of 0.27 mg/L, and confirms that air stripping was not a significant factor in accounting for any \( \text{NH}_4^+ - N \) losses, as this represents less than 2.6% of the \( \text{NH}_4^+ - N \) in any of the SBRs.
4.1.2.5 Discussion  90% of the CODf in the AWW was removed, and this was unaffected over the range of HRTs studied. However, an unexpected CODf removal profile was observed, whereby 57% of the CODf was captured by the end of fill without a concurrent PO₄³⁻ - P release. This is attributed to the high DO in the AWW (≥ 8 mg/L), and is not expected to be representative of normal residential wastewater. The CODf removed during fill is suspected to be RBCOD which would otherwise have been available for use by the PAOs. The relationship between NO₃⁻ - N denitrified during fill, and PO₄³⁻ - P release indicates that the PO₄³⁻ - P release observed in this study was limited by a shortage of available RBCOD. Denitrification further reduced the RBCOD, and hence caused the reduction in PO₄³⁻ - P release during the anoxic period. Since denitrification occurred in SBRs 5 and 6 prior to fill and they both achieved higher PO₄³⁻ - P release than the other SBRs it can be concluded that shortage of endogenous stores was not a factor in PO₄³⁻ - P release, nor were endogenous stores significantly involved in denitrification during the anoxic cycle.

Nitrification was not dependent on HRT and was easily achieved within the aeration time provided. On average 70% of the applied N in the AWW was removed. During the course of the experiments it was thought that the CODf captured represented CODf which was not easily degraded, and hence was not able to play a role in the PO₄³⁻ - P release. Subsequent analysis has led to the conclusion that other causes discussed above were at least partly responsible. Had there been adequate time during the experiments the question of RBCOD availability could have been resolved.
by testing the AWW for short chain fatty acids, or RBCOD. Additionally, measures to maintain a low DO in the AWW (< 1 mg/L) could have been employed. It may be that the complex AWW used simply contained less RBCOD than normal residential wastewater.

In order to operate at steady-state and produce an effluent with low PO$_4^{3-}$ - P concentration it is necessary to remove sludge or ML from the system after the PO$_4^{3-}$ - P uptake is completed (Section 2.2.2.2), and before any subsequent release commences. The problems encountered in achieving PO$_4^{3-}$ - P release coupled with time constraints prevented this from being accomplished in these studies.

The ability of SBRs 5 and 6 to completely denitrify after decant and prior to fill without significant PO$_4^{3-}$ - P release represents a phenomenon which merits further study due to the favorable effect on PO$_4^{3-}$ - P release. It is not understood why this did not occur to the same extent in the other SBRs.

4.2 Starvation

The SBRs were tested for CODf, NH$_4$ - N, NO$_3^-$ - N, and PO$_4^{3-}$ - P several times during the course of starvation. Each of the parameters characterized is considered in one of the following sections. It should be remembered that SBRs 4 and 5 were not stirred during the sampling process so as not to jeopardize the overall non-mixed experimental status. Therefore the parameters measured in these SBRs may represent non-homogeneous conditions.
4.2.1 CODf During Starvation

The CODf rose significantly within the initial 2 to 3 days of starvation as can be seen in plots of the CODf readings for batteries 1 and 2 (Figures 18 and 19 respectively). In Battery 1 higher levels of CODf were reached and maintained in SBR 2 (aerated and decanted) than in SBR 1 (stirred and decanted). The levels in SBR 3 (stirred, but not decanted) were similar to those in SBR 1, indicating that CODf concentration was a function of DO availability. When factored to account for the fact that the SBRs were decanted, the levels in 1 and 2 were lower than those in 3. The overall upward trend in CODf level with increased starvation time for SBRs 1 and 3 appears to confirm that diffusion supplied DO was the limiting factor.

In Battery 2 the CODf levels for SBR 5 showed an initial spike, then dropped (this may not have been a representative sample due to no mixing), and increased over time, whereas the levels in SBR 4, and 6 dropped slightly with time after the initial increase.
Figure 18 Battery 1 CODf During Starvation

Figure 19 Battery 2 CODf During Starvation
The overall general trend of CODf with duration of starvation which appears in Figures 18 and 19 and is summarized in Figure 20 is that CODf starts at approximately the same level (avg. 24 mg/L) in all SBRs, and except for SBR 4, reaches a maximum level of 70 to 100 mg/L. Note that SBRs 1 and 2 were decanted prior to the onset of starvation, and given the resulting higher biomass concentration at the start, they will therefore possibly have higher CODf concentrations than would be the case if they had not been decanted.

4.2.2 \( \text{NH}_4^+ \) - N and \( \text{NO}_3^- \) - N During Starvation

During the course of starvation nitrate concentrations increased in all SBRs. Concentrations in SBRs 1 and 2 (decanted) were much higher than in the others, with SBR 2 having the highest concentration of all. Data shown in Figures 21 and 22 suggest that the production of nitrate is limited by either the internal stores of available
N and/or pH, and is a function of the availability of oxygen. After observing the rapid nitrate formation and an associated drop in pH in SBRs 1 and 2, additional alkalinity was added to SBR 3 (500 mg/L of both Na$_2$CO$_3$ and NaHCO$_3$) at the onset of starvation. This was excessive and raised the pH to 9.1 which is above the optimal level for nitrification. During the course of starvation and associated nitrification the pH did not drop below 8.5 in SBR 3.

![Figure 21 NO3 - N During Starvation (Battery 1)](image)

The nitrate reading for SBR 6 of 28.2 mg/L at the end of its 84 day starvation period was similar to the 21 mg/L for SBR 3 after 71 days starvation. However the concentrations in SBRs 5 and 6 at 55 days starvation were only 4 mg/L and 4.5 mg/L respectively. Whereas the concentration in SBR 6 increased thereafter, that in SBR 5
was reduced to 0 after 78 days. Concurrent with this NH\textsubscript{4} - N levels were 4 mg/L in SBR 5 and 1.3 mg/L in SBR 6. The reasons for this nitrification/denitrification difference between these SBRs is thought to be due to SBRs 4 and 5 not being mixed. In battery 2 the pH remained near 7.

![Figure 22 NO\textsubscript{3} - N During Starvation (Battery 2)](image)

**Figure 22 NO\textsubscript{3} - N During Starvation (Battery 2)**

4.2.3 PO\textsubscript{4}\textsuperscript{3-} P during starvation

All SBRs exhibited PO\textsubscript{4}\textsuperscript{3-} P release after the onset of starvation. The PO\textsubscript{4}\textsuperscript{3-} P concentration reached a maximum shortly after starvation commenced, and there was virtually no increase thereafter. This is shown clearly for Batteries 1 and 2 in Figures 23 and 24.
Figure 23 PO4 - P During Starvation (Battery 1)

Figure 24 PO4 - P During Starvation (Battery 2)
The \( \text{PO}_4^{3-} \) - P release rate for SBR 2 of 0.8 mg/L.hr was double that of 0.4 mg/L.hr for SBR 1. Both SBRs 1 and 2 had a total P content of approximately 88 mg/L before decant, which converts to after decant total concentrations of 176 mg/L for SBR 1, and 234 mg/L for SBR 2. The maximum \( \text{PO}_4^{3-} \) - P measured in SBR 1 was 17.6 mg/L, or 10% of the total P. The maximum \( \text{PO}_4^{3-} \) - P measured in SBR 2 was 119 mg/L or just over 50% of the total P. The average \( \text{PO}_4^{3-} \) - P for all SBRs except SBR 3 at the end of starvation was 13.3 mg/L.

The progress of the changes in \( \text{PO}_4^{3-} \) - P concentrations for SBRs 1 and 2 in relation to their \( \text{NO}_3^- \) - N concentration is compared in Figure 25. The \( \text{PO}_4^{3-} \) - P concentration in SBR 2 increased at roughly the same rate as that of the \( \text{NO}_3^- \) - N, and reached its maximum concurrent with the \( \text{NO}_3^- \) - N concentration starting to level off. \( \text{NO}_3^- \) - N formation in SBR 1 is subject to a lag of 8 days before measurable concentrations are observed.

It is not known why the concentration of \( \text{PO}_4^{3-} \) - P in SBR 2 was so much higher than in all of the others. It would appear that this difference is related to aeration. One possibility is that a precipitate is forming in all SBRs at a slow rate, and the \( \text{PO}_4^{3-} \) - P release rate in SBR 2 is much greater than this rate. The \( \text{PO}_4^{3-} \) - P concentration simply overtakes the precipitate formation. Later on when the \( \text{PO}_4^{3-} \) - P formation has ceased, the precipitate formation continues and the \( \text{PO}_4^{3-} \) - P concentration is gradually reduced until it equilibrates at the same level as in the other SBRs.
4.2.4 Status at end of Starvation

Prior to restarting the SBRs the characteristics of the ML were recorded before the addition of substrate. These were as shown in Table 11.

**Table 11 Mixed Liquor Prior to Restarting SBRs**

<table>
<thead>
<tr>
<th>SBR #</th>
<th>DAYS STARVED, (T)</th>
<th>CODf mg/L</th>
<th>NH$_4$ - N mg/L</th>
<th>NO$_3$ - N mg/L</th>
<th>PO$_4$ - P mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>58</td>
<td>28</td>
<td>3.8</td>
<td>0.2</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>72</td>
<td>70</td>
<td>&lt; 0.1</td>
<td>21.1</td>
<td>1.2</td>
</tr>
<tr>
<td>5</td>
<td>78</td>
<td>67</td>
<td>4</td>
<td>&lt; 0.1</td>
<td>14.2</td>
</tr>
<tr>
<td>6</td>
<td>84</td>
<td>79</td>
<td>1.3</td>
<td>28.2</td>
<td>15.3</td>
</tr>
<tr>
<td>2</td>
<td>95</td>
<td>103</td>
<td>2.8</td>
<td>163</td>
<td>10.8</td>
</tr>
<tr>
<td>1</td>
<td>117</td>
<td>100</td>
<td>0.2</td>
<td>134</td>
<td>13</td>
</tr>
</tbody>
</table>
4.2.5 Discussion

Endogenous decay occurred during starvation and was dependent on availability of DO as was evident from the progression of CODf and NO₃⁻ - N concentrations with time. NO₃⁻ - N formation was highest in the decanted SBRs (1 and 2), lower in the mixed SBRs (3 and 6), and lowest in the non-mixed SBRs (4 and 5). It is likely that the low pH in SBRs 1 and 2 was inhibitory to further nitrification, and possibly to other endogenous processes. The high pH in SBR3 may have also had an inhibitory effect. PO₄³⁻ - P release appeared to be a function of endogenous processes, however the total quantity of PO₄³⁻ - P released was not determined.

4.3 Starvation Recovery

4.3.1 CODf During Starvation Recovery

When the SBRs were restarted, the rate at which the effluent CODf stabilized varied among the SBRs as shown in Figure 26, and Table 12 which summarizes the key points. The effluent CODf from the decanted SBRs started at a higher level and was reduced at a slower rate than the others (excluding SBR 5, see 4.3.2).

The temperature difference between Batteries 1 and 2 appears to have been the other determining factor in the rate of recovery of CODf removal capability. Starvation at the 5°C higher temperature (Battery 1) resulted in generally slightly higher CODf levels during the recovery period. Although SBR 3 reached its prestarvation CODf level after the second day, this was a high level as this SBR had
been converted from a series of other cycles 2 weeks prior to the onset of starvation and had not stabilized. At the end of starvation it had a CODf of 70 mg/L, which was 6 mg/L below its level at the start of starvation.

From the standpoint of recovery of CODf removal capability SBR 4 performed the best, followed by SBR6, and then SBR5 which experienced aeration problems (Section 4.3.2).

![Figure 26 Effluent CODf](image)

**Table 12 CODf Removal During Starvation Recovery**

<table>
<thead>
<tr>
<th></th>
<th>SBR 1</th>
<th>SBR 2</th>
<th>SBR 3</th>
<th>SBR 4</th>
<th>SBR 5</th>
<th>SBR 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>days to 2.5X prestarvation level</td>
<td>9</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>days to 40 mg/L level</td>
<td>9</td>
<td>10</td>
<td>4</td>
<td>1</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>days to 30 mg/L level</td>
<td>NR</td>
<td>15</td>
<td>NR</td>
<td>1</td>
<td>14</td>
<td>8</td>
</tr>
</tbody>
</table>

NR - not reached.
4.3.2 \( \text{NH}_4^+ - \text{N and NO}_3^- - \text{N} \) During Starvation Recovery

During the period when starvation recovery was monitored neither SBR 1, nor SBR 2 recovered the ability to nitrify (Figure 27). However, during a tracking study done after 14 days of recovery, SBR 1 showed a 2 mg/L reduction in \( \text{NH}_4^+ - \text{N} \) which may have partly been due to growth, and an \( \text{NO}_3^- - \text{N} \) concentration of 0.5 mg/L. SBR 3 showed decreased \( \text{NH}_4^+ - \text{N} \) on the 6th day, and \( \text{NO}_3^- - \text{N} \) was measurable on the 7th day. By the 19th day nitrification was complete for both SBRs 3 and 4. SBRs 4, 5, and 6 (Figure 28) showed ability to nitrify on the first cycle after restart. Unfortunately a blizzard delayed receipt of testing reagents and there is a 12 day gap in the effluent data for SBR 4. After 14 days the effluent nitrate level had reached 2.4 mg/L, and after 17 days was removing virtually all of the \( \text{NH}_4^+ - \text{N} \) (0.2 mg/L). SBR 5 was found to exhibit erratic \( \text{NH}_4^+ - \text{N} \) effluent levels. This was eventually traced to a problem in the timer which activated the aerator. Its battery was low, and although it showed the time properly, it occasionally did not trip the relay to start the aerator as scheduled, resulting in varying \( \text{NH}_4^+ - \text{N} \) concentrations in the effluent. This also probably affected the COD discussed in the previous section. SBR 6 demonstrated immediate \( \text{NH}_4^+ - \text{N} \) removal capability which gradually increased after a 3 day lag, until after the 10th day \( \text{NH}_4^+ - \text{N} \) levels were at 1.8 mg/L, and \( \text{NO}_3^- - \text{N} \) was at 6 mg/L.
Figure 27 Effluent NH4 - N & NO3 - N (Battery 1)

Figure 28 Effluent NH4 - N & NO3 (Battery 2)
4.3.3 PO\textsubscript{4}\textsuperscript{3-} - P during starvation recovery

It was decided to restart SBR 4 first because of its pre-starvation performance wherein it had shown negligible PO\textsubscript{4}\textsuperscript{3-} - P release or uptake, but had shown good organics removal as well as nitrification-denitrification (Figure 14). During starvation recovery PAO activity in this SBR became evident during a tracking study of the 7th cycle (2 days). This is discussed more fully in Section 4.3.4.

![Graph showing effluent PO\textsubscript{4} - P during starvation recovery](image)

**Figure 29 Effluent PO\textsubscript{4} - P During Starvation Recovery**

None of the SBRs in Battery 1 exhibited effluent PO\textsubscript{4}\textsuperscript{3-} - P reduction to low levels during the period monitored (Figure 29). However, SBR 1 did show a differential of 1.8 mg/L PO\textsubscript{4}\textsuperscript{3-} - P between the end of anoxic mix and the end of
aeration in a tracking study after 14 days recovery. SBR 2 showed a reduction in effluent $\text{PO}_4^{3-} - \text{P}$ commencing on the 15th day, and a tracking study on the 20th day revealed a differential of 6.8 mg/L $\text{PO}_4^{3-} - \text{P}$ between the end of anoxic mix and the end of aeration. SBR 3 showed no evidence of $\text{PO}_4^{3-} - \text{P}$ release or uptake in effluent or in tracking studies.

All of the SBRs in Battery 2 exhibited reduced $\text{PO}_4^{3-} - \text{P}$ concentrations in their effluent (Figure 29) during the course of starvation recovery. After 2 days SBR 4 showed a $\text{PO}_4^{3-} - \text{P}$ differential of 2.5 mg/L between the end of anoxic mix and the end of aeration in a tracking study, and after 14 days effluent $\text{PO}_4^{3-} - \text{P}$ was reduced to 0.4 mg/L. SBR 5 showed reduced levels of $\text{PO}_4^{3-} - \text{P}$ in the effluent after 2 days and reached a level of 1.9 mg/L after 5 days, after which the level commenced to rise. SBR 6 showed reduced effluent $\text{PO}_4^{3-} - \text{P}$ after 1 day of operation, thereafter levels dropped progressively until the end of the study (10 days) when a level of 0.68 mg/L $\text{PO}_4^{3-} - \text{P}$ was reached.

4.3.4 Nitrate Attenuation of $\text{PO}_4^{3-} - \text{P}$ Removal Capability

During the starvation recovery studies it was observed that $\text{PO}_4^{3-} - \text{P}$ release and uptake was achieved and then gradually lost, particularly in SBRs 4 and 5. Whereas the problems with the aerator control in SBR 5 make it difficult to be certain as to the cause of the variations, there was no such problem with SBR 4, and the loss of $\text{PO}_4^{3-} - \text{P}$ removal capability was observed in a series of tracking studies (Figure 30). These studies clearly show the recovery of the capability to release and subsequently
take up $\text{PO}_4^{3-}$ - P, and thereafter the gradual decline in this capability.

![Graph showing concentration over time for SBR 4, PO4 - P During Starvation Recovery]

**Figure 30** SBR 4, PO4 - P During Starvation Recovery

The progression of $\text{NO}_3^-$ - N and $\text{PO}_4^{3-}$ - P concentrations for days 14, 18, and 36 is shown in Figure 31.

As with the prestarvation studies it is evident that as the $\text{NO}_3^-$ - N concentration to be denitrified increased, the $\text{PO}_4^{3-}$ - P released decreased. In addition the length of time taken for denitrification increased as the $\text{NO}_3^-$ - N concentration increased. This served as a further demonstration that the systems studied were short of RBCOD available for PAO use.
4.3.5 Discussion

There were two main factors which affected the ability of the SBRs to recover organic and nutrient removal capabilities after starvation.

(1) Endogenous substrate utilization, which was dependent on the availability of DO, was detrimental to recovery of CODf removal capability, nitrification, and $\text{PO}_4^{3-}$ - P release and uptake capability. This was demonstrated in SBR 2 which was decanted and aerated, and to a lesser extent in SBR 1 which was left in the decanted state, and would have therefore had a higher DO concentration than SBR 3 which was in the same battery, but was not decanted and recovered quicker. SBR 3 responded less
quickly in developing COD\textsuperscript{f} removal capability, but very much the same for NH\textsubscript{4} - N removal as did SBR 4 which was 5°C cooler, but otherwise on the same cycle.

(2) SBR 6 had a longer anoxic period prior to starvation than did SBR 4, and this appears to have selected for nitrifiers which better survived starvation. This longer anoxic cycle also appeared to enhance the ability to recover PO\textsubscript{4}\textsuperscript{3-} - P release and uptake as SBR 6 recovered removal capability quicker than any other SBR.

The attenuation of PO\textsubscript{4}\textsuperscript{3-} - P release and uptake observed prior to the onset of starvation (Section 4.1.2.2) was observed to develop in SBR 4 during recovery from starvation. The study clearly showed that as nitrification development proceeded (it commenced not long prior to the study on day 14), there was a concurrent loss of PO\textsubscript{4}\textsuperscript{3-} - P release and uptake capability, and an increase in PO\textsubscript{4}\textsuperscript{3-} - P in the effluent.
CHAPTER 5
SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary

HRT did not have a measurable effect on effluent quality over the range studied (10.7 - 32 hr). All of the SBRs were capable of removing at least 90% of applied CODf and producing an effluent with a CODf of 18.4 mg/L. 70% of the applied NH₄-N was removed and the remainder was completely nitrified in the effluent which had an average NO₃⁻N concentration of 8.4 mg/L. The HRT did not influence the NO₃⁻N concentration in the effluent.

There was a shortage of RBCOD in the anoxic stage. This is believed to have been partly a consequence of high DO in the AWW, which led to 57% of the CODf being captured during fill without a concurrent PO₄³⁻-P release. Due to the RBCOD shortage, the presence of nitrate in the ML before fill was found to have a severe negative impact on the ability to release and take up phosphate. PO₄³⁻-P released was reduced by 2.34 mg/mgNO₃⁻-N denitrified during anoxic fill. There was no relationship between PO₄³⁻-P released and HRT.

During starvation endogenous processes were dependent on the availability of oxygen. Consequently those SBRs which were not decanted recovered more quickly than those which were decanted. The non-decanted SBRs were all capable of reducing effluent CODf to below 40 mg/L within 4 days. The recovery of nitrification capability
took considerably longer. SBRs with 3.5 hr anoxic cycles appeared to recover complete nitrification capability more quickly (10 d) than did those with 2 hr cycles (17d-19d). The same effect was observed for recovery of PO$_4^{3-}$ - P release and uptake capability which took 10 days to develop in SBR 6 (3.5 h) vs 14 days in SBR 4 (2h).

5.2 Conclusions

An SBR operated to treat AWW in a manner which will result in the removal of organics, and nutrients is capable of recovering its process capability after periods of starvation up to at least 84 days at 22°C.

The survival capability of the SBR treatment process depends to a large extent on the condition which the SBR is left in at the onset of starvation and to a lesser extent on the operating sequence of the SBR prior to starvation. Quiescent conditions during starvation allowed for the most rapid recovery of treatment capability, and a longer anoxic stage in the operating cycle was observed to enhance this effect.

5.3 Recommendations for Further Study

Research to resolve the following issues needs to be completed in order to enhance the applicability of this technology.

(1) Determine the effect of temperature on starvation recovery over the ranges encountered in typical field applications.

(2) The effects of longer HRTs employing longer anoxic and aeration stages during restart need to be known in order to reduce the time taken for full process
recovery. This could assist in the optimization of an operating strategy which would result in the release of a high quality effluent after restart.
REFERENCES


APPENDIX A

EXPERIMENTAL DATA
### Table 1: SOLIDS DATA AT TIME OF TRACKING STUDY SBR 1

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