Investigation of Biological Phosphorus Removal
for the Treatment of a
Cellulose Acetate Manufacturing Wastewater

by

Thomas C. Pully

Thesis submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of
Master of Science
in
Environmental Engineering

APPROVED:

Clifford Randall, Chairman

John Novak

Daniel Gallagher
LD 5655
V855 1997 P855 0.52
Investigation of Biological Phosphorus Removal for the Treatment of a Cellulose Acetate Manufacturing Wastewater

by

Thomas C. Pully
Dr. Clifford Randall, Chairman
Environmental Engineering

... (ABSTRACT)

The use of a two stage Biological Phosphorus Removal (BPR) system to treat a high strength, low pH industrial wastewater was evaluated. A laboratory scale BPR system was continuously operated and fed the industrial wastewater. Effective utilization or removal of carbonaceous material as measured by Chemical Oxygen Demand (COD) was attained, but BPR was not achieved. Other advantages of BPR and its anaerobic-aerobic sequencing were also monitored. While the loading on the aerobic zone was reduced 10 to 20% by the anaerobic zone, there was no noticeable improvement in secondary settling or effluent quality.

Efforts to supplement the industrial wastewater influent with phosphorus, nitrogen, potassium and iron did not produce any significant improvements. Mixtures of the industrial wastewater, municipal wastewater, and supplements were also used as influent to the system. This indicated that the laboratory BPR system was capable of BPR when provided with an appropriate influent.

Information characterizing the influent and system operation were collected. Loading and operating parameters were computed with these data. When compared with phosphorus removal data, little was revealed and the results varied. The information did indicate that pH was an important factor in achieving BPR. The industrial wastewater pH of 4.4 to 4.8 resulted in a pH of 5.5 in the anaerobic zone, and this prevented the establishment of BPR.
ACKNOWLEDGEMENTS

The author would like to thank the Sussman Foundation for providing financial assistance for this investigation. For their cooperation and assistance, the author would like to thank Hoeshct Celanese. The author would also like to express his appreciation to Dr. Clifford Randall for his expertise, input and patience during the course of this investigation. Great gratitude is extended to Julie Petruska and Marilyn Grendor for their advice and assistance in the laboratory. Special thanks is given to my wife and family, Trina Mastran and Sandra Davidson for their encouragement, help and humor.
TABLE OF CONTENTS

I. INTRODUCTION ......................................................... 1

II. LITERATURE REVIEW ................................................ 5

   Overview .............................................................. 5

   Biological Phosphorous Removal .................................. 6
      Anaerobic Conditions .............................................. 6
      Metabolic Pathways of Bacteria .................................. 10
      Role of pH .......................................................... 13
      Influent Characteristics ......................................... 15
      Operational Parameters .......................................... 19

   Selector Technology ................................................. 21

III. METHODS AND MATERIALS .......................................... 24

   Overview .............................................................. 24

   Experimental Design ............................................... 24

   Laboratory System Description ................................... 25
      Configuration ...................................................... 25
      Equipment ........................................................ 28
         Reactors ......................................................... 28
         Pumping ........................................................ 29
      Influent ........................................................... 30

   Operation of Laboratory BPR System ............................. 32
      Process Control .................................................. 34
      Start Up ............................................................ 34
      Sludge Wasting ................................................... 35
      Procedures ......................................................... 36
      Maintenance ....................................................... 38

   Sampling and Analyses .............................................. 39
      Overview ............................................................ 39
      Samples and Analyses ............................................. 39
      Analytical and Testing Procedures ............................. 42
      In-situ Testing .................................................... 44
## V. RESULTS

Overview .......................................................... 46

Laboratory BPR Treatment
System Performance ............................................. 46

Process Control ....................................................... 46
Substrate Utilization ............................................... 49
Anaerobic Substrate Removal .................................... 55
Phosphorous Removal ............................................... 63
Biomass Production and Concentrations ......................... 72
Settleability ........................................................... 81

Laboratory BPR Treatment System
Operating Conditions .............................................. 90

Dissolved Oxygen Concentrations and Utilizations and Oxidation Reduction Potential .......... 90
pH Measurements ................................................... 90
Nitrogen Compounds .............................................. 97
Other Inhibitors and Supplements ............................... 104
Influent Ratios and Operating Parameters ..................... 107

## V. DISCUSSION

Biological Phosphorus Removal .................................. 114

Industrial Wastewater ............................................ 114
Municipal Wastewater with COD Supplement .................. 118
Municipal Wastewater and 50/50 Municipal/Industrial Mixture ........................................ 119

Anaerobic Substrate Removal .................................... 121

Factors Affecting Sludge Settleability .......................... 125

## V. CONCLUSIONS .................................................. 127

REFERENCES ...................................................... 130

APPENDIX FIGURES ................................................ 133

APPENDIX TABLES .................................................. 140
LIST OF FIGURES

Figure 1. Schematic of a two-stage biological phosphorus removal system ........................................... 7

Figure 2. Schematics of BPR systems used by Wentzel et al. (1988) in their investigation .......................... 17

Figure 3. Schematic of the laboratory two-stage BPR system used for this investigation ............................ 26

Figure 4. Schematic of the laboratory three-stage BPR system used for this investigation ............................ 27

Figure 5. Location and description of the sample points for monitoring operation and performance of the laboratory system . 40

Figure 6. Mean cell residence time (MCRT) and hydraulic retention time (HRT) of the laboratory system during the investigation ............................................................... 48

Figure 7. Mass of Chemical Oxygen Demand (COD) removed by the laboratory system during the investigation ........... 51

Figure 8. Specific rate of COD removal per unit of MLVSS (SSUR) and food to mass (F:M) loading ratio of the laboratory system during the investigation ................................................. 53

Figure 9. Influent and effluent Chemical Oxygen Demand (COD) concentrations for the laboratory system during the investigation ................................................................. 54

Figure 10. Anaerobic zone COD removal rate per unit MLVSS_{Ana} by the laboratory system during the investigation ................................................................. 58

Figure 11. Comparison of system COD removal with the sum of COD removal in the anaerobic and aerobic zones . 59

Figure 12. TP and TSP concentrations of influent and effluent samples from the laboratory system during the investigation .... 65

Figure 13. The %P of the aerobic MLVSS_{Aer} of the laboratory system during the investigation ...................... 66
Figure 14. Comparison of the amount of TP removed by the system with the P requirement of the system ........................................... 67

Figure 15. The amount of TP removed by the system and the calculated P requirement of the system during the investigation ...... 68

Figure 16. Mass of total phosphorus fed to the laboratory system in the influent compared to mass of Total Phosphorus removed from the laboratory system in the waste activated sludge and the effluent ............................................. 70

Figure 17. Phosphate concentrations of samples from the influent, anaerobic zones, anoxic zone, aerobic zone and effluent of the laboratory system during the investigation (analyzed using ion chromatograph) ................................................. 74

Figure 18. Comparison influent PO₄ concentrations determined by colorimetric method and the ion chromatograph ................. 75

Figure 19. Comparison effluent PO₄ concentrations determined by colorimetric method and the ion chromatograph ................. 76

Figure 20. Biomass produced or lost in the effluent by the system compared to the influent TCOD of the system during the investigation .......................................................... 77

Figure 21. The observed yield (Y_{obs}) of the laboratory system during the investigation ......................................................... 78

Figure 22. The total suspended solids (TSS) concentrations of samples from the anaerobic and the aerobic zones of the laboratory system during the investigation .............................. 80

Figure 23. The TCOD of samples from the aerobic zone of the laboratory system during the investigation ................................. 83

Figure 24. The average effluent TSS concentration for each experiment of the investigation .................................................. 84

Figure 25. Effluent TSS concentrations of samples from the laboratory system during investigation ................................. 85

Figure 26. The solids loading rate (SLR) on the clarifier of the laboratory system during the investigation ........................... 86
Figure 27. Total suspended solids (TSS) concentrations of the aerobic zone biomass and the effluent from the laboratory system during the investigation ........................................ 88

Figure 28. Sludge volume Index (SVI) and Zone Settling Velocity (ZSV) of the aerobic zone biomass from the laboratory system during the investigation ........................................ 89

Figure 29. Dissolved oxygen concentrations of the influent, the reactor zones, and the clarifier surface of the laboratory system during the investigation ............................. 92

Figure 30. The oxygen utilization rate (OUR) and specific oxygen utilization rate (SOUR) of the aerobic biomass of the laboratory system during the investigation ......................... 93

Figure 31. The pH of the influent, the reactor zones, and the effluent of the laboratory system during the investigation ................... 98

Figure 32. The estimated concentration of nitrogen in the influent and the calculated concentration of nitrogen needed for the growth requirements of the laboratory system biomass during the investigation .................................................. 99

Figure 33. Nitrate (NO₃-N) concentration of samples from the anaerobic zone and the RAS of the laboratory system during the investigation ............................................. 102

Figure 34. The concentration of potassium supplemented into the influent and the calculated concentration of potassium needed for the growth requirements of the laboratory system biomass during the investigation .................................................. 109

Figure 35. The concentration of iron supplemented into the influent and the calculated concentration of iron needed for the growth requirements of laboratory system biomass during the investigation ............................................. 110

Figure 36. The %P of the MLVSSₐer compared to the influent TCOD to TP ratio of the laboratory system during the investigation .................................................. 113

Figure 37. Comparison of the average %P of the MLVSSₐer biomass with the average pH of the anaerobic zone for each experiment of the investigation ............................................. 122
Figure 38. Comparison of the average %P of the MLVSS$_{AER}$ and average mass of excess TP removed per influent COD loading with the average influent COD loading for each experiment of the investigation.
LIST OF TABLES

Table 1. Description of laboratory system operation for each experiment of the investigation .................. 33

Table 2. Description of laboratory system influent and average COD loading for each experiment of the investigation .... 47

Table 3. Averages, standard deviations and variances of the mean cell residence time (MCRT) and the system hydraulic retention time (HRT) of the laboratory system for each experiment of the investigation ................................................. 50

Table 4. Averages of the system food to mass loading ratio (F:M) and system specific rate of COD removal (SSUR) for each experiment of the investigation ...................... 52

Table 5. Averages of system and anaerobic % COD removals, influent, anaerobic and effluent COD concentrations, and anaerobic mass fraction (AMF) and hydraulic retention time (HRT_{ANA}) for each experiment of the investigation ............... 57

Table 6. Averages of system, anaerobic, and aerobic COD removals, anaerobic COD removal rate, and aerobic COD utilization for each experiment of the investigation ............ 60

Table 7. Averages of system COD removal, aerobic COD utilization, COD of wasted MLVSS_{AER} (COD_{w}), system total oxygen utilization (TOR), the oxygen required for oxidation of NH_{3}-N to NO_x-N (NOD), and anaerobic stabilization (AnS) for each experiment of the investigation ............ 62

Table 8. Summary of average influent and effluent TP concentrations, average TP removals, % TP removed, %TP in the aerobic MLVSS, P requirement of the biomass, TP removed per influent COD, and COD removed per TP removed for each experiment .................................................. 64

Table 9. Averages of ferric chloride (FeCl_3) dosage and the calculated mass of P precipitated at that dosage for each experiment of the investigation ........................................ 71

Table 10. Average phosphate (PO_4-P) concentrations from different points and zones of the laboratory system for each experiment of the investigation .............................. 73
Table 11. Averages of biomass production ($\Delta X$) and the observed yield ($Y_{obs}$) of the laboratory system for each experiment of the investigation ........................................ 79

Table 12. Averages of total suspended solids (TSS) and % volatiile suspended solids (%VSS) for different zones of the laboratory system for each experiment of the investigation ........................................ 82

Table 13. Averages of dissolved oxygen concentration from different points and zones of the laboratory system for each experiment of the investigation ...................... 91

Table 14. Averages of oxygen utilization rate (OUR) and specific oxygen utilization rate (SOUR) for the aerobic biomass from the laboratory system for each experiment of the investigation ........................................ 94

Table 15. Averages of oxidation reduction potential (ORP) in different zones of the laboratory system for each experiment of the investigation ............................... 95

Table 16. Averages of pH for the influent, anaerobic zones, anoxic zone, aerobic zone and effluent of the laboratory system for each experiment of the investigation ............... 96

Table 17. Total Kjeldahl Nitrogen (TKN) concentrations of influent and effluent samples from the laboratory system .................. 101

Table 18. Averages of nitrate (NO$_3$-N) and nitrite (NO$_2$-N) concentrations of samples from the influent, anaerobic zones, anoxic zone, aerobic zone, and effluent of the laboratory system for each experiment of the investigation .................. 103

Table 19. Average sulfate concentrations of samples from the influent, anaerobic zones, anoxic zone, aerobic zone, and effluent of the laboratory system for each experiment of the investigation ............................ 105

Table 20. Magnesium (Mg$^{2+}$) concentrations of influent and effluent samples from the laboratory system during the investigation 106

Table 21. Potassium (K$^+$) concentrations of influent and effluent samples from the laboratory system during the investigation 108
Table 22. Averages of the influent TCOD loading to the mass of MLSS\textsubscript{ana}, the influent TCOD to MLSS\textsubscript{ana} ratio, and the influent TCOD to influent TP ratio for each experiment of the investigation ................. 112
CHAPTER I. INTRODUCTION

The use of suspended growth Biological Nutrient Removal (BNR) systems to treat municipal wastewater is recognized internationally. These mixed culture systems have been demonstrated and practically applied at treatment facilities of various sizes. Numerous BNR process configurations exist. Each one is as unique as the influent wastewater that is treated and the effluent discharge limitations that must be attained. These systems oxidize the carbonaceous materials in wastewater while also biochemically utilizing and removing the various forms of the nutrients, nitrogen and phosphorus.

Operators, engineers, and researchers have found that BNR systems offer other advantages and benefits in addition to phosphorus and nitrogen removal. They have shown that BNR has the potential to produce savings in operating expenses and improve operational performance. These benefits come in the form of reduced chemical and energy costs, improved sludge settleability, and decreased sludge production and disposal costs.

Over the past two decades, world-wide application of BNR has progressively increased. This is especially true in South Africa, North America, Europe, Japan and other areas where drinking water resources and water quality have been affected by the discharge of nitrogen and phosphorus.

Increasingly, the requirement for nutrient removal is being imposed in point source discharge limitations in the United States. Promulgation of the Clean Water Act and its reauthorizations has resulted in a wave of comprehensive regulations. These regulations focus on preventing antidegradation, protecting or reestablishing water body beneficial uses, and eliminating the discharge of toxins. Convincing evidence has linked the point and non-point discharges of nitrogen and phosphorus to the degradation of water quality and subsequent loss of beneficial uses of water resources.

Poor water quality also means higher potable water treatment costs. Other beneficial uses such as recreation and commercial fishing also suffer. Maintaining in-stream nutrient concentrations at levels which limit the amount
available prevents the proliferation of aquatic vegetation and protects valuable natural and drinking water resources.

With the potential of BNR so attractive and the need to protect water quality and resources so crucial, BNR should be considered as a wastewater treatment alternative to activated sludge processes, physical-chemical treatment, and other advanced pollutant removal systems. Even though application of BNR is currently somewhat limited to municipal sewage and lab prepared substrates, the possibility of applying it to other, e.g., industrial, waste streams should not be overlooked or discredited.

Promotion of BNR begins with its ability to biologically remove nutrients in excess amounts relative to cellular growth requirements. Next, there are the potential benefits of less sludge production, reduced dependence on chemical additions, and savings in the amount of energy required for microbial oxidation-reduction reactions. BNR has also been demonstrated as a selector of favorably settling activated sludge. Compared with other pollutant and nutrient removal systems, BNR should have a decided economical, operational and environmental advantage.

The selection of a wastewater treatment system is usually economically based. The major items which are evaluated and given consideration are the following:

- Capital costs
- Annual operation and maintenance expenses
- Waste and process compatibility
- Reliable process performance
- Degree of pollutant removal required
- Retrofit of existing structures

BNR systems could rate favorably in all these categories. Properly designed, operated, and maintained BNR systems have the capacity to produce a high quality effluent at competitive or reduced operating costs. These potential savings are significant enough to justify the cost of a study to determine if BNR
may be applicable. Even if nitrogen or phosphorus removal is not required, BNR could prove to be a cost effective alternative.

Realization of these advantages is not guaranteed. BNR systems require extensive process control and monitoring. Like other activated sludge processes, BNR is susceptible to hydraulic washout and toxic shock loads. Ultimately, BNR success depends on the constituents and characteristics of the influent wastewater. While provisions may be made to handle some unfavorable or uncertain conditions, it is imperative that BNR’s applicability be examined and investigated prior to final design and construction.

The influent waste that was tested in this investigation was a high strength industrial wastewater. A laboratory scale BNR system was continuously fed the industrial wastewater and the operating conditions were varied. The wastewater was seemingly idea for biological phosphorus removal because it typically contained about 1000 milligrams/liter (mg/L) of acetic acid and more than 30 mg/L of total phosphorus. However, the high acetic acid caused a wastewater pH of 4.5 and lower. The testing results were evaluated to assess the effect of an anaerobic zone on the activated sludge treatment of this industrial wastewater. The high strength wastewater used as influent for the laboratory BNR unit was from a cellulose acetate manufacturing plant. Its influent characteristics present a unique, less researched situation for BNR.

The industrial wastewater contained several organic compounds in addition to acetic acid and was usually nitrogen deficient for activated sludge metabolism. Known organics present other than acetic acid, in order of concentration, were isopropyl alcohol, acetone, methyl ethyl ketone, methyl cyanide, isopropyl acetate, and traces of benzene and mesityl oxide. The nitrogen compounds were probably from wood fibers, but their exact nature was unknown. The wastewater also contained low concentrations (< 0.50 mg/L) of copper, chromium, and nickel.

The purpose of the investigation was to determine if this wastewater could be successfully treated using BPR technology. Subjecting the BPR process to different waste characteristics could assist in more fully under-
standing BPR and could foster continued interest in the application of BNR processes to wastewaters other than domestic.

BNR systems have been used to treat municipal and mixtures of municipal and industrial wastewaters. BNR may be applicable to a range of different wastewaters, but past experience has shown that influent wastewater characteristics figure prominently into the performance of BNR. Ample amounts of essential micro- and macro-nutrients must be present along with a readily biodegradable substrate.

Experience to date has indicated that the higher the proportion of substrate loading to nutrient loading, the better the nutrient removal efficiencies. However, high substrate loadings have not been thoroughly explored or defined beyond municipal sewage or prepared substrates of similar concentrations. Use of this industrial wastewater as influent for a BNR system could help determine the limits of BNR. It could also serve as a way to identify favorable or detrimental influent organic components for BNR.

The major objective of this investigation was to determine if BPR could be achieved with the industrial wastewater as influent to the laboratory BPR system. Consequent objectives were to evaluate the laboratory BPR system for the removal of carbonaceous material, the amount of substrate utilization in the anaerobic zone, and the effects of an anaerobic selector on sludge settleability. In addition, information on the influent and laboratory system operating conditions could be compared to system performance. The objective being to identify influent characteristics and loading parameters which impacted BPR or treatment.
CHAPTER II. LITERATURE REVIEW

OVERVIEW

There are numerous ways to arrange the anaerobic, anoxic and aerobic zones of BNR processes. These systems are single-sludge activated sludge systems but with the addition of anaerobic and anoxic zones in series with an aerobic zone(s). The purpose of these non-aerated, well-mixed zones is to create environmental conditions which promote excess biological phosphorus and nitrate removal.

The excess biological phosphorus removal (BPR) process may be combined with nitrification and denitrification processes in various combinations. These processes may function together in the same system to remove phosphorus, ammonia and nitrates. It should be noted that these reductions in nutrients are greater than the amounts required for the normal metabolic requirements of activated sludge systems.

Depending on the influent waste characteristics and the effluent discharge permit limitations, phosphorus removal may be dependent or independent of total nitrogen removal. The requirement for nitrification of ammonia and the amount of oxidized nitrogen present in the influent and recycle sludge streams dictate whether an anoxic zone is necessary. Effective denitrification is often directly related to successful BPR. If little or no ammonia is present in the influent or there is not an ammonia effluent permit limitation, a BPR system may be operated without nitrification, and consequently, without denitrification.

In this investigation, the application of BPR for treatment of a high strength industrial wastewater was tested. The wastewater organics were readily biodegradable and consisted primarily of acetic acid, the best organic for the stimulation of BPR (Abu-ghararah and Randall, 1991). The microbial culture which grew and survived on this waste stream was dependent on the waste’s constituents and the environmental conditions to which the culture was subjected. However, utilization of the mixed microorganism cultures which are unique to BPR processes requires an understanding of the mechanisms
responsible. The current state of knowledge about these microorganisms and mechanisms is summarized in the following sections.

**BIOLOGICAL PHOSPHORUS REMOVAL**

It is generally agreed that phosphorus is removed through incorporation or removal with the solids which make up the biomass or activated sludge under aerobic conditions. As BPR applications increase and research progresses, the specific mechanisms which are responsible for BPR are becoming more fully understood. There are a number of factors which affect the performance of the BPR process. Theses include,

- Anaerobic conditions
- Metabolic pathways of bacteria
- Role of pH
- Influent characteristics
- Operational parameters

Understanding the role of these factors in relation to BPR is imperative. Information about these can be useful in the operation and evaluation of the BPR system as a treatment alternative.

**Anaerobic Conditions**

The presence of a non-aerated, anaerobic zone is a known prerequisite for BPR (Barnard, 1975). This zone must be devoid of dissolved oxygen (DO) and oxidized nitrogen. In its most basic form (see Figure 1), the system is described as two-stage BPR. The anaerobic zone receives influent wastewater and the underflow or return activated sludge from the secondary clarifier.

Generally, it has been found that when the anaerobic environment was established, there was a release of phosphate and an uptake of substrate in the anaerobic zone. Following this release and uptake, there was a corresponding uptake of phosphate in the aerobic zone. While the amount of phosphorus which was taken up in the aerobic zone was important and the objective of treatment, the key features of BPR were the release of phosphorus and the uptake of a carbon source in the anaerobic zone.
Figure 1. Schematic of a two-stage biological phosphorus removal system.
Buchanan et al. (1984) explained that this stored carbon would supply energy for the exclusive use of the microorganisms which would uptake excess phosphate in the aerobic zone. They noted that the readily biodegradable chemical oxygen demand (RBCOD) was partitioned or stored under anaerobic conditions by consuming polyphosphate reserves. Due to its importance to BPR, methods were developed (Dold et al., 1980) to quantify this RBCOD fraction.

Koch and Oldham (1985) found in their investigations that the measurement of oxidation-reduction potential (ORP) was useful in BNR. They determined that ORP was a good indicator of the presence of nitrates in the anaerobic zone. It was also suggested that there was a relationship between phosphate release and ORP. From their efforts, it was shown that ORP levels less than -150 millivolts (mV) indicate anaerobic conditions (absence of nitrates). This level is not considered absolute since it varies with the different BNR processes and measurement methods. Koch and Oldham (1985) found through testing that the combination perphric platinum ring electrodes filled with the silver-silver chloride reference gel worked best in anaerobic conditions. They utilized these commercially available electrodes to test in-situ conditions.

The release of phosphate in the anaerobic zone does not necessarily assure BPR. Comeau et al. (1987) have shown that there can be a release of phosphorus under anaerobic conditions for other reasons. Adjustment to a high pH, addition of a 2,4-dinitrophenol solution, and the bubbling of carbon dioxide (CO₂) and hydrogen sulfide (H₂S) gas were shown to cause the anaerobic release of phosphate. It was further indicated that this release of phosphate was not favorable to BPR. There was no accompanying storage of carbon substrates which allow subsequent phosphate uptake in the aerobic zone.

In addition to the non-aerated, anaerobic conditions that must be maintained, attention has also been given to the physical characteristics of the anaerobic zone. The staging of the anaerobic zone was demonstrated to be important to BPR (Daigger et al., 1987 and Buchan et al., 1984). Their evaluations indicated that subdividing the anaerobic zone into a number of
compartments in series optimizes the operation of this zone. More effective adsorption of the RBCOD was observed in a compartmentalized system when compared to a single stage system (Buchan et al., 1984).

Consideration should also be given to the hydraulic retention time (HRT) and the mass fraction of the anaerobic zone. Randall et al. (1992) recommended that when designing this zone the secondary release of phosphates should be avoided. Secondary release of phosphate was defined as the release of phosphates without a corresponding storage of a substrate for energy under subsequent aerobic conditions. They explained that when this secondary release dominated, there were not sufficient carbon reserves available for the uptake of the released phosphates in the aerobic zone.

Randall et al. (1992) recommended that the HRT in the anaerobic zone be limited to prevent secondary phosphate release. They noted, however, that with stronger or partially fermented waste, longer HRTs could be beneficial compared to those used for weaker or completely fermented wastewaters. In this instance, they indicated that longer anaerobic retention time could improve BPR performance. Randall et al. (1992) concluded from their communications and observations that fermentation in the anaerobic zone should not be relied upon for many wastewaters.

Cases were noted where higher anaerobic mass fractions proved to be beneficial. Comeau et al. (1987) noted that during their pilot operations with a three-stage Bardenpho process being fed an acetate substrate, doubling the anaerobic mass fraction doubled the phosphorus removal.

Randall et al. (1992) stated that while the anaerobic mass fraction has an effect on BPR, the anaerobic hydraulic retention time is more important to BPR performance. To obtain the desired effluent phosphorus concentration, they recommended making the size of the anaerobic zone variable or adjustable. When the influent already contains sufficient fermentation products, they noted that anaerobic HRT only needs to be long enough for the storage of the carbon substrate as poly-B-hydroxybutyrate (PHB) or some similar energy form. As
they later noted, these examples indicate that care should be used in sizing and providing for fermentation in the anaerobic zone.

Wentzel et al. (1988) found that care must be taken to ensure that the bio-P bacteria in the anaerobic zone are not overloaded with acetate. They determined that the anaerobic mass fraction must be large enough to prevent the leakage of acetate to the aerobic zone. They theorized that this caused the bio-P bacteria to lose their competitive advantage in the system. This allowed other microorganisms to become dominant.

Pattarkine (1991) noted in his research that phosphorus removal or uptake did not start in the aerobic zone until all of the volatile fatty acids (VFA) had been removed from solution. Pilot studies using a municipal sewage have also indicated the importance of aerobic HRT (Fukase et al., 1985). Fukase et al. (1985) concluded that removal of P improved at shorter aerobic HRTs. This was conditioned on the appropriate anaerobic parameters being maintained in the anaerobic zone.

Metabolic Pathways of Bacteria

Since the phenomenon of excess phosphorus removal was first reported, many possible reasons for this removal have been postulated. Fuhs and Chen (1975) were able to demonstrate that phosphate was released from intracellular polyphosphate granules by the action of certain types of bacteria. Nicholls and Osborn (1978) were the first to propose that under anaerobic conditions, polyphosphate (poly-P) serves as an energy source to facilitate the storage of carbon sources such as acetate. The anaerobic release of phosphates into solution accompanies this storage of carbon. The simple carbon compounds are stored as PHB inside the bacterial cells. Under subsequent aerobic conditions, the PHB is metabolized to provide energy for the uptake of all available orthophosphate.

Marais et al. (1983) explained in their review that the consumption and utilization of poly-P reserves provides energy to partition influent RBCOD for the exclusive use of the bacteria which accumulate phosphate in the aerobic zone.
Comeau *et al.* (1986) referred to the bacteria which are responsible for BPR and capable of storing poly-P and carbon substrates as bio-P bacteria.

Efforts have been made over the last few years to model BPR. Work by researchers has led to the formulation of some significant relationships. In their investigations into the kinetics of BPR, Wentzel *et al.* (1985) determined that with municipal sewage:

- the release of P with respect to RBCOD conformed to a first order type of reaction at a relatively slow rate
- the rate of release of P was governed by the rate of conversion of RBCOD to short-chain fatty acids (SCFA)
- the mass of P released appeared to be proportional to the mass of RBCOD converted

With the addition of acetate in anaerobic batch tests, they found:

- the release of P with respect to acetate conformed to a zero order reaction at a relatively fast rate
- the total mass of P released was proportional to the mass of acetate taken up

When subjected to aerobic conditions, they established:

- the mass of P taken up was proportional to the mass of P released in the anaerobic reactor
- The mass of P taken up was not significantly affected by system sludge age; therefore, it was concluded that the poly-P organisms have a very low endogenous mass loss rate

In their model for BPR by *Acinetobacter*, Comeau *et al.* (1986) proposed that the internal ATP/ADP ratio of the bio-P bacteria and the regulation of the pH gradient across their inner membrane could explain phosphorus uptake and release. According to their model, poly-P was degraded by the bio-P bacteria to provide energy for the transport and storage of simple carbon substrates like acetate into the bacterial cells under anaerobic conditions. Within the cell, orthophosphate accumulated as poly-P was utilized. The orthophosphate was released from the cell into solution with the aid of a pH influenced transporter.
They calculated that one proton ion (H⁺) was discharged across the cell membrane for every unit of non-ionic acetic acid which was transferred into the membrane. In the aerobic zone, the stored carbon was consumed for the uptake of phosphate to produce poly-P. Their theory proposed acetyl-CoA and the tricarboxylic acid (TCA) cycle as necessary for the synthesis of PHB.

From Randall et al. (1992), it was learned that Wentzel et al. (1987) built upon the Comeau et al. (1986) model. For their modifications, they theorized that ATP/ADP and NADH/NAD ratios control poly-P and PHB synthesis and degradation. In the anaerobic zone, they concluded that without a terminal electron acceptor, the NADH/NAD ratio increases while the ATP/ADP ratio decreases. This ensured that under aerobic conditions, the degradation of PHB was controlled by a decrease in the NADH/NAD ratio. PHB degradation provided the cell with energy and it increased the ATP/ADP ratio which aided in the formation of poly-P. This model was based on the TCA and glyoxylic cycles being activated by the aerobic NADH/NAD decrease. It was the NADH/NAD decrease which led to poly-P synthesis.

In contrast, Mino et al. (1987) implicated the Embden-Meyerhof-Parnas (EMP) pathway for the generation of NADH₂ for PHB creation under anaerobic conditions. They noted that acetyl-CoA was not oxidized to NADH₂ under anaerobic conditions through the TCA cycle. Thus, the production of PHB would be prevented.

Randall et al. (1992) added that the presence of nitrates in the anaerobic zone could provide some bacteria with a terminal electron acceptor. This prevented the RBCOD from being converted to SCFA which were needed for the formation of PHB. Past research and operation experience indicated that when the concentration of oxidized nitrogen exceeded 0.20 mg NO₃⁻/L in the RAS to the anaerobic zone, BPR was not successful. As a result, under aerobic conditions there was not enough accumulated carbon for the bio-P bacteria to use as an energy source to take up phosphates.

Tracy and Flammino (1987) proposed a three-step biochemical pathway for BPR. They, like those previously mentioned, noted the anaerobic transport
and storage of organic substrates, and the subsequent aerobic metabolism which drove phosphate uptake. They also emphasized the key importance of the enzyme, polyphosphate kinase. They believed it catalyzed the anaerobic generation of ATP and the aerobic replenishment of the poly-P pool. They also added that high aerobic metabolic rates and the generation of sufficient ATP were necessary to reverse the reaction catalyzed by this enzyme. Randall et al. (1992) summarized Lotter's explanation of the role of polyphosphate kinase. This review indicated a similar role for the enzyme.

Role of pH

Most bacteria have an optimum pH at which they function at their best. For most it is near neutral, with some being able to survive at a minimum of 5 and a maximum around 9 (Gaudy and Gaudy, 1988). There does not seem to be much literature on the effects of pH on BPR, especially related to non-aerated, anaerobic conditions. One would believe that bio-P bacteria and their metabolism would be strongly influenced by pH. Gaudy and Gaudy (1988) pointed out that in the anaerobic treatment of industrial waste or sewage sludge the control of pH is important because of the narrow pH range where treatment is effective. They explained that this is even more so for anaerobic than for aerobic treatment.

An anaerobic batch test was conducted to test the effect of lowering the pH on BPR (Comeau et al., 1987). Sludge was taken from the aerobic zone of a BNR pilot plant, then subjected to non-aerated conditions for 6.5 hours to ensure complete denitrification. The pH was then adjusted to 4.0 with a 0.5 N hydrochloric acid (HCl) solution. Only a slight increase in phosphate was observed over 4 hours of anaerobic conditions in comparison with the control. The air was then turned on for 3.5 hours to simulate aerobic conditions. No phosphate uptake was observed except in the control reactor. They noted that this indicated that the metabolic activity of the biomass in the pH-lowered reactor was probably inhibited.

Testing by Tracy et al. (1988) showed that the uptake of phosphorus in the aerobic zone is strongly influenced by pH. Their results indicated that the
maximum uptake rate was obtained over a pH range of about 6.8 to 7.4. The uptake rate dropped off significantly below pH 6.0 and it appeared to approach zero at a pH of less than 5.4. As Randall et al. (1992) noted, with the mechanisms proposed to be responsible for BPR, more energy will be required to take up acetates against a higher proton (H\(^+\)) concentration in the anaerobic zone. Gaudy and Gaudy (1988) explained that the effects of pH on the transport of materials across the cell membrane was very important and perhaps the determining factor influencing growth. Proton-motive force was responsible for this transport of materials into the cell. It was defined as an electrochemical gradient across the cell membrane which is caused by the translocation of H\(^+\). The establishment of this gradient, they explained, provided for the conveyance of a variety of ions and organic compounds across the membrane. They added that the pH gradient across the membrane was affected by the pH of the surrounding aqueous environment which in turn influenced the driving force for transport.

The microorganisms also affected the pH of the surrounding environment. Gaudy and Gaudy (1988) pointed out that the internal pH of the cell was not determined solely by the environment. They noted that microorganisms have some ability to control the entry or exit of ions, like H\(^+\), into and out of their cells. They found that changes in the pH of the surroundings could be brought about by the microorganisms. This was accomplished by cells transporting alkaline products into the environment or by the cell removing certain ions from the environment.

Gaudy and Gaudy (1988) also mentioned that pH has other indirect effects. Besides altering the actual pH gradient, pH controlled the configuration and activity of the cell membrane's proteins which bind with compounds and transport them across the membrane. The external pH also figured into the determination of the ionization state of nutrients required by the cells. This surrounding pH helped define the ionization state of compounds which may be toxic and inhibitory to the cells. Gaudy and Gaudy (1988) pointed out that cells were more permeable to nonionized compounds and nutrients than to
ionized compounds and nutrients. Therefore, a compound may be toxic or inhibitory to the microorganisms’ growth and survival only at a pH at which it is not ionized.

**Influent Characteristics**

Since the inception of BNR processes, operators and researchers have attempted to quantify components of the influent wastewater which lead to successful BNR operation. Through these efforts, it was established that the performance of BNR was influenced and even dependent on the composition of the influent wastewater.

In their compilation, Buchan *et al.* (1984) detailed municipal wastewater characteristics which govern the design of BNR and the effluent quality that may be produced. Some of the important influent parameters they used were as follows:

- Total Chemical Oxygen Demand (TCOD)
- RBCOD fraction
- Total Kjeldahl Nitrogen (TKN) to TCOD ratio, TKN/TCOD
- Maximum specific growth rate of nitrifiers @ 20°C
- Maximum and minimum temperature
- Total Phosphorus (TP) to TCOD ratio, TP/TCOD

They stressed that the success of BPR was dependent on the RBCOD content of the influent. Another consideration that figured prominently in BPR performance was the exclusion of nitrates from the anaerobic zone. They suggested that the TP/TCOD ratio should be less than 0.017 to 0.020 to achieve effluent phosphorus concentrations down to 0.5 mg TP/L. If nitrogen removal was required, they advised that the TKN/TCOD ratio should be less than 0.08 for complete denitrification. It should be understood that these ratios were developed using municipal wastewaters in South Africa.

In their work to develop an enhanced culture of bio-P bacteria, Wentzel *et al.* (1988) found that optimizing BPR required the selection of the appropriate influent constituents. They were able to develop enhanced bio-P cultures by feeding an influent of mixed substrates and nutrients to the anaerobic zone of
BNR systems as depicted in Figure 2. The influent which was fed to these systems consisted of 500 mg COD/L of acetate, a mixture of inorganic nutrients, and yeast extract.

It appears that BPR is also influenced by the type of organic compound available for bio-P bacteria metabolism. Randall et al. (1992) pointed out that phosphorus removal varied with the amount of stored substrate contained in the bio-P bacteria when they enter the aerobic zone. They explained that the bio-P bacteria need SCFA to form stored organics like PHB. Abu-ghararah and Randall (1991) fed different VFAs and municipal sewage to the anaerobic zone of a BPR pilot plant operating at a mean cell residence time (MCRT) of 13 days. Their data indicated that the phosphorus removal efficiency varied with the specific organic compound being used. Acetic acid was found to be the most efficient organic for BPR. With acetic acid, they obtained the following results:

**Acetic Acid - Substrate**

- mg/L P Uptake per mg/L COD utilized = 0.37
  (TP uptake in aerobic zone)
- mg CODUtilized per mg P Removed = 16.8
  (COD utilized and P removed in total system)

Randall et al. (1992) discussed the results of testing conducted by Gerber. The batch tests by Gerber consisted of feeding both nitrates and various carbon substrates to the activated sludge of a BNR plant under non-aerated conditions. When using acetic acid as the substrate, it was found that as the nitrates and substrate were being consumed, substrate must have also been available for uptake by the bio-P bacteria. This was concluded because of the rapid release of phosphates which accompanied the consumption of nitrates and acetic acid. When the acetic acid was depleted, a much slower or secondary release of phosphate continued.

Randall et al. (1992) concluded that BPR must be either phosphorus or storable organics limited for all municipal wastewaters. When BPR was phosphorus limited, all the readily available organics could not be stored or taken up in the anaerobic zone by the bio-P bacteria. They noted that if the
Three-stage Bardenpho System

University of Cape Town System

Figure 2. Schematics of BPR systems used by Wentzel et al. (1988) in their investigation.
soluble organics entered the aerobic zone, it could permit the growth of non bio-P bacteria. The resulting percent P in the aerobic mixed liquor would be relatively low, 4% to 6%. On the plus side, the soluble effluent phosphorus should be very low (<0.5 mg P/L) unless secondary phosphate release occurred in the aerobic zone or the secondary clarifier.

They also found that where the influent was COD limited, there was not enough storable organics available to facilitate phosphorus removal to the low concentrations. While the bio-P bacteria were dominant and the percent P in the MLVSS was high, the effluent phosphorus tended to be high (>1.5 mg P/L). They further recognized that COD could be limiting for a couple of reasons. One limitation could be that there were not enough total organics present in the influent to achieve the phosphorus removal required. The other possibility could have been that the wastewater organics were not present as SCFAs.

Information on the inhibition of the metabolic mechanisms of bio-P bacteria by metals or other compounds was limited (Randall et al., 1992). They found only one instance where BPR was prevented due to the toxic nature of a component of the wastewater. The specific compound was not identified, but the responsible sidestream was isolated. They noticed that in the majority of the cases of full scale operation, nitrification would also be inhibited. As discussed earlier, the determining factor on whether or not a substance prevents BPR seems to depend on whether the substance un-ionized or ionized. Even if a potentially toxic compound was present in the influent substrate, it might not be toxic or restricting to the growth of the microbial culture if it was in a non-ionic form. In this form the substance would not pass through the microorganisms' cell membrane and get assimilated.

The presence of cations in the influent wastewater was implicated as an important ingredient for successful BPR. Wentzel et al. (1988) found in their research efforts to develop an enhanced polyphosphate organism culture that certain cations were needed in the influent. They noted the effects of magnesium (Mg), calcium (Ca), and potassium (K) on BPR. Improvements in
P removal were seen with the addition of these cations. Comeau et al. (1987) observed that Mg, Ca, and potassium K were co-transported with phosphate during anaerobic and aerobic conditions. The metallic cations Mg and K were identified as essential for bio-P bacteria and BPR by Pattarkine (1991). His research showed that both Mg and K must be available to the bio-P bacteria for phosphate release and uptake to be accomplished. Randall et al. (1992) noted that the minimum amounts required for each mole of P removed is 0.25 moles of Mg and 0.23 moles of K.

Operational Parameters

Once the BPR system has been designed and constructed, operation may prove relatively simple. That is, if the influent has been sufficiently characterized and some flexibility has been provided in process configuration and zone volumes. Experiences with full scale and pilot plant operations have allowed operators and researchers to define operational parameters which affect BPR performance.

The researchers for Air Products and Chemicals, Inc. indicated that unfavorable substrate, as measured by biochemical oxygen demand (BOD), to phosphorus loadings (BOD/P) may be overcome by high soluble substrate (food) to biomass (F_s/M) ratios (Tracy et al., 1988). They used twelve years of operating experience to develop a model which can use the relationship between BOD/P and F_s/M to predict effluent phosphorus concentration. They claimed that their model demonstrated that as the influent BOD/P increased, the minimum system F_s/M needed to achieve a certain effluent quality decreased. They contended that the two-stage BPR which they had patented as the Anaerobic/Oxic (A/O) process was favored by operation at high system F/M. It was noted that this model was only applicable for BOD/P from 10 to 15, and F_s/M range of 0.10 to 0.40.

In other research by A/O promoters, it was found that at F/M organic loading rates of greater than 0.50 g BOD/g VSS-day, the phosphorus removal deteriorated (Tracy et al., 1986). The percent phosphate removal went from
80 to 55 and 46 when the F/M was increased from 0.46 to 0.76 and 0.98, respectively. The following should be noted for this experiment:

- three batch-fill and draw units were operated
- cycle time for each unit consisted of 1 hour of non-aerated mixing, 5.5 hours of aeration and 1.5 hours of settling, decanting, degassing and recycle mixing
- nitrification was inhibited by addition of allylthiourea
- the feed for the high loadings was a mixture of brewery and cheese plant wastewater and municipal sewage
- the feed for the lower loading was a more typical municipal wastewater

Other researchers have conducted similar research in an effort to determine favorable ranges for other operational parameters. In their pilot study, Fukase et al. (1985) obtained information which demonstrated the importance of several parameters. In the anaerobic zone, BOD loading rates and BOD to biomass ratios were concluded to have an impact on phosphorus removal. In their study, a two-stage BPR was fed a municipal sewage. From their results, they concluded that when the anaerobic zone BOD loading rates were maintained below 0.20 kg BOD/kg MLSS-day and BOD to MLSS ratios were kept less than 0.10 kg BOD/kg MLSS, the lowest effluent phosphate levels were achieved.

Fukase et al. (1985) believed these results agreed with some generally accepted theories. One was that the substrate loading rate to the anaerobic zone must be lower than the BOD uptake rate of the microorganisms. The other was that the amount of substrate available should not exceed the microorganism’s capacity for BOD storage. In their testing to determine the affect of aerobic HRT on BPR, the aerobic zone HRT was varied over a range of 3 to 6 hours. Their results enabled them to determine that the shorter HRT produced the greatest phosphorus removal. The influence of MCRT and BOD loading rates were also examined during this part of the study. They deduced that these parameters were not as important as HRT and MLSS concentration.
The importance of sludge age, solids retention time (SRT) or MCRT on BPR has also been downplayed by other researchers. Randall et al. (1992), citing case studies reported by Barnard, noted that BPR systems are practically independent of SRT over the range of two to forty days. They explained that because of how the percent phosphorus in the mixed-liquor volatile suspended solids (MLVSS) varied as a function of the influent COD:P ratio, BPR was independent of SRT. They found that the percent P in the MLVSS increased up to a maximum capacity without affecting the P removal when the MCRT was increased.

The increase in the percent P, Randall et al. (1992) reasoned, was due to the increase in the fraction of bio-P bacteria in the biomass. They theorized that the percent P increased until either available P or available COD became limiting. Using data collected from full-scale demonstrations and pilot studies of BPR systems, they compared the influent substrate to phosphorus ratios to the percent P of the MLVSS and the effluent phosphorus levels. As noted earlier, their graphs confirmed that the influent ratio of COD to P is inversely related to effluent P and percent P of the MLVSS.

SELECTOR TECHNOLOGY

In their study on the control of activated sludge settling, Tracy et al. (1986) defined an anaerobic selector as "a zone in which influent wastewater and biomass are contacted in the absence of supplied oxygen or oxidized nitrogen". They like several others, have associated improved sludge settleability and prevention of filamentous bulking with the addition of an anaerobic zone ahead of the aerobic or oxic reactor in the activated sludge process. Whether it was the elimination of filamentous organisms, the anaerobic utilization of available substrate, or the creation of a substrate gradient, it has been seen that anaerobic selectors improve sludge settling.

Earlier reports and observations of the advantages of the two-stage BPR process culminated in experiments by Wanner et al. (1987) and Tracy et al. (1986). They recognized that good sludge settling and the elimination of filamentous bulking could be linked to the use of an anaerobic selector. Pitt
and Jenkins (1988) even attributed the reduction of Nocardia foaming to the use of an anaerobic selector. Some researchers have concluded that there is a relationship between favorable settling characteristics and the anaerobic utilization or uptake of substrate (Wanner et al., 1987).

While Randall et al. (1992) cautioned against the use of sludge volume index (SVI) as a measure of sludge settleability, it is generally considered to be the most practical and widely used method. As they noted, in cases where the aerobic mixed-liquor suspended solids (MLSS) concentration are greater than 3500 to 4000 mg/L, SVI values are not a good indication of sludge settling. This is the MLSS range of most BNR systems, especially those that nitrify. In lieu of any other common methods of gauging sludge settling, SVI has been used predominately in wastewater treatment and research.

Microscopic examinations and Neisser staining were utilized by Wanner et al. (1987) to characterize the biomass floc, identify filamentous bacteria, and locate polyphosphate granules. These combined with SVI determinations demonstrated that anaerobic conditions have positive effects on sludge settling. Their observations indicated that filamentous microorganisms were not as common in the anaerobic selector systems as in the aerobic control unit. The bulking was repressed because of metabolic principles, they reasoned. It was concluded that heterotrophic filamentous microorganisms do not have sufficient substrate available for their survival in the subsequent aerobic zone.

Wanner et al. (1987) found that effective selection of favorably settling bacteria occurred when most of the substrate available was utilized in the anaerobic zone. Tracy et al. (1986) cited Eikelboom as confirming that a selector is effective only if 50 to 70 percent of the influent COD is taken up in the initial zone. They added that while a high substrate gradient and the staging of the anaerobic zone was advantageous to phosphorus removal, it was not required for eliminating filamentous bulking. They further determined that the configuration of the anaerobic zone was not responsible for suppressing the growth of filamentous bacteria. Their work proved, they contended, that the
selection pressure in an anaerobic zone is based on a metabolic difference between most filamentous and non-filamentous microorganisms.

Others have claimed that an initial contact zone with a high substrate gradient and nonlimiting DO prevent the widespread growth of filamentous microorganisms (Chudoba et al., 1973). Experiments by Tracy et al. (1986) using batch-fill-and-draw units showed that initial low DO conditions provided for both BPR and the growth of filamentous microorganisms. They found that an initial DO level of at least 1.0 mg/L was needed to produce a good settling, non-filamentous sludge. Their work also indicated that an anaerobic selector process improved sludge settling. They reported that the anaerobic selector process could handle a wide range of high organic loadings and still produce a sludge with good settling properties. As noted earlier, BPR was not sustained under these high loadings. Biological phosphorus removal resumed when the lower loading to the anaerobic zone was re-established. This, they felt, implied that the poly-P survival mechanism could persist and the poly-P bacteria had a competitive advantage during the higher loadings.

The work discussed in this section indicates that a two-stage BPR process may improve process performance. The benefits which were noted included lower SVI values, reduced effluent TSS concentrations, and less susceptibility to sludge bulking. From previous investigations, it also appears that there are unfavorable conditions for anaerobic selectors. At high organic loadings, Wentzel et al. (1988) reported that sludge settleability deteriorated. While they attributed this bulking to the lack of some essential nutrient, the high organic loading could have contributed to the decline in sludge settling.

Because the most critical part of all activated sludge systems is the solid-liquid separation or secondary clarification step, the use of a process which utilizes an anaerobic selector should be considered. The anaerobic zone has been shown to stabilize sludge settling and help prevent filamentous bulking.
CHAPTER III. METHODS AND MATERIALS

OVERVIEW

During this investigation a laboratory scale BPR system was continuously fed the high strength industrial wastewater. This chapter describes the experimental systems used to simulate the BPR process. It also specifies the equipment and analytical methods used to evaluate system performance. Information describing the treatment system, testing, and sampling will be presented.

EXPERIMENTAL DESIGN

The most effective way to determine if a treatment system can achieve the desired objectives is by application. Full scale operation would be ideal, but it may be impractical and too expensive. This is especially true for existing plants that cannot be easily retrofitted or have other physical constrictions. Pilot testing of the treatment process is another alternative, but it can also be expensive. Use of a small, lab scale, continuously fed unit is more practical. By simulating the different conditions on a small scale, the BPR process could be evaluated. The results obtained from the experiments helped determine if the waste was treatable by BPR and if the proposed benefits of BPR could be realized. In addition, the experience operating the lab scale unit provided information that would be helpful for full scale operation and design.

The lab scale unit simulated the various zone conditions of BPR. Selection of the unit’s zone volumes, feed and recycle pump rates, and MCRT was based on previous experiences with BPR operation. Utilizing system set ups and configurations from successful BPR applications, the lab unit was developed. Monitoring and operating the lab scale BPR system provided information which indicated whether the desired conditions were being attained.

The small scale of the lab unit allowed this system to be flexible. Adjustments were made to the operating conditions by physically modifying equipment or changing a process parameter. The zone reactor volumes, feed and recycle pump rates, rate of aeration or DO level, and degree of mixing
could be regulated over a limited range. These could be increased or decreased to change the unit’s HRT, MCRT, and aerobic zone DO, and process configuration. By being flexible, the lab system could maintain BPR operation over a wide range of operating conditions and influent characteristics.

LABORATORY SYSTEM DESCRIPTION

Configuration

The laboratory scale BPR system consisted of anaerobic, anoxic, and aerobic reactors in series. The mixing regime of each reactor was intended to simulate a completely mixed continuous-flow reactor. Figure 3 illustrates the initial system configuration of the lab scale unit. This unit is described as a two-stage BPR process. It consisted of anaerobic and aerobic reactors. Later in the investigation, the anoxic reactor was added to account for the change of influent characteristics. This three-stage BPR process is depicted in Figure 4.

The experimental reactors were sized to provide greater detention times than are generally used for municipal BNR systems. Since the system was treating a high strength industrial wastewater, sizing of the reactors was conservative. The target HRT of the anaerobic zone was initially selected to be 6 hours with an aerobic zone HRT of 42 hours. The total HRT for the BPR system was 2 days or 48 hours. Later, the target anaerobic detention time was increased to almost 9 ½ hours with the total system HRT remaining at 48 hours. When the anoxic zone was created, the non-aerated HRT was doubled to 19 hours. The aerobic HRT was decreased to 29 hours. The reactors provided a total HRT similar to that used by the cellulose acetate manufacturer for full-scale wastewater treatment.

To limit nitrification and the recycle of oxidized nitrogen to the anaerobic zone, a target MCRT of 8 days was selected. This insured that the industrial wastewater was contacted with an adequate concentration of active biomass. The lab unit was operated with the room environment maintained at approximately 20° C. While nitrification can be accomplished at this temperature, the low MCRT was expected to prevent significant nitrification.
Figure 3. Schematic of the laboratory two-stage BPR system for this investigation.
Figure 4. Schematic of the laboratory three-stage BPR system for this investigation.
given the high organic strength. The industrial waste contained ammonia-nitrogen, but it was usually low and variable in concentration.

The feed and return activated sludge (RAS) recycle pump rates were maintained at a constant rate and at a 1:1 ratio. When the anoxic zone was used, there was a nitrified recycle which pumped 2 to 3 times the feed and RAS rate. The goal was to approach steady state operating conditions and allow for the contact of a sufficient amount of biomass with the influent. While this did not simulate the dynamic nature of full scale BPR, it permitted evaluation of the process as a treatment alternative. Once it could be shown that BPR could be achieved, additional investigation could test the effects of different operating conditions.

**Equipment**

**Reactors.** The lab unit consisted of separate compartments which served as the anaerobic, anoxic and aerobic zones. These zones were interconnected by polyethylene tubing. Together these formed a continuous flow system.

A circular, 2.05 liter (L), high density polyethylene (HDPE) container with screw-on top served as the anaerobic zone. It was approximately 10 centimeters (cm) in diameter and 25.5 cm tall. The pumped influent and recycle streams merged at a wye fitting before entering the top of the reactor 4 cm below the water surface. The anaerobic zone effluent exited 4 cm from the bottom to limit short circuiting. When the anaerobic HRT was increased, a staging effect was created by adding another container of the same description. Its flow pattern was the opposite of the first reactor with its effluent overflowing into the aerobic zone.

The contents of these reactors were thoroughly agitated by a flat, two-blade paddle. The paddle was suspended in the center of the reactor and it was rotated by an electric motor with an output of 50 revolutions per minute (RPM).

Later, other mixing equipment with variable speed control became available and was utilized. The output RPM range of these mixers was 0 - 100. The mixer RPM was set to insure well-mixed conditions. Care was taken to
prevent air entrainment.

The aerobic zone was a rectangular tank made from 5 mm thick acrylic. The aerobic zone volume varied from 12.5 to 16.5 L during the investigation. Porous diffuser stones attached to rigid glass tubing were placed on the tank bottom. Flexible tubing then connected to the laboratory compressed air system. Flow was regulated with a stop valve. This provided oxygen and mixing to simulate aerobic conditions.

Two diffusers were placed on the tank bottom. This coarse bubble system provided for thorough mixing of the reactor contents. A throttling valve on the air supply piping was utilized to control the air flow rate and thus, the DO concentration in the aerobic zone.

The anoxic zone was created by partitioning approximately one-quarter of the aerobic reactor with a vertical baffle. The sludge passed around the submerged baffle edges. This 4 L zone approached completely-mixed conditions with the same mixing equipment as used in the anaerobic reactors. The top of this zone was uncovered.

The aerobic tank effluent overflowed into the secondary clarifier. Solids-liquid separation was provided by a circular glass clarifier with a conical bottom. It consisted of a center feed to mid-depth, a continuously scraped bottom cone, RAS underflow from the center of the bottom, and effluent overflow from a single 15 millimeter (mm) opening on the side. This 2.4 L, 15.25 cm diameter settling tank had a length to width ratio of approximately one, and an HRT which varied from 5.6 to 6.2 hours.

**Pumping.** The feed and recycle pumping were provided by Masterflex® peristaltic pumps. These pumps were manufactured by Cole-Parmer Instrument Company, Chicago, Illinois. They consisted of a pump head and an electrically powered drive with solid state speed control. The RAS and feed pump heads were able to share a drive since their flow rates were equal. These heads were model number 7014-20. The drive used 115 voltage alternating current (VAC), had an adjustable range of 0 - 100 %, and it was model number 7553-30. When the system was nitrifying, the nitrified recycle was 2 to 3 times the
influent feed rate. It was driven by a separate drive, but utilized the same model number pump head. The drive was powered like the other pumps' drive, but its model number was 7553-50.

Transport of the influent and recycle flows was accomplished through polyethylene tubing. The inside diameter of this tubing was 1.5 mm. Glass fittings and HDPE were used at the connections and intersections of the tubing and the reactors. The inter-connections between the reactors and the clarifier were made with the same type tubing, but a larger diameter. The flow through these connections was either gravity flow or the result of head pressure. The larger diameter tubing helped prevent clogging and permitted cleaning.

**Influent**

The influent feed for the lab scale unit was collected at the manufacturing facility and transported back to the Virginia Polytechnic Institute and State University (VPI & SU), Environmental Engineering and Sciences laboratories in Blacksburg, Virginia. Round trip time was approximately two hours. The feed was collected in 24 L carboys from the effluent discharge of the equalization basin at the waste treatment plant. On most occasions, there was a small sample pump available to fill the carboys. After a piping failure, the feed had to be collected by lowering a bucket into the equalization basin effluent well. This repetitive dipping tended to pick up more floatables than the pump.

Feed collection usually averaged about once per week. The carboys were stored in a refrigerator in the laboratory until they were needed. The temperature during storage was generally around 0° - 4° C. When being fed, the influent was at the ambient room conditions noted earlier. No biological activity was expected considering the waste’s constituents, the storage time and the sample preservation.

During part of the investigation, a municipal wastewater was used as feed for the BPR. This feed was obtained from a sanitary manhole on the VPI & SU campus. The manhole was located on a large diameter interceptor sewer on the downstream side of the campus. A sump pump was placed in the pipe
invert. It was used to pump the wastewater into the carboy. A new carboy
was collected every other day. The time of collection varied from mid-morning
to early evening.

With the influent flow rates which were used, one carboy lasted 48
hours. Prior to being fed to the lab unit, the industrial wastewater was
supplemented with essential nutrients. This was also true for the mixtures of
municipal and industrial wastewater.

Crystalline forms of essential macro nutrients were dissolved in the feed
carboy. These nutrients were required for bacteria growth and survival. By
calculating the mass of biomass produced per day and the observed yield of the
system, the required concentrations of nitrogen, phosphorus, potassium and
iron were calculated. The following were taken from Benefield and Randall
(1980) to determine the amount of macro nutrients needed.

\[ Y_{obs} = \frac{(\text{Mass of Biomass Produced per day})}{(\text{Mass of COD Utilized per day})} \]

\[ Y_{obs} = \left[ (Q_w \times X_{MLVSS}) + \left( (Q_f - Q_p) \times X_{VSS_{eff}} \right) / (\text{TCOD}_{\text{INF}} - \text{SCOD}_{\text{INF}}) \right] \times Q_c \]

\[ \Delta X = \text{Mass of Biomass Produced per day} \]

\[ \Delta X = Y_{obs} \times Q_p \times (\text{TCOD}_{\text{INF}} - \text{SCOD}_{\text{INF}}) \]

\[ N_{req} = 0.122 \times \Delta X / Q_p; \quad \text{Nitrogen 12.2\% of microbial composition} \]

\[ P_{req} = 0.023 \times \Delta X / Q_p; \quad \text{Phosphorus 2.3\% of microbial composition} \]

\[ K_{req} = (1/14) \times N_{req} \]

\[ F_{req} = (0.20/14) \times N_{req} \]

Taking the supplements' other constituents into consideration, the
weight of the nutrient containing compound was calculated. With the known
feed start volume, the required nutrient concentrations could be prepared.

When the macro-nutrients were added, the following laboratory grade
chemicals were used.

- Nitrogen - Urea, NH₂CONH₂ (Fisher Scientific; CAS Number 57-13-6)
- Phosphorus - Potassium phosphate monobasic, KH₂PO₄ (Fisher Scientific;
  CAS Number 7778-77-0)
- Potassium - Potassium Hydroxide, KOH (Fischer Scientific; CAS Number
  1310-58-3)
- Iron - Ferric Chloride, FeCl₃ (Fisher Scientific; CAS Number 10025-77-1)
Some of the macro-nutrients were present in the industrial waste in the form of various compounds. It was assumed that the majority of these compounds were not readily available for use by the bacteria. In addition, there were not sufficient quantities to provide for complete bacterial synthesis and growth functions. To prevent this deficiency from being limiting to the success of BPR, the nutrients were supplemented.

The concentration of phosphorus was purposely selected to be higher than what was required. This served to evaluate the P removal capacity of the system. Potassium was also supplemented in excessive amounts. Initially, it was decided to have potassium in the influent at a concentration of 25 mg/L to insure it was not limiting. Based on the above noted calculations, this was more than enough potassium. Later, when KH$_2$PO$_4$ was used to supplement phosphorus, the KOH was not needed.

During the later part of the investigation, the influent was supplemented with sodium acetate. This solution was obtained by taking glacial acetic acid, (CH$_3$COOH, Fischer Scientific; CAS 64-19-7) and neutralizing it with a stock solution of 1 N sodium hydroxide (NaOH) to a pH of near 7. It was added to the influent carboy to insure there was sufficient substrate available for the bacteria to accomplish BPR.

**OPERATION OF LABORATORY BPR SYSTEM**

The lab scale BPR system was set up and operated to determine if BPR treatment objectives and benefits could be achieved. When evaluation and monitoring indicated that BPR was not being attained, the influent composition, the process configuration and the operating parameters were altered. Based on observations and test results, the operating conditions of the system were changed. These efforts concentrated on creating the conditions required for BPR and eliminating physical restrictions associated with the laboratory system. Table 1 describes the operation of the BPR system during the course of the investigation. The changes to the influent and process configuration are noted.

Efforts were initially focused on adjusting some of the operating conditions of the two stage BPR system and adding essential nutrients to the
Table 1. Description of laboratory system operation for each experiment of the investigation

<table>
<thead>
<tr>
<th>EXP</th>
<th>DAYS OF EXPERIMENT</th>
<th>INFLUENT</th>
<th>SYSTEM CONFIGURATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 - 62</td>
<td>100% Industrial waste; NH$_2$CONH$_2$, KOH (Day 1 - Day 47), KH$_2$PO$_4$ (Day 48 - Day 62) &amp; FeCl$_3$ supplemented</td>
<td>Two-stage BPR system</td>
</tr>
<tr>
<td>2</td>
<td>63 - 97</td>
<td>100% Industrial waste; NH$_2$CONH$_2$, KH$_2$PO$_4$ &amp; FeCl$_3$ supplemented</td>
<td>Two-stage BPR system, Anaerobic HRT doubled &amp; staged into two compartments</td>
</tr>
<tr>
<td>3</td>
<td>98 - 135</td>
<td>50% Industrial waste &amp; 50% Municipal sewage; NH$_2$CONH$_2$, KH$_2$PO$_4$, &amp; FeCl$_3$ supplemented</td>
<td>Two-stage BPR system, same as in Phase II</td>
</tr>
<tr>
<td>4</td>
<td>136 - 151</td>
<td>100% Municipal sewage; KH$_2$PO$_4$ supplemented</td>
<td>Three-stage BNR system, Anoxic zone &amp; nitrified recycle added, 2-3Q</td>
</tr>
<tr>
<td>5</td>
<td>152 - 161</td>
<td>100% Municipal sewage; Sodium Acetate &amp; KH$_2$PO$_4$ supplemented</td>
<td>Three-stage BNR system, same as in Phase IV</td>
</tr>
<tr>
<td>6</td>
<td>162 - 167</td>
<td>Municipal sewage with 500 mg/L as COD of industrial waste; KH$_2$PO$_4$ supplemented</td>
<td>Three-stage BNR system, same as in Phases IV &amp; V</td>
</tr>
<tr>
<td>7</td>
<td>168 - 185</td>
<td>100% Industrial waste; NH$_2$CONH$_2$ &amp; KH$_2$PO$_4$ supplemented</td>
<td>Three-stage BNR system, same as in Phases IV, V &amp; VI</td>
</tr>
</tbody>
</table>
influent. The lab-scale BPR unit did not respond as desired to these adjustments. Attention was then directed to the physical configuration of the system. It needed to be shown that the laboratory system could achieve the treatment results expected from a BPR system.

The influent composition was altered, and then the treatment system configuration was modified to account for the different influent characteristics. BPR has been proven with this type of system configuration and municipal sewage of similar characteristics. Using this set-up eliminated the influent feed as a limitation to BPR and allowed the assessment of the laboratory system. After the bio-P bacteria were established with this arrangement and the addition of sodium acetate, the amount of the industrial waste in the influent was increased.

**Process Control**

The laboratory BPR system was operated to maintain steady state conditions. Under these conditions, BPR was most likely to occur. While true steady state conditions were difficult to achieve and sustain, operating conditions were kept as uniform and constant as possible. Process control was critical to operation at steady state. For the lab scale BPR unit, process control was limited to the feed:RAS ratio, the pump flow rates, the MCRT, and the aerobic zone DO concentration. Target values for these parameters were selected. They were attained except for some minor variations. This permitted the laboratory BPR system to be evaluated as a treatment alternative.

**Start Up**

To prevent having to develop or acclimate an activated sludge culture to treat the industrial waste, the activated sludge from the existing full scale treatment system was utilized. The RAS of this extended aeration system was collected in a 24 L carboy on the initial trip to collect the industrial waste. The seed sludge was taken from the influent end of the aeration basin just before the RAS entered the basin.

The anaerobic and aerobic reactors of the lab BPR units were filled to approximately 80% with mixed liquor. The balance of the reactor volumes was
made up with the industrial waste. Mixing and aeration were then initiated. The feed and RAS pumps were then started and set to achieve the target HRT. At certain junctures of this investigation, waste activated sludge (WAS) was taken from other BNR systems and substituted into the laboratory BPR system. This was an attempt to inoculate the microbial culture with bio-P bacteria. The lab and pilot scale systems which supplied the WAS were removing phosphorus biologically in excessive amounts. Bio-P bacteria were known to exist in their sludge.

The influent being fed to these donor BNR systems was considerably different. The amount of influent substrate as measured by COD was approximately one-quarter of the industrial waste. In addition, the influent pH for these systems was near neutrality. Inoculation with BNR WAS was limited. It was done on three occasions. No more than 2.5 L was used each time to prevent shocking the BPR system. There was no net loss of biomass volume. The biomass was removed from the laboratory system aerobic zone. The seed WAS was taken out of the aerobic zones of the donor BNR systems.

**Sludge Wasting**

Consistent achievement of the target MCRT meant an unique microorganism population would be developed. The MCRT directly influenced the viability of the biomass. This operational parameter was used to describe and quantify the treatment system biomass.

MCRT was defined as the average time a unit of biomass spends in the activated sludge reactors of the treatment system. At steady state, the rate at which biomass is produced in the system equals the rate at which biomass is wasted from the system (Benefield and Randall, 1980). By calculating the mass of active biomass in the reactors and dividing by the mass of active biomass withdrawn daily from the system, the MCRT was determined. The following equation was used:

\[
MCRT = \frac{(MLVSS \times V_{\text{AER}})}{(Q_w \times MLVSS) + ((Q_f - Q_w) \times VSS_{\text{eff}}))}
\]

The MCRT was selected, the zone volumes were known and the feed flow rate and solids concentrations were determined by monitoring and testing.
Substituting and rearranging the equation allowed calculation of the amount of solids to be wasted daily.

**Procedures**

The laboratory system's operating conditions were checked daily. Several tasks were done on a routine basis to confirm that the operational objectives were maintained. Verification and support of operations consisted of:

- Preparing the influent waste for feeding
- Checking pump rates
- Wasting sludge or biomass
- Monitoring mixing and DO concentrations in system zones

After the influent was collected and stored as described earlier, a new batch of influent was prepared. First, the influent feed carboy was taken out of service. The influent pump’s suction tubing was removed from the carboy and inserted into a small container of influent while the new batch of feed was prepared. The time the switch was made was recorded. The influent remaining in the carboy which was taken out of service was then measured and recorded. This information on the time in use and volume fed permitted the calculation of the feed rate and subsequently, the average HRT. Usually the switch was made in the morning, anywhere from 7 a.m. to 12 Noon, every other day.

A known volume of influent was poured into the feed carboy. The side of the carboy had been marked to show where this level was. After the supplements were added, the carboy was placed back into service. This process took about 15 minutes. During a portion of the investigation, the influent was purged with compressed, industrial grade nitrogen gas for approximately 5 - 10 minutes. This was an attempt to strip DO and oxidized nitrogen from the influent feed. It was accomplished by using a 9" x 55" cylinder, a single-stage regulator, polyethylene and glass tubing, and a diffuser stone.

With the pumps operating and the new influent carboy in-service, a
check of the pump flow rates was conducted. This was accomplished by removing the pump’s discharge tubing at its destination and having it discharge into a 50 mL graduated cylinder. The graduated cylinder was placed near the intended discharge point to maintain similar pump head conditions. The pump discharged into the cylinder for a known period of time in the 3 - 5 minute range. Converting to mL per minute, the target and actual flow rates were compared. Adjustments were made accordingly, and the flow rates rechecked until within 0.5 mL of each other. The contents of the cylinder, whether influent or biomass, were dumped into the aerobic zone.

The amount of biomass to be removed daily was determined as noted earlier. With a small laboratory system like this, the functions of the microorganisms were very sensitive to sludge wasting. Biomass was removed to prevent upset and decrease the effects on performance. Depending on how much needed to be wasted, the biomass was removed in batches. Accounting for that removed for test samples, measured volumes of biomass were taken from the aerobic zone. Wasting was done anytime from noon to midnight. Usually, when the volume was less than one liter, the WAS was withdrawn in one batch. The volume of WAS was measured with a one liter graduated cylinder.

The degree of mixing and the DO concentrations in the zones of the laboratory system were regulated to provide:

- Homogeneous, well-mixed conditions in each reactor
- An anaerobic DO of less than 0.10 mg/L
- An aerobic DO of greater than 2.0 mg/L

While these objectives sometimes conflicted, a balance was sought where each was achieved. The program developed for the operation of the laboratory unit attempted to confirm that these conditions were being consistently maintained. Daily visual observations coupled with in-situ measurements of the reactors’ DO provided information on whether adjustment was needed. Adjustment was limited to regulation of the air flow rate to the aerobic zone and the mixer motor output RPM.
Maintenance

General upkeep work was performed on the laboratory system daily. Besides the daily observations to verify unimpaired system function and intactness, the laboratory unit was subjected to routine cleaning and equipment maintenance. This program helped prevent equipment failures and pump tubing leaks. It also reduced the growth and accumulation of slime and other attached growth microorganisms in the system. In all, more consistent operation was provided.

Using a mixer shaft with flat paddle blade, the insides of the aerobic zone were scraped daily. A small test tube brush was used to clean the interior, wetted surfaces of the anaerobic reactor and the clarifier. The diffuser stones in the aerobic reactor were also cleaned with the brush. Due to the disruption in operation, these system components were cleaned weekly. No material or growth was removed from the unit during these operations except for the anaerobic reactor. A thin layer of white material which will be discussed later was removed from the reactor water surface periodically.

To prevent the build up of material on the inside of the influent and RAS tubing, the suction and discharge sections were flushed and bleached periodically. This was accomplished by stopping system operations, disconnecting the tubing, and isolating where the tubing was attached. The suction end was placed into containers. The influent tubing was placed into a mild bleach solution. For the RAS tubing, hot water was used to prevent shocking the biomass with bleach. The discharge ends of the tubing were placed into another container to collect the water and bleach. With the pumps running, the tubing was flushed. After the influent tubing was bleached, it was flushed again with potable water to prevent shocking the biomass. Operation was stopped 30 minutes for this process.

Equipment maintenance consisted of replacing the pump tubing, cleaning the mixers and the pumps, and lubricating the pumps. The laboratory system was temporarily shut down for these tasks. Aeration was left on when possible. It usually took less than 30 minutes to do this work. The pump
tubing was replaced on a biweekly rotation. Cleaning and lubrication of the mixers and pumps was instigated by malfunctions. As part of the repair, the equipment was checked, cleaned and lubricated with an appropriate lubricant.

**SAMPLE COLLECTION AND ANALYSIS**

**Overview**

To evaluate the laboratory BPR system’s performance, a testing program was developed. These quantitative and qualitative measurements of the influent, reactor contents and effluent provided the information needed for this investigation. The testing was also important to the unit’s operation and process control. Accepted practices and procedures were utilized for all test methods. After the various tests were completed, the results were checked. The quality control/quality assurance (QC/QA) program was limited, but adequate for the purposes of this investigation.

The amount of testing increased as the investigation progressed. It was found that additional data were needed for system evaluation. The number of sample points and tests were increased to better define system performance and its characteristics.

**Samples and Analyses**

The sample points were selected to provide results which indicated whether phosphorus removal and good settling were attained. Collection and preparation of the various samples were the next steps in producing data. The points where the samples were taken are shown and described in Figure 5. The different samples were gathered and handled according to the tests which needed to be performed. Using the descriptions in Figure 5, the following lists the tests which were being performed on each of the samples at the end of the investigation.

**List of Sample Tests**

1. Influent - pH, DO, ORP, SCOD, TCOD, TOC, TSS, VSS, PO₄-P, NO₂-N, NO₃-N, SO₄-S, TP, STP, TKN
2. First stage anaerobic reactor - pH, DO, ORP, SCOD, TSS, VSS, PO₄-P, NO₂-N, NO₃-N, SO₄-S
Figure 5. Location and description of the sample points for monitoring operation and performance of the laboratory BPR system.
3. Second stage anaerobic reactor - pH, DO, ORP, SCOD, TSS, VSS, PO₄-P, NO₂-N, NO₃-N, SO₄-S
4. Anoxic reactor - pH, DO, ORP, SCOD, TSS, VSS, PO₄-P, NO₂-N, NO₃-N, SO₄-S
5. Aerobic reactor - pH, DO, ORP, SCOD, TSS, VSS, PO₄-P, NO₂-N, NO₃-N, SO₄-S, TP, STP, TKN, STKN, OUR, SVI, ZSV
6. Secondary Settling - DO
7. Final Effluent - pH, SCOD, TCOD, STOC, TSS, VSS, PO₄-P, NO₂-N, NO₃-N, SO₄-S, TP, STP, STKN
8. Return Activated Sludge - DO, SCOD, TSS, VSS, PO4-P, NO₂-N, NO₃-N, SO₄-S

Grab type samples were collected for all testing. The samples were collected first thing in the morning to minimize the affects of the previous day’s sludge wasting. The grab samples were representative of the system’s treatment performance and characteristics.

The effluent sample was obtained first. The tube attached to the clarifier overflow port was removed. A polyethylene container was placed under the opening to catch the effluent as it flowed out. About 500 mL of effluent was collected. The influent sample was obtained by pouring approximately 500 mL out of the feed carboy into a polyethylene container. The carboy was shaken prior to sampling to get a representative sample.

Samples from the different reactor contents were also placed in polyethylene containers. Samples for these were gathered using 10 mL wide-mouthed pipets. The pipet was dipped repetitively until 50 - 100 mL were collected. A different pipet was used for each reactor to prevent any sample contamination. The RAS sample was collected by placing the RAS pump discharge in a polyethylene container. About 20 - 30 mL were pumped before the tubing was returned to its connection.

All sample containers were acid-washed in a 15 % HCl bath and rinsed with distilled water prior to sampling. Analyses were initiated right after the samples were collected. No sample preservation was usually needed.
Sometimes due to test equipment availability, samples were refrigerated at 0° - 4°C. These samples were stored for no more than 6 hours before analysis.

**Analytical and Testing Procedures**

The samples collected from the laboratory BPR system were analyzed in the VPI & SU, Environmental Engineering and Sciences laboratory facilities in Blacksburg, Virginia. Automated instrumentation and electronic equipment were utilized to perform the analyses and measurements noted in the previous section.

After the samples were assembled, the samples were tested to determine TSS and VSS. These were performed in accordance with Section 2540 D., Total Suspended Solids Dried at 103 - 105°C and Section 2540 E., Fixed and Volatile Solids Ignited at 550°C, *Standard Methods for the Examination of Water and Wastewater* (1989). The filtrate produced was filtered through a 0.45 micron (µm) membrane filter. This partitioned the dissolved or soluble fraction of the sample for analyses of soluble chemical oxygen demand (SCOD), total organic carbon (TOC), phosphate (PO₄), nitrite (NO₂), nitrate (NO₃), sulfate (SO₄), soluble total phosphorus (STP), and soluble total Kjeldahl nitrogen (STKN). The COD tests were then set up as described in Section 5220 C., Closed Reflux, Titrimetric Method, *Standard Methods for the Examination of Water and Wastewater* (1989).

The soluble samples were analyzed using a Dionex Ion Chromatograph (Dionex Corporation; Sunnyvale, CA), Model 2010i, with an AS9 column, to determine the concentrations of NO₂-N, NO₃-N, PO₄-P, and SO₄-S. Due to the high concentration of SO₄ in the industrial waste, the samples had to be diluted 10:1 and the output range of the chromatograph maximized to 300. The dilution required decreased as the percentage of the industrial waste in the influent decreased. When the influent was 100% municipal wastewater, no dilution was needed and the output range was 100.

Digestion and colorimetric analysis as outlined in Section 4500-P E., Ascorbic Acid Method, *Standard Methods for the Examination of Water and Wastewater* (1989) were done to determine the TP and STP concentrations of
the selected samples. A Beckman DU-6 Spectrophotometer was used. Using the absorbance produced with standards of 0, 0.25, 0.50, 0.75, and 1.00 mg ortho-PO₄/L, a linear regression was performed. The y-intercept was made to equal zero. This produced the equation of the best fit line for the data. The absorbance was the dependent variable and the concentration was the independent variable. After determining the sample absorbance, the sample concentration was computed with this equation. A standard equation was developed for each sample event. The samples were diluted so that their absorbance were within curve range (0 - 1.0 mg/L).

In the latter part of the investigation, samples were analyzed for TKN and STKN. This was an effort to determine the amount of nitrogen in the influent and if there was any nitrification occurring. These tests were performed in accordance with Section 4500-Norg C., Semi-Micro-Kjeldahl Method, Standard Methods for the Examination of Water and Wastewater (1999).

The BPR system’s aerobic oxygen utilization rate (OUR) was calculated from data collected on the biomass’s utilization of DO. This testing was performed as described in Section 2710 B., Oxygen-Consumption Rate, Standard Methods for the Examination of Water and Wastewater (1989). The OUR was calculated from an x-y plot of DO over time. A straight line portion of the plot was selected and an equation for this line was determined. The slope of this line was the OUR. This provided a measurement of the DO consumption by the biomass. This along with the MLSS of the aerobic reactor were used to calculate the specific oxygen utilization rate (SOUR).

The sludge settling of the BPR system was characterized using the parameters sludge volume index (SVI) and zone settling velocity (ZSV). While these measurements have their deficiencies, they provided methods to gauge if sludge settling was changing. The procedure used to determine SVI was Section 2710 D., Sludge Volume Index, Standard Methods for the Examination of Water and Wastewater (1989). The ZSV was determined by taking the initial sludge blanket height and subtracting the height after 30 minutes. This distance was divided by 30 minutes to produce the settling rate, length over
time.

On several occasions, during the middle of the investigation, metals analyses were performed on the industrial waste and the bench-scale BPR unit effluent. The Total Recoverable Metals; Mg and K were determined using EPA 600/4-79-020, *Methods for Chemical Analysis of Water and Wastes*. This was done to verify that these micronutrients were present in sufficient quantities and to determine the amount being utilized. Due to high concentrations of these metals, serial dilutions were made. The Mg samples had to be diluted to 1:1100 and the K samples to 1:200.

The Perkin Elmer (Norwalk, CT) Flame Atomic Absorption (FAA) Spectrophotometer Model 703 was used to quantify the metals, K and Mg. To prevent interferences by other sample constituents, lanthanum (La) and cesium (Cs) were added when analyzing for Mg and K, respectively.

**In-situ Testing**

Some sample locations of the laboratory BPR system were also monitored for pH, DO and ORP. This testing provided additional data to help characterize the system and its operation. These measurements were made without removing samples. The different probes utilized were placed in the reactors or locations as the unit operated. Sufficient time was provided at the sample points to allow the probes to equilibrate. Placement of the probe was selected to be representative of reactor contents. This was verified by initially moving the probes around to different areas in the reactors. All measurements were made in a sequential order, starting with the effluent and ending with the influent.

The pH measurements were obtained with a Fisher Scientific (Springfield, NJ) Accumet® pH meter. This combination meter with an ion-combination probe was used to measure solution pH. Prior to use, the instrument and probe were calibrated using standard buffer solutions of known pH.

DO measurements were made with an analog type meter and probe from Yellow Springs Instrument Company (Yellow Springs, Ohio). The probe, Model 5750, and meter, Model 5400, were calibrated and maintained according to the
manufacturer's instructions. The membrane on the probe was the regular type. It was replaced weekly. DO levels expected in the nonaerated zones were below the sensitivity of the instrument and probe, but it helped indicate if DO was present.

The ORP was measured in mV. The measurements of CRP were made with the above noted pH meter set to mV and a probe outfitted with reference and oxidation-reduction electrodes. The electromotive force between a silver-silver chloride reference electrode and a platinum oxidation-reduction electrode was measured when immersed in solution. Calibration consisted of placement of electrodes into a Ferrous-Ferric reference solution and adjustment of meter mV potential to the known reference solution potential. Measurement and calibration were done in accordance with D 1498 - 76, Standard Practice for Oxidation-Reduction Potential of Water, Annual Book of American Society of Testing and Materials Standards, Volume 11.01, 1981.
CHAPTER IV. RESULTS

OVERVIEW

The data collected during the course of this investigation are presented in this chapter. Initially, efforts focused on determining whether or not BPR could be attained during treatment of the industrial wastewater. Later, the investigation concentrated on whether the laboratory system was capable of BNR (BPR plus biological nitrogen removal).

Evaluation of the performance of the laboratory system was made using test results and data which described the operation of the system. Presentation of this information was oriented around tracking system performance with respect to BPR, substrate or COD removal, and effluent settleability. Influent data were also utilized to characterize the influent and its effects on the performance of the laboratory system.

The influent was changed during the course of the investigation in an effort to achieve BPR with the laboratory system. As noted previously, the investigation was divided into experiments which corresponded with the influent changes.

Initially, the influent was the industrial wastewater. When BPR was not accomplished, municipal wastewater was added. Still unsuccessful, the influent was changed to 100% municipal wastewater. Finally, to obtain efficient BPR, sodium acetate was added to the municipal wastewater influent. With the system achieving BPR, the industrial wastewater was substituted for the sodium acetate. The system continued to realize BPR until the influent was changed back to 100% industrial wastewater. The influent compositions and the average influent COD loadings for the experiments are provided in Table 2.

LABORATORY BPR TREATMENT SYSTEM PERFORMANCE

Process Control

The laboratory system was operated to maintain a target MCRT of eight days for each experiment of the investigation. Based on the MCRT and HRT values displayed in Figure 6, fairly consistent process control and operation were accomplished throughout the investigation. The methods used to
Table 2. Description of laboratory system influent and average COD loading for each experiment of the investigation

<table>
<thead>
<tr>
<th>EXPERIMENT</th>
<th>DAYS OF EXPERIMENT</th>
<th>INFLUENT</th>
<th>System COD LOADING mg COD/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 - 62</td>
<td>100% Industrial waste; NH₂CONH₂, KOH (Day 1 - Day 47), KH₂PO₄ (Day 48 - Day 62) &amp; FeCl₃ supplemented</td>
<td>22,656</td>
</tr>
<tr>
<td>2</td>
<td>63 - 97</td>
<td>100% Industrial waste; NH₂CONH₂, KH₂PO₄ &amp; FeCl₃ supplemented</td>
<td>23,612</td>
</tr>
<tr>
<td>3</td>
<td>98 - 135</td>
<td>50% Industrial waste &amp; 50% Municipal sewage; NH₂CONH₂, KH₂PO₄, &amp; FeCl₃ supplemented</td>
<td>14,087</td>
</tr>
<tr>
<td>4</td>
<td>136 - 151</td>
<td>100% Municipal sewage; KH₂PO₄ supplemented</td>
<td>3,866</td>
</tr>
<tr>
<td>5</td>
<td>152 - 161</td>
<td>100% Municipal sewage; Sodium Acetate &amp; KH₂PO₄ supplemented</td>
<td>6,880</td>
</tr>
<tr>
<td>6</td>
<td>162 - 167</td>
<td>Municipal sewage with 500 mg/L as COD of Industrial waste; KH₂PO₄ supplemented</td>
<td>7,728</td>
</tr>
<tr>
<td>7</td>
<td>168 - 185</td>
<td>100% Industrial waste; NH₂CONH₂ &amp; KH₂PO₄ supplemented</td>
<td>25,621</td>
</tr>
</tbody>
</table>

System COD Loading, mg COD/day = TCOD_{INF} x Q_{INF}
Figure 6. Mean cell residence time (MCRT) and hydraulic retention time (HRT) of the laboratory system during the investigation.
calculate MCRT and regulate HRT provided reinforcement that system operating conditions were maintained.

The average, variance and standard deviation of MCRT and HRT for all the experiments are included in Table 3. These further support that process control was adequate. Besides a few exceptions, there were no large variations from the target MCRT and HRT. Low MCRTs (5 - 7 days) were experienced in the first twenty days of experiment one. These were due to the high effluent suspended solids of the system at the time of grab sampling.

**Substrate Utilization**

Treatment performance with respect to the removal of organics was assessed based on mg of COD utilized or removed per day, effluent COD concentration and percent COD removal. The results indicated that the laboratory BPR system provided effective COD removal. The system was operated at sufficient MCRT and HRT for COD removal.

Figure 7 tracks the mass of COD removed per day by the system over the course of the investigation. As expected, the system removed four to five times more COD per day when fed the higher COD industrial wastewater. On a mass basis, the laboratory system removed roughly 22,000 mg per day of COD when fed the industrial wastewater in experiments #1, #2 and #7. This amount of COD should have been more than adequate to support the substrate requirements of BPR.

The system food to mass (F:M) ratio and the specific rate of COD removal per unit MLVSS (SSUR) were calculated and averaged for each experiment. As seen in Table 4, the averages for experiments #1 - #3, #5 and #6 varied little. The ratios and rates for experiments #4 and #7 were influenced by the large changes in influent COD. The system was adjusting to the changes during these experiments, but the substrate removal rate closely paralleled the loading rate. All the ratios and rates which were calculated are plotted in Figure 8.

In Figure 9, the influent TCOD and effluent SCOD concentrations are shown for the investigation. The low effluent SCOD results for experiments
Table 3. Averages, standard deviations and variances of the Mean Cell Residence Time (MCRT) and the system Hydraulic Retention Time (HRT) of the laboratory system for each experiment of the investigation.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>MCRT, Days</th>
<th>s</th>
<th>s²</th>
<th>HRT, Days</th>
<th>s</th>
<th>s²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.7</td>
<td>0.66</td>
<td>0.44</td>
<td>2.02</td>
<td>0.09</td>
<td>0.009</td>
</tr>
<tr>
<td>2</td>
<td>8.0</td>
<td>0.08</td>
<td>0.01</td>
<td>2.03</td>
<td>0.17</td>
<td>0.027</td>
</tr>
<tr>
<td>3</td>
<td>8.0</td>
<td>0.13</td>
<td>0.02</td>
<td>2.02</td>
<td>0.09</td>
<td>0.008</td>
</tr>
<tr>
<td>4</td>
<td>8.1</td>
<td>0.18</td>
<td>0.03</td>
<td>2.04</td>
<td>0.12</td>
<td>0.016</td>
</tr>
<tr>
<td>5</td>
<td>8.0</td>
<td>0.31</td>
<td>0.10</td>
<td>1.96</td>
<td>0.01</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>7.9</td>
<td>0.06</td>
<td>0.004</td>
<td>1.96</td>
<td>0.04</td>
<td>0.002</td>
</tr>
<tr>
<td>7</td>
<td>7.6</td>
<td>0.06</td>
<td>0.004</td>
<td>1.81</td>
<td>0.08</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Target MCRT and HRT for all the experiments were 8.0 days and 2.0 days, respectively.
Figure 7. Mass of Chemical Oxygen Demand (COD) removed by the laboratory system during the investigation.
Table 4. Averages of the system food to mass loading ratio (F:M) and system specific rate of COD removal (SSUR) for each experiment of the investigation.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>System F:M Ratio</th>
<th>SSUR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg COD/mg MLVSS • day</td>
<td>mg COD/mg MLVSS • day</td>
</tr>
<tr>
<td>1</td>
<td>0.49</td>
<td>0.48</td>
</tr>
<tr>
<td>2</td>
<td>0.48</td>
<td>0.51</td>
</tr>
<tr>
<td>3</td>
<td>0.53</td>
<td>0.54</td>
</tr>
<tr>
<td>4</td>
<td>0.24</td>
<td>0.25</td>
</tr>
<tr>
<td>5</td>
<td>0.57</td>
<td>0.59</td>
</tr>
<tr>
<td>6</td>
<td>0.48</td>
<td>0.56</td>
</tr>
<tr>
<td>7</td>
<td>0.81</td>
<td>0.92</td>
</tr>
</tbody>
</table>

\[
F:M = \frac{(TCOD_{INF} \times Q_{INF})}{((V_{ANA} \times MLVSS_{ANA}) + (V_{AER} \times MLVSS_{AER}))}
\]

\[
SSUR = \frac{(TCOD_{INF} - SCOD_{EFF}) \times Q_{INF} \times ((V_{ANA} \times MLVSS_{ANA}) + (V_{AER} \times MLVSS_{AER}))}{(V_{ANA} \times MLVSS_{ANA}) + (V_{AER} \times MLVSS_{AER})}
\]
Figure 8. Specific rate of COD removal per unit of MLVSS (SSUR) and food to mass (F:M) loading ratio of the laboratory system during the investigation.
Figure 9. Influent and effluent Chemical Oxygen Demand (COD) concentrations for the laboratory system during the investigation.
one through six (\(<\ 70\)) indicate effective COD removal. There was only a small decrease in SCOD\textsubscript{eff} with the lower COD influents. In experiments \#1 - \#3, the SCOD\textsubscript{eff} was only 20 - 30 mg COD/L greater than in experiments \#4 - \#6.

In experiments \#3 through \#6, the composition of the influent was varied to determine if the laboratory system was capable of BPR. The influent was altered to wastewaters which have been successfully used by others for BPR. Thus, the possibility that BPR was not attained due to limitations of the laboratory system were eliminated.

The system specific growth rate (SGR), day\(^{-1}\), and the SCOD\textsubscript{eff} to TCOD\textsubscript{inf} ratio were calculated. A tabulation of experiment averages of these are presented in Table A-1 of the appendix.

The results of TOC analyses were also evaluated. Influent and effluent concentrations of TOC are shown in Figure A-1. The influent and effluent COD:TOC ratios for the investigation were also calculated and plotted in Figure A-2. These influent and effluent ratios give a rough indication of the oxidative state of the organic matter. As Gaudy and Gaudy (1988) generalized, the higher the ratio, the less oxidized the material. The averages of the TOC concentrations and ratios for each period are found in Table A-2.

From the data on the influent and effluent ratios it is clear that oxidation occurred in the laboratory system for the different influent wastewaters. The influent COD:TOC ratio is higher, or less oxidized than the effluent ratio for all experiments.

The use of TOC as a substitute for COD as a measure of sample organic matter was also considered. If a consistent correlation could be established, TOC analysis could be used instead of COD. However, the variability of these ratios did not permit use of TOC in place of COD.

**Anaerobic Substrate Removal**

Efforts to determine if there would be any treatment benefits or energy savings produced by the addition of the anaerobic zone were limited. The results indicated that the COD loading to the aerobic zone could be significantly
reduced using an anaerobic reactor with a HRT between 6 and 9 hours and an anaerobic mass fraction (AMF) of 11% to 19%.

Starting in experiment #2, data were collected to determine the amount of COD removed in the anaerobic zone. These were used to quantify substrate uptake or removal in the anaerobic zone. The experiment averages of SCOD$_{Ana}$, percent COD removed in the anaerobic zone and mass of COD removed in the anaerobic zone are listed in Table 5. Between 10 to 20% of the COD was removed in the anaerobic zone when the influent was 100% industrial waste or a 50/50 mix of the industrial and municipal wastewaters in experiments #2 and #3. Between 40 to 50% of the influent COD was removed from solution in the anaerobic zone in experiments #5 and #6. When the influent consisted of 100% municipal wastewater in experiment #4, over 70% of the COD was removed in the anaerobic zone.

The mass of COD removed in the anaerobic zone was divided by the MLVSS$_{Ana}$ to calculate the COD uptake rate of the biomass in the anaerobic reactor. The plot of these results in Figure 10 indicates that the COD uptake rate varied with the influent changes. The highest uptakes were seen in experiments #5 and #6, but they were only slightly larger than in experiments #2 and #3. The lowest rates were observed in experiment #4 when the influent was the municipal wastewater, i.e., when the COD loading was less.

As a check of the COD data, the COD removed by the system was compared to the sum of the observed removals in the anaerobic and aerobic zones. The sum of the anaerobic and aerobic removals is plotted versus the system removal in Figure 11. The results of the linear regression of these data are provided on the figure. The removal averages for each experiment are provided in Table 6. The averages and the figure show that the COD data was fairly accurate and useable.

The COD of the MLVSS$_{Aer}$ (COD$_{w}$) and the total oxygen utilization of the system (TOR) were used with other system data to estimate aerobic COD utilization in the laboratory system. The experimental averages of these are included in Table 6 for comparison. The aerobic COD removal was greater than
Table 5. Averages system and anaerobic % COD removals, influent, anaerobic, and effluent COD concentrations, and anaerobic mass fraction (AMF) and hydraulic retention time (HRT\textsubscript{ANA}) for each experiment of the investigation.

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>% COD Removed</th>
<th>COD, mg/L</th>
<th>Anaerobic Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SYS</td>
<td>ANA</td>
<td>INF</td>
</tr>
<tr>
<td>1</td>
<td>97</td>
<td>*</td>
<td>2,445</td>
</tr>
<tr>
<td>2</td>
<td>98</td>
<td>13</td>
<td>2,333</td>
</tr>
<tr>
<td>3</td>
<td>97</td>
<td>18</td>
<td>1,381</td>
</tr>
<tr>
<td>4</td>
<td>94</td>
<td>67</td>
<td>388</td>
</tr>
<tr>
<td>5</td>
<td>97</td>
<td>51</td>
<td>658</td>
</tr>
<tr>
<td>6</td>
<td>96</td>
<td>42</td>
<td>740</td>
</tr>
<tr>
<td>7</td>
<td>93</td>
<td>16</td>
<td>2,345</td>
</tr>
</tbody>
</table>

Notes: The effluent and anaerobic zone COD concentrations were soluble COD.

* There was no soluble COD data on the contents of the anaerobic zone for this experiment.
Figure 10. Anaerobic zone COD removal rate per unit MLVSS\textsubscript{ANA} by the laboratory system during the investigation.
Figure 11. Comparison of system COD removal with the sum of COD removal in the anaerobic and aerobic zones.
Table 6. Averages of system, anaerobic and aerobic COD removals, aerobic COD utilization and anaerobic COD removal rate for each experiment of the investigation.

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>COD Removed System, mg COD/day</th>
<th>COD Removed Anaerobic, mg COD/day</th>
<th>COD Removed Aerobic, mg COD/day</th>
<th>COD Utilized Aerobic, mg COD/day</th>
<th>Anaerobic COD Removal Rate, mg COD/mg VSS • day</th>
<th>COD Removed Anaerobic + Aerobic, mg COD/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22,205*</td>
<td>*</td>
<td>*</td>
<td>14,029</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>2</td>
<td>23,051</td>
<td>3,194</td>
<td>20,054</td>
<td>19,466</td>
<td>0.4</td>
<td>23,248</td>
</tr>
<tr>
<td>3</td>
<td>12,935</td>
<td>2,381</td>
<td>11,154</td>
<td>10,765</td>
<td>0.5</td>
<td>13,694</td>
</tr>
<tr>
<td>4</td>
<td>3,572</td>
<td>2,547</td>
<td>1,091</td>
<td>4,970</td>
<td>0.7</td>
<td>3,638</td>
</tr>
<tr>
<td>5</td>
<td>6,629</td>
<td>3,274</td>
<td>3,408</td>
<td>6,617</td>
<td>1.8</td>
<td>6,682</td>
</tr>
<tr>
<td>6</td>
<td>7,409</td>
<td>3,269</td>
<td>4,210</td>
<td>8,343</td>
<td>1.2</td>
<td>7,479</td>
</tr>
<tr>
<td>7</td>
<td>23,329</td>
<td>4,263</td>
<td>19,735</td>
<td>21,885</td>
<td>0.6</td>
<td>23,997</td>
</tr>
</tbody>
</table>

* There was no soluble COD data on the contents of the anaerobic zone for this period.

# Calculated using SCOD\text{EFF} \text{COD Removal, System} = (TCOD_{\text{INF}} \times Q_{\text{INF}}) - (TCOD_{\text{EFF}} \times (Q_{\text{INF}} - Q_{\text{WAS}}))

TCOD_{\text{INF}} = \frac{(TCOD_{\text{INF}} + SCOD_{\text{RAS}})/2 - SCOD_{\text{ANA}} \times (2 \times Q_{\text{INF}})}{SCOD_{\text{ANA}} \times (2 \times Q_{\text{INF}}) - (SCOD_{\text{AER}} \times (2 \times Q_{\text{INF}})}$

COD Util., Aerobic = (SOUR \times (MLVSS_{\text{AER}} \times V_{\text{AER}})) + ((TCOD_{\text{AER}}/MLVSS_{\text{AER}}) \times \Delta X + ((Q_{\text{INF}} - Q_{\text{WAS}}) \times VSS_{\text{EFF}}))

Anaerobic COD Removal Rate = (COD Removal, Anaerobic)/(V_{\text{ANA}} \times MLVSS_{\text{ANA}})
the aerobic COD utilization in experiments #2 and #3, but the removal was less than the utilization in experiments #4, #5, and #6. The averages were within 1,500 mg/day in experiments #2, #3 and #7 (TCOD_{inf} high). When the TCOD_{inf} was considerably less in experiments #4, #5 and #6, the aerobic COD utilization was roughly twice the aerobic COD removal.

Other efforts were made to estimate any possibility of anaerobic stabilization (AnS) in the laboratory system. For experiments #1 - #3, it was assumed that there was no nitrogenous oxygen demand (NOD), i.e., stabilization of COD in an anoxic zone because the wastewater was thought to be nitrogen deficient. The anaerobic stabilization (AnS) was calculated by subtracting the aerobic COD utilization from the system COD removal. In experiment #1, there was roughly 8,100 mg/day of COD anaerobically stabilized. In experiments #2 and #3, this decreased to about 3,600 and 2,100 mg COD/day, respectively.

The NOD was included in the calculation of AnS for experiments #4 - #6. The additional oxygen demand was required for nitrification. Limited TKN and NO_x data confirmed nitrification was occurring, but did not allow accurate quantification of its oxygen requirement. Despite this, the TKN and NO_3 data were used to estimate nitrogenous oxygen demand (NOD). The NOD values calculated with the TKN data were added to system COD removals. The TKN NOD was used because it provided a better estimate. There was still little or no AnS calculated for experiments #4 - #6. The averages obtained for each experiment are displayed in Table 7.

In experiments #1 and #2, there were growths observed on the water surface of the anaerobic reactors. These thin, predominately white cultures were generally identified as some type of mold or fungi. The pH of these reactors and the fact that fungi prefer an acid environment, support the assumption that the growths were some type of fungi. These growths were removed during the routine cleaning of the system.
Table 7. Averages of system COD removal, aerobic COD utilization, COD of wasted MLVSS\textsubscript{AER} (COD\textsubscript{w}), system total oxygen utilization (TOR), the oxygen required for oxidation of NH\textsubscript{3}-N to NO\textsubscript{x}-N (NOD), and anaerobic stabilization (AnS) for each experiment of the investigation.

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>COD Removed System, mg COD/day</th>
<th>COD Utilized Aerobic, mg COD/day</th>
<th>COD\textsubscript{w} mg COD/day</th>
<th>TOR Measured mg O\textsubscript{2}/day</th>
<th>AnS mg O\textsubscript{2} eq./day</th>
<th>NOD mg O\textsubscript{2}/day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NO\textsubscript{x}-N</td>
</tr>
<tr>
<td>1</td>
<td>22,205*</td>
<td>14,029</td>
<td>6,028</td>
<td>7,578</td>
<td>8,176</td>
<td>*</td>
</tr>
<tr>
<td>2</td>
<td>23,051</td>
<td>19,466</td>
<td>6,817</td>
<td>12,261</td>
<td>3,585</td>
<td>*</td>
</tr>
<tr>
<td>3</td>
<td>12,935</td>
<td>10,765</td>
<td>3,535</td>
<td>6,258</td>
<td>2,170</td>
<td>218</td>
</tr>
<tr>
<td>4</td>
<td>3,572</td>
<td>4,970</td>
<td>2,841</td>
<td>3,008 (846)</td>
<td>294</td>
<td>552</td>
</tr>
<tr>
<td>5</td>
<td>6,629</td>
<td>6,617</td>
<td>2,130</td>
<td>4,528</td>
<td>237</td>
<td>147</td>
</tr>
<tr>
<td>6</td>
<td>7,409</td>
<td>8,343</td>
<td>3,016</td>
<td>5,009 (687)</td>
<td>+</td>
<td>245</td>
</tr>
<tr>
<td>7</td>
<td>23,329</td>
<td>21,885</td>
<td>6,527</td>
<td>12,514</td>
<td>2,428</td>
<td>+</td>
</tr>
</tbody>
</table>

ND = No data
* System was not nitrifying
+ System was denitrifying, could not calculate
# Calculated using SCOD\textsubscript{EFF}

COD Removed, System = (TCOD\textsubscript{INF} x Q\textsubscript{INF}) - (TCOD\textsubscript{EFF} x (Q\textsubscript{INF} - Q\textsubscript{WAS}))

COD Util., Aerobic = TOR + COD\textsubscript{w} + (Q\textsubscript{INF} - Q\textsubscript{WAS}) x VSS\textsubscript{EFF}

COD\textsubscript{w} = (TCOD\textsubscript{AER}/MLVSS\textsubscript{AER}) x \Delta X

TOR = (SOUR x (MLVSS\textsubscript{AER} x V\textsubscript{AER}))

NOD, NO\textsubscript{x}-N = (4.57 x NO\textsubscript{3}-N\textsubscript{EFF}) + (3.43 x NO\textsubscript{2}-N\textsubscript{EFF})

NOD, NH\textsubscript{3}-N = ((TKN\textsubscript{INF} x Q\textsubscript{INF}) - (TKN\textsubscript{EFF} x (Q\textsubscript{INF} - Q\textsubscript{WAS})) - (.11 x \Delta X)) x 4.57

AnS = COD Removed, System + NOD - COD Util., Aerobic
Phosphorus Removal

Data which describe BPR and would help assess whether it had been attained were collected. The averages of influent and effluent TP, %TP of the aerobic biomass, and the percent and mass of TP removed by the system for each experiment of the investigation are presented in Table 8. The influent and effluent TP and TSP data for the investigation are shown in Figure 12. The %P of biomass which was calculated from aerobic zone TP and MLVSS data is tracked for the investigation in Figure 13. Figures A-3 and A-4 are plots of the concentration of TP removed by the system and the %TP removal by the system, respectively, during the investigation.

The amount of P required for cellular growth requirements was calculated to compare to the observed TP removal of the system. The P required for growth is plotted versus the observed TP removal in Figure 14. The amount of P required and the observed TP removal are tracked together in Figure 15. The average P requirement of the system for each experiment is included in Table 8. Also included in Table 8 are the experiment averages of the mg TP removed by the system per mg TCOD\textsubscript{INF} and the mg COD removed per mg TP removed by the system. These values characterize P removal with respect to TCOD\textsubscript{INF} and system COD removal.

Figures 14 and 15 and the averages of P required and observed TP removal indicate that the observed TP removals were greater than the biomass phosphorus requirements in all the experiments except #1. The greatest phosphorus removals and the largest differences between required and observed were achieved in experiments #2, #5 and #6. The average differences for these experiments ranged from 140 to 160 mg/day. The differences in experiments #3, #4, and #7 ranged from 30 to 70 mg/day.

Despite the scatter of data in Figure 12, the fluctuations of TP\textsubscript{INF} and TSP\textsubscript{EFF} are evident. These variations were attributed to several conditions. One was the amount of phosphorus which was added to the influent feed. The amount of P which was added in the influent is shown in Figure 12. The influent TP concentrations reflect the P in the wastewater and the P added to
Table 8. Summary of average influent and effluent concentrations, average TP removals, % TP removed, % TP in the aerobic MLVSS, P requirement of biomass, TP removed per influent COD, and COD removed per TP removed for each experiment of the investigation.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Concentration, mg TP/L</th>
<th>% TP in Aerobic MLVSS</th>
<th>% TP Removed by System</th>
<th>TP Removed per TCOD\textsubscript{INF}</th>
<th>COD Removed per TP Removed</th>
<th>mg P required per day for biomass</th>
<th>mg TP removed per day by System</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33.0</td>
<td>9.9</td>
<td>1.05</td>
<td>15.6</td>
<td>0.004</td>
<td>290</td>
<td>142</td>
</tr>
<tr>
<td>2</td>
<td>64.7</td>
<td>17.6</td>
<td>2.48</td>
<td>27.1</td>
<td>0.008</td>
<td>83</td>
<td>144</td>
</tr>
<tr>
<td>3</td>
<td>46.7</td>
<td>8.3</td>
<td>2.52</td>
<td>17.4</td>
<td>0.006</td>
<td>202</td>
<td>79</td>
</tr>
<tr>
<td>4</td>
<td>27.9</td>
<td>2.0</td>
<td>5.02</td>
<td>7.3</td>
<td>0.006</td>
<td>42</td>
<td>52</td>
</tr>
<tr>
<td>5</td>
<td>29.0</td>
<td>12.9</td>
<td>5.51</td>
<td>44.5</td>
<td>0.017</td>
<td>45</td>
<td>34</td>
</tr>
<tr>
<td>6</td>
<td>28.6</td>
<td>17.8</td>
<td>7.27</td>
<td>62.2</td>
<td>0.024</td>
<td>35</td>
<td>47</td>
</tr>
<tr>
<td>7</td>
<td>43.7</td>
<td>10.7</td>
<td>3.36</td>
<td>23.3</td>
<td>0.005</td>
<td>144</td>
<td>106</td>
</tr>
</tbody>
</table>

TP Removed, mg TP/L = TP\textsubscript{INF} - TSP\textsubscript{EFF}  
%TP = (TP\textsubscript{AER} - TSP\textsubscript{AER})/MLVSS\textsubscript{AER} x 100  
Mass TP Removed, System = (Q\textsubscript{INF} x TP\textsubscript{INF}) - ((Q\textsubscript{INF} - Q\textsubscript{WAS}) x TSP\textsubscript{EFF})  
Mass TP Required by biomass = 0.023 x ΔX  
COD Removed per TP Removed = (TCOD\textsubscript{INF} - SCOD\textsubscript{EFF}) x Q\textsubscript{INF})/Mass TP Removed  
TP Removed per TCOD\textsubscript{INF} = (TP\textsubscript{INF} - TSP\textsubscript{EFF})/TCOD\textsubscript{INF}
Figure 12. TP and TSP concentrations of influent and effluent samples from the laboratory system during the investigation.
Figure 13. The %P of the aerobic MLVSS\textsubscript{AER} of the laboratory system during the investigation.
Figure 14. Comparison of the amount of TP removed by the system with the P requirement of the system.
Figure 15. The amount of TP removed by the system and the calculated P requirement of the system during the investigation.
the influent. Another contributor was the P concentration in the industrial wastewater as opposed to the municipal wastewater. While the municipal wastewater had a range of 4 - 8 mg P/L, the industrial wastewater TP concentration varied considerably. It ranged from 1 to 21 mg/L. Data presented in Table A-3 were obtained from the industry. It was used to help characterize the industrial waste prior to the addition of supplements.

As an overall check of the TP data accuracy, the masses of TP entering and leaving the bench scale unit were used. The results of the TP analyses and operations data were used to calculate the masses of TP in the influent, effluent and WAS. The comparison of the masses of TP in and out of the system is shown in Figure 16. A simple linear regression was performed on the data used for the figure and the results are provided on the figure. Ideally, the mass of influent TP should equal the sum of the masses of effluent and WAS TP.

Figure 16 indicates that the TP results were reasonably accurate and acceptable for the purposes of this investigation. As seen on the figure, there are a few data points which are a significant distance from the desired response. Nonetheless, the $R^2$ value was fairly close to one and the data produced a slope or x coefficient which was close to one.

One of the feed supplements, FeCl₃, is known and used to precipitate phosphorus. Due to this possibility, the amount of FeCl₃ added in the feed was adjusted several times during the first three experiments, then eliminated after the first day of experiment #4. The dose of FeCl₃ ranged from 0.90 to 0.50 mg/L in experiments #1, #2 and #3.

The FeCl₃ had little impact on the mass of TP removed. Using the stoichiometric ratio of 5.2 grams of FeCl₃ required per gram of P removed (Sedlak et al., 1991), the amount of P which theoretically would have been precipitated was calculated. The results of these calculations were averaged and these are provided in Table 9. As indicated, the amount of P removed by FeCl₃ was negligible.
Figure 16. Mass of total phosphorus fed to the laboratory system in the influent compared to mass of Total Phosphorus removed from the laboratory system in the waste activated sludge and the effluent.
Table 9. Averages of ferric chloride (FeCl₃) dosage and the calculated mass of P precipitated at that dosage for each experiment of the investigation.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>FeCl₃ Dosage (mg FeCl₃/L)</th>
<th>Mass of P Precipitated (mg P/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.87</td>
<td>1.5</td>
</tr>
<tr>
<td>2</td>
<td>0.50</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>0.50</td>
<td>1.0</td>
</tr>
<tr>
<td>4</td>
<td>0.50*</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* FeCl₃ added on first day of experiment only
The release of PO\textsubscript{4} in the anaerobic zone and the subsequent aerobic uptake of PO\textsubscript{4} are known characteristics and indicators of BPR. The PO\textsubscript{4} data which were collected were analyzed for these traits. Table 10 lists the average PO\textsubscript{4} concentrations of the different samples which were analyzed for each experiment. The PO\textsubscript{4} data for the investigation are plotted in Figure 17.

Since the instrument output range (300) was high and the peaks were small, the results of the ion chromatograph were checked. Limited colorimetric phosphate analyses were done. This method also required a 10:1 dilution. As shown in Figures 18 and 19, the colorimetric method consistently produced higher influent and effluent concentrations than the ion chromatograph. The difference between the two decreased from the end of experiment #1 (day 47) to experiment #4 when the last colorimetric analysis was done.

It is difficult to determine which phosphate results more closely predicted the actual concentrations and were reliable. Since there were more results produced with the chromatograph over the course of the investigation, it was used in evaluation of BPR.

**Biomass Production and Concentrations**

Biomass production, ΔX, was calculated to characterize system biomass activity. As expected, ΔX was dependent on the amount of TCOD\textsubscript{INF}. Calculated ΔX values and TCOD\textsubscript{INF} results for the experiments are shown in Figure 20. This figure shows that biomass production roughly tracked TCOD\textsubscript{INF} for all the experiments. There were some tubing and mechanical failures, but they did not adversely impact the amount of biomass.

The amount of ΔX per mass of TCOD\textsubscript{INF} removed by the laboratory system, or Y\textsubscript{OBS}, was calculated. This was the measured yield of the system. Figure 21 is a plot of these results. These seem to show that the Y\textsubscript{OBS} of the system was fairly consistent for most of the experiments. The high values seen in experiment #4 were caused by the decrease in TCOD\textsubscript{INF}. The experiment averages for Y\textsubscript{OBS} and ΔX are provided in Table 11.

The process zone MLSS concentrations during the investigation are plotted in Figure 22. As with ΔX, the MLSS\textsubscript{AER} and MLSS\textsubscript{ANA} varied in
Table 10. Average phosphate (PO$_4$-P) concentrations from different points and zones of the laboratory system for each experiment of the investigation.

<table>
<thead>
<tr>
<th>Exp</th>
<th>INF</th>
<th>ANA</th>
<th>2ANA</th>
<th>ANX</th>
<th>AER</th>
<th>EFF</th>
<th>RAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.8</td>
<td>6.4</td>
<td>*</td>
<td>*</td>
<td>4.3</td>
<td>4.4</td>
<td>#</td>
</tr>
<tr>
<td>2</td>
<td>27.9</td>
<td>#</td>
<td>30.6</td>
<td>*</td>
<td>24.4</td>
<td>25.2</td>
<td>#</td>
</tr>
<tr>
<td>3</td>
<td>28.3</td>
<td>#</td>
<td>32.1</td>
<td>*</td>
<td>38.2</td>
<td>30.9</td>
<td>#</td>
</tr>
<tr>
<td>4</td>
<td>23.3</td>
<td>29.1</td>
<td>31.8</td>
<td>26.1</td>
<td>22.1</td>
<td>23.5</td>
<td>22.9</td>
</tr>
<tr>
<td>5</td>
<td>23.8</td>
<td>27.4</td>
<td>28.5</td>
<td>25.7</td>
<td>13.0</td>
<td>13.8</td>
<td>13.5</td>
</tr>
<tr>
<td>6</td>
<td>20.0</td>
<td>33.8</td>
<td>37.6</td>
<td>32.9</td>
<td>5.0</td>
<td>6.0</td>
<td>5.3</td>
</tr>
<tr>
<td>7</td>
<td>18.4</td>
<td>24.6</td>
<td>26.6</td>
<td>26.7</td>
<td>25.0</td>
<td>23.0</td>
<td>28.0</td>
</tr>
</tbody>
</table>

Note: All analytical results produced using the ion chromatograph
# No sample analyzed from this stream or zone
* Zone did not exist during the period of the investigation

<table>
<thead>
<tr>
<th>Exp</th>
<th>INF</th>
<th>ANA</th>
<th>2ANA</th>
<th>ANX</th>
<th>AER</th>
<th>EFF</th>
<th>RAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>0</td>
<td>14</td>
<td>0</td>
<td>14</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>
Figure 17. Phosphate concentrations of samples from the influent, anaerobic zones, anoxic zone, aerobic zone and effluent of the laboratory system during the investigation (analyzed using ion chromatograph).
Figure 18. Comparison influent PO₄ concentrations determined by colorimetric method and the ion chromatograph.
Figure 19. Comparison of effluent $\text{PO}_4$ concentrations determined by colorimetric method and the ion chromatograph.
Figure 20. Biomass produced or lost in the effluent by the system compared to the influent TCOD of the system during the investigation.
Figure 21. The observed yield ($Y_{obs}$) of the laboratory system during the investigation.
Table 11. Averages of biomass production ($\Delta$) and the observed yield ($Y_{OBS}$) of the laboratory system for each experiment of the investigation.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>$\Delta X$ (mg MLVSS/day)</th>
<th>$Y_{OBS}$ (mg MLVSS/mg COD • day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6,183</td>
<td>0.28</td>
</tr>
<tr>
<td>2</td>
<td>6,259</td>
<td>0.28</td>
</tr>
<tr>
<td>3</td>
<td>3,431</td>
<td>0.27</td>
</tr>
<tr>
<td>4</td>
<td>2,273</td>
<td>0.66</td>
</tr>
<tr>
<td>5</td>
<td>1,493</td>
<td>0.25</td>
</tr>
<tr>
<td>6</td>
<td>2,065</td>
<td>0.28</td>
</tr>
<tr>
<td>7</td>
<td>4,629</td>
<td>0.20</td>
</tr>
</tbody>
</table>

$\Delta X = (MLVSS_{AER} \times Q_w) + \left( VSS_{EFF} \times (Q_{INF} - Q_w) \right)$

$Y_{OBS} = \frac{\Delta X}{(Q_{INF} \times (TCOD_{INF} - SCOD_{EFF}))}$
Figure 22. The total suspended solids (TSS) concentrations of samples from the anaerobic and the aerobic zones of the laboratory system during the investigation.
experiments #1 and #2, and tracked with TCOD_{INF} when it decreased in experiments #3 and #4 and increased in experiments #5 - #7. Table 12 contains the average MLSS for each reactor for each experiment. This table reveals that MLSS_{AER} was slightly greater than in the other zones. The %VSS was 80% - 85% of the MLSS_{AER} for all experiments except #6 which had an average of 75%.

The measured COD values of the aerobic biomass during the experiments are illustrated in Figure 23. It shows that the industrial waste had some effect on the COD of the biomass. The biomass COD was 1.1 to 1.2 for the industrial waste and 1.3 to 1.4 for the municipal wastewater. Other researchers have found that this ratio was in range of 1.3 - 1.5 mg COD per mg VSS for biomass under various conditions (Gaudy and Gaudy, 1988).

**Settleability**

Characterization of the biomass solid-liquid separation in secondary clarification was made using the parameters TSS_{EFF}, ZSV and SVI. The test results were used to gauge biomass settling. The use of the BPR configuration did not produce any noticeable improvements in sludge settling. Marginal to poor settling was demonstrated. Good settling, as measured by effluent TSS concentrations, was seen in experiments #4, #5, and #6.

The average TSS_{EFF} for each experiment is shown as a bar chart in Figure 24. This figure indicates that the average TSS_{EFF} was less than 10 in experiments #4, #5 and #6. During the other experiments the average TSS_{EFF} ranged from 39 to 185. The TSS_{EFF} data during the investigation are tracked in Figure 25. Initially, during experiments #1, #2 and #3, TSS_{EFF} results were erratic and poor with a range of 20 - 500 mg TSS/L.

The TSS_{EFF} improved in experiments #4, #5, and #6 and this was accompanied by a decrease in the solids loading on the clarifier. In experiments #4, #5 and #6, the solids loading rate (SLR) was approximately one-third of the SLR in periods #1 and #2. The SLRs which were determined during testing are graphed in Figure 26.
Table 12. Averages of total suspended solids (TSS) and % volatile suspended solids (%VSS) for different zones of the laboratory system for each experiment of the investigation.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>1st Aerobic mgTSS/L</th>
<th>%VSS</th>
<th>2nd Anaerobic mgTSS/L</th>
<th>%VSS</th>
<th>Anoxic mgTSS/L</th>
<th>%VSS</th>
<th>Aerobic mgTSS/L</th>
<th>%VSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2890</td>
<td>90</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>3110</td>
<td>84</td>
</tr>
<tr>
<td>2</td>
<td>2510</td>
<td>82</td>
<td>2530</td>
<td>82</td>
<td>*</td>
<td>*</td>
<td>3140</td>
<td>81</td>
</tr>
<tr>
<td>3</td>
<td>1470</td>
<td>86</td>
<td>1670</td>
<td>86</td>
<td>*</td>
<td>*</td>
<td>1580</td>
<td>85</td>
</tr>
<tr>
<td>4</td>
<td>1030</td>
<td>87</td>
<td>1110</td>
<td>87</td>
<td>1030</td>
<td>84</td>
<td>1060</td>
<td>82</td>
</tr>
<tr>
<td>5</td>
<td>460</td>
<td>89</td>
<td>550</td>
<td>89</td>
<td>660</td>
<td>89</td>
<td>770</td>
<td>81</td>
</tr>
<tr>
<td>6</td>
<td>850</td>
<td>84</td>
<td>820</td>
<td>86</td>
<td>900</td>
<td>84</td>
<td>1110</td>
<td>75</td>
</tr>
<tr>
<td>7</td>
<td>1920</td>
<td>83</td>
<td>1800</td>
<td>84</td>
<td>1850</td>
<td>83</td>
<td>2180</td>
<td>83</td>
</tr>
</tbody>
</table>

* No sample, zone was not present in laboratory system
Figure 23. The TCOD of samples from the aerobic zone of the laboratory system during the investigation.
Figure 24. The average effluent TSS concentration for each experiment of the investigation.
Figure 25. Effluent TSS concentrations of samples from the laboratory system during investigation.
Figure 26. The solids loading rate (SLR) on the clarifier of the laboratory system during the investigation.
The TSS$_{\text{eff}}$ also affected the biomass concentration. As seen in Figure 27, high TSS$_{\text{eff}}$ caused the MLSS$_{\text{AER}}$ to decrease. This was most evident in experiment #1 when erratic TSS$_{\text{eff}}$ caused the MLSS$_{\text{AER}}$ to drop from around 2800 mg/L to about 2200 mg/L. In experiment #2, improved and more consistent TSS$_{\text{eff}}$ did not provide for a more consistent MLSS$_{\text{AER}}$. In this period, MLSS$_{\text{AER}}$ varied from 3400 to 2600 mg/L. These increases and decreases in the amount of biomass were attributed to both the varying TCOD$_{\text{inf}}$ and TSS$_{\text{eff}}$. 

The SVI and the ZSV determinations further reinforced that settling was poor throughout most of the investigation. The SVI and ZSV which were recorded during the investigation are shown in Figure 28. While there was some improvement in experiment #3, effluent settling was not as good as the SVI indicated in experiment #1. The low SVIs may be misleading. The MLVSS$_{\text{AER}}$ were high and in the range where they strongly limit SVI values. Observations also revealed that straggler floc was left in the clear zone after rapid settling.

Seeding with other laboratory and pilot BPR biomasses seemed to improve settling only momentarily in most cases. These systems' settling characteristics were neither maintained nor improved in the experimental laboratory system. When the municipal wastewater was added in experiment #3 and a municipal pilot plant seed sludge was used, settling improved. This was sustained for over thirty days.

Sludge bulking problems became evident in experiment #7. The sludge blanket in the clarifier was at the water surface. The presence of large filaments was confirmed microscopically. This problem was treated by dosing the aerobic zone effluent line with household bleach (4 - 6% NaOCl). This was done twice. The aliquots of bleach used were 50 and 100 mL.

While filamentous microorganisms were probably present to some degree throughout the investigation, their population probably begin to increase with changes in TCOD$_{\text{inf}}$ beginning in experiment #3 and ending with the drastic increase of TCOD$_{\text{inf}}$ in experiment #7.
Figure 27. Total suspended solids (TSS) concentrations of the aerobic zone biomass and the effluent from the laboratory system during the investigation.
Figure 28. Sludge Volume Index (SVI) and Zone Settling Velocity (ZSV) of the aerobic zone biomass from the laboratory system during the investigation.
LABORATORY BPR TREATMENT SYSTEM OPERATING CONDITIONS
Dissolved Oxygen Concentrations and Utilizations and Oxidation Reduction Potentials

The DO concentrations in the reactors and components of the BPR system were monitored. The readings that were obtained in the different zones are averaged for each period in Table 13. Using the tabulation and the plot of all the DO data in Figure 29, several observations were made.

First, there was sufficient DO in the aerobic zone for the uptake of P and utilization of COD in all periods. The average DO_{aer} was greater than 3.0 mg DO/L during testing. As impairments to BPR, the influent and RAS recycle contributed oxygen loadings to the anaerobic zone. The average DO_{ana} was greater than 0.20 mg DO/L (0.50) in periods #1 and #3. With nearly the same DO inputs, the DO_{ana} was equal to or less than 0.20 mg DO/L in periods #2, #4, #5, #6 and #7. It should be noted that these low levels were at the bottom or below the range of the instrument.

The results of testing to determine OUR and SOUR of the aerobic biomass are found in Figure 30 and Table 14. These were calculated using DO measurements and MLVSS data. The figure indicates that SOUR increased only slightly over the course of the investigation.

As another monitor of anaerobic conditions, ORP measurements were made. Table 15 provides the averages of the readings in the reactors for each experiment. There are a couple of reference electrodes and different calibration methods used to measure ORP. This makes comparison of these readings to those obtained by others difficult. It does appear that the data provide a favorable indication that anaerobic redox potential was achieved in experiments #2, #3, #4, #5 and #6. These ORP were close to the anaerobic ORPs reported by Koch and Oldham (1985). The reference electrode which was used was the same type that they utilized in their work.

pH Measurements

The average pH of the influent, effluent, and in the BPR system's zones for each experiment are presented in Table 16. The averages show that
Table 13. Averages of dissolved oxygen concentration from different points and zones of the laboratory system for each experiment of the investigation.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Dissolved oxygen concentration, mg DO/L</th>
<th>INF</th>
<th>1ANA</th>
<th>2ANA</th>
<th>ANX</th>
<th>AER</th>
<th>RAS</th>
<th>Top of Clarifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>1.0</td>
<td>0.5</td>
<td>*</td>
<td>*</td>
<td>5.4</td>
<td>#</td>
<td>1.9</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>#</td>
<td>0.2</td>
<td>0.2</td>
<td>*</td>
<td>5.5</td>
<td>0.2</td>
<td>2.8</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>1.5</td>
<td>0.5</td>
<td>0.5</td>
<td>*</td>
<td>4.7</td>
<td>1.1</td>
<td>1.6</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>0.9</td>
<td>0.2</td>
<td>0.1</td>
<td>0.9</td>
<td>5.1</td>
<td>1.8</td>
<td>3.2</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>1.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>5.4</td>
<td>2.1</td>
<td>3.7</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>1.3</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>4.3</td>
<td>1.6</td>
<td>3.0</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>1.9</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>3.4</td>
<td>0.5</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Note: DO measurements were made in-situ. The laboratory system was monitored without disturbing operation.

# Zone or stream not monitored
* Reactor or zone not present in laboratory system
Figure 29. Dissolved oxygen concentrations of the influent, the reactor zones, and the clarifier surface of the laboratory system during the investigation.
Figure 30. The oxygen utilization rate (OUR) and specific oxygen utilization rate (SOUR) of the aerobic biomass of the laboratory system during the investigation.
Table 14. Averages of oxygen utilization rate (OUR) and specific oxygen utilization rate (SOUR) for the aerobic biomass from the laboratory system for each experiment of the investigation.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Dissolved Oxygen consumption by the system biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OUR, mg DO/L-hr</td>
</tr>
<tr>
<td>1</td>
<td>19.2</td>
</tr>
<tr>
<td>2</td>
<td>31.3</td>
</tr>
<tr>
<td>3</td>
<td>15.7</td>
</tr>
<tr>
<td>4</td>
<td>7.6</td>
</tr>
<tr>
<td>5</td>
<td>11.3</td>
</tr>
<tr>
<td>6</td>
<td>11.7</td>
</tr>
<tr>
<td>7</td>
<td>34.8</td>
</tr>
</tbody>
</table>
Table 15. Averages of oxidation reduction potential (ORP) in different zones of the laboratory system for each experiment of the investigation.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; ANA</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; ANA</th>
<th>ANX</th>
<th>AER</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>2</td>
<td>-236</td>
<td>-273</td>
<td>#</td>
<td>182</td>
</tr>
<tr>
<td>3</td>
<td>-127</td>
<td>-152</td>
<td>#</td>
<td>125</td>
</tr>
<tr>
<td>4</td>
<td>-161</td>
<td>-152</td>
<td>5</td>
<td>278</td>
</tr>
<tr>
<td>5</td>
<td>-305</td>
<td>-283</td>
<td>-202</td>
<td>233</td>
</tr>
<tr>
<td>6</td>
<td>-242</td>
<td>-261</td>
<td>-99</td>
<td>239</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>58</td>
<td>23</td>
<td>129</td>
</tr>
</tbody>
</table>

* ORP was not monitored
# This reactor or zone was not in the BPR system
Table 16. Averages of pH for the influent, anaerobic zones, anoxic zone, aerobic zone and effluent for each experiment of the investigation.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>INF</th>
<th>1&lt;sup&gt;st&lt;/sup&gt;ANA</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt;ANA</th>
<th>ANX</th>
<th>AER</th>
<th>EFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.43</td>
<td>5.47</td>
<td>*</td>
<td>*</td>
<td>8.03</td>
<td>8.04</td>
</tr>
<tr>
<td>2</td>
<td>4.42</td>
<td>5.44</td>
<td>5.45</td>
<td>*</td>
<td>8.05</td>
<td>8.08</td>
</tr>
<tr>
<td>3</td>
<td>4.57</td>
<td>5.54</td>
<td>5.57</td>
<td>*</td>
<td>7.83</td>
<td>7.76</td>
</tr>
<tr>
<td>4</td>
<td>6.54</td>
<td>6.83</td>
<td>6.83</td>
<td>7.04</td>
<td>7.15</td>
<td>7.26</td>
</tr>
<tr>
<td>5</td>
<td>6.60</td>
<td>7.13</td>
<td>7.10</td>
<td>7.54</td>
<td>8.01</td>
<td>8.15</td>
</tr>
<tr>
<td>6</td>
<td>5.27</td>
<td>6.42</td>
<td>6.46</td>
<td>6.96</td>
<td>7.66</td>
<td>7.92</td>
</tr>
<tr>
<td>7</td>
<td>4.35</td>
<td>5.33</td>
<td>5.35</td>
<td>6.23</td>
<td>7.59</td>
<td>7.82</td>
</tr>
</tbody>
</table>

* The zone did not exist in the laboratory BPR system
influent pH was one to two orders of magnitude less in experiments #1, #2 and #3, than in experiments #4, #5 and #6. The industrial wastewater used as influent for experiments #1, #2, and #7 was acidic (pH 4.5). When the influent was the 50/50 mix in experiment #4, the influent was only slightly higher (pH 4.6). The influent was close to neutrality (pH 6.5) for experiments #4 and #5. In experiment #6, the average influent pH was roughly 5.3. The influent was mostly municipal wastewater for experiments #4, #5 and #6. All the pH data for each experiment of the investigation are presented in Figure 31.

The large difference from the influent to effluent pH in experiments #1, #2, and #3 was also noted. The pH increased three orders of magnitude from the influent to the effluent of the laboratory system. In experiments #4 and #5, the pH only increased one to two orders of magnitude. In experiment #6 the increase was two to three orders of magnitude, but the average anaerobic pH was near 6.4 as opposed to a pH of less than 5.6 for experiments #1, #2, and #3. The average pH in the anaerobic zone was just greater than 6.8 for experiments #4 and #5.

Nitrogen Compounds

Nitrogen and nitrogen containing compounds were important in two respects. First, nitrogen in a readily available form is required for the growth and synthesis of BPR biomass. Its concentration in the feed needed to at least match the nutritional requirements of the microorganisms. Oxidized nitrogen compounds like NO$_3$ and NO$_2$ are detrimental to BPR if present in the anaerobic zone. Their presence in the anaerobic zone reduces the amount of substrate stored for subsequent uptake in the aerobic zone.

Nitrogen in the form of urea was added to the influents in all the experiments except #4 and #5. The amount of nitrogen added plus the amount estimated to be present in the influent are compared to biomass requirement in Figure 32. Except for portions of experiment #1, the nitrogen requirements were met during all of the experiments.

From the data characterizing the industrial wastewater in Table A-3, it seems that on an average 85% of the TKN in the industrial waste was in an
Figure 31. The pH of the influent, the reactor zones, and the effluent of the laboratory system during the investigation.
Figure 32. The estimated concentration of nitrogen in the influent and the calculated concentration of nitrogen needed for the growth requirements of the laboratory system biomass during the investigation.
organic form. Ammonia, the urea supplement, and NO$_x$ were the other nitrogen containing compounds present in the influent for experiments #1, #2 and #7. For experiments #4 and #5, the amount and forms of nitrogen present were more typical of municipal wastewaters. Since the influent was a mixture of the industrial and municipal wastewaters in experiments #3 and #6, the amount and forms of nitrogen were a blend of the two.

Selected samples were analyzed for TKN during experiments #4, #5, and #6. These limited results are listed in Table 17. Even though the laboratory system was operated at a relatively short MCRT to limit nitrification, the reduction from TKN$_{INF}$ to TKN$_{EFF}$ indicated that there was nitrification or partial nitrification in experiments #4, #5, and #6.

Realizing the detrimental effect of NO$_x$ compounds in the anaerobic zone to BPR performance, they were monitored at different points of the laboratory system at various times. Oxidized nitrogen data in the anaerobic zone along with some limited analysis of the RAS are seen in Figure 33. Averages of all the NO$_x$ data which were collected are provided in Table 18.

The data indicate there were oxidized nitrogen compounds present in the anaerobic zone in experiments #1, #2 and #3. The sources of these appeared to be the RAS and the industrial wastewater. The average RAS NO$_3$ was 4.0 mg NO$_3$-N/L in experiment #1. With an average influent NO$_3$ of 2.5 mg NO$_3$-N/L (from Table A-3), the total loading approached 3.2 mg NO$_3$-N/L. This did not include the varying amounts of nitrites which were found to be present in the RAS. For experiment #2, there was no RAS NO$_3$ data, but the influent NO$_3$ and the anaerobic zone NO$_3$ indicate the loading was significant. To a lesser extent, this also appears to be the case for experiment #3.

For experiments #4, #5 and #6, the NO$_x$ concentrations are evidence that reduced conditions could be attained in the anaerobic zone. The anaerobic NO$_x$ concentration was 0.20 mg/L or less in experiments #4 and #5 and 0.70 mg/L in experiment #6. The NO$_3$ in the anaerobic zone in experiment #6 seems to be a result of the NO$_3$ in the influent and RAS.
Table 17. Total Kjeldahl Nitrogen (TKN) concentrations of influent and effluent samples from the laboratory system.

<table>
<thead>
<tr>
<th>Date</th>
<th>Day</th>
<th>TKN, mg TKN-N/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total Influent</td>
</tr>
<tr>
<td>10/11/90</td>
<td>141</td>
<td>32.9</td>
</tr>
<tr>
<td>10/19/90</td>
<td>149</td>
<td>30.2</td>
</tr>
<tr>
<td>10/21/90</td>
<td>151</td>
<td>22.3</td>
</tr>
<tr>
<td>10/27/90</td>
<td>157</td>
<td>21.8</td>
</tr>
<tr>
<td>10/29/90</td>
<td>159</td>
<td>24.3</td>
</tr>
<tr>
<td>10/31/90</td>
<td>161</td>
<td>19.0</td>
</tr>
<tr>
<td>11/4/90</td>
<td>165</td>
<td>24.7</td>
</tr>
<tr>
<td>11/6/90</td>
<td>167</td>
<td>30.9</td>
</tr>
<tr>
<td>11/8/90</td>
<td>169</td>
<td>55.8</td>
</tr>
</tbody>
</table>
Figure 33. Nitrate (NO$_3$-N) concentration of samples from the anaerobic zone and the RAS of the laboratory system during the investigation.
Table 18. Averages of nitrate (NO$_3$-N) and nitrite (NO$_2$-N) concentrations of samples from the influent, anaerobic zones, anoxic zone, aerobic zone and effluent of the laboratory system for each experiment of the investigation.

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>INF</th>
<th>ANA1</th>
<th>ANA2</th>
<th>ANX</th>
<th>AER</th>
<th>EFF</th>
<th>RAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO$_3$</td>
<td>NO$_2$</td>
<td>NO$_2$</td>
<td>NO$_3$</td>
<td>NO$_2$</td>
<td>NO$_3$</td>
<td>NO$_2$</td>
</tr>
<tr>
<td>1</td>
<td>#</td>
<td>#</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>2</td>
<td>3.6</td>
<td>0</td>
<td>#</td>
<td>#</td>
<td>3.6</td>
<td>0.6</td>
<td>*</td>
</tr>
<tr>
<td>3</td>
<td>2.2</td>
<td>0</td>
<td>#</td>
<td>#</td>
<td>2.0</td>
<td>0</td>
<td>*</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
<td>2.7</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0.7</td>
<td>0</td>
<td>0.7</td>
<td>0</td>
<td>0.7</td>
<td>0</td>
<td>0.7</td>
</tr>
<tr>
<td>7</td>
<td>3.0</td>
<td>0.3</td>
<td>2.9</td>
<td>0</td>
<td>2.8</td>
<td>0</td>
<td>2.7</td>
</tr>
</tbody>
</table>

# Sample not analyzed for nitrate or nitrite.

* Zone did not exist in the laboratory system during this period.
Besides preventing the establishment of anaerobic or reduced conditions, NO$_3$ is known to consume COD when it is denitrified. This effect was examined by taking the averages of influent and RAS NO$_X$ in experiments #1, #2 and #3 and calculating the mass of COD which would be consumed for denitrification. For denitrification, 3.7 mg of COD are needed per mg of NO$_3$ denitrified (Sedlak et al., 1991). The following calculation was used to estimate the amount of COD that may be consumed:

$$\text{COD, mg/day} = (\text{NOX}_{\text{INF}} + \text{NOX}_{\text{RAS}}) \times Q_{\text{ted}} \times 3.7$$

The results reveal that 260, 325 and 230 mg of COD/day would be required for the denitrification of NO$_X$ in experiments #1, #2 and #3, respectively.

**Other Inhibitors and Supplements**

It was known that the industrial waste contained other substances which can affect BPR performance. High concentrations of SO$_4$ and elemental Mg were measured in the influent feed. Other elements, Fe and K were also monitored. The Mg, Fe, K, and the nutrients N and P are essential for biomass cellular growth. All but Fe have been identified as essential nutrients needed for BPR (Pattarkine, 1991).

As seen in Table 19, there were substantial amounts of SO$_4$ present in the system in experiments #1, #2, #3 and #7. The effluent results were higher than the influent in experiments #1, #2 and #3. If anything, it appears that the BPR system generated some SO$_4$ during the first three periods. Inorganic sources of sulfur could have been formed from organic compounds and then oxidized to SO$_4$ under aerobic conditions. The sulfate present in the influent could also serve as an electron acceptor for some bacteria (Gaudy and Gaudy, 1988), but the results indicate this was insignificant in these experiments.

The influent and effluent Mg results are presented in Table 20. The industrial waste had an average influent concentration in experiments #1 and #2 of 1,068 and 852 mg Mg, respectively. This trace element is needed in very small amounts for bacteria growth. It is also co-transported (release and uptake) with P in BPR. Comeau et al. (1986) summarized reported molar ratios of cations co-transported with P from different references. The molar ratios of
Table 19. Average sulfate concentrations of samples from the influent, anaerobic zone, anoxic zone, aerobic zone and effluent of the laboratory system for each experiment of the investigation.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>INF</th>
<th>1ANA</th>
<th>2ANA</th>
<th>ANX</th>
<th>AER</th>
<th>EFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3780</td>
<td>3962</td>
<td>*</td>
<td>*</td>
<td>4197</td>
<td>4544</td>
</tr>
<tr>
<td>2</td>
<td>3464</td>
<td>#</td>
<td>3456</td>
<td>*</td>
<td>3557</td>
<td>3703</td>
</tr>
<tr>
<td>3</td>
<td>1447</td>
<td>#</td>
<td>1436</td>
<td>*</td>
<td>1989</td>
<td>1609</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>14</td>
<td>30</td>
<td>25</td>
<td>55</td>
<td>111</td>
</tr>
<tr>
<td>5</td>
<td>14</td>
<td>15</td>
<td>14</td>
<td>20</td>
<td>25</td>
<td>23</td>
</tr>
<tr>
<td>6</td>
<td>464</td>
<td>442</td>
<td>439</td>
<td>232</td>
<td>434</td>
<td>429</td>
</tr>
<tr>
<td>7</td>
<td>2472</td>
<td>2335</td>
<td>2342</td>
<td>2267</td>
<td>2239</td>
<td>2199</td>
</tr>
</tbody>
</table>

* The zone did not exist in the laboratory BPR system
# Sample was not analyzed for sulfate
Table 20. Magnesium (Mg\(^{2+}\)) concentrations of influent and effluent samples from the laboratory system during the investigation.

<table>
<thead>
<tr>
<th>Date</th>
<th>Total Influent</th>
<th>Soluble Influent</th>
<th>Total Effluent</th>
<th>Soluble Effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/26/90</td>
<td>858</td>
<td>-</td>
<td>-</td>
<td>869</td>
</tr>
<tr>
<td>7/2/90</td>
<td>773</td>
<td>-</td>
<td>-</td>
<td>1632*</td>
</tr>
<tr>
<td>7/3/90</td>
<td>1442</td>
<td>-</td>
<td>-</td>
<td>5200*</td>
</tr>
<tr>
<td>7/4/90</td>
<td>2021*</td>
<td>-</td>
<td>-</td>
<td>1867*</td>
</tr>
<tr>
<td>7/13/90</td>
<td>1175</td>
<td>-</td>
<td>-</td>
<td>1238</td>
</tr>
<tr>
<td>7/14/90</td>
<td>1148*</td>
<td>-</td>
<td>-</td>
<td>1252*</td>
</tr>
<tr>
<td>7/17/90</td>
<td>1385*</td>
<td>-</td>
<td>-</td>
<td>1437*</td>
</tr>
<tr>
<td>8/8/90</td>
<td>711</td>
<td>-</td>
<td>-</td>
<td>1203*</td>
</tr>
<tr>
<td>8/10/90</td>
<td>#</td>
<td>1125*</td>
<td>-</td>
<td>1211*</td>
</tr>
<tr>
<td>8/16/90</td>
<td>895</td>
<td>878</td>
<td>900</td>
<td>905</td>
</tr>
<tr>
<td>8/20/90</td>
<td>915</td>
<td>887</td>
<td>874</td>
<td>893</td>
</tr>
<tr>
<td>8/22/90</td>
<td>954</td>
<td>887</td>
<td>10,822*</td>
<td>#</td>
</tr>
<tr>
<td>8/28/90</td>
<td>777</td>
<td>724</td>
<td>#</td>
<td>831</td>
</tr>
<tr>
<td>9/7/90</td>
<td>471</td>
<td>458</td>
<td>555</td>
<td>555</td>
</tr>
<tr>
<td>9/17/90</td>
<td>340</td>
<td>322</td>
<td>-</td>
<td>339</td>
</tr>
<tr>
<td>9/21/90</td>
<td>446</td>
<td>501</td>
<td>375</td>
<td>462</td>
</tr>
<tr>
<td>9/25/90</td>
<td>417</td>
<td>402</td>
<td>415</td>
<td>424</td>
</tr>
</tbody>
</table>

* Out of range of calibration
# Sample produced negative (-) absorbance
- No results, sample not analyzed
Mg that were given ranged from 0.26 to 0.32. Results in experiments #1, #2 and #3 indicate that it was present in amounts much greater than required for growth and BPR combined.

Potassium like Mg has a dual purpose in BPR. It is needed by the biomass for growth and it is co-transported with P. The molar ratios of K which have been reported range from 0.20 to 0.34 (Comeau et al., 1986). The influent and effluent K results are presented in Table 21. Unlike Mg, K was not present in the industrial wastewater. It was added to the influent to prevent it from being limiting to BPR. The biomass potassium requirement, the concentration of K added in the feed and the measured influent K is compared in Figure 34. This figure shows that adequate amounts of K were available to the system except for several occasions in experiment #1.

To determine the maximum amount of K required for co-transport with P, the molar ratio of 0.34 and the highest average influent TP concentration (experiment #2) were used to calculate the required K concentration. It was estimated that the K concentration needed to be 29 mg/L in experiment #2 which was the worse case situation. As seen in Figure 34, the K concentration supplemented into the influent was 31 mg/L during this experiment. Only in experiment #1 would there have been occasions when the concentration was not adequate (avg. TP = 33 mg/L; K req’d = 15 mg/L).

Iron presented a different situation. While iron is required for biomass growth, it is not necessary for BPR. It is responsible for the precipitation of phosphorus in its ferric form. As discussed previously, it was limited and then eliminated for this reason. Overall, iron was not present in the influent in sufficient amounts as dictated by cell metabolic requirements. The Fe supplemented in the feed and Fe required by the biomass are compared in Figure 35.

Influent Ratios and Operating Parameters

System influent loading ratios and operating parameters were monitored during testing. These ratios and parameters were compared with the data characterizing BPR. Some loading and operating conditions yielded BPR. Other
Table 21. Potassium (K\(^+\)) concentrations of influent and effluent samples from the laboratory system during the investigation.

<table>
<thead>
<tr>
<th>Date</th>
<th>Total Influent</th>
<th>Soluble Influent</th>
<th>Total Effluent</th>
<th>Soluble Effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/26/90</td>
<td>22.2</td>
<td>-</td>
<td>-</td>
<td>7.6</td>
</tr>
<tr>
<td>7/2/90</td>
<td>22.0</td>
<td>-</td>
<td>-</td>
<td>15.9</td>
</tr>
<tr>
<td>7/3/90</td>
<td>17.0</td>
<td>-</td>
<td>-</td>
<td>24.4</td>
</tr>
<tr>
<td>7/4/90</td>
<td>15.6</td>
<td>-</td>
<td>-</td>
<td>14.7</td>
</tr>
<tr>
<td>7/13/90</td>
<td>33.3</td>
<td>-</td>
<td>-</td>
<td>16.9</td>
</tr>
<tr>
<td>7/14/90</td>
<td>35.3</td>
<td>-</td>
<td>-</td>
<td>34.5</td>
</tr>
<tr>
<td>7/17/90</td>
<td>39.4</td>
<td>-</td>
<td>-</td>
<td>36.7</td>
</tr>
<tr>
<td>8/8/90</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>26.2</td>
</tr>
<tr>
<td>8/16/90</td>
<td>#</td>
<td>-</td>
<td>-</td>
<td>20.8</td>
</tr>
<tr>
<td>8/20/90</td>
<td>16.1</td>
<td>91.3(^*)</td>
<td>16.6</td>
<td>82.8(^*)</td>
</tr>
<tr>
<td>8/22/90</td>
<td>15.2</td>
<td>88.3(^*)</td>
<td>91.3(^*)</td>
<td>#</td>
</tr>
<tr>
<td>8/28/90</td>
<td>108.1</td>
<td>81.8(^*)</td>
<td>-</td>
<td>73.7(^*)</td>
</tr>
<tr>
<td>9/7/90</td>
<td>119.2</td>
<td>122.1</td>
<td>139.2</td>
<td>114.7</td>
</tr>
<tr>
<td>9/17/90</td>
<td>225.1</td>
<td>230.8</td>
<td>-</td>
<td>194.8</td>
</tr>
<tr>
<td>9/21/90</td>
<td>36.6</td>
<td>39.1</td>
<td>32.8</td>
<td>33.8</td>
</tr>
<tr>
<td>9/25/90</td>
<td>36.4</td>
<td>37</td>
<td>35.6</td>
<td>34.3</td>
</tr>
</tbody>
</table>

\(^*\) Out of range of calibration
# Sample produced negative (-) absorbance
- No results, sample not analyzed
Figure 34. The concentration of potassium supplemented into the influent and the calculated concentration of potassium needed for the growth requirements of the laboratory system biomass during the investigation.
Figure 35. The concentration of iron supplemented into the influent and the calculated concentration of iron needed for the growth requirements of laboratory system biomass during the investigation.
loadings, expected to improve BPR performance, did not achieve BPR. The information also indicated that there were varying results produced at the same loadings and operating conditions.

Randall, et al. (1992) have reported that BPR is a function of the TCOD$_{INF}$ to TP$_{INF}$ ratio for municipal wastewater. The ratio of TCOD$_{INF}$ to TP$_{INF}$ was examined for each experiment. The different influents used and the amount of phosphorus supplemented in the feed produced ratios ranging from 10 to 110. The average TCOD$_{INF}$:TP$_{INF}$ ratio for each experiment is provided in Table 22.

Comparison of the TCOD$_{INF}$:TP$_{INF}$ ratio with TP removal or TP$_{EFF}$ indicated little and was meaningless. The TCOD$_{INF}$:TP$_{INF}$ ratio was plotted with the %P of the aerobic zone biomass in Figure 36. The %P was highest when the TCOD$_{INF}$:TP$_{INF}$ ratio was in the 20 - 30 range. Figure 36 gives a subtle indication that at the higher influent ratios, the %P in the aerobic biomass was less than at the lower ratios.

The operating and loading conditions of the anaerobic zone were examined. The TCOD$_{INF}$:TSS$_{ANA}$ ratio and the TCOD$_{INF}$ loading on the mass of TSS$_{ANA}$ were calculated. The averages for each experiment are given in Table 22. The TCOD$_{INF}$:TSS$_{ANA}$ ratio and the TCOD$_{INF}$ loading on the mass of TSS$_{ANA}$ are plotted for the investigation in Figures A-5 and A-6, respectively.
Table 22. Averages of the influent TCOD loading to the mass of MLSS\textsubscript{ANA}, the influent TCOD to MLSS\textsubscript{ANA} Ratio, and influent TCOD to influent TP ratio for each experiment of the investigation.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>TCOD\textsubscript{INF} Loading to MLSS\textsubscript{ANA}, mgTCOD/mgTSS*day</th>
<th>TCOD\textsubscript{INF}: MLSS\textsubscript{ANA} Ratio, mgTCOD/mgTSS</th>
<th>TCOD\textsubscript{INF}:TP\textsubscript{INF} Ratio, mgTCOD/mgTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.7</td>
<td>0.41</td>
<td>78</td>
</tr>
<tr>
<td>2</td>
<td>2.4</td>
<td>0.47</td>
<td>37</td>
</tr>
<tr>
<td>3</td>
<td>2.3</td>
<td>0.46</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>1.0</td>
<td>0.20</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>3.4</td>
<td>0.64</td>
<td>26</td>
</tr>
<tr>
<td>6</td>
<td>2.3</td>
<td>0.44</td>
<td>26</td>
</tr>
<tr>
<td>7</td>
<td>4.0</td>
<td>0.75</td>
<td>55</td>
</tr>
</tbody>
</table>

\[ \text{TCOD}_{\text{INF}} \text{ to MLSS}_{\text{ANA}} = \frac{(\text{TCOD}_{\text{INF}} \times Q_{\text{INF}})}{\text{(Vol. ANA Zone} \times \text{MLSS}_{\text{ANA}})} \]

\[ \text{TCOD}_{\text{INF}}:\text{MLSS}_{\text{ANA}} \text{ Ratio} = \frac{\text{TCOD}_{\text{INF}}}{\text{MLSS}_{\text{ANA}}} \]

\[ \text{TCOD}_{\text{INF}}:\text{TP}_{\text{INF}} \text{ Ratio} = \frac{\text{TCOD}_{\text{INF}}}{\text{TP}_{\text{INF}}} \]
Figure 36. The %P of the MLVSS_{AER} compared to the influent TCOD to TP ratio of the laboratory system during the investigation.
CHAPTER V. DISCUSSION

In this chapter, the results of the experiments are analyzed to interpret the operation and performance data of the laboratory BPR system. Relationships were developed and comparisons were made to provide a better understanding of the experimental responses.

BIOLOGICAL PHOSPHORUS REMOVAL DURING THE EXPERIMENTS

Data that characterize BPR were examined for all experiments. Information on influent and effluent phosphorus concentrations, mass of TP removed, anaerobic PO$_4$ release, and aerobic PO$_4$ uptake were evaluated and utilized. Calculations and comparisons were made to characterize and describe system operation with respect to BPR. These were used to determine whether the laboratory system exhibited the traits of BPR while treating the different wastewaters and mixtures.

**Industrial Wastewater**

With the industrial wastewater as influent, the laboratory system did not exhibit the characteristics of BPR that have been demonstrated by others using municipal wastewater and prepared influents. The effluent phosphorus concentrations were high, the %P of aerobic MLVSS were average to less than average, and anaerobic release and aerobic uptake of PO$_4$ were insignificant.

When the mass of TP required for the biomass growth functions is compared to the mass of TP removed by the laboratory system, there is a different indication. In experiment #2, the observed mass of TP removed was over twice the amount required for cellular growth. That is, an average of 150 mg/day of TP were removed in excess of the biomass' growth requirement. Otherwise, the system displayed no features which were indicative of BPR. The average concentrations of PO$_4$ in the anaerobic and aerobic zones were not representative of a BPR system. The average anaerobic PO$_4$ was only 3 mg/L greater and the average aerobic PO$_4$ was only 3 mg/L less than the influent PO$_4$. The P release and uptake are usually much more pronounced. As commonly seen in the works of others with BPR systems, the concentration of PO$_4$ in the anaerobic zone is usually at least twice the influent PO$_4$ and the
aerobic PO₄ is significantly less than the influent. The average %P of the aerobic MLVSS was 2.5%. This is only slightly greater than what is found in a municipal, conventional (fully aerobic) activated sludge biomass. While the %P in BPR systems typically range anywhere from 3 to 8%, %P in itself does not indicate BPR.

The average concentration of TP removed by the system in experiment #2 was close to 20 mg/L. The high average effluent TP concentration of 47 mg/L reveals there was some limitation on the amount which was removed. The average effluent PO₄ of 25 mg/L was also high and substantiates there was additional P to be utilized. The average concentration of PO₄ removed was considerably less than seen with TP. The average effluent concentrations were just 3 mg/L less than the average influent concentrations.

With the 0.5 mg FeCl₃/L dosage which was used in experiment #2, only 1 mg of P per day would have been removed by chemical precipitation. This supports that the mass of TP removed by the laboratory system was accomplished by the biomass. Less than 0.4% of the observed mass of TP removed, on an average, could have been chemically precipitated.

During experiment #1, there is no question that the laboratory system did not accomplish BPR. The observed mass of TP removed was less than the amount required for growth. The anaerobic release and aerobic uptake of phosphate were minimal. There was less than 2 mg/L difference between the average anaerobic and aerobic phosphate concentrations and the average influent phosphate concentration. The average %P of the aerobic MLVSS was 1.05% which is less than half of the 2.3% normally found in a municipal, conventional activated sludge biomass. Comparison of the average influent and effluent TP and PO₄ concentrations further reinforce that BPR was not established. The average concentrations of TP and PO₄ removed were less than 10 mg/L and less than 2 mg/L, respectively.

Comparison of the results which were obtained in experiments #1 and #2 with the work of others is difficult. The industrial wastewater and its high COD are not easily compared to the results of others. Others have used mainly
municipal wastewaters and prepared influents. Wentzel et al. (1988) reported that their Phoredox (A/O) system removed 20 mg P/L at a rate of 0.02 mg P/mg influent COD. The influent consisted of 1000 mg COD/L of sodium acetate and inorganic macro-nutrients. The average rates for the laboratory system in experiments #1 and #2 were 0.004 and 0.008 mg P/mg influent COD, respectively. These are five to two and a half times less than the rates Wentzel et al. (1988) obtained. The higher TCOD\textsubscript{inf} accounts for most of the difference, but there was still P available and it was clearly not limiting to additional P removal. Tracy et al. (1986) reported in their batch tests with a high strength wastewater (BOD = 1070 mg/L) that there was 28 mg/L of ortho-P removed, but the effluent concentration was 16 mg/L. They also noted that no P release or uptake of organics occurred during anaerobic conditions.

Abu-ghararah and Randall (1991) and others have found that acetic acid is the most efficient organic for BPR. They reported that 16.8 mg of COD were utilized per mg of P removed by their system. In experiments #1 and #2, the industrial wastewater must not have been a very good substrate for BPR with 290 and 83 mg COD/mg P, respectively, obtained.

With a knowledge of the mechanisms responsible and conditions required for BPR, data on the operating conditions of the laboratory system were scrutinized. These conditions were mainly a result of the industrial wastewater. While some of its characteristics are seen as favorable to BPR, others were detrimental.

As noted in Chapter II., pH is important to BPR and the anaerobic release of P. As discussed in this section, BPR was not established when the low pH industrial waste was used as the influent. While influent pH may not be solely responsible for the failure of BPR, it was confirmed that BPR did not occur with the laboratory system in experiments #1 and #2 when the influent pH was in the 4.4 to 4.6 range.

The pH of the anaerobic reactors was directly related to the pH of the influent. In experiments #1 and #2, the anaerobic pH was only 5.5 to 5.6. The results reported by Tracy, et al. (1988) show that BPR is very inefficient.
at this pH. As described by Comeau, et al. (1986) in their modelling efforts of the bacterial bioenergetics in BPR, the proton motive force (pmf) may be affected by low pH. In earlier batch tests with bio-P mixed liquor, a low pH of 4.0 did not produce significant P release under non-aerated, denitrified conditions. The acidic influent in experiments #1 and #2 is speculated to have reduced the pmf which is composed of pH and charge gradients. Without the pH gradient or its reestablishment, the amount of substrate transported into the bacterial membrane and stored would be limited. There was no or minimal release of PO$_4$ because there was no or not enough polyphosphate to reestablish the pH gradient or serve as an energy source for the storage of carbon substrates. The pH gradient not being reestablished may also explain why less than 20% of the TCOD$_{inf}$ was utilized in the anaerobic zone during experiment #2.

Oxidized nitrogen in the anaerobic zone will suppress BPR (Barnard, 1975). The amount of NO$_3$ in the anaerobic zone was greater than 3.0 mg/L for experiments #1 and #2. This concentration indicates the anaerobic zone had oxidized conditions which would prevent BPR. From NO$_3$ data which was collected, it appears that the RAS recycle and the industrial wastewater were responsible for the loading of NO$_3$ to the anaerobic zone. The data from the industry, Table A-3, confirms the industrial waste was a source of NO$_x$ for the anaerobic zone.

The NO$_x$ from the inputs to the anaerobic zone could be denitrified provided there was sufficient anaerobic zone HRT and influent substrate. Assuming all or most of the TCOD$_{inf}$ in experiments #1 and #2 was readily available organics, there should have been more than enough for complete denitrification and BPR. Estimation of the COD required for denitrification of NO$_x$ in the influent and RAS for experiments #1 and #2 indicated over 80% of the TCOD$_{inf}$ would remain for BPR. The SCOD$_{ana}$ data from experiment #2 demonstrates that enough COD was removed for complete denitrification, but NO$_3$ was measured in the anaerobic zone.
It could also be speculated that some of the inorganics or organics in the industrial wastewater may have been inhibiting BPR. The anaerobic zone may also have been overloaded with substrate. bleed through of significant amounts of available organics or soluble COD would permit the growth of non bio-P bacteria (Randall et al., 1992). While this occurred in experiments #1 and #2, the reason the COD was not utilized is uncertain. There may be reasons, other than has been stated, that the mechanisms of BPR were prevented. Regardless, something about or in the industrial wastewater was responsible and the laboratory system could not handle it.

Municipal Wastewater with COD Supplement

It was determined that the laboratory system was capable of BPR. The characteristics of BPR were exhibited in experiments #5 and #6 when the influent consisted of municipal wastewater and a 500 mg/L COD supplement. The COD supplement was sodium acetate in experiment #5 and the industrial wastewater in experiment #6. Even though the duration and data were limited, the results for these experiments establish that BPR could be achieved by the laboratory system. Phosphorus removals were greater than that required for cellular growth, the %TP of the aerobic MLVSS was 5% to 7%, and the concentrations of PO₄ in the anaerobic and aerobic zones were more typical of BPR.

In experiments #5 and #6, there was 140 and 160 mg/day of TP, respectively, removed in excess of the biomass growth requirement. The system removed just over five times what was needed in experiment #5 and almost four and one-half times what was needed in experiment #6. Additionally, anaerobic PO₄ release and aerobic PO₄ uptake occurred in these experiments. In experiment #5, the anaerobic PO₄ release was just 5 mg/L greater than the influent PO₄, but the aerobic uptake of PO₄ was 10 mg/L less than the influent PO₄. The amount of release and uptake increased in experiment #6. The anaerobic PO₄ was nearly twice the influent PO₄ and the aerobic PO₄ was 15 mg/L less than the influent PO₄.
The %P of the aerobic MLVSS in experiments #5 and #6 provided additional verification that the laboratory system demonstrated BPR. The average %P was 5.5% for experiment #5 and 7.3% for experiment #6. The concentrations of TP removed were similar to those in experiment #2, but unlike experiment #2, the effluent PO₄ concentrations in experiment #5 and especially #6 demonstrate there was aerobic uptake taking place. It is believed that if the influent had not been changed for experiment #7 these results would have improved. The bio-P bacteria would have become more dominant, the poly-P reserves would have built up, the P removals would have increased and the BPR characteristics would have more distinct.

The pH of the anaerobic zone during these experiments was close to neutrality with experiment #6 having the lowest average of 6.5. There were low concentrations of NO₃ present in the anaerobic zones. The NO₃ averages of the second anaerobic stage were 0 and 0.7 mg/L for experiments #5 and #6, respectively. The anaerobic pH and low NO₃ which the laboratory system exhibited are known to provide good conditions for BPR. The parameters which were used to measure BPR indicate that the system was just beginning to realize BPR under these conditions during experiments #5 and #6.

Municipal Wastewater and 50/50 Municipal/Industrial Mixture

With either the municipal wastewater or the 50% municipal and 50% industrial wastewater mixture as influent, the laboratory system was unable to establish BPR. In experiment #4, the influent was municipal wastewater like for experiments #5 and #6, but it did not contain the CCD (acetate/acetic acid or industrial wastewater) supplement. The influent in experiment #3 was a mixture of 50% municipal wastewater and 50% industrial wastewater, by volume.

The mass of TP removed exceeded the biomass P requirements in both of these experiments. There was an average of 50 and 40 mg/day of excess P removal in experiments #3 and #4, respectively. These amounts were not significant as the average influent and effluent PO₄ and TP concentrations reflected only a small amount of removal with high effluent concentrations of
P remaining. The average %P of the biomass was a typical 2.5% for experiment #3. This was essentially the same as what was obtained in experiment #2. When the influent was changed for experiment #4, the average %P doubled to 5.0% which is more indicative of BPR.

Review of PO$_4^-$ data on the anaerobic and aerobic zones supports that BPR was not achieved. In experiment #3, there was some PO$_4^-$ release, but no aerobic PO$_4^-$ uptake. With the municipal wastewater, there was an even larger PO$_4^-$ release. Like experiment #3, there was minimal aerobic PO$_4^-$ uptake in experiment #4. It is likely there would have been more aerobic PO$_4^-$ uptake had the TCOD$_{INF}$ been higher in experiment #4. The laboratory system appears to have been COD limited in this experiment. As noted by Randall et al. (1992), it could have been insufficient total organics or the organics were not present as SCFAs. The average influent TCOD to TP ratios of 30 and 13 for these experiments was low to marginal for large amounts of P removal.

The laboratory system was experiencing some nitrification in these two experiments. The NO$_3^-$ recycled to the anaerobic zone reduced the COD which was available for bio-P bacteria. Using average NO$_3^-$ concentrations of the RAS and influent, it was calculated that the available COD was reduced 37 and 67 mg/L for experiments #3 and #4, respectively. This was not significant for experiment #3, but it was to the low TCOD$_{INF}$ of experiment #4. Nearly 20% of the TCOD$_{INF}$ went to denitrification.

The pH in the anaerobic zone during experiment #3 was also unfavorable. Its average pH was just less than 5.6. The average pH for experiment #4 was 6.8. The industrial wastewater and its pH were a big part of why BPR was not accomplished in experiment #3. In experiment #4, it seems the low TCOD$_{INF}$ and the utilization of COD for nitrification in the anaerobic zone were factors in BPR not being attained.

In summary, the influent TCOD loading to the laboratory system and the pH in the anaerobic zone were identified as factors which influenced BPR with the industrial wastewater. The results of the investigation which best support
this are the %P of the aerobic biomass and the excess TP removed per mass of influent TCOD loading.

A graph of the average %P of the MLVSS$_{AER}$ versus the average anaerobic pH for each experiment is provided in Figure 37. It shows that the highest %P was obtained between pH 6.3 and 7.2. The industrial wastewater was responsible for the anaerobic zone pH being below 5.5 which was where the resulting %P was low.

Using averages from each experiment, a comparison of the %P of the MLVSS$_{AER}$ and mass of excess TP removed per mass of influent TCOD loading with the influent TCOD loading was made. This plot, Figure 38, indicates that the highest amounts of excess TP removal per influent TCOD loading and the greatest %P occurred when the influent TCOD loading was between 5,000 and 10,000 mg/day.

**ANAEROBIC SUBSTRATE REMOVAL**

As the data and figures confirm, there was some anaerobic removal of substrate or soluble COD in all the experiments. However, the COD loading and type of influent seemed to affect the COD removal in the anaerobic zone. It was speculated that when the industrial wastewater was being applied, the anaerobic zone biomass was overloaded with substrate, or, a portion of the influent COD was not available for anaerobic BPR, and/or the influent contained an inhibitory substance.

It was noted that the highest average anaerobic COD removals, 3,274 and 3,269 mg COD/day, occurred during experiments #5 and #6 when BPR was occurring. It was also during these experiments that the anaerobic COD removals accounted for almost half the system COD removal. Only in experiment #4 was the average anaerobic removal greater with roughly 70% of the COD removed in the anaerobic zone. While the COD loadings for experiments #2 and #3 were at least three times the loadings in experiments #5 and #6, the anaerobic uptake rates and removals were less. The anaerobic zone was capable of higher rates and removals, but something about or in the industrial wastewater seemed to prevent it.
Figure 37. Comparison of the average %P of the MLVSS\textsubscript{AER} biomass with the average pH of the anaerobic zone for each experiment of the investigation.
Figure 38. Comparison of the average %P of the MLVSS_{AER} and average mass of excess TP removed per influent COD loading with the average influent COD loading for each experiment of the investigation.
Review of the results of COD/oxygen-utilization mass balance equation which was used to estimate the stabilization of organic substrate indicates there was a considerable amount of COD anaerobically stabilized in experiments #1, #2 and #3. As done by Randall et al. (1992b) with the data from Lan et al., the measured TOR was compared to the predicted TOR. Using the averages in Table 6, the following were obtained for these experiments:

**Experiment #1**

\[
\text{TOR}_{\text{predicted}} = 22,205 - 6,028 = 16,177 \text{ mg/day}
\]
\[
\text{TOR}_{\text{measured}} = 7,578 \text{ mg/day}
\]
\[
\text{AnS} = 8,599 \text{ mg/day (TOR}_{\text{predicted}} - \text{TOR}_{\text{measured}}\)
\]
\[
\text{AnS (Table 7)} = 8,176 \text{ mg/day}
\]

**Experiment #2**

\[
\text{TOR}_{\text{predicted}} = 23,051 - 6,817 = 16,234 \text{ mg/day}
\]
\[
\text{TOR}_{\text{measured}} = 12,261 \text{ mg/day}
\]
\[
\text{AnS} = 3,973 \text{ mg/day (TOR}_{\text{predicted}} - \text{TOR}_{\text{measured}}\)
\]
\[
\text{AnS (Table 7)} = 3585 \text{ mg/day}
\]

**Experiment #3**

\[
\text{TOR}_{\text{predicted}} = 12,935 - 3,535 = 9,400 \text{ mg/day}
\]
\[
\text{TOR}_{\text{measured}} = 6,258 \text{ mg/day}
\]
\[
\text{AnS} = 3,142 \text{ mg/day (TOR}_{\text{predicted}} - \text{TOR}_{\text{measured}}\)
\]
\[
\text{AnS (Table 7)} = 2,170 \text{ mg/day}
\]

**Experiment #4**

\[
\text{TOR}_{\text{predicted}} = 3,572 + 552 - 2,841 = 1,283 \text{ mg/day}
\]
\[
\text{TOR}_{\text{measured}} = 3,008 \text{ mg/day}
\]
\[
\text{AnS} = -1,725 \text{ mg/day (TOR}_{\text{predicted}} - \text{TOR}_{\text{measured}}\)
\]
\[
\text{AnS (Table 7)} = -846 \text{ mg/day}
\]

**Experiment #5**

\[
\text{TOR}_{\text{predicted}} = 6,629 + 225 - 2,130 = 4,724 \text{ mg/day}
\]
\[
\text{TOR}_{\text{measured}} = 4,528 \text{ mg/day}
\]
\[
\text{AnS} = 196 \text{ mg/day (TOR}_{\text{predicted}} - \text{TOR}_{\text{measured}}\)
\]
\[
\text{AnS (Table 7)} = 237 \text{ mg/day}
\]
Experiment #6
\[ \text{TOR}_{\text{predicted}} = 7,409 + 245 - 3,016 = 4,638 \text{ mg/day} \]
\[ \text{TOR}_{\text{measured}} = 5,009 \text{ mg/day} \]
\[ \text{AnS} = -371 \text{ mg/day} (\text{TOR}_{\text{predicted}} - \text{TOR}_{\text{measured}}) \]
\[ \text{AnS (Table 7)} = -687 \text{ mg/day} \]

Experiment #7
\[ \text{TOR}_{\text{predicted}} = 23,329 + 984 - 6,527 = 17,786 \text{ mg/day} \]
\[ \text{TOR}_{\text{measured}} = 12,514 \text{ mg/day} \]
\[ \text{AnS} = 5,272 \text{ mg/day} (\text{TOR}_{\text{predicted}} - \text{TOR}_{\text{measured}}) \]
\[ \text{AnS (Table 7)} = 2,428 \text{ mg/day} \]

Interestingly, these show that anaerobic stabilization was substantial when the industrial wastewater was a major component. In the other experiments, there was very little to zero stabilization. When compared with the anaerobic stabilization calculated in Table 6, the two are fairly close except for experiment #7. However, in experiments #1, #2, #3 and #7, the AnS was greater than the anaerobic COD removals. This is not possible and there is probably an error in the measurements. In experiments #4, #5 and #6, all or most of the COD removal could be attributed to oxygen uptake or cell synthesis.

**FACTORS AFFECTING SLUDGE SETTLEABILITY**

Mixed performance in regards to effluent TSS concentrations was experienced in the different experiments. The effluent quality in terms of TSS_{eff} was not acceptable when the influent was the industrial wastewater. The laboratory system did not appear to function as a selector of favorably settling activated sludge when it was fed this wastewater. The TSS_{eff} decreased and improved results were achieved in experiments when the majority of the influent was municipal wastewater. Operating conditions and not attaining BPR are believed to have contributed to the less than desirable effluent quality when the industrial wastewater was the influent. These conditions caused a large amount of floc to be left in clear zone or supernatant of the secondary clarifier.
Foremost appears to be the laboratory system not realizing BPR in experiments #1 and #2. When the average TSS$_{\text{eff}}$ was less than 10 mg/L in experiments #4, #5 and #6, the laboratory system was indicating signs of BPR. When compared to the TSS$_{\text{eff}}$, the SVI and ZSV data appear contradictory in regards to settleability. Generally, the SVI results did not correlate with TSS$_{\text{eff}}$ results. The SVI was lower when the higher TSS$_{\text{eff}}$ were experienced and vice versa. As noted earlier, SVI is not the best indicator of sludge settling.

The failure to attain reduced or anaerobic conditions in experiments #1, #2 and #3 may have prevented the suppression of filamentous bulking. Tracy et al. (1986) claimed that their batch reactor tests demonstrated that this bulking could be prevented with BPR. When anaerobic conditions were achieved in experiments #4, #5 and #6, the TSS$_{\text{eff}}$ improved. While this may not be the sole reason, it may have played a part.

The amount of SCOD$_{\text{ana}}$ entering the aerobic zone was also seen as significant. The growth of filamentous microorganisms has been shown to be suppressed when most of the available COD is utilized in the anaerobic zone (Wanner et al., 1987) of a BPR system. In their experiments, Wanner et al., (1987) reported the lowest SVIs (135 - 60) when the soluble anaerobic COD was 35 - 45 mg/L. The lowest SCOD$_{\text{ana}}$ concentrations were in experiments #4, #5 and #6. The TSS$_{\text{eff}}$ were less than 10 mg/L, but the SVIs indicative of poor settling sludge.

The filamentous bulking in experiment #7 was disastrous to the laboratory system. The reason for the presence of the filaments was thought to be increased soluble COD loading to the aerobic zone resulting from the large increase in TCOD$_{\text{inf}}$ from experiment #6 to #7.

The variable and high TSS$_{\text{eff}}$ which were experienced in experiments #1, #2 and #3 were a result of the amount of floc left above the solids-liquid interface. Observations made during settling test (SVI and ZSV) substantiated this assertion.
CHAPTER VI. CONCLUSIONS

The main objective of this study was to investigate the utilization of BPR for the treatment of a high acetic acid industrial wastewater. Secondary objectives of the investigation were related to the other potential benefits of BPR. Information on carbonaceous substrate removal, anaerobic substrate utilization, and secondary settling was collected. Influent characteristics and the effects of operating conditions of the laboratory BPR system on system performance were also evaluated. Parameters describing these were compared to BPR performance data.

The high strength, low pH industrial manufacturing wastewater was fed to the laboratory BPR system. Evaluation of the results led to the following conclusions:

1) BPR was not established in the laboratory BPR system when the influent was the low pH, high acetic acid wastewater.
2) BPR was established when the laboratory system was fed municipal wastewater supplemented with sodium acetate.
3) The low pH (4.3 - 4.6) of the industrial wastewater contributed to not achieving BPR because only partial neutralization occurred in the anaerobic zone, resulting in a mixed liquor pH of 5.5 even though the aerobic effluent pH was 8.0.
4) Denitrification was not established when the laboratory system was fed the industrial wastewater. The DO concentrations were 0.20 mg/L or less and the ORP was less than -200 mV in the anaerobic zone, indicating that anaerobic conditions had been achieved, but the average NO$_3$-N concentration was 3.6 mg/L.

The results of testing to determine the affects of anaerobic-aerobic sequencing on the removal of carbonaceous material, anaerobic substrate utilization, and solid-liquid separation were evaluated. These results led to the following conclusions:
1) The laboratory BPR system provided effective removal of carbonaceous material as measured by COD from the industrial wastewater, and excellent pH neutralization occurred in the aerobic zone.

2) The anaerobic zone always removed some soluble COD and reduced the COD loading to the aerobic zone. However, the percent removal was very poor (10 - 20%) when the industrial wastewater was being treated, and much higher (40 - 50%) when BPR was established while treating the supplemented municipal wastewater.

3) For the conditions of these experiments, the influent COD loading did not significantly affect the mass of anaerobic COD removal. Roughly the same mass of COD was anaerobically removed for the different experimental influent loadings. However, this was coincidental considering the very different influent COD and MLSS concentrations.

4) Solid-liquid separation was marginal to poor when the laboratory BPR system was fed the industrial wastewater. Effluent TSS concentrations were as much as four to six times higher than typical of municipal activated sludge systems. Favorable settling characteristics were not achieved because anaerobic conditions were not established and the COD loading to the aerobic zone was higher than acceptable (anaerobic COD removal less than required).

Comparison of influent characteristics and laboratory BPR system operating parameters with phosphorus removal data led to the following conclusions:

1) The high influent TCOD to TP ratio of the industrial wastewater did not produce the expected phosphorus removals. Even with the influent supplements of nitrogen, phosphorus and potassium, the industrial wastewater does not appear to be a potential influent for BPR.

2) There were no operating parameters or influent ratios identified as having a definable relationship with phosphorus removal during these experiments. The ratios would only be useful when the system is accomplishing BPR. For most of the experiments, the laboratory system did not provide BPR.
The latter part of the investigation focused on providing the laboratory BPR system with an influent which would show that the system was capable of BPR. Results of these efforts led to the following conclusions:

1) The laboratory BPR system achieved BPR when operated with an influent of municipal wastewater with a TCOD:TP ratio of 26. At a COD loading rate of 6,800 to 7,800 mg/day the effluent TSP concentration averaged 10 to 16 mg/L.

2) Biological phosphorus removal could be attained by the laboratory BPR system with an influent consisting of up to 20% (by volume) of the industrial wastewater. The corresponding COD loading rate was 7,800 mg/L.
REFERENCES


Tracy, K. D. and Flammino, A. Biochemistry and Energetics of Biological Phosphorus Removal. *Proceedings, IAWPRC International Specialized Conference, Biological Phosphate Removal from Wastewater, Rome, Italy, Biological Phosphate Removal from Wastewater: 15-25 (1987).*


APPENDIX FIGURES
Figure A-1. Total organic carbon concentrations of influent and effluent samples from the laboratory system during the investigation.
Figure A-2. COD:TOC ratios of the influent and effluent of the laboratory system during the investigation.
Figure A-3. Total phosphorus concentration removed by the laboratory system during the investigation.
Figure A-4. The %TP removed by the laboratory system during investigation.
Figure A-5. The COD loading to the mass of anaerobic solids, MLSS_{ANA} of the laboratory system during the investigation.
Figure A-6. The influent COD to anaerobic MLSS$_{Ana}$ ratio of the laboratory system during the investigation.
Table A-1. Averages of Specific Growth Rate (SGR), effluent COD to influent COD ratio and influent COD for each experiment of the investigation.

<table>
<thead>
<tr>
<th>Period</th>
<th>SGR day^{-1}</th>
<th>Eff SCOD/Inf TCOD Ratio</th>
<th>Influent TCOD mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.130</td>
<td>26</td>
<td>2445</td>
</tr>
<tr>
<td>2</td>
<td>0.125</td>
<td>21</td>
<td>2333</td>
</tr>
<tr>
<td>3</td>
<td>0.124</td>
<td>30</td>
<td>1381</td>
</tr>
<tr>
<td>4</td>
<td>0.124</td>
<td>64</td>
<td>388</td>
</tr>
<tr>
<td>5</td>
<td>0.125</td>
<td>29</td>
<td>658</td>
</tr>
<tr>
<td>6</td>
<td>0.127</td>
<td>41</td>
<td>740</td>
</tr>
<tr>
<td>7</td>
<td>0.132</td>
<td>69</td>
<td>2345</td>
</tr>
</tbody>
</table>

SGR = \frac{1}{MCRT}, \text{day}^{-1}
Table A-2. Average Total Organic Carbon (TOC) concentrations and COD to TOC ratios of the influent and effluent for each period of the investigation.

<table>
<thead>
<tr>
<th>Period</th>
<th>TOC concentrations mg TOC/L</th>
<th>COD/TOC Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Influent</td>
<td>Effluent</td>
</tr>
<tr>
<td>1</td>
<td>1034</td>
<td>51</td>
</tr>
<tr>
<td>2</td>
<td>860</td>
<td>38</td>
</tr>
<tr>
<td>3</td>
<td>549</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>119</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>249</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>287</td>
<td>33</td>
</tr>
<tr>
<td>7</td>
<td>833</td>
<td>68</td>
</tr>
</tbody>
</table>

Note: Unfiltered influent and filtered effluent samples were analyzed for TOC.
# This reactor or zone was not in the BPR system.
Table A-3. Data characterizing the nitrogen compounds and total phosphorus in the cellulose acetate manufacturing waste prior to the addition of supplements.

<table>
<thead>
<tr>
<th>Influent Nitrogen Compounds</th>
<th>Ammonia NH$_3$-N mg/L</th>
<th>Nitrite NO$_2$-N mg/L</th>
<th>Nitrate NO$_3$-N mg/L</th>
<th>Total Kjeldahl Nitrogen TKN, mg/L</th>
<th>Total Nitrogen TN, mg/L</th>
<th>Total Phosphorus TP, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVG</td>
<td>2.18</td>
<td>2.01</td>
<td>2.53</td>
<td>14.0</td>
<td>18.0</td>
<td>12.6</td>
</tr>
<tr>
<td>MIN</td>
<td>0.01</td>
<td>0.64</td>
<td>0.22</td>
<td>10.0</td>
<td>10.0</td>
<td>0.83</td>
</tr>
<tr>
<td>MAX</td>
<td>8.63</td>
<td>4.08</td>
<td>10.5</td>
<td>29.0</td>
<td>28.8</td>
<td>21.8</td>
</tr>
</tbody>
</table>

Note: The test results of forty-one (41) samples were utilized. These samples were collected and analyzed by the owner of the industry.

The results cover the period of January 1, 1990 to May 23, 1990.
VITA

Thomas Pully was born on July 15, 1962 in Richmond, Virginia. He graduated from Virginia Military Institute in Lexington, Virginia in May 1985 with a Bachelor of Science degree in Civil Engineering. After working several years for an engineering consulting firm, he returned to graduate school at Virginia Polytechnic Institute & State University to earn a Master of Science degree in Environmental Engineering with an emphasis on water and wastewater treatment. Since November 1990, he has been working as a Process Engineer at the Henrico County Wastewater Treatment Facility.