# ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL USING A SEQUENCING BATCH RBC

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# Abstract

The objective of the research program was to demonstrate the technical feasibility of removing phosphorus, by the enhanced biological phosphorus removal mechanism, from domestic wastewater using a laboratory scale Sequencing Batch Rotating Biological Contactor (SBRBC). The rotating discs of the RBC were subjected to alternating anaerobic/aerobic conditions by varying the water level in the reaction vessel. At the start of the treatment cycle, the RBC reactor would be filled submerging the rotating discs and ensuring anaerobic conditions in the RBC biofilm. Acetate would be added to the reaction vessel at this time. Following the batch anaerobic react period part of the reactor contents were decanted to either the sewage feed tank or a separate holding vessel to later become part of the influent for the next treatment cycle. With the rotating discs of the RBC partially submerged oxygen was available to the bacteria in the RBC biofilm.

Three operating schedules were tried with the above process. Each operating schedule differed in the way the decanted wastewater from the anaerobic phase was handled.

Batch tests were conducted weekly to determine the nature of the biological reactions taking place in each of the batch anaerobic and aerobic phases.

The SBRBC process showed promise for enhanced biological phosphorus removal from domestic wastewater. Carbon removal and nitrification of the wastewater were secondary benefits to this process. The success of the process was found to be dependent on the attainment of proper anaerobic conditions at the start of each treatment cycle.

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# Chapter 1

#### Introduction

Population growth, intensified agriculture, and industrial development have increased the nutrient loading on many waterbodies, resulting in an acceleration of the eutrophication process. Control of run-off, better agricultural practises, and removal of nutrients from point source discharges have been practised to rectify the problem.

Many experts agree that the removal of the nutrient phosphorus from wastewaters is the key to the treatment of point source discharges. The other major nutrients, carbon and nitrogen, are often readily available in the environment. Carbon is naturally abundant in organic compounds and the bicarbonate ion. The required forms of nitrogen, nitrate and ammonia, can be brought into a system by nitrogen fixing organisms like blue-green algae. The only natural source of phosphorus is from the earth's crust.

The majority of domestic wastewater plants removing phosphorus do so using chemical precipitation with alum, lime or ferric salts. In the last twenty years a technology referred to as 'excess biological phosphorus removal' has emerged as a viable alternative to chemical precipitation.

Biological phosphorus removal is induced when a culture of 'acclimatized' microorganisms is subjected to alternating anaerobic (no oxygen) and aerobic (aerated) conditions. Under non-aerated conditions, the bacteria release phosphorus into the bulk liquid, and store available short chain fatty acids. Under aerated conditions, the bacteria will use the carbon stored in the anaerobic phase to trigger storage of phosphorus in excess of metabolic requirements. To date the above technology has been practised

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using modifications of the activated sludge process. Due to the recent advances in the understanding of the biochemical mechanisms responsible for biological phosphorus removal, the application of processes other than activated sludge now appears feasible.

It was the purpose of this research to demonstrate the ability of a rotating biological contactor to remove phosphorus by the biological phosphorus removal mechanism, when operated in a sequencing batch mode.

The literature review discusses those articles which supplied the necessary information for the design of the experimental unit. The methodology section describes the methods and analytical techniques followed.

All findings are presented in the results and discussion sections, and conclusions and recommendations for further research are proposed.

# Chapter 2

# Literature Review

This research deals with the combined application of enhanced biological phosphorus removal, Sequencing Batch Reactor (SBR), and Rotating Biological Contactor (RBC) technologies. A brief overview of some of the available literature covering these three areas is presented in sections 2.1, 2.2, and 2.3.

# 2.1 Excess Biological Phosphorus Removal

Comprehensive literature reviews of excess biological phosphorus removal have been presented by Comeau (1984) and others. Only a brief review of biological phosphorus removal research is presented here. Special attention is given to recent research which discusses the biochemical model for biological phosphorus removal.

Excess biological phosphorus removal was first reported by Srinath et al. (1959) in India. No explanation for why the phenomenon occurred was reported.

The nature of the above phenomenon was investigated by Levin and Shapiro (1965) using mixed liquor from the District of Columbia sewage treatment plant. The authors concluded that the prospects of achieving reduction of dissolved inorganic orthophosphate from sewage, using a modified activated sludge process, were promising.

In the late sixties and early seventies, a number of activated sludge plants in the United States were removing phosphorus in excess of metabolic requirements. A number of researchers, such as Vacker (1967), Wells (1969), and Milbury (1971) studied the above plants. The exact reasons for the observation of excess phosphorus removal were

not given. The mechanism appeared to be biological in nature.

Barnard (1974) reported on an activated sludge process referred to as the 'Bardenpho' process. The process was tested for 18 months in a pilot plant at the Daspoort Sewage Treatment Works in Pretoria, South Africa. The plant consisted of four completely mixed activated sludge basins in series followed by a clarifier from which sludge was returned to the first basin. The first and third basins were only stirred to keep the solids in suspension, while the second and fourth basins were aerated. Complete nitrification was achieved in the second basin, and mixed liquor from that basin was returned to the first basin. The author reported nitrogen removals of 90-95%, and phosphorus removals of 97%. Low concentrations of nitrate in the effluent coincided with good phosphorus removal. Barnard postulated that it was essential for the mixed liquor to pass through an anaerobic period during some stage of the process followed by a well aerated stage to ensure good phosphate removal.

In (1975) Fuhs and Chen isolated a number of bacterial species from activated sludge plants. The two researchers attributed excess phosphorus storage to microorganisms of the Acinetobacter genus. These organisms were found to accumulate poly- $\beta$ -hydroxybutyrate (a carbonaceous reserve material) and poly-phosphate ( a phosphate storage material). They hypothesized that the poly- $\beta$ -hydroxybutyrate might serve as a storage of energy for excess phosphorus uptake.

It was further discovered by the two researchers that the Acinetobacter organisms, isolated from enhanced biological phosphorus removal plants, were unable to use glucose as a substrate. They required short chain fatty acids such as acetate, and succinate, to proliferate. The authors concluded that the purpose of the anaerobic-aerobic sequence was to allow the establishment of a facultatively anaerobic microflora to produce the short chain fatty acids required by the Acinetobacter as a carbon source.

Barnard (1976) reviewed biological phosphorus removal in the activated sludge process. Using the results from his own experiments, as well as the published results of other researchers, he concluded that the mixed liquor or return sludge in an activated sludge plant, to successfully remove phosphorus, must go through a period of anaerobiosis during which phosphates are released, followed by an aerobic period in which phosphates are taken up. He further concluded that the presence of nitrates in the anaerobic zone would raise the oxidation-reduction potential above the minimum value required for anaerobic conditions. Barnard's hypothesis was used to explain the findings of Vacker et al., Milbury et al., and Garber. He then suggested a modification of his Bardenpho process which included an anaerobic zone at the head end receiving both the sludge recycle and the influent stream. The new process was referred to as the Modified Bardenpho Process or Phoredox process.

Simpkins and McLaren (1978) did pilot scale experiments with the Phoredox process. The authors concluded that the physical-chemical precipitation of phosphorus was small compared to the removal by biological uptake, confirming the findings of other researchers. Simpkins and McLaren found the anaerobic release of phosphate to be in direct proportion with the COD input. Rapid 'absorption' of soluble COD was also observed in the anaerobic zone. The authors also found that the denitrification rates in the secondary anoxic zone were much lower than those in the primary anoxic basin. It was suggested that the secondary anoxic and re-aeration zones be eliminated from the Phoredox process and the primary anoxic zone be enlarged.

In (1979) Nicholls and Osborn examined sludges from the Olifantsvlei Works and Alexandra sewage treatment plants in Johannesburg, South Africa. Both plants were removing phosphorus in excess of metabolic requirements.

Poly-phosphate (poly-P) and poly- $\beta$ -hydroxybutyrate (PHB) were found to be present

in abundance in the above sludges. Nicholls and Osborn suggested that PHB and poly-P may play a mutually interdependent role in assisting aerobic bacteria to survive a period of anaerobic stress.

It was hypothesized that under anaerobic conditions, substrate in the wastewater could be metabolized to acetyl-CoA and electrons and protons by aerobic bacteria. However, due to the absence of a terminal electron acceptor (oxygen) the electrons and protons could not be used to generate ATP via the electron transport chain. Acetyl CoA, however, would act as an electron and proton sink by its reduction to PHB, and as an energy source for ATP synthesis via deactivation to acetic acid. Poly-P was proposed to serve as a source of P for the formation of ATP above. The ATP generated would then be utilized for cell maintenance via hydrolysis to ADP + phosphate (Pi). Under aerobic conditions, the hydrogen ions stored temporarily as PHB could pass back through acetyl-CoA as an intermediary and enter the normal Krebs cycle once more. PHB was thought to serve not only as an energy source for cell function but also for poly-P generation and storage.

Using the nitrification-denitrification model developed at the University of Cape Town, Rabinowitz and Marais (1980) defined a parameter known as the anaerobic potential. The anaerobic potential was defined as the difference between the denitrification capacity of the anaerobic reactor and the mass of nitrate entering the reactor. The two researchers suggested a modification of the Phoredox process to eliminate nitrate from the anaerobic zone. As suggested by Simpkins and McLaren, the secondary anoxic and re-aeration zones were eliminated. The sludge recycle and mixed liquor from the aerobic zone would be discharged to the anoxic basin. An additional recycle would be added to the anoxic basin to transfer mixed liquor to the anaerobic zone. By adjusting the recycle ratio from the aerobic to the anoxic zone, the nitrate concentration in the anoxic, and therefore the anaerobic zone, could be reduced. The above process became known as the UCT process.

Marias et al. (1983) traced the development of biological excess phosphorus removal from its discovery in 1959 up until 1983. The biochemical models of Fuhs and Chen (1975) and Nicholls and Osborn (1979) were reviewed. The authors hypothesized that poly-P accumulation serves as an energy reservoir, to sustain the organisms during the anaerobic stressed state, but more importantly to gain an advantage over non-P accumulating organisms by partitioning of readily biodegradible COD ( in the lower fatty acid form) in the anaerobic state for its exclusive use subsequently in the aerobic state.

The authors hypothesized that during anaerobic conditions the internal ATP/ADP ratio of poly-P organisms would be low. It was also suggested that the above ratio may act as a feedback mechanism activating ployphosphatase to catalyze the breakdown of poly-P to Pi and energy for ATP production. Energy from ATP would then be used to bring substrate into the cell and convert it to a lipid form available for storage as aceto-acetate and/or poly- $\beta$ -hydroxybutyrate.

The fate of both short chain fatty acids, e. g. acetate, and a relatively 'high energy' substrate, which can be used by facultative and aerobic organisms to yield energy through anaerobic glycolysis, was also considered. It was hypothesized that acetate entering the cell during the anaerobic period is complexed by the enzyme CoA to form acetyl CoA, which requires the input of two ATP. Pi and energy required for ATP production could be derived from the breakdown of the poly-P chain. Complexation of the acetate to acetyl CoA would reduce the acetate concentration within the organism, thus allowing an osmotic pressure to be created for further entry of substrate into the cell. Due to the limited supply of CoA in the cell, storage as acetoacetate or PHB was considered likely. The high concentration of Pi in the cell, resulting from poly-P breakdown, would lead to Pi diffusion out of the cell.

Two possibilities for glucose utilization, in the anaerobic zone, were proposed. The authors suggested that if the poly-P organisms are unable to use substrates such as glucose directly, facultative organisms will break them down via the Embden-Meyerhof pathway to acetate which the poly-P organisms can store as discussed previously. Poly-P organisms able to use glucose would break the substrate down via the Embden-Meyerhof pathway to form pyruvate. A pathway utilizing electrons as NAD to transform pyruvate to PHB was proposed.

It was suggested that poly-P organisms entering an aerobic or anoxic region would utilize their stored substrate immedately, giving rise to an increase in the ATP/ADP ratio. An increase in the above ratio may act as a feedback mechanism, activating poly-P kinase to catalyze poly-P production in order to refill the poly-P pool.

Comeau et al. (1986) proposed a biochemical model for enhanced biological phosphorus removal based on experimental observations and principals of bacterial energetics and membrane transport. Batch tests were conducted using aerobic mixed liquor obtained from the University of British Columbia biological phosphorus removal pilot plant. Acetate addition to the mixed liquor, under anaerobic conditions, resulted in P release in proportion to the amount of acetate added. Carbon storage as PHB was also observed. Subsequent nitrate addition resulted in P uptake by the biomass until all the oxidized nitrogen had disappeared from solution. The addition of 2,4 dinitrophenol (DNP), sodium hydroxide (high pH),  $H_2S$  gas, and  $CO_2$  gas was also found to cause P release.

The release of P due to  $CO_2$  addition was noted to be consistent with the observations of other researchers (Fuhs and Chen 1975, and Deinima 1985). Because  $CO_2$  will not likely be stored in carbon reserves, the previous proposal of Marais et al. (1983) that polyphosphate degradation supplies energy for carbon storage was not considered entirely correct by the authors.

To explain the above descrepancy, Comeau et al. proposed that polyphosphate reserves could be used to supply energy to maintain the proton motive force of the bio-P bacteria, in addition to its role in carbon storage. Using the above as a basis, a biochemical model was proposed using acetate as a substrate.

Comeau et al. hypothesized that the addition of acetate to a bio-P biomass, under anaerobic conditions, would result in the formation of the ionic form of the above (for pH values greater than 6.5, more than 99% of the acetate added will be in the ionic form). Due to the requirement for electroneutrality when crossing the cell membrane, the acetate will combine with hydrogen ions outside of the cell before being transported inside. The result will be a reduction of the pH gradient of the proton motive force of one hydrogen ion for each acetate ion transported into the cell. If the pH gradient is not restored, acetate uptake, and the corresponding ability to increase carbon storage as PHB, would cease. To expel hydrogen ions and re-establish the pH gradient, it was argued that poly-phosphate may expel protons across the cytoplasmic membrane using a translocating enzyme. The resulting phosphate accumulation inside the cell, due to poly-phosphate breakdown, would give rise to a phosphate release to the outside of the cell, along the concentration gradient. The presence of a pH sensitive phosphate carrier protein in the cell wall was also hypothesized. For unfavorable pH gradients (anaerobic conditions), inorganic P could not be used for the synthesis processes and would therefore be released at high concentrations. When the pH gradient is favorable (aerobic conditions), the carrier will be inoperable. It was concluded that the extent of PHB accumulation would therefore be limited by the availability of poly-phosphate for the re-establishing of the pH gradient. This fact was confirmed by batch tests that indicated P release would not increase beyond a certain value regardless of how much extra acetate was added.

In the aerobic zone, the bacteria would contain PHB reserves and reduced amounts

of poly-P. In the presence of oxygen, the electron transport chain would be operational and the internal ATP/ADP ratio would increase. The bio-P bacteria would degrade their internal PHB reserves for energy, and possibly synthesis, and rebuild their poly-phosphate reserves using phosphorus in solution in the process. Nitrate reducing bacteria would also take up P in solution according to this model, due to the operation of the electron transport chain.

To explain the release of P when  $H_2S$  and  $CO_2$  gas were added to the mixed liquor, the authors suggested that the diffusion of  $H_2S$  or  $CO_2$  and their subsequent intracellular dissociation would decrease both the outer and inner pH to such an extent that the pH gradient would be reduced, resulting in P release.

Wentzel et al. (1986) stated that the biochemical model of Comeau et al. is incomplete because it does not provide quantitative information on the pathways and control mechanisms governing biochemical reactions under different sets of imposed conditions of substrate and oxygen tension. The authors further stated that the mechanism proposed for maintaining the pmf (proton motive force) in the anaerobic zone gives rise to charge and proton imbalances across the cytoplasmic membrane. A modified biochemical model was provided in an attempt to overcome the above objections. Fundamental to the model's development was the effect of the anaerobic and aerobic phases on the intracellular NADH/NAD and ATP/ADP ratios, and the influence of these ratios on the biochemical regulation of carbon and phosphorus metabolic pathways. Acetate was the substrate considered when developing the proposed biochemical model.

In the anaerobic zone, the cells were proposed to have a high NADH/NAD ratio and a low ATP/ADP ratio, due to the lack of oxidative phosphorylation. The external concentration of acetate in the anaerobic zone would allow passive diffusion of this substrate into the cell. Once in the cell, acetate is converted to acetyl-CoA by ATP hydrolysis. The ATP/ADP ratio is reduced to such an extent that ATP formation

via poly-P degradation is stimulated. The intracellular concentration of P, and of the cations stabilizing the negative charges of the poly-P chain, increases, resulting in their eventual release into the bulk solution. PHB synthesis results when NADH is oxidized to NAD, with electrons and protons reducing acetoacetyl-CoA to  $\beta$ -hydroxybutyl-CoA. The decrease in the NADH/NAD ratio stimulates the TCA cycle, which generates more NADH. The formation of PHB lowers the intracellular concentration of acetate, resulting in more acetate diffusion into the cell.

The authors further proposed that phosphate release occurs via a hydroxyl mediated antiport protein carrier with Pi moving out of the cell as  $H_2PO_4$ . Cation release occurs via a proton mediated antiport protein carrier, and acetic acid will be taken up by passive diffusion. Both the proton motive force, and charge neutrality, were shown to be maintained using this model.

The authors suggested that oxidative phosphorylation will take place in the aerobic zone resulting in a reduction of the NADH/NAD ratio and an increase in the ATP/ADP ratio. The decrease in the NADH/NAD ratio would stimulate further PHB degradation to acetate, providing carbon and energy for cell function. The high ATP/ADP ratio would stimulate poly-P synthesis, enabling the organism to establish any required pmf and utilize ATP for molecule translocation. A similar discussion of anoxic conditions was presented.

#### 2.2 Sequencing Batch Reactors

A great deal of literature has been published on sequencing batch reactor (SBR) technology. Only a brief review of recent research on the application of SBR technology to biological phosphorus removal is presented here.

Alleman and Irvine (1980) traced the development of the SBR to the classic study

of Ardern and Lockett in the early 1900's. The authors stated that interest in the fill-and-draw mode of wastewater treatment declined in the 1920's. However, recently, interest in the SBR process had been rekindled. The SBR process was described as consisting of five distinct phases including Fill, React, Settle, Drain, and Idle. During the fill period, raw sewage is brought into the reactor. Once the reactor is filled, a set amount of time is allowed for the mixed liquor suspended solids to degrade the wastewater organics. The mixers and aerators are then shut off, allowing the mixed liquor to settle, and the treated supernatant is decanted during the drain phase. During the idle phase, sludge wasting is practised as required. Alleman and Irvine concluded that the opportunity to follow the metabolism of the substrate species during the react period was one of the advantages of the SBR.

Manning and Irvine (1985) used bench scale SBR's, treating synthetic wastewater, to remove phosphorus biologically. The basic cycle used included a two hour fill period, four hour react period, and a two hour period for draw, settle and idle. Six operating strategies were tested. The feed addition pattern, feed addition time, and mixer status during fill times differed for each strategy. The strategies which eliminated DO and NOx-N during the fill period, resulted in biological phosphorus removal. The authors concluded that biological phosphorus removal could be achieved in an SBR with a relatively low COD/TKN ratio.

Ketchum et al. (1987) reported results of phosphorus removal experiments using a full scale SBR at Culver, Indiana. The SBR used is a fill and draw activated sludge system in which each basin in the system is filled and then aerated in a batch treatment mode. Five discrete periods, consisting of Fill, React, Settle, Draw, and Idle, were identified for each cycle. Both chemical and enhanced biological phosphorus removal were tried at this facility. The authors concluded that the full scale use of an SBR for enhanced biological phosphorus removal was quite effective. Okada et al. (1987) used a laboratory scale SBR to clarify the behavior of microorganisms responsible for phosphorus removal and to remove nitrogen, phosphorus, and BOD. Operations where anoxic/anaerobic reactions were introduced into the fill period gave the best performance.

Appeldoom and Deinema (1987) used a laboratory scale fill-and-draw system to study biological phosphorus removal. A six hour operating schedule, consisting of two hours and forty-five minutes of aerobic time, one hour and fifteen minutes of anaerobic time, and two hours settling time, was used. When the system was run with acetic acid and ten percent glucose, the sludge was found to contain more than 60% Acinetobacter. When fifty percent glucose was used in the feed 20% fewer Acinetobacter were observed than in the first run. The authors concluded that fill-and-draw systems could be used to explain the discrepancies in the behavior of pure cultures of Acinetobacter and those of sludge from P-removal plants.

# 2.3 Rotating Biological Contactors (RBC's)

The development of the RBC process began in Germany and the United States in the 1920's using wooden discs. By 1959, J. Conrad Stangelin was manufacturing two and three meter diameter polystyrene discs in West Germany, and by the late sixties a number of small West German communities were using RBC's for their wastewater treatment. The capital cost of these original units was quite high. However, the maintenance and energy costs proved to be low. The above is still a selling point used by RBC manufacturers. In 1972, the American company Autotrol developed a more compact disc for the RBC process and RBC's soon became cost competitive with other treatment processes. A cost-effectiveness analysis was done by Lundberg and Pierce (1980) to compare air-drive and mechnical drive RBC processes with air and

pure oxygen activated sludge processes. For the range of design flows studied (3-50 MGD) RBC's were found to require 40-60% of the power needed for the pure oxygen activated sludge facilities and from 23-60% of the power needed for air activated sludge processes. The authors concluded that RBC processes were a viable alternative to activated sludge in the 3-50 MGD design range.

The RBC literature provided in this section has been subdivided into the two main areas considered pertinent to this research: RBC biofilm studies; and nutrient removal using the RBC process.

# 2.3.1 RBC Biofilm Studies

Alleman et al. (1982) used scanning electron microscopy to evaluate the stratified composition of an RBC biofilm. Biofilm samples were taken from the Alexandria, Virginia RBC facility. The biofilm was found to contain a stratified layering of morphologically diverse organisms. The top layer contained filamentous bacteria and the lower layer contained comma shaped rods. The authors hypothesized that the aerobic top layer contained Beggiatoa, which are able to use sulphide as an energy source. The lower anaerobic layer was thought to contain Desulphovibria, able to reduce sulphate to sulphide.

Hoag et al. (1983) investigated the types of organisms associated with RBC biofilms. Both laboratory and full scale units were used in their study. Biofilm samples were microscopically examined and microfauna present were identified using taxonomic keys. A succession of microfauna from stage to stage was observed in both full and laboratory scale RBC's. Filamentous bacteria were found to dominate the biofilms in the first stage of the RBC's tested. Attached ciliates were the most frequently dominant microfaunal group in the second stage laboratory and first stage full-scale units. Rotifers and Sarcodinians were the dominant group in the third and fourth stage of lab units and the third through sixth stage of full scale units. The authors related population levels of four microfaunal groups to the concentration of COD and ammonia.

Kinner and Maratea (1985) examined biofilm bacteria from an RBC to obtain detailed information on the microflora present. Organic loading rate was varied and the effect on bacterial morphology was investigated.

A great number and variety of cells was observed in the most heavily loaded RBC compartments. The distribution between gram-positive and gram-negative bacteria appeared to be equal. The large gram-positive cells contained fewer inclusions than similarly sized gram-negative cells. The smaller bacteria were observed to store large amounts of PHB-like material. Polyphosphate and possibly sulfur inclusions were also observed.

Kinner and Maratea found that the lower organic loading rates resulted in a predominantly gram-negative population, and less diversity of cell types. The amounts of PHB and polyphosphate were reduced and no sulphur inclusions were observed.

Bacteria resembling nitrifiers were found in the final compartment of the RBC operating at the lowest loading rate  $2gTOC/m^2 - hr$ . The above bacteria contained extensively convoluted cytomembranes, were often enclosed in common capsules, and often contained polyphosphate-like inclusions. PHB was not stored in any of the above cells.

#### 2.3.2 Nutrient Removal using RBC processes

RBC processes have been used extensively in the past for carbon oxidation and nitrification. The use of RBC's for denitrification has been limited to date. To the authors knowledge no RBC plant has been purposefully operated for enhanced biological phosphorus removal as of yet. However, a number of plants have incorporated chemical phosphorus removal into their process trains. The reason for the above is that many

researchers hold the same view as Strom and Chung (1985) that it would be difficult to employ RBC's for the luxury uptake of phosphorus. Presented below is a literature review of research on nutrient removal using RBC's. Although by no means complete it is felt that some of the most pertinent information has been presented.

Pretorius (1971) used a laboratory scale RBC to treat anaerobically pretreated domestic sewage. The author used an inoculum from an activated sludge plant to seed the process, and observed a good biological film on the discs after twenty-one days of operation. The rotating discs nearest the inlet were covered with a thick filamentous growth of sphaerotilus and beggiatoa. The remaining discs consisted of a slimy indefinable film, made up of very fine filamentous cells together with a zoogleal mass. Good COD removal and nitrification were reported. Pretorius reported very poor phosphorus removal using this system. In particular the TP concentration decreased slowly from 13.2 to 10.6 mg/l across the RBC unit. The orthophosphate concentration remained virtually unchanged.

Antonie et al. (1974) reported on the results of a study done at the Pewankee, Wisconsin wastewater treatment plant. The above plant was the first full-scale American facility using the RBC process. Primary clarification, two banks of four 3.1 m diameter RBC units, secondary clarification, and aerobic digestion comprised the process train of the 0.5 mgd plant. The authors investigated rotational disk velocity, hydraulic loading, and effect of climatic conditions on treatment efficiency. A high degree of BOD and SS removal and nitrification was reported. Stable operation under fluctuating hydraulic and organic loading and wastewater temperature was observed. Low power, maintenance, and sludge handling costs were also cited as advantages of the RBC process.

Davies and Pretorius (1975) studied the denitrification performance of an anaerobic RBC, with respect to carbon requirements, pH and temperature. Their study

was divided into four stages including adaptation of the RBC unit to denitrification, determination of the lowest carbon to nitrogen ratio necessary for complete denitrification and the determination of the effects of pH and temperature on denitrification rate. The carbon to nitrogen (C:N) ratio was varied by changing the methanol feed rate. Once the optimum C:N ratio was found the effects of pH and temperature variations were studied. The optimum C:N ratio for denitrification was found to be 2.6:1. The optimum pH for denitrification was between 7 and 8.5. Values on either side of the reported range gave less than complete denitrification. The optimum temperature range for denitrification was found to be between 10° and 30° C. All the above values were reported as being similar to those observed for activated sludge denitrification units. The authors concluded that the mechanical simplicity and ease of operation of the RBC make it a system worth considering for denitrification.

Hao et al. (1975) reported on the results of a pilot study at the Columbus, Indiana sewage treatment plant. An RBC unit was used for carbonaceous BOD, SS, and ammonia nitrogen removals. Phosphate removal was accomplished by chemical addition. Both liquid alum and ferric chloride were tried. Chemical addition to and after the fourth stage was investigated. The addition of liquid alum directly to the fourth stage appeared to affect effluent BOD and SS adversely. The authors hypothesized that the chemical coating on the media decreased the biological treatment efficiency, and that chemical addition resulted in poor solids settleability. Addition of phosphorus precipitating chemicals after the fourth stage resulted in improved BOD removals. When no chemicals were added the authors reported phosphorus removals between 20 and 40 percent. The authors concluded that the RBC system provides good removals of BOD, SS, and ammonia nitrogen for the Columbus wastewater under an hydraulic loading of  $1.5qpd/ft^2$ .

Murphy et al. (1977) compared the denitrification capabilities of a suspended growth

system, a submerged RBC unit, and four upflow submerged packed columns. The denitrification rate for the RBC was found to be independent of nitrate and nitrite concentrations. Efficient and predictable removals at all temperatures normally encountered were reported. The effluent from the RBC was found to be quite low in suspended solids, indicating the possibility of eliminating further clarification.

Odegaard and Rusten (1980) studied nitrogen removal using RBC's. The experimental unit consisted of a submerged RBC for denitrification followed by a semisubmerged RBC for nitrification. Nitrified effluent was recycled from the nitrifying unit to the denitrifying unit. Raw wastewater was used as the carbon source for denitrification. Three different types of raw wastewater were used in the experiments including synthetic sewage, municipal sewage, and landfill leachate. The authors found that it was unnecessary to cover the free water surface of the submerged RBC, as denitrification took place at  $O_2$  concentrations in the water of 1 mg  $O_2/l$ . The proposed process was demonstrated to give good nitrogen removals for all three wastewaters. Organic matter was found to be consumed at a rate of 3g COD/g NOx-N removed. To achieve complete denitrification the ratio of soluble COD to NOx-N should be greater than seven. The authors concluded that the main advantages of the process were that no external carbon source was required, and that the process would be economical from both an investment and operations view point.

Noss et al. (1980) studied wastewater recarbonation using an RBC. Pilot studies were conducted using domestic sewage. Raw degritted wastewater was pumped to a rapid mix tank where lime was added for phosphorus removal purposes. The wastewater then flowed through a flocculation basin, a primary settling tank, an RBC unit, and finally a secondary clarifier. The concentration of phosphorus in the wastewater was reduced from values as high as 10.5 mg/l to values of 2 mg/l or less. The RBC unit successfully recarbonated the high pH wastewater with no deleterious effects on the biofilm organisms being reported. Advantages to the above process included reduced RBC surface area requirements for BOD removal due to the BOD reduction achieved during chemical precipitation, and the production of an excellent environment for nitrification in the later RBC stages due to biological oxidation of organics in the initial RBC stages.

Ouyang (1980) attempted to characterize the sludge from two conventional RBC pilot facilities. One facility had two stages, while the other had four. He found that biofilm thickness was dependent on the organic loading and rotational speed for both units. He attempted to compare the solids concentration in the RBC with the conventional activated sludge plant using a value he referred to as the equivalent suspended solids concentration (ESS). The ESS was defined as the dry weight of solids on the disks in  $g/m^2$  divided by the liquid volume to disk surface areas as  $l/m^2$ . Ouyang found the ESS to vary with the organic loading. He also found an average ESS of 9,000 mg/l for the 4 stage process when the BOD loading ranged from 10-641 g  $BOD/m^2$  day. The 2 stage process had an average ESS of 11,000 mg/l for the same loading. It was argued that the high values of ESS explained the higher efficiencies of RBC's compared to the activated sludge process. Using the ESS and surface loading rates, the author estimated the F/M ratio of the RBC process to be 0. 2 - 0. 4 g BOD/g ESS day, which is similar to that of the activated sludge process.

Analysis of the biofilm was done and Ouyang reported an average volatile fraction of 74% with a water content of 95%. The reported chemical composition was  $C_{4.2}H_8N_{0.6}O_2$ . RBC sloughed solids were reported to settle quickly. The treatability of the RBC solids using aerobic or anaerobic treatment was reported to be better than that of activated sludge.

Hynek and Lemura (1980) looked at phosphorus and nitrogen removal using the RBC. In particular the authors looked at nutrient removal using Autotrols' Bio-Surf

Process. Both conventional and submerged units were used. Conventional RBC units were used for nitrification and BOD removal. Submerged RBC units were used for denitrification. Methanol was the carbon source used for denitrification. Phosphorus removal was achieved by alum or ferric chloride addition. The authors reported a minimum phosphorus removal of 80% using the Autotrol process.

Knoetz et al. (1980) studied the inhibitory effects of various heavy metal ions and organic inhibitors. Laboratory scale activated sludge and RBC units were used in their study. The authors found that nitrifying and denitrifying rotating disc units, and nitrifying-denitrifying activated sludge units had completely different responses to inhibitory substances. The denitrifying RBC showed greater resistance to inhibition by  $Cr^{6+}$  and  $Ni^{+2}$  than did the nitrifying RBC and activated sludge units. However, nitrifying discs and activated sludge units tolerated  $Zn^{+2}$  and  $Cd^{+2}$  salts much better than the denitrifying disc units. The activated sludge units were not affected as greatly by mercury salts as were both rotating disc units.

Of all the organics studied only dithane and dichlorophenol had significantly different effects on the units studied. The denitrifying discs had a much higher tolerance for dithane, while the nitrifying discs had a much higher tolerance for dichlorophenol.

Smith and Khettry (1980) reported on the results of a pilot study done by the Ontario ministry of the Environment using RBC's for complete nitrogen removal. The pilot plant consisted of two RBC's in series. The first unit nitrified the incoming wastewater. A second RBC was used for wastewater denitrification. The second RBC was operated in both the submerged and semi-submerged modes to determine the effect of disc submergence on denitrification efficiency. Close to full ammonia oxidation was attained in the nitrification RBC module. The DO concentration in the nitrifying RBC was found to increase with increasing disc rpm at the expense of nitrate removal in the denitrification module. When the denitrification module was operated in the

full submergence mode a nitrate removal efficiency of 91% was attained. The semisubmerged mode of operation resulted in much lower nitrate removals.

Singhal (1980) reported on phosphorus and nitrogen removal at the Cadillac, Michigan wastewater treatment plant. RBC's were used for ammonia nitrogen removal and addition of ferric chloride to the aeration system was used for phosphorus removal. The author found high concentrations of heavy metals in the ferric chloride being used for phosphorus removal. The heavy metals were found to accumulate in the biofilm of the nitrifying RBC and it was hypothesized that they may inhibit nitrification to some degree.

The Japanese company Kubota (1980) made a Japanese patent application for an RBC system to provide nitrification and denitrification in the same reactor. Nitrification would be carried out in the RBC unit when the discs were partially immersed in wastewater. Denitrification would be carried out when the discs were completely submerged in the wastewater. The advantages claimed for this system included the fact that nitrification and denitrification could be carried out in the same RBC unit, installation costs are saved, the addition of a carbon source for the denitrification process is not required, and the amount of alkali matter used in the nitrification process is decreased due to the increase in alkalinity in the denitrification procedure.

Masuda et al. (1983) used a completely closed RBC unit to investigate simultaneous nitrification and denitrification (SND) in an RBC biofilm. Vent holes in the reactor were closed and the experimenters monitored the concentrations of oxygen, nitrogen, and total gas with time. The removal rate of nitrogen due to simultaneous nitrification and denitrification was found to be dependent upon ammonia loading, organic loading, mean cell residence time, and the pressure of oxygen in the gas phase. A long mean cell residence time accompanied by low concentrations of ammonia and organic matter resulted in increased removal of nitrogen by simultaneous nitrification and denitrification.

High ammonia and organic loadings and short mean cell residence time resulted in little nitrogen removal by SND. Experiments were conducted in which ammonia and organic loadings, as well as the partial pressure of oxygen were varied. The rate limiting step for SND was goverened by the partial pressure of oxygen. When the partial pressure of oxygen was low, nitrification limited SND in the biofilm. As the partial pressure of oxygen increased, the diffusion of organic matter to the denitrifying bacteria limited SND. The value of partial pressure governing the rate limiting step was dependent on the organic loading. Experiments were carried out to determine the optimum C/N ratio for SND.

Masuda et al. developed a biofilm model to explain their results. It was proposed that the biofilm was comprised of an outer heterotrophic layer, an autotrophic layer, and an inner anaerobic layer. The gradients of organic carbon, dissolved oxygen, and ammonia would determine the dominant bacterial species. The rate limiting step in SND would also be determined by the above gradients. The authors concluded that it was theoretically possible to accomplish organic oxidation, nitrification, and denitrification in the same RBC reactor.

Much of the research discussed in the above literature review lends credence to the concept of using an RBC, operated in a sequencing batch mode, to remove phosphorus from wastewater by the enhanced biological phosphorus removal mechanism. With the recent advances made in the understanding of the biochemical mechanisms governing enhanced biological phosphorus removal, the application of this technology to treatment processes, other than activated sludge, appears possible. Research done by Irvine and others has shown that SBR's can be used for biological phosphorus removal at both lab and full scale. The patent application brought forth by Kubota (1980) suggests RBC's can be operated successfully in a sequencing batch mode with alternating aerobic and anaerobic conditions. The discovery of both poly-P and PHB inclusions in the biofilm

bacteria of RBC's by Kinner and Maratea (1985) suggests that the proper 'bio-P' bacteria can be encouraged to grow in a fixed-film culture.

The primary objective of this research was to prove that a lab scale sequencing batch RBC could in fact be used for enhanced biological phosphorus removal. It was hoped that knowledge gained with the lab scale reactor could be used to provide guidelines for the design of a pilot-scale facility.

## Chapter 3

## Methodology

This section describes the rationale for the proposed process, the individual process components, system operation, seeding, sewage characteristics, sampling procedures, and analytical techniques used.

3.1 The Experimental Unit

#### 3.1.1 System Rationale

Due to recent advances in the understanding of the mechanisms governing biological phosphorus removal, the application of this technology to fixed film wastewater treatment systems now appears possible. In January 1987 an investigation into the use of an RBC system for biological phosphorus removal was started. The RBC was chosen due to its low operating and maintenance costs.

For enhanced biological phosphorus removal to take place 'acclimatized microorganisms' must be subjected to alternating anaerobic and aerobic conditions. The presence of short chain fatty acids and absence of nitrates in the anaerobic phase is also essential.

To achieve the necessary anaerobic/aerobic sequence, the rotating discs of a laboratory scale RBC were designed to be subjected to varying degrees of submergence. Initially, the reactor was filled to ensure the rotating discs were completely submerged.

## Chapter 3. Methodology

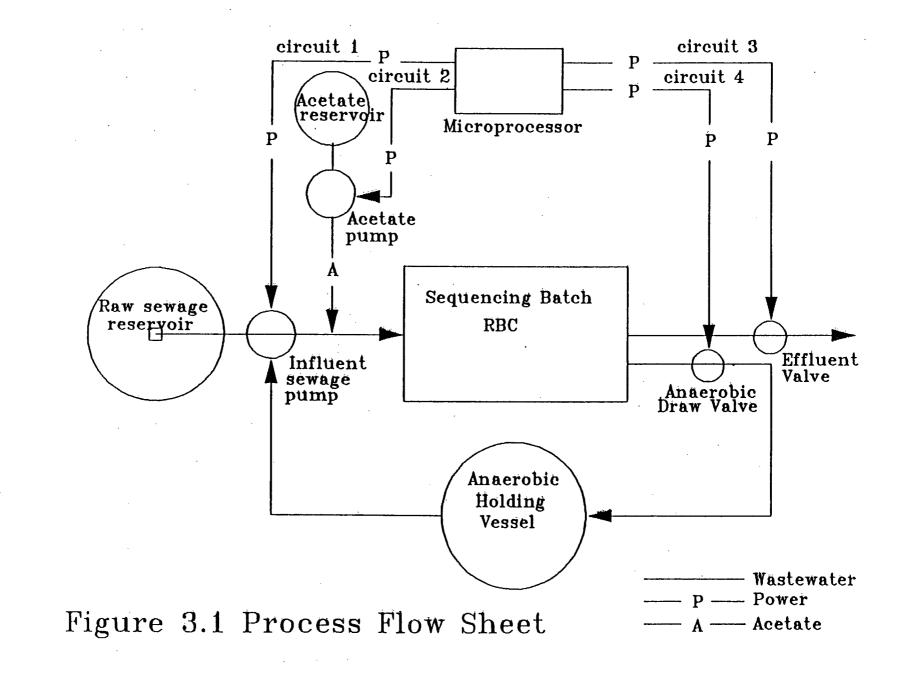
During the fill period, acetate was added to the influent line of the reactor. Following the fill period the reactor was operated in the batch mode. Enough time was allowed for the necessary anaerobic processes to take place. Theoretically, the bacteria attached to the RBC discs would take up the added acetate for carbon storage and release phosphorus into the bulk solution from their poly-P-reserves.

After the anaerobic react period part of the reactor contents were then drawn off into a holding vessel to later become part of the influent for the next cycle. The partially submerged discs were then rotated during the batch aerobic react period. Oxygen would be available from the atmospheric air via the bulk liquid. With the availability of oxygen, the biofilm bacteria would be able to use the carbon stored during the anaerobic period to store phosphorus. Any nitrification of the wastewater was considered to be a secondary benefit of the proposed process.

Following the aerobic period treated effluent and sloughed solids were decanted to a liquid/solid separation unit. Removal of accumulated phosphorus from the system coincided with the removal of sloughed solids in the effluent draw. The end of the effluent draw signaled the beginning of the next fill period.

Raising and lowering the rotating discs was considered as an alternative to the above mode of operation. This option was not pursued in this research due to the difficulty of constructing such a system for laboratory scale operation.

Process control was achieved using an electronic timer/microprocessor. Predetermined times for pumping, decanting, and reaction could be programmed into the individual circuits. Solenoid valves were used to control the water level in the reactor. The disc rotational speed was kept constant at all times. A process flow sheet of the experimental unit is shown in Figure 3.1. Table 3.1 shows the operating schedule with the desired reactions.



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Process Step	Microprocessor status	Desired Reaction
1. pump	circuits 1 and 2 on circuits 3 and 4 off	Sewage pumped from the raw sewage reservoir and anaerobic holding vessel into the RBC. Rotating discs submerged to create anaerobic conditions. Acetate added during pump phase.
2. Anaerobic react	all circuits off	Submerged rotating discs. Biofilm bacteria store added acetate as PHB and release phosphorus.
3. Anaerobic decant	circuit 4 on all other circuits off	Partially treated wastewater drawn into anaerobic holding vessel. Gravity flow when anaerobic valve on. Draw stops at draw line level. Discs now 50% submerged.
4. Aerobic react	all circuits off	Biofilm bacteria now exposed to oxygen. Stored PHB used to store phosphorus as poly-P. Nitrification and majority of COD reduction take place.
5. Effluent decant	circuit 3 on all other circuits off	Treated effluent and P rich sloughed solids decanted for solid/liquid separation.

# Table 3.1: Operating Schedule

Prior to the start-up of this system a number of concerns of peers with respect to the potential success of the process were given special consideration. Paramount among their concerns was the ability of such a system to remove phosphorus in the sloughed solids at a rate equal to the rate of phosphorus accumulation. Other concerns brought forth included the difficulty of achieving anaerobic conditions in the laboratory unit. The above concerns were kept in mind in the design of the system prototype.

# 3.1.2 Reactor and Rotating Discs

The laboratory-scale reactor consisted of three main pieces including the reaction vessel, the shaft and attached discs, and a plexiglass cover with its attached bearing supports.

The 20 litre reaction vessel was rectangular in plan with a cylindrical bottom. Plexiglass construction was used throughout. The reaction vessel was formed in two parts due to the difficulty of forming long plexiglass pieces. Inlet, anaerobic draw, and effluent pipes were 6 mm in diameter.

Thirty four, 19.5 cm diameter polyethylene discs were attached to a 6 mm diameter stainless steel shaft. The shaft was 46 cm long. The discs were fitted to the shaft in two groups of 17 discs. Each group of discs was spaced 3.5 cm from the end of the shaft center. Individual discs were spaced 1 cm apart on centers. A 25 mm diameter stainless steel sprocket was welded to the shaft at a distance of 1 cm offset from center. A 6 mm inch pitch was used for the drive sprocket.

The cover and attached bearing supports were made of 1 cm thick plexiglass. Each bearing support was 19.5 by 22 cm. A teflon friction bearing was set into each support 16 cm from the top of the plexiglass cover. The cover was constructed with two 5 centimeter diameter air vents, a 1.5 by 7 cm slot for the drive chain, a 14 by 18 cm plexiglass pad on which to mount the drive motor, and a 2 cm diameter hole at the influent end of the RBC reaction vessel to accommodate an ORP probe. The entire

reactor assembly is shown in Figure 3.2.

The drive motor was bolted to the plexiglass pad. Due to the structural flexibility of the plexiglass bearing supports the disc/shaft assembly could be easily fitted into the Teflon bearings. The drive chain was then attached to the stainless steel sprockets. The entire assembly including the discs and the shaft would fit into the reaction vessel, and be supported by the side walls of the reaction vessel, and the center compartment wall. A slot was cut into the center wall to accommodate the shaft, drive sprocket, and chain. The assembly is shown in Figure 3.2. The above construction allowed for easy shaft and media maintenance.

Special metal clips were constructed to support two floating covers for the reaction vessel. The styrofoam covers were provided to minimize the surface exchange of oxygen between the atmosphere and the bulk liquid during the anaerobic phase. The metal clips allowed the styrofoam covers to rest 6 cm above the rotating discs during the aerobic react period. Figure 3.3 shows the details of the metal clips and floating covers.

Prior to the anaerobic react period the reaction vessel would be filled to a level above that of the metal clips allowing the covers to float.

#### 3.1.3 Disc Drive Unit

The mechanical drive unit consisted of a DC gearmotor, a variable speed controller, two stainless steel sprockets and 6 mm pitch stainless steel chain.

A 1/8 hp Dayton gearmotor model 4Z135B was used to rotate the shaft and attached discs. The motor was connected to a Dayton SCR Controller model 60648. This configuration allowed for motor rotational speeds from 0 to 100 RPM. A 2.5 cm diameter stainless steel sprocket was welded to the gear motorshaft. A stainless steel chain was connected between the 2.5 cm sprocket and the 5 cm sprocket on the RBC shaft. The 2:1 sprocket ratio allowed for disc rotational speeds between 0 and 50 rpm.

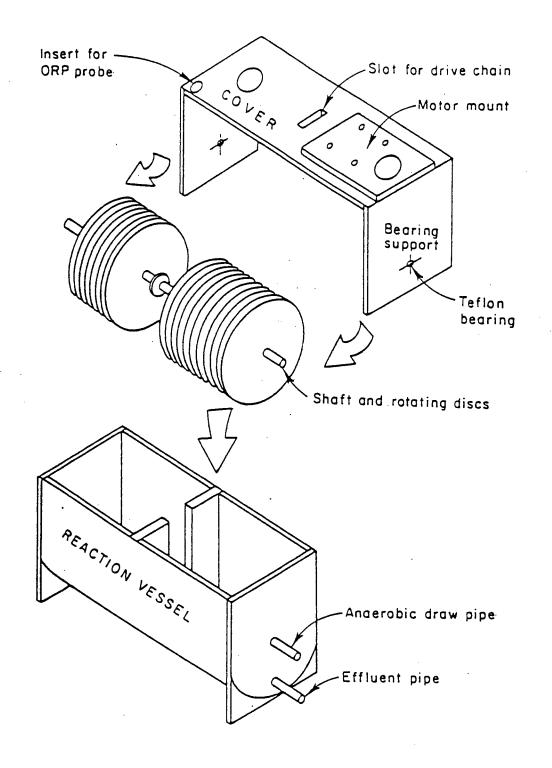
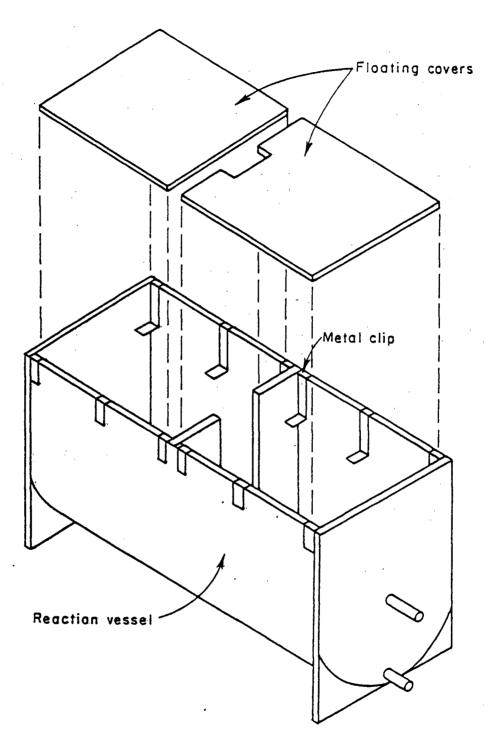


FIG. 3.2. REACTOR ASSEMBLY.



# FIG. 3.3. FLOATING COVERS.

Stainless steel sprockets and drive chain were used due to their resistance to corrosive agents present in domestic sewage. The ability to vary the disc rotational speed over such a wide range gave more process flexibility.

# 3.1.4 Raw Sewage Reservoir and Anaerobic Holding Vessel

At the start of each treatment cycle raw wastewater and anaerobically treated wastewater would be used to fill the reaction vessel. The raw wastewater was stored in a 10 gallon plastic garbage can. The anaerobically treated wastewater was excess wastewater from the anaerobic react period. A five gallon plastic garbage can was used to store this liquid. Both reservoirs were covered and mixed to keep solids in suspension.

The raw sewage reservoir was mixed with a model 4Z135B 1/8 hp Dayton gearmotor. The mixing shaft was a 13 mm diameter stainless steel shaft. A propeller shaped mixer was attached to the end of the mixing shaft. A Dayton model 2Z807 12 RPM gearmotor was used to keep the contents of the anaerobic holding vessel in suspension.

#### 3.1.5 Pumps

Two pumps were used in the RBC process. One pump was used to pump the raw and anaerobically treated wastewater into the reaction vessel at the start of each treatment cycle. A chemical feed pump was used to inject acetate solution into the influent line. Both pumps were operated from separate microprocessor circuits.

The influent sewage pump was a Masterflex model 7520-00 variable speed unit. Two number 15 Masterflex pump heads were used for this pump. One pump head was used to pump from each of the raw sewage and anaerobic holding vessels. A Cole-Palmer model C-1714SP chemical feed pump was used for acetate addition. A 2 litre graduated cyclinder served as the reservoir for the concentrated acetate solution.

# 3.1.6 Timer/Microprocessor and Solenoid Valves

Process control was achieved using an electronic timer/microprocessor and two solenoid valves.

The microprocessor used was a Chrontrol Model CD03 4 circuit microprocessor. The two solenoid valves and all pumps were operated by the microprocessor. All pumps used one circuit each. Each solenoid valve was connected to a seperate circuit. Control programs were written for the three circuits used to control pumping, react, and decanting times.

Two Asco 13 mm model 8030A17 Solenoid valves were used to control disc submergence in the reaction vessel.

# 3.1.7 Miscellaneous

Six millimeter diameter Tygon tubing was used for all flow connections.

3.2 System Operation

The three main process operations of fill, react, and decant were time controlled by the microprocessor. Time requirements for filling and decanting were calibrated prior to system start-up. The amount of time allowed for reaction is discussed in section 3.2.2.

At the beginning of the treatment cycle the circuits controlling pumping were activated. All other microprocessor circuits were off at this time. Equal volumes of raw and anaerobically treated wastewater were pumped from the respective reservoirs into the reaction vessel. Acetate solution was injected into the influent line at a predetermined rate and concentration to give the desired concentration in the reaction vessel. During the anaerobic react period all microprocessor circuits were turned off.

At the end of the anaerobic react period, the circuit controlling the solenoid valve for the draw line at the reactor midpoint was activated. A portion of the reactor contents were decanted into the anaerobic holding vessel to form part of the influent for the next cycle.

With the discs partially submerged, the necessary aerobic reactions could take place. At the end of the aerobic time period, the circuit controlling the solenoid valve for the effluent draw line, at the bottom of the reaction vessel, was opened. Treated effluent and phosphorus rich sloughed solids were then decanted to an effluent bucket.

At the end of the effluent decant period, the pump circuit was activated once more, to signal the start of the next treatment cycle.

# 3.2.1 Disc Rotational Speed

The U.S. Environmental Protection Agency design manual for rotating biological contactors suggests that the peripheral velocity of the rotating discs of an RBC unit be 0.3 m/s for good oxygen transfer and biofilm management. For the 19.5 cm diameter discs employed in these experiments this corresponds to a disc rotational speed of 30 RPM. Prior to the installation of floating covers, a disc rotational speed of 10 RPM was employed. The lower speed was chosen in an attempt to strike a balance between the requirements of the anaerobic and aerobic phases. It was hoped the lower rotational speed would result in lower surface turbulence, and therfore reduced oxygen transfer, in the anaerobic phase. However, the lower rotational speed resulted in an undesirable lag in the time required to go from anaerobic to aerobic conditions following the anaerobic decant period. Biofilm bridging between discs also resulted due to the inadequate shear given at the lower rotational speed. Following the installation of the floating covers, the disc rotational speed was increased to the suggested value and the above was corrected.

# 3.2.2 Length of Reaction Phases

The length of anaerobic and aerobic time required for the necessary reactions to take place was chosen on the basis of the past experiences of other researchers with activated sludge. An anaerobic react period of one and a half hours and an aerobic react period of three hours were used. Batch tests proved these times to be quite conservative. In retrospect, these times could have been shortened considerably.

# 3.2.3 Acetate Addition

Acetate was added to the reactor to give a concentration of 25 mg/l as COD during the anaerobic react phase. The results of Comeau (1984) and others have shown that a concentration of 15 mg/l would be more than adequate for lab scale activated sludge units. The higher concentration was chosen to overcome any mass transfer limitations that might be present. Again, the choice proved to be very conservative.

## 3.2.4 Flow Management

Due to the unusual shape of the reaction vessel, more wastewater entered the anaerobic holding vessel during the anaerobic decant period than was reintroduced to the RBC from this vessel during the reactor fill period. The resulting flow accumulation in the anaerobic holding vessel of 11. 5 liters per day was transferred to the raw wastewater reservoir at the head end of the plant. This situation was unavoidable due to the difficulty of predicting the amount of fluid displaced by the biomass at any period of time. To alleviate the problem, a more sophisticated process control system would have been required. One of the methods which was attempted to alleviate this problem included pumping the anaerobic holding vessel dry during each fill period. This practise increased the proportion of anaerobically treated wastewater to raw wastewater in the influent, and also introduced oxygen into the anaerobic phase.

# 3.3 Seeding

Seeding of the RBC reactor was undertaken to reduce reactor start-up time, and increase the likelihood of fixing the desired micro-organisms to the rotating discs.

Fletcher (1979) discussed the attachment of bacteria to surfaces in aquatic environments. She found that an increase in either culture concentration or exposure time resulted in an increase in the number of bacteria attached to a surface.

Using Fletcher's results as a guide, a seeding program was devised. Thickened sludge was obtained from the pilot-scale enhanced biological phosphorus removal plant at the University of British Columbia. The MLSS concentration of the thickened sludge was approximately 5,000 mg/l. A 50:50 mixture of raw sewage and thickened sludge was added to the reaction vessel. At this time the reactor was operated in a batch mode, at a disc rotational speed of 10 RPM. An acetate slurry was added to the reaction vessel four times daily to supplement carbon levels. The concentrations of C, N, and P were monitored daily to ensure nutrient levels were adequate.

Each day, the disc rotation was stopped and the 'mixed liquor' in the reaction vessel was allowed to settle. The supernatant was decanted and the removed volume was replaced with fresh sewage. This practise ensured adequate concentrations of N, P, and trace elements daily. Supplementing carbon levels with acetate kept measured bacterial growth rates at accelerated levels. Nilsson and Dostálek (1984) showed that the ability of a pure culture of Pseudomonas putida to form a biofilm increased with increasing growth rate.

Characteristic	Concentration Range (mg/l)				
COD	100-300				
SS	NA				
TP	3.5-4.5				
TKN	15-25				

# Table 3.2: Typical Characteristics of UBC bio-P plant Influent Sewage

#### 3.4 Sewage Characteristics

Raw sewage was obtained from the U. B. C. biological phosphorus removal pilot plant inflow. The plant treats domestic sewage from a two thousand person residential complex. The typical composition of the raw sewage during the experimental period is given in Table 3.2.

#### 3.5 Sampling Procedure

Sampling was carried out twice weekly. One sample run consisted of raw, influent (raw wastewater plus wastewater from the anaerobic holding vessel), anaerobic draw (wastewater decanted to the anaerobic holding vessel immediately after the anaerobic react period), and effluent samples. Total and soluble COD, NOx, TKN, TP, and orthophosphate analysis were run on all of the above samples. Raw and effluent samples were also analyzed for suspended solids concentration. A biofilm sample was taken for a % P determination once weekly. The above sampling procedure gave a quick scan of the process treatment performance.

Batch testing was conducted during the second weekly sampling run. In addition to the regular raw wastewater, influent and effluent samples volatile fatty acid, soluble COD, orthophosphate, and NOx samples were drawn from the reaction vessel at predetermined times, during the anaerobic and aerobic react phases. These samples

were taken using a 60 ml syringe. Batch testing results gave the concentration of the bulk liquid soluble species with time. A strong phosphorus release during the anaerobic period, followed by a subsequent phosphorus uptake, during the aerobic period would be considered as confirmation of enhanced biological phosphorus removal. ORP measurements were also taken during all batch tests.

# 3.6 Analytical Techniques

## 3.6.1 Nonfilterable Residue (Suspended Solids)

Nonfilterable residue (SS) was determined by filtering a known volume of sample through a standard glass fiber filter (Whatman 934AH), and drying the sample to a constant weight at 104° C. The difference in the weight of the dried filter before and after sample filtration divided by the filtered volume was taken as the concentration of nonfilterable residue in mg/l.

# 3.6.2 Nitrogen (N)

# (a) Nitrate plus Nitrite-Nitrogen (NOx)

NOx was analyzed using the Technicon Method 100-70W (1973) with the Technicon Autoanalyzer II. In the above procedure, nitrate is reduced to nitrite by a coppercadmium reductor column. Nitrite ion reacts with sulfanilamide under acidic conditions to form a diazo compound. A reddish purple azo dye is formed after the coupling of diazo compound with N-1-naphthylethylenediamine dihydrochloride. A detection limit of 0.04 mg  $NO_3$ -N/l is achieved.

# (b) Total Kjeldahl Nitrogen (TKN)

TKN samples were digested, prior to sample analysis, using the block digestor

method with sulfuric acid. Sample analysis was carried out on the autoanalyzer according to Technicon autoanalyzer II, Method No. 146/71A (1972).

## **3.6.3** Oxidation-Reduction Potential (ORP)

ORP was measured during all batch tests. A Broadley James Corp. Ag/AgCl probe was used for all measurements.

#### 3.6.4 Phosphorus

Ortho and total phosphorus were both measured. The percentage of phosphorus in the biofilm was also measured.

### (a) Orthophosphate $(PO_4)$

Samples were filtered using a 0.45 micron membrane filter. The automatic ascorbic acid reduction method (Technicon Autoanalyzer II, Method No. 94-70W, 1973) was used to analyze filtered samples for ortho-phosphate. The detection limit is 0.2 mgP/l.

#### (b) Total Phosphorus

Total Phosphorus samples were digested prior to sample analysis, using the block digestor method with sulfuric acid. Sample analysis was carried out on the Autoanalyzer according to Technicon Autoanalyzer II, Method No. 327-73W (1974).

## 3.6.5 Biofilm Phosphorus Content

Biofilm samples were scraped from the rotating discs once weekly. Scrapings were dried to a constant weight at 104° C, and then ground into a powder. A weighed aliquot of the above powder was then analyzed for total phosphorus using the method described above. Results were expressed as (mass P/mass SS) on a percentage basis.

## 3.6.6 Volatile Fatty Acids (VFA's)

Concentrations of three VFA's were determined: acetic, propionic, and butyric acids. Samples were preserved by filtration and freezing. Sample analysis was done using the Hewlett-Packard HP 5880 gas chromatograph with a flame ionization detector (FID). The analysis procedure followed is outlined in Supelco Bulletin 751E (1982).

Sample injection was carried out using microsyringes (Hamilton Model 75N, #87900, 5 micro liters). The gas chromatograph column was 0.91 m long with a 4 mm internal diameter. Sulpeco 60/80 Carbopack C/0.3% Carbowax 20M/0.1%  $H_3PO_4$  was used to pack the column. Prior to sample injection, the pH was adjusted to below 3 by adding phosphoric acid giving a 1% acid solution. The experimental conditions for the chromatagraph were: injection port temperature=150° C, detector temperature=110° C, and carrier gas flowrate=20ml/min. The standards were prepared using reagent grade acetic (99.9%), propionic (96%), and butyric (98%) acids.

#### 3.6.7 Chemical Oxygen Demand (COD)

COD was determined according to method 220 of Standard Methods for the Examination of Water and Wastewater 13th edition (1971).

#### 3.6.8 Disc Solids

Biofilm was washed from the discs and onto  $74 \times 48 \times 4$  cm drying dishes, at the end of the experiments. The drying dishes were placed in a fume hood for 48 hours to allow the majority of the water to evaporate. The biofilm cake was then scraped into a two litre pyrex beaker and dried to a constant weight at 104° C, to determine the total biofilm weight. The pyrex beaker was then fired at 550° C in a muffle oven to destroy all volatile compounds. The above allowed the determination of both total and organic solids present in the biofilm.

#### Chapter 4

#### **Results and Discussion**

In this chapter experimental results are presented first. Data analysis is presented in the second section of the chapter. Finally, the results are discussed in the context of the data analysis provided.

The experimental results are presented in three separate sections each corresponding to a different time period. The first time period was from September 1, 1987 to February 1, 1988. For the first time period the anaerobic holding vessel was included in the process. The second time period ran from February 6, 1987 to February 16, 1987. During the second time period the anaerobic holding vessel was taken off line and the anaerobic draw was returned directly to the raw sewage vessel. During the third time period the anaerobic holding vessel was put back on line. The pumping rate from this vessel was adjusted to double the value it had been during the September 1,1987 to February 1,1988 time period. The increase in pumping rate ensured the anaerobic holding vessel was pumped dry during each reactor fill period.

#### 4.1 Results

#### 4.1.1 Phase One (September 1,1987- February 1, 1988)

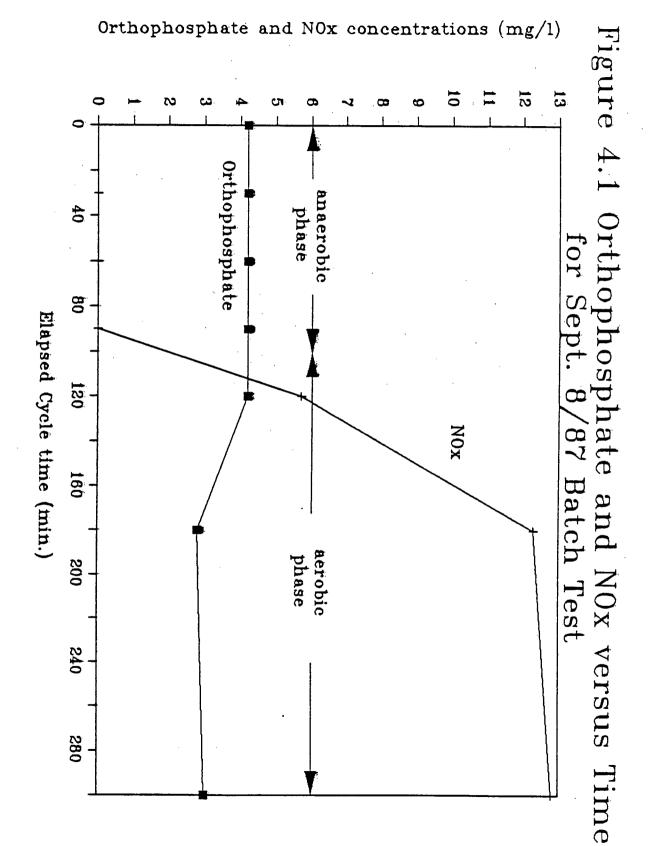
Seeding of the reactor was started on July 30th, 1987. A good biofilm was evident on the discs by mid August.

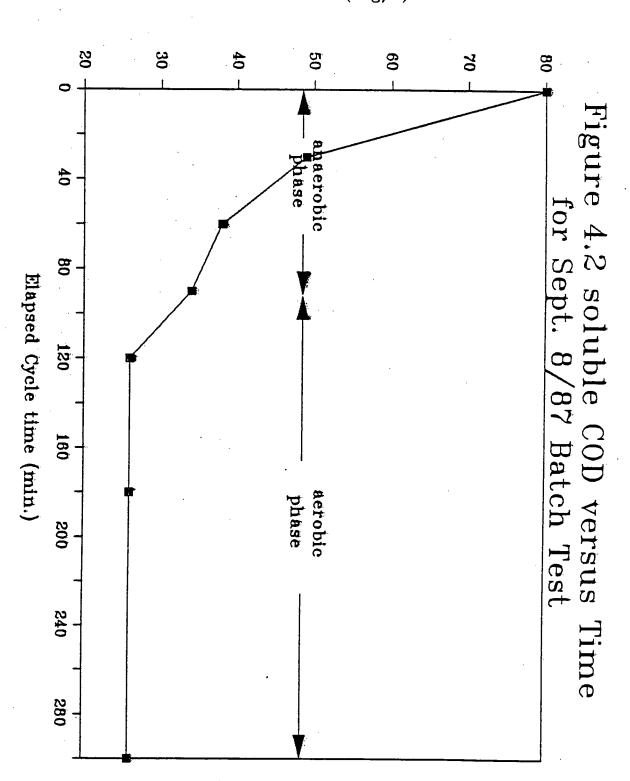
Sampling was started September 1st, 1987. Initially, phosphorus removal in excess

of growth requirements was not observed. The results of a batch test conducted on September 8/87 are shown in Figures 4.1 and 4.2. No anaerobic phosphorus release was observed at this time. It was suspected that true anaerobic conditions were not being achieved.

A shaft failure on September 19/87 supplied the opportunity to retrofit the system with floating covers for the RBC reaction vessel, the raw sewage reservoir, and the anaerobic holding vessel. It was hoped that the above changes would drastically reduce any surface exchange of oxygen between the atmosphere and the liquid. The system was put back on line on September 22/87. Effluent soluble phosphorus levels of less than 1 mg/l were observed on September 29 and 30. A batch test was run on October 5. The results for the above test are shown in Figures 4.3,4.4, and 4.5. The sudden decrease in the reactor  $PO_4$ , and increases in  $NO_x$  concentration and ORP at ninety minutes coincides with the draw off of a portion of the reactor contents to expose the discs for aerobic conditions. Bulk liquid ORP reached a level of -120 mV (vs. Ag/AgCl) during the anaerobic phase of the treatment cycle. The ORP value in the immediate vicinity of the biofilm was probably much lower. A phosphorus release of 1.6 mg/l of anaerobic volume was observed in the anaerobic phase. Uptake in the aerobic phase was 5.6 mg/l of aerobic volume. Overall percent phosphorus removal was 93%. Twenty-five milligrams per litre of acetate as COD was added to the anaerobic volume. A second shaft failure on Octobe<del>r</del> 7 resulted in the system being taken off line for mechanical design modifications.

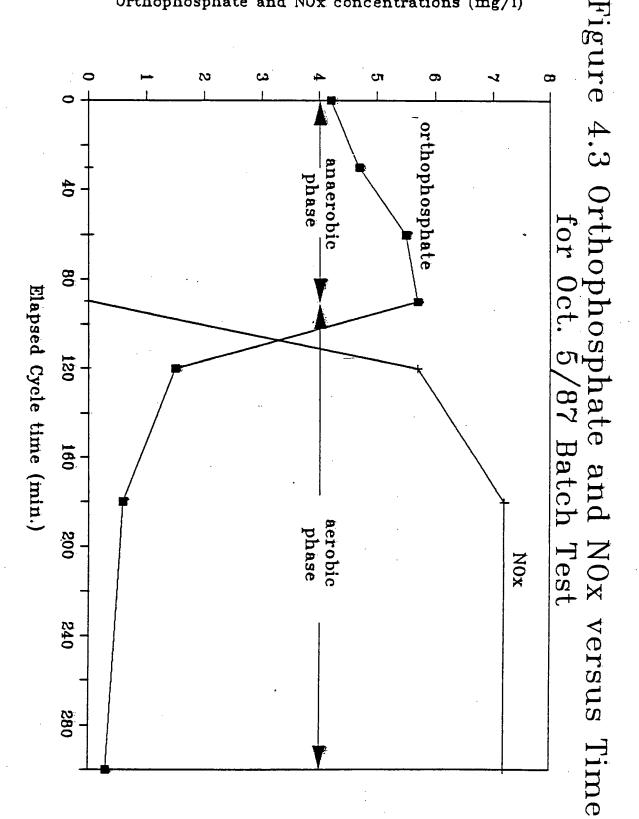
By October 15, 1987 the system was again back on line and seeding of the reactor was initiated. By November 1 a healthy biomass covered the rotating discs. Effluent sampling was started on November 3, and phosphorus removal in excess of seventy percent was observed by November 10, 1987. A batch test run on November 14 confirmed the removal mechanism to be 'enhanced biological phosphorus removal'. During the

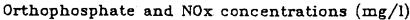


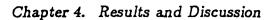


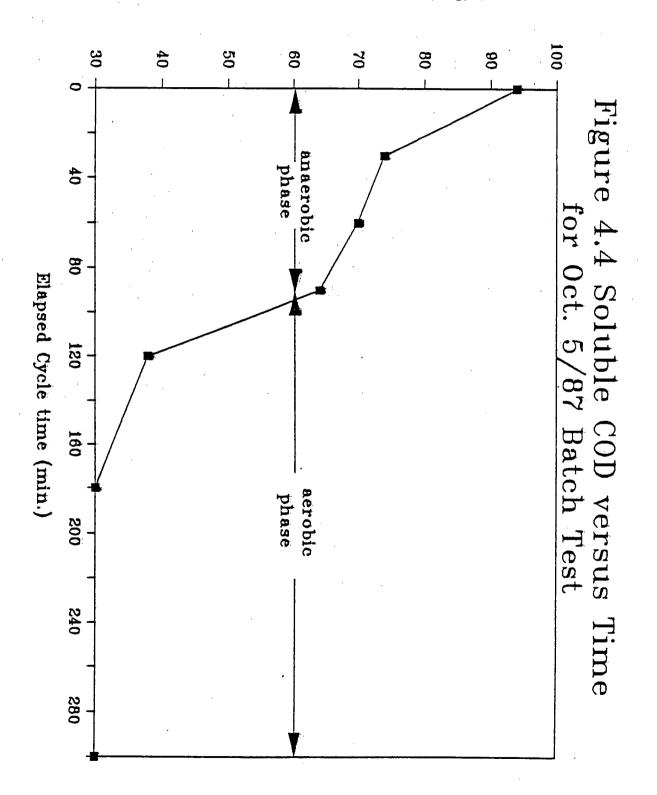
Soluble COD (mg/l)

Chapter 4. Results and Discussion

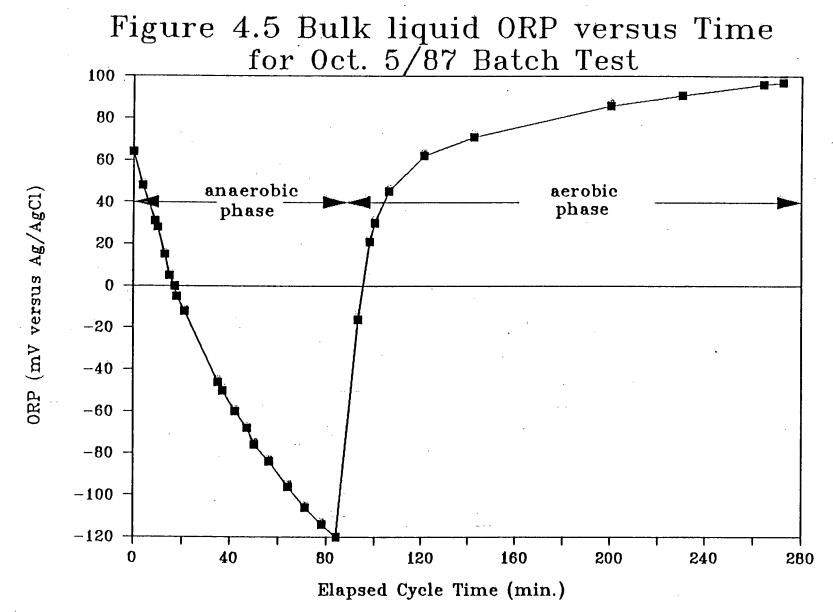








Soluble COD concentration (mg/l)



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Results and Discussion Chapter 4.

above run a phosphorus release of 1.4 mg/l of anaerobic volume was observed during the anaerobic phase. Eighty-three percent phosphorus removal was achieved at this time. Very little nitrification was evident. By November 18, 1987 P removals were consistently above 90%.

Percent COD and phosphorus removals for the period from November 11/87 to February 1/88 are shown in Figures 4.6 and 4.7. Percent nitrification for the same time period is shown in Figure 4.8. Percent phosphorus removals were calculated using raw total phosphorus (TP) and effluent orthophosphate ( $PO_4$ ) values. COD removal was calculated on the basis of raw total COD and effluent soluble COD. Percent nitrification was calculated as the ratio of effluent NOx to raw TKN expressed as a percent. It is likely that some simultaneous nitrification- denitrification took place in the biofilm during the aerobic phase of each treatment cycle, although this was difficult to quantify. The calculation method chosen for percent nitrification was used only to get a relative measure of this parameter between treatment cycles. November 11, 1987 is referred to as day zero of the above time period.

The decline in percent phosphorus removal on day 19 (October 30/87) of the experimental run coincided with a malfunction of the acetate feed pump. No acetate had been added to the anaerobic phase of the treatment cycle for three cycles prior to sampling. The average characteristics of the raw sewage, the reactor combined influent, the anaerobic draw, and the effluent for the time period November 11/87-February 1/88 are shown in Table 4.1. Figures 4.9 to 4.11 give the results of a typical batch test for the above time period. Table 4.2 shows the observed values for percent phosphorus content of the biofilm.

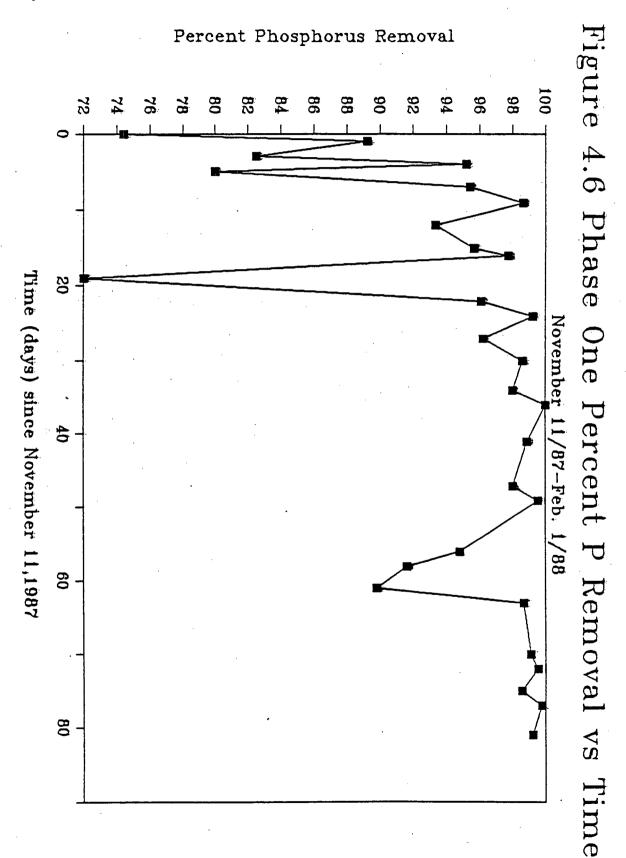
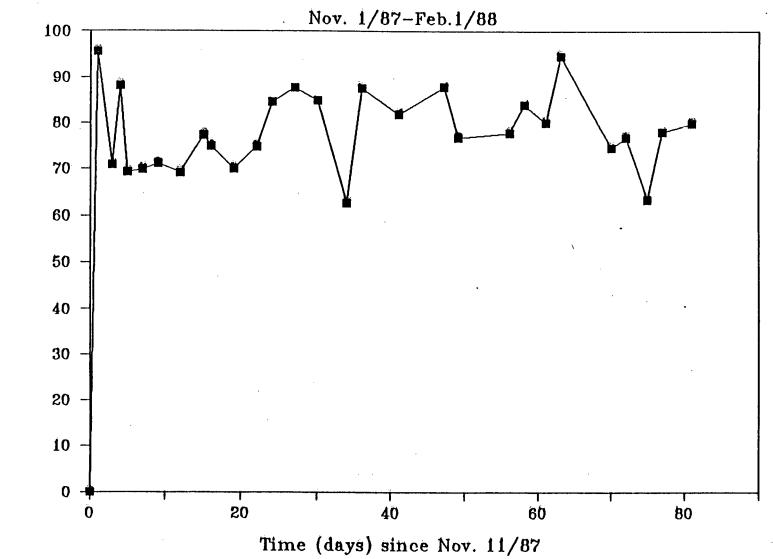




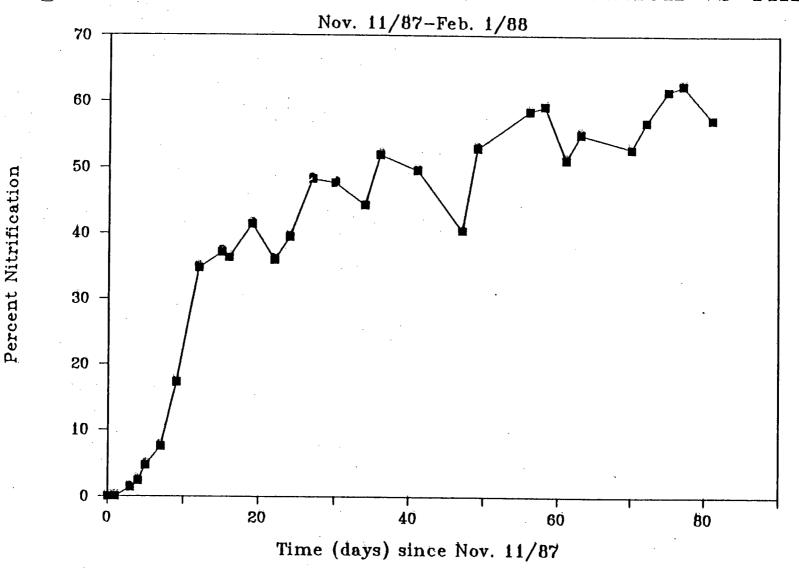
Fig. 4.7 Phase One Percent COD Removal vs Time



Percent COD Removal

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Fig. 4.8 Phase One Percent Nitrification vs Time



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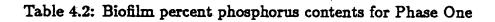
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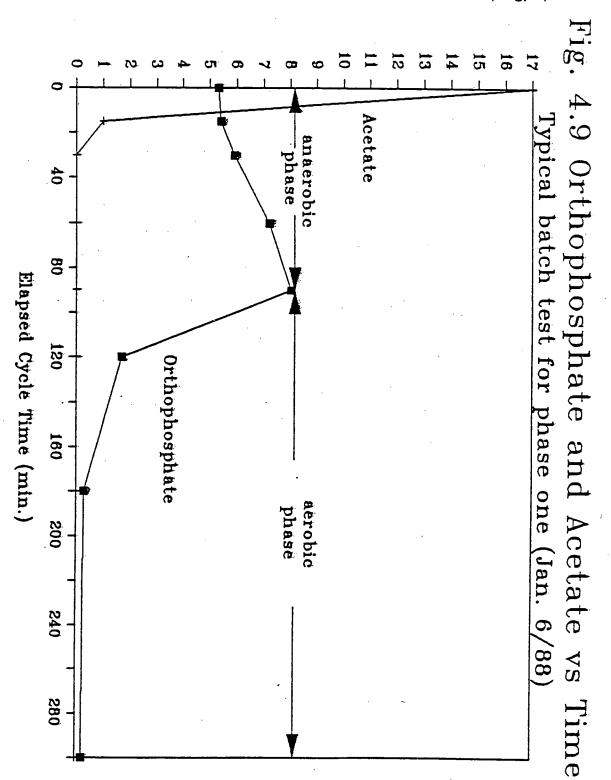
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Raw Sewage								
Concentration (mg/l)								
Value	C	DD	P		] ]	TSS		
	sol.	tot.	PO <sub>4</sub>	TP	NOx	TKN		
Avg.	74	154	NA	4.6	0	20.5	50	
Min.	35	67	NA	3.0	0	15.2	10	
Max.	220	504	NA	6.7	0	30	86	
	Influent Sewage							
		Coi	icentra	tion (1	ng/l)			
Avg.	62	121	5.4	6.6	0	17.6	NA	
Min.	35	53	3.2	4.5	0	12.2	NA	
Max.	101	194	9.0	10.1	0.2	22.5	NA	
		I	Inaero	bic Dr	aw			
		Cor	icentra	tion (1	ng/l)	-		
Avg.	45	NA	8.0	9.3	0	14.9	NA	
Min.	<b>26</b>	NA	4.3	5.7	0	5.0	, <b>NA</b>	
Max.	<b>69</b>	NA	13.1	20.0	0	30.0	NA	
	Effluent							
Concentration (mg/l)								
Avg.	<b>29</b>	NA	0.2	1.4	7.7	7.9	61	
Min.	9	NA	0.0	0.3	0.0	2.2	14	
Max.	54	NA	1.4	5.2	14.5	18.5	186	

Table 4.1: Average Characteristics and Ranges for Nov. 11/87-Feb.1/88

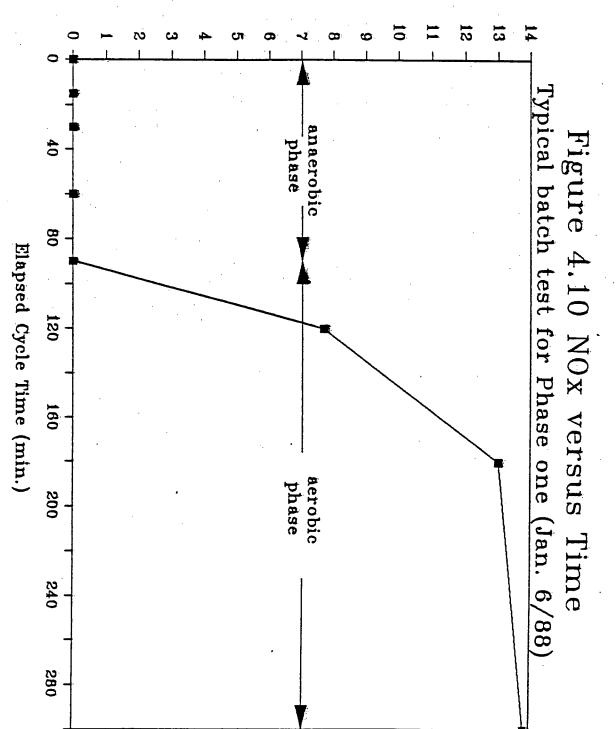
Date	Biofilm %P content	Biofilm %P content			
	(total solids basis)	(volatile solids basis)			
Dec. 17/87	1.84	3.29			
Jan. 6/88	2.04	3.64			
Jan. 13/88	2.39	4.27			
Jan. 20/88	2.30	4.11			
Jan. 25/88	2.27	4.05			
Feb. 1/88	2.00	3.57			





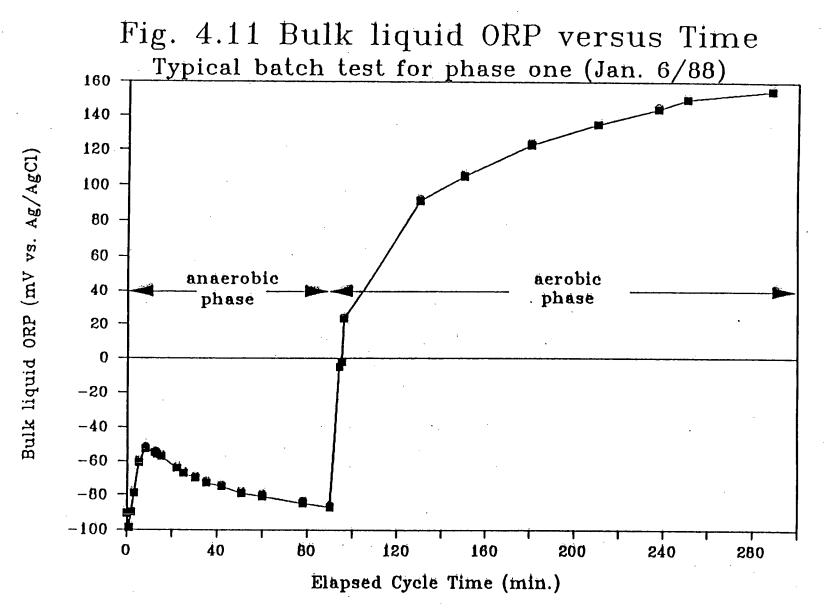
Orthophosphate and Acetate concentrations (mg/l)

Chapter 4. Results and Discussion



NOx concentration (mg/l)

<del>. . . . . . .</del>



Results and Discussion Chapter 4.

#### Chapter 4. Results and Discussion

Due to the large draw of wastewater from the reaction vessel following the anaerobic phase, more fluid was returned to the anaerobic holding vessel than was pumped from this vessel during the influent period. Up until February 1, 1988 approximately 11.5 litres of P rich sewage accumulated in the anaerobic holding vessel daily. To alleviate the problem, excess anaerobically treated sewage was transfered to the raw sewage vessel as required. The above practise lead to large fluctuations in the raw and influent sewage characteristics reported in Table 4.1. Influent P levels as high as 10.1 mg/l were observed during the above time period with no apparent effect on effluent quality. Even higher influent P levels may have been observed if both the raw and anaerobic holding vessels had not been emptied and cleaned weekly.

# 4.1.2 Phase Two (February 6, 1988 - February 16, 1988)

In an attempt to streamline process operation, and eliminate the flow accumulation problem, the anaerobic holding vessel was taken off line on February 6, 1988. Thereafter, the anaerobic draw line returned directly to the raw sewage tank. The rationale behind the above change was that, if returning the accumulated flow from the anaerobic holding vessel to the raw sewage vessel did not affect effluent quality, neither would direct return of the excess anaerobic draw volume.

In fact, the above modification proved to be detrimental to effluent quality. Tables 4.3 and 4.4 show the raw sewage and effluent characteristics for the time period February 6 to February 11, 1988. Daily sampling of the feed tank (raw sewage vessel) was carried out before and after each sewage addition. Samples for effluent phosphate and soluble COD were taken during the treatment cycles prior to raw sewage addition. Treated effluent was collected, between raw sewage additions to the feed tank, to obtain composite samples for TP, TKN, and TSS. Figure 4.12 shows the progressive increase in the total phosphorus concentration of the feed tank between February 6/88 and

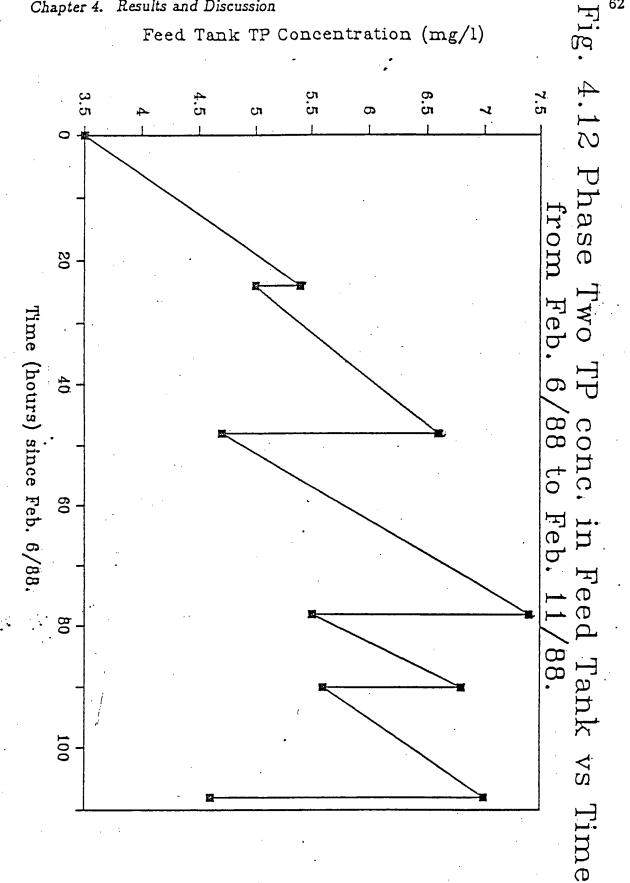
February 11/88. The sharp drops in TP concentration correspond to the addition of fresh sewage to the feed tank. Figure 4.13 shows the steady increase in effluent phosphate concentrations for the same time period.

Feed Tank Sewage Composition								
			Concentration (mg/l)					
Date	Time elapsed since feed tank cleaned (days)	Time when sample taken before(b) or after(a) fresh sewage add	COD(tot. )	PO <sub>4</sub> -3	TP	TKN	TSS	
Feb. 6/88	0	а.	130	2.6	3.5	16.3	25	
Feb. 7/88	1	Ъ	70	4.8	5.4	16.8	25	
Feb. 7/88	1 .	<b>a</b> .	116	3.4	5	15.5	60	
Feb. 8/88	2	Ъ	64	5.6	6.6	12.9	38	
Feb. 8/88	2	3.	110	3.4	4.7	20	37	
Feb. 9/88	3	b	176	5.1	7.4	18.1	119	
Feb. 9/88	3.	<b>a</b> .	· 181	3.8	5.5	16.8	86	
Feb. 10/88	4	Ъ	115	4.9	6.8	18.7	67	
Feb. 10/88	. 4	a	123	4.0	5.6	18.7	65	
Feb. 11/88	5	Ъ	75	NA	7.0	12.2	38	
Feb.11/88	5	a	119	NA	4.6	17.1	64	

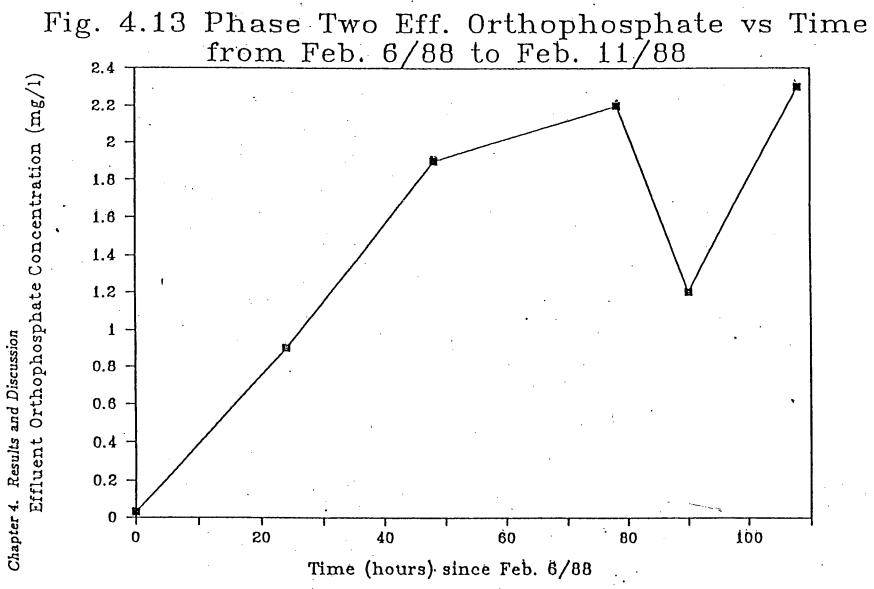
Table 4.3: Reactor Feed Composition Phase Two (Feb.6/88-Feb.11/88)

Effluent Characteristics								
		Concentration (mg/l)						
Date	Time elapsed since feed tank cleaned (days)	COD(sol. )	PO <sub>4</sub> -3	TP	NO <sub>≖</sub>	TKN	TSS	
Feb. 6/88	0	18	0.3	0.7	8.7	2.5	18	
Feb. 7/88	1	55:	0.9	1.7	9.5	2.7	23	
Feb. 8/88	2	26	1.9	2.2	8.2	3.6	44	
Feb. 9/88	3	18	2.2	2.4	8.0	2.7	25	
Feb. 10/88	4	22	1.2	2.6	8.0	4.0	57	
Feb. 11/88	5	22	2.3	2.7	8.7	4.0	42	

Table 4.4: Effluent Composition Phase Two (Feb.6/88-Feb.11/88)



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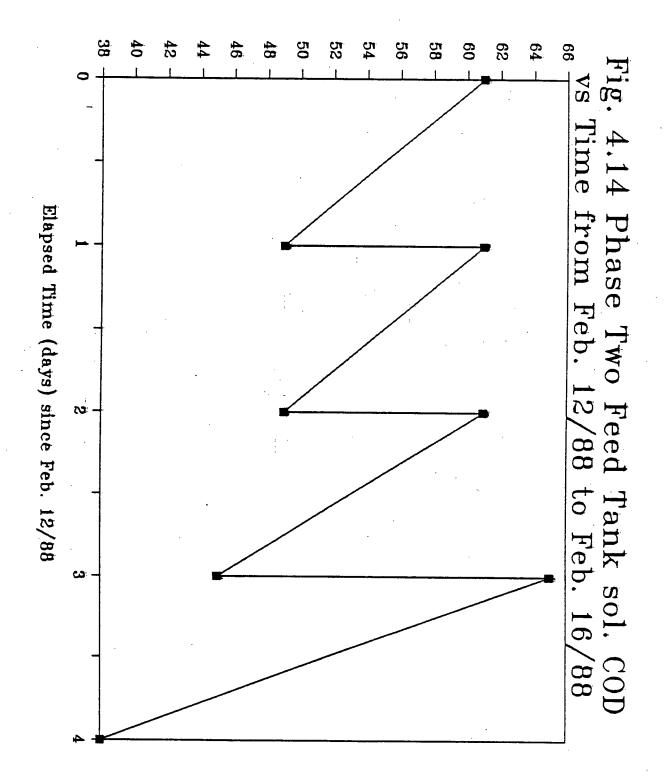
On February 12, 1988 the feed tank was cleaned and refilled with fresh sewage. The raw sewage and effluent characteristics for the time period February 12 - February 16/88 are shown in Tables 4.5 and 4.6. Figure 4.14 shows the concentration of soluble COD in the feed tank with time. The sharp increases in soluble COD correspond to the addition of fresh sewage to the feed tank. The concentrations of TP and  $PO_4$  in the feed tank versus time are shown in Figure 4.15. Again, the sharp decreases in TP and  $PO_4$  concentrations coincide with the daily addition of fresh sewage to the feed tank. Effluent  $PO_4$  concentrations for the Feb.12 -Feb.16/88 time period are shown in Figure 4.16. Figure 4.17 shows the corresponding percent phosphorus removals.

Feed Tank Sewage Composition							
			Concentration (mg/l)				
Date	Time elapsed since feed tank cleaned (days)	Time when sample taken before(b) or after(a) fresh sewage add	COD(sol. )	PO <sub>4</sub> <sup>-3</sup>	TP	TKN	TSS
Feb. 12/88	0	3	61	2.1	2.9	21	65
Feb. 13/88	1	b	49	3.2	4.3	18.5	81
Feb. 13/88	1	а.	61	3.3	3.9	19	46
Feb. 14/88	2	Ъ	49	3.5	4.4	13.7	30
Feb. 14/88	2	а.	61	2.6	4.3	17.6	50
Feb. 15/88	3	<b>b</b> .	45	5.2	5.7	12.2	24
Feb. 15/88	3	а.	65	3.7	4.8	18	61
Feb. 16/88	4	b	38	5.3	6.1	13.7	50

Table 4.5: Reactor Feed Composition Phase Two (Feb.12/88-Feb.16/88)

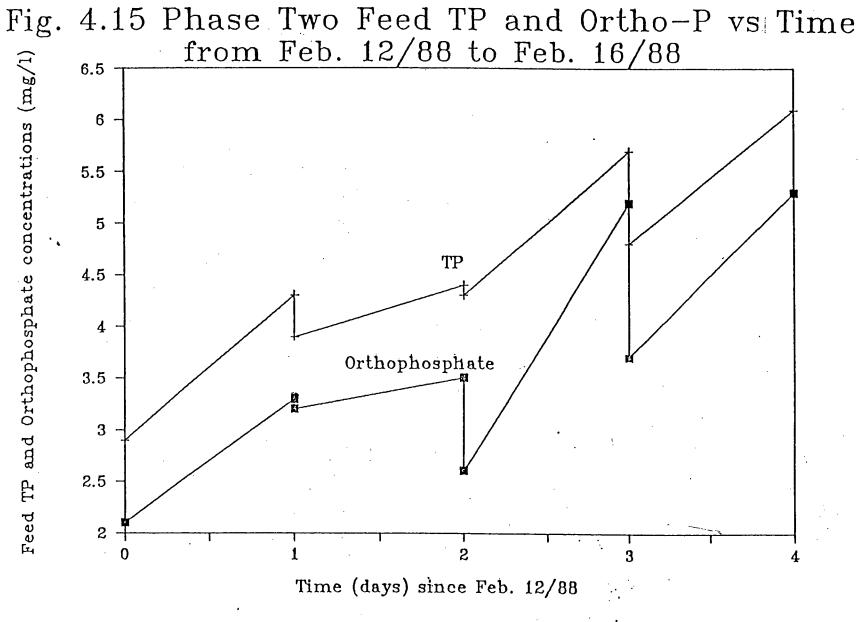
Effluent Characteristics							
· · · · ·		Concentration (mg/l)					
Date	Time elapsed since feed tank cleaned (days)	COD(sol. )	PO <sub>4</sub> <sup>-3</sup>	TP	NO <sub>z</sub>	TKN	TSS
Feb. 12/88	0	20	0.1	NA	13.3	NA	NA
Feb. 13/88	1	24	0.3	1.0	11.1	4	31
Feb. 14/88	2	20	0.9	2.3	8.9	4	65
Feb. 15/88	3	20	1.3	2.6	8.9	4	39
Feb. 16/88	4	22	2.2	2.9	8.3	4	56

Table 4.6: Effluent Composition Phase Two (Feb.12/88-Feb.16/88)



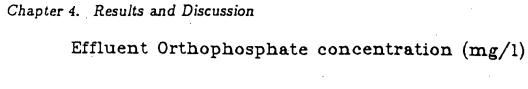
Feed Tank soluble COD concentration (mg/l)

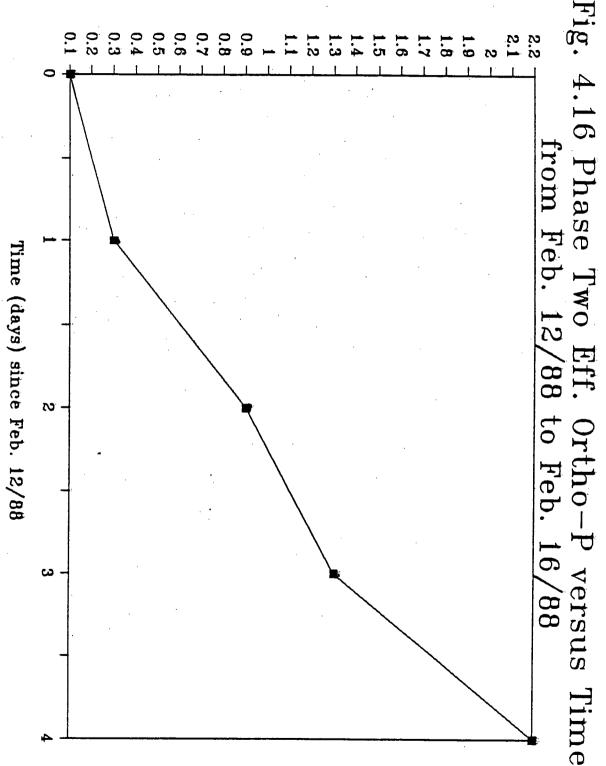


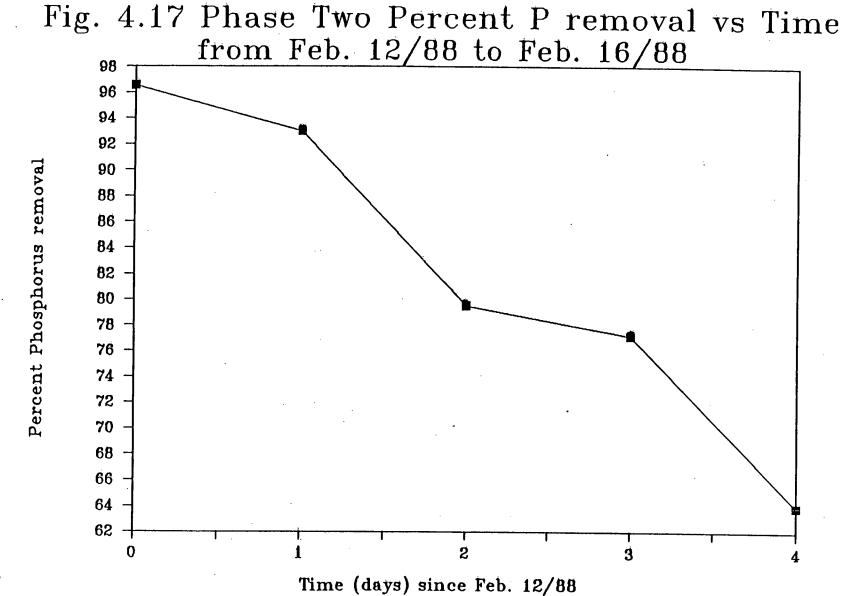


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Unfortunately, no batch test data is available for the time period from Feb. 6 to Feb. 16, 1988. A biofilm scraping was taken on Feb. 10 and a %P value of 2.3% (total solids basis) was recorded.

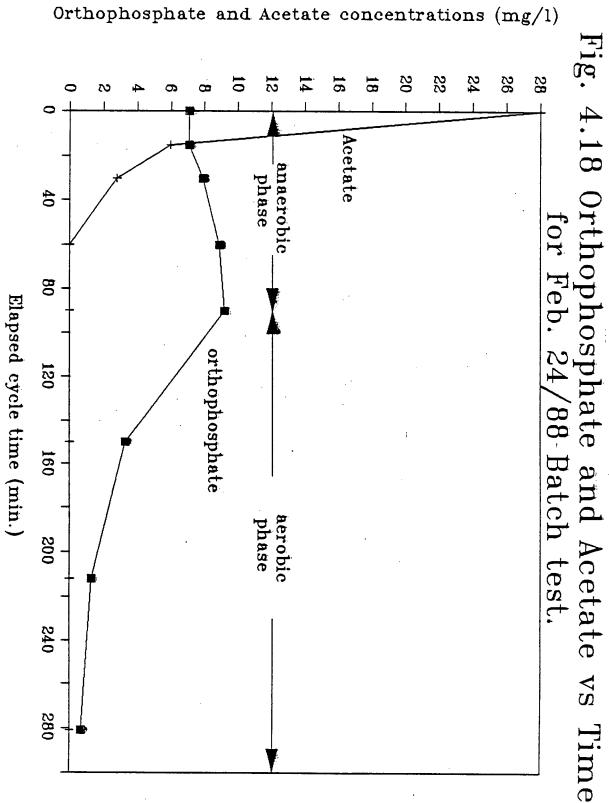
### 4.1.3 Phase Three (February 17 - March 17, 1988)

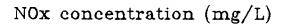
On February 17, 1988 the anaerobic holding vessel was again put back on line. However, the pumping rate from this tank was now double of what it had been in phase one. The increase in pumping rate ensured that the entire contents of the anaerobic holding vessel were emptied during the start of each treatment cycle, thus eliminating any flow accumulation in this tank. The anaerobic holding vessel now supplied 66% of the combined influent to the reaction vessel, as opposed to the previous 50%.

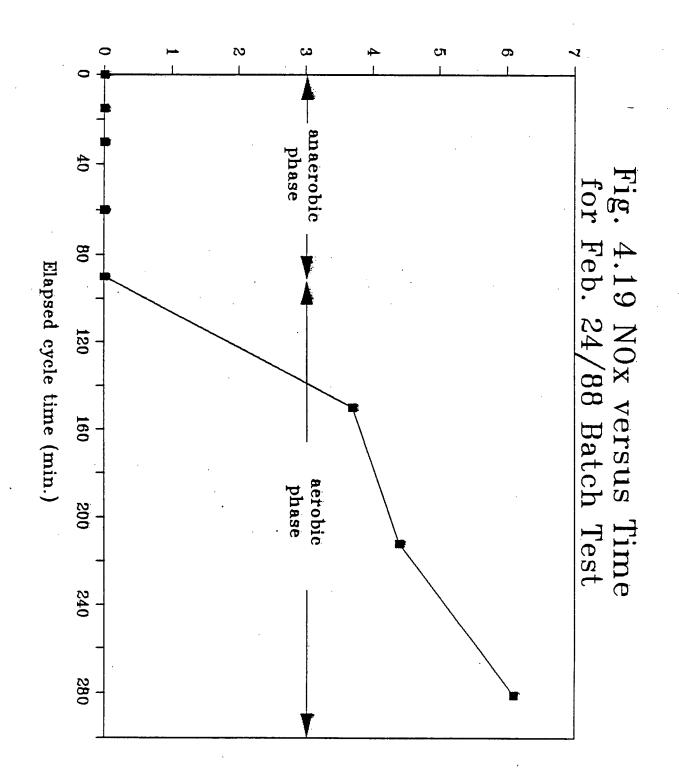
Percent phosphorus removals increased following the above change. Phosphorus removals of 75 to 95% were realized from Feb. 19 to Feb. 25, 1988. A batch test was run on Feb. 24/88 and the results are shown in Figures 4.18 to 4.20. Mean effluent  $PO_4$  for the above run was 0.7 mg/l as P. The initial increase in bulk liquid ORP, observed at the start of the anaerobic phase of the Feb. 24 treatment cycle, was believed to be caused by influent air entrainment.

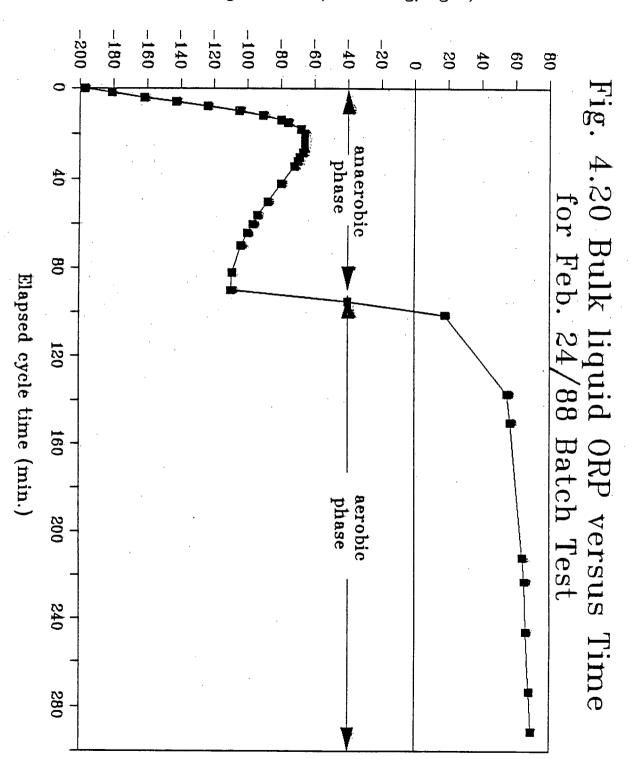
On Feb. 25, 1988 both the raw sewage and anaerobic holding vessels were cleaned. Percent phosphorus removals from Feb. 26-March 1, 1988 ranged from 17 to 50%. On March 1, 1988 a new batch of sewage was collected. Again, the raw sewage and anaerobic holding vessels were cleaned, and refilled.

The results of a batch test conducted on March 2 are presented in Figures 4.21 to 4.23. Eighty to ninety percent phosphorus removal was achieved at this time and continued up until March 8. A batch test run on March 9 showed that percent phosphorus removal had decreased to 70%. However, the characteristic anaerobic P release and aerobic P uptake associated with biological phophorus removal was still observed.









Bulk liquid ORP (mV vs Ag/AgCl)

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Orthophosphate concentrations (mg/l)

and

Acetate

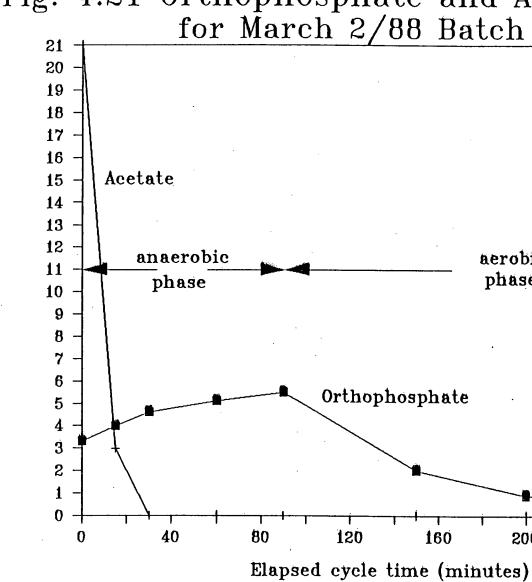


Fig. 4.21 Orthophosphate and Acetate vs Time for March 2/88 Batch Test

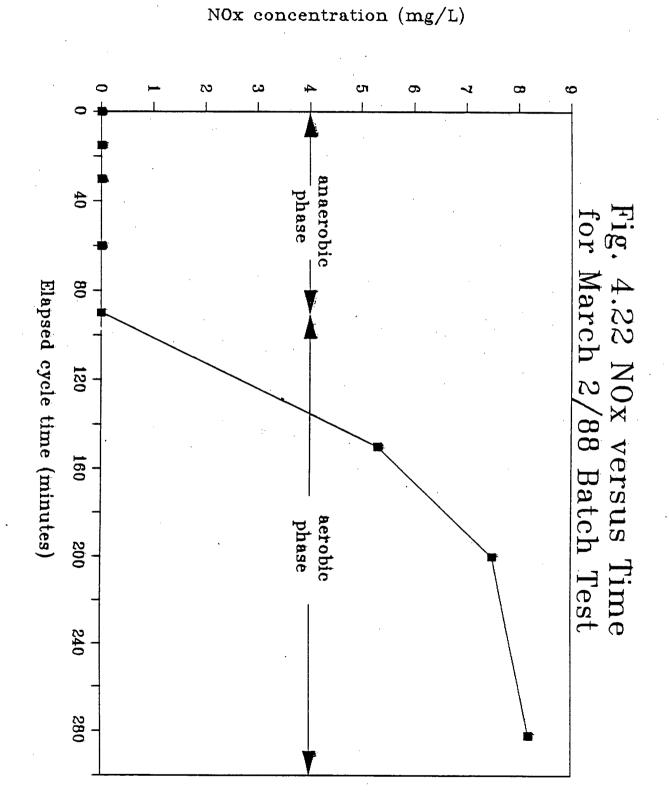
aerobic

phase

200

240

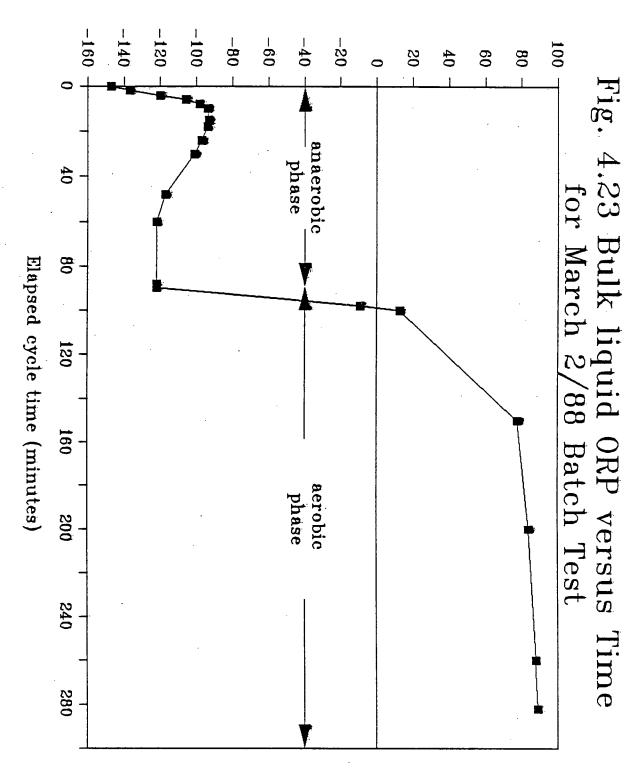
280



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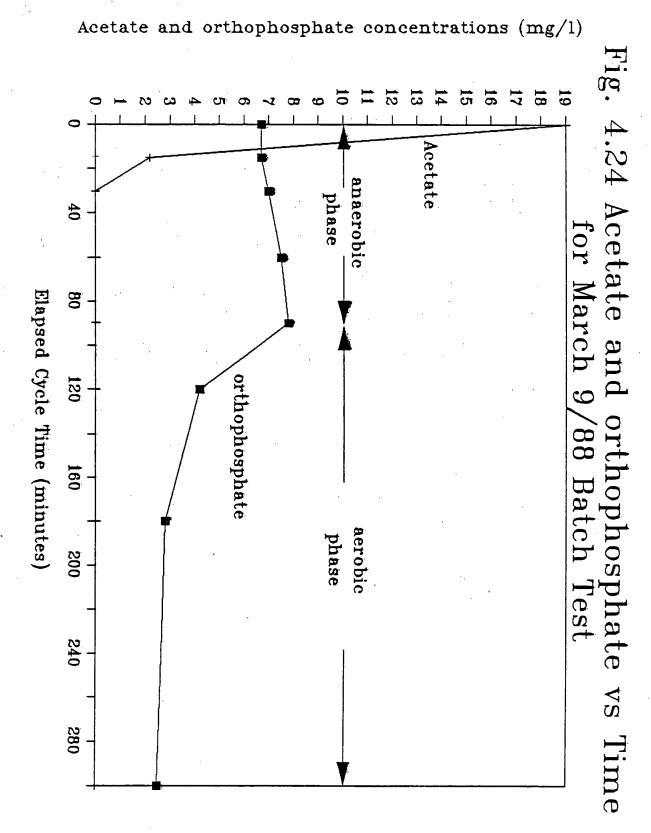


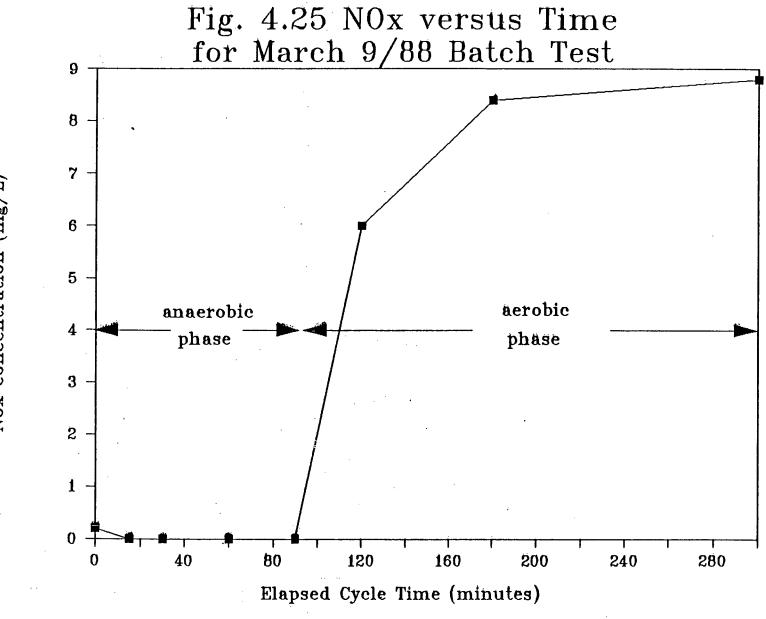
Bulk liquid ORP (mV vs Ag/AgCl)

Figures 4.24 to 4.26 show the batch test results for March 9, 1988.

Percent phosphorus removals between 45 and 62% were observed up until March 14, 1988. At that time, both the raw sewage and anaerobic holding vessels were cleaned. An effluent sample was taken one cycle after both tanks had been cleaned.Percent phophorus removal had increased to 75%. Mean effluent  $PO_4$  was 0.7 mg/l as P.

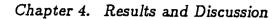
A percent phosphorus removal of 35% was observed by March 15. A batch test was run on March 16. An anaerobic phosphorus release of only 0.6 mg/l of anaerobic volume was observed. The presence of nitrates in the influent was also observed. Figures 4.27 to 4.29 show the results from the March 16 batch test. Sampling was ceased on March 17, 1988. Tables 4.7, 4.8, and 4.9 show the raw sewage, anaerobic holding vessel, and effluent characteristics for the time period February 19, 1988 to March 17, 1988. Figures 4.30 to 4.33 show the anaerobic holding vessel and raw  $PO_4$  concentrations versus time, the influent and effluent  $PO_4$  concentrations versus time, the influent and raw soluble COD concentrations versus time, and the percent phosphorus removal versus time, for the time period February 27 to March 17, 1988.

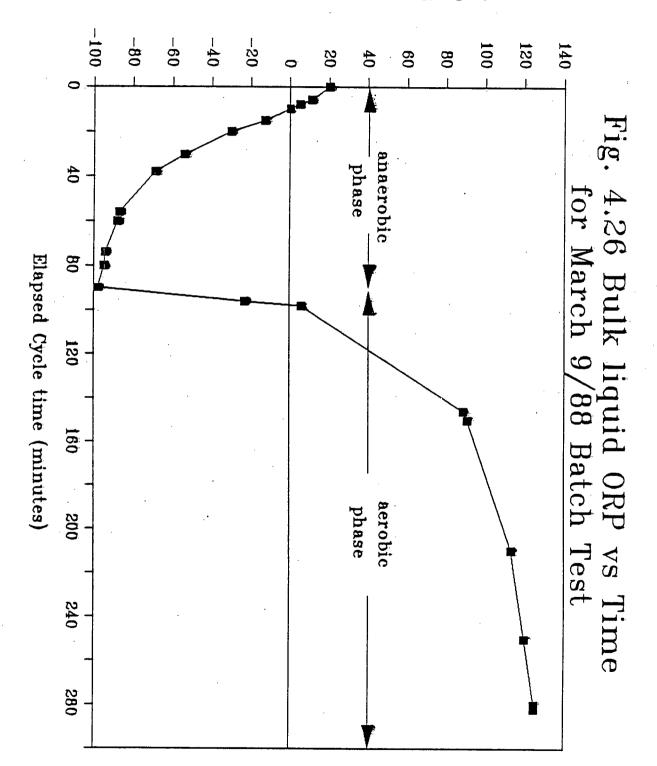




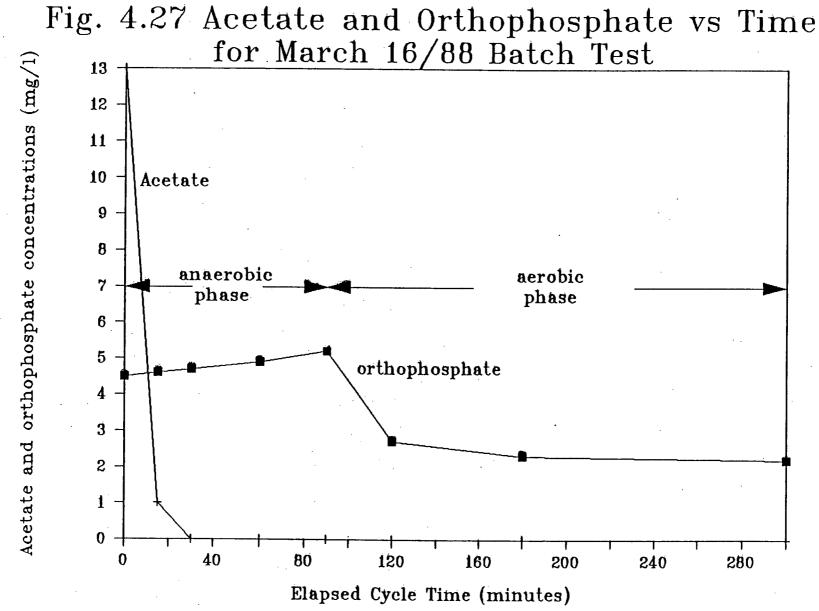
NOx concentration (mg/L)

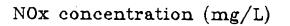
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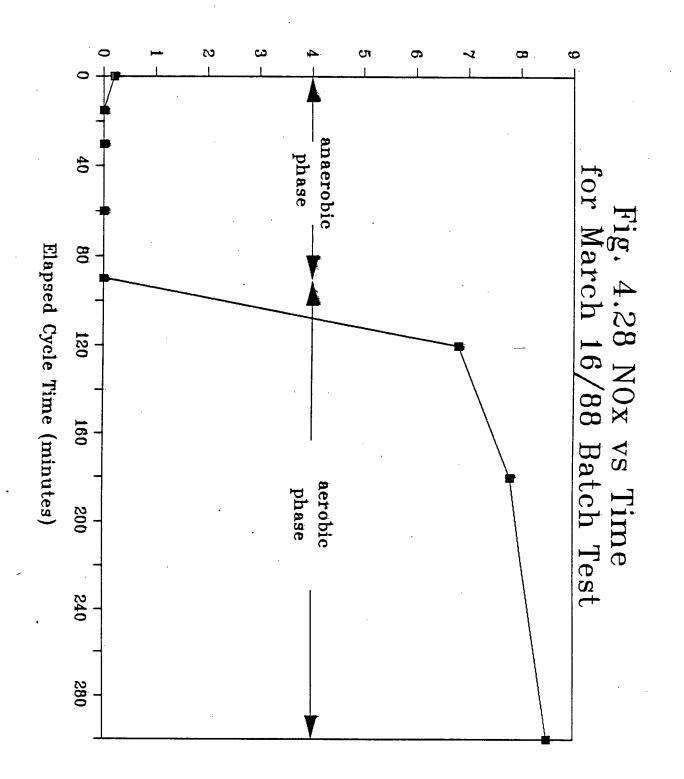


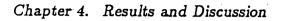


Bulk liquid ORP (mV vs Ag/AgCl)

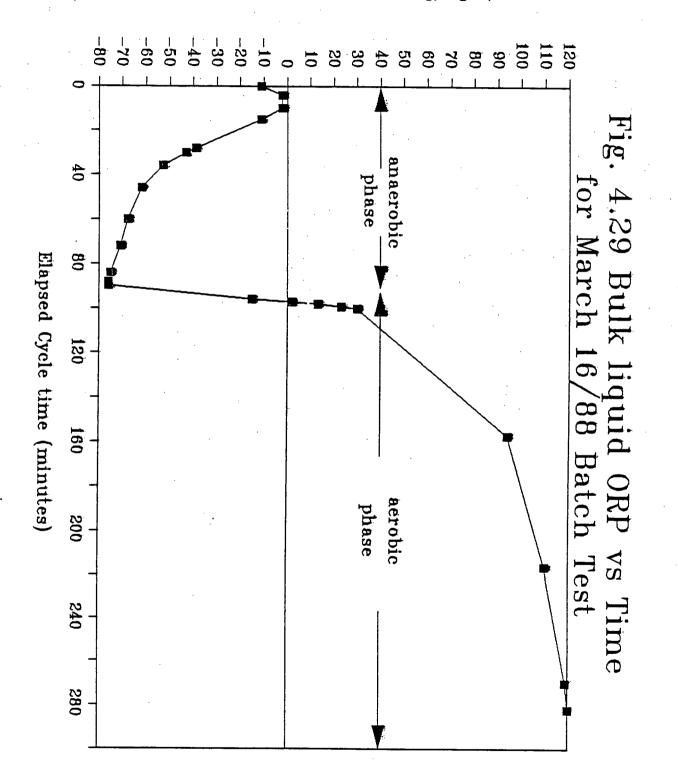








Bulk liquid ORP (mV vs Ag/AgCl)



Raw Sewage Characteristics							
Date	Concentrations (mg/l)						
	COD(sol.)	COD(tot.)	PO4	TP	NO <sub>x</sub>	TKN	TSS
Feb.19/88	83	143	2	3.1	0	19.2	30
Feb.20/88	103	135	.2	2.9	0	18.4	NA
Feb.21/88	82	163	2.2	3	0	18.8	NA
Feb.22/88	91	115	2.2	2.8	0	18	32
Feb.23/88	95	135	2.1	2.9	0	18.4	39
Feb.24/88	71	104	2.1	2.9	0	18.4	33
Feb.25/88	79	136	2.1	3.1	0	20	59
Feb.26/88	83	118	2.2	3.1	0	19.2	44
Feb.27/88	79	106	2.1	2.7	0	14.5	23
Feb.28/88	84	128	2.1	2.9	0	14.5	46
Feb.29/88	94	126	2.2	2.9	0	14.1	35
Mar.1/88	111	181	2.4	3.6	0	16.9	99
Mar.2/88	65	132	2.3	3.6	· 0 ·	16.9	67
Mar.3/88	67	130	2.7	3.7	0	22.2	62
Mar.4/88	86	136	2.8	3.5	0	20.4	54
Mar.5/88	76	132	2.7	3.5	0	20.7	48
Mar.6/88	120	209	2.7	3.5	0	20	28
Mar.7/88	56	115	2.7	3.5	0	20.4	63
Mar.8/88	56	91	2.7	3.2	0	18.5	28
Mar.9/88	53	132	2.7	3.4	0	18.9	55
Mar.10/88	55	100	2.8	3.3	0	20	35
Mar.11/88	47	123	1.8	3	0	20	74
Mar.12/88	51	107	2	2.8	0	19.6	<b>53</b> -
Mar.13/88	<b>53</b> <sup>-</sup>	94	1.8	2.9	0	19.6	46
Mar.14/88	53	115	1.8	2.8	0	19.2	49
Mar.15/88	51	135	1.8	2.8	0	19.6	66
Mar.16/88	<b>59</b>	121	2	2.8	0	18.1	56
<b>Mar.17/88</b>	66	127	2	2.7	0	18.5	51

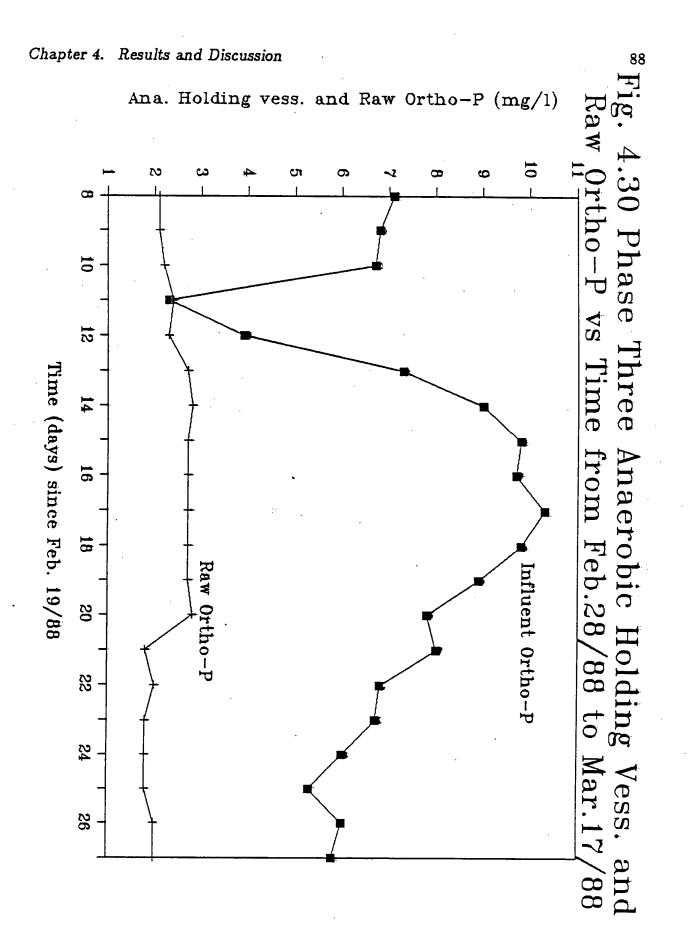
Table 4.7: Phase Three Raw Sewage Data (Feb.19/88-Mar.17/88)

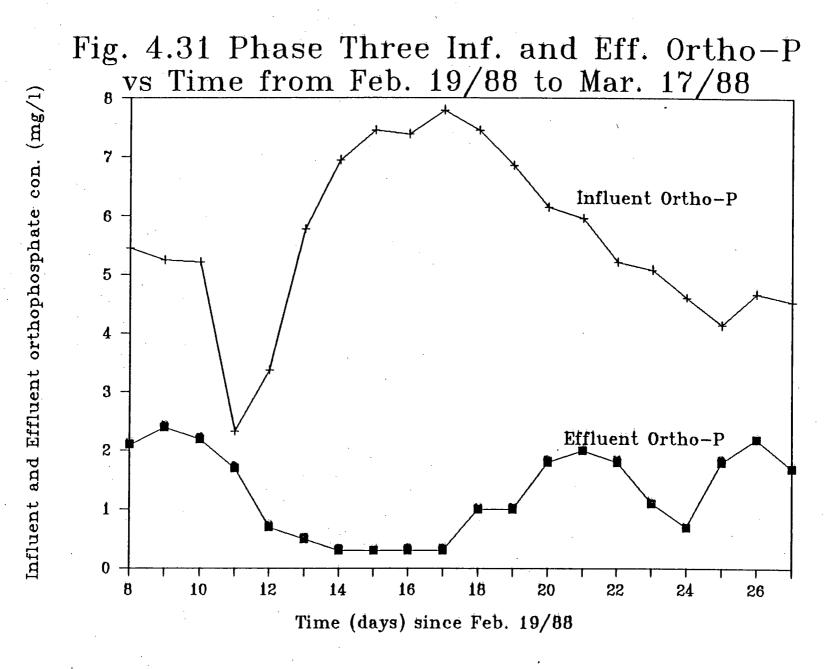
Anaerobic Holding Vessel Characteristics						
Date	Concentrations (mg/l)					
	COD(sol.)	COD(tot.)	PO4	TP	$NO_{x}$	
Feb.19/88	83	143	2	3.1	0	
Feb.20/88	NA	NA	NA	NA	0	
Feb.21/88	32	NA ·	8	8.9	0	
Feb.22/88	<b>NA</b>	NA	NA	NA	0	
Feb.23/88	NA.	NA	NA	NA	0	
Feb.24/88	NA	NA.	NA	NA	0	
Feb.25/88	79	136	2.1	3.1	0	
Feb.26/88	NA	NA	NA	NA	0	
Feb.27/88	35	NA	7.1	NA	0	
Feb.28/88	49	NA	6.8	NA	0	
Feb.29/88	47	NA	6.7	NA	0	
Mar.1/88	53	NA	2.3	NA	0	
Mar.2/88	36	NA	3.9	NA	0	
Mar.3/88	37	NA	7.3	NA	0	
Mar.4/88	39	NA	9	NA.	0	
Mar.5/88	70	NA	9.8	NA	0	
Mar.6/88	43	NA	9.7	NA	0	
Mar.7/88	31	NA	10.3	NA	0	
Mar.8/88	37	NA	9.8	NA	0	
Mar.9/88	31	NA	8.9	NA	0	
Mar.10/88	31	NA	7.8	NA	0	
Mar.11/88	29-	NA	8	NA	0	
Mar.12/88	31	NA	6.8	NA	0	
Mar.13/88	41	NA	6.7	NA	0	
<b>Mar.14/88</b>	53	NA	6	NA	0	
Mar.15/88	53	NA	5.3	NA	0	
Mar.16/88	66	NA	6	NA	0	
Mar.17/88	41	NA	5.8	NA	0	

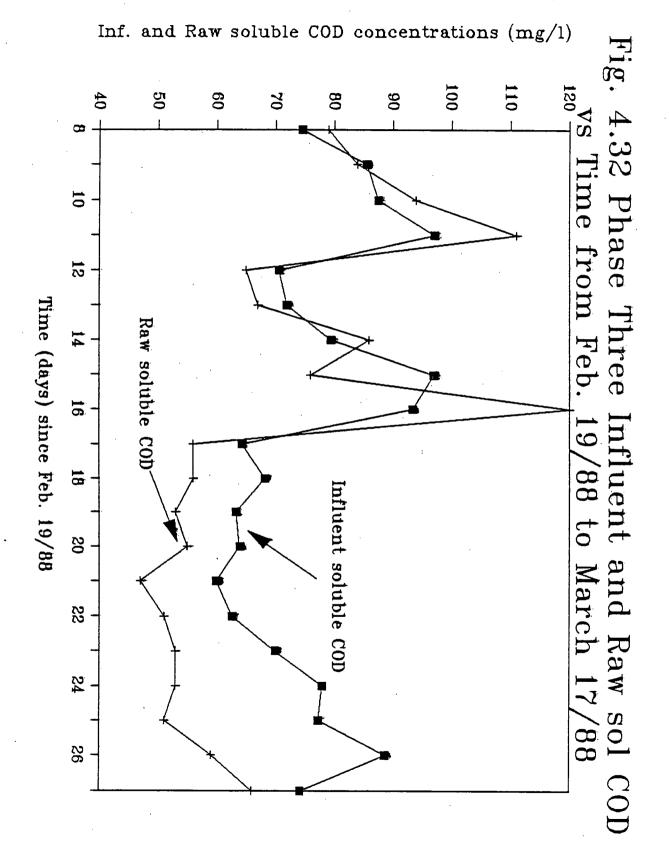
Table 4.8: Phase Three Anaerobic Holding Vessel Data (Feb.19/88-Mar.17/88)

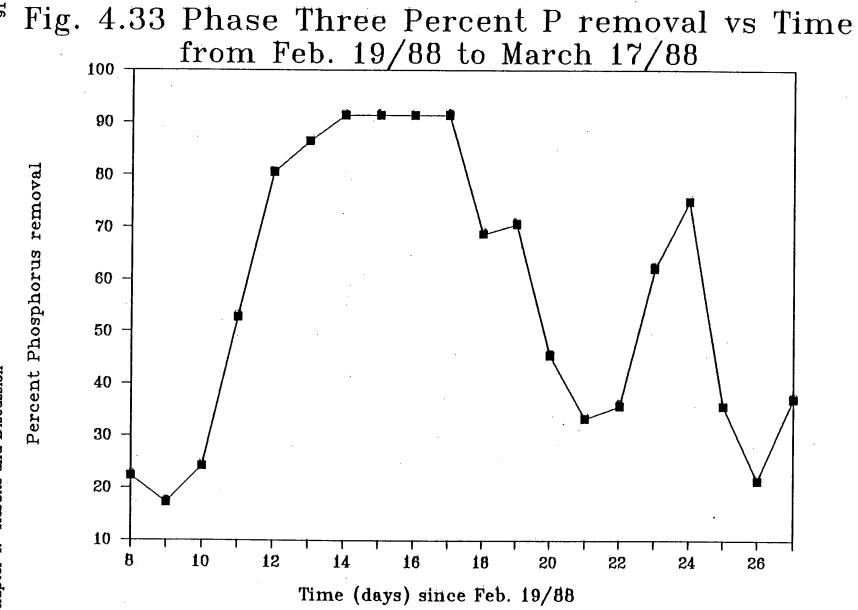
Effluent Characteristics						
Date	Concentrations (mg/l)					
	COD(sol.)	PO4	TP		TKN	TSS
Feb.20/88	26	0.15	1	7.5	2.9	NA
Feb.21/88	20	0.35	1	7.5	2.5	NA
Feb.22/88	26	0.2	1.2	8.3	2.5	NA
Feb.23/88	18	0.3	1	8.3	3.3	36
Feb.24/88	39	0.7	1.2	7.2	2.9	29
Feb.25/88	35	0.6	1.9	8.3	4	49
Feb.26/88	22	1.8	2.9	8.5	3.3	42
Feb.27/88	35	2.1	3.4	8.5	4.8	33
Feb.28/88	29	2.4	3.4	9.3	3.7	40
Feb.29/88	31	2.2	3.4	9	4.1	55
Mar.1/88	30	1.7	2.9	7.3	3.6	49
Mar.2/88	18	0.7	2.3	8.2	4.1	72
Mar.3/88	31	0.5	0.9	8.5	2.5	36
Mar.4/88	31	0.3	1.3	8.2	2.9	40
Mar.5/88	29	0.3	0.9	8.3	2.9	36
Mar.6/88	25	0.3	3.2	8.6	3.6	58
Mar.7/88	27	0.3	1.5	7.9	3.6	48
Mar.8/88	25	1	2.7	8.2	4.4	79
Mar.9/88	NA	1	NA	8.6	NA	NA
Mar.10/88	31	1.8	2.8	8.4	2.5	34
Mar.11/88	20	2	3.3	9	5.4	56
Mar.12/88	25	1.8	3.3	8.4	2.5	41
<b>Mar.13/88</b>	25	1.1	2.2	8.2	2.9	49
<b>Mar.14</b> /88	20	0.7	1.6	8	2.5	37
<b>Mar.15</b> /88	25	1.8	3.2	7.8	4	61
Mar.16/88	29	2.2	4.3	8.5	9.6	136
Mar.17/88	33	1.7	2.2	8.3	2.2	34

Table 4.9: Phase Three Effluent Data (Feb.20/88-Mar.17/88)









Following the experimental runs, the biofilm was scraped from the discs and weighed. The dried biofilm weight was 66 grams. Thirty-seven grams (56%) of the total mass of the biomass was volatile. Table 4.10 shows %P contents of the biofilm for the entire experimental period.

Date	Biofilm %P content	Biofilm %P content
	(total solids basis)	(volatile solids basis)
Dec. 17/87	1.84	3.29
Jan. 6/88	2.04	3.64
Jan. 13/88	2.39	4.27
Jan. 20/88	2.30	4.11
Jan. 25/88	2.27	4.05
Feb. 1/88	2.00	3.57
Feb. 10/88	2.3	4.11
Feb. 23/88	2.3	4.11
Mar. 1/88	2.27	4.05
Mar. 8/88	2.4	4.29

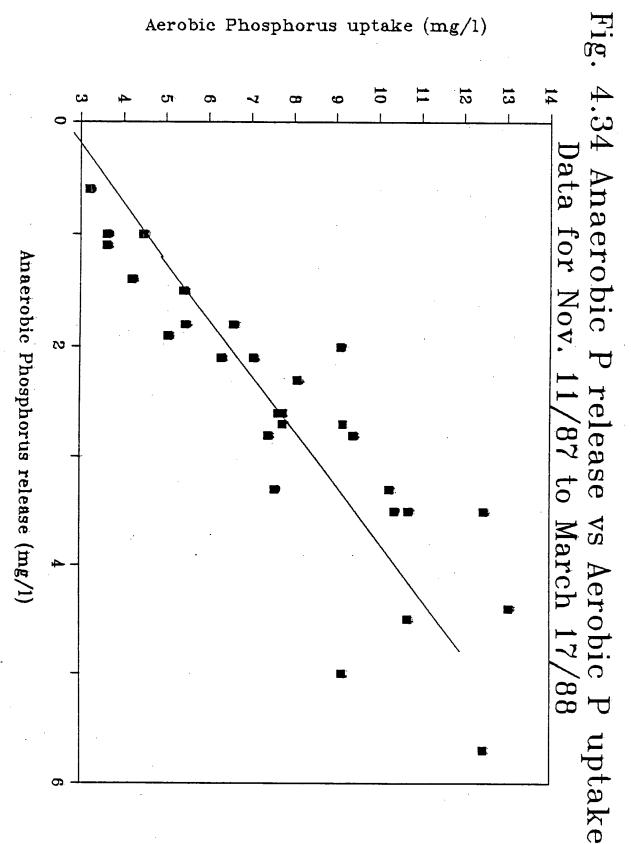
Table 4.10: Biofilm percent phosphorus contents for experimental period

### 4.2 Data Analysis

The computer package SAS (statistical analysis system) version 5 was used to get a correlation matrix of all the parameters measured in these experiments. Parameters having high correlation coefficients were then used to explain the results obtained.

From the correlation matrix, a relationship was found to exist between anaerobic P release and aerobic P uptake. Multiple regression analysis showed a linear relationship between influent TP, anaerobic ORP, and anaerobic P release.

Figure 4.34 shows the scatter plot for anaerobic phosphorus release versus aerobic phosphorus uptake. The best fit line for the data is also provided. Both anaerobic P release and aerobic P uptake are calculated as the difference in initial and final  $PO_4$ concentrations during the respective phases of the treatment cycle.  $R^2$  for the best fit line was found to be 0.765. In other words, 76.5% of the aerobic P uptake observed during these experiments could be explained by anaerobic P release. The higher the anerobic P release, the higher the subsequent P uptake under aerobic conditions. Anaerobic P release has been reported by Barnard (1976) and others to be one of the prerequisites for good phosphorus removal. Previous research into the mechanism of biological phophorus removal has determined anaerobic P release to be dependent on the availability of simple carbons, and the absence of nitrates and oxygen in the anaerobic phase. The correlation matrix generated using SAS showed weak correlations between influent NOx and anaerobic P release, and influent acetate COD and anaerobic P release. It was felt that the poor correlations above were due in part to the small ranges of NOx and influent acetate concentrations observed in these experiments. It is also felt that, in the range of acetate concentrations used in these experiments, acetate was never limiting to the biological phophorus removal mechanism. Therefore, small variations in influent acetate concentration would not effect anaerobic P release.



Influent TP gave the best correlation of all the chemical data provided.  $R^2$  for P release versus influent TP (total phosphorus) was 0.41. The above suggests that P was in fact limiting to the biological phosphorus mechanism for the amount of acetate added to the anaerobic phase.

Influent NOx,  $PO_4$ , COD, TP, TKN and acetate concentrations were correlated with anaerobic P release. The  $R^2$  value for the above multiple linear regression was found to be 0.556. Again, the most significant parameter was influent TP.

Batch test ORP values were then correlated with anaerobic P release. The ORP values after 15, 30, 60 and 90 minutes of anaerobic time were used. The correlation matrix showed an  $R^2$  value of 0.45 between the ORP after 30 minutes and anaerobic P release. The ORP readings at 60 and 90 minutes gave similar correlation cofficients. The correlation between P release and ORP after 15 minutes was not as good.

A multiple linear regression was then run for P release versus influent TP and anaerobic ORP.  $R^2$  for the above regression was found to be 0.654. Considering the facts that the ORP values were taken with two different probes, and only sixteen data points were available for the regression, this was considered to be a reasonable fit to the data.

To summarize, the correlation coefficient for anaerobic phosphorus release and aerobic phosphorus uptake was found to be 0.765. The correlation coefficient for influent TP and anaerobic ORP versus anaerobic phosphorus release was 0.654. Considering the facts that the above parameters were not purposely varied to determine their interrelationships, and that the number of data points was small, the above correlations are quite good.

## 4.3 Discussion

The data collected between September 1/87 and February 1/88 suggests that consistent enhanced biological phosphorus removal is possible using a Sequencing Batch Rotating Biological Contactor(SBRBC). However, data collected from Feb.6/88-March 17/88 appears to contradict the above claim. The discussion section provides 'reasonable explanations' for the data observed.

The average percent phosphorus removal between November 11/87 and Feb. 1/88 was 94.8%. One hundred percent phosphorus removal was observed on occasion. The average value includes the data collected on November 30 following three cycles with no acetate addition, as well as, the time period from November 11 to November 20, 1988, when it appeared that steady state with respect to phosphorus removal had not yet been reached. Even without acetate addition for three cycles, phosphorus removal was 72%. This suggests that some volatile fatty acids were being provided by the sewage or that extra stored PHB's were available for more than one cycle.

Steady state with respect to COD removal appeared to be reached more quickly than for phosphorus removal (see Figure 4.7). The fluctuations in COD removals may have been caused by the variability in raw total COD values.

It is difficult to tell whether nitrification reached steady-state during phase one of these experiments (Sept. 1/87-Feb. 1/88). Figure 4.8 appears to show an increasing trend in percent nitrification over time. However, percent nitrification for the remainder of the experiments did not increase appreciable above the levels observed during phase one. Since the reactor contents were emptied after each aerobic period, the presence of  $NO_x$  in the anaerobic phase of the treatment process was not expected. It is likely that lower biofilm layers did not receive any oxygen during the aerobic portion of the treatment cycle, due to oxygen depletion in the upper biofilm layers. With  $NO_x$ 

available, denitrification likely resulted. It would be difficult however, to determine how much denitrification actually took place in the biofilm.

The one fact that appears to come out of the results up until February 1, 1988 is that enhanced biological phosphorus removal using a 'fixed film process' is definitely possible on a short-term basis. The batch test results presented in Figures 4.3 to 4.6 and Figures 4.9 to 4.11, were typical for the above time period. Each batch test showed an anaerobic phosphorus release upon acetate addition followed by an aerobic uptake in excess of metabolic requirements. Looking at the plots of  $PO_4$  versus time (Figures 4.3 and 4.9) it is apparent that the values of bulk liquid  $PO_4$  at 60 minutes and 90 minutes of the anaerobic phase did not vary appreciable. The bulk liquid  $PO_4$  concentrations at the 180 minute and 280 minute marks for the above plots did not vary appreciable either. The above facts suggest that the anaerobic time might have been shortened by 30 minutes and the aerobic time by 100 minutes, with treatment efficiences for phosphorus similar to the ones observed.

In enhanced biological phosphorus removal treatment plants, phosphorus is removed from the system with the phosphorus rich biomass. In the activated sludge process, controlled sludge wasting is practised to remove the phosphorus from the system. In a fixed-film system such as the SBRBC biofilm sloughing provides the required solids wasting. However, unlike the sludge wasting in activated sludge plants, biofilm sloughing is uncontrolled and is dependent on sewage strength, oxygen availabilty, and disc rotational speed. It is this lack of control that worried some my colleagues prior to the start up of this project. In particular, the concern was that the required steady state biofilm %P, at the low wasting rate, would be in excess of the phosphorus storage capacity of the bacteria responsible for biological phosphorus removal, resulting in system failure.

The accumulation of phosphorus beyond the systems capacity did not appear to

be a problem. One of the reasons for this could be the apparent P limitation that was indicated by the statistical analysis. Influent TP gave the best correlation with anaerobic P release. Higher levels of influent TP corresponded to higher levels of anaerobic P release. Influent acetate COD did not correlate well with anaerobic P release. As discussed in the data analysis section of this thesis, the poor correlation may have been due to the fact that the amount of acetate added was in excess of that required to remove the amount of P present in the bulk liquid.

In fact, lower than expected values of biofilm percent P were observed in these experiments, when percent P was measured on a total solids basis. When percent P in the biofilm was reported on a volatile solids basis, the 3.5 to 4.5 % phosphorus content expected was observed. It is believed that the RBC biofilm acted as an inorganic solids trap. The measurement of the total and volatile solids contents of the biofilm supports the above argument. Only 56% of the total biofilm mass was volatile, as opposed to a typical value of 80% for activated sludge systems operating in a phosphorus removal mode. Therefore, it appears that the biofilm percent P for these experiments is comparable to values expected for enhanced biological phosphorus removal activated sludge plants, when the comparison is made on a volatile solids basis.

Data collected during phase two (Feb. 6-Feb. 16/88) and phase three (Feb. 19-March 17/88) appear to contradict the claim for consistent enhanced biological phosphorus removal using an SBRBC. Each time period gave inconsistent removals for apparently slightly different reasons.

During the second phase of the experiments the anaerobic draw flow was returned directly to the raw sewage vessel. The sewage vessel was cleaned on Feb. 6 and again on Feb. 12, 1988. Percent phosphorus removal was high after the above vessel was cleaned and refilled with fresh sewage. However, percent phosphorus removal declined with time following cleaning of the raw sewage vessel. Referring to Figure 4.14, showing the

influent soluble COD versus time for the Feb. 12 to Feb. 16 portion of phase two, a steady decrease in soluble COD concentration is observed after each sewage addition. Between Feb. 15 and Feb. 16 the above decrease was almost 30 mg/l. The soluble COD data for the raw sewage vessel between Feb. 6 and Feb. 11 is not available. However, the total COD data for this time period shows a similar trend to the influent soluble COD data for Feb12 to Feb. 16/88. Part of the reason for the decrease in feed vessel (raw sewage vessel) soluble COD, between raw sewage additions, was likely the dilution effect caused by returing the anaerobic draw volume directly to this vessel. Aerobic COD reduction likely took place at the start of the anaerobic phase, due to the presence of air entrained in the influent during the fill period. Influent air entrainment is indicated by all of the batch test ORP plots provided. For each ORP trace, there was a brief period at the beginning of the anaerobic cycle in which the ORP value was either positive or increased prior to the steady drop in ORP expected. Storage of soluble COD and some anaerobic fermentation also reduced the COD of the anaerobic draw being returned to the feed after each anaerobic time period. Some bacterial reduction of COD in the feed tank may have also taken place. The source of the above bacteria was believed to be the sloughed solids returned to the feed tank in the anaerobic draw.

An increase in the phosphate concentration in the feed tank between raw sewage additions was also observed during phase two. This was as expected, for phosphorus would be released during the anaerobic phase of the treatment cycle. The anaerobically treated wastewater returned to the feed tank would therefore have a higher phosphate concentration than the wastewater entering the RBC reactor at the beginning of the treatment cycle.

The hypothesized sequence of events responsible for the progressive decrease in percent phosphorus removal, following each feed tank cleaning during phase two, is as follows. At time zero, the feed tank was cleaned and filled with raw sewage. Percent

phosphorus removal for the first two or three treatment cycles was quite good. At the start of each treatment cycle, some aerobic COD reduction would likely take place in the RBC reaction vessel due to the presence of entrained air introduced during the fill period. Soluble COD storage by 'bio-P' bacteria and some anaerobic fermentaion would also reduce the COD of the bulk liquid in the reaction vessel. Soluble COD storage by 'bio-P' bacteria would result in phosphorus release. Biofilm sloughing would also take place during this phase. The anaerobic draw volume reintroduced into the feed tank following the anaerobic phase, would have a lower COD concentration, and higher phosphate concentration than the wastewater originating from the feed vessel during the time the RBC reaction vessel was filled. The dilution of carbon strength and concentration of phosphate in the feed tank would be the result. The introduction of sloughed solids to the feed tank, during the anaerobic draw period, could only contribute to the above. Sloughed solids introduced into the feed vessel could use up more soluble COD for carbon storage with resulting phosphorus release. Some anaerobic fermentation in the feed tank may also have taken place. Over time, the oxygen demanding capability of the RBC influent sewage would be reduced as a result of the dilution of carbon strength. The proportion of the influent soluble COD being used by the aerobic bacteria to remove entrained air, introduced during the pumping cycle, would increase. Therefore, less soluble COD would be available for carbon storage by 'bio-P' bacteria at a time when the influent loading of phosphorus was increasing. When the feed vessel was cleaned and refilled with fresh sewage, the carbon strength was once again high enough to satisfy the demands of influent air entrainment depletion, and of necessary anaerobic carbon storage. However, the progressive dilution of the feed tank carbon strength would result in the decrease in the oxygen demanding capability of the influent, and therefore percent phosphorus removal. When comparing this situation to that encountered in phase one of these experiments, it is noted that only 11.5 liters of anaerobic draw was returned to the raw sewage vessel daily in the phase one case. In phase two, more than four times the 11.5 liters was returned to the feed tank daily. An increase in the volume of anaerobic draw returned to the feed tank would result in a subsequent increase in the number of sloughed solids in this tank, in addition to the dilution of carbon strength.

The reader is also referred to the model for anaerobic P release formulated in the data analysis section. Anaerobic phosphorus release was found to be dependent on influent TP and anaerobic ORP. The presence of entrained air at the beginning of the treatment cycle would explain the poor correlation of influent soluble COD versus anaerobic P release.

The data collected during phase three of the experiments can be explained in a similar manner to the above. To avoid the problem of flow accumulation in the anaerobic holding vessel, the pumping rate from this tank was doubled. Pumping the anaerobic holding vessel 'dry' ensured a degree of aeration in the reactor prior to the anaerobic phase. Influent samples taken on March 9 and March 16, 1988 showed NOx concentrations of 0.2 mg/l. Figures 4.26 and 4.29 show a slow rate of ORP decrease for both the above batch tests. In fact, both tests showed very low anaerobic phosphorus release, indicating poor anaerobic conditions. It is therefore suspected that poor anaerobic conditions were again set up during the above times, accounting for the resultant decrease in phosphorus removal.

Figures 4.30 to 4.33 lend even more credence to the above argument. In Figure 4.30, both influent and raw phosphate concentrations are plotted with time. The concentration of phosphate in the raw sewage vessel appears relatively constant. The variation in influent phosphate concentration can therefore be explained by the variation in the concentration of this species in the anaerobic holding vessel. The concentration of

phosphate in the anaerobic holding vessel would be governed by the degree of anaerobic phosphorus release taking place in the reaction vessel during the anaerobic phase of the treatment cycle.

Figure 4.31 shows that the lowest effluent phosphate concentrations correspond to the highest influent concentrations. The reader is referred to the argument in the above paragraph. Since the variation in influent phosphate concentration was due to the variation in the concentration of phosphate in the anaerobic holding vessel, and therefore the degree of anaerobic P release, the poor phosphorus removals can once again be traced to poor anaerobic P release. Periods of high anaerobic P release would result in carbon storage as PHB and an increase in the concentration of phosphate in the anaerobic holding vessel. The good anaerobic P release would result in good aerobic P uptake, as discussed in the data analysis section of this thesis.

Figure 4.32 shows influent and raw soluble COD values with time. It is noted here that the high values of influent COD correspond to the high percent phosphorus removals. High influent COD gives a high immediate oxygen demand and good anaerobic conditions. Good anaerobic conditions, and available volatile fatty acids result in good phosphorus release. As discussed above, good anaerobic phosphorus release, with it's resulting carbon storage, results in good aerobic phosphorus uptake later.

To summarize, the proposed SBRBC process appears to hold promise for biological phosphorus removal from wastewater. In each test situation in which high effluent  $PO_4$  values were observed, the cause of the problem could be traced to improper anaerobic conditions during the anaerobic phase. The problems caused in phase two seem to be due to the progressive dilution of the feed tank carbon strength by the direct return of the anaerobic draw to this tank. The introduction of sloughed solids into the feed tank may have also caused some problems. The results from phase three of these experiment show variable results. Poor phosphorus removals corresponded to low

influent soluble COD. The extra air entrained when the anaerobic holding vessel was pumped dry in this phase may have caused many of the problems during this time period. Nitrates were found in the reactor influent, at the start of the anaerobic phase, on ocassion. Batch tests showed that the runs with poor overall phosphorus removal exhibited poor anaerobic phosphorus release. The data analysis supports the above arguments. Anaerobic phosporus release correlated well with aerobic phosphorus uptake. Good anaerobic phosporus release was dependent on influent TP and anaerobic ORP.

#### Chapter 5

#### **Conclusions and Recommendations**

Conclusions drawn from this research are presented in the first section of this chapter. Recommendations for future research and for process improvements are then discussed.

#### 5.1 Conclusions

The main objective of this research from the outset was to study the technical feasibility of using a Sequencing Batch Rotating Biological Contactor (SBRBC) for 'enhanced biological phosphorus' removal. It was felt that, if the proposed RBC process proved to be technically feasible, that some of the 'economic benefits' inherent to RBC technology might be realizable for the removal of phosphorus as well as carbon. The above research project appears to have been reasonably successful, considering the fact that this was a first attempt at the above problem. Below is a list of the major conclusions that have been derived from this research.

(1) Enhanced biological phosphorus removal is definitely possible using a Sequencing Batch Rotating Biological Contactor (SBRBC).

(2) The attainment of proper anaerobic conditions during the anaerobic phase of the process appeared to be the key to making the process work. Air entrained during the influent period of the treatment cycle enabled aerobic bacteria to use added acetate and influent soluble COD. The utilization of these materials by aerobic bacteria left less for carbon storage by 'bio-P' bacteria, and resulted in lower percent phosphorus removals.

#### Chapter 5. Conclusions and Recommendations

(3) Anaerobic biofilm sloughing may cause problems if the anaerobic draw is returned to the sewage feed tank.

(4) An alternative to the anaerobic holding vessel appears to be necessary to minimize operating problems with the SBRBC.

(5) Carbon removal and nitrification are secondary benefits to the SBRBC process for phosphorus removal.

(6) Greater anaerobic phosphorus release resulted in greater aerobic phosphorus uptake.

(7) Anaerobic phosphorus release was dependent on influent total phosphorus and anaerobic ORP in these experiments. The facts that influent TP correlated with anaerobic P release, and influent acetate did not, suggested that P was limiting in these experiments.

#### 5.2 Recommendations

The most important question regarding SBRBC technology appears to have been answered. The process appears to work. The next logical step for future research is to provide alternatives to the anaerobic holding vessel. Pilot scale studies could be conducted to investigate the possible economic advantages of the above process. It is felt by the author that the SBRBC technology may share many of the economic advantages of the conventional RBC — namely lower operating costs, less sludge production, and ease of operation. The above have yet to be proven.

The solution to the flow accumulation problem and the use of an anaerobic holding vessel could be solved using two SBRBC units. The actual raising and lowering of the RBC discs, instead of flow recycle, may be a worthwhile alternative. Both the above alternatives would use a microprocessor, solenoid valves, and possibly level switches to achieve process control. Other modifications to the SBRBC process are possible, and might be worthwhile investigating.

In the process option involving two SBRBC units, both units would be operated in a sequencing batch mode. One unit would be operated under anaerobic conditions at the same time as the other unit was being operated under aerobic conditions. After a predetermined reaction time in the aerobic RBC, a valve would be opened to remove the treated effluent from the reactor. Following the effluent decant, part of the contents of the anaerobic reactor would be transfered to the now empty aerobic reactor. The aerobic reactor would then be filled to capacity, submerging the rotating discs, with raw sewage. The past aerobic reactor would now become the anaerobic reactor. The past anaerobic reactor, with its partially submerged discs, would now become the aerobic reactor. The above series of events would continue with each reactor alternating between anaerobic and aerobic conditions. Acteate or some other suitable volatile fatty acid source would be added to the anaerobic phases as required. All valves and pumps would be controled by a microprocessor.

The experience gained in these experiments could also be very useful from a process operation point of view. To reduce the likelihood of experiencing the same problems encountered in these experiments, during the anaerobic period of the treatment cycle, it is recommended that acetate be added to the anaerobic phase only after the ORP in this phase has reached a predetermined value, indicating true anaerobic conditions have been attained. The above would also prevent added acetate from being used by aerobic bacteria, present in the biofilm, in the presence of reactor influent entrained air.

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# Appendix A

### **Statistical Analysis**

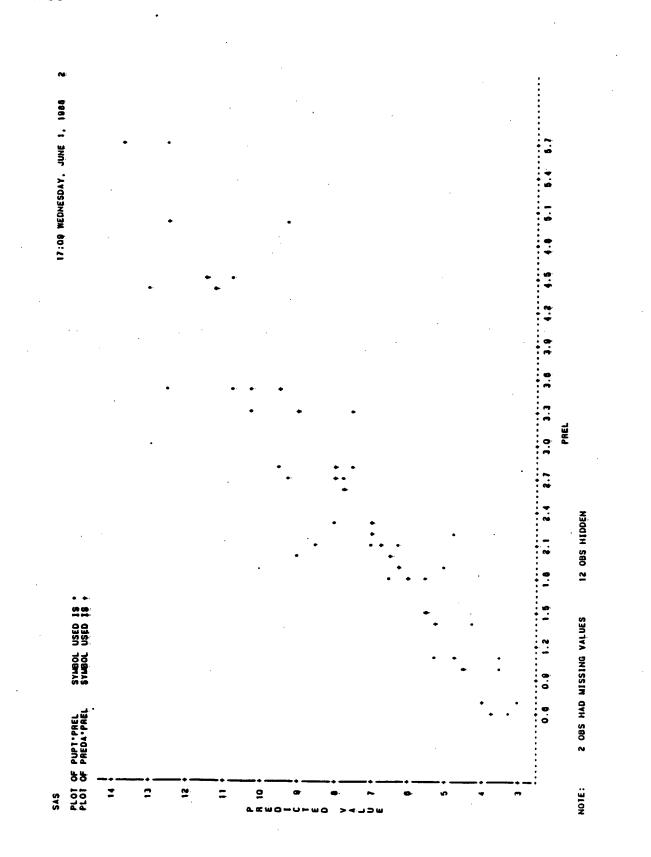
The statistical analysis for this research was done using version five of the SAS computer package. SAS (Statistical Analysis System) is a software system for information storage and retrieval, data modification and programming, report writing, statistical analysis, and file handling. The statistical analysis procedures range from simple descriptive statistics to complex multivariate techniques. The SAS output for the linear regression of anaerobic P release (PREL) versus aerobic P uptake (PUPT) is provided in the following pages. The output generated for the multiple linear regression of anaerobic P release (APREL), influent TP (ITP), and anaerobic ORP (ORP30) is also provided. , SAS

DEP VARIABLE: PUPT ANALYSIS OF VARIANCE

SOURCE	DF	SUM OF	MEAN SQUARE	F VALUE	PROB>F
MODEL Error C Total	1 30 31	189.66414 58.14728204 247.81142	189.66414 1.93824273	97.854	0.0001
	MSE EAN	1.392208 7.481562 18.60852	R-SQUARE Adj R-Sq.	0.7854 0.7575	•

PARAMETER ESTIMATES

VARIABLE	DF	PARAMETER ESTIMATE	STANDARD ERROR	T FOR HO: Parameter=0	PROB >  T
INTERCEP	1	2.55408283	0.55560443	4.597	0.0001
PREL	1	1.98089635	0.20025032	9.892	



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DEP VARIABLE: APREL ANALYSIS OF VARIANCE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PROB>F
MODEL Error C'Total	2 9 11	8.29338055 4.38328611 12.67666667	4.14889028 0.48703179	8.514	0.0084
ROOT MSE DEP MEAN C.V.		0.8978786 3.518687 19.84483	R-SQUARE Adj R-Sq	0.8542	

#### PARAMETER ESTIMATES

VARIABLE	DF	PARAMETER ESTIMATE	STANDARD ERROR	T FOR HO: Parameter=0	PROB >  T
INTERCEP	1	2.80297057	1.14337460	2.451	0.0367
ORP30	1	-0.02953982	0.007191805	-4.107	0.0025
ITP	1	-0.20450558	0.18301380	-1.255	0.2412