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NITRATE UTILIZATION AS THE FINAL ELECTRON ACCEPTOR IN A BIOLOGICAL PHOSPHORUS REMOVAL SYSTEM

by

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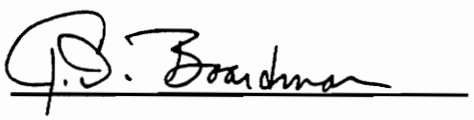
APPROVED:

C.W. Randall, Chairman



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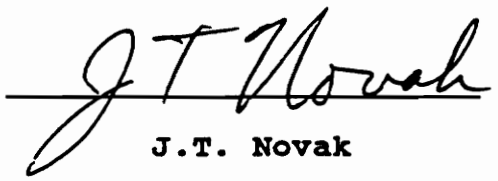

J.T. Novak

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(ABSTRACT)

The study of nitrate utilization as the final electron acceptor in biological phosphorus removal systems was investigated. The objectives of the study were (1) to determine whether polyphosphate (polyP) microorganisms can use nitrate as the final electron acceptor, and (2) to evaluate and compare polyP accumulation in the biomass of the system using nitrate as the terminal electron acceptor to the system using oxygen as the terminal electron acceptor. Two lab-scale biological phosphorus removal systems were operated as the A/O Process under the same conditions except for the terminal electron acceptor involved. The first system, System I, was operated as an Anaerobic/Anoxic process and the other, System II, was operated as an Anaerobic/Anoxic process. Both systems were operated at a 5-day sludge age and the same nominal

hydraulic retention time of 9.1 hours (2.9 hours anaerobic, 6.2 hours anoxic or aerobic). The sludge recycle flow rate was equal to the influent flow rate. The two systems were fed with the same domestic wastewater spiked with sodium acetate and potassium phosphate to give the wastewater a COD concentration of 300-400 mg/L and a phosphorus concentration of 13-14 mg/L as P. Nitrate was fed to the second reactor of System I, while the second reactor of System II was aerated.

The results showed that polyP microorganisms can use nitrate as the final electron acceptor. In this research, the Anaerobic/Anoxic system removed more phosphorus (74 mg P/day) from solution than the Anaerobic/Aerobic system (64 mg P/day). The phosphorus content of the sludge in the Anaerobic/Anoxic system was greater than that of the Anaerobic/Aerobic system, i.e. 6.5% as compared to 5.6%.

The above evidence strongly confirms that polyP microorganisms can use nitrate as the final electron acceptor and that excess biological phosphorus uptake occurs under anoxic condition. The implication is that COD stored in the anaerobic reactor can be used to simultaneously remove nitrogen and phosphorus, which can substantially reduce the amount of COD required for combined nutrient removal.

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CHAPTER ONE: INTRODUCTION

Phosphorus is often removed from wastewater to avoid or reduce eutrophication in receiving waters. Historically, many treatment plants have been designed or developed to remove phosphorus by chemical precipitation. Recently, the enhanced biological phosphorus removal process has been developed, and it has been the subject of much research. The new process of phosphorus removal provides an alternative to conventional chemical precipitation and offers the advantage of not requiring chemical addition, which reduces the volumes of sludge produced.

A simplified process for biological phosphorus removal systems includes 2 stages: anaerobic and aerobic, in that order. This system has become known as the anaerobic-oxic (A/O) process in this country. In the first stage, phosphorus is released from the biomass and soluble organic matter is simultaneously removed under anaerobic conditions. Anaerobic conditions are defined as an environment where oxygen and nitrate are absent, which means the oxidation-reduction potential is low. In the subsequent aerobic stage, phosphorus uptake occurs. The completion of this aerobic step leads to the phenomenon of "luxury" uptake of phosphorus by a large variety of microorganisms, referred to

collectively as poly-phosphate microorganisms. The results of this step are that there is a higher concentration of phosphorus in the biomass and a lower concentration in the liquid medium than there would be in the conventional activated sludge process. Phosphorus-rich sludge is wasted from this aerobic reactor to accomplish phosphorus removal.

Many Biological Phosphorus Removal (BPR) processes are also designed to remove nitrogen as well. The University of Cape Town (UCT) process is one example. It is designed to achieve nitrification in the aerobic zone and denitrification by the addition of an anoxic zone. An anoxic zone is one where nitrate is present as an electron acceptor for heterotrophic metabolism, but oxygen is not. Nitrogen removal is accomplished when nitrate is reduced to nitrogen gas during organic matter stabilization.

The conditions in the three zones of a biological nutrient removal process need to be thoroughly defined and understood. The **anoxic** stage is the stage in which oxygen is absent but nitrate is present and serves as the final electron acceptor in the electron transport system during denitrification. The **anaerobic** stage is the stage where oxygen and nitrate are both absent. The **aerobic** stage is the stage where dissolved oxygen is present. These three terms will be used throughout this entire thesis.

The anaerobic zone, where phosphate release takes place, is a prerequisite condition to subsequent aerobic phosphate uptake. Nicholls and Osborn (1979) proposed that under anaerobic conditions, polyphosphate serves as an energy source for storing simple carbon compounds in the form of poly-ß-hydroxybutyrate (**PHB**). The simple carbon compounds, or readily biodegradable COD, are short-chain fatty acids. Polyphosphates (polyP) serve as the energy supply for the partitioning of readily biodegradable COD into PHB by conversion of polyP to orthophosphate (PO_4^-) via ATP and ADP.

In the aerobic zone the stored PHB is utilized for the dissimilation and assimilation functions of the organisms, i.e., the PHB accumulated during the anaerobic condition serves as the energy source for growth of the polyP microorganisms in the aerobic zone, and also for accumulation of phosphates in the polyphosphates form.

It is obvious that orthophosphate uptake can also occur during anoxic conditions (Gerber et al., 1987; Hascoet et al., 1985; Koch and Oldham, 1984; and Osborn and Nicholls, 1978). Such anoxic uptake of orthophosphate may be associated with polyP microorganisms, considering the large number of denitrifying Acinetobacter strains as reported by Lötter (1985). However, no known literature has demonstrated that a biological phosphorus removal system can

be operated using only nitrate as the final electron acceptor (anoxic condition). In other words, there is no proof that polyP microorganisms can use nitrate as the only final electron acceptor during continuous flow-through treatment.

For this research, two lab-scale biological phosphorus removal systems were operated according to the A/O Process under the same conditions except for the terminal electron acceptors involved. One was operated with oxygen available in the final (aerobic) zone whereas the other was operated with only nitrate available in the final zone. Therefore, the objectives of this study were to:

(1) Determine whether polyP microorganisms can use nitrate as the final electron acceptor,

(2) Evaluate and compare polyP accumulation in the biomass of the system using nitrate as the terminal electron acceptor to the system using oxygen as the terminal electron acceptor.

The additional objectives of the investigation were to gain further knowledge of the effects of nitrate respiration on a biological phosphorus removal process, and to relate the results to BPR process design.

CHAPTER TWO: LITERATURE REVIEW

The excess biological phosphorus removal phenomenon has received a great deal of attention for the past two decades. Extensive research has been conducted in an attempt to understand the phenomenon in order to enable the design of full-scale wastewater treatment plants and take advantage of the benefits. This is because phosphorus removal from municipal and industrial wastewaters has been recognized as essential treatment where the receiving water bodies are in danger of becoming eutrophic due to excess phosphorus. As opposed to biological phosphorus removal, chemical precipitation techniques are currently in use in various parts of the world, but the biological process offers more cost-effectiveness than chemical precipitation because chemical addition is costly and generates higher volumes of sludge, which adds cost to the sludge disposal process. Despite extensive research in this field and the application of biological phosphorus removal in a number of full-scale wastewater treatment plants, some mechanisms related to the phenomenon of luxury uptake are still not clearly understood. This includes the possibility of anoxic uptake of phosphorus using nitrate as the final electron acceptor. In order to understand the mechanisms of excess biological

phosphorus removal, a thorough review of the literature will be presented in this chapter.

2.1 CONCEPTS OF EXCESS BIOLOGICAL PHOSPHORUS REMOVAL

McCarty (1970) proposed the molecular formula, $C_{60}H_{87}O_{23}N_{12}P$, to describe the biochemical composition of the biomass from conventional activated sludge. Using this formula, it may be seen that phosphorus represents 31/1374 or 0.023 (2.3%) of the cell mass. Although phosphorus content in the biomass constitutes only a small fraction of cellular biomass, it is important with respect to cellular metabolism. Therefore, under normal aerobic growth in the conventional activated sludge treatment, phosphorus content will account for 2-3% of the dry cellular biomass.

Contrary to the "normal" phosphorus removals which have been observed in wastewater treatment plants, it has been repeatedly proven that excess phosphorus removal (well over that required for the synthesis of cellular material) can be accomplished without the addition of chemicals. It is the illustration of this mechanism of enhanced biological phosphorus removal that has been the objective of numerous investigations during the past twenty years.

2.2 HISTORICAL BACKGROUND : OBSERVATIONS SUPPORTING BIOLOGICAL PHOSPHORUS REMOVAL

Srinath et al. (1959) apparently were the first who reported on excess biological phosphorus removal. They conducted experiments by combining raw wastewater with mixed liquor from a treatment plant. After a period of aeration the concentration of water-soluble phosphorus dropped from an initial concentration of 7.5 mg P/L to 0.8 mg P/L in two hours of aeration.

Alarcon (1961) quoted by Levin and Shapiro (1965) also did similar experiments by aerating mixed liquor samples from activated sludge plants. He observed drop of filtrate (soluble) phosphate from about 5.4 mg P/L to near zero in six hours, almost 100% removal.

Levin and Shapiro (1965) after reviewing the work of Srinath et al. (1959) and Alarcon (1961) conducted the experiments at the Washington, D.C. Sewage Treatment Plant Research Laboratory, using influent sewage to the plant. The treatment plant was a "high-rate" aeration plant, the following observations were reported:

a) "Luxury" uptake of dissolved orthophosphate by activated sludge organisms, that is, uptake without growth, was demonstrated.

b) Soluble orthophosphate uptake occurred during aeration, orthophosphate release occurred under unaerated conditions.

c) The addition of succinate and glucose to mixed liquor enhanced dissolved orthophosphate uptake more than without the addition.

d) The rate of orthophosphate uptake was maximum under a certain aeration rate but insufficient aeration led to less uptake.

e) The addition of an uncoupler of oxidative phosphorylation, 2,4-dinitrophenol, inhibited orthophosphate uptake. Since only oxidative phosphorylation is affected by this uncoupler, not the substrate level phosphorylation, the results showed that oxidative phosphorylation played an important role in phosphorus uptake.

f) The optimum pH range for orthophosphate uptake was 7-8. In fact, at pH 9 uptake was lower than at the 7-8 range.

g) Pure oxygen application to the mixed liquor evoked markedly better orthophosphate uptake than did the application of equal quantities of oxygen-in-air.

Based on the results and conclusions, they suggested that excess phosphorus may be stored in volutin granules contained in certain microorganisms.

Subsequent studies by Shapiro (1967) and Shapiro et al. (1967) on factors affecting phosphorus release can be summarized as follows:

a) There were indications that the release of phosphate is stimulated by low dissolved oxygen or low redox (oxidation-reduction) potential (ORP) or both. The phenomenon was reversible.

b) Temperature affects orthophosphate release. There was more orthophosphate release at 30°C than at 25°, 20° and 10°C, respectively.

c) The nature of phosphate release is not due to decomposition of the sludge or to lysis of the microorganisms, since phosphate release was not accompanied by an increase in soluble biochemical oxygen demand (BOD) or by a significant rise in dissolved TKN, and the release and uptake of phosphate were reversible.

As a result of the work done by Shapiro and co-workers, many investigations have been performed in the area of biological phosphorus removal. This led to intensive studies of the possible applications of this phenomenon for the removal of phosphate in activated sludge plants and also of the mechanisms involved in the phenomenon. The studies in the sixties and early seventies were reported by Vacker et al. (1967), Connell and Vacker (1971), Milbury et al.

(1971), and Yall et al. (1970). Similarly, all the plants that successfully removed phosphorus were high rate, non-nitrifying, plug flow activated sludge plants with sludge recycle ratios ranged from 0.25:1 to 0.50:1 (Siebritz et al., 1983). Barnard (1985) pointed out that the low sludge ages served to suppress nitrification.

Although the reversibility of release and uptake had been reported (Shapiro, 1967), a well-defined release and uptake phenomenon was not proposed until the research done by Barnard (1974) and Fuhs and Chen (1975). Fuhs and Chen (1975) observed on the nature of release and uptake of phosphate in a laboratory unit operated on anaerobic/aerobic cycles. Fuhs and Chen (1975) also isolated an organism belonging to the genus Acinetobacter which plays an important role in phosphorus release and uptake phenomenon.

Fuhs and Chen (1975), Nicholls and Osborn (1979), Marais et al. (1983), and Comeau et al. (1985) indicated that the P release/uptake pheromonon is mediated by a group of microorganisms which can store phosphate in the form of polyphosphate (polyP) in quantities larger than the amount of phosphorus associated with cellular biosynthesis. This group of microorganisms is called 'polyP microorganisms', which Fuhs and Chen (1975) identified that they belong to a genus Acinetobacter.

PolyP microorganisms in anaerobic conditions release phosphorus from the stored polyP chains into the liquid solution of anaerobic reactors. Simultaneously, readily availaable organics (short chain fatty acid, e.g. acetate, propionate, butyrate, etc.) are transported from the liquid solution into the cells. Once in the cells, organics are converted into a storage product which is believed to be PHB (poly-ß-hydroxybutyrate). Comeau et al. (1985) have postulated biochemical models to discribe the mechanism of excess biological P removal.

2.3 TYPE OF BIOLOGICAL PHOSPHORUS REMOVAL (BPR) SYSTEMS

A number of different types of treatment plant processes have been developed to achieve biological phosphorus removal. Considerable literature has reviewed different schemes of BPR and BNR (Biological Nutrient Removal) Processes. This includes Arvin (1985), Barnard (1985), Brannan (1986), and Siebritz (1983). Some of the major schemes are summarized here.

2.3.1 Phoredox Process

Barnard (1975a) proposed the Phoredox Process based on experience with the Bardenpho Process with sufficiently large anoxic basins. He, after observing phosphorus removal in the Bardenpho Process, came up with the idea of the Phoredox Process. The Phoredox Process concept may be used with or without nitrification. In its simplest form, the Phoredox consists of two basins, the first being anaerobic, the second an aeration basin, followed by a clarifier (Barnard 1983). When combined with nitrification, some denitrification reactors (anoxic) must be added. This could be achieved by the process shown in Figure 2.1. More recently, the Phoredox system for nitrogen and phosphorus removal is termed "Modified Bardenpho Process." In addition, it is worth mentioning that the "Modified Phoredox" refers to a Phoredox system in which the secondary anoxic reactor and the reaeration reactor have been eliminated. The Modified Phoredox Process can also be called the 3-Stage Phoredox and the Phoredox is referred to as the 5-Stage Phoredox. According to Marais et al. (1983) the maximum limit of TKN/COD ratio is 0.08 mg N/mg COD before the nitrate concentration in the effluent will recycle back via the sludge recycle to the anaerobic reactor.

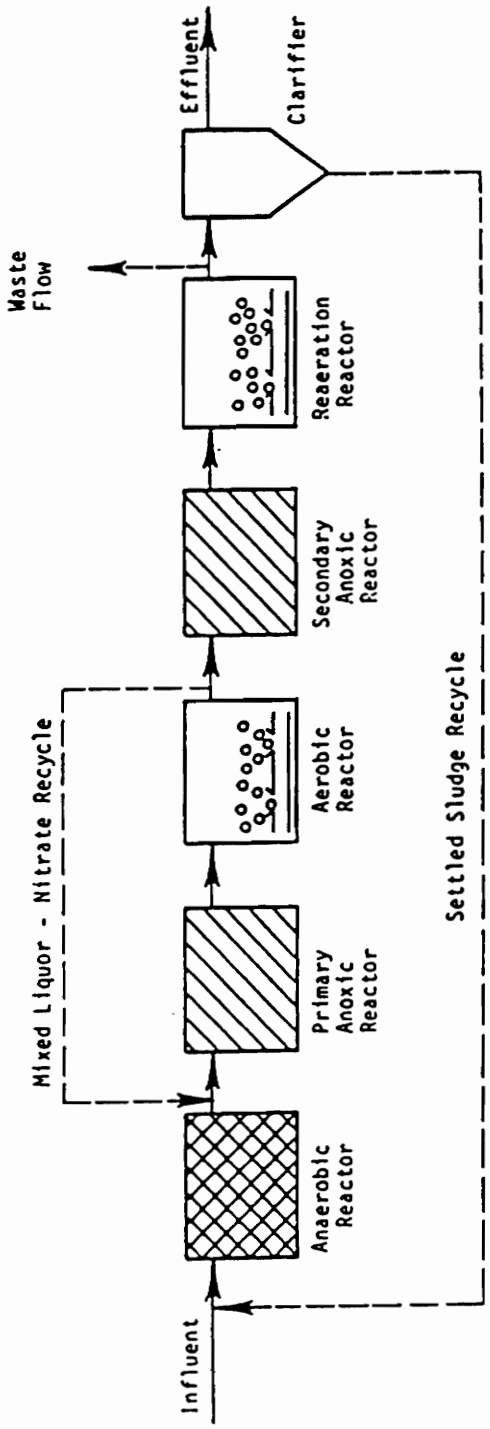


Figure 2.1 Schematic diagram of the Phoredox process for biological nitrogen and phosphorus removal.

2.3.2 UCT Process

Researchers at the University of Cape Town (UCT) (Siebritz et al., 1983 and Ekama et al. (1983) developed the UCT process, shown in Figure 2.2. The concept of the process is to help prevent nitrate from entering the anaerobic reactor through sludge recycle. In the UCT process, the settling tank underflow recycle (sludge recycle) as well as the mixed liquor-nitrate recycle (recycle a) are discharged back to the anoxic reactor and an additional mixed liquor (r) recycle from the anoxic reactor to the anaerobic reactor is introduced. The nitrate recycle to the anoxic reactor can be controlled by appropriately adjusting the mixed liquor-nitrate recycle such that the nitrate concentration in the outflow of the anoxic reactor remains approximately zero. In consequence, the mixed liquor r-recycle from the anoxic to the anaerobic reactor will contain very little or no nitrate and the anaerobic conditions in the anaerobic reactor will be optimal. Siebritz et al. (1983) proposed, based on the experimental data, that a maximum TKN/COD ratio of 0.14 mg N/mg COD (at 14^{o}C and 25 days sludge age) can be treated by the process without significantly affecting phosphorus removal.

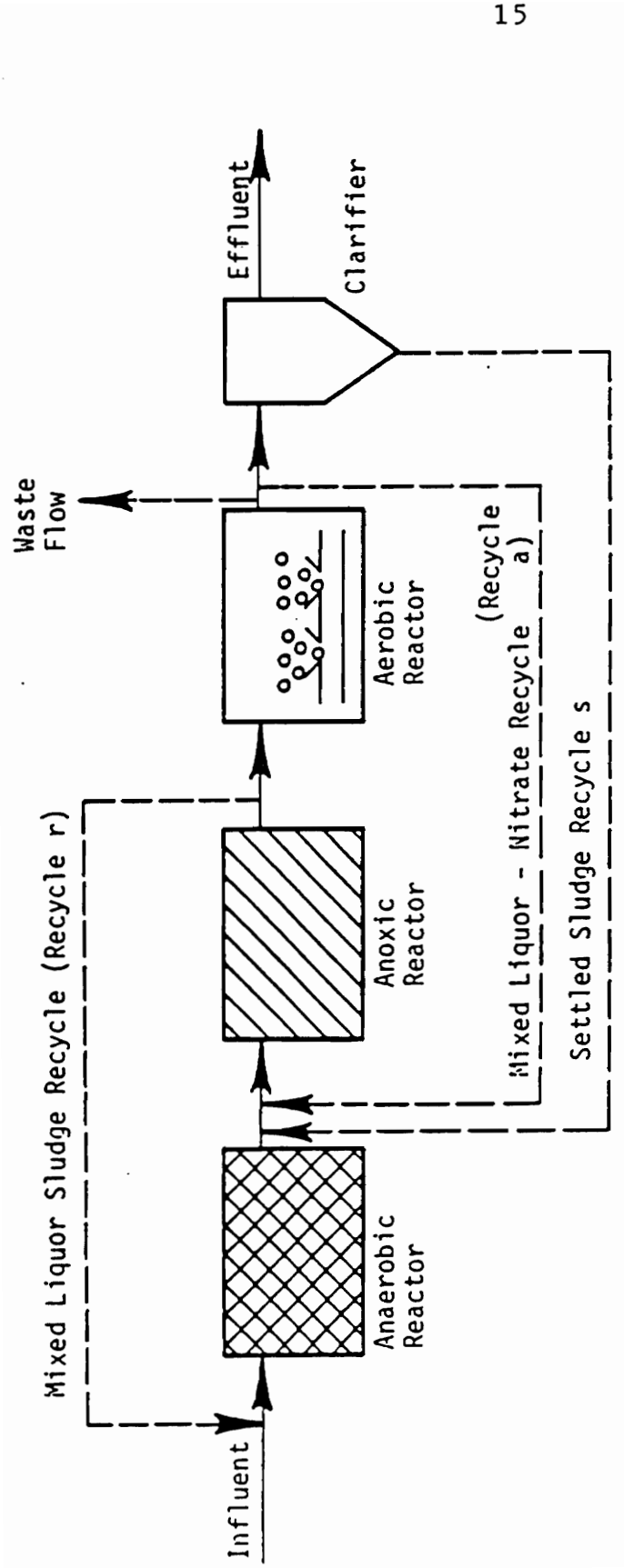


Figure 2.2 Schematic diagram of the UCT process for biological nitrogen and phosphorus removal. After Siebritz et al. (1983).

2.3.3 Modified UCT Process

The group of UCT researchers (Siebritz et al., 1983 and Ekama et al., 1983) found that, in the UCT Process, as TKN/COD ratio increases, the a-recycle ratio needs to be reduced. A reduction in the a-recycle causes an increase in the actual anoxic retention time. It was also found that for high influent COD concentrations (> 500 mg COD/L) and TKN/COD ratio greater than 0.11 mg N/mg COD, the actual anoxic retention time was greater than 1 hour. They claimed that as the actual anoxic retention time increased above 1 hour, the settleability of the mixed liquor sharply declined. Therefore, they developed the Modified UCT Process, as shown in Figure 2.3

The Modified UCT Process has two separate anoxic reactors. With these two separate anoxic reactors, a-recycle can be maintained at a higher rate so the actual anoxic retention time is less than 1 hour, while ensuring a nitrate free discharge to the anaerobic reactor. The first anoxic reactor receives the underflow s-recycle and the r-recycle to the anaerobic reactor is taken from it. The second anoxic reactor receives the mixed liquor-nitrate recycle (a-recycle) from the aerobic reactor. The Modified UCT Process can be operated successfully for phosphorus removal with influent TKN/COD ratios up to 0.11 mg N/mg COD.

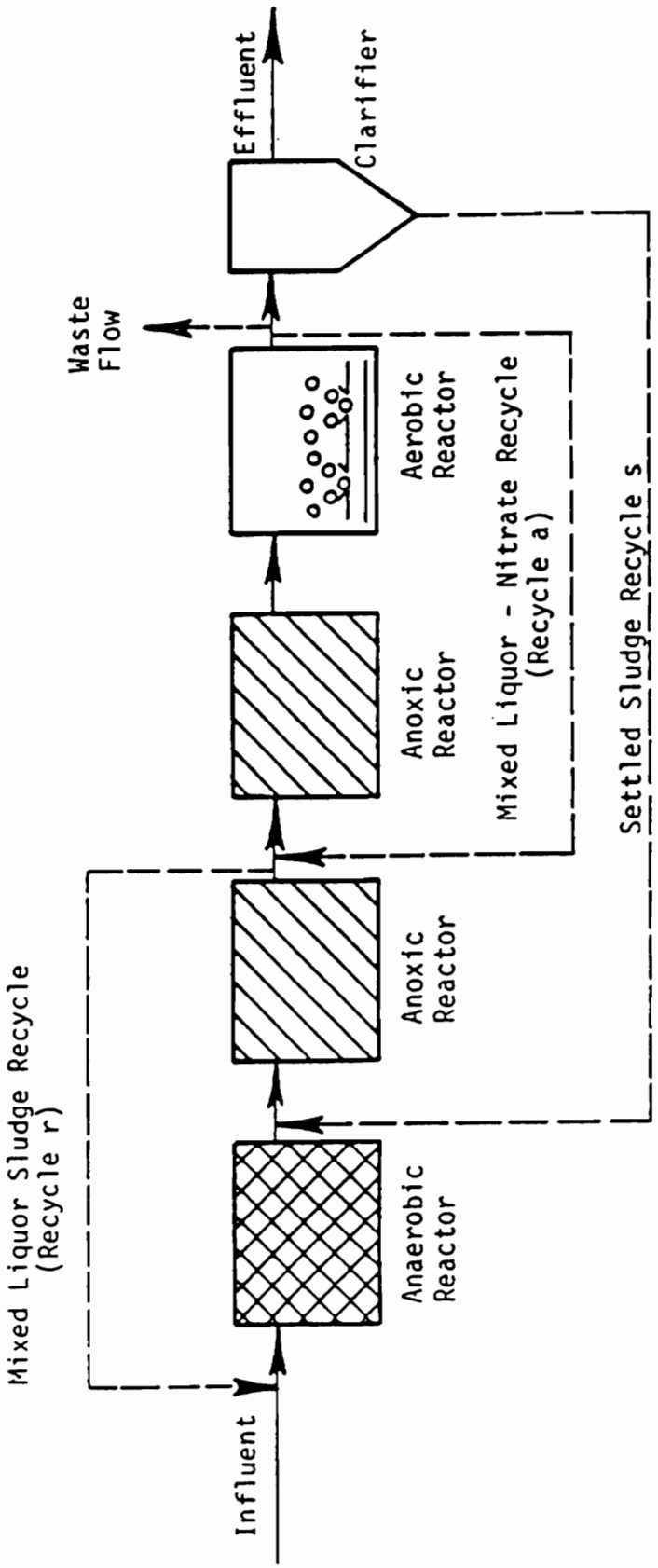


Figure 2.3 Schematic diagram of the modified UCT process for biological nitrogen and phosphorus removal. After Siebritz et al. (1983).

2.3.4 A/O Process

The A/O process has been patented by Air Products and Chemicals, Inc., Allentown, Pennsylvania, USA. It was designed to achieve biological phosphorus removal through anaerobic-aerobic (oxic) sequencing in a continuous flow, single-sludge activated sludge system. If operated at a low sludge age, nitrification is suppressed, therefore, no nitrate will be recycle back to the anaerobic reactor. The A/O process is shown in Figure 2.4. However, the same type of process was proposed by Barnard (1975a, 1976, 1982, 1983) as he referred to the simplest form of Phoredox process which consists of two basins, the first being anaerobic, the second an aerobic basin, followed by a clarifier with sludge recycle back to the anaerobic basin, which is the same as the A/O process. The A/O process when operating at a high sludge age will promote nitrification and nitrates are formed. In this case, denitrification is required by the modification as shown in Figure 2.5 (Hong et al., 1984), which is the same as a 3-Stage Phoredox process. This process is called the Modified A/O or A^2/O process. The typical A/O designs and operating parameters are shown in Table 2.1 (Hong et al., 1984).

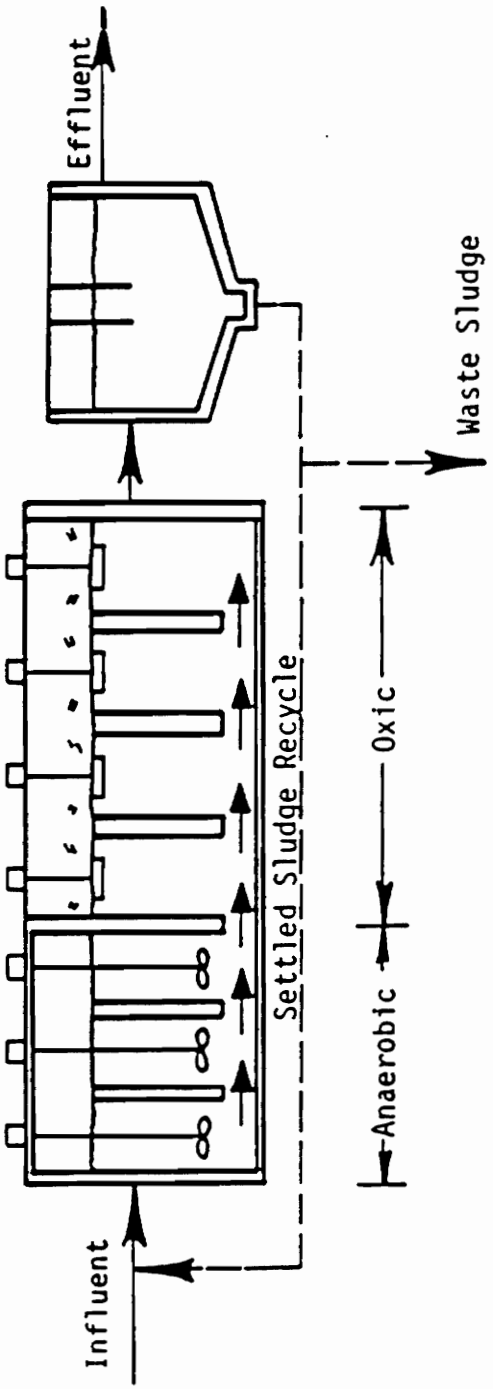


Figure 2.4 Schematic diagram of the A/O process. After Hong et al. (1984).

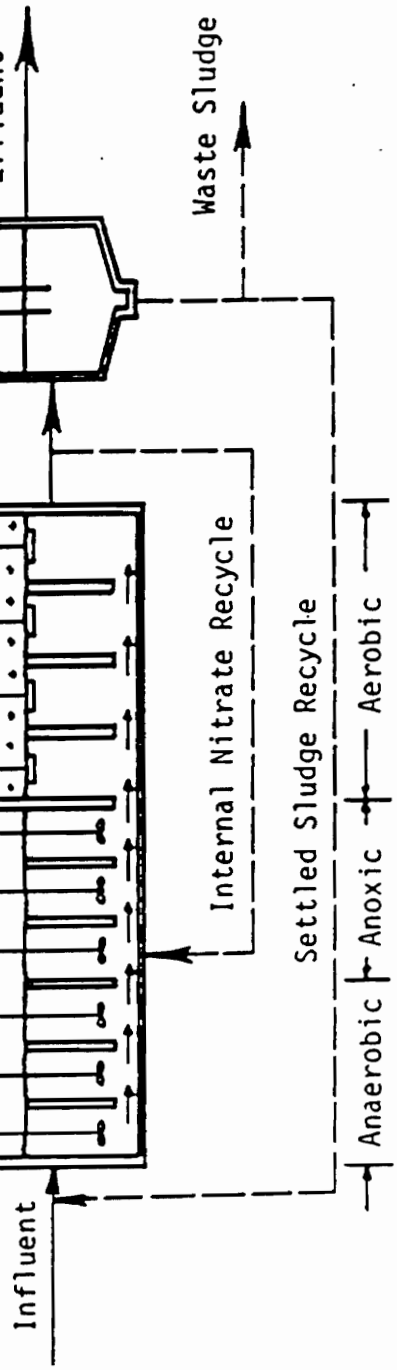


Figure 2.5 Schematic diagram of the A/O system for BOD and phosphorus removal with nitrification and denitrification. After Hong et al. (1984).

Table 2.1 Typical A/O Designs and Operating Parameters (Hong et al., 1984).

	Detention Time, hr
Anaerobic	0.5 - 1.0
Anoxic	0.5 - 1.0
Non-nitrifying	1.8 - 2.5
Nitrifying	3.5 - 6.0
F:M	0.15 - 0.7
F_s:M	0.08 - 0.4
MLSS, mg/L	2000 - 4000

Basin Configuration

	No. of Stages
Anaerobic/Anoxic/Aerobic	3/3/4
Anaerobic/Aerobic	3/4

2.4 EFFECTS OF NITRATE ON BIOLOGICAL PHOSPHORUS REMOVAL

2.4.1 Effects of Nitrate on Phosphorus Release

Barnard (1975a, 1976) proposed that the Phoredox system could be applied to any kind of wastewater, provided that nitrates could be maintained at a sufficiently low level for an anaerobic stage to be formed in the reactor. He further pointed out that an important condition for phosphorus removal was to eliminate nitrates in the effluent which could be done by denitrification or by suppressing nitrification in the plant. Barnard (1982) suggested that for good phosphorus removal, there should be enough substrate for the reduction of nitrates formed during nitrification. He collected data from 11 wastewater treatment plants in South Africa operated as the Phoredox process and found that the influent wastewater contained COD:TKN ratios equal to or greater than 10:1, which is favorable for the removal of phosphorus.

The effects of nitrates on the biological phosphorus removal process are controversial. Koch and Oldham (1985) studied the relationship between ORP (Oxidation-Reduction Potential), nitrate, and orthophosphate. They found that orthophosphate release did not occur until the nitrate concentration had dropped to

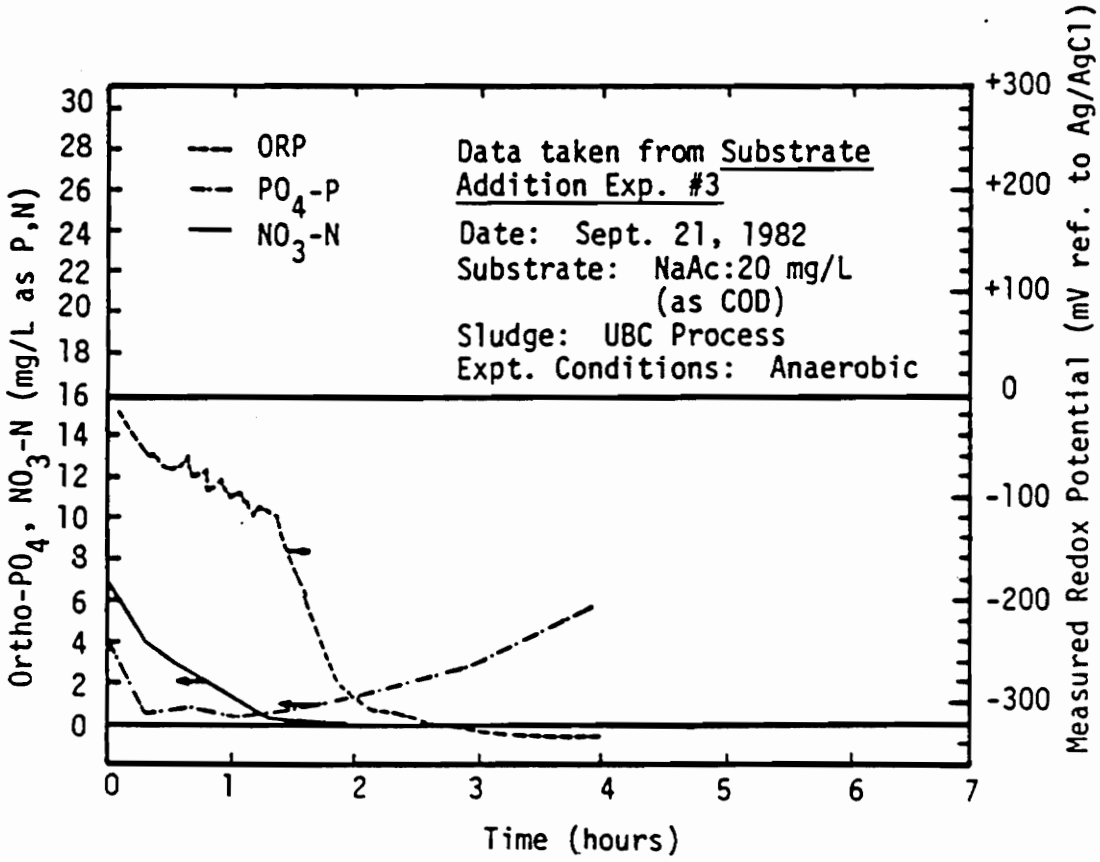


Figure 2.6 Soluble orthophosphate, nitrate, and ORP measured during a batch test under anaerobic conditions. After Koch and Oldham (1985).

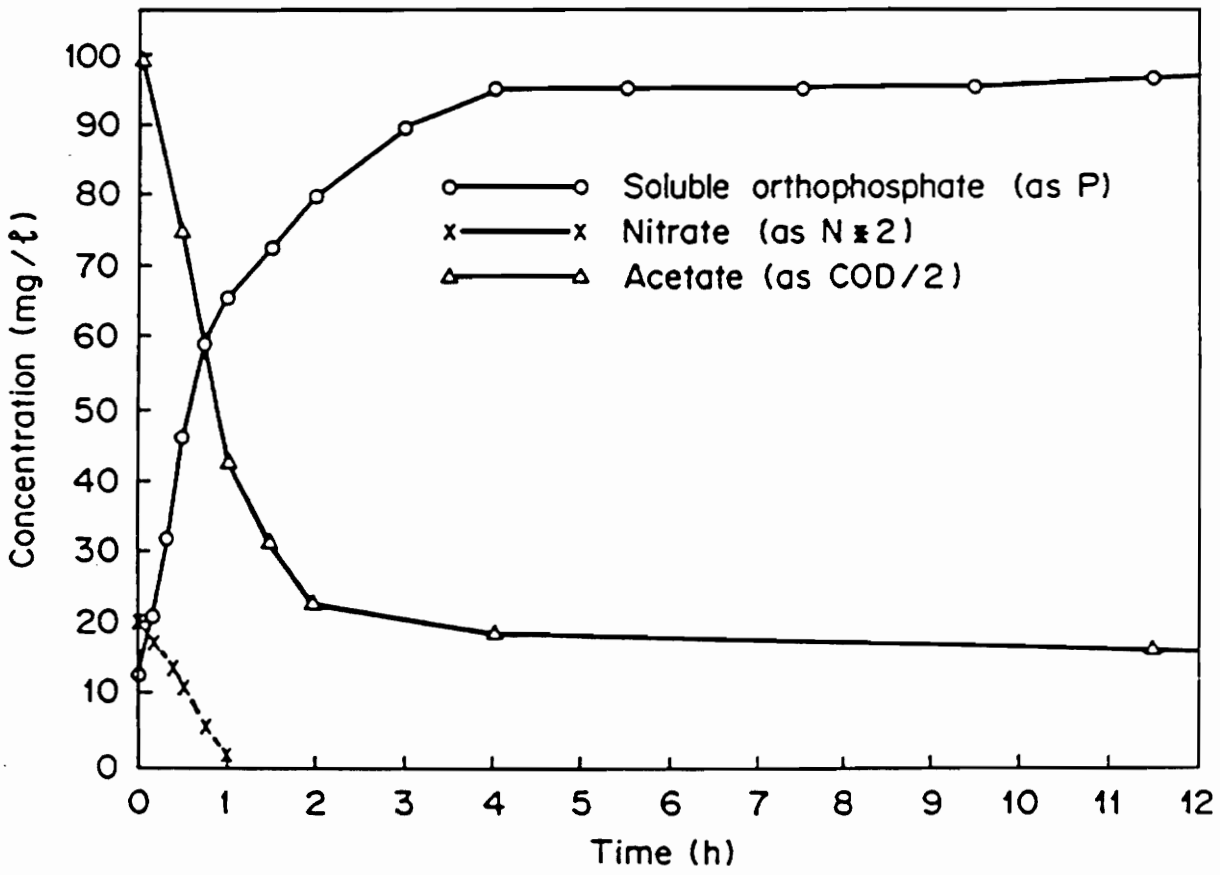
near zero (see Figure 2.6). This evidence goes along with Barnard (1974, 1975a, 1975b, and 1982) that nitrate in the anaerobic stage can reduce or prevent the release of phosphorus which, as a result, can affect uptake of phosphorus and deplete phosphorus removal.

Taking into consideration that nitrate will destroy the anaerobic condition, the sludge recycle to the anaerobic portion should contain no or non-significant amounts of nitrate. Ekama et al. (1983) and Siebritz et al. (1983) proposed design considerations for various kinds of BPR processes to achieve complete denitrification in the anoxic stage. One of the significant factors is the TKN:COD ratio which is shown in Table 2.2.

Gerber et al. (1987) used batch experiments to study the biological phosphorus removal mechanisms and found that P release occurred in the presence of various nitrate concentrations. The results are shown in Figure 2.7. It is obvious that phosphorus release can occur in the presence of nitrate. However, subsequent aeration of the sludge did not lead to an excess P uptake. As can be seen from Figure 2.7, the final P concentrations are all higher than the initial P concentrations, except the one without nitrate addition. This suggests that failure to achieve anaerobic conditions does not affect P release but it leads to considerable loss of excess phosphorus uptake capability.

Table 2.2 Design Considerations in various processes in TKN:COD ratio for maximum nitrogen removal (at 14°C and 25 days sludge age).

Process	TKN:COD ratio
Phoredox	$\leqslant$ 0.08
UCT	$\leqslant$ 0.14
Modified UCT	$\leqslant$ 0.11



Origin of sludge : Baviaanspoort ; MLSS of reaction mixture = 3400 mg/ℓ

Figure 2.7 Illustration of phosphate release when nitrate is consumed before complete utilization of substrate. After Gerber et al. (1987).

Hascoet et al. (1985) reported results showing P release occurring in the presence of nitrates. The results of the batch experiments with and without 50 mg NO_3-N/L present at various COD concentrations by Hascoet et al. (1985) is shown in Figure 2.8. As can be seen from Figure 2.8, P release occurred at all level of COD concentrations with the highest P release at the highest COD concentration. Hascoet et al. (1985) proposed two hypotheses to explain the anoxic release of phosphorus:

(a) The addition of high amounts of organic matter had masked the presence of nitrates and the P release might partially originate in the part of biomass which was not able to reduce nitrates.

(b) Substrate addition might cause a drop in the oxidation-reduction potential which was low enough to initiate the P release.

2.4.2 Anoxic Uptake of Phosphorus

As mentioned earlier, Hascoet et al. (1984) observed the uptake of orthophosphate in the presence of nitrates (anoxic uptake) following phosphorus release in batch experiments (see Figure 2.8). Simpkins and McLaren (1978), Osborn and Nicholls (1978), Nicholls et al. (1986) and Gerber et al. (1987) also observed anoxic uptake of orthophosphate. But, none of the above literature either identified the denitrifying organisms responsible

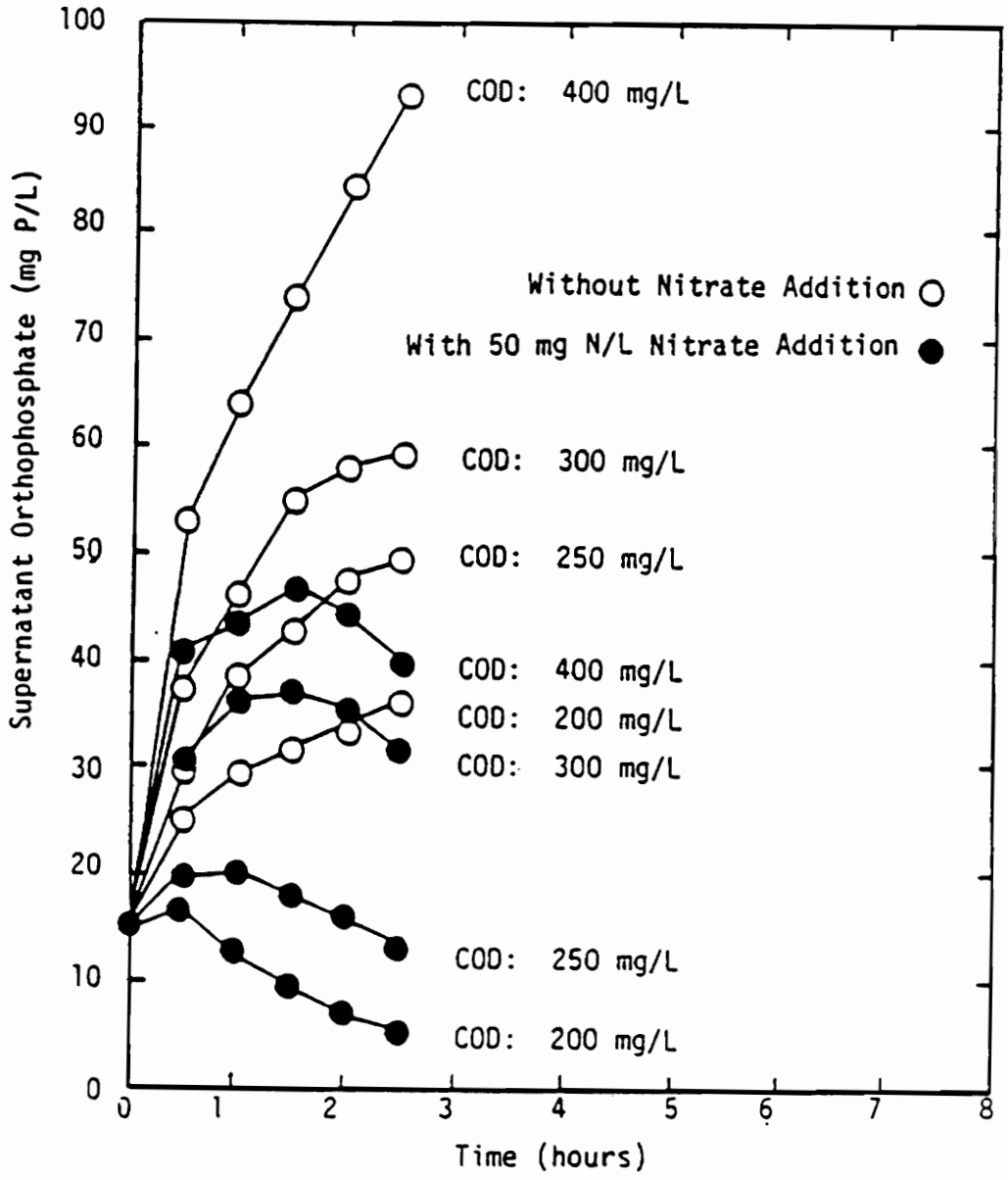


Figure 2.8 Orthophosphate concentration measured during batch tests conducted under non-aerated conditions with and without the addition of 50 mg/L nitrate nitrogen for various initial COD concentrations. After Hascoet et al. (1985).

for such P uptake in anoxic condition or examined polyphosphate accumulation in the sludge. However, according to Lötter (1985), she found that 52 out of 100 Acinetobacter bacteria strains isolated were capable of reducing nitrate to nitrogen gas. This is a significant finding which indicates a possibility of oxidative metabolism in an anoxic zone, which implies the potential for uptake of phosphorus in an anoxic zone by Acinetobacter bacteria or other poly P microorganisms.

In subsequent experiments, Lötter et al. (1986) showed that a number of Acinetobacter strains can reduce nitrate to nitrite only. However, Fuhs and Chen (1975) found that their Acinetobacter strains could not reduce nitrate. Meganck et al. (1985) pointed out that most organisms of the Moraxella group can use nitrate as a final electron acceptor. Moraxella group is capable of accumulating polyphosphate (Deinema et al., 1980).

Osborn and Nicholls (1978) did an experiment on phosphate uptake under anoxic conditions. They found that even though phosphate removal under anoxic conditions occurred, the rate of P uptake was not as fast as under fully aerobic conditions and ceased when denitrification was complete. Phosphorus release took place after that.

Gerber et al. (1987) demonstrated that phosphorus release and uptake happened under anoxic conditions, followed by secondary release under anaerobic conditions after nitrate was used up. Figures 2.9 and 2.10 illustrate this phenomenon. This confirms that anoxic uptake of phosphorus is possible and that nitrate does not stimulate phosphorus release.

2.5 EFFECTS OF OXIDATION-REDUCTION POTENTIAL (ORP) ON BIOLOGICAL PHOSPHORUS REMOVAL

Shapiro et al. (1967) suggested that low ORP may trigger the release of phosphorus from cells. However, Randall et al. (1970) studied the relationship between ORP and P release and concluded that P release did not necessarily depend on ORP. Recent studies by Koch and Oldham (1985) of ORP in excess biological phosphorus removal processes in batch experiments under anaerobic and anoxic conditions showed that phosphate release occurred when ORP was low (see Figure 2.6). Koch and Oldham (1985) indicated that there was a breakpoint in the ORP curve which coincided with the end of denitrification activity. The value of the ORP at the breakpoint varied typically between -40 and -140 mV (reference to Ag/AgCl) but, on occasion, breakpoint values were seen to be outside of the range. Koch and Oldham (1985) found that the ORP in the anaerobic zone ranged between -125 to -275 mV. Although the data were

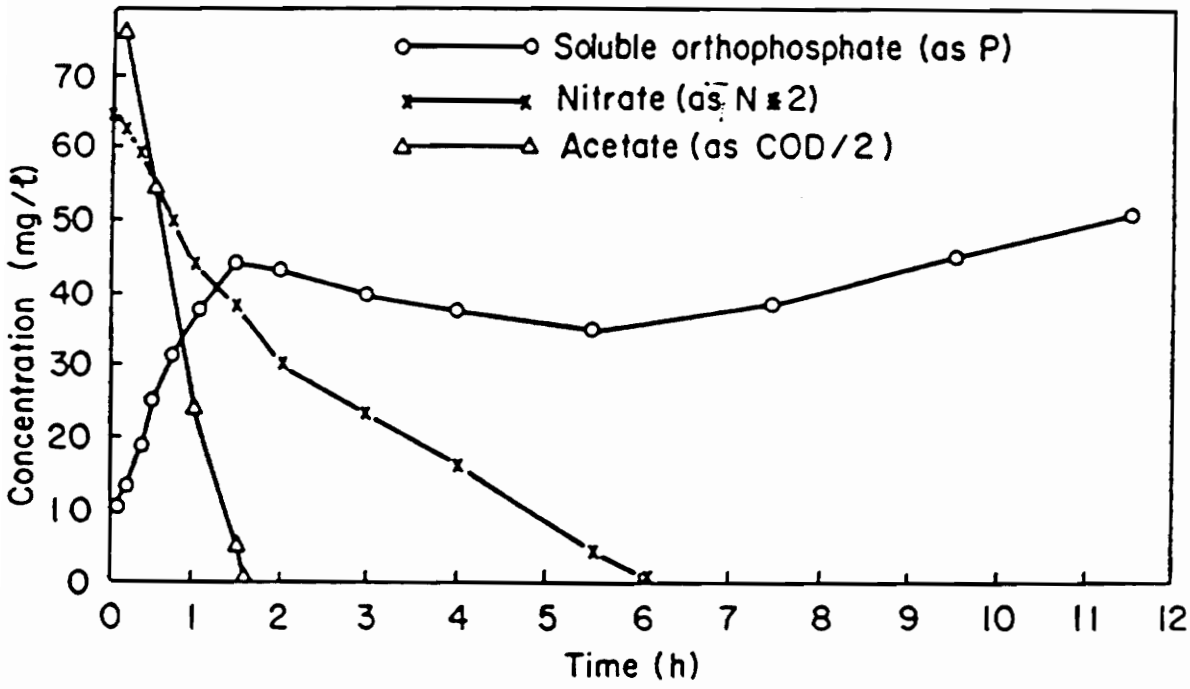


Figure 2.9 Illustration of sequential periods exhibiting net phosphate release and uptake under anoxic conditions, followed by secondary release under anaerobiosis. After Gerber et al. (1987).

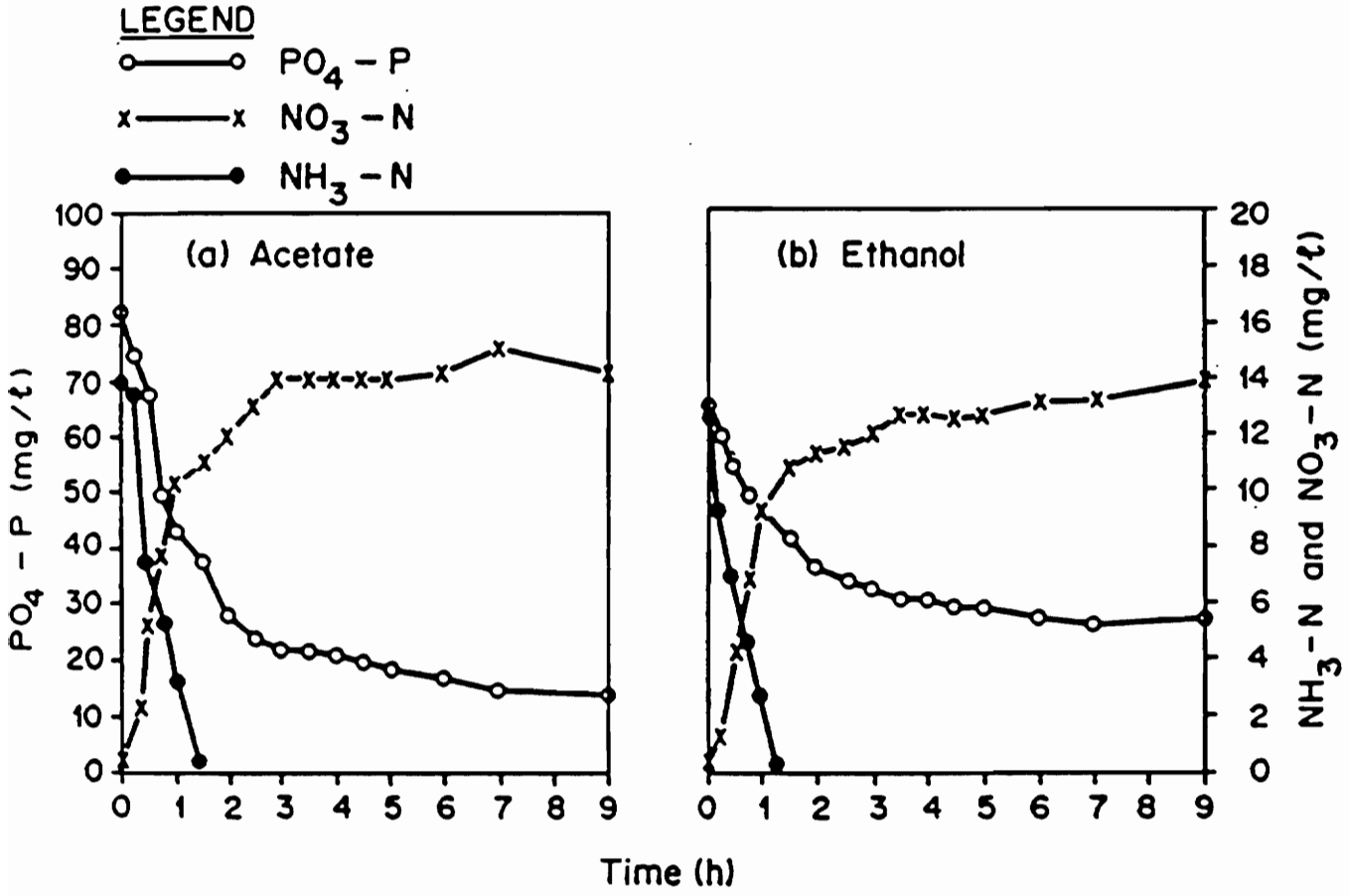


Figure 2.10 Dependence of aerobic phosphate uptake on the nature of the organic substrate introduced during anoxic/anaerobic. After Gerber et al. (1987).

scattered, they claimed that optimum anoxic ORP was at -100 mV.

2.6 CONCEPTS OF DENITRIFICATION

Denitrification is the reduction of nitrate nitrogen (NO_3-N) as it serves as the final electron acceptor for microbial 'anaerobic respiration' (respiration in the absence of molecular oxygen). Generally, there are two kinds of nitrate reduction, assimilatory nitrate reduction and dissimilatory nitrate reduction. The reduction of nitrate to ammonium for protein synthesis during the reproduction of new cells is called '<u>assimilatory nitrate reduction</u>.' The microbial reduction of nitrate to nitrite and further to nitrogen gas (N_2) is called '<u>dissimilatory nitrate reduction</u>.' The '<u>denitrification process</u>' in wastewater treatment process refers only to dissimilatory nitrate reduction. Throughout this thesis the term denitrification refers only to dissimilatory nitrate reduction.

2.6.1 <u>Energetics of Denitrification</u>

The amount of free energy released by the flow of an electron from electron donors to nitrate (NO_3) as the final electron acceptors is 17.128 kcal/mole electron when the final product is N_2 gas. In the same manner, when using

nitrite (NO_2) as the final acceptor the energy release is even higher, that is 22.263 kcal/mole electron. However, the energy release in the aerobic condition by the reduction of O_2 to H_2O is 18.675 kcal/mole electron (McCarty 1972). The energy release by reduction of O_2 gas to H_2O is a little more than energy release by reduction of nitrate to N_2 gas. Surprisingly, energy release by reduction of nitrite to N_2 gas is the highest among the three. Free energies for the three half reactions are shown below.

	ΔG^o, kcal/mole electron	
$1/3\ NO_2^- + 4/3\ H^+ + C^- \rightarrow 1/6\ N_2 + 2/3\ H_2O$	- 22.263	(2.1)
$1/4\ O_2 + H^+ + e^- \rightarrow 1/2\ H_2O$	- 18.675	(2.2)
$1/5\ NO_3^- + 6/5\ H^+ + e^- \rightarrow 1/10\ N_2 + 3/5\ H_2O$	- 17.128	(2.3)
$1/2\ NO_3^- + H^+ + e^- \rightarrow 1/2\ NO_2^- + 1/2\ H_2O$	- 9.425	(2.4)

Because they obtain more energy utilizing oxygen gas, denitrifiers prefer to use oxygen rather than nitrate as the final electron acceptor. Whatley (1981) stated that Paracoccus denitrificans when cultured in media with both oxygen and nitrate, started using nitrate only after the oxygen was used up. The pathway from nitrate to N_2 gas in nitrate respiration is summarized below.

$NO_3^- \rightarrow NO_2^- \rightarrow (NO) \rightarrow N_2O \rightarrow N_2$ (Payne, 1973).

The pathway above suggests that denitrifiers can be grown with NO_3^-, NO_2^- or N_2O as a terminal electron acceptor.

Because NO_3^--N serves as the final electron acceptor in denitrification its concentration can also be expressed on an oxygen equivalence basis. From equations (2.3) and (2.4) above, it can be seen that 1/5 mole of NO_3^--N is equivalent to 1/4 mole of O_2. When converted to a mass basis each milligram of NO_3^--N used is equivalent to 2.86 mg of O_2.

If all of the organic matter added to an anoxic reactor were converted to carbon dioxide and water it would be easy to calculate the amount (expressed as total oxygen demand) required to reduce all of the NO_3^--N to N_2 gas. It would be equal to 2.86 times the NO_3^--N concentration. However, some organic matter is converted into cell material and is not oxidized completely so that the amount required is always greater than that value.

Denitrification is a producer of alkalinity. Theoretically, it produces 3.57 mg of alkalinity as $CaCO_3$ per mg NO_3^--N reduced. Alkalinity production based on a nitrate removal basis might be lower than the theoretically predicted amount of 3.57 mg because assimilatory nitrate reduction for cellular synthesis was not taken into account.

Using methanol as the sole carbon source, the energy reaction may be represented by the following equations (Metcalf and Eddy, 1972):

$$6NO_3^- + 2CH_3OH \rightarrow 6NO_2^- + 2CO_2 + 4H_2O \quad (2.5)$$

$$6NO_2^- + 3CH_3OH \rightarrow 3N_2 + 3CO_2 + 3H_2O + 6OH^- \quad (2.6)$$

Overall: $6NO_3^- + 5CH_3OH \rightarrow 5CO_2 + 3N_2 + 7H_2O + 6OH^-$ (2.7)

A typical synthesis reaction as given by McCarty (1969) is:

$$3NO_3^- + 14CH_3OH + CO_2 + 3H^+ \rightarrow 3C_5H_7O_2H + H_2O \quad (2.8)$$

On an experimental basis, McCarty (1969) developed the following empirical equation to describe the overall nitrate removal reaction.

Overall nitrate removal:

$$NO_3^- + 1.08\ CH_3OH + H^+ \rightarrow 0.065\ C_5H_7O_2N + 0.47\ N_2 + 0.76\ CO_2 + 2.44\ H_2O \quad (2.9)$$

The optimum pH for denitrification is about 7-8 (Dawson and Murphy, 1972). Denitrification will be inhibited by extremes of pH, temperature, low organic loadings, presence of dissolved oxygen, and the presence of toxic materials (Dawson and Murphy, 1972).

2.6.2 Bacteria Involved in Denitrification

Microorganisms that can denitrify are known as facultative microorganisms rather than strict aerobes. Denitrification can be accomplished by a large number of genera commonly found in wastewater treatment systems,

including Achromobacter, Aerobacter, Alcaligenes, Bacillus, Chrombacter, Corynebacterium, Flavobacterium, Micrococcus, Propionobacterium, Proteus, and Pseudomonas (Christensen and Harremoes, 1977).

Some bacterial species are able to use nitrate for dissimilatory denitrification but cannot reduce it further than nitrite. The species in the following genera, Escherichia, Spirillum, Propionobacterium, Corynebacterium, and Streptococcus produce nitrite in this way. Others are able to denitrify nitrate all the way to nitrogen gas, but they have the ability to accumulate nitrite (NO_2^-) temporarily and later produce N_2 gas. Examples of this group include species of Paracoccus, Thiobacillus, Pseudomonas, Hyphomicrobium, Alcaligenes, and Bacilllus (Whatley, 1981).

2.6.3 Enzyme Induction During Denitrification

The enzymes of denitrification are only formed where conditions for dissimilatory denitrification are present, i.e., they are inducible. Anaerobic conditions alone will not induce all the enzymes of dissimilatory denitrification, but the presence of nitrate or nitrite intensifies the forming of enzymes nitrate and nitrite reductases. The conditions for production of nitrate and nitrite reductases vary from species to species. Paracoccus

denitrificans and Escherichia coli form nitrate reductase (and nitrite reductase) when oxygen is absent. Most denitrifiers are facultative aerobes, if offered the choice between oxygen or nitrate it is clear that both Paracoccus and E. coli prefer to use oxygen (John and Whatley, 1977).

CHAPTER THREE: MATERIALS AND METHODS

In this chapter, details will be given on the experimental approach and experiments, start up and operation of the experimental systems, and the methods of sampling and monitoring of the systems. Specific details of the analytical methods and equipment used will be presented.

3.1 EXPERIMENTAL APPROACH

The primary objective of this study was to determine whether polyP organisms can use nitrate as the final electron acceptor in a continuous flow-through activated sludge system. To accomplish the objective, two identical sets of continuous flow reactors were operated similar to the A/O Process. One set used oxygen as the final electron acceptor in the second stage and the other used nitrate as the only final electron acceptor in the second stage. The two systems were operated at the same Biological Solids Retention Time (BSRT); i.e., sludge age, of 5 days. Domestic waste from the primary clarifier effluent of the Blacksburg-VPI Sanitation Authority Treatment Plant was used as feed and sodium acetate and potassium dihydrogenphosphate (KH_2PO_4) were added to the wastewater. Sodium acetate was added because, according to much of the literature, acetate enhances phosphorus release and uptake.

The operating BSRT was achieved by wasting the desired mixed liquor volumes from the reactors. This approach considered biomass loss from the system in the effluent and also variations in mixed liquor suspended solids. The actual BSRT was calculated from the equations that will be mentioned later.

3.2 EXPERIMENTAL EQUIPMENT

The two experimental systems, shown in Figure 3.1, each consisted of 2 reactors and a clarifier. The first reactor was operated anaerobically, the second reactor was operated either anoxically or aerobically. The anaerobic/anoxic system was called "System I" and the anaerobic/aerobic system was called "System II", which was the control system. Construction material for the reactor was 9.5 millimeter (mm) plexiglass. Pertinent design information is provided in Table 3.1. The influent flow rate was 17 L/d and the sludge recycle flow rate was the same for a 1:1 ratio. Both systems were operated at the same nominal hydraulic retention time of 9.1 hours.

Removable covers were provided for all reactors except the aerobic reactor. A small hole was drilled at the center of each cover through which a stainless steel paddle rod extended. The two systems were operated in a constant temperature room which maintained temperature at $20 \pm 1^{o}C$.

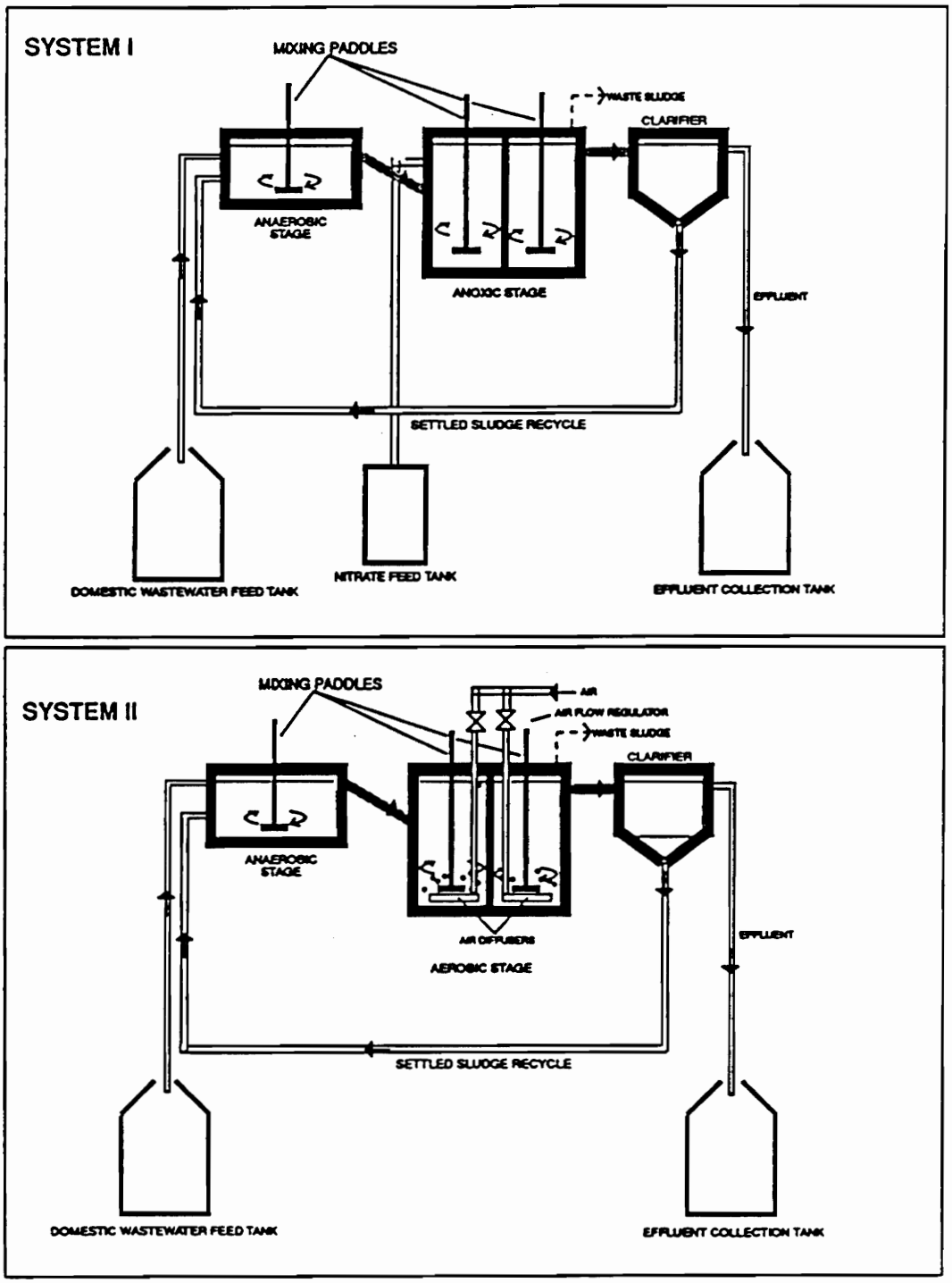


Figure 3.1 Experimental Laboratory-scale Systems. System I: Anaerobic-Anoxic; System II: Anaerobic-Aerobic.

Table 3.1 Design Parameters for the Experimental Reactors.

Reactor	Volume (liters)	Hydraulic Retention Time[1] (hours)	Approximate Length:Width Ratio
Anaerobic of each System	2.05	2.9	2:1
Anoxic of System I	4.4	6.2	2:1
Aerobic of System II	4.4	6.2	2:1

[1] Values are based on an influent flow rate of 17 L/day. Recycle rate is not included in the calculations.

Nitrogen gas was continuously purged into the anaerobic reactors to keep the anaerobic condition as complete as possible. Three pumps were needed for operation of the systems. Domestic wastewater feed was pumped from the two 18 liter calibrated Nalgene plastic carboys, one to each of the anaerobic reactors, using a Cole-Parmer Masterflex Model No. 7520-30 pump and Cole-Parmer Masterflex Model No. 7014 pump heads. The sludge recycle line of each system was operated using a Cole-Parmer Masterflex Model No. 7553-30 with 7015 Cole-Parmer Masterflex pump heads. The sludge recycle flow rate was kept constant at a 1:1 ratio with the influent throughout the entire study. Sludge recycles were pumped to the heads of the systems, that is to the anaerobic reactors of the two systems. Tygon tubing was used for all of the above pump lines.

The two reactors of each system were connected by 2.5 cm tubing. The elevation differential between the two reactors was sufficient to allow rapid transfer of the sludge between reactors and to prevent sedimentation. The detention time of the sludge in the tubes was insignificant. Paddle stirrers were utilized to provide mixing for all four reactors. A mixing speed of approximately 40 revolutions per minute (rpm) was maintained for anaerobic units and 60 revolutions per minute for anoxic and aerobic reactors.

An air supply was used to help achieve complete mixing and transfer oxygen in the aerobic unit. The in-house air supply was connected to porous diffuser stones placed in an aerobic reactor. An air-flow meter was connected between the air regulator and the aerobic reactor to obtain more precise control of the air flow rate.

3.3 FEED

Primary clarifier effluent domestic wastewater was used as the feed for the entire study. Domestic wastewater was hauled every week from the Lower Stroubles Creek Treatment Plant of the Blacksburg-VPI Sanitation Authority located on Prices Fork Road. The reactors had been seeded with activated sludge obtained from the aerobic zone of the York River Wastewater Treatment Plant which was being operated as a biological nutrient removal system. It is owned and operated by the Hampton Roads Sanitation District, and located at Seaford, Virginia.

A sodium acetate solution was added directly to the feed to increase the influent concentration by 200 mg/L as COD. Phosphorus was also added to the feed in the form of KH_2PO_4 to increase the influent concentration by 10 mg P/L.

A potassium nitrate solution was pumped at a rate of approximately 0.3 L/d from a calibrated glass container through No. 14 Tygon tubing directly to the anoxic reactor

using a peristaltic pump (Harvard Apparatus Co., Inc., Millis, Mass., Model No. 1201).

Nitrogen gas was contineously purged into domestic wastewater feed to reduce all the dissolved oxygen down to zero before feeding.

3.4 CHEMICAL ADDITIONS TO FEED

3.4.1 Acetate

As previously stated, acetate was added to the domestic wastewater feed to increase the COD concentration by 200 mg/L. Acetate was selected as a key component of the substrate for the following reasons:

(a) It is a readily biodegradable substrate.

(b) According to much of the literature, acetate addition will enhance phosphorus release and uptake.

(c) Acetate is a product of fermentation which is not available to fermenters in the anaerobic stage but is available for the exclusive use of poly P organisms under anaerobic conditions.

Acetate used in the experiment was in the form of sodium acetate.

3.4.2 Phosphorus

Phosphorus added was in the form of potassium dihydrogenphosphate, KH_2PO_4. The amount of KH_2PO_4 added increased the feed phosphorus concentration by 10 mg/L. The stock P solution of 10,000 mg/L as P was prepared and 18 ml of stock P solution was added to each 18 L of domestic wastewater.

The composition of the influent domestic wastewater fed to the systems after added acetate and phosphorus is shown in Table 3.2.

3.5 START UP AND OPERATION

The reactors were seeded with activated sludge from the aerobic zone of the York River wastewater treatment plant. The two systems, I and II, were operated without sludge wastage for the first fifteen days of operation. Samples were taken regularly to test whether the phosphorus removal processes had been established within the systems.

The Biological Solid Retention Time (BSRT), also known as the Mean Cell Residence Time (MCRT) or sludge age (θ_c) is the average length of time the mixed liquor volatile suspended solids (MLVSS) remain in the reactor. BSRT will be used in this thesis. Biological Solids Retention Time (BSRT) can be defined as,

Table 3.2 Composition of the Influent Domestic Wastewater Feed After Addition of Acetate and Phosphorus Solutions.

Constituent	Average Concentration* (mg/L)
COD	341±48
Total Phosphorus, as P	13.9±0.1
Orthophosphate, as P	12.4±0.7
TKN, as N	23.4±2.9
Ammonia, as N	15.1±1.2
Nitrate, as N	0.0
Nitrite, as N	0.0
pH	7.0±0.1
Alkalinity, as $CaCO_3$	215±33

* Except for pH which is expressed in standard units.

$$\theta_c = \frac{\text{Mass of Total Active Biomass in the System}}{\text{Mass of Active Biomass Lost from system per day}} \quad (3.1)$$

(Lawrence and McCarty, 1970)

For a completely mixed activated sludge system with recycle and wastage from the aeration tank, the BSRT, θ_c, is

$$\theta_c = \frac{VX}{Q_w X + (Q-Q_w)X_e} \quad (3.2)$$

where θ_c = biological solids retention time, days

V = volume of aeration tank, L

X = average biomass concentration in the two reactors, mg/L

Q = influence flow rate, L/day

θ_w = sludge wastage flow rate, L/day

X_e = biomass concentration in the effluent, mg/L.

From the above equation, it is obviously shown that if X_e is relatively small compared to X, then the $(Q-Q_w)X_e$ term approaches zero, and BSRT can be approximated as

$$\theta_c = \frac{V}{Q_w} \quad (3.3)$$

An average biomass concentration, X, can be calculated as follows:

$$X = \frac{X_1 V1 + X_2 V_2}{V_1 + V_2} \quad (3.4)$$

where X_1 = biomass, MLVSS, in an anaerobic reactor,

X_2 = biomass, MLVSS, in an anoxic or aerobic reactor,

V_1 = volume of an anaerobic reactor,

V_2 = volume of an anoxic or aerobic reactor.

The operating BSRT was achieved by wasting the desired mixed liquor volumes from the reactors by calculating the volume of sludge wasting according to equation (3.2). This approach considered biomass lost from the system in the effluent and also variations in mixed liquor volatile suspended solids in the various reactors of the systems.

Since the sludge was wasted from the last reactors (anoxic for System I or aerobic for System II), the actual waste sludge was calculated by the equation (3.5).

$$\text{Actual Waste Sludge Volume (L/day)} = \frac{Q_w * X}{X_2} \qquad (3.5)$$

where Q_w = theoretically sludge wastage flow rate, L/day

X = average MLVSS concentration calculated by equation (3.4), mg/L

X_2 = MLVSS concentration in the last reactor (anoxic or aerobic), mg/L

The actual BSRT differed slightly from the operational BSRT when bulking sludge and wash-out occurred. The

experiment was conducted under steady state conditions, so it was necessary to insure that consistent performance had been achieved before any samples had been taken. Theoretically, steady state is the state where all parameters remain constant for a certain period of time. In reality, true steady state conditions are difficult to achieve and even more difficult to maintain over an extended period of time. Steady state operation is affected by many factors, such as fluctuations in domestic wastewater characteristics, changes in air flow rates in the aerobic reactor, oxygen leakage into anaerobic reactors, and population shifts in the microbial species present in the reactors.

Steady state operation can be influenced by such occurrences as attachment and growth of microorganisms on tubing, reactor walls and paddles, and microbial growth in the feed tanks, etc. Thus, proper maintenance of the system can be important to achieve consistent operation.

3.6 SYSTEM MONITORING AND SAMPLES COLLECTION

Once steady state conditions were attained, a rigorous schedule of sampling and analysis was followed. All samples were collected weekly. Wastewater feed samples were collected on the Monday before and after phosphorus and acetate were added. The feed samples were analyzed for

total phosphorus, orthophosphate, TKN, ammonia, nitrite and nitrate, COD, alkalinity, and pH. On Tuesday, samples were collected from both reactors and clarifier effluent of each system. The collected samples were analyzed for the same parameters as above. All parameters monitored weekly have been summarized in Table 3.3.

The dissolved oxygen concentration (DO) of each reactor and clarifiers was measured in situ using a submersible dissolved oxygen probe.

A well-mixed portion of effluent collected in the effluent carboy over the 8-hour period was taken for effluent samples.

Samples that needed to be filtered, especially mixed-liquor samples, were pre-filtered with No. 4 qualitative Whatman filters (Whatman Limited, Maidstone, England). Filtered samples were then filtered a second time with 47 mm diameter 0.45 micrometer membrane filters (Gelman TCM-450, Ann Arbor, Michigan). In some cases, where it was difficult to filter due to high MLSS concentration, an intermediate filtering step using Whatman No. 2 qualitative glass fiber filter was made prior to the final filtering step with the 0.45 micrometer membrane filter described above.

Some samples were analyzed immediately, some that were not analyzed immediately were then stored in HDPE (High

Table 3.3 Parameters monitored weekly for each system during the experiment.

Parameter	Influent	Reactor 1	Reactor 2	Effluent
TP	x	x	x	x
Orthophosphate	x	x	x	x
Nitrite	x	x	x	x
Nitrate	x	x	x	x
Total Alkalinity	x	-	-	x
pH	x	x	x	x
COD	x	x	x	x
TKN*	x	-	-	x
NH_4*	x	-	-	x
MLSS	-	x	x	x
MLVSS	-	x	x	x
ORP	-	x	x	x
DO	x	x	x	x

*Performed every week for the first two weeks, performed every two weeks after that.

Density Polyethylene) bottles at freezing temperature. The bottles had been acid washed with 10% HCl overnight, rinsed with distilled water and dried prior to use.

The pH and dissolved oxygen of the feed were measured every Monday morning after the feed was taken into the laboratory. Samples for COD, Ammonia and TKN were acidified to pH less than 2 with concentrated sulfuric acid and stored at 4^{o}C when analyses could not be performed immediately.

3.7 ANALYTICAL PROCEDURES

The analytical procedures used in this study are described below.

3.7.1 Chemical Oxygen Demand (COD)

The chemical oxygen demand for the unfiltered wastewater feed samples, filtered reactor and clarifier samples was determined by the Dichromate Closed-Reflux Titrimetric Method as outline in Section 508 B Standard Methods for the Examination of Water and Wastewater (1985).

3.7.2 Total Kjeldahl Nitrogen (TKN) and Ammonia

TKN analyses were performed on unfiltered wastewater feed samples, filtered reactors and clarifiers samples, in accordance with section 420 A, Macro-Kjeldahl

Method, Standard Methods (1985). An acidimetric titration was used to complete the procedure.

3.7.3 Orthophosphate Phosphorus (PO_4-P), Nitrite-Nitrogen (NO_2-N), Nitrate-Nitrogen (NO_3-N).

Orthophosphate phosphorus, NO_3-N, and NO_2-N were determined on filtered samples using a Dionex 2010i Ion Chromatograph (Dionex Corporation, Palo Alto, CA) equipped with an anion separator column, HPIC-AS4A (Dionex Corp.), an anion fiber suppressor, and a conductivity detector. The eluent was 1.8 mM sodium carbonate (Na_2CO_3) flowing at 1.7 ml/min. The suppressor anion micromembrane was continuously regenerated using 0.025 N Na_2SO_4 flowing at a rate of 3.0 ml/min. The output range was set at 30 ÌS full scale and the signal was recorded with a Spectra-Physics 4270 integrator.

3.7.4 Total Phosphorus (TP)

Digestion of sludge samples from reactors, unfiltered influent wastewater feed samples, and filtered reactor samples was performed using the persulfate digestion method as outlined in Section 424 C (III), Standard Methods (1985). Digested samples were then analyzed for orthophosphate using the Ascorbic Acid Method outlined in Section 424 F, Standard Methods (1985). Unfiltered wastewater influent and filtered reactors and clarifier

samples were analyzed for total phosphorus and total soluble phosphorus, respectively. Samples were diluted before analysis so that the measured values would be less than 1 mg/L as P. A blank and four phosphorus standards were carried along with sample analysis for constructing a standard phosphorus curve. All samples and standards were analyzed spectrophotometrically using a Beckman (Irvine, California) DU-6 UV-visible spectrophotometer.

3.7.5 Dissolved Oxygen

The dissolved oxygen concentrations were measured with a YSI Model 54A (Yellow Springs Instrument Company, Yellow Springs, Ohio) oxygen meter. A YSI Model 5739 submersible probe was used with the meter for taking dissolved oxygen measurements in the reactors. The probe was moved to various locations and depths in each reactor to determine the average concentration. A probe with a stirring mechanism designed for use in a 300 ml BOD bottle was used in determining the oxygen uptake rate.

3.7.6 Specific Oxygen Uptake Rate

The oxygen uptake rate (OUR) was measured in reactor 2 (aerobic reactor) of the control system using the procedure outline in Section 213 A, Oxygen Consumption Rate, Standard Methods (1985). Mixed-liquor from the aerobic reactor was well mixed and analyzed using a BOD bottle and

direct measurement of dissolved oxygen concentration, using YSI probe as described earlier, as a function of time. The oxygen uptake rate was divided by the mixed liquor volatile suspended solids concentration, from the same reactor, to determine the specific oxygen uptake rate (SOUR).

3.7.7 Mixed Liquor Suspended Solids (MLSS) and Mixed Liquor Volatile Suspended Solids (MLVSS)

The mixed liquor suspended solids (MLSS) concentration was determined as total nonfilterable residue in accordance with Section 209 C, Total Suspended Solids dried at 103-105^{o}C, Standard Methods (1985). The mixed liquor volatile suspended solids (MLVSS) concentration was determined as total volatile residue after heating at 550^{o}C in accordance with Section 209 D, Standard Methods (1985). In addition, the total suspended solids (TSS) and volatile suspended solids (VSS) of the effluent were determined in the same manner as MLSS and MLVSS, respectively. Whatman 934-AH glass microfiber filters (Whatman Limited, Maidstone, England). Volumes of 20 to 25 milliliters were used for MLSS/MLVSS determinations while volumes of up to 200 ml were used for TSS/VSS determinations, depending on the difficulty of filtering the sample.

3.7.8 Alkalinity

Samples of unfiltered feed solution and filtered reactor and clarifier samples were analyzed for total alkalinity in accordance with section 403, Alkalinity, Standard Methods (1985). However, standardized 0.02 N sulfuric acid was used in potentiometric titration instead of 1 N. Alkalinity concentrations were reported in terms of mg/L as calcium carbonate.

3.7.9 pH

The pH of the influent domestic wastewater, reactor and effluent samples were measured in situ using a Model 610A Fisher (Springfield, NJ) pH meter. The pH probe was standardized before being used by standard buffer solutions, pH 4, 7 and 10 (Fisher Scientific).

3.7.10 ORP (Oxidation-Reduction Potential)

The ORP of the two systems was monitored throughout the investigation using the ORP probe, platinum indicator, Ag/AgCl reference, Fisher Cat. No. 13-620-82. The ORP probe was standardized before being used by standard Zobell's solution. All reported measurements are referenced to the Ag/AgCl cell.

The ORP measurements was done as in situ monitoring. The probe was left for two hours in each reactor before being read. In the anaerobic reactor, a hole

was made to exactly fit the ORP probe. When not in use, this hole was closed by a rubber stopper.

CHAPTER FOUR: RESULTS

The results obtained throughout this investigation were organized to accomplish the objectives of this research. The two lab-scale biological phosphorus removal systems were operated for approximately eight months. The anaerobic/ anoxic system was called "System I" and the anaerobic/ aerobic system was called "System II".

The nitrate feed to the anoxic reactor was maintained at the level that produced approximately 2 mg/L NO_3-N in the clarifier effluent. The dissolved oxygen concentration in the aerobic reactor was maintained at approximately 2-3 mg/L. Both nitrate and oxygen concentrations were maintained at these levels to avoid recycling back the two components to the respective anaerobic reactors. If either nitrate or oxygen were recycled back to the anaerobic reactors, they would affect anaerobic conditions by increasing the redox potential in the reactors and would reduce P release (Ekama et al., 1983).

Most P release/P uptake studies have reported P release/P uptake in the form of orthophosphate, based on the theory that P release and uptake is in the form of orthophosphate. However, in this study both orthophosphate and total phosphorus were measured on unfiltered feed

samples and filtered reactor samples. The procedures emphasized total phosphorus measurements, because these are necessary for mass balance analysis of phosphorus in the sludge.

To accomplish the objectives of this investigation, excess biological phosphorus removal had to be established. Based on the literature review, the following observations should be seen in a system in which excess biological phosphorus removal occurs:

(a) A high release of orthophosphate should take place in the anaerobic stage and a high uptake of orthophosphate should take place in the aerobic stage.

(b) The phosphorus content of the sludge in the aerobic stage should be in excess of that typically reported for conventional activated sludge systems.

(c) There should be significant removal of readily biodegradable substrate during the anaerobic stage of treatment.

System I, Anaerobic/Anoxic System, showed strong evidence of excess biological phosphorus removal by all criteria. However, System II, Anaerobic/Aerobic System, showed stronger evidence in P release. System I performed

better in P removal and had a higher sludge P content. All of the evidence will be discussed later.

4.1 PHOSPHORUS RELEASE AND UPTAKE

Profiles of average total phosphorus concentrations measured in each treatment stage are plotted in Figure 4.1. The influent phosphorus concentrations are shown both in the patterns of phosphorus concentration measured in the feed and by mass balance.

The total phosphorus of the feed and the total soluble phosphorus in the reactors and clarifiers were measured. The results of the average phosphorus release/uptake patterns for both systems (I and II) are shown in the forms of total phosphorus and are summarized in Tables A-1 and A-2 (Appendix A). The influent phosphorus concentration of both systems are shown in both measured and mass balance concentrations. For System I, Anaerobic/Anoxic system, there is an anaerobic P release pattern when all P changes are analyzed by mass balance technique, even though the reactor concentration was less than the influent concentration. System II shows a P release pattern both by concentration increase and mass balance analysis. There appeared to be phosphorus release from the sludge back into the bulk solution in the clarifiers of both systems

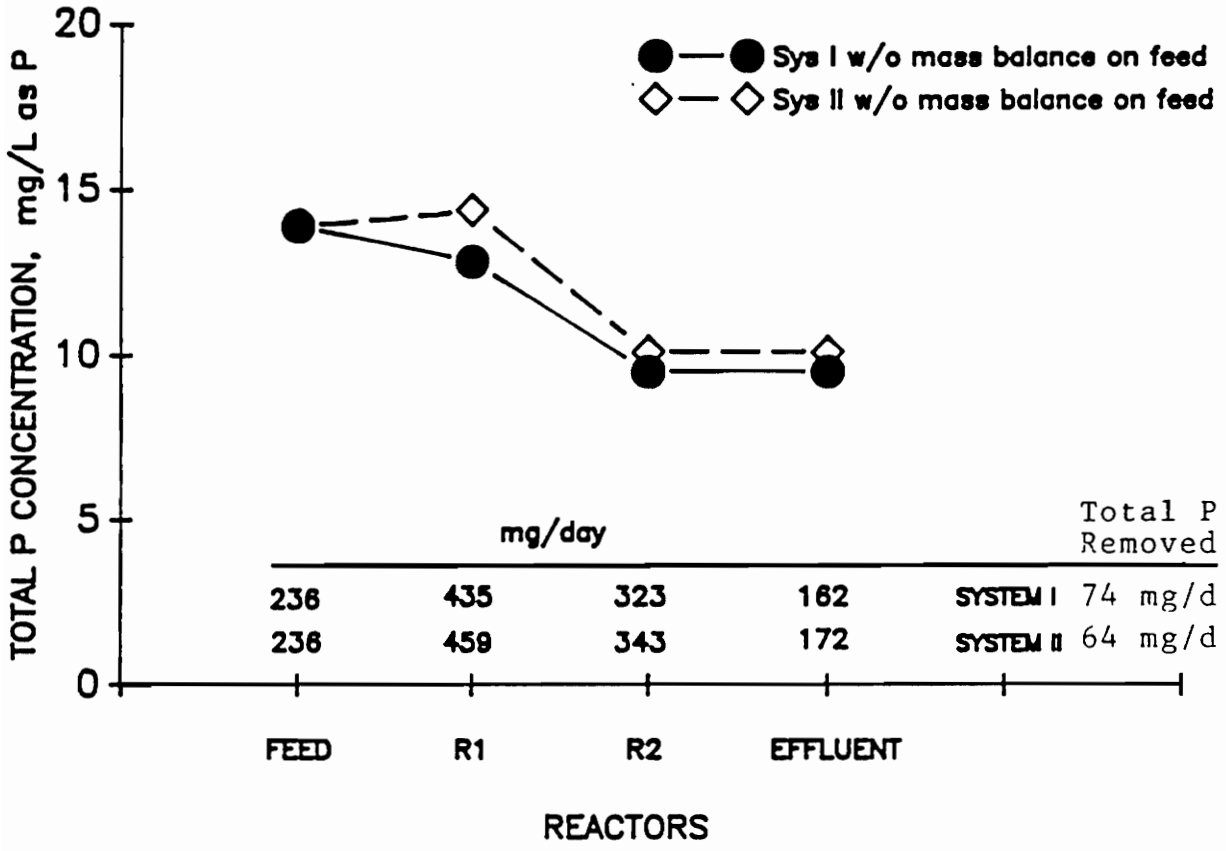


Figure 4.1 Total Phosphorus Release and Uptake Patterns in the Two Systems.

according to some data points recorded in Tables A-1 and A-2.

System I, Anaerobic/Anoxic, with nitrate as a final electron acceptor, performed better than System II, Anaerobic/Aerobic, with oxygen as a final electron acceptor in removing phosphorus from the feed and also in sludge P uptake.

4.2 PHOSPHORUS CONTENT OF SLUDGE

The average phosphorus content of sludge in the last reactors (Anoxic and Aerobic), expressed as a percentage of phosphorus in MLVSS, is shown in Table 4.1 for both systems. Phosphorus content of sludge was expressed both in the forms of measurement and mass-balance technique. Phosphorus contents of sludge by mass-balance technique were calculated by dividing the concentration of phosphorus removal by biomass production (in term of MLVSS). The sludge phosphorus contents were clearly higher than the 2.3 percent typically observed in conventional activated sludge.

The differences of phosphorus content of sludge between measurement and mass-balance come from the difficulties in analysis techniques used in analysing ktotal phosphorus in the sludge. These difficulties are:

Table 4.1 Phosphorus Content of Sludge.

	Average Percentage of Phosphorus in Sludge (MLVSS) (mg P/L per mg/L of MLVSS) x 100	
	Measurement	Mass-Balance Technique(1)
System I	4.7 ± 1.5	6.5 ± 1.6
System II	3.9 ± 1.2	5.6 ± 1.4

(1) Used mass balance to calculate these numbers, i.e., mg of P removed by system per day divided by the mg of MLVSS production per day.

1) The lack of uniform distribution of sludge in the mixed liquor samples.
2) The dilutions of sludge by 1:50 or 1:100 to meet the final concentration of TP μ 1 mg P/L, because of the limitation of spectrophotometer, makes it very difficult to get the good value to represent the whole volume of sludge sample.

4.3 COD REMOVAL IN THE ANAEROBIC STAGE

Most of the COD removed in the system was removed in the anaerobic stage followed by a smaller percentage of removal in the anoxic or aerobic stage, as can be seen in Figure 4.2. It appears that System II (Anaerobic/Aerobic System) worked a little better than System I (Anaerobic/Anoxic System) in COD removal but the difference was not significant. Also, the anaerobic reactor of System II worked better for COD removal than its System I counterpart. System II removed an average of 91% of the influent COD which 84% of the total COD removed occurred in the anaerobic reactor; 12.8% and 3.2% were removed in the aerobic reactor and clarifier, respectively. The average COD removal of System I was 89% of which 81.1% of the total COD removal occurred in the anaerobic reactor, and 15.5% and 3.4% occurred in the anoxic reactor and clarifier, respectively. Figure 4.2 shows the amounts of COD removal

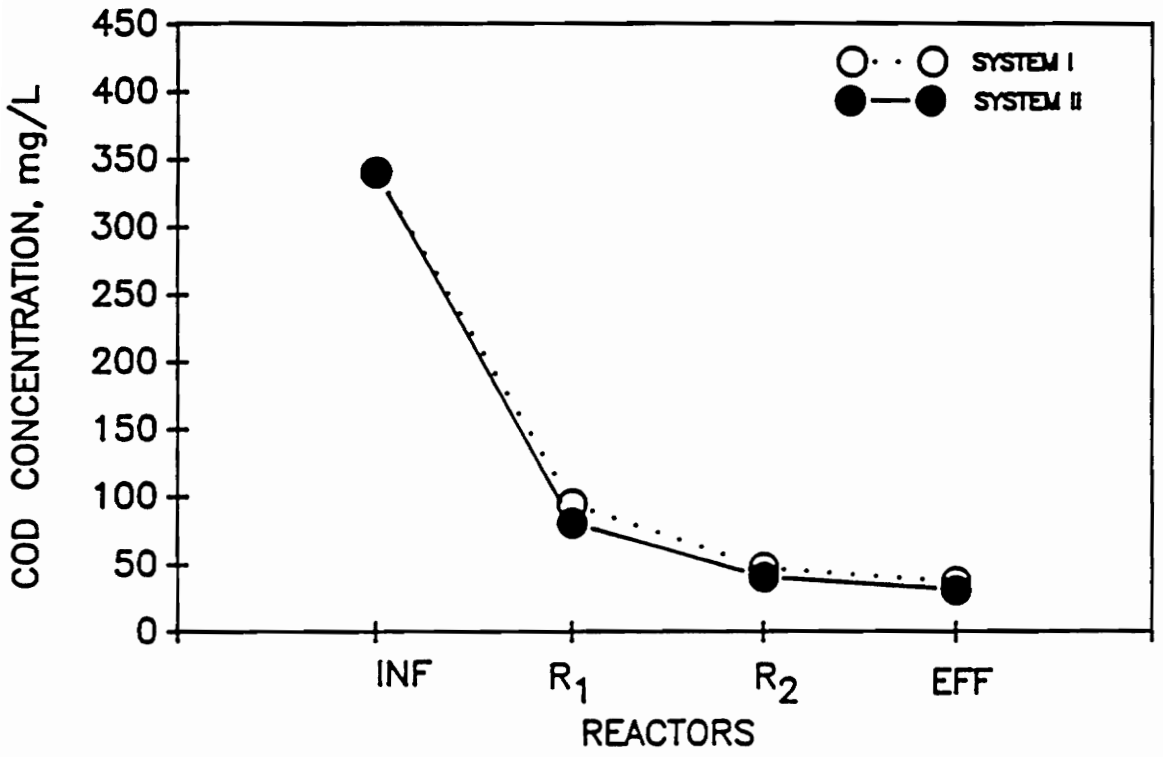


Figure 4.2 Comparison of COD removal in systems.
R_1 = Anaerobic reactor of each system.
R_2 = Anoxic reactor for System I, or
Aerobic reactor for System II.

in each stage for both systems. Figure 4.3 shows the percentage of total COD removal for both systems.

4.4 BIOLOGICAL TREATMENT PERFORMANCE OF EXPERIMENTAL SYSTEMS

Table 4.2 shows the performance characteristics of the two systems. The two systems are very close in COD removal. System II, anaerobic/aerobic system, removed a little more COD than System I, anaerobic/anoxic system. The F:M ratio of System II seems to be high, and it is a little higher than System I.

4.5 OXYGEN UTILIZATION VS. NITRATE UTILIZATION

Average specific oxygen uptake rates of the aerobic reactor of System II are shown in Table A-6 and nitrate utilization by System I is shown in Table 4.3. It can be seen that the anoxic reactor in System I used 228.2 mg NO_3-N per gram of MLVSS per day as compared to utilization of 358.8 mg O_2 per gram of MLVSS per day in System II. Thus, the ratio of O_2 used per gram of MLVSS to NO_3-N use was 1.57, whereas the theoretically ratio should have been 2.86 by stoichiometry. This is because the Anaerobic/Anoxic System (System I) did not have complete denitrification of nitrate to nitrogen gas since nitrite was detected most of the time in the clarifier effluent.

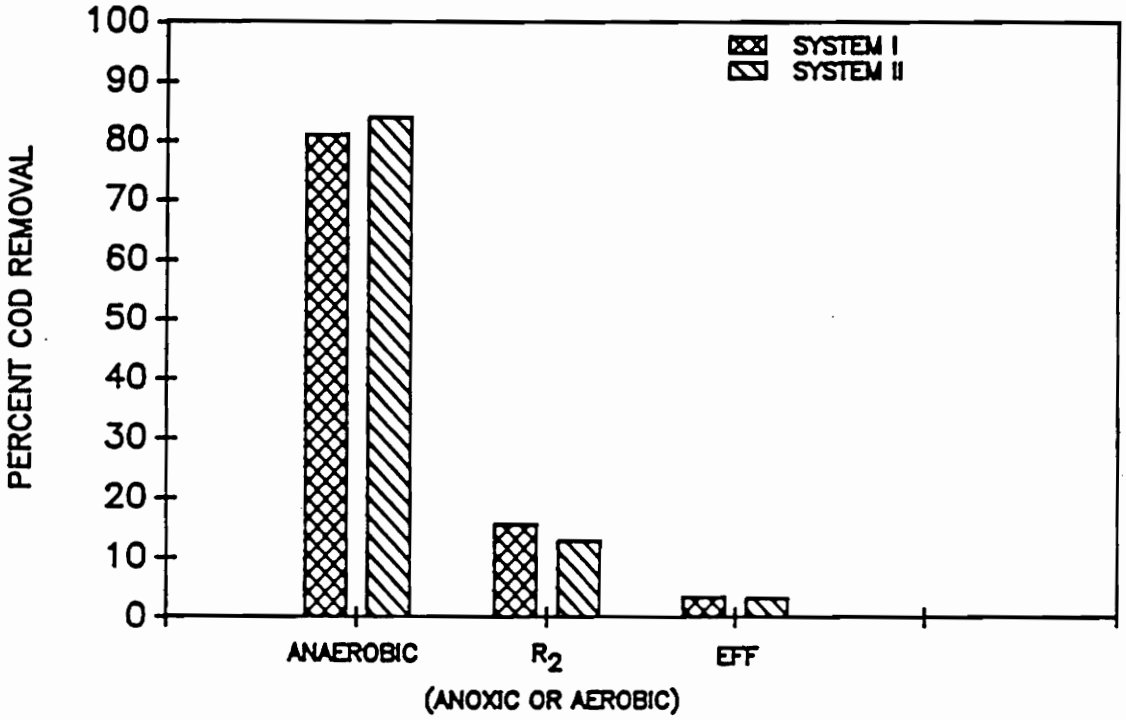


Figure 4.3 Average percentage of COD removed in each treatment stage for both systems.
R_2 = Anoxic reactor for System I, or Aerobic reactor for System II.

Table 4.2 Average Biological Treatment Performance Data for Experimental System.

System No.	Actual BSRT (days)	System COD Removal[1] (%)	F:M Ratio[2] (day^{-1})	Y_{obs}[3]	System TKN Conversion[4] (%)	System NH_4 Conversion[5] (%)
I	5	89.15	1.00	0.233	46.88	36.39
II	5	90.95	1.09	0.230	97.43	100.00

1 $[(COD_{influent}-COD_{effluent})/COD_{influent}]*100$

2 Total $COD_{influent}$/Total MLVSS in system; $F:M=\frac{mgCOD\ applied}{mg\ MLVSS.day}$

3 Observed Growth Yield = $\frac{Sludge\ (MLVSS)\ Production}{COD\ Utilized\ in\ System}$

4 $(TKN_{influent}-TKN_{effluent})/TKN_{influent}$

5 $(NH_4\ influent-NH_4\ effluent)/NH_4\ influent$

Table 4.3 Average nitrate uptake rate in the anoxic reactor of System I (anaerobic/anoxic).

Parameters	Average
NO_3 used, mg NO_3-N/L	55.04 ± 5.09
, mg NO_3-N/day	935.7 ± 88.61
MLVSS , mg/L	931 ± 0.20
, g[1]	4.1 ± 0.9
NO_3 used : MLVSS, mg/g[2]	62.6 ± 16.9
, mg/g/day[3]	228.2 ± 65.2

(1) MLVSS (g/L) x Reactor Volume (4.4 L)
(2) NO_3 used (mg NO_3-N/L)/MLVSS (g/L)
(3) NO_3 used (mg NO_3-N/day)/MLVSS (g)

4.6 NITROGEN IN THE SYSTEMS

Monitoring the utilization of nitrogen and the production of oxidized nitrogen was important in the evaluation of the systems performances. Since, both systems need to be controlled to have only 2-3 mg N/L in the forms of oxidized nitrogen (nitrite and nitrate). Otherwise, if they recycled back into the anaerobic zone, it would affect the anaerobic condition and subsequently affect phosphorus uptake.

As shown in Table 4.2, System I removed 47%, while System II removed 97.4% of the influent TKN. Removal in System I was only the amount wasted in the waste sludge.

4.7 SOLIDS CONCENTRATION (MLVSS)

It is important to determine the MLVSS concentrations in each stage since they were used in calculating the amount of sludge wasted from the systems. MLVSS was also used in mass balance analysis of P content in sludge and to calculate the F:M ratio. Average MLVSS concentrations measured in each reactor of both systems are presented in Figure 4.4. System I produced about the same amount of sludge as Systems II did, (see Table A-12). There were some differences in the concentration of MLVSS in each reactor of each system. However, it is clear that the MLVSS

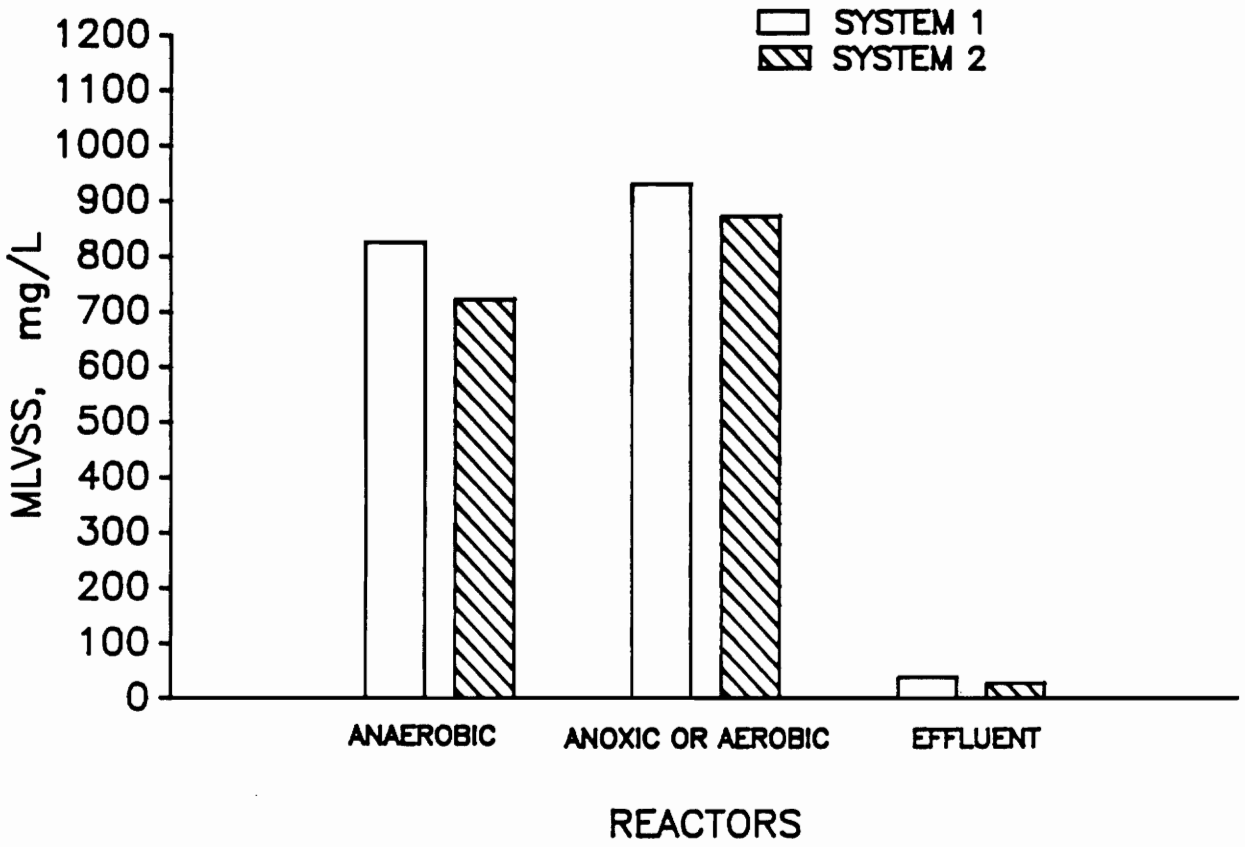


Figure 4.4 Average MLVSS concentrations in experimental systems.

concentration in the aerobic and anoxic reactors were always higher than in the anaerobic ones.

4.8 OXIDATION REDUCTION POTENTIAL (ORP)

The ORP for all reactors of both systems are presented in Figure 4.5. All ORP values were referenced to the Ag/AgCl electrode.

ORP in the Anaerobic Zone

As was expected, ORP was lower in this zone of both systems than in any other zones becausee anaerobic conditions were maintained. On average, the anaerobic zone of System I had a lower ORP than System II. The range of ORP values in this zone was -250 to -400 mV with an average of -320þ45 mV for System I and -180 to -320 mV with an average of -240þ47 mV for System II.

ORP in the Anoxic Zone

The observed ORP values of the anoxic zone were more variable than these in the anaerobic zone with values ranging from -100 to -330 mV with an average of -185þ66 mV. The values were typical for zones dominated by the presence of nitrates.

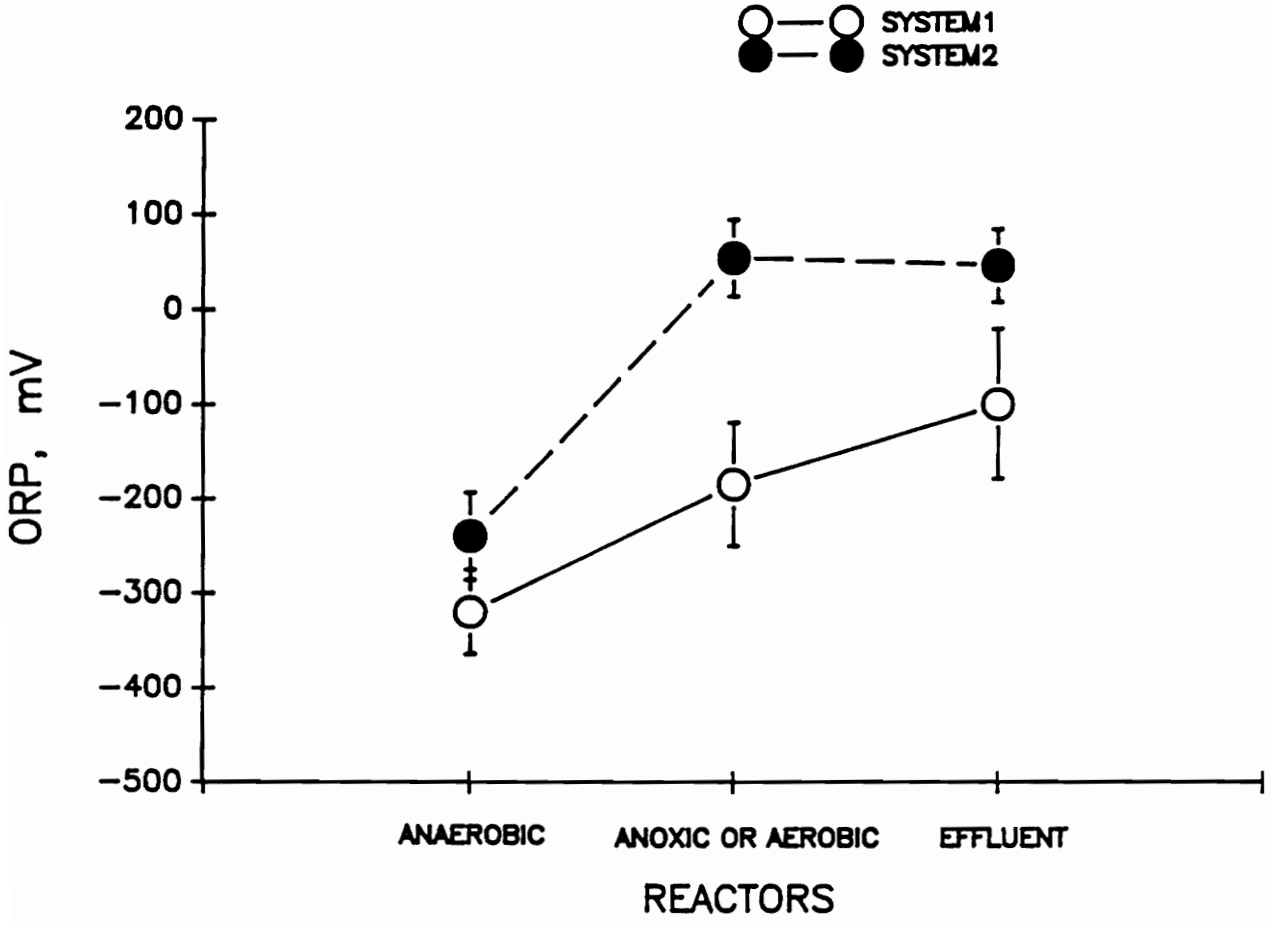


Figure 4.5 Average values of the ORP for all stages of both systems (Ag/AgCl Reference).

ORP in the Aerobic Zone

The ORP of the aerobic zone exhibited as would be expected. The ORP values were higher than in any other zones. It ranged from -10 to +120 mV with an average of +54þ41 mV. This was expected for a zone dominated by the presence of dissolved oxygen.

4.9 ALKALINITY AND pH

Alkalinity production occurred in the anoxic reactor of System I by the process of denitrification. However, alkalinity destruction in the aerobic condition of System II did not really occur. This was because nitrification hardly takes place with a low sludge age such as the 5-day sludge age used in this experiment. Alkalinity production in the anoxic system correlated with the pH increase. Alkalinity changes are shown in Figure 4.6.

The pH changes in the reactors of both systems are shown in Figure 4.7. System I showed high pH value in all reactors due to alkalinity production while System II did not change much in alkalinity. The high pH in the anaerobic reactor of System I (pH 8.2) seemed to have had negative effect on phosphate release similar to observations by Potgieter and Evans (1983). This effect is shown in Figure 4.1.

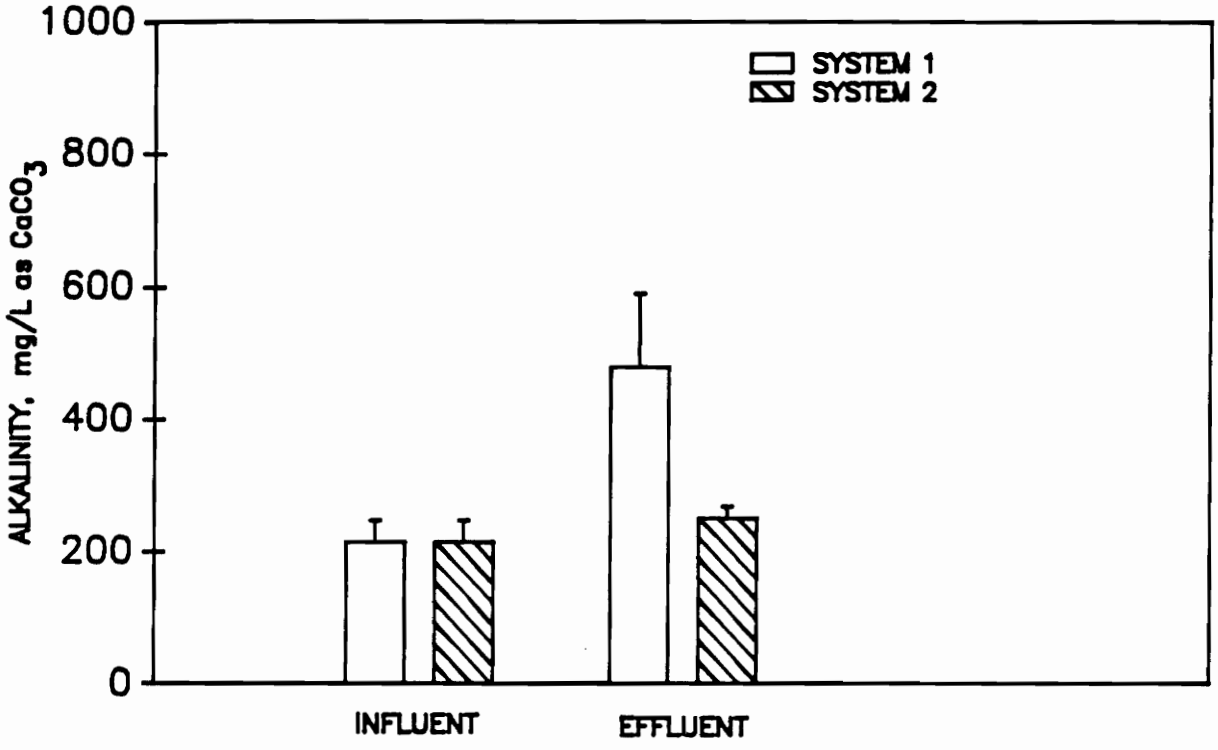


Figure 4.6 Average Alkalinity changes in both systems.

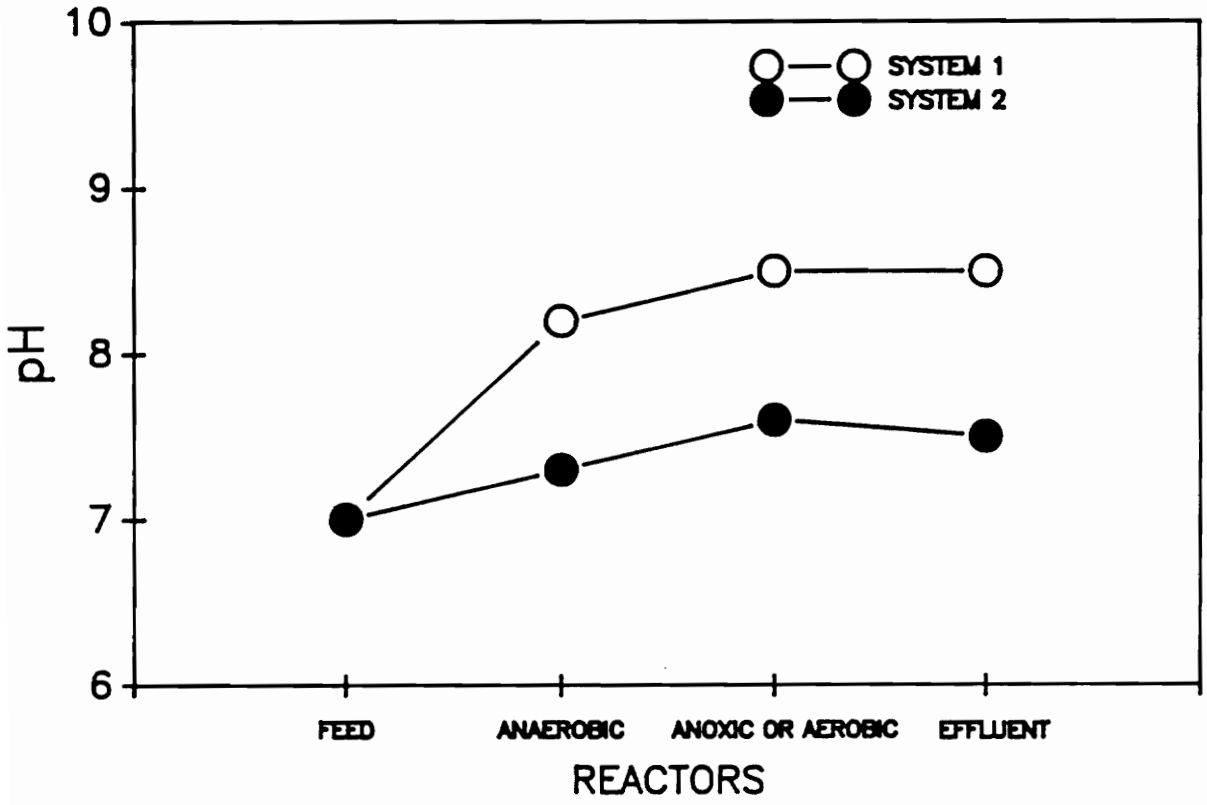


Figure 4.7 The average pH changes in the reactors of both systems.

4.10 DISCUSSION OF RESULTS

4.10.1 Evidence of Excess Biological Phosphorus Removal

Evidence that excess biological phosphorus removal was established in the two systems was provided in the previous sections of this chapter. The conclusion that excess biological phosphorus removal had taken place in both systems resulted from the following observations:

(i) Phosphorus release and uptake occurred in both systems.

(ii) Large amounts of COD were removed in the anaerobic section of the two systems.

(iii) The sludge P contents in the anoxic stage of System I and aerobic stage of system II were significantly greater than expected for conventional activated sludge plants.

These results agreed with much of the literature, e.g. Nicholls and Osborn (1979), Marais, et al. (1983), Comeau et al. (1985), and Brannan et al. (1986). Experimental evidence by those authors indicated that the P release/P uptake phenomenon is mediated by a group of microorganisms which can store phosphate in the form of polyphosphate in quantities larger than the amount of phosphorus associated

with cellular biosynthesis. This group of microorganisms is called polyP microorganisms.

The amount of phosphorus uptake must be high enough for polyP microorganisms to store P in their cells and also for cell growth of both poly P and non-polyP microorganisms. Table 4.4 illustrates the actual P removal in Systems I and II. The analysis indicates excess P removal in both systems as compared to conventional activated sludge systems. The amount of P removed was about 2 to 3 times greater than that of conventional activated sludge. This evidence supports the high sludge phosphorus contents listed in Table 4.1, which are 6.5 percent for the Anaerobic/Anoxic System (System I) and 5.6 percent for the Anaerobic/Aerobic System (System II), compared to 2.3 percent for a conventional activated sludge system.

The experimental systems also performed well in COD removal in the anaerobic stage as shown in Figures 4.2 and 4.3.

All the evidence above proves that a BPR system can be operated using nitrate as the terminal electron acceptor and can perform as well, or even better than, a BPR system using oxygen as the final electron acceptor.

4.10.2 Effluent Quality And System Performance

The Anaerobic/Anoxic System (System I) removed more TP than the Anaerobic/Aerobic System (System II), as shown in Table 4.4 (78.3 mg P/day as compared to 67.8 mg P/day). This has an effect on the effluent TP concentration. Table 4.5 illustrates that effluent TP of System I was 9.5 mg P/L as compared to 10.1 mg P/L of System II. However, System II removed more COD than System I did (91% as compared to 89%). It can be seen in Table 4.5 that System I's effluent had higher soluble COD concentrations than System II's effluent. This indicates that System II, utilizing oxygen as the final electron acceptor, performed COD removal more efficiently using oxygen for cell synthesis and energy metabolism than System I, which utilized nitrate as the final electron acceptor, did for cell synthesis and energy metabolism.

Table 4.6 summarizes System I's performance as compared to System II's performance. Even though System I's average MLVSS was higher than System II's average MLVSS, the sludge production showed that System II produced more sludge than System I. The apparent contradiction occurred because wash-out of biomass from System II occurred on day 10/11 of the experiment (see Table A-12). This unintentionally increased the amount of sludge loss from the system and lowered the BSRT. As a result, the amount of sludge

Table 4.4 Comparison of Actual Phosphorus Removal in Experimental System with Theoretical Phosphorus Removal in a Conventional Activated Sludge System.

System	Actual Average MLVSS Wasted and Loss from System (mg/day)	Theoretical P Fraction of MLVSS in Conventional Activated Sludge*	Theoretical P Removal by Conventional Activated Sludge System (mg/day)(1)	Actual Average P Removal by the System (mg/day)(2)
I	1204	0.023	27.6	78.3
II	1211	0.023	27.9	67.8

* See Chapter Two "Concepts of Excess Biological Phosphorus Removal"

(1) MLVSS Production (Column 2) * Theoretical % P in sludge (Column 3)

(2) MLVSS production (Column 2) * Actual % P in sludge (Table A-3)

Table 4.5 Effluent Quality of Both Systems.

Parameters	Average Concentration, mg/L*	
	System I	System II
TP as P	9.5	10.1
COD	37	31
TSS	44	32
TVSS	39	28
pH	8.5	7.5

* Except for pH which is expressed in standard units.

Table 4.6 Comparison of System I Performance to System II Performance.

Parameters	Average Concentration	
	System I	System II
TP Removed, mg P/L	4.4	3.8
P Content of Sludge, %	6.5	5.6
F:M Ratio	1.00	1.09
Y_{obs}	0.233	0.230
MLVSS, mg/L	898	825
Sludge Production, mg/day	1204	1211
COD Removed, mg/L	304	310
, %	89	91
COD Removed : P Removed		
, mole : mole	68	81
, mg/L : mg P/L	69	82

production in System II increased while the MLVSS concentration decreased. This is also the reason why the F:M ratio of System I was lower than that of System II. System I also has a lower COD removed : P removed ratio than System II.

CHAPTER FIVE: CONCLUSIONS

The following conclusions were developed based on an analysis of the results of this research:

1. Poly P microorganisms can use nitrate as the final electron acceptor for excess biological phosphorus removal. In this research, the Anaerobic/Anoxic system (System I) removed more phosphorus (74 mg P/day) from solution than the Anaerobic/Aerobic system (System II) (64 mg P/day) operated under similar conditions, and the MLVSS of the Anaerobic/Anoxic system had a greater phosphorus content, i.e. 6.5% as compared to 5.6% of the Anaerobic/Aerobic system by mass balance technique.
2. The mechanisms of organic and phosphorus removal in the Anaerobic/Anoxic system were apparently the same as the mechanisms in the Anaerobic/Aerobic system. In both systems, most of the organic matter, measured as COD, was removed from solution in the anaerobic section, and phosphorus was released simultaneously. Phosphorus uptake then occurred in the aerobic section.

3. There were notable differences in the effluent quality of the two systems. The Anaerobic/Aerobic effluent was lower in COD, 31 to 37 mg/L, lower in TSS, 32 to 44 mg/L, and lower in pH by 7.5 to 8.5. However, the Anaerobic/Anoxic system was lower in effluent total phosphorus by 0.6 mg P/L in spite of the higher TSS concentration.
4. The performances of the two systems were very similar as measured by sludge yield, organic removals, and sludge settleability.

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APPENDIX

Table A-1 Total phosphorus concentrations in System I (Anaerobic/ Anoxic), mg/L as P.

Concentration	Dates								
in mg P/L	9/27	10/4	10/11	10/18	10/25	11/1	11/8	11/15	Average
Feed	14.0	13.8	14.0	14.0	14.0	13.7	13.8	14.0	13.9±0.1
Anaerobic	12.0	13.0	11.5	12.8	14.5	14.8	11.3	12.8	12.8±1.2
Anoxic	9.7	9.4	10.0	10.1	9.2	9.9	8.5	9.1	9.5±0.5
Effluent	9.2	9.5	9.7	10.4	9.3	9.9	9.0	9.3	9.5±0.4
P uptake in Anoxic(1)	4.3	4.4	4.0	3.9	4.8	3.8	5.3	4.9	4.4±0.5
System P removal(2)	4.8	4.3	4.3	3.6	4.7	3.8	4.8	4.7	4.4±0.4

(1) P uptake in Anoxic = Feed - Anoxic
(2) System P removal = Feed - Effluent

Table A-2 Total phosphorus concentrations in System II (Anaerobic/ Aerobic), mg/L as P.

Concentration	Dates								
in mg P/L	9/27	10/4	10/11	10/18	10/25	11/1	11/8	11/15	Average
Feed	14.0	13.8	14.0	14.0	14.0	13.7	13.8	14.0	13.9±0.1
Anaerobic	21.0	14.5	14.3	12.5	15.4	11.9	13.0	12.6	14.4±2.7
Aerobic	10.5	9.4	10.0	10.7	10.2	10.5	9.5	10.0	10.1±0.4
Effluent	10.4	9.5	9.7	10.8	10.4	10.2	9.7	10.0	10.1±0.4
P uptake in Aerobic(1)	3.5	4.4	4.0	3.3	3.8	3.2	4.3	4.0	3.8±0.4
System P removal(2)	3.6	4.3	4.3	3.2	3.6	3.5	4.1	4.0	3.8±0.4

(1) P uptake in Aerobic = Feed - Aerobic
(2) System P removal = Feed - Effluent

Table A-3 Phosphorus Content of Sludge.

Date	Total P in sludge / MLVSS x 100 (% P)			
	System I		System II	
1988	Measurement	Mass-Balance Technique	Measurement	Mass-Balance Technique
9-27	3.4	5.6	4.7	4.8
10-4	6.8	6.5	4.5	6.4
10-11	6.4	7.9	3.3	4.2
10-18	3.6	4.2	3.3	4.7
10-25	4.1	6.9	2.7	4.6
11-1	3.2	4.2	2.8	5.3
11-8	3.4	7.7	3.4	6.2
11-15	6.4	8.7	6.4	8.7
AVG.	4.7	6.5	3.9	5.6
S.D.	±1.5	±1.6	±1.2	±1.4

Table A-4 COD Data for both systems.

	COD (mg/l)							
Date	System I				System II			
1988	Feed	Anaerobic	Anoxic	Eff	Feed	Anaerobic	Aerobic	Eff
9-27	323	87	50	37	323	46	29	29
10-4	357	113	53	53	357	93	24	24
10-11	316	94	49	47	316	82	61	25
10-18	321	72	37	25	321	118	48	33
10-25	293	50	23	18	293	41	29	26
11-1	331	126	51	17	331	70	33	29
11-8	460	128	52	44	460	112	32	32
11-15	324	84	64	56	324	80	68	48
AVG.	341	94	47	37	341	80	41	31
S.D.	±48	±25	±11	±14	±48	±26	±15	±7

Table A-5 Nitrate Utilization in the Anoxic Reactor (System I).

	Concentration, mg/L as N			
Date 1988	(1) Nitrate Feed	(2) $NO_2.N + NO_3.N$ in Anoxic Reactor ($NO_2.N + NO_3.N$) =	(3) $NO_2.N + NO_3.N$ in Effluent ($NO_2.N + NO_3.N$) =	(4)[a] Nitrate Used in Anoxic Reactor
9-27	54.6	(2.3 + 0.1) = 2.4	(0.1 + 0) = 0.1	52.2
10-4	67.0	(1.7 + 0) = 1.7	(3.4 + 0) = 3.4	65.3
10-11	67.0	(7.7 + 0.2) = 7.9	(8.5 + 0.1) = 8.6	59.1
10-18	78.0	(11.2 + 11.3) = 22.5	(9.1 + 17.2) = 26.3	55.5
10-25	58.0	(9.2 + 0) = 9.2	(10.7 + 0.1) = 10.8	48.8
11-1	58.0	(10.8 + 0.2) = 11.0	(9.6 + 0.1) = 9.7	47.0
11-8	58.0	(0.1 + 0.6) = 0.7	(0.1 + 0.6) = 0.7	57.3
11-15	58.0	(3.0 + 0) = 3.0	(1.9 + 0) = 1.9	55.0
AVG.	62.3	7.3	7.7	55.0
S.D.	±7.3	±6.8	±8.1	±5.5

[a] (4) = (1) - (2) : Nitrate Feed - ($NO_2.N + NO_3.N$ in the Anoxic Reactor)

Table A-6 Specific Oxygen Uptake Rates in the Aerobic Reactor (System II).

Date 1985	(1) Oxygen Uptake Rate (OUR) (mg/L/hr)	(2) MLVSS in the Aerobic Reactor (g/L)	(3)[a] Specific Oxygen Uptake Rate (SOUR) (mg/g/hr)	(mg/g/day)
9-27	12.0	0.83	14.46	347.0
10-4	12.3	0.96	12.81	307.5
10-18	14.4	1.014	14.20	340.8
11-15	12.0	0.655	18.32	439.7
AVG.	12.7	0.865	14.95	358.8
S.D	±1.0	±0.138	±2.05	±49.1

[a] (3) = (1) - (2)

Table A-7 Alkalinity Data for both systems.

Date 1988	Alkalinity (mg/l as CaCO3)			
	System I		System II	
	Feed	Clarifier	Feed	Clarifier
9-27	249.5	439.1	249.5	234.5
10-4	237.5	432.1	237.5	249.5
10-11	227.1	495.4	227.1	245.9
10-18	247.5	439.1	247.5	273.4
10-25	215.6	426.1	215.6	227.5
11-1	221.6	442.1	221.6	234.5
11-8	163.7	767.4	163.7	280.4
11-15	157.7	401.2	157.7	264.5
AVG.	215.0	480.3	215.0	251.3
S.D.	±33.3	±111.3	±33.3	±18.3

Table A-8 MLSS and TSS Data for Both Systems.

Date	MLSS in Reactors and TSS in Effluent (mg/l)					
	System I			System II		
1988	Anaerobic	Anoxic	Effluent	Anaerobic	Aerobic	Effluent
9-27	850	1715	41	1435	905	17
10-4	670	1000	79	720	1140	18
10-11	880	860	26	560	775	118
10-18	1415	1230	40	735	1137	17
10-25	1000	1000	57	1020	1227	12
11-1	1430	1390	26	790	1055	5
11-8	995	1000	51	760	925	40
11-15	655	945	35	525	715	31
AVG.	987	1143	44	818	985	32
S.D.	±279	±268	±17	±273	±172	±34

Table A-9 MLVSS and TVSS Data for Both Systems.

Date 1988	MLVSS in Reactors and TVSS in Effluent (mg/l)					
	System I			System II		
	Anaerobic	Anoxic	Effluent	Anaerobic	Aerobic	Effluent
9-27	650	1320	34	1245	830	14
10-4	550	770	66	670	960	17
10-11	735	695	26	480	670	103
10-18	1215	1045	35	640	1014	13
10-25	910	885	54	890	1093	10
11-1	1210	1150	23	720	940	5
11-8	790	815	41	665	820	33
11-15	545	770	31	470	655	29
AVG.	826	931	39	723	873	28
S.D.	±250	±205	±14	±234	±147	±30

Table A-10 pH Data for Both Systems

	pH							
Date	System I				System II			
1988	Feed	Anaerobic	Anoxic	Effluent	Feed	Anaerobic	Aerobic	Effluent
9-27	7.0	8.1	8.3	8.4	7.0	7.3	7.7	7.7
10-4	6.9	8.1	8.7	8.7	6.9	7.1	7.5	7.5
10-11	7.0	8.4	8.4	8.4	7.0	7.4	7.4	7.4
10-18	6.9	8.6	8.5	8.5	6.9	7.4	7.5	7.3
10-25	7.2	8.4	8.4	8.5	7.2	7.3	7.6	7.6
11-1	7.0	8.0	8.7	8.6	7.0	7.3	7.4	7.4
11-8	7.0	8.3	8.8	8.8	7.0	7.4	7.7	7.6
11-15	7.0	8.0	8.1	8.1	7.0	7.1	7.6	7.6
AVG.	7.0	8.2	8.5	8.5	7.0	7.3	7.6	7.5
S.D.	±0.1	±0.2	±0.2	±0.2	±0.1	±0.1	±0.1	±0.1

Table A-11 ORP Data for Both Systems

	ORP					
Date	System I			System II		
1988	Anaerobic	Anoxic	Effluent	Anaerobic	Aerobic	Effluent
9-27	-300	-160	-120	-180	+120	+100
10-4	-360	-330	-240	-210	+ 30	+ 20
10-11	-250	-200	-150	-220	+ 40	+ 40
10-18	-300	-100	0	-290	+ 90	+ 80
10-25	-280	-110	- 30	-200	+100	+100
11-1	-350	-200	-140	-280	+ 40	+ 30
11-8	-400	-200	-140	-320	- 10	- 10
11-15	-300	-180	0	-210	+ 30	+ 10
AVG.	-320	-185	-100	-240	+ 55	+ 46
S.D.	±45	±66	±80	±47	±41	±39

Table A-12 Amount of Sludge Production in Both Systems.
Sludge Production = Amount of Sludge Wastage + Amount of Sludge Loss in Effluent

Date 1988	System I			System II		
	Sludge Wastage (mg/day)	Sludge Loss in Effluent (mg/day)	Sludge Production (mg/day)	Sludge Wastage (mg/day)	Sludge Loss in Effluent (mg/day)	Sludge Production (mg/day)
9-27	877	578	1455	1026	238	1264
10-4	0	1122	1122	847	289	1136
10-11	489	442	931	0	1715	1751
10-18	850	595	1445	947	221	1168
10-25	249	918	1167	1168	170	1338
11-1	1140	391	1531	1043	85	1128
11-8	363	297	1060	558	561	1119
11-15	391	527	918	290	493	783
AVG.	545	659	1204	735	476	1211
S.D.	±354	±232	±227	±386	±505	±254

Table A-13 Nitrite and Nitrate Data for System I

Date 1988	Concentration, mg/L as N			
	Feed	Anaerobic	Anoxic	Effluent
	$NO_2 + NO_3 =$	$NO_2 + NO_3 =$	$NO_2 + NO_3 =$	$NO_2 + NO_3 =$
9-27	0+0 = 0	0+0.1 = 0.1	2.3+0.1 = 2.4	0.1+0 = 0.1
10-4	0+0 = 0	0+0 = 0	1.7+0 = 1.7	3.4+0 = 3.4
10-11	0+0 = 0	0+0.1 = 0.1	7.7+0.2 = 7.9	8.5+0.1 = 8.6
10-18	0+0 = 0	0+0 = 0	11.2+11.3 = 22.5	9.1+17.2 = 26.3
10-25	0+0 = 0	1.8+0 = 1.8	9.2+0 = 9.2	10.7+0.1 = 10.8
11-1	0+0 = 0	0.4+0 = 0.4	10.8+0.2 = 11.0	9.6+0.1 = 9.7
11-8	0+0 = 0	0+0 = 0	0.1+0.6 = 0.7	0.1+0.6 = 0.7
11-15	0+0 = 0	0+0 = 0	3.0+0 = 3.0	1.9+0 = 1.9
AVG.	0	0.3	7.3	7.7
S.D.	±0	±0.6	±6.8	±8.1

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