Evaluation Of Prefermentation As A Unit Process Upon Biological Nutrient Removal Including Biokinetic And Wastewater Parameters

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EVALUATION OF PREFERMENTATION AS A UNIT PROCESS UPON BIOLOGICAL NUTRIENT REMOVAL INCLUDING BIOKINETIC AND WASTEWATER PARAMETERS

by

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ABSTRACT

The objective of this dissertation was to provide a controlled comparison of identical continuous flow BNR processes both with and without prefermentation in order to provide a stronger, more quantitative, technical basis for design engineers to evaluate the potential benefits of prefermentation to EBPR in treating domestic wastewater. In addition, the even less understood effect of prefermentation on denitrification kinetics and anoxic phosphorus (P) uptake was studied and quantified. Other aspects of BNR performance, which might change due to use of prefermentation, will also be addressed, including anaerobic stabilization. Potential benefits to BNR processes derived from prefermentation are compared and contrasted with the more well-known benefits of primary clarification. Finally, some biokinetic parameters necessary to successfully model both the activated sludge systems and the prefermenter were determined and compared for the prefermented versus the non-prefermented system.

Important findings developed during the course of this dissertation regarding the impact of prefermentation upon the performance of activated sludge treatment systems are summarized below:

- For a septic COD-limited (TCOD:TP < 40:1) wastewater, prefermentation was found to enhance EPBR by 27.7% at a statistical significance level of $\alpha=0.05$ (95% confidence level).

- For septic P-limited (TCOD:TP > 40:1) wastewaters, prefermentation was not found to improve EBPR at a statistical significance level of $\alpha=0.05$ (95% confidence level).
• The increased anaerobic P release and aerobic P uptakes due to prefermentation correlated with greater PHA formation and glycogen consumption during anaerobiosis of prefermented influent.

• Improvements in biological P removal of septic, non-P limited wastewater occurred even when all additional VFA production exceeded VFA requirements using typical design criteria (e.g. 6 g VFA per 1 g P removal).

• Prefermentation increased RBCOD content by an average of 28.8% and VFA content by an average of 18.8%, even for a septic domestic wastewater.

• Prefermentation increased specific anoxic denitrification rates for both COD-limited (14.6%) and P-limited (5.4%) influent wastewaters. This increase was statistically significant at $\alpha=0.05$ for COD-limited wastewater, but not for P-limited wastewater.
ACKNOWLEDGMENTS

I deeply appreciate the past 11 years of support and encouragement from Dr. Andrew A. Randall. My academic and professional careers have been profoundly influenced by the education, assistance, and advice that Dr. Randall has graciously and willingly provided. I am particularly grateful for the encouragement to complete this dissertation.

I would like to thank my committee, John D. Dietz, Debra Reinhart, Cherie Geiger, and Linda Malone for their support, review, and participation in this effort.

This research would not have been possible without the funding provided by the National Science Foundation (Award #9616144), for which I am eternally grateful. I would also like to thank the Orange County Utilities Eastern Water Reclamation Facility personnel and the Plant Manager, Tim Madhanagopal, P.E., DEE, QEP for allowing the pilot plants to be operated at their facility.

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<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>APT</td>
<td>Activated Primary Tank</td>
</tr>
<tr>
<td>ARCY</td>
<td>Anaerobic Recycle</td>
</tr>
<tr>
<td>BNR</td>
<td>Biological Nutrient Removal</td>
</tr>
<tr>
<td>C</td>
<td>Celsius</td>
</tr>
<tr>
<td>CASS</td>
<td>Control Activated Sludge System</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical Oxygen Demand</td>
</tr>
<tr>
<td>EBPR</td>
<td>Enhanced Biological Phosphorus Removal</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic Retention Time</td>
</tr>
<tr>
<td>IMUC</td>
<td>Intermittently Mixed Upflow Clarifier</td>
</tr>
<tr>
<td>MLSS</td>
<td>Mixed Liquor Suspended Solids</td>
</tr>
<tr>
<td>MLVSS</td>
<td>Mixed Liquor Volatile Suspended Solids</td>
</tr>
<tr>
<td>NARCY</td>
<td>Nitrate Recycle</td>
</tr>
<tr>
<td>NSF</td>
<td>National Science Foundation</td>
</tr>
<tr>
<td>P</td>
<td>Phosphorus</td>
</tr>
<tr>
<td>PASS</td>
<td>Prefermented Activated Sludge System</td>
</tr>
<tr>
<td>PHA</td>
<td>Polyhydroxyalkanoate</td>
</tr>
<tr>
<td>RAS</td>
<td>Return Activated Sludge</td>
</tr>
<tr>
<td>SOP</td>
<td>Soluble Ortho Phosphorus</td>
</tr>
<tr>
<td>SRT</td>
<td>Solids Retention Time</td>
</tr>
<tr>
<td>SVI</td>
<td>Sludge Volume Index</td>
</tr>
<tr>
<td>TCOD</td>
<td>Total Chemical Oxygen Demand</td>
</tr>
<tr>
<td>TKN</td>
<td>Total Kjeldahl Nitrogen</td>
</tr>
<tr>
<td>TP</td>
<td>Total Phosphorus</td>
</tr>
<tr>
<td>TSS</td>
<td>Total Suspended Solids</td>
</tr>
<tr>
<td>UCT</td>
<td>University of Cape Town</td>
</tr>
<tr>
<td>VFA</td>
<td>Volatile Fatty Acids</td>
</tr>
<tr>
<td>VSS</td>
<td>Volatile Suspended Solids</td>
</tr>
<tr>
<td>WEF</td>
<td>Water Environment Federation</td>
</tr>
<tr>
<td>ZSV</td>
<td>Zone Settling Velocity</td>
</tr>
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</table>
CHAPTER 1 INTRODUCTION

Prefermentation

Biological Nutrient Removal (BNR) systems remove nitrogen and/or phosphorus from influent wastewater in addition to biodegradable materials. The phosphorus and nitrogen must be removed from the effluent of wastewater treatment plants because the presence of these nutrients in the effluent accelerates the growth of algae and other photosynthetic aquatic life in receiving water bodies. This can eventually result in excessive loss of dissolved oxygen (DO) in the receiving water body, causing undesirable changes in the aquatic environment. Nitrogen or phosphorus is typically the limiting nutrient in aquatic ecosystems. Thus, minimizing effluent concentrations of nitrogen and phosphorus into the aquatic environment is essential in maintaining good environmental water quality.

BNR processes developed in the 1960s, initially with nitrogen and phosphorus removal practiced separately. One of the earliest BNR processes employed a series of separate suspended growth systems to accomplish removal of organic matter and nitrogen sequentially (i.e. the first reactor removed organic matter, the second reactor was designed nitrification, and the third reactor focused on denitrification). This sequential method did not become popular because of high capital and operational costs. Another approach was to use a single sludge reactor for nitrogen and carbon removal, with separate aerobic and anoxic zones within the single sludge reactor in order to achieve both nitrification and denitrification. This concept of subdividing a single sludge reactor into separate treatment zones was expanded in the 1970s when it was discovered that enhanced biological phosphorus removal (EPBR) can occur if the single sludge
reactor has an initial anaerobic zone followed by an aerobic zone. Prior to the 1970s, phosphorus removal was achieved by chemical precipitation through the addition of lime, alum, or iron salts.

The simultaneous biological removal of carbon, nitrogen, and phosphorus in a single BNR system can be achieved through a combination of anaerobic, anoxic, and aerobic treatment zones located within single sludge systems. Examples of contemporary BNR designs include the five-stage Bardenpho system, the University of Cape Town (UCT) process, and the Modified University of Cape Town (MUCT) process. If the BNR system is properly designed and operated, it can be more stable and generate a better quality effluent than the conventional aerobic plug-flow activated sludge processes (Randall et al, 1992). BNR process can be further enhanced through the use of influent prefermentation. Prefermentation alters the characteristics of the raw influent to enable superior biological removal of both phosphorus and nitrogen (Van Muench, et al, 1996).

**Problem Statement**

EBPR requires the presence of VFAs in the anaerobic zone of any BNR wastewater treatment system. Unless the sewage is strong and septic (i.e. the influent already has a high VFA concentration) VFAs must be produced. This VFA production is accomplished either within the anaerobic zone of the BNR system or it is done prior to the BNR system in a separate anaerobic process called prefermentation in which hydrolysis and acidogenic fermentation takes place, producing VFAs in a separate step. Prefermenters as a unit process were developed by Dr. James Barnard in South Africa along with researchers at the University of Cape Town in the mid 1970s when BNR systems were first developed at full scale. The simplest prefermenters are
primary clarifiers with a high sludge blanket, referred to as “static” prefermenters. With the addition of a recycle to elute the VFAs in the sludge blanket, the term activated primary tank (APT) is used (Van Muench and Koch, 1997). Either of these prefermenters are commonly referred to as “on-line” prefermenters since the entire wastewater stream is treated. Sidestream prefermenters receive underflow from normally operated primary clarifiers and consist of completely or partially mixed reactors in which acidogenic fermentation of the primary solids takes place. In some cases the prefermented solids and supernatant are fed to the anaerobic zone of the BNR plant. In other cases sidestream prefermenters may have dedicated thickeners and only the VFA rich supernatant may go to the anaerobic zone. Examples of sidestream prefermenters include the complete mix fermenter, the single stage fermenter/thickener, and the 2-stage complete mix/thickener fermenter (Barnard, 1994).

Design practice in Canada, South Africa, and Australia is such that prefermenters are frequently used for BNR processes in a significant number of plants, even in warm climates (Van Muench et al., 1996; VanMunch and Koch, 1997). In the United States, prefermenters have rarely been considered outside the Northwest (where the Canadian influence has been significant) even when they might arguably have been advantageous. Only in recent times have prefermenters actually been constructed in the United States, with two prefermenters currently operating in Florida, and another prefermenter being operated in North Carolina, among other locations. Because of the very few quantitative comparisons of identical systems with and without prefermenters, design engineers often disagree on the necessity of a prefermenter and make decisions based on their prior experience. For example, a 500 ML/day BNR plant located in Calgary, Canada, involving both U.S. and Canadian BNR design experts, is a good example of a large full scale plant where there is still considerable disagreement over whether or not
prefermenters, which were built at significant capital cost, were necessary to meet effluent requirements and were ultimately cost effective.

**Statement of Objectives**

The objective of this research was to provide a controlled comparison of identical continuous flow BNR processes both with and without prefermentation in order to provide a stronger, more quantitative, technical basis for design engineers to determine the potential benefits to EBPR. In addition the even less understood effect of prefermentation on denitrification kinetics and anoxic P uptake was to be studied and quantified. Other aspects of BNR performance (e.g. settleability, etc…) which might change due to use of prefermentation were also addressed. In order for a complete study upon the potential effects of prefermentation on BNR performance to be conducted, influent characteristics of the wastewater must be varied (e.g. septic vs. non-septic and COD vs. P/N limited; Randall et al., 1992; Water Environment Federation, 1998). For example one of the few controlled comparisons isolating prefermentation as a variable in the literature is Danesh and Oleszkiewicz (1997), who studied the effect of prefermentation on lab scale sequencing batch reactor performance for EBPR. Effects on biological nitrogen removal were not addressed and only one non-septic (4 mg/L volatile fatty acids or VFAs) wastewater with a TCOD:TP of 67.8 was studied. Thus this research was meant to generate information for both EBPR and biological nitrogen removal for the four basic wastewater categories with respect to EBPR (e.g. septic vs. non-septic and COD vs. P/N limited; Randall et al., 1992; Water Environment Federation, 1998). The information in this study was intended to provide a more rational and objective basis from which design engineers might
determine if prefermentation is: a) essential, b) advisable, c) unnecessary, or d) inadvisable for a
given site treating domestic wastewater.

In order to facilitate the comparisons between identical continuous flow BNR processes
both with and without prefermentation, this study was split into two distinct stages. In the first
stage of the study, two parallel bench scale activated sludge wastewater treatment systems, with
a total reactor volume of 15 liters, were constructed, along with a static prefermenter (also called
an intermittently mixed upflow clarifier, or IMUC), as shown in Figures 3.1 and 3.2 in Chapter
3. The systems were located at a local full-scale plant (East Orange County Water Reclamation
Facility, a 5-stage Bardenpho plant removing both nitrogen and phosphorus). The purpose of the
bench scale system was to evaluate the effect of prefermentation upon the removal of both
phosphorus and nitrogen from influent domestic wastewater and to generate data necessary for
the design of a larger pilot scale system. The flow configuration selected for the bench-scale
activated sludge systems was the University of Cape Town (UCT) configuration for biological
nutrient removal. For more information concerning the bench scale WWTP system, including
design and operation and maintenance procedures, please see Chapter 3 Experimental Methods
and Procedures.

The results obtained from the bench scale BNR systems yielded information that was
used to construct the second phase of the study, which was a larger pilot scale BNR process. The
pilot scale system initially consisted of three, parallel 3-stage modified University of Capetown
(MUCT) systems. The total reactor volume of each of the trains was slightly less than 100 liters.
Two of the systems received prefermented wastewater, and one served as a control system. The
two separate prefermented trains allowed the evaluation of a step-feed modification in which half
of the prefermented influent was routed to the anoxic zone. For more information concerning the
operation and maintenance of the pilot scale WWTP system, please see Chapter 3 Experimental Methods and Procedures of this document. Later in the study, the pilot scale system was reduced to two trains with a reduced number of reactors, as shown in Figure 3.3, in order to reduce the analytical load and improve operational reliability.

The pilot scale wastewater treatment systems were designed to meet a series of three separate research objectives. Each of these research objectives help in the determination of the effect of primary influent prefermentation upon all aspects of the performance of BNR systems. The three research objectives of the pilot study are summarized below:

1) An evaluation of the impact of differing influent wastewater characteristics upon prefermentation and the BNR activated sludge systems.

2) A comparison of the impacts on the BNR, activated sludge oxygen requirements, and WAS production with a primary clarifier vs. a prefermenter.

3) The effect of prefermentation and other variables on the biokinetic parameters necessary for the modeling of the activated sludge system.
References


CHAPTER 2 LITERATURE REVIEW

Prefermentation

The anaerobic sequestration of short-chain volatile fatty acids (SCVFAs) is of critical importance to the phenomena of enhanced biological phosphorus removal (EBPR). These SCVFAs that are necessary to EBPR are produced through the fermentation of organic substrates and particulate matter found within domestic wastewater. Indeed, the primary purpose of the initial anaerobic stage typical in BNR wastewater treatment plants is to create an environment in which fermentative bacteria, which are strictly anaerobic, can convert complex organic molecules and particulate matter to the SCVFAs that are crucial to successful EBPR. The presence of oxygen, or even nitrate, will halt the fermentation process by allowing other types of faster-growing bacteria to out-compete fermentative bacteria for valuable substrates.

Fermentation can occur not only in anaerobic zones of BNR plants, but also in sewage collection systems, particularly for sewage collection systems with high temperatures and long retention times. The most common fermentation products found in domestic wastewater are acetic and propionic acids. Acetic acid most commonly comprises between 70 to 85% of the total SCVFAs present within domestic wastewater, with propionic acid typically consisting of between 10 to 20% of the total SCVFAs. In some domestic wastewaters, this ratio can drop to 50% acetic acid and 40% propionic acid, with greater molecular weight SCVFAs such as butyric, valeric, or isovaleric acids making up the remainder (Speece, 1996). A septic sewage, typically found in collection systems with high temperatures and long retention times, may already have between 30 to 50 mg/L of SCVFAs before the wastewater even enters a wastewater treatment
plant. In contrast, a non-septic sewage, typical in colder climates, may have no measurable SCVFAs (Barnard, et al, 1992).

An alternative to relying upon an anaerobic zone to produce SCVFAs within a BNR wastewater treatment plant is to instead construct an independent unit process, called a prefermenter. The function of the prefermenter is solely to promote the generation of fermentation products, namely SCVFAs. The SCVFA laden prefermenter supernatant is then sent to the anaerobic zone of a BNR plant where the SCFVAs are sequestered by polyphosphate accumulating organisms (PAOs). The primary purpose of this study is to explore potential benefits of the prefermentation of influent wastewater to the operation of BNR treatment systems.

**Fermentation**

The fermentation of complex organic substrates and particulate matter found in domestic wastewater to SCVFAs is merely a step in a larger biological process called anaerobic digestion. The fermentation products required for successful EBPR are actually the products of an incomplete anaerobic digestion. Anaerobic digestion can be divided into three distinct phases: hydrolysis, fermentation, and methanogenesis. For successful EBPR, it is desired to maximize the fermentation products available to the activated sludge while minimizing the generation of methane. The three stages of anaerobic digestion are described in further detail below.

Hydrolysis, the first step of anaerobic digestion, is the breaking down of complex organic substrates and particulate matter into smaller molecules through the incorporation of a water molecule. Simple sugars, amino acids, and long-chain fatty acids are some examples of
hydrolysis products. Enzymes secreted by bacteria outside the boundaries of the cell catalyze hydrolysis reactions. Hydrolysis reactions are necessary because the cell cannot directly utilize the complex organic molecules and particulate matter present within wastewater as sources of carbon and energy (Madigan et al, 1997).

The second phase of anaerobic digestion is acidogenesis, also called the fermentation phase. During acidogenesis, the simple sugars, amino acids, and long-chain fatty acids produced during hydrolysis are utilized as both carbon and energy sources by fermentative bacteria. Depending upon the initial substrate, various end products are possible, including (Madigan et al, 1997):

1. Acetic acid
2. Propionic acid
3. Butyric acid
4. Formic acid
5. Lactic acid
6. Hydrogen

The third phase of anaerobic digestion is methanogenesis, or the production of methane. The fermentation products produced during the acidogenic phase of anaerobic digestion are in turn utilized as carbon and energy sources by methanogenic bacteria, producing methane gas. In fact, acetate is a prime precursor of methanogenesis in anaerobic digesters. As much as 70% of the total volume of methane produced in an anaerobic digester comes from acetate (Speece, 1996). Acetoclastic (acetate-utilizing) methanogens produce methane through the decarboxylation of acetate and the carbon dioxide with hydrogen gas (Madigan et al, 1997).
It should be clear that the production of methane in any prefermentation system is detrimental to successful EBPR, and must be avoided. The production of methane from fermentation products, particularly acetic acid, by methanogenic bacteria results in less acetate available for EBPR. Fortunately, a number of methods that can potentially limit the growth of methanogenic bacteria exist. One method of controlling methanogenesis is to operate prefermenters at an SRT lower than that commonly found in anaerobic digesters. This method works because methanogenic bacteria grow much more slowly than fermentative bacteria. A second method that can control the growth of methanogenic bacteria in prefermenters is through periodic aeration. Methanogenic bacteria are strict anaerobes, implying that the presence of oxygen can kill methanogenic bacteria (Madigan, et al, 1997).

**Prefermenter Configurations**

Four predominant prefermenter types can be found in the literature:

1. Activated Primary Tank (APT)
2. Complete Mix Fermenter
3. Single Stage Fermenter/Thickener
4. 2-Stage Complete Mix/Thickener Fermenter

An ideal prefermenter is one that consistently produces SCVFAs, is inexpensive, and is simple to operate. The degree to which the various prefermenter configurations meet these criteria, as well as their advantages and disadvantages, are discussed below.
The activated primary tank (APT) is the simplest type of prefermenter. Primary sludge from the primary clarifier is recycled to the inlet of the clarifier, either directly or through an elutriation tank, such that a sludge blanket of fermenting bacteria is formed on the clarifier floor. As the sludge is recycled to the inlet of the primary clarifier, the fermenting bacteria contact the incoming particulate matter from the influent, thus initiating the fermentation process. The recycling of the sludge also allows for the elutriation of the SCVFAs that were produced within the sludge blanket into the primary clarifier effluent. The major advantage of this prefermenter configuration is its simplicity and the fact that existing primary clarifiers can be easily reconfigured into an APT (Barnard, 1994).

Despite the fact that APTs have been successfully utilized in BNR operations, several disadvantages of this type of prefermentation configuration exist. First, successful operation of an APT results in high solids loading to the primary clarifier, which in turn typically results in additional solids loading to the BNR process. Secondly, SRT is extremely difficult to control in an APT. The best that can be done is to maintain a constant sludge blanket height through the wasting of primary solids. If the SRT gets too high, methane and sulfide formation can occur, especially in warmer climates. This methane production in turn leads to reduced SCVFA yields. Third, the fact that the SCVFAs are not discharged directly to the BNR process, but instead to the primary clarifier effluent, can lead to the volatilization or the aerobic metabolization of the SCVFAs during transport between the APT and the BNR process. A fourth disadvantage of APTs is that the continual recycling of primary solids leads to a build-up of fibrous material and plastics, which could lead to maintenance problems with the recycle pumps (Rabinowitz, 1994).
The major parameter used in the design of APTs is the sludge age, or SRT. The SRT is typically between 2 and 4 days for successful operation. The wastage rate is selected in order to maintain a certain sludge blanket height above the clarifier floor, typically 1.5 to 2 meters. The primary sludge recirculation rates are commonly 5 to 10% of the average dry weather flow to the plant (Rabinowitz, 1994).

**Complete Mix Fermenter**

The complete mix fermenter, similar in concept to the APT, was initially proposed by Rabinowitz et al (1987). Sludge from the primary clarifier is sent to a separate completely mixed tank where fermentation occurs. Tank overflow is returned by gravity to the primary clarifier, where mixing with the incoming wastewater occurs. The primary effluent is then sent to the BNR process. The complete mix fermenter HRT is determined by the tank volume and the SRT is determined by the sludge wastage rate. Surplus primary sludge is wasted from the fermenter. The primary advantage of a complete mix fermenter over an APT is that the completely mixed tank allows for greater control over the SRT, which in turn allows greater control over the amount of methane generation that occurs (Rabinowitz, 1994).

The disadvantages of a complete mix fermenter configuration are similar to those experienced by the APT. To summarize, those disadvantages included a higher solids loading to the BNR process and the potential of stripping and/or aerobic metabolization in the passage of the SCVFAs through the primary clarifier. In addition, the “roping” of fibrous material around the mixers in the completely mixed tank is also a problem, along with the other operational problems that are encountered with APTs.
Complete mix fermenters are typically designed to have an HRT of between 6 to 12 hours and an SRT of 4 to 8 days. Solids concentrations within the completely mixed fermenter range from 1 to 2%. SCVFA concentrations between 300 and 500 mg/L have been reported within the bulk liquid of the completely mixed tank, resulting in an increase of 15 to 30 mg/L of SCVFAs entering the BNR process. The complete mix fermenters are designed to handle between 5 and 10% of the average dry weather flow to the plant. The use of slow speed mixers (between 5 and 10 W/m$^3$) is also required to prevent the entrainment of oxygen within the bulk liquid of the fermenter (Rabinowitz, 1994).

**Single Stage Fermenter/Thickener**

The single stage fermenter/thickener is a gravity thickener with increased side water depth to allow for the storage of fermenting primary solids on the thickener floor. Primary sludge is pumped into a center well and allowed to settle and thicken in the unit. Thickened primary sludge is drawn from the bottom of the fermenter, typically at solids concentrations of 5 to 8 percent, and wasted to the solids handling system. Solids are wasted at a controlled rate, in order to maintain a consistent SRT within the fermenter. The major advantage of a single stage fermenter/thickener is that the SCVFA-rich supernatant can be discharged directly into the anaerobic zone of the BNR process, thus allowing for optimal use of this substrate.

Sludge ages typically found in single stage fermenter/thickeners are between 4 and 8 days, depending upon temperature. Side water depths of 3.5 to 5 meters are used in order to ensure that the required sludge inventory can be maintained. The loading rate of primary solids
to the fermenter/thickener is usually on the order of 25 to 40 kg/m²/d, which is significantly lower than the solids loading rate typically used for gravity thickeners (Rabinowitz, 1994).

2-Stage Complete Mix/Thickener Fermenter

The 2-stage complete mix/thickener prefermenter consists of a complete mix tank and a gravity thickener in series. Primary sludge is pumped into the completely mixed tank, and the overflow flows by gravity into the gravity thickener. Thickened sludge from the thickener bottom is recycled to the complete mix tank, with a portion being wasted to maintain the desired SRT. The SCVFA-rich supernatant is conveyed directly to the anaerobic zone of the bioreactor.

This type of fermenter has typical SRTs of 4 to 8 days, and a solids concentration of between 1.5 and 2 percent in the complete mix tank. The thickened sludge recycle rate from the thickener to the complete mix tank is usually around half of the primary sludge pumping rate. The mixing energy is the same as the previously considered complete mix fermenter, between 5 to 10 W/m³ (Rabinowitz, 1994).

Advantages and Disadvantages of Prefermenters

Potential advantages in the use of dedicated prefermenters include (Barnard, 1994):

1. The extra SCVFAs produced from particulate matter in prefermenters can result in improved EBPR performance, to the point that the use of chemicals, such as alum, to polish effluent wastewater phosphorus concentrations to levels less than 1 mg/L may no longer be necessary.
2. The use of a prefermenter can result in a reduction of the anaerobic reactor volume required for successful EBPR – to as little as 5% of the net reactor volume. The reason for this reduction in required anaerobic volume is that the fermentation of complex organic substrates and primary solids to SCVFAs takes much more time than does the sequestration of SCVFAs by PAOs. Therefore, the function of an anaerobic zone in a BNR plant with a prefermenter is to serve merely as a contacting chamber between SCVFAs and PAOs.

3. The high SCVFA production improves sludge settling characteristics.

Potential disadvantages in the use of dedicated prefermenters include (Barnard, 1994):

1. The capital costs incurred through construction of the prefermenter.

2. Unwanted secondary phosphorus release (release of phosphorus without SCVFA uptake) from SCVFA production in excess of BNR requirements can result effluent phosphorus concentrations exceeding statutory limitations.

3. Prefermenters, like most anaerobic treatment technologies, operate in hostile environments that require reliable equipment and robust design features. Potential design problems include variable wastewater solids degradability, solid-liquid separation problems, float formation, grit accumulation with the associated equipment wear, and hazardous gas production and odors (Skalsky et al, 1995).

**Anaerobic Stabilization**

Anaerobic Stabilization (AnS) is defined as the difference between actual and theoretical oxygen use in activated sludge systems with anaerobic zones. Barker and Dold (1995) report
than COD balances on EBPR systems were consistently lower than those for conventional
activated sludge systems, with some EBPR systems showing COD balances of less than 70%
(thereby leaving 30%+ of the disappearance of the influent mass of COD unexplained). The
average % agreement of COD mass balances for EBPR systems treating domestic wastewater
was 78%, with enhanced culture EBPR systems fed with acetate achieving an average COD
balance of 91%. Studies conducted by Wable and Randall (1992 and 1994) and Randall (1994)
indicate that AnS values of 15 – 55% of the theoretical oxygen requirement were measured in
laboratory and pilot-scale studies.

One possible explanation of AnS is the production of reduced gases in the anaerobic
zone, such as hydrogen (H$_2$) or methane (CH$_4$). Clearly if these gases were produced in
significant quantities, this could help explain the phenomena of AnS. However, Wable and
Randall (1994) developed a method to measure H$_2$ and CH$_4$ production in the anaerobic zone of
EBPR systems, and found that less than 1% of measures AnS values were attributed to H$_2$ and
CH$_4$ production. Only in a system with influent feed supplemented with formate was CH$_4$
generation found to be significant.

A second theory explaining the phenomena of AnS is the hypothesis that fermentation in
the anaerobic reactor results in the production of volatile compounds, which are then released
from the system under aerobic conditions. However, it seems unlikely that this hypothetical
volatilization mechanism is responsible for AnS, as these volatile fermentation products are
typically readily biodegradable, and should be removed from the system prior to the aerobic zone
(Barker and Dold, 1995).
A third explanation for AnS is that an external oxidant, other than oxygen, enters the system as a dissolved gas, such as nitrogen (involved in nitrogen-fixation) and carbon dioxide (involved in carbon-fixation) (Wable and Randall, 1994).

A fourth explanation for AnS involves the limitations of the COD test to accurately measure all reduced species. Wable and Randall (1994) show evidence that some reduced species, such as NADH, can effectively resist oxidation by the dichromate oxidant under the COD test conditions. It is also speculated that a fraction of the incoming COD might be oxidizable by the COD test, but not during the standard 2-hr duration of the COD test.
References


CHAPTER 3 EXPERIMENTAL METHODS AND PROCEDURES

Experimental Design and Operation

Bench Scale System Design and Operation

In the first stage of the study, two parallel bench scale activated sludge wastewater treatment systems were constructed, along with a static prefermenter (also called an intermittently mixed upflow clarifier, or IMUC). The purpose of the bench scale system was to evaluate the effect of prefermentation upon the removal of both phosphorus and nitrogen from influent wastewater. This effect of the prefermenter was isolated for comparison by the fact that one of the systems had an IMUC online, while the other system did not. The experimental system with the IMUC online was called the prefermenter activated sludge (PAS) system while the other system was called the control activated sludge (CAS) system, as shown in Figure 3.1 on the next page. A split-feed flow configuration in which half the influent was shunted from the anaerobic zone to the anoxic zone was investigated in phase 2 of the bench scale study, and is shown in Figure 3.2. Both the PAS and CAS systems share a common influent tank that was filled daily with raw domestic wastewater from the East Orange County Water Reclamation Facility (Orange County, FL). The influent to the PAS system was passed first through the prefermenter before entering the PAS system, while the influent to the CAS system was routed directly from the influent tank.
Note: NARCY – Nitrate Recycle from Aerobic Zone, ARCY – Biomass Recycle from Anoxic Zone, RAS – Return Activated Sludge from Clarifier

Figure 3.1 Schematic of the Bench-Scale System During Phase 1

Note: NARCY – Nitrate Recycle from Aerobic Zone, ARCY – Biomass Recycle from Anoxic Zone, RAS – Return Activated Sludge from Clarifier

Figure 3.2 Schematic of the Bench-Scale System During Phase 2 with Step Feed
The flow configuration selected for both activated sludge systems was the University of Cape Town (UCT) configuration for biological nutrient removal, again shown in Figures 3.1 and 3.2. The UCT configuration consisted of an activated sludge reactor divided into three distinct zones (namely the anaerobic, anoxic, and aerobic zones), followed by a secondary clarifier which returned biomass back to the anoxic zone of the activated sludge system via the return activated sludge (RAS) recycle line. In addition to the RAS recycle line, there were also two internal recycle lines. The nitrate recycle line (NARCY) returned the nitrates produced in the aerobic zone during nitrification to the anoxic zone. The anaerobic recycle (ARCY) line returned sludge from the anoxic zone to the anaerobic zone. This configuration of recycle lines allowed for the protection of the anaerobic zone from both oxygen and nitrate, while a low oxygen / high nitrate environment was maintained within the anoxic zone.

The bench scale systems were operated within the East Orange County Water Reclamation Facility (Orange County, Florida) in an enclosed room with access to a tap with raw domestic wastewater. Fresh influent was provided for the systems daily by filling a 180-liter cylindrical polyethylene tank. At the end of a daily cycle, any remaining influent was dumped and the sides of the influent tank were scrubbed prior to the addition of fresh influent. A single submersible pump (Little Giant Pump Co., Oklahoma City, OK) provided the mixing energy necessary to keep the influent tank sufficiently mixed without aerating the influent. Peristaltic pumps manufactured by Cole-Parmer Instrument Company (Vernon Hills, IL) were used to maintain design flow rates for the influent line and all recycle lines. Mixing energy for both the anaerobic and anoxic zones of the activated sludge systems was provided by 50-rpm gear motors (Grainger, Lake Forest, IL). Mixing energy for the aerobic zones, as well as the aeration
capacity, was provided by aquarium aerators (Rena, Annecy, France). Both the secondary clarifiers and the IMUC have surface skimmers and bottom scrapers powered by 1-rpm gear motors (Grainger, Lake Forest, IL). The secondary clarifiers were constructed from 6-inch diameter PVC and an 8-inch diameter funnel glued together. The IMUC was constructed from 5-inch diameter PVC and Plexiglas. The activated sludge reactor was constructed from a Plexiglas manufacturer (Precision Plastics, Orlando, Florida) with notches cut in the sides which allowed for baffles to be inserted. These baffles allowed for the creation of the anaerobic, anoxic, and aerobic zones within the reactor.

Four operators, who sampled and monitored the systems seven days per week, maintained the bench scale plant. After an initial start-up period in which the operators learned how to maintain a constant SRT, the bench scale systems were operated in two distinct phases. Phase 1 consisted of eight months of data in which a constant SRT was maintained. Figure 3.1 shows the flow configuration utilized in Phase 1. In Phase 2, a process change was made in which half the influent flow was routed to the anoxic zones, instead of directly to the anaerobic zones, as shown in Figure 3.2.

**Pilot Scale System Design and Operation**

In the second stage of the study, three parallel pilot scale activated sludge wastewater treatment trains were constructed, along with a prefermentation unit. Three different prefermentation units (a complete mix fermenter, an APT, and an IMUC) were constructed, with the IMUC yielding the best results. The pilot scale systems were nearly an order of magnitude larger than the previous bench scale systems. The purpose of the pilot scale system was to further
evaluate the effect of prefermentation upon the removal of both phosphorus and nitrogen from influent wastewater. Two of the trains received effluent from an online IMUC, while the third system received its influent directly from the influent tank. The control train, which received its influent directly from the influent tank, was called the control activated sludge (CAS) system. Of the two experimental trains that received flow from the online IMUC, one of the trains received all of its flow from the IMUC in the anaerobic zone (the prefermented activated sludge – PAS – system). The third train was operated in a split-feed mode, with half of the IMUC flow going into the first anaerobic zone and the other half of the flow going into the second anoxic zone (the split-feed activated sludge – SAS – system). All three systems share a common influent tank that was filled daily with raw domestic wastewater from the East Orange County Water Reclamation Facility (Orange County, FL). The influent to the PAS and SAS systems were passed first through the prefermenter before entering those systems, while the influent to the CAS system was routed directly from the influent tank.

The flow configuration selected for all three activated sludge systems of the pilot scale WWTP was the Modified University of Cape Town (MUCT) configuration for biological nutrient removal. The MUCT configuration is similar to that of the UCT configuration, with the exception that an extra anoxic zone is included. The first anoxic zone receives the RAS, while the second anoxic zone received the NARCY recycle line. The ARCY recycle line returns biomass from the first anoxic zone to the anaerobic zone.

The purpose of the first anoxic zone is to provide extra protection to the anaerobic zone by further depleting the oxygen and nitrates which might be present in the RAS line. The actual pilot scale system, as constructed, had two anaerobic zones, four anoxic zones, and three aerobic
zone in each train. The purpose of the extra tankage was to further delineate the kinetics of BNR.

The pilot scale systems were operated within the East Orange County Water Reclamation Facility (Orange County, Florida) in an enclosed room with access to a tap with raw domestic wastewater. Fresh influent was provided for the systems daily by filling an 800-liter cylindrical polyethylene tank. At the end of a daily cycle, any remaining influent was dumped and the sides of the influent tank were scrubbed prior to the addition of fresh influent. A single submersible pump (Little Giant Pump Co., Oklahoma City, OK) provided the mixing energy necessary to keep the influent tank sufficiently mixed without aerating the influent. Peristaltic pumps manufactured by Cole-Parmer Instrument Company (Vernon Hills, IL) were used to maintain design flow rates for the influent line and all recycle lines. Mixing energy for both the anaerobic and anoxic zones of the activated sludge systems was provided by 50-rpm gear motors (Grainger, Lake Forest, IL). Mixing energy for the aerobic zones, as well as the aeration capacity, was provided by aquarium aerators (Rena, Annecy, France). Both the secondary clarifiers and the IMUC had surface skimmers and bottom scrapers powered by 1-rpm gear motors (Grainger, Lake Forest, IL). The secondary clarifiers and the primary clarifier were constructed from 50-liter cylindrical tanks with a conical bottom. The IMUC was constructed from a 100-liter barrel-shaped polyethylene storage container. The anaerobic and anoxic zones of the activated sludge reactor were constructed from 8-inch square polyethylene reactors, with each reactor having a liquid volume of approximately 7 liters. The aerobic zone activated sludge reactors were constructed from 20- liter cylindrical polyethylene reactors. The entire activated sludge system was hard-plumbed with 1-inch diameter schedule 40 PVC. A series of 1-inch ball valves allowed for the rerouting of flows to multiple locations, as desired by the operators.
These ball valves allowed for multiple recycle line exit points, a bypass line for the first anaerobic zone, and split-feed lines.

Cleaning techniques were also found to be of tremendous importance in maintaining steady operation of the pilot system. Specifically, a daily scrubbing of the side walls of all reactors of the activated sludge system, especially the aerobic tank, was necessary to prevent the build-up of a biofilm. The side walls of the secondary clarifiers were also gently scraped above the sludge blanket on a daily basis. This was necessary in order to maintain a more steady effluent solids concentration. Specifically, if the side walls of the secondary clarifier were not scraped daily, a biofilm would accumulate on the side walls, and would eventually slough off, thereby elevating the effluent solids concentration. It was also important to clean the 1-inch PVC lines connecting the anaerobic, anoxic, and aerobic tanks together, as biofilms could easily grow in those lines. To prevent clogging, the barb fitting where the 1-inch PVC was connected to the 3/8 inch ID neoprene tubing was periodically brushed clean.

Three operators, who sampled and monitored the systems seven days per week, maintained the bench scale plant.

**Modified Pilot Scale System Design and Operation**

Due to operational problems encountered during the pilot scale study (see Shah, 2001), the initial pilot scale system design was modified. The problems with successful influent prefermentation encountered in the pilot study were solved in the modified pilot scale study by developing an off-line prefermenter operated in a batch mode. Primary solids taken from the only operational full scale municipal WWTP primary clarifier in Central Florida (Altamonte
Springs Water Reclamation Facility, Altamonte Springs, FL) was used to feed the experimental off-line prefermenter. In order to equalize COD loading between control and prefermented activated sludge trains, prefermented primary solids were added to PAS train influent, while an equal volume fresh non-prefermented primary solids were added to the control influent. The second major change implemented during the modified pilot scale study was a reduction in tankage volume. Specifically, the number of trains, and the number of reactors in each train, was reduced in the modified pilot scale study (see Figure 3.3) in order to devote additional sampling efforts to explaining the disagreement in COD and N mass balances found in the previous pilot scale study (Shah, 2001). In all other respects, the modified pilot plant was similar to the initial pilot plant in both design and operation.

**ACRONYMS**

- AN Anaerobic
- AX Anoxic
- AE Aerobic
- RAS Return Activated Sludge
- ARCY Anaerobic Recycle
- NARCY Nitrate Recycle

**Figure 3.3 Schematic of Pilot Scale System**
The modified pilot scale study was divided into 3 distinct phases. Phase 1 focused on evaluating the effects of prefermentation on a septic, COD-limited wastewater. The normally P-limited raw influent wastewater was supplemented with additional phosphorus in order to make the influent COD-limited. Phase 2 explored the potential tradeoffs between primary clarification and prefermentation for a septic, COD-limited wastewater. Prefermented primary solids were added to PAS train influent, while an equal volume of fresh non-prefermented primary solids were added to the control influent in order to equalize COD loadings to the two systems. Phase 3 evaluated the effects of prefermentation on a septic, P-limited wastewater.

**Sample Collection and Monitoring**

During all phases of this research project, activated sludge trains were operated until steady state conditions were met. Mass balance sampling events took place between one and three times per week. Parameters that were analytically determined included TSS, VSS, COD, TP, SOP, NO₃, TKN/SKN, NH₄, VFAs, PHAs, and glycogen. Both the influent tank and the wet well that provided a 10-minute retention time for the IMUC effluent had composite samplers (Isco Inc., Lincoln, NE). All other samples were grab samples. All sample analyses were conducted within 24 hours after sampling (most within 4 hours), so beyond refrigeration, no sample storage protocols were established (i.e. no acid additions). All samples were filtered immediately upon removal from the bioreactor. Samples were first centrifuged on site immediately after sampling, then filtered with Whatman 934 AH glass fiber filters, and finally membrane filtered with 0.45 µm membrane filters. Field parameters, such as DO, pH, temperature, SVI, ZSV, and OUR were typically run within 12 hours of the mass balance
sampling event during the bench scale study, and concurrently with sampling events during the pilot scale and modified pilot scale studies.

**Analytical Methods**

**Solids**

Total suspended solids (TSS) and volatile suspended solids (VSS) were determined in accordance with the method described Standard Methods section 2540 D and E (1995). The filters utilized in both TSS and VSS analyses were Whatman 934/AH glass fiber filters with an average pore size of 0.45 µm, as specified by Standard Methods. Aluminum planchets and 4.25 cm diameter glass fiber filters were used when only TSS values were to be determined. Smaller diameter glass fiber filters, 2.5 cm, were necessary when determining VSS because of the ceramic Gooch crucibles used during the analysis. For both TSS and VSS, the glass fiber filters were initially rinsed with DI water in order to make the pore sizes on the glass fiber filter more uniform, and placed in an oven. For TSS, the filters were heated for one hour at 105°C. In the determination of VSS, the ceramic crucibles and filters were heated at 550°C for twenty minutes. After the completion of the initial drying cycle, the filters for both TSS and VSS were placed in a dessicator and then weighed. For TSS determinations, just the filter was weighed, where in the case of VSS, both the ceramic Gooch crucible and the filter were weighed.
Chemical Oxygen Demand

Chemical oxygen demand (COD) is a measure of the amount of organic carbon found within a sample. The closed reflux titrimetric method, as specified in Standard Methods (Section 5220 C, 1995), was used during the course of this study. The Ferrous Ammonium Sulfate concentration used during the titration was 0.0125 M, an order of magnitude less than the concentration specified in Standard Methods. This change was made in order to achieve greater precision in titrations. Blanks and potassium hydrogen phthalate (KHP) standards were run every time wastewater samples were run. CODs were run on both unfiltered samples, referred to hereafter as total COD (TCOD), and filtered samples, referred to hereafter as soluble COD (sCOD). TCODs were typically run on influent, effluent, and on occasional aerobic MLSS sample. Soluble COD profiles across the entire plant, from the influent through the effluent, were also run. At all times, at least 20% of all samples were duplicates.

Biochemical Oxygen Demand

Biochemical Oxygen Demand (BOD) is a laboratory technique (Standard Methods 5210 B, C, 1995) used to determine the relative oxygen requirements of various wastewater samples. Both 5-day and 20-day BOD tests were conducted during the course of the study. However, these tests were only run periodically, in order to establish a ratio of BOD to COD in the influent and effluent.
Phosphorus

Similar to COD, phosphorus samples can also be split into total and soluble fractions. In wastewater samples, phosphorus can be found in the form orthophosphate (PO$_4^{3-}$), condensed phosphate molecules, and incorporated into solids. A variety of colorimetric methods have been developed that react with orthophosphorus. The colorimetric method selected for this study was the vanadomolybdophosphoric acid colorimetric method, as specified in Standard Methods (Section 4500-P C, 1995). This method was selected over other colorimetric methods because of its wider linear range. The amount of sample volume used for low soluble orthophosphate (SOP) concentration samples was increased to make sure the minimum detection limit of 1 mg/L PO$_4^{3-}$-P was exceeded. The absorbance was measured at a wavelength of 420 nm, using a spectrophotometer, model Spec 20 D+ (Spectronic Instruments, Rochester, NY).

In order to calculate the total phosphorus of a sample, the sample must first undergo a digestion process that converts the phosphorus bound in solids to orthophosphate. This is necessary because all colorimetric phosphorus tests react only with orthophosphate. The persulfate digestion method, as specified in Standard Methods (Section 4500-P B5, 1995), was used during the duration of this study. Upon completion of the persulfate digestion, the vanadomolybdophosphoric acid colorimetric method, as described in the preceding paragraph, was utilized.

All samples were filtered immediately with 0.45 µm membrane filters on-site. Standard curves were run during every analysis for both total phosphorus (TP) and SOP. Approximately 10% of all wastewater samples were duplicated, and an additional 5% of all samples were spiked with known additions in order to obtain percent recoveries.
Nitrogen

The nitrogen forms of interest in this study were organic nitrogen, ammonia, and nitrate. The method selected for determining organic nitrogen concentrations was the macro Kjehldahl method, as specified by Standard Methods (Section 4500-Norg B, 1995). Samples containing organic nitrogen were divided into total and soluble fractions, called total Kjehldahl nitrogen (TKN) and soluble Kjehldahl nitrogen (SKN). The soluble samples were filtered on-site using 0.45 µm glass fiber filters.

Ammonia concentrations were determined in a distillation step as specified in Standard Methods (Section 4500-NH4 C, 1995).

Over the life of the study, nitrate concentrations were determined using three different methods. The first method used to quantify nitrate concentrations was through the use of ion chromatography. Specifically, a Dionex 2000 I/SP ion chromatograph (Sunnyvale, CA) with a CDM-3 conductivity detector and a 4270 integrator was utilized. The column used was an AG4A ground column and an AS4A analytical column. The element used was 1.8 mM Na2CO3-1.8 mM NaHCO3 and the element flow rate was 2 µL/min. The reagent used was 50 mM H2SO4 and the sample loop volume was 50 mL. The ion chromatograph gave good analytical results, but due to the large expenses incurred during analysis, other methods were developed.

The second method developed to determine nitrate concentrations was the cadmium reduction method, as specified by Standard Methods (Section 4500-NNO3, 1995). This analytical method also gave credible results, but was dropped in favor of method developed by Hach (Loveland, CO) because the cadmium reduction method takes much longer to complete than does the Hach method. Note that the Hach method is simply a modification of the cadmium
reduction method, as they both use the same reagents. The main difference is that multiple samples can be run using the Hach method, while only one sample at a time can be run using the cadmium reduction column. Relative percent differences (RPDs) for both the cadmium reduction column method and the Hach method were comparable, both typically less than 10%. Spiked samples with known additions were also comparable for both methods. All samples were immediately filtered with 0.45 µm membrane filters on-site. During the modified pilot study, the method to determine nitrate concentrations was switched back from the Hach method to the IC. A guard column was placed on the IC, thereby eliminating the expensive prefiltration steps required earlier in the study.

**Sludge Volume Index**

Sludge Volume Index (SVI) is the volume in milliliters occupied by 1 g of a suspension of aerobic activated sludge after 30 minutes of settling in a 1 liter graduated cylinder. This test was conducted as specified in Standard Methods (Section 2710 D, 1995).

**Zone Settling Velocity**

The zone settling velocity (ZSV) was determined as specified in Standard Methods (Section 2710 E, 1995). At high concentrations of suspended solids, suspensions enter in the zone-settling regime. This type of settling takes place under quiescent conditions and is characterized by a distinct sludge interface between the supernatant liquid and the sludge zone. The height of this distinct interface is measured with time as the solids settle.
Oxygen Uptake Rate

Oxygen uptake rate (OUR) was determined as specified by Standard Methods (Section 2710 B, 1995). A BOD bottle was filled with aerobic sludge and DO measurements were taken using a BOD bottle probe and dissolved oxygen meter (YSI, Yellow Springs, Wyoming). DO measurements were taken over time to determine the rate of oxygen consumption in the aerobic zones. Specific OURs (SOUR) will be calculated by dividing the OUR by the MLVSS concentration.

Volatile Fatty Acids

Liquid samples were analyzed for short-chain volatile fatty acids (SCVFAs) following Supelco Bulletin 856B (1995). SCVFAs have carbon skeletons containing between two and five carbon atoms. The SCVFAs of particular interest in this study were acetic acid and propionic acid, since they are the most common SCVFAs found in municipal wastewater in the United States. In other parts of the world, such as Japan, both isovaleric and valeric acids are found in measurable quantities in municipal wastewater. A Shimadzu gas chromatograph model 14-A equipped with a flame ionization detector (FID) was utilized to conduct the analysis. A 3 mm inner diameter glass column with 60/80 Carbopack C/0.3% Carbowax 20M/0.1%H3PO4 packing (Supelco Inc., Bellefonte, PA) was used to separate the various SCVFAs. Helium, at approximately 30 mL/min, was selected as the carrier gas. The injection port and the FID were maintained at 200°C. The oven of the gas chromatograph was programmed to begin sample analysis at 105°C, remaining at 105°C for two minutes, before increasing at a rate of 5°C per minute to 150°C, and to hold at 150°C for an additional two minutes, resulting in a total run time
of 13 minutes per sample. The sample injection volume was 2 µL, double the volume specified in Supelco Bulletin 856B (1995). The injection volume was doubled in order to improve the reliability of the analysis at low concentrations. A Shimadzu automatic sampler AOC-20I injected the samples into the gas chromatograph. A Shimadzu Chromatopac CR501 integrated the resultant peaks that were separated by the gas chromatograph.

Calibration curves were established for acetic and propionic acids by using both pure reagents purchased from Fisher Scientific (Pittsburgh, PA) and neat standards purchased from Supelco (Bellefonte, PA). Calibrations curves typically had coefficient of determination ($R^2$) values greater than 0.995. A fresh calibration curve was run with every sample analysis. Fresh standards were prepared when the peak areas for the standards showed significant decline, typically 2 weeks. Standards were stored at 4°C. For the purposes of quality control 10% of all matrix samples were replicates and an additional 5 percent of all samples were spiked with a known addition. In addition, every liquid sample vial, both standards and matrix samples were injected onto the column twice before moving to the next vial.

All samples were filtered with a 0.45 µm membrane filters prior to analysis. Samples were filtered on-site and placed into 1.5 mL gas chromatography vials with no head space. The vials were then sealed with teflon-lined septum and screw caps and stored at 4°C. Immediately prior to analysis, 150 mL of 3% $H_3PO_4$ was added to each sample in order to drop the pH to approximately 3. This sample acidification allows for better analyte separation by the column packing. The reason that $H_3PO_4$ was not added prior to any extended storage was because acetic and propionic acids are much more volatile at low pH values. This approach differs from that of Chu (email correspondence, 1999), who acidified samples with $H_3PO_4$ prior to storage and then stored samples for up to 2 weeks prior to analysis. The Supelco Bulletin 856B (1995) specified
only to acidify samples prior to injection. Analysis of SCVFAs following Chu’s method resulted in lower peak areas for both acetic and propionic acids, especially after extended storage times (greater than 2 days). Samples were stored at 4°C prior to analysis. Since the length of storage time also has an impact on decreasing peak areas, all samples during the course of this study were run with 48 hours, and almost always within 12 hours.

**Prefermentation Potential**

The prefermentation potential of a given wastewater was determined following a method developed by Liu and Welander (1991). Prefermentation potential is a parameter that can determine the amount of short-chain volatile fatty acids (SCVFAs) that can potentially be fermented from any given wastewater. To briefly summarize the fermentation process, primary solids within the wastewater are hydrolyzed and converted to SCVFAs by acidogenic bacteria naturally present within the wastewater. To determine the prefermentation potential, raw wastewater was placed within a 120 mL amber glass Wheaton serum bottle. The serum bottles were crimp sealed with aluminum crimp seals and unlined butyl rubber septum. The serum bottles were sampled for SCVFAs, following the procedure outlined previously in this document, until SCVFA production stopped, typically 6-10 days. The difference between the initial SCVFA value and the final stabilized SCVFA value was the prefermentation potential of the wastewater. This test was run in triplicate each time each time the prefermentation potential of the wastewater was to be determined. The serum bottles were typically maintained at room temperature. However, during the course of this study, it was found that elevating the temperature to 30°C decreased the amount of time required to reach a stable endpoint, while
resulting in only slightly higher prefermentation potential values. Municipal wastewaters may have significant variation in prefermentation potential. In highly septic wastewaters, such as those found in Florida, most of the fermentation has already occurred in the collection system, resulting in relatively high SCVFA concentrations in the wastewater. In colder climates such as Canada, however, significant SCVFA concentrations are rarely found in municipal wastewaters, implying that little fermentation occurs within the collection system. Intuitively, a prefermenter as a unit process should have a greater impact on wastewaters with high prefermentation potential values.

PHAs

Poly hydroxy alkanoates (PHAs) were measured using a DB-1 capillary column and a Shimadzu (Tokyo, Japan) 14A gas chromatograph equipped with a flame ionization detector. A Shimadzu automatic sampler AOC-20I injected the samples into the gas chromatograph. A Shimadzu Chromatopac CR501 integrated the resultant peaks that were separated by the gas chromatograph. The carrier gas, helium was maintained at a velocity of 2 ml/min and as the make up gas (25 ml/min). The procedure for determing PHAs was based on that of Liu (2001). The injection port and detector were maintained at a temperature of 230 °C. The column temperature started at 100 °C for 2 minutes, was increased by 20 °C per minute to 160 °C, and maintained at 160 °C for an additional 2 minutes. Prior to injection, sludge samples must be freeze-dried using a lyophilizer and then run through a digestion. About 0.15 grams of dry sludge was put into 5.0 ml Wheaton V vials. 2 ml of benzoic acid in chloroform was added to the vial for use as an internal standard and solvent, respectively. Next, 2 ml of 20% H₂SO₄ in
methanol was added as the digestion/esterification reagent (methyl esters of the PHA are what is actually extracted into the chloroform phase). The vials were then placed inverted into a 100 °C oven for 18 hours. After cooling to room temperature, the chloroform phase was removed from the vial and placed into a 1.5 ml GC vial.

**Glycogen**

The anthrone method (Murray, 1981) was used to determine the glycogen content of sludges during this study. After an initial ice water bath, 5 ml of anthrone reagent was added to each sample and boiled for exactly 10 minutes, and returned to the ice water bath. After color development, absorbance at 625 nm was measured using a Spec 20 D+ (Spectronic Instruments, Rochester, NY).

**Rapidly and Slowly Biodegradable Chemical Oxygen Demand**

Rapidly biodegradable chemical oxygen demand (RBCOD) and slowly biodegradable chemical oxygen demand (SBCOD) are influent fractions important in the modeling of activated sludge systems. Techniques developed both by Ekama et al (1986) and Wentzel et al (1995) were used during this study. A BOD bottle probe and dissolved oxygen meter (YSI, Yellow Springs, Wyoming) were used in Ekama’s method, while an automatic OUR meter (High Tech Microsystems, Capetown, South Africa) was used for Wentzel’s method. In both cases, the tests revolve around OURs taken over time for a given sample. The changes in the slope of the OUR measurements assist in determining values for RBCOD.
References


CHAPTER 4 CHANGES IN ANOXIC DENITRIFICATION RATE DUE TO PREFERMENTATION OF A SEPTIC, PHOSPHORUS LIMITED, WASTEWATER

Abstract

A preliminary bench scale study of parallel University of Cape Town (UCT) biological nutrient removal (BNR) systems showed improvement in anoxic denitrification rates due to prefermentation of a septic (i.e. high volatile fatty acid or VFA content), phosphorus limited (i.e. TCOD:TP<40:1), wastewater. Net phosphorus (P) removals due to Enhanced Biological Phosphorus Removal (EBPR) were only improved marginally by prefermentation in spite of significant increases in anaerobic phosphorus release, polyhydroxyalkanoate (PHA) formation, and higher anoxic and aerobic uptakes. This was probably due to the high VFA: total phosphorus (TP) ratio in the raw influent relative to the VFA requirements for EBPR, since enough VFAs were already present for P removal prior to prefermentation. An additional assessment of prefermentation using parallel UCT systems with step feed of 50% of the influent to the anoxic zone was completed. This second phase quantified the effect of prefermentation in a step feed scenario which prioritized prefermentation use to enhance denitrification rather than EBPR. While specific denitrification rates in the anoxic zone were significantly improved by prefermentation high denitrification in the clarifiers and aerobic zones (simultaneous denitrification) made definitive conclusions concerning the potential improvements in total system nitrogen removal questionable. The prefermented system always showed superior zone settling velocity (ZSV) and sludge volume index (SVI) values and the improvement became increasingly statistically significant when the prefermenter was performing well.
Keywords

Biological nutrient removal, phosphorus, prefermentation, volatile fatty acids, denitrification, enhanced biological phosphorus removal, biological nitrogen removal.

Introduction

Prefermentation of wastewater or primary solids is a common practice associated with Biological Nutrient Removal facilities in many parts of the world although it is only used in a few full scale installations in the United States to date. Prefermentation technology is associated in the minds of many engineers exclusively with cold climates as an enhancement solely for Enhanced Biological Phosphorus Removal (EBPR) for non-septic wastewaters. It is true that prefermentation technology is used broadly in Canada for that purpose. However prefermentation is practiced widely in Australia (Keller and Hartley, 1997), South Africa, and other temperate or even tropical climates, and has been used or investigated in many other countries including some parts of the United States.

Prefermenters can be either on-line (the entire wastewater stream is treated) or sidestream (only primary clarifier underflow is treated). The most basic on-line prefermenter is simply a primary clarifier operated with a very high sludge blanket, commonly referred to as a Static Prefermenter. These prefermenters are not very efficient, often elevating influent VFAs less than more sophisticated prefermenters (VanMunch et al., 1996). Static Prefermenters were improved with a recycle to elute VFAs from the sludge blanket and this configuration is referred to as an Activated Primary Tank or APT. Sidestream Prefermenters are reactors which receive the primary clarifier underflow instead of fermenting the entire wastewater flow. They can consist
Traditionally the function of prefermenters has been to convert a large portion of the slowly degradable influent chemical oxygen demand (COD) into readily available substrate (e.g. VFAs) to drive EBPR in the anaerobic zone. In plants in Western Canada, where prefermentation is very common, consistent effluents of 0.5 mg/L and lower are claimed without chemical polishing for some wastewaters. Reliably going below 1 mg/L without chemical polishing is anecdotally described as routine. However there are obvious disadvantages to prefermentation. One is that the capital costs of primary clarification are incurred while many of the benefits may be lost (i.e. no direct reduction in oxygen demand or secondary waste sludge production although increased denitrification may mitigate this). In addition in countries where there is a phosphate detergent ban such as the United States, it is not as difficult to meet effluent standards and chemical polishing costs can be significantly less than in countries with significantly higher influent phosphorus concentrations. Further in the southern United States, and seasonally in the north, raw wastewater is often at least partially septic, and in Florida it is very septic and raw wastewater concentrations may routinely exceed 50 mg/L total VFAs even in the winter. As a result it is often presumed that there will be little benefit to prefermentation in a warm climate.

Prefermenters have historically been an unusual unit process because they are frequently used with BNR plants by some design communities, while other design communities have not (at least in the past) seriously considered them as an option. Part of the reason for this is the absence of a single tank which may or may not be completely mixed, or of a complete mix tank followed by a dedicated thickener. BNR facilities may receive both prefermented solids and liquid from a Sidestream Prefermenter, or may receive only the supernatant, depending on which configuration is used.
of quantitative information on the process and effluent changes resulting from prefermentation for a variety of wastewaters and climates. Most information is from full scale applications and is anecdotal (e.g. we have a plant with prefermentation that always meets 0.5 mg/L P, we have a plant without prefermentation that always goes below 1 mg/L P, etc…), with only a few direct comparisons existing in the literature (e.g. Danesh and Oleskiewica, 1997).

This bench scale study was conducted with two basic objectives:

- To conduct controlled comparisons isolating prefermentation as an experimental variable using parallel BNR processes with prefermentation, and without prefermentation, for a variety of wastewater conditions.
- To determine if prefermentation might be beneficial in niches for which it has not traditionally been used; i.e. to enhance denitrification kinetics as opposed to the normal niche of enhancing biological P removal, or for septic wastewaters in warm climates as opposed to the normal niche of fresh/non-septic wastewaters in cold or temperate climates.

**Methods and Materials**

Two bench scale, 15 liter liquid volume, Biological Nutrient Removal (BNR) systems were run simultaneously at a solids retention time of 12 days to determine the enhancement of anoxic zone denitrification rates using a prefermenter in combination with a BNR system (Figure 4.1).
Both systems were three zone (i.e. anaerobic, anoxic, aerobic) University of Cape Town (UCT) systems but one system was preceded by an on-line prefermenter. The other system did not have a prefermenter and served as a control system.

Influent wastewater consisted of a septic, phosphorus (P) limited domestic wastewater (TCOD=428 mg/L; VFA without prefermentation=46 mg/L; TCOD:TP=58:1; i.e. phosphorus limited in that TCOD:TP>40; WEF, 1998). However the wastewater had significant influent total suspended solids (TSS - 121 mg/L) with an associated COD demand of 1.8 mg COD/mg TSS. This meant that significant prefermentation potential remained since relatively unstable primary solids were still present. Early in the study several prefermentation potential tests (Lie
and Welander, 1997) using serum bottles confirmed 20 mg/L or more of additional VFA could potentially be produced from the wastewater.

The effect of an on-line intermittently mixed upflow clarifier (IMUC) with a 2.1 to 2.4-hour hydraulic retention time (HRT) used for the retention and prefermentation of influent primary solids was analyzed. Since the wastewater was strong, with a high VFA content, according to current design methodology (WEF, 1998) there was enough readily biodegradable COD already present in the influent for efficient Enhanced Biological Phosphorus Removal (EBPR) prior to prefermentation, especially since the TCOD:TP ratio was high. Changes in anoxic zone specific denitrification rates while maintaining P removal was thus the focus of this preliminary study. In the first phase the prefermented activated sludge system (PASS) was compared to the control activated sludge systems (CASS) in a standard UCT configuration (Figure 4.1). However in Phase 2 the influents to both systems were divided 50:50 between the anaerobic and anoxic zones to shunt more of the VFAs to drive denitrification rather than EBPR (Figure 4.2).

In both phases all recycles were operated at one times the influent flow since it was anticipated that the anoxic zone would be undersized with respect to protecting the anaerobic zone. As a result no attempt was made to optimize the system performance as a whole (i.e. quantifying zone performance and comparing the PASS and CASS under identical conditions were the objectives, not producing a good final effluent quality).
Note: NARCY – Nitrate Recycle from Aerobic Zone, ARCY – Biomass Recycle from Anoxic Zone, RAS – Return Activated Sludge from Clarifier

Figure 4.2 Schematic of the Bench-Scale System During Phase 2 with Step Feed

**Results and Discussion**

**IMUC Performance**

Influent VFAs were elevated by the IMUC from 54 to 61 mg/L in Phase 1 and 41 to 65 mg/L in Phase 2. In addition the ratio of acetic to propionic acid was increased by prefermentation in both phases (in some cases propionic acid concentrations actually decreased, probably due to fermentation to acetic acid). Part of the reason for the superior IMUC performance in Phase 2 may have been due to an increase in average influent temperatures from
28 to 32 degrees C and an increase in solids loading since the influent flow rate was 40.7 L/day in Phase 1 and 48 L/day in Phase 2. IMUC solid (SRT) and hydraulic (HRT) retention times were equal to 4 days and just over 2 hours, respectively, in both phases. The IMUC was aerated for 2-3 minutes/day to suppress methanogens through oxygen toxicity.

**Effect of Prefermentation on EBPR**

The results showed that in both phases every parameter associated with EBPR in the anaerobic and anoxic zone was significantly increased by prefermentation, but the overall process net P removal was not significantly increased. The average change in anaerobic soluble ortho-phosphorus (SOP) was 19 to 33% greater with prefermentation (Table 4.1).

Table 4.1 Soluble Ortho-Phosphorus Concentrations for Phase 1 & 2

<table>
<thead>
<tr>
<th>Parameters (mg/L)</th>
<th>Phase 1</th>
<th>Phase 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PASS</td>
<td>CASS</td>
</tr>
<tr>
<td>TP influent</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Anaerobic SOP</td>
<td>13.3</td>
<td>11.2</td>
</tr>
<tr>
<td>Anoxic SOP</td>
<td>6.2</td>
<td>6.0</td>
</tr>
<tr>
<td>Aerobic SOP</td>
<td>1.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Clarifier SOP</td>
<td>1.1</td>
<td>1.5</td>
</tr>
<tr>
<td>% P removal</td>
<td>83</td>
<td>78</td>
</tr>
<tr>
<td>Apparent Anaerobic P Release</td>
<td>5.3</td>
<td>3.2</td>
</tr>
<tr>
<td>Apparent Anoxic P Uptake</td>
<td>7.1</td>
<td>5.2</td>
</tr>
<tr>
<td>Aerobic P Uptake</td>
<td>4.9</td>
<td>4.4</td>
</tr>
<tr>
<td>Net P Uptake (excluding clarifier)</td>
<td>6.7</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Polyhydroxyalkanoate (PHA) content (only observed for phase 1) was 22 % greater for the PASS, and the decrease in SOP concentration from the anaerobic to the anoxic zones (apparent anoxic P uptake) was 37 to 72% greater for the PASS. However net P removals were
not significantly different (only 2 to 5% greater for the PASS) and aerobic zone SOP concentrations differed by a disproportionately small amount compared to the other EBPR relevant parameters. In Phase 2, where effluent SOP values were well below 1 mg/L, this could be attributed to the low concentrations of P in the aerobic zone which might limit aerobic P uptake even though greater uptake potential existed. In Phase 1 this explanation doesn't seem as plausible although, at bench scale, it still may have been the reason for the very small difference in net P uptake observed.

No trade-off was observed between biological nitrogen removal and EBPR during Phase 2 when 50% of the influent bypassed the anaerobic zone. This might have been expected to reduce EBPR significantly, but anaerobic SOP concentrations actually increased over values observed in Phase 1. Anaerobic mass fraction only differed by 1 or 2% between Phases 1 and 2 so this cannot explain the anaerobic SOP increase. However anaerobic contact time in the anaerobic zone was almost doubled by going to step feed, from 1.5 hours in Phase 1 to 2.6 hours in Phase 2 (this was because only 50% of the influent flow entered into the anaerobic zone, overall system HRTs and anoxic/aerobic contact times were similar in both phases if weighted for flow passing through each zone). Subsequent net P removals remained almost constant in both systems (Phase 1 PASS 6.7 mg/L vs. 6.1 mg/L Phase 2; Phase 1 CASS 6.4 mg/L vs. 6.0 mg/L Phase 2) however effluent SOP was lower in Phase 2 since influent TP was only 6.8 mg/L compared to 8.0 mg/L in Phase 1. So, probably because this was a P limited wastewater, there was no significant loss of EBPR resulting from the split flow to enhance denitrification. However, it would be inappropriate to conclude that split flow wouldn't be detrimental to EBPR for a COD limited (TCOD:TP<40) wastewater.
A mass balance analysis was more revealing than analysis of reactor concentrations where recycles could dilute the observed anaerobic or anoxic zone concentrations (thus the label "apparent" P release or uptake in Table 4.1). Table 4.2 clearly shows that the decrease in SOP concentrations in the anoxic zone was due to dilution (i.e. from the nitrate recycle) for the control system and that in fact there was anoxic P release.

Table 4.2  Phosphorus Mass Flux Values for Phase 1 & 2 (with influent TCOD flux)

<table>
<thead>
<tr>
<th>Parameters (mg/day)</th>
<th>Phase 1</th>
<th>Phase 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PASS</td>
<td>CASS</td>
</tr>
<tr>
<td>TCOD influent</td>
<td>17308</td>
<td>17039</td>
</tr>
<tr>
<td>TP influent</td>
<td>325</td>
<td>320</td>
</tr>
<tr>
<td>Anaerobic SOP Release</td>
<td>635</td>
<td>431</td>
</tr>
<tr>
<td>Anoxic SOP Uptake</td>
<td>118</td>
<td>-34</td>
</tr>
<tr>
<td>Net SOP Release</td>
<td>517</td>
<td>465</td>
</tr>
<tr>
<td>Aerobic SOP Uptake</td>
<td>774</td>
<td>715</td>
</tr>
<tr>
<td>Net SOP Uptake</td>
<td>257</td>
<td>250</td>
</tr>
<tr>
<td>∆P in wasted solids</td>
<td>282</td>
<td>259</td>
</tr>
<tr>
<td>∆P in wasted solids -</td>
<td>59</td>
<td>59</td>
</tr>
<tr>
<td>Net ∆P in waste solids &gt;</td>
<td>223</td>
<td>200</td>
</tr>
<tr>
<td>%P in MLSS</td>
<td>8.6</td>
<td>7.8</td>
</tr>
</tbody>
</table>

For both systems the step feed either decreased the anoxic P uptake (PASS) or increased the anoxic P release (CASS, a dramatic increase in this case). However the mass balances confirm that while prefermentation affected the distribution of P release and uptake between the anaerobic and anoxic zones, the net P release for these two zones hardly varied due to prefermentation in either phase. This change in distribution presumably would have to do with the fate of slowly biodegradable or fermentable influent COD (e.g. SBCOD). In the preferfermented system much of this influent fraction was converted to VFAs, and was sequestered.
to form a greater PHA content in the anaerobic zone (as observed in phase 1), and since VFAs are not being produced by fermentation there is anoxic P uptake. However a plausible explanation for the anoxic P release in the CASS system is that there were VFAs still available or being produced in the anoxic zone for sequestration. It has been observed in the literature that the presence of VFAs (e.g. acetic acid) will cause P release even in anoxic and sometimes aerobic conditions (Meinhold et al., 1998). Batch experiments with the PASS and CASS biomass under anoxic conditions showed that both the raw and prefermented influent initially induced P release simultaneous with very high denitrification rates. This was then followed by anoxic P uptake and lower denitrification rates, presumably after the easily sequestered COD such as VFAs had been taken up.

Prefermentation resulted in a marginally higher P content of the mixed liquor solids (MLVSS/MLSS ratios in both systems were almost equal at 0.78 for the PASS and 0.77 for the CASS in both phases). Regardless of how the data were analyzed, whether excluding the clarifier effects or for the systems as a whole, the P removals were very close to equal with only a marginal improvement due to prefermentation. The significant effect of the prefermenter was in distributing the system P release entirely into the anaerobic zone rather than being distributed between the anaerobic and anoxic zone as it was in the CASS.

**Effect of Prefermentation on Biological Nitrogen Removal**

Influent total Kjeldahl nitrogen TKN averaged 43.8 mg/L-N. Nitrification rates and extent were similar in both systems (slightly higher in the PASS) and in both phases (Table 4.3).
Influent TCOD:TKN averaged 10, a ratio indicative of sufficient COD to drive denitrification for most domestic wastewaters (WEF, 1998). Assimilated N was calculated using the assumption that the average bacteria in the system has a molecular formula of C₅H₇O₂N, resulting in a nitrogen content in the biomass of 12.4% (Tchobanoglous and Burton, 1991). The data in Table 4.3 shows simultaneous denitrification was very significant in these bench scale systems, and this made definitive conclusions concerning the effect of prefermentation on biological nitrogen removal for the system as a whole problematic. Aerobic zone oxidation-reduction potentials ranged from 0 to +30 mV during the study and while this does not necessarily imply that floc centers were anoxic it was on the low end of the range expected and was certainly not inconsistent with possible simultaneous denitrification. Excluding the clarifier the PASS system seemed to show a slight improvement in dissimilative nitrogen removal (assimilated nitrogen removals were virtually equal in both phases) but this difference decreased when step feed was used for both systems. However it would be hard to conclude these results are meaningful for a full scale system since the levels of simultaneous denitrification were very high for these systems. Part of the reason for these high simultaneous denitrifications was probably because the nitrogen recycle was only being operated at one times the influent flow rate (since denitrification was not complete in the anoxic zones denitrification rates could be quantified without transporting more nitrate to the zone; it was close to complete in Phase 2 but with values of 0.2 to 0.5 mg/L-N vs. a method limit close to 0.1 mg/L-N, so still detectable). This resulted in very high nitrate concentrations in the aerobic zone and in the effluent (also explaining the high denitrification in the clarifiers). The high aerobic zone nitrate concentrations might have created more of a driving force for nitrate diffusion deep into possible anoxic zones in aerobic flocs.
Table 4.3 Nitrogen Mass Flux Values for Phase 1 & 2

<table>
<thead>
<tr>
<th>Parameters (mg/day)</th>
<th>Phase 1</th>
<th></th>
<th>Phase 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PASS</td>
<td>CASS</td>
<td>PASS</td>
<td>CASS</td>
</tr>
<tr>
<td>TKN influent</td>
<td>1813</td>
<td>1746</td>
<td>1955</td>
<td>1954</td>
</tr>
<tr>
<td>Assimilated N</td>
<td>312</td>
<td>313</td>
<td>341</td>
<td>352</td>
</tr>
<tr>
<td>Available N</td>
<td>1501</td>
<td>1433</td>
<td>1614</td>
<td>1602</td>
</tr>
<tr>
<td>Nitrate Produced</td>
<td>1342</td>
<td>1226</td>
<td>1398</td>
<td>1359</td>
</tr>
<tr>
<td>% Nitrification</td>
<td>89.4</td>
<td>85.6</td>
<td>86.6</td>
<td>84.8</td>
</tr>
<tr>
<td>Nitrate Load to Anoxic Zone</td>
<td>660</td>
<td>635</td>
<td>845</td>
<td>667</td>
</tr>
<tr>
<td>Nitrate Load leaving Anoxic Zone</td>
<td>226</td>
<td>269</td>
<td>69</td>
<td>57</td>
</tr>
<tr>
<td>Anoxic Zone Denitrification</td>
<td>434</td>
<td>366</td>
<td>777</td>
<td>610</td>
</tr>
<tr>
<td>Specific Denitrification Rate in the Anoxic Zone</td>
<td>43.8</td>
<td>39.9</td>
<td>67.4</td>
<td>53.3</td>
</tr>
<tr>
<td>Simultaneous Denitrification(^1)</td>
<td>674</td>
<td>565</td>
<td>237</td>
<td>331</td>
</tr>
<tr>
<td>Clarifier Denitrification</td>
<td>88</td>
<td>112</td>
<td>57</td>
<td>196</td>
</tr>
<tr>
<td>System Denitrification (without clarifier)</td>
<td>1108</td>
<td>931</td>
<td>1014</td>
<td>941</td>
</tr>
<tr>
<td>Total System Denitrification</td>
<td>1196</td>
<td>1043</td>
<td>1071</td>
<td>1137</td>
</tr>
</tbody>
</table>

\(^1\)Simultaneous denitrification was calculated by assuming total system nitrogen removal that could not be accounted for by direct mass balances on all other zones and the clarifier occurred in the aerobic zone. Simultaneous denitrification cannot be observed directly by normal mass balance techniques. Possible denitrification in the anaerobic zone would only lower the estimated values by 10% or less, and the magnitude was too large to explain easily by analytical error.

The anoxic zone data from the bench scale system did not share these liabilities however. The anoxic zone specific denitrification rates in both phases showed higher rates with prefermentation. This was also observed for the overall mass of nitrate removed in the anoxic zones. However the Phase 1 data is actually close enough, and showed sufficient variability, that it cannot be concluded there was a difference between PASS anoxic denitrification rates and CASS anoxic denitrification rates. In Phase 2 however the difference was probably significant (26.5 % greater). This also correlated with the improved prefermenter performance in Phase 2 (in Phase 1 influent VFA was increased by 7 mg/L compared to 24 mg/L in Phase 2).
probably occurred because the prefermented influent shunted to the anoxic zone in Phase 2 was much more readily degradable. It is well known that VFAs such as acetic acid result in higher specific denitrification rates than more complex compounds. In addition the significantly higher PHA content in the PASS biomass could have provided an internal source of carbon for denitrification resulting in higher rates. It can be seen from Table 4.2 that the CASS anoxic zone still had significant P release occurring (and presumably anoxic PHA formation competing with anoxic PHA degradation) suggesting that at least part of the biomass in the CASS anoxic zone was still active sequestering VFA from fermented readily biodegradable COD, while in the prefermented system the fermentation had already occurred, allowing all the VFA sequestration and P release (Table 4.2) to occur in the anaerobic zone.

**Sludge Settleability**

Differences in the settleability of the biomasses were also observed. The PASS biomass always had superior average zone settling velocities (ZSVs) than the CASS throughout the study (Table 4.4).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Phase 1</th>
<th>Phase 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PASS</td>
<td>CASS</td>
</tr>
<tr>
<td>ZSV (in/hr)</td>
<td>32</td>
<td>17</td>
</tr>
</tbody>
</table>

SVIs were consistent in both phases at 177 ml/gVSS for the PASS and 212 ml/gVSS for the CASS. Thus both systems had SVIs that were quite high compared to full scale systems. It was noticed that the statistical confidence level (i.e. P value) of the difference between the PASS
and the CASS increased during periods when the prefermenter was performing well in terms of elevating PASS influent VFAs over the CASS influent concentration. However confidence levels rarely exceeded 80 to 90% in statistical comparisons of settleability data. The test conducted was a simple t test for paired observations (Steel and Torrie, 1960). Calculations were done using Microsoft Excel and a table of t test values from Steel and Torrie (1960).

**Inter-Phase Comparisons**

Comparing the Phase 2 PASS to the Phase 1 PASS, or similarly with the CASS, can only be done with great caution since these are not controlled comparisons like single intra-phase comparisons for the PASS versus CASS. It seems probable that the dramatic increases in anoxic zone denitrification rates (Table 4.3) in Phase 2 compared to Phase 1 were probably significantly impacted by going to step feed mode. However there was also a 4 degree C increase in temperature from Phase 2 to Phase 1, and depending on the $\Theta$ value chosen many popular design equations would suggest that much of the increase in denitrification rate was due to temperature. It is certain that step feed did not harm EBPR however, and it seems likely that there was some benefit with respect to the anoxic zone denitrification rates. This suggests that for a P limited wastewater, depending on the P effluent requirements, it may make more sense to shunt available carbon to the anoxic zone rather than the anaerobic zone where it is not needed to meet treatment objectives. However this is a somewhat speculative conclusion since the step feed strategy has not yet been studied in isolation.
Conclusions

Prefermentation caused greater anoxic zone specific denitrification rates but only very slightly improved EBPR, both with and without step feed. There may be benefits to prefermentation even in a septic wastewater, but these benefits may be more significant with respect to anoxic denitrification rates and biological nitrogen removal rather than EBPR. These preliminary results suggest that for a septic, P limited, wastewater:

1. Anoxic zone specific denitrification rates were increased by prefermentation. However the data was ambiguous with respect to reaching conclusions concerning the significance of the increased anoxic zone denitrification rate increase in terms of improving overall system biological nitrogen removal.

2. Step feed to the anoxic zone was used without detrimental effects to EBPR for this P limited wastewater.

3. Prefermentation resulted in significant redistribution of P release and uptake between the anaerobic and anoxic zones but this did not significantly change the net P removal of the system for this P limited wastewater.

4. The advantages of prefermentation for a septic, P limited, wastewater may be enhanced by step feed, but this is a tentative finding since only prefermentation was isolated as an experimental variable.
Acknowledgements

This research was funded by the National Science Foundation, Award #9616144. In addition the assistance of the Orange County Utilities Eastern Water Reclamation Facility personnel and the Plant Manager, Tim Madhanagopal, P.E., DEE, QEP, is gratefully acknowledged.
References


CHAPTER 5 EVALUATION OF INFLUENT PREFERMENTATION AS A UNIT PROCESS UPON BIOLOGICAL NUTRIENT REMOVAL

Abstract

The objective of this NSF sponsored research was to provide a controlled comparison of identical continuous flow biological nutrient removal (BNR) processes both with and without prefermentation in order to provide a stronger, more quantitative, technical basis for design engineers to determine the potential benefits of prefermentation to EBPR in treating domestic wastewater. Specifically, this paper focused upon the potential impacts of primary influent prefermentation upon BNR processes treating septic domestic wastewater. This study can be divided into two distinct phases – an initial bench-scale phase which treated septic P-limited (TCOD:TP>40) wastewater and a subsequent pilot-scale phase which treated septic COD-limited (TCOD:TP<40) wastewater. The following conclusions can be drawn from the results obtained to date:

- Prefermentation increased RBCOD, SBCOD and VFA content of septic domestic wastewater.
- Prefermentation resulted in increased biological P removal for a highly septic, non-P limited (TCOD:TP<40:1) wastewater. However, in septic, P-limited (TCOD:TP>40:1) wastewater, changes in net P removal due to prefermentation were suppressed by limited P availability, even though P release and PHA content were affected.
Prefermentation increased specific anoxic denitrification rates for both COD and P-limited wastewaters, and in the pilot (COD-limited) study also coincided with greater system N removal.

**Key Words**

Wastewater, Prefermentation, Biological Nutrient Removal, EBPR

**Introduction**

Prefermentation of wastewater or primary solids is a common practice associated with Biological Nutrient Removal facilities in many parts of the world although it is only used in a few full scale installations in the United States to date. Prefermentation technology is associated in the minds of many engineers exclusively with cold climates as an enhancement solely for Enhanced Biological Phosphorus Removal (EBPR) for non-septic wastewaters. It is true that prefermentation technology is used broadly in Western Canada for that purpose. However prefermentation is practiced widely in Australia (Keller and Hartley, 1997), and to varying degrees in other temperate or even tropical climates, including some parts of the United States.

Prefermenters can be either on-line (the entire wastewater stream is treated) or sidestream (only primary clarifier underflow is treated). The most basic on-line prefermenter is simply a primary clarifier operated with a very high sludge blanket, commonly referred to as a Static Prefermenter. These prefermenters are not very efficient, often elevating influent VFAs less than more sophisticated prefermenters (VanMunch et al., 1996). Static Prefermenters were improved with a recycle to elute VFAs from the sludge blanket and this configuration is referred to as an
Activated Primary Tank or APT. Sidestream Prefermenters are reactors which receive the primary clarifier underflow instead of fermenting the entire wastewater flow. They can consist of a single tank which may or may not be completely mixed, or of a complete mix tank followed by a dedicated thickener. BNR facilities may receive both prefermented solids and liquid from a Sidestream Prefermenter, or may receive only the supernatant, depending on which configuration is used.

Traditionally the function of prefermenters has been to convert a large portion of the slowly degradable influent COD into readily available substrate (e.g. VFAs) to drive EBPR in the anaerobic zone. In plants in Western Canada, where prefermentation is very common, consistent effluents of 0.5 mg/L and lower are claimed without chemical polishing for some wastewaters. Reliably going below 1 mg/L without chemical polishing is anecdotally described as routine. However there are obvious disadvantages to prefermentation. One is that the capital costs of primary clarification are incurred while many of the benefits may be lost (i.e. no direct reduction in oxygen demand or secondary waste sludge production although increased denitrification may mitigate this). In addition in countries where there is a phosphate detergent ban such as the U.S., it is not as difficult to meet effluent standards and chemical polishing costs can be significantly less than in countries with significantly higher influent phosphorus concentrations. Further in the southern U.S., and seasonally in the north, raw wastewater is often at least partially septic, and in Florida it is very septic and raw wastewater concentrations may routinely exceed 50 mg/L total VFAs even in the winter. As a result it is often presumed that there will be little benefit to prefermentation in a warm climate.

Prefermenters have historically been an unusual unit process because they are frequently used with BNR plants by some design communities, while other design communities have not (at
least in the past) seriously considered them as an option. Part of the reason for this is the absence of quantitative information on the process and effluent changes resulting from prefermentation for a variety of wastewaters and climates. Most information is from full scale applications and is anecdotal (e.g. we have a plant with prefermentation that always meets 0.5 mg/L P, we have a plant without prefermentation that always goes below 1 mg/L P, etc…), with only a few direct comparisons existing in the literature (e.g. Danesh and Oleskiewica, 1997).

This portion of the current National Science Foundation (NSF) funded study is being conducted with two basic objectives:

1. To conduct controlled comparisons of BNR processes with prefermentation, and without prefermentation, for a variety of wastewater conditions.
2. To determine if prefermentation might be beneficial in niches for which it has not traditionally been used; i.e. to enhance denitrification kinetics, or for septic wastewaters in warm climates.

**Methods and Materials**

In order to meet the research objectives listed above, the performance characteristics of an activated sludge train augmented with the effluent of a prefermenter was operated in parallel with a control activated sludge train that did not receive prefermenter effluent. The data presented in this document was collected in two distinct phases; an initial bench-scale phase that treated a septic, P-limited influent wastewater, and a larger pilot-scale phase that treated a septic, COD-limited influent wastewater. The two phases are described in detail below.
In the initial phase of this study, two bench-scale, 15 liter liquid volume, Biological Nutrient Removal (BNR) systems were run simultaneously at a solids retention time of 12 days and an average hydraulic retention time of 8.3 hours in order to determine the effect of influent prefermentation upon the performance characteristics of activated sludge treatment systems (Figure 5.1). Both systems were three zone (i.e. anaerobic 16.7% of the total volume, anoxic 33.3%, and aerobic 50.0%) University of Cape Town (UCT) systems. One system was preceded by an on-line prefermenter, served as the prefermented activated sludge (PAS) system. The other system, which did not have a prefermenter, served as the control activated sludge (CAS) system.

![Figure 5.1 Schematic of the Bench-Scale System](image-url)
During the latter part of the bench-scale phase, both activated sludge trains were operated in a split-feed mode in which the influents to both trains were divided equally between the anaerobic and anoxic zones to shunt more of the VFAs to drive denitrification rather than EBPR. The prefermenter in operation during the bench-scale phase was an on-line intermittently mixed upflow clarifier (IMUC) with a 2.1 to 2.4 hour hydraulic retention time (HRT) and a solids retention time of 4 days was used for the retention and prefermentation of influent primary solids.

Influent wastewater during the bench-scale phase consisted of a strong, septic, phosphorus (P) limited domestic wastewater (TCOD=428 mg/L; VFA without prefermentation=46 mg/L; TCOD:TP=58:1; i.e. phosphorus limited in that TCOD:TP>40; WEF, 1998). However the wastewater had significant influent TSS (121 mg/L) with an associated COD demand of 1.8 mg COD/mg TSS. This meant that significant prefermentation potential remained since relatively unstable primary solids were still present. Early in the study several prefermentation potential tests (Lie and Welander, 1997) using serum bottles confirmed 20 mg/L or more of additional VFA could potentially be produced from the wastewater. The prefermenter increased the average total VFA content of the already septic wastewater by 15.5 mg/L.

The pilot-scale system consists of two parallel 4 stage modified UCT systems. The flowsheet for the pilot-scale system is similar to that shown in Figure 5.1, with the exception that the anoxic zone was split into two separate reactors. The NARCY recycle line in the pilot plant flowed into the second anoxic zone, while the RAS returned into the first anoxic zone. The purpose of this revised flow configuration was to minimize nitrate loading into the anaerobic zones. Both trains had a total tankage of approximately 35 L, with volume fractions of 9.6%
anaerobic, 39.4% anoxic, and 51.0% aerobic. Both trains were operated at solids retention time of 8.5 days and a hydraulic retention time of 3.5 hours, deliberately low in order to test which system would fail first. The prefermented activated sludge system (PAS) was augmented with the effluent from a completely mixed sidestream prefermenter operated at a 10 day SRT, while the control activated sludge system (CAS) received an equal amount of fresh, unprefermented primary solids.

Influent wastewater during the pilot-scale phase consisted of a strong, septic, COD-limited domestic wastewater (TCOD=381 mg/L; VFA without prefermentation=55 mg/L; TCOD:TP=31.8. The prefermenter increased the average total VFA content of the already septic wastewater by 18.2 mg/L. Both influent tanks were also supplemented with 6 mg/L of phosphorus (in the form of K$_2$HPO$_4$) to increase the PO$_4$-P in the influent to 12.0 mg/L in order to insure that excess phosphorus was present, ensuring that influent wastewater was COD-limited. Both the pilot-scale and the bench-scale systems were located at a local full-scale BNR plant (a 5 stage Bardenpho plant removing both nitrogen and phosphorus).

**Results and Discussion of the Septic, P-Limited Phase**

The results from the bench-scale septic, P-limited phase have been documented in the literature (Randall et al, 2000). The most important differences between the PAS system and the CAS system for the bench-scale septic, P-limited phase are listed below:

- Prefermentation resulted in a greater PHA content for the biomass in the PAS system as compared to the CAS system.
• Prefermentation resulted in significant redistribution of P release and uptake between the anaerobic and anoxic zones, but this did not significantly change either the net P release nor the net P removal of the system for this P limited wastewater.

• Prefermentation increased specific denitrification rates for the PAS system as compared to the CAS system.

• A step feed modification to the anoxic zone in which half the influent was routed from the anaerobic zone to the anoxic zone, increasing denitrification rates, was used without detrimental effects to EBPR for both the PAS and CAS systems utilizing this P limited wastewater.

Results and Discussion of the Septic, COD-Limited Phase

Influent VFAs in the domestic wastewater averaged 55 mg/L during the pilot study. The completely mixed prefermenter utilized during the pilot phase consisted of a hydraulically isolated completely mixed reactor. Primary solids were collected from a full-scale primary clarifier and added to the prefermenter in sufficient quantity to maintain a 10 day SRT. During the daily operation of the pilot plants, 1 liter of solids from the prefermenter was added to the influent tank for the prefermented system. At the same time, an equal volume of fresh primary solids from the full-scale primary clarifier was added to the influent tank of the control system in order to equalize the solids loading to both of the trains. Composite samplers on the influent tanks measured an average total VFA content of 75.0 mg/L in the control influent tank and 93.2 mg/L in the prefermented influent tank.
The impact of prefermentation upon the phosphorus removal characteristics of the activated sludge systems are shown in Tables 5.1 and 5.2. Comparison of effluent P shows that prefermentation lead to an effluent 2.1 mg/L lower than the control system (CAS; Table 5.1). Looking at the mass balance data, a significantly higher anaerobic release was observed due to prefermentation, and also a significantly higher release in the first anoxic zone. The net P release in the prefermented system (PAS) was over 80% higher than the control system, which was surprising since the influent VFA increase of 18.2 mg/L due to prefermentation was less than a 20% increase in the very high influent VFA levels found in the septic wastewater typical of Central Florida.

Table 5.1 Pilot-Scale Phosphorus Concentrations

<table>
<thead>
<tr>
<th>Parameters (mg/L)</th>
<th>PAS</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP influent</td>
<td>11.6</td>
<td>12.4</td>
</tr>
<tr>
<td>Anaerobic SOP</td>
<td>36.7</td>
<td>27.3</td>
</tr>
<tr>
<td>Anoxic I SOP</td>
<td>41.7</td>
<td>33.0</td>
</tr>
<tr>
<td>Anoxic II SOP</td>
<td>12.7</td>
<td>10.5</td>
</tr>
<tr>
<td>Aerobic SOP</td>
<td>4.2</td>
<td>6.3</td>
</tr>
<tr>
<td>Clarifier SOP</td>
<td>4.0</td>
<td>6.7</td>
</tr>
<tr>
<td>% P removal</td>
<td>64.2</td>
<td>49.3</td>
</tr>
<tr>
<td>Apparent Anaerobic P Release</td>
<td>25.1</td>
<td>14.9</td>
</tr>
<tr>
<td>Apparent Anoxic I P Release</td>
<td>5.0</td>
<td>5.7</td>
</tr>
<tr>
<td>Apparent Anoxic II P Uptake</td>
<td>29.0</td>
<td>22.5</td>
</tr>
<tr>
<td>Aerobic P Uptake</td>
<td>8.6</td>
<td>4.2</td>
</tr>
<tr>
<td>Net P Uptake (excluding clarifier)</td>
<td>7.4</td>
<td>6.1</td>
</tr>
</tbody>
</table>
Table 5.2  Pilot-Scale Phosphorus Mass Flux Values

<table>
<thead>
<tr>
<th>Parameters (mg/day)</th>
<th>PAS</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP influent</td>
<td>2794</td>
<td>2995</td>
</tr>
<tr>
<td>Anaerobic SOP Release</td>
<td>4834</td>
<td>2254</td>
</tr>
<tr>
<td>Anoxic I SOP Release</td>
<td>9009</td>
<td>7349</td>
</tr>
<tr>
<td>Anoxic II SOP Uptake</td>
<td>4239</td>
<td>4050</td>
</tr>
<tr>
<td>Net Anoxic SOP Release</td>
<td>4770</td>
<td>3299</td>
</tr>
<tr>
<td>Net SOP Release</td>
<td>9604</td>
<td>5553</td>
</tr>
<tr>
<td>Aerobic SOP Uptake</td>
<td>11356</td>
<td>7093</td>
</tr>
<tr>
<td>Net SOP Uptake (excludes 1840)</td>
<td>1840</td>
<td>1377</td>
</tr>
<tr>
<td>%P in MLSS (via Mass Balance)</td>
<td>10.5</td>
<td>8.8</td>
</tr>
</tbody>
</table>

Nitrogen data also indicated a significantly higher rate of both nitrification and denitrification in the PAS system (Tables 5.3 and 5.4). Higher PHA content could explain the higher denitrification rates, but the nitrification results are unexplained. Respirometric data in batch experiments are being conducted to determine: a) if the higher denitrification rate observed in Table 5.4 is also consistent with a higher rate of nitrate/nitrite respiration when the MLSS is taken from the system and observed in batch mode, and b) if SOUR is significantly different in the aerobic zone MLSS from the two systems and this corresponds to the observed differences in nitrification and simultaneous denitrification (Table 5.4).

Table 5.3  Pilot-Scale Nitrogen Concentrations

<table>
<thead>
<tr>
<th>Parameters (mg/L)</th>
<th>PAS</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TKN Influent</td>
<td>41.4</td>
<td>35.2</td>
</tr>
<tr>
<td>SKN Influent</td>
<td>34.0</td>
<td>32.1</td>
</tr>
<tr>
<td>Ammonia Influent</td>
<td>30.8</td>
<td>28.7</td>
</tr>
<tr>
<td>Nitrate Influent</td>
<td>0.27</td>
<td>0.19</td>
</tr>
<tr>
<td>TKN Effluent</td>
<td>7.3</td>
<td>9.4</td>
</tr>
<tr>
<td>SKN Effluent</td>
<td>6.5</td>
<td>7.8</td>
</tr>
<tr>
<td>Ammonia Effluent</td>
<td>5.1</td>
<td>6.7</td>
</tr>
<tr>
<td>Nitrate Effluent</td>
<td>5.19</td>
<td>2.10</td>
</tr>
</tbody>
</table>
A table showing both glycogen content and PHA concentrations in the two activated sludge pilot-scale trains is shown in Table 5.5. There was a 2.5 fold increase in glycogen degradation from the anaerobic zone to the first anoxic zone and a 15.6% increase in glycogen production from the second anoxic zone to the aerobic zone in the PAS train as compared to the CAS train. The greater glycogen consumption (anaerobic, anoxic I) and biosynthesis (anoxic II, aerobic) corresponded to greater PHA formation in the PAS train. PHA concentrations were greater in the PAS train as compared to the CAS train in every reactor. Despite similar PHA concentrations in the anaerobic zones, there was 16.5% greater PHA concentration in the first anoxic zone, with a corresponding 39.1% increase in PHA production from the anaerobic zone to the first anoxic zone. In the second anoxic zone, however, a 200% increase in PHA degradation from the first to the second anoxic zones in the CAS train as compared to the PAS train, even though anoxic II P uptakes were similar (Table 5.2). This observation may be an artifact since the PHA and glycogen data is much more preliminary than the N and P data (fewer repetitions).
<table>
<thead>
<tr>
<th>Parameters (mg/day)</th>
<th>PAS</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TKN influent</td>
<td>9987</td>
<td>8526</td>
</tr>
<tr>
<td>Assimilated N</td>
<td>1633</td>
<td>1484</td>
</tr>
<tr>
<td>Available N</td>
<td>8354</td>
<td>7042</td>
</tr>
<tr>
<td>Nitrate Produced</td>
<td>6792</td>
<td>5178</td>
</tr>
<tr>
<td>% Nitrification</td>
<td>80.9</td>
<td>73.2</td>
</tr>
<tr>
<td>Nitrate Load to Anaerobic Zone</td>
<td>99</td>
<td>80</td>
</tr>
<tr>
<td>Nitrate Load leaving Anaerobic Zone</td>
<td>66</td>
<td>72</td>
</tr>
<tr>
<td>Anaerobic Zone Denitrification</td>
<td>33</td>
<td>7</td>
</tr>
<tr>
<td>Nitrate Load to Anoxic I</td>
<td>958</td>
<td>434</td>
</tr>
<tr>
<td>Nitrate Load leaving Anoxic I</td>
<td>96</td>
<td>93</td>
</tr>
<tr>
<td>Anoxic I Denitrification</td>
<td>862</td>
<td>341</td>
</tr>
<tr>
<td>Nitrate Load to Anoxic II</td>
<td>6116</td>
<td>2131</td>
</tr>
<tr>
<td>Nitrate Load leaving Anoxic II</td>
<td>3891</td>
<td>727</td>
</tr>
<tr>
<td>Anoxic II Denitrification</td>
<td>2225</td>
<td>1403</td>
</tr>
<tr>
<td>Specific Denitrification Rate in the Anoxic I (mgNO₃/gVSS*day)</td>
<td>55.1</td>
<td>20.4</td>
</tr>
<tr>
<td>Specific Denitrification Rate in the Anoxic II (mgNO₃/gVSS*day)</td>
<td>96.9</td>
<td>81.7</td>
</tr>
<tr>
<td>Simultaneous Denitrification</td>
<td>1992</td>
<td>2900</td>
</tr>
<tr>
<td>Clarifier Denitrification</td>
<td>509</td>
<td>57</td>
</tr>
<tr>
<td>System Denitrification (without clarifier)</td>
<td>5112</td>
<td>4653</td>
</tr>
<tr>
<td>Total System Denitrification</td>
<td>5621</td>
<td>4709</td>
</tr>
</tbody>
</table>
Table 5.5 Intracellular Storage Products

<table>
<thead>
<tr>
<th></th>
<th>PAS AN</th>
<th>PAS AX I</th>
<th>PAS AX II</th>
<th>PAS AE</th>
<th>CAS AN</th>
<th>CAS AX I</th>
<th>CAS AX II</th>
<th>CAS AE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen (mg Glycogen/g MLSS)</td>
<td>96.0</td>
<td>86.0</td>
<td>97.3</td>
<td>104.7</td>
<td>91.8</td>
<td>89.0</td>
<td>90.2</td>
<td>96.6</td>
</tr>
<tr>
<td>PHB Concentration (mg/L)</td>
<td>141.4</td>
<td>253.4</td>
<td>149.8</td>
<td>0.0</td>
<td>138.5</td>
<td>220.5</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>PHV Concentration (mg/L)</td>
<td>50.7</td>
<td>83.2</td>
<td>85.9</td>
<td>0.0</td>
<td>46.6</td>
<td>68.5</td>
<td>41.7</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Respirometry and other experimental techniques are currently being used to quantify the biokinetic parameters used in dynamic modeling (e.g. ASM1 and 2d) using an OUR meter purchased from South Africa (it was developed by the UCT research group led by George Ekama and now commercially available). Preliminary data obtained using techniques similar to those of Wentzel et al. (1995) indicate that PAS influent contained 10% more of both RBCOD and SBCOD than the influent from the control system. Other biokinetic parameters, including the heterotrophic maximum specific growth rate on RBCOD ($\mu_{\text{maxH}}$), the heterotrophic maximum specific growth rate on SBCOD ($K_{\text{MP}}$), and the heterotrophic active biomass concentration ($Z_{\text{BH}}$) will be discussed during the presentation of this paper at the conference.

**Conclusion**

- Prefermentation increased both RBCOD, SBCOD, and VFA content of septic domestic wastewater.
- Prefermentation resulted in increased biological P removal for a highly septic, non-P limited (TCOD:TP<40:1) wastewater. However, in septic, P-limited (TCOD:TP>40:1) wastewater.
wastewater, changes in net P removal due to prefermentation were suppressed, in spite of elevated PHA content, due to limited P availability.

- Prefermentation increased specific anoxic denitrification rates, and in the pilot (COD-limited) study also coincided with greater system N removal.
- The increased anaerobic P release, aerobic P uptakes, and greater specific denitrification rates correlated with greater PHA formation and glycogen consumption during anaerobiosis of prefermented influent.
- Improvements in biological P removal of septic, non-P limited wastewater occurred even when all additional VFA production exceeded VFA requirements using typical design criteria (e.g. 6 g VFA per 1 g P removal).

**Acknowledgements**

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References


CHAPTER 6 IMPROVED P REMOVAL OF COD-LIMITED, SEPTIC, WASTEWATER VIA PREFERMENTATION

Abstract

The potential benefits prefermentation can provide to biological nutrient removal (BNR) are measured and contrasted in septic wastewaters that are both COD-limited (TCOD:TP ratio greater than 40:1) and P-limited (TCOD:TP ratio less than 40:1). For a septic COD-limited wastewater, prefermentation was found to enhance EPBR by 27.7% at a statistical significance level of $\alpha=0.05$ (95% confidence level). However, for septic P-limited wastewaters, prefermentation was not found to increase EBPR. Prefermentation increased specific anoxic denitrification rates for both COD-limited (14.6%) and P-limited (5.4%) wastewaters. This increase in denitrification was statistically significant at $\alpha=0.05$ for COD-limited wastewaters, but not for P-limited wastewaters. Prefermentation increased RBCOD content by 28.8% and VFA content by 18.8%, even though the influent was already highly septic, with initial VFAs as high as 71 mg/L. Additionally, the data collected during this study suggests that anaerobic stabilization is potentially significant when treating warm, septic influent wastewater.

Keywords

BNR, EBPR, prefermentation, volatile fatty acids, denitrification, anaerobic stabilization.
Introduction

Enhanced Biological Phosphorus Removal (EBPR) requires the presence of volatile fatty acids (VFAs) in the anaerobic zone of any biological nutrient removal (BNR) wastewater treatment system. Unless the sewage is strong and septic (i.e. the influent already has a high VFA concentration) VFAs must be produced. This VFA production is accomplished either within the anaerobic zone of the BNR system or it is done prior to the BNR system in a separate anaerobic process called prefermentation. In prefermentation hydrolysis and acidogenic fermentation take place, producing VFAs in a separate step. Prefermenters as a separate unit process were developed by Dr. James Barnard in South Africa along with researchers at the University of Cape Town in the mid 1970s when BNR systems were first developed at full scale. In the United States, however, prefermenters have until recently rarely been considered even when they might arguably have been advantageous. Because of the very few quantitative comparisons of identical systems with and without prefermenters, design engineers often disagree on the necessity of a prefermenter and make decisions based on their prior experience.

Prefermentation of wastewater or primary solids is a common practice associated with Biological Nutrient Removal facilities in many parts of the world although it is only used in a few full-scale installations in the United States to date. Prefermentation technology is associated in the minds of many engineers exclusively with cold climates as an enhancement solely for Enhanced Biological Phosphorus Removal (EBPR) for non-septic wastewaters. It is true that prefermentation technology is used broadly in parts of Canada for that purpose. However prefermentation is practiced widely in Australia (Keller and Hartley, 1997), to some extent in South Africa, and other temperate or even tropical climates.
Prefermenters can be either on-line (the entire wastewater stream is treated) or sidestream (only primary clarifier underflow is treated). The basic on-line prefermenter is simply a primary clarifier operated with a very high sludge blanket, commonly referred to as a Static Prefermenter. These prefermenters are not very efficient, often elevating influent VFAs less than more sophisticated prefermenters (Van Meunch et al., 1996). Static Prefermenters were improved with a recycle to elute VFAs from the sludge blanket and this configuration is referred to as an Activated Primary Tank or APT. Sidestream Prefermenters are reactors that receive the primary clarifier underflow instead of fermenting the entire wastewater flow. They can consist of a single tank, which may or may not be completely mixed, or of a complete mix tank followed by a dedicated thickener. BNR facilities may receive both prefermented solids and liquid from a Sidestream Prefermenter, or may receive only the supernatant, depending on which configuration is used.

Traditionally the function of prefermenters has been to convert a large portion of the slowly degradable influent chemical oxygen demand (COD) into readily available substrate (e.g. VFAs) to drive EBPR in the anaerobic zone. In plants in Western Canada, where prefermentation is very common, consistent effluents of 0.5 mg/L and lower are claimed without chemical polishing for some wastewaters. Reliably going below 1 mg/L without chemical polishing is anecdotally described as routine. However there are obvious disadvantages to prefermentation. One is that the capital costs of primary clarification are incurred while many of the benefits may be lost (i.e. no direct reduction in oxygen demand or secondary waste sludge production although increased denitrification may mitigate this). In addition in countries where there is a phosphate detergent ban such as the United States, it is not as difficult to meet effluent standards and chemical polishing costs can be significantly less than in countries with
significantly higher influent phosphorus concentrations. Further in the southern United States, and seasonally in the north, raw wastewater is often at least partially septic. In Florida domestic wastewater is very septic and raw wastewater concentrations may routinely exceed 50 mg/L total VFAs even in the winter. As a result it is often presumed that there will be little benefit to prefermentation in a warm climate, and this will be addressed in this paper.

Prefermenters have historically been an inconsistently utilized unit process because they are frequently used with BNR plants by some design communities, while other design communities have not (at least in the past) seriously considered them as an option. Part of the reason for this is the absence of quantitative information on the process and effluent changes resulting from prefermentation for a variety of wastewaters and climates. Most information is from full scale applications and is anecdotal (e.g. we have a plant with prefermentation that always meets 0.5 mg/L P, we have a plant without prefermentation that always goes below 1 mg/L P, etc…), with only a few direct comparisons existing in the literature (e.g. Danesh and Oleskiewicz, 1997).

This pilot scale study was conducted with two basic objectives:

1. To conduct controlled comparisons isolating prefermentation as an experimental variable using parallel BNR processes with prefermentation, and without prefermentation, for both COD-limited and P-limited wastewaters.

2. To determine if prefermentation might be beneficial in niches for which it has not traditionally been used; i.e. to enhance denitrification kinetics as opposed to the normal niche of enhancing biological P removal, or enhancement for septic wastewaters in warm climates as opposed to the normal niche of fresh/non-septic wastewaters in cold or temperate climates.
Materials and Methods

Pilot Scale System

In order to explore the potential benefits of prefermentation to BNR, two parallel pilot scale activated sludge wastewater treatment trains were constructed. The prefermented activated sludge (PAS) train, received raw influent augmented with prefermented primary solids from an off-line static prefermenter. Primary solids taken from the only operational full scale municipal wastewater treatment plant (WWTP) primary clarifier in Central Florida (Altamonte Springs Water Reclamation Facility, Altamonte Springs, FL) were used to feed the experimental off-line prefermenter. The off-line prefermenter was maintained at an SRT of 10 days. The control train, which did not receive prefermented solids, was called the control activated sludge (CAS) system. In order to equalize COD loading between control and prefermented activated sludge trains, prefermented primary solids were added to the PAS train influent, while an equal volume of fresh non-prefermented primary solids were added to the control influent.

The flow configuration selected for the activated sludge systems of the pilot scale WWTP was the Modified University of Cape Town (MUCT) configuration for biological nutrient removal (Figure 6.1). The MUCT configuration is similar to that of the UCT configuration, with the exception that an extra anoxic zone is included. The first anoxic zone receives the RAS, while the second anoxic zone received the NARCY recycle. The ARCY recycle returns biomass from the first anoxic zone to the anaerobic zone. The purpose of the first anoxic zone is to provide extra protection to the anaerobic zone by further depleting the oxygen and nitrates which
might be present in the RAS. The pilot scale system, as constructed, had one anaerobic zone, two anoxic zones, and one aerobic zone (Figure 6.1).

Figure 6.1 Schematic of Pilot Scale System

The pilot scale systems were operated within the East Orange County Water Reclamation Facility, or EOCWRF (Orange County, Florida) in an enclosed room with access to a tap with raw domestic wastewater. Fresh influent was provided for the systems daily and placed into two separate polyethylene tanks, one for the PAS train and one for the CAS train. At the end of a daily cycle, any remaining influent was dumped and the sides of the influent tank were scrubbed prior to the addition of fresh influent. A single submersible pump (Little Giant Pump Co., Oklahoma City, OK) provided the mixing energy necessary to keep the influent tanks sufficiently mixed without aerating the influent. Peristaltic pumps manufactured by Cole-Parmer Instrument Company (Vernon Hills, IL) were used to maintain design flow rates for the influent line and all recycle lines. Mixing energy for both the anaerobic and anoxic zones of the activated
sludge systems was provided by 50-rpm gear motors (Grainger, Lake Forest, IL). Aquarium aerators (Rena, Annecy, France) provided mixing energy for the aerobic zones, as well as aeration. The secondary clarifiers had surface skimmers and bottom scrapers powered by 1-rpm gear motors (Grainger, Lake Forest, IL), and were constructed from 50-liter cylindrical tanks with a conical bottom. The off-line prefermenter was constructed from a 50-liter cylindrical polyethylene storage container. The anaerobic and anoxic zones of the activated sludge reactor were constructed from 8-inch square polyethylene reactors, with each reactor having a liquid volume of approximately 7 liters. The aerobic zone activated sludge reactors were constructed from 20-liter cylindrical polyethylene reactors. The entire activated sludge system was hard-plumbed with 1-inch diameter schedule 40 PVC. A series of 1-inch ball valves allowed for the rerouting of flows to multiple locations, as desired by the operators. These ball valves allowed for multiple recycle line exit points, a bypass line for the first anaerobic zone, and split-feed lines to allow for step feeding.

Cleaning techniques were also found to be of tremendous importance in maintaining steady operation of the pilot system. Specifically, a daily scrubbing of the sidewalls of all reactors of the activated sludge system, especially the aerobic tank, was necessary to prevent the build-up of a biofilm. The sidewalls of the secondary clarifiers were also gently scraped above the sludge blanket on a daily basis. This was necessary in order to maintain a more steady effluent solids concentration. Specifically, if the sidewalls of the secondary clarifier were not scraped daily, a biofilm would accumulate on the sidewalls, and would eventually slough off, thereby elevating the effluent solids concentration. It was also important to clean the 1-inch PVC lines connecting the anaerobic, anoxic, and aerobic tanks together, as biofilms could easily grow in those lines. To prevent clogging in recycle lines, the barb fitting where the 1-inch PVC
was connected to the 3/8 inch ID neoprene tubing was periodically brushed clean. This connection was located where the neoprene tubing passed through the peristaltic pump head.

In its natural unaugmented condition, the raw wastewater at East Orange County is septic (total VFA content averaging 74.8 mg/L prior to prefermentation) and P limited (i.e. TCOD: TP ratio was greater than 40:1 – WEF, 1998). In order to conduct research on a COD limited wastewater, sufficient phosphorus in the form of potassium diphosphate was added to the raw influent in order to decrease the TCOD: TP ratio to 29.9 from its unaugmented value of 49.9. In this paper the results obtained from the P-limited phase are contrasted with those from the COD-limited phase.

Difficulties in primary solids separation for the EOCWRF influent led to the development of an off-line prefermenter during this study. Primary solids were taken from a full-scale primary clarifier located at the Altamonte Springs Water Reclamation Facility (Altamonte Springs, FL) and added on a daily basis to a 20-liter cylindrical prefermentation tank which was maintained at 10 day solids retention time (SRT). Prefermented primary solids were then transferred to the 600-liter influent tank of the prefermented activated sludge (PAS) train at a rate of 2 liters per day. In order to equalize COD loading between the PAS and control activated sludge (CAS) trains, an equivalent amount of the same unprefermented primary solids fed to the prefermenter were also added to the CAS influent tank on a daily basis.

**Chemical Analysis**

Total suspended solids (TSS) and volatile suspended solids (VSS) were determined according to Standard Methods (APHA et al., 1995). Total phosphorus (TP) samples underwent
persulfate digestion as outlined in Standard Methods 4500-P B(5), followed by the
vanadomolybdophosphoric acid colorimetric method 4500-P C (APHA, et al., 1995). Soluble
orthophosphorus (SOP) were determined using the vanadomolybdophosphoric acid colorimetric
method 4500-P C in Standard Methods (APHA, et al., 1995). Chemical oxygen demand (COD)
was determined by following section 5220 C in Standard Methods (APHA, et al., 1995).
Organic nitrogen (both total Kjeldahl nitrogen and soluble Kjeldahl nitrogen) and ammonia
nitrogen were analyzed by methods 4500-Norg A and 4500-NH₃ C, respectively, of Standard
Methods (APHA et al., 1995). Nitrate was determined using a Dionex 2000 I/SP ion
chromatograph (Sunnyvale, CA) with a CDM-3 conductivity detector and a 4270 integrator
using a method similar to that found in Standard Methods 4500-NO₃- C (APHA, et al., 1995).
Samples were analyzed for short-chain volatile fatty acids (SCVFAs) following Supelco Bulletin
Scientific Instruments, Inc., Columbia, MD) equipped with a flame ionization detector (FID) was
utilized to conduct the analysis. A 3 mm inner diameter glass column with 60/80 Carbopack
C/0.3% Carbowax 20M/0.1%H₃PO₄ packing (Supelco Inc., Bellefonte, PA) was used to separate
the various SCVFAs. Helium, at approximately 30 mL/min, was selected as the carrier gas. The
injection port and the FID were maintained at 200 °C. The oven of the gas chromatograph was
programmed to begin sample analysis at 105 °C, remaining at 105 °C for two minutes, before
increasing at a rate of 5 °C per minute to 150 °C, and to hold at 150 °C for an additional two
minutes, resulting in a total run time of 13 minutes per sample. PHAs were analyzed by a gas
chromatographic method (Liu, 2001) using a DB-1 capillary column. The carrier gas, helium,
was maintained at a velocity of 2 ml/min and as the make up gas (25 ml/min). The injection port
and detector were maintained at a temperature of 230 °C. The column temperature started at 100
°C for 2 minutes, was increased by 20 °C per minute to 160 °C, and maintained at 160 °C for an additional 10 minutes, resulting in a run time of 15 minutes. Prior to injection, sludge samples were freeze-dried using a lyophilizer and then run through a digestion. About 0.15 grams of dry sludge was put into 5.0 ml Wheaton V vials. 2 ml of benzoic acid (50 mg/100 mL) in chloroform was added to the vial for use as an internal standard and solvent, respectively. Next, 2 ml of 20% \( \text{H}_2\text{SO}_4 \) in methanol was added as the digestion/esterification reagent (methyl esters of the PHA are what is actually extracted into the chloroform phase). The vials were then placed inverted into a 100 °C oven for 18 hours. It is also advisable to retighten the vial caps early during the digestion (within 2 hours of starting), and to run duplicates, as approximately 10% of the vials develop leaks during the digestion process. After cooling to room temperature, 1 mL of deionized was added to the vial and shaken with a vortexer for 5 minutes. After the washing step was completed, the chloroform phase was removed from the vial with a 10 \( \mu \)L syringe and placed into a 1.5 ml GC vial. Carbohydrates were determined by the anthrone method (ASM, 1981). Readily biodegradable chemical oxygen demand (RBCOD) was determined following techniques developed both by Ekama et al. (1986) and Wentzel et al. (1995).

**Sample Collection and Monitoring**

During all phases of this research project, activated sludge trains were operated until steady state conditions were met (e.g. greater than 3 MCRTs). Mass balance sampling events took place between one and three times per week. Composite samplers (Isco Inc., Lincoln, NE) were used on influent samples. All other samples taken during the study were grab samples. All sample analyses were conducted within 24 hours after sampling (most within 4 hours), so
beyond refrigeration, no sample storage protocols were established (e.g. no acid additions). All samples were filtered immediately upon removal from the activated sludge system. Mixed liquor reactor samples were first centrifuged on site immediately after sampling, then filtered with Whatman 934 AH glass fiber filters, and finally membrane filtered with 0.45 µm membrane filters. Field parameters, such as DO, pH, temperature, SVI, ZSV, and both in-situ and ex-situ OURs were run concurrently with sampling events during the pilot scale study.

Results

Effects of Prefermentation on Influent Characteristics

Composite samplers (Isco Inc., Lincoln, NE) on both the influent tanks allowed for the impact of prefermentation upon influent characteristics to be measured. Specifically, prefermentation was found to increase the VFA content within the PAS influent by 14.4 mg/L as COD (an increase of 18.8%). Note that the control influent wastewater was already highly septic, with a VFA content averaging 74.8 mg/L as COD. The only VFAs detected in the influent tanks were acetate and propionate. Prefermentation was not found to significantly alter the ratio of acetate to propionate within the influent in this study, with acetate content averaging about 70% of the VFAs as COD for both the PAS and CAS influents. Additionally, prefermentation was also found to significantly increase the RBCOD content found within the influent wastewater. Prefermentation increased the RBCOD content of the PAS influent by 28.2% (from 110 mg/L in the CAS influent to 141 mg/L for the PAS influent) in the COD-
limited phase of the study and by 29.3% (from 99 to 128 mg/L) for the P-limited phase of the study.

**Effects of Prefermentation on EBPR**

One of the major results of the pilot scale study was that septic, COD-limited wastewaters prefermentation increased the net P removal, which is the ultimate objective of EBPR. Figure 6.2 compares the soluble ortho phosphorus (SOP) profiles of the PAS and CAS trains for the COD-limited and P-limited phases of the pilot study.

![Figure 6.2 SOP Profile for the COD-Limited and P-Limited Phases](image-url)
Using a paired difference test between two population means, it can be shown that the effluent phosphorus concentration for the PAS train (3.4 mg/L) was superior to that of the CAS train (4.7 mg/L) during the COD-limited phase, and statistically significant at an $\alpha$ value of 0.05 (Mendenhall and Sincich, 1995). However, at $\alpha=0.05$, there was no significant statistical difference between the effluent SOP concentration from the PAS (0.8 mg/L) and CAS (1.0 mg/L) trains for the P-limited phase. This implies that for septic wastewaters, the initial ratio of TCOD:TP in the influent will determine if prefermentation has a significant impact upon net P removal. For wastewaters that are non-septic (i.e. have little initial VFA content), the potential benefits of prefermentation would probably be significantly greater. Also, it is important to keep in mind that this wastewater was already highly septic (74.8 mg/L total VFA as COD) prior to prefermentation, and that prefermentation was still able to improve EBPR. The literature indicates that a VFA:TP ratio of between 4 to 10 mg VFA per mg P is necessary for good phosphorus removal. Metcalf and Eddy (2003) cites a conservative 10:1 ratio of VFA:P, while Daigger et. al. (1993) and anecdotal suggestions specify VFA:TP ratios of 7:1 and 4:1, respectively. Much of the seeming contradictions in the literature may be due to temperature. Generally, the 4:1 ratio has been ascribed by practitioners in western Canada where there are cold but stable temperatures allowing for psychrophilic EBPR. The temperatures found in this study were quite elevated in contrast. Table 6.1 shown below displays the ratio of VFA:TP found during both the COD-limited and P-limited phases:

<table>
<thead>
<tr>
<th>Table 6.1 VFA:TP Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS COD-limited</td>
</tr>
<tr>
<td>VFA:TP ratio</td>
</tr>
</tbody>
</table>
The superior P removal found during the COD-limited phase implies that improved P removal could still be realized by increasing the VFA:TP ratio from 6.6 to 7.8. However there was no net improvement in EBPR from increasing the VFA:TP ratio from 10.6 to 12.3. Thus the data implies that for this wastewater the optimal VFA:TP ratio was between 7.8 and 10.6.

An analysis of the mass flux of phosphorus through the individual reactors of the pilot systems yields additional insight into the potential of prefermentation to increase P removal. Table 6.2 shows the results of this mass flux analysis on phosphorus:

<table>
<thead>
<tr>
<th>Parameters (mg/day)</th>
<th>COD-limited PAS</th>
<th>COD-limited CAS</th>
<th>P-limited PAS</th>
<th>P-limited CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP influent</td>
<td>2917</td>
<td>2905.1</td>
<td>1680.8</td>
<td>1683.7</td>
</tr>
<tr>
<td>Anaerobic SOP Release</td>
<td>4746</td>
<td>2910.2</td>
<td>2801.4</td>
<td>1956.0</td>
</tr>
<tr>
<td>Anoxic I SOP Release</td>
<td>8567</td>
<td>7178.5</td>
<td>6359.7</td>
<td>6643.5</td>
</tr>
<tr>
<td>Anoxic II SOP Uptake</td>
<td>5481</td>
<td>3450.5</td>
<td>1355.4</td>
<td>1069.4</td>
</tr>
<tr>
<td>Net SOP Anoxic Release</td>
<td>3086</td>
<td>3728.0</td>
<td>5004.3</td>
<td>5574.1</td>
</tr>
<tr>
<td>Total SOP Release</td>
<td>13313</td>
<td>10088.7</td>
<td>9161.1</td>
<td>8599.5</td>
</tr>
<tr>
<td>Aerobic SOP Uptake</td>
<td>10120</td>
<td>8461.1</td>
<td>9250.0</td>
<td>8924.3</td>
</tr>
<tr>
<td>Clarifier SOP Uptake</td>
<td>-211</td>
<td>-84.8</td>
<td>41.6</td>
<td>42.0</td>
</tr>
<tr>
<td>Total SOP Uptake</td>
<td>15390</td>
<td>11826.8</td>
<td>10647.0</td>
<td>10035.7</td>
</tr>
<tr>
<td>SOP Uptake:SOP Release Ratio</td>
<td>1.16</td>
<td>1.17</td>
<td>1.16</td>
<td>1.17</td>
</tr>
<tr>
<td>Net SOP Uptake</td>
<td>2077</td>
<td>1738</td>
<td>1486</td>
<td>1436</td>
</tr>
<tr>
<td>%P in MLSS as calculated via MB</td>
<td>10.0</td>
<td>8.7</td>
<td>6.5</td>
<td>6.3</td>
</tr>
</tbody>
</table>

When comparing the %P in MLSS as calculated via a mass balance, it can be seen that prefermentation increased the %P content of MLSS for a COD-limited wastewater (10.0% vs. 8.7%) but not for a P-limited wastewater (6.5% vs. 6.3%). The result of these changes may also be observed in Figure 6.2. Of further interest is the marked difference in SOP release and uptake.
between the PAS and CAS trains for both the COD-limited and P-limited phases. The PAS trains had greater SOP release in the anaerobic zone for both phases. This correlated with the greater amount of VFAs found within the PAS train due to prefermentation. In addition, superior SOP uptake in both Anoxic II and the Aerobic zone of the PAS trains was noted for both phases. During the COD-limited phase, a 32.0% increase in the total SOP release and a 30.1% increase in the total SOP uptake was found in the PAS train as compared to the CAS train. During the P-limited phase, only a 6.5% increase in total SOP release and a 6.1% increase in SOP uptake was noted in the PAS train as compared to the CAS train. However, for both the COD-limited and P-limited phases, the SOP Uptake: SOP Release ratio remained consistent between the two trains, as shown in Table 6.2.

Other parameters of importance to EBPR were also measured, including PHAs (both PHB and PHV) and glycogen. For both COD-limited and P-limited phases, PHA and glycogen concentrations were higher in the PAS train as compared to the CAS train. Figures 6.3 and 6.4 show the PHA profiles of the COD-limited phase and the P-limited phase, respectively. Figure 6.5 shows the glycogen profile for the COD-limited phase. The glycogen profile for the P-limited phase was similar. Note that the apparent increase in the concentration of glycogen from the Anaerobic zone to Anoxic I is an artifact of the MUCT flow configuration. A mass flux analysis of glycogen indicated there is glycogen depletion across both the Anaerobic zone and Anoxic I, which corresponds to the increase in PHA concentrations illustrated in Figures 6.3 and 6.4.
Figure 6.3 PHA Profile for the COD-Limited Phase

Figure 6.4 PHA Profile for the P-Limited Phase
Nitrogen forms, including nitrate (NO$_3$-N), ammonia (NH$_4$-N), soluble Kjeldahl nitrogen (SKN), and total Kjeldahl nitrogen (TKN), were measured during the course of this study. All phases had similar nitrogen profiles, with differences coming only in the absolute values of the measured parameters. The greatest difference in concentration of N-forms measured during this study was found in the effluent nitrate values. In the COD-limited phase, the PAS train had an effluent nitrate concentration of 12.3 mg/L NO$_3$-N, as compared to a 10.6 mg/L value for the CAS train. In the P-limited phase, the PAS train had an effluent nitrate value of 11.9 mg/L NO$_3$-N, while the CAS nitrate effluent averaged 11.4 mg/L. For both phases, the CAS train had a
statistically significant lower measured nitrate effluent value than those from the PAS train, using a paired difference test between two population means and an $\alpha$ value of 0.05 (Mendenhall and Sincich, 1995).

Nitrogen mass balances were conducted upon the data generated during this study in order to verify the quality of the data collected using the equation (1).

\[
\Sigma TN_{\text{influent}} = \Sigma \Delta NO_3 \text{ denitrified} + N_{\text{assimilated}} + \Sigma SN_{\text{effluent/WAS}}
\]  

(1)

where:

$\Sigma TN_{\text{influent}} = \text{sum of total nitrogen in the influent, mg/d}$

$\Sigma \Delta NO_3 \text{ denitrified} = \text{sum of nitrate denitrified in unaerated zones, mg/d}$

$N_{\text{assimilated}} = \text{nitrogen assimilated into growth of new biomass, mg/d}$

$\Sigma SN_{\text{effluent/WAS}} = \text{sum of soluble nitrogen in the effluent and waste activated sludge, mg/d}$

Table 6.3 shows the results of nitrogen mass balances conducted during this study.

Of particular interest is the good agreement found in the nitrogen mass balances, with % agreement values ranging from 88.8% to 100.6%. Three of the four nitrogen mass balances shown in Table 6.3 show good agreement, easily within the error of the measurements. The COD-limited phase CAS train had a N mass balance disagreement of 1168 mg/day, or 11.2% of the balance. This difference was either due to simultaneous denitrification, or due to analytical error.
Anoxic denitrification rates were enhanced by prefermentation in both the COD-limited and P-limited phases of the pilot study. Table 6.4 compares specific anoxic denitrification rates measured in the second anoxic zone of the pilot systems.

Table 6.4 Specific Anoxic Zone Denitrification Rates in the Pilot Scale Study (mg NO₃⁻ / g VSS*Day)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PAS</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD-limited</td>
<td>82.9</td>
<td>72.3</td>
</tr>
<tr>
<td>P-limited</td>
<td>77.6</td>
<td>73.6</td>
</tr>
</tbody>
</table>

Statistically, at α = 0.05, paired difference tests indicate that the anoxic denitrification rate measured in the PAS train was statistically superior to that measured in the CAS train during the COD-limited phase of the pilot study. However, at the same α level, no difference could be detected between the two trains during the P-limited phase data (Mendenhall and Sincich, 1995).
Mass balances conducted around Anoxic II on PHA, glycogen, and soluble COD were not able to identify the substrate driving the superior denitrification rates found in Anoxic II during the COD-limited phase, as the depletion of these three compounds were similar for both the PAS and CAS trains during the COD-limited phase. This may imply that there were differences in the biodegradability of the sCOD.

An analysis of Tables 6.3 and 6.4 reveals an apparent contradiction – the statistically significant (95% confidence interval) difference in denitrification rates for the COD-limited phase corresponds to a higher soluble nitrogen in the effluent of the PAS train (Table 6.3). A comparison of the nitrogen forms in the effluent for the PAS and CAS trains for the COD-limited phase revealed that the nitrogen forms were similar with the exception of nitrate, with 16.3% more nitrate being found in the PAS effluent. Further analysis of Table 6.3 reveals that the nitrogen mass balance for the CAS train during the COD-limited phase did not agree as well as the other phases of the study (88.8% agreement for the CAS COD-limited phase vs. an average of 99% agreement for the other phases), indicating that sampling or analytical error may have occurred in the COD-limited CAS train. It is also possible that the CAS train experienced simultaneous denitrification, and the other trains did not. This would also explain how the PAS could have superior denitrification rates but a higher effluent NO₃.

Effects of Prefermentation on COD Mass Balances

In order to further test the continuity of the data generated from the pilot system, a mass balance on chemical oxygen demand (COD) was conducted. The object of this mass balance was to verify that the mass of COD entering the system was accounted for, either through
various biological activities of the microorganisms in the activated sludge system, or through leaving the system via the effluent and waste activated sludge (WAS). This particular COD mass balance was conducted on a system wide basis, with the boundary conditions encompassing the entire pilot plant. Equation 2 provides the framework from which this COD mass balance was conducted:

\[
M_{\text{COD, influent}} = M_{\text{COD, effluent}} + M_{\text{COD, WAS}} + M_{\text{COD, oxidized}} \quad (2)
\]

Where:

\[
M_{\text{COD, influent}} = \text{mass of COD in the system influent, mg COD/d}
\]

\[
M_{\text{COD, effluent}} = \text{mass of COD in the system effluent, mg COD/d}
\]

\[
M_{\text{COD, WAS}} = \text{mass of COD in the waste sludge, mg COD/d}
\]

\[
M_{\text{COD, oxidized}} = \text{mass of COD oxidized in the system, mg COD/d}
\]

Further defining some of the above terms:

\[
M_{\text{COD, effluent}} = (TCOD_{\text{effluent}}) (Q_{\text{effluent}}) \quad (3)
\]

\[
M_{\text{COD, WAS}} = (Q_{\text{WAS}}) (MLVSS_{\text{WAS}}) (f_{CV}) \quad (4)
\]

Where:

\[
TCOD_{\text{effluent}} = \text{concentration of total COD in the effluent, mg COD/L}
\]

\[
Q_{\text{effluent}} = \text{flow rate of effluent, L/d}
\]

\[
Q_{\text{WAS}} = \text{flow rate of waste activated sludge, L/d}
\]
MLVSS\textsubscript{WAS} = MLVSS of the waste activated sludge, mg VSS/L

\( f_{CV} \) = ratio of COD:VSS of waste activated sludge, 1.42 mg COD/mg VSS

In order to determine the mass of COD oxidized in the system, it must be recognized that the total quantity of oxygen consumed in the aerobic reactors consists of both carbonaceous and nitrogenous oxygen demand. The carbonaceous oxygen demand occurs as a result of the complete oxidation of reduced organics present in the pilot plant influent to \( \text{CO}_2 \) and \( \text{H}_2\text{O} \), with \( \text{O}_2 \) serving as the terminal electron acceptor. The nitrogenous oxygen demand occurs as a result of nitrification, in which \( \text{NH}_4^+ \) is biologically transformed to \( \text{NO}_3^- \) in an aerobic environment, thereby resulting in an oxygen demand. The nitrogenous oxygen demand is calculated by determining the mass of nitrate produced in the aerobic zone, and then multiplying the mass of nitrate produced by 4.57, which is the mass in \( \text{O}_2 \) (mg) required to produce each mg of nitrate via nitrification. The carbonaceous oxygen demand is then determined by subtracting the nitrogenous oxygen demand from the oxygen uptake rates measured in the aerobic zone. Note that equation 5 assumes that simultaneous denitrification was negligible.

\[
M_{\text{NO}_3^- \text{ produced}} = \Sigma M_{\text{NO}_3^- \text{ exiting aerobic zone}} - \Sigma M_{\text{NO}_3^- \text{ entering aerobic zone}} \tag{5}
\]

\[
M_{\text{COD, aerobic}} = (\text{OUR}_{\text{aerobic}}) (V_{\text{aerobic}}) - (M_{\text{NO}_3^- \text{ produced}}) (4.57) \tag{6}
\]

Where

\( M_{\text{COD, aerobic}} = \) carbonaceous oxygen demand, mg COD/d

\( \text{OUR}_{\text{aerobic}} = \) oxygen uptake rate measured in the aerobic zone, mg O/L/d

\( V_{\text{aerobic}} = \) volume of the aerobic reactor, L
Additionally, since the MUCT design of this pilot plant also allows for denitrification in the two separate anoxic zones, one must account for the oxygen equivalents of the amount of organic matter that would be oxidized during the denitrification process in which NO$_3^-$ is used as the terminal electron acceptor. Quantitatively this is done through the use of the conversion factor 2.86 mg O$_2$ per mg NO$_3^-$ denitrified (see equation 7).

\[
M_{\text{COD, denitrified}} = (M_{\text{NO}_3^- \text{denitrified}}) (2.86) \quad (7)
\]

Where

- $M_{\text{COD, denitrified}}$ = mass of COD oxidized during denitrification, mg COD/d
- $M_{\text{NO}_3^- \text{denitrified}}$ = mass of nitrate denitrified in anoxic zones, mg NO$_3^-$/d

Combining equations 5, 6, and 7 to determine the total amount of COD oxidized:

\[
M_{\text{COD, oxidized}} = (\text{OUR}_{\text{aerobic}})(V_{\text{aerobic}}) - (M_{\text{NO}_3^- \text{produced}})(4.57) + (M_{\text{COD, denitrified}}) * (2.86) \quad (8)
\]

In order to calculate the % agreement of the COD mass balance, equation 9 can be utilized:

\[
\% \text{ COD agreement} = \frac{\text{COD output}}{\text{COD input}} = \frac{(M_{\text{COD, effluent}} + M_{\text{COD, WAS}} + M_{\text{COD, oxidized}})}{(M_{\text{COD, influent}})} \quad (9)
\]
Based on equations 2 through 9 presented in the preceding discussion, Table 6.5 shows the terms in a COD mass balance as illustrated in equation 2, and Table 6.6 shows the % agreement of the COD mass balances for the COD-limited and P-limited phases of this study.

Table 6.5  COD Mass Balance

<table>
<thead>
<tr>
<th></th>
<th>COD-limited PAS</th>
<th>COD-limited CAS</th>
<th>P-limited PAS</th>
<th>P-limited CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_{\text{COD, influent}}$ mg/d</td>
<td>87336</td>
<td>86458</td>
<td>82483</td>
<td>85472</td>
</tr>
<tr>
<td>$M_{\text{COD, effluent}}$ mg/d</td>
<td>13452</td>
<td>12178</td>
<td>14258</td>
<td>13893</td>
</tr>
<tr>
<td>$M_{\text{COD, WAS}}$ ¹ mg/d</td>
<td>17396</td>
<td>17179</td>
<td>19162</td>
<td>18691</td>
</tr>
<tr>
<td>$M_{\text{COD, oxidized}}$ ² mg/d</td>
<td>30635</td>
<td>26520</td>
<td>30554</td>
<td>29086</td>
</tr>
<tr>
<td>COD Loss mg/d</td>
<td>25853</td>
<td>30581</td>
<td>18509</td>
<td>23802</td>
</tr>
</tbody>
</table>

¹ assumes $f_{CV} = 1.42$

² includes oxygen inputs from recycle lines, oxidation in the secondary clarifier, and diffusion from atmosphere

Table 6.6  COD Mass Balance % Agreement¹

<table>
<thead>
<tr>
<th></th>
<th>% COD Agreement PAS</th>
<th>% COD Agreement CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I (COD-limited)</td>
<td>70.4</td>
<td>64.6</td>
</tr>
<tr>
<td>Phase III (P-limited)</td>
<td>77.6</td>
<td>72.2</td>
</tr>
</tbody>
</table>

¹ assumes $f_{CV} = 1.42$

As can be seen from Table 6.6, the COD mass balance % agreements do not approach 100% for either the COD-limited phase or the P-limited phase. The values shown in Table 6.6
also take into account oxygen inputs from both internal recycle lines and from the liquid/atmosphere interface. Two recycle lines, the NARCY and the RAS, input oxygen into non-aerated zones. This oxygen input was calculated by multiplying the relevant flow rate by the dissolved oxygen (DO) measured in the reactor from which the recycle line originated. For example, the NARCY flow rate was multiplied by the DO measured in the aerobic zone to determine the oxygen input from the NARCY into Anoxic II. Oxygen input from the atmosphere/liquid interface was determined by a batch test in which the DO increase of tap water within the reactors used in the pilot study was measured vs. time. The tap water was spiked with sodium sulfite to drop the initial DO level, along with cobalt (a catalyst facilitating the initial DO drop). Results indicated that oxygen input from the atmosphere/liquid interface was trivial, averaging just under 300 mg/day per reactor.

The potential of $f_{cv}$, the COD content of MLVSS, to significantly impact the COD mass balance, was investigated (i.e. the sensitivity of the mass balance to $f_{cv}$). It is possible that $f_{cv}$ could be slightly different from reactor to reactor, and that difference could potentially impact the COD mass balance. After performing theoretical calculations, however, it can be shown that this impact is minimal. For example, the difference between using $f_{cv}$ values of 1.42 vs. 1.48 results in only a 4% impact on COD mass balances. An additional calculation was conducted to determine the $f_{cv}$ value that would be required to account for the discrepancy in the COD mass balance. This required value for $f_{cv}$ averaged 3.1 for both phases of this study, clearly an unrealistic number when compared to the $f_{cv}$ value of 1.42 which is commonly used in the literature for activated sludge systems operating at steady state.

The lack of agreement found within the system-wide COD mass balances for both the COD-limited and the P-limited phases indicates one of two possible problems:
1. Analytical error

2. The existence of some phenomena which accounts for the observed loss of COD

It would be easy to speculate that analytical error was the cause of the discrepancy but the very tight N mass balances in Table 6.3 make this seem less likely. In addition, extensive QA/QC validated the COD data, including blanks, standard curves, replicates, and % recoveries. So assuming analytical error was not to blame, what evidence is there in the data collected that points to some phenomena causing the observed COD loss?

After reviewing the literature, one possible explanation of the discrepancies found within the COD mass balances is anaerobic stabilization. Anaerobic Stabilization (AnS) can be defined as COD removal (transfer to the gas phase or anaerobic oxidation) due to biological activity in the anaerobic zone of a BNR system. Barker and Dold (1995) report than COD balances on EBPR systems were consistently lower than those for conventional aerobic activated sludge systems, with some EBPR systems showing COD balances of less than 70% (thereby leaving 30%+ of the disappearance of the influent mass of COD unexplained). Specifically, anaerobic-anoxic-aerobic flow configurations, such as Phoredox, 3-stage Bardenpho, Johannesburg, UCT, and MUCT, resulted in an average % COD mass balance agreement value of 78%, with a minimum of 61% and a maximum of 89%. The average percentage agreement of COD mass balances for EBPR systems treating domestic wastewater was 78%, with enhanced culture EBPR systems fed with acetate achieving an average COD balance of 91%. In the same study, parallel trains without anaerobic zones consistently gave COD mass balances approaching 100%. Studies conducted by Wable and Randall (1992 and 1994) and Randall et al (1994) indicate that
AnS values of 15 – 55% of the theoretical oxygen requirement were measured in laboratory and pilot-scale studies. One possible explanation of AnS is the production of reduced gases in the anaerobic zone, such as hydrogen (H\textsubscript{2}) or methane (CH\textsubscript{4}). Clearly if these gases were produced in significant quantities, this could help explain the phenomena of AnS. However, Wable and Randall (1994) developed a method to measure H\textsubscript{2} and CH\textsubscript{4} production in the anaerobic zone of EBPR systems, and found that less than 1% of the measured AnS values were attributed to H\textsubscript{2} and CH\textsubscript{4} production. Only in a system with influent feed supplemented with formate was CH\textsubscript{4} generation found to be significant. A second theory explaining the phenomena of AnS is the hypothesis that fermentation in the anaerobic reactor results in the production of volatile compounds, which are then released from the system under aerobic conditions. However, it seems unlikely that this hypothetical volatilization mechanism is responsible for AnS, as these volatile fermentation products are typically readily biodegradable, and should be removed from the system prior to the aerobic zone (Barker and Dold, 1995). A third potential explanation for AnS is that an external oxidant, other than oxygen, enters the system as a dissolved gas, such as nitrogen (involved in nitrogen-fixation) and carbon dioxide (involved in carbon-fixation) (Wable and Randall, 1994). A fourth possible explanation for AnS involves the limitations of the COD test to accurately measure all reduced species. Wable and Randall (1994) showed evidence that some reduced species, such as NADH, can effectively resist oxidation by the dichromate oxidant under the COD test conditions. It also speculated that a fraction of the incoming COD might be oxidizable by the COD test, but not during the standard 2-hr duration of the COD test.

Evidence for anaerobic stabilization within the data collected during this study is circumstantial in nature, as no attempts to directly measure anaerobic stabilization, or its potential mechanisms, were made. The first circumstantial piece of evidence can be found in the
N mass balances. The N mass balances were much tighter (i.e. closer to 100% agreement) than the COD mass balances, as can be seen in reviewing tables 3 and 5. This fact, while it may increase faith in the analytical measurements reported in this study, is not evidence in and of itself of anaerobic stabilization. Two other calculations were conducted- cell yield calculations and a mass balance of PHAs, glycogen, and COD around the anaerobic reactor - which make a stronger case for anaerobic stabilization.

Typical values of biomass yield (Y) for aerobic heterotrophic biomass growing on carbohydrates varies between 0.48 and 0.72 mg biomass as COD per utilized substrate COD (Grady, et al., 1999). For bacteria utilizing domestic wastewater, these yields are lower, typically in the range of 0.3 - 0.6 mg biomass as COD per utilized substrate COD (Metcalf & Eddy, 2003). The left side of Table 6.7 shows the yields calculated for both phases during this study. Note that they are significantly lower than what would ordinarily be expected. However, if anaerobic stabilization were a real phenomena, and COD is lost in some manner prior to being incorporated into new biomass or energy for cell maintenance, the calculated yield values would increase into a more acceptable range. To explain, the denominator of yield calculations is substrate utilized, determined by subtracting the total COD in the effluent from the total influent COD. If anaerobic stabilization were a real phenomenon, and that COD loss is not utilized to drive growth, then this COD loss must also be subtracted from the total influent COD in the yield calculations. The right side of Table 6.7 shows the results of yield calculation that assume AnS is real. Note that these yield values, while within acceptable ranges, are still on the low end of the ranges reported by Metcalf and Eddy.
Another piece of circumstantial evidence in favor of AnS stems from a mass balance calculation conducted around the anaerobic zone of the pilot systems. Specifically, this mass balance tracked concentrations of PHA, glycogen, and COD. Theoretically, the consumption of COD and glycogen in the anaerobic zone, on a COD basis, should be equal to PHA formation measured in the anaerobic zone. AnS would be indicated if the sum of COD and glycogen consumption in the anaerobic zone were greater than the PHA formation. This concept is shown algebraically below

$$\text{AnS} = \Delta \text{COD} + \Delta \text{GLY} - \Delta \text{PHA} \quad (10)$$

Where

$\text{AnS} = \text{anaerobic stabilization, mg COD/d}$

$\Delta \text{COD} = \text{consumption of COD across the anaerobic zone, mg COD/d}$

$\Delta \text{GLY} = \text{consumption of glycogen reserves in the anaerobic zone, mg COD/d}$
\[ \Delta \text{PHA} = \text{formation of PHAs in the anaerobic zone, mg COD/d} \]

Table 6.8 shows the results of this mass balance around the anaerobic zone, consistent with equation 10. Of particular interest is the similarity between the AnS predicted by using equation 10 and the AnS calculated in the COD mass balances. While not proof of anaerobic stabilization, this similarity does provide solid circumstantial evidence that AnS could be a real phenomenon.

Table 6.8 Mass Balance on COD, Glycogen, and PHA Around Anaerobic Zone

<table>
<thead>
<tr>
<th></th>
<th>ΔCOD mg</th>
<th>ΔGLY mg COD/d</th>
<th>ΔPHA mg</th>
<th>AnS mg</th>
<th>AnS from COD MB mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I (COD-limited)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAS</td>
<td>54359</td>
<td>14090</td>
<td>43830</td>
<td>24620</td>
<td>26336</td>
</tr>
<tr>
<td>CAS</td>
<td>52020</td>
<td>14547</td>
<td>38562</td>
<td>28004</td>
<td>29429</td>
</tr>
<tr>
<td>Phase III (P-limited)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAS</td>
<td>56442</td>
<td>5340</td>
<td>43594</td>
<td>18188</td>
<td>16916</td>
</tr>
<tr>
<td>CAS</td>
<td>53333</td>
<td>6367</td>
<td>37780</td>
<td>21920</td>
<td>21325</td>
</tr>
</tbody>
</table>

**Conclusions**

The following bulleted list summarizes the important findings developed from the presented experimental data:

- For a septic COD-limited (TCOD:TP < 40:1) wastewater, prefermentation was found to enhance EPBR by 27.7% at a statistical significance level of \( \alpha = 0.05 \) (95% confidence level).
• For septic P-limited (TCOD:TP > 40:1) wastewaters, prefermentation was not found to improve EBPR at a statistical significance level of \( \alpha = 0.05 \) (95% confidence level).

• The increased anaerobic P release and aerobic P uptakes due to prefermentation correlated with greater PHA formation and glycogen consumption during anaerobiosis of prefermented influent.

• Prefermentation increased RBCOD content by an average of 28.8% and VFA content by an average of 18.8%, even for a septic domestic wastewater.

• Prefermentation increased specific anoxic denitrification rates for both COD-limited (14.6%) and P-limited (5.4%) influent wastewaters. This increase was statistically significant at \( \alpha = 0.05 \) for COD-limited wastewater, but not for P-limited wastewater.

• The data suggests that anaerobic stabilization is potentially significant when treating warm, septic influent wastewater.

Acknowledgements

Credits

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References


CHAPTER 7 CONTRASTING THE BENEFITS OF PRIMARY CLARIFICATION VS. PREFERMENTATION IN ACTIVATED SLUDGE BNR SYSTEMS

Abstract

The potential benefits prefermentation can provide to biological nutrient removal (BNR) are measured and compared to the costs of excess oxygen consumption and sludge production incurred by an activated sludge system that utilizes prefermentation, instead of primary clarification. Prefermentation was found to produce superior performance in regards to enhanced biological phosphorus removal, or EBPR. A lower soluble ortho-phosphorus (SOP) effluent value (3.2 mg/L for the prefermented activated sludge (PAS) train vs. 4.6 mg/L for the control train with primary clarification, or PCAS) and a higher percent phosphorus (% P) content of the biomass (9.0% for the PAS train vs. 7.8% for the PCAS train) were both found to be statistically significant (P-values of 4.26 x 10^-5 and 0.0082, respectively). In addition statistically significant improvements in denitrification rates and reduced observed yields were observed due to prefermentation. However statistically significant increases in solids inventory and in particular oxygen uptake rates offset these improvements. Waste activated sludge production was slightly higher in the PAS train but was not found to be statistically significant.

Keywords

Wastewater, Biological Treatment, Phosphorus, Nitrification, Denitrification, Oxygen Demand
Introduction

The benefits that primary clarification can provide to wastewater treatment are well known in the literature. Efficiently designed and operated primary clarifiers should remove between 50 to 70 percent of the suspended solids and 25 to 40 percent of the biochemical oxygen demand (BOD) found in the influent (Metcalf and Eddy, 2003). This reduction in solids and BOD loading to an activated sludge process result in lower oxygen consumption, less sludge production, and reduced capital costs. The primary solids removed via primary clarification are sent through the solids-handling system and disposed. These primary solids, however, could potentially have a beneficial use to wastewater treatment via the process of prefermentation.

Enhanced Biological Phosphorus Removal (EBPR) requires the presence of volatile fatty acids (VFAs) in the anaerobic zone of any biological nutrient removal (BNR) wastewater treatment system. Unless the sewage is strong and septic (i.e. the influent already has a high VFA concentration) VFAs must be produced. This VFA production is accomplished either within the anaerobic zone of the BNR system or it is done prior to the BNR system in a separate anaerobic process called prefermentation in which hydrolysis and acidogenic fermentation takes place, producing VFAs in a separate step. Prefermenters as a separate unit process were developed by Dr. James Barnard in South Africa along with researchers at the University of Cape Town in the mid 1970s when BNR systems were first developed at full scale. In the United States, however, prefermenters have until recently rarely been considered even when they might arguably have been advantageous. Because of the very few quantitative comparisons of identical systems with and without prefermenters, design engineers often disagree on the necessity of a prefermenter and make decisions based on their prior experience.
Prefermentation of wastewater or primary solids is a common practice associated with Biological Nutrient Removal facilities in many parts of the world although it is only used in a few full-scale installations in the United States to date. Prefermentation technology is associated in the minds of many engineers exclusively with cold climates as an enhancement solely for Enhanced Biological Phosphorus Removal (EBPR) for non-septic wastewaters. It is true that prefermentation technology is used broadly in western Canada for that purpose. However prefermentation is practiced widely in Australia (Keller and Hartley, 1997), to some extent in South Africa, and other temperate or even tropical climates.

Prefermenters can be either on-line (the entire wastewater stream is treated) or sidestream (only primary clarifier underflow is treated). The most basic on-line prefermenter is simply a primary clarifier operated with a very high sludge blanket, commonly referred to as a Static Prefermenter. These prefermenters are not very efficient, often elevating influent VFAs less than more sophisticated prefermenters (Van Munch et al., 1996). Static Prefermenters were improved with a recycle to elute VFAs from the sludge blanket and this configuration is referred to as an Activated Primary Tank or APT. Sidestream Prefermenters are reactors that receive the primary clarifier underflow instead of fermenting the entire wastewater flow. They can consist of a single tank, which may or may not be completely mixed, or of a complete mix tank followed by a dedicated thickener. BNR facilities may receive both prefermented solids and liquid from a Sidestream Prefermenter, or may receive only the supernatant, depending on which configuration is used. Note that a BNR facility receiving only supernatant flow from a prefermenter will retain some of the benefits of primary clarification (e.g. primary solids removed by the primary clarifier) while still retaining the enhanced VFA benefits.
Traditionally the function of prefermenters has been to convert a large portion of the slowly degradable influent chemical oxygen demand (COD) into readily available substrate (e.g. VFAs) to drive EBPR in the anaerobic zone. In plants in Western Canada, where prefermentation is very common, consistent effluents of 0.5 mg/L and lower are claimed without chemical polishing for some wastewaters. Reliably going below 1 mg/L without chemical polishing is anecdotally described as routine. However there are obvious disadvantages to prefermentation. One is that the capital costs of primary clarification are incurred while many of the benefits may be lost (i.e. no direct reduction in oxygen demand or secondary waste sludge production although increased denitrification may mitigate this). In addition in countries where there is a phosphate detergent ban such as the United States, it is not as difficult to meet effluent standards and chemical polishing costs can be significantly less than in countries with significantly higher influent phosphorus concentrations. Further in the southern United States, and seasonally in the north, raw wastewater is often at least partially septic, and in Florida it is very septic and raw wastewater concentrations may routinely exceed 50 mg/L total VFAs even in the winter. As a result it is often presumed that there will be little benefit to prefermentation in a warm climate.

Prefermenters have historically been frequently used with BNR plants by some design communities, while other design communities have not (at least in the past) seriously considered them as an option. Part of the reason for this is the absence of quantitative information on the process and effluent changes resulting from prefermentation for a variety of wastewaters and climates. Most information is from full scale applications and is anecdotal (e.g. we have a plant with prefermentation that always meets 0.5 mg/L phosphorus (P), we have a plant without
prefermentation that always goes below 1 mg/L P, etc…), with only a few direct comparisons existing in the literature (e.g. Danesh and Oleszkiewicz, 1997).

This pilot scale study was conducted with the basic objective of quantifying benefits to BNR of prefermentation and contrasting them with increased oxygen consumption and sludge production one would expect when compared to a system that utilized primary clarification.

**Materials and Methods**

**Pilot Scale System**

In order to compare and contrast the potential benefits of prefermentation to BNR against the well known benefits of primary clarification (e.g. lower oxygen consumption rates, less secondary waste sludge production, etc.), two parallel pilot scale activated sludge wastewater treatment trains were constructed. The prefermented activated sludge (PAS) train, received raw influent augmented with prefermented primary solids from an off-line static prefermenter. Primary solids taken from a full scale municipal wastewater treatment plant (WWTP) primary clarifier in Central Florida (Altamonte Springs Water Reclamation Facility, Altamonte Springs, FL) were used to feed the experimental off-line prefermenter. The off-line prefermenter, which had a liquid volume of 20 liters, was maintained at an SRT of 10 days. The second activated sludge pilot train, which did not receive any additional primary solids, was called the primary clarification activated sludge (PCAS) system. The lack of primary solids addition to the PCAS system was intended to resemble an influent that received primary clarification, when compared...
to the PAS train influent, which contained extra primary solids COD that passed through the off-line static prefermenter.

The flow configuration selected for the activated sludge systems of the pilot scale WWTP was the Modified University of Cape Town (MUCT) configuration for biological nutrient removal, and is shown in Figure 7.1 for the PAS pilot train.

The MUCT configuration is similar to that of the University of Cape Town (UCT) configuration, with the exception that an extra anoxic zone is included. The first anoxic zone receives the return activated sludge (RAS), while the second anoxic zone received the nitrate recycle (NARCY) recycle. The anaerobic recycle (ARCY) recycle returns biomass from the first anoxic zone to the anaerobic zone. The purpose of the first anoxic zone is to provide extra protection to the anaerobic zone by further depleting the oxygen and nitrates which might be present in the RAS. Note that the PCAS train is identical to the PAS train, except for the lack of primary solids addition from the off-line static prefermenter. Influent flows averaged 247.2 L/d for the PAS train, and 248.3 L/d for the PCAS train. Recirculation rates were 1Q for the ARCY
(anaerobic recycle), 3.1Q for the NARCY (nitrate recycle), and 0.7Q for the RAS (return activated sludge). The PAS train was operated at an SRT of 9.0 days, and an HRT of 3.4 hr, while the PCAS train was operated at an SRT of 8.8 days, and an HRT of 3.5 hr.

The pilot scale systems were operated within the East Orange County Water Reclamation Facility or EOCWRF (Orange County, Florida) in an enclosed room with access to a tap with raw domestic wastewater. Fresh influent was provided for the systems daily by two separate polyethylene tanks, one for the prefermented activated sludge (PAS) train and one for the primary clarifier activated sludge (PCAS) train, with raw influent wastewater. Two Liters per day of prefermented primary solids was added to the PAS influent tank. Sufficient phosphorus was added to both influents to make them COD-limited (Total COD:Total P ratio less than 40:1), instead of the wastewater’s natural P-limited state (TCOD:TP ratio greater than 40:1), thus making differences in enhanced biological phosphorus removal (EBPR) easier to identify for this septic (e.g. high VFA content) wastewater (WEF, 1998). At the end of a daily cycle, any remaining influent was dumped and the sides of the influent tank were scrubbed prior to the addition of fresh influent. A single submersible pump (Little Giant Pump Co., Oklahoma City, OK) provided the mixing energy necessary to keep each influent tank sufficiently mixed. Peristaltic pumps manufactured by Cole-Parmer Instrument Company (Vernon Hills, IL) were used to maintain design flow rates for the influent line and all recycle lines. Mixing energy for both the anaerobic and anoxic zones of the activated sludge systems was provided by 50-rpm gear motors (Grainger, Lake Forest, IL). Aquarium aerators (Rena, Annecy, France) provided mixing energy for the aerobic zones, as well as aeration. The secondary clarifiers had surface skimmers and bottom scrapers powered by 1-rpm gear motors (Grainger, Lake Forest, IL), and were constructed from 50-liter cylindrical tanks with a conical bottom. The off-line prefermenter
was constructed from a 20-liter cylindrical polyethylene storage container. The anaerobic and anoxic zones of the activated sludge reactor were constructed from 8-inch square polyethylene reactors, with each reactor having a liquid volume of approximately 7 liters. The aerobic zone activated sludge reactors were constructed from 20-liter cylindrical polyethylene reactors. The entire activated sludge system was hard-plumbed with 1-inch diameter schedule 40 PVC. A series of 1-inch ball valves allowed for the rerouting of flows to multiple locations, as desired by the operators. These ball valves allowed for multiple recycle line exit points, a bypass line for the first anaerobic zone, and split-feed lines to allow for step feeding.

Cleaning techniques were also found to be of tremendous importance in maintaining stable operation of the pilot system. Specifically, a daily scrubbing of the sidewalls of all reactors of the activated sludge system, especially the aerobic tank, was necessary to prevent the build-up of a biofilm along the walls of the reactors. The sidewalls of the secondary clarifiers were also gently scraped above the sludge blanket on a daily basis. This was necessary in order to maintain a more consistent effluent solids concentration. Specifically, if the sidewalls of the secondary clarifier were not scraped daily, a biofilm would accumulate on the sidewalls, and would eventually slough off, thereby elevating the effluent solids concentration. It was also important to clean the 1-inch PVC lines connecting the anaerobic, anoxic, and aerobic tanks together, as biofilms could easily grow in those lines. To prevent clogging, the barb fitting where the 1-inch PVC was connected to the 3/8 inch ID neoprene tubing was periodically brushed clean. This connection was located where the neoprene tubing passed through the peristaltic pump head.
Chemical Analysis

Total suspended solids (TSS) and volatile suspended solids (VSS) were determined according to Standard Methods (APHA et al., 1995). Total phosphorus (TP) samples underwent persulfate digestion as outlined in Standard Methods 4500-P B(5), followed by the vanadomolybdophosphoric acid colorimetric method 4500-P C (APHA, et al., 1995). Soluble orthophosphorus (SOP) were determined using the vanadomolybdophosphoric acid colorimetric method 4500-P C in Standard Methods (APHA, et al., 1995). Chemical oxygen demand (COD) was determined by following section 5220 C in Standard Methods (APHA, et al., 1995). Organic nitrogen (both total Kjeldahl nitrogen and soluble Kjeldahl nitrogen) and ammonia nitrogen were analyzed by methods 4500-Norg A and 4500-NH$_3$ C, respectively, of Standard Methods (APHA et al., 1995). Nitrate was determined using a Dionex 2000 I/SP ion chromatograph (Sunnyvale, CA) with a CDM-3 conductivity detector and a 4270 integrator using a method similar to that found in Standard Methods 4500-NO$_3$- C (APHA, et al., 1995). Samples were analyzed for short-chain volatile fatty acids (SCVFAs) following Supelco Bulletin 856B (1995) using gas chromatography. A Shimadzu gas chromatograph model 14-A (Shimadzu Scientific Instruments, Inc., Columbia, MD) equipped with a flame ionization detector (FID) was utilized to conduct the analysis. A 3 mm inner diameter glass column with 60/80 Carbopack C/0.3% Carbowax 20M/0.1%H$_3$PO$_4$ packing (Supelco Inc., Bellefonte, PA) was used to separate the various SCVFAs. Helium, at approximately 30 mL/min, was selected as the carrier gas. The injection port and the FID were maintained at 200 °C. The oven of the gas chromatograph was programmed to begin sample analysis at 105 °C, remaining at 105 °C for two minutes, before increasing at a rate of 5 °C per minute to 150 °C, and to hold at 150 °C for an additional two
minutes, resulting in a total run time of 13 minutes per sample. Polyhydroxyalkanoates, or PHAs, were analyzed by a gas chromatographic method (Liu, 2001) using a DB-1 capillary column. The predominant forms of PHA that were measured were poly-β-hydroxybutyrate (PHB) and poly-β-hydroxyvalerate (PHV). The carrier gas, helium was maintained at a velocity of 2 ml/min and as the make up gas (25 ml/min). The injection port and detector were maintained at a temperature of 230 °C. The column temperature started at 100 °C for 2 minutes, was increased by 20 °C per minute to 160 °C, and maintained at 160 °C for an additional 10 minutes, resulting in a run time of 15 minutes. Prior to injection, sludge samples were freeze-dried using a lyophilizer and then run through a digestion. About 0.15 grams of dry sludge was put into 5.0 ml Wheaton V vials. 2 ml of benzoic acid in chloroform (50 mg/100 mL) was added to the vial for use as an internal standard and solvent, respectively. Next, 2 ml of 20% H₂SO₄ in methanol was added as the digestion/esterification reagent (methyl esters of the PHA are what is actually extracted into the chloroform phase). The vials were then placed inverted into a 100 °C oven for 18 hours. Early during the digestion (within 2 hours of starting), vial caps were retightened, in order to minimize the chance of leakage. Additionally, duplicates were run of all samples, as approximately 10% of the vials develop leaks during the digestion process. After cooling to room temperature, 1 mL of deionized water is added to the vial, and the contents of the vial are shaken using a vortexer (Fisher Scientific (Hampton, NH) for 5 minutes. Once the 5-minute washing phase was completed, the chloroform phase was removed from the vial and placed into a 1.5 ml GC vial for injection. Carbohydrates were determined by the anthrone method (ASM, 1981). Readily biodegradable chemical oxygen demand (RBCOD) was determined following techniques developed both by Ekama et al. (1986) and Wentzel et al. (1995).
Sample Collection and Monitoring

During all phases of this research project, activated sludge trains were operated until steady state conditions were met (i.e. greater than three mean cell residence times, or MCRTs). The data presented in this manuscript reflects the results of 9 separate sampling events conducted over a three-week period. Composite samplers (Isco Inc., Lincoln, NE) were used on influent samples. All other samples taken during the study were grab samples. All sample analyses were conducted within 24 hours after sampling (most within 4 hours), so beyond refrigeration, no sample storage protocols were established (e.g. no acid additions). All samples were filtered immediately upon removal from the activated sludge system. Mixed liquor reactor samples were first centrifuged on site immediately after sampling, then filtered with Whatman 934 AH glass fiber filters, and finally membrane filtered with 0.45 µm membrane filters. Field parameters, such as dissolved oxygen (DO), pH, temperature, sludge volume index (SVI), zone settling velocity (ZSV), and both in-situ and ex-situ oxygen uptake rates (OURs) were run concurrently with sampling events during the pilot scale study.

The results of the analytical tests were statistically analyzed using a paired difference test in which the means of various parameters were compared between the two trains (Mendenhall and Sincich, 1995). Differences were assumed to be significant if the p-values were less than 0.1. However, along with any statements of statistical significance, the actual p-value is also reported.

In all figures, error bars with +/- 1 standard deviation are shown.
Results

Effects upon Influent Characteristics

Composite samplers (Isco Inc., Lincoln, NE) on both the influent tanks allowed for the impact of prefermentation upon influent characteristics to be compared to an influent that underwent primary clarification. Specifically, prefermentation was found to increase the VFA content within the prefermented AS train influent by 17.7 mg/L as COD (an increase of 26.4%). Note that the control train with primary clarification influent wastewater was already highly septic, with a VFA content averaging 67.0 mg/L as COD. The only VFAs detected in the influent tanks were acetate and propionate. Prefermentation was not found to significantly alter the ratio of acetate to propionate within the influent in this study, with acetate content averaging approximately 66% of the VFAs as COD for both the prefermented train and the control train influents. Additionally, prefermentation was also found to significantly increase the RBCOD content found within the influent wastewater. Prefermentation increased the RBCOD content of the PAS influent by 31.9% (from 94 mg/L in the PCAS influent to 124 mg/L for the PAS influent).

Effects upon EBPR

One of the major results of the pilot scale study was that for this septic, COD-limited wastewater prefermentation increased the net P removal when compared to a control train with primary clarification, which is the ultimate objective of EBPR. Figure 7.2 compares the soluble ortho phosphorus (SOP) profiles of the PAS and PCAS.
Figure 7.2  SOP Profile for the PAS and PCAS Trains

The effluent soluble ortho phosphorus for the control train with primary clarification (PCAS) was nearly 44% higher than that of the prefermented train (PAS). Using a paired difference test between two population means, it can be shown that the effluent phosphorus concentration for the PAS train (3.2 mg/L) was statistically superior to that of the PCAS train (4.6 mg/L) with a p-value of 4.26 x 10^{-5} (Mendenhall and Sincich, 1995). This result is not surprising, as the PAS train received influent that was richer in both VFA and RBCOD content than the PCAS train.

Both trains had sufficient VFA content to drive EBPR. The literature indicates that a VFA:TP ratio of between 4 to 10 mg VFA per mg P is necessary for good phosphorus removal. Metcalf and Eddy (2003) cites a conservative 10:1 ratio of VFA:P, while Daigger, et. al. (1993)
and anecdotal suggestions specify VFA:TP rations of 7:1 and 4:1, respectively. Much of the seeming contradictions in the literature may be due to temperature. Generally, the 4:1 ratio applies to western Canada where there are cold but stable temperatures allowing for psychrophilic EBPR. The temperatures found in this study were quite elevated in contrast, averaging 28.0 °C. In this study, the VFA:TP ratio was observed to be 7.1 for the PAS train, and 5.7 for the CAS train. Since increasing the VFA:TP ratio from 5.7 to 7.1 resulted in improved EBPR, the data was more consistent with the mid-range or high ratios in the literature (i.e. a VFA:TP ratio greater than 5.7:1 provides benefits to EBPR).

An analysis of the mass flux of phosphorus through the individual reactors of the pilot systems yields additional insight to the potential of prefermentation to increase P removal when compared to an activated sludge system that has a primary clarifier. Table 7.1 shows the results of this mass flux analysis on phosphorus:

<table>
<thead>
<tr>
<th>Parameters (mg/day)</th>
<th>PAS Train</th>
<th>PCAS Train</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP influent</td>
<td>2912.0</td>
<td>2901.3</td>
</tr>
<tr>
<td>Anaerobic SOP Release</td>
<td>3693.7</td>
<td>2584.9</td>
</tr>
<tr>
<td>Anoxic I SOP Release</td>
<td>9629.8</td>
<td>7865.5</td>
</tr>
<tr>
<td>Anoxic II SOP Uptake</td>
<td>5023.2</td>
<td>3320.8</td>
</tr>
<tr>
<td>Net SOP Anoxic Release</td>
<td>4606.6</td>
<td>4544.7</td>
</tr>
<tr>
<td>Total SOP Release</td>
<td>13323.5</td>
<td>10450.4</td>
</tr>
<tr>
<td>Aerobic SOP Uptake</td>
<td>10502.1</td>
<td>8934.2</td>
</tr>
<tr>
<td>Clarifier SOP Release</td>
<td>77.6</td>
<td>27.9</td>
</tr>
<tr>
<td>Total SOP Uptake</td>
<td>15447.7</td>
<td>12227.1</td>
</tr>
<tr>
<td>SOP Uptake:SOP Release Ratio</td>
<td>1.16</td>
<td>1.17</td>
</tr>
<tr>
<td>Net SOP Uptake</td>
<td>2124.2</td>
<td>1776.7</td>
</tr>
<tr>
<td>%P in MLSS as calculated via mass balance</td>
<td>9.0</td>
<td>7.8</td>
</tr>
</tbody>
</table>
When comparing the %P in MLSS as calculated via a mass balance, it can be seen that prefermentation increased the %P content of MLSS (9.0% vs. 7.8% for the control train, which is a statistically significant difference with a P-value of 0.0082). This correlated with the lower effluent SOP profiles shown in Figure 7.2. Of further interest is the marked difference in SOP release and uptake between the PAS and PCAS trains. The PAS trains had 42.8% greater SOP release in the anaerobic zone than the PCAS train. This correlated with the greater amount of VFAs found within the PAS train due to prefermentation. In addition, superior SOP uptake in both Anoxic II and the Aerobic zone of the PAS train when compared to the PCAS train was noted. Specifically, a 27.5% increase in the total SOP release and a 26.3% increase in the total SOP uptake was found in the PAS train as compared to the PCAS train. However, despite the differences in phosphorus release and uptake between the two trains, the SOP Uptake: SOP Release ratios were remarkably similar (1.16 for the PAS train and 1.17 for the PCAS train), as shown in Table 7.1.

Other parameters of importance to EBPR were also measured, including polyhydroxyalkanoates, or PHAs (both PHB and PHV), and glycogen. Both PHA and glycogen concentrations were higher in the PAS train as compared to the PCAS train. Figures 7.3 and 7.4, respectively, show the PHA and glycogen profiles for both the PAS and PCAS trains.

Note that the apparent increase in the concentration of glycogen from the Anaerobic zone to Anoxic I is an artifact of the MUCT flow configuration. A mass flux analysis of glycogen indicated there is glycogen depletion across both the Anaerobic zone and Anoxic I, which corresponds to the increase in PHA concentrations illustrated in Figure 7.3.
Figure 7.3 PHA Profile for the PAS and PCAS Trains
Figure 7.4 Glycogen Profile for the PAS and PCAS Trains
Effects of Prefermentation on Denitrification and N Mass Balances

Nitrogen forms, including nitrate (NO$_3$-N), ammonia (NH$_4$-N), soluble Kjeldahl nitrogen (SKN), and total Kjeldahl nitrogen (TKN), were measured during the course of this study. All phases had similar nitrogen profiles for all nitrogen (N) forms, with differences coming only in the absolute values of the measured parameters. The greatest difference in concentration of N-forms measured during this study was found in the effluent nitrate values. Figure 7.5 shows a comparison of the nitrate profiles of the two trains.

![Nitrate Profile](image)

Figure 7.5 Nitrate Profile
The prefermented (PAS) train had an effluent nitrate concentration of 11.4 mg/L NO$_3$-N, as compared to a 12.5 mg/L value for the control train with primary clarification (PCAS) train, which amounts to only a 5% difference. However, despite the small absolute value of the difference in the effluent nitrate concentration between the two trains, the difference between the two means had statistical significance, with a p-value of 4.26 x 10$^{-5}$ (Mendenhall and Sincich, 1995).

Nitrogen mass balances were conducted upon the data generated during this study in order to verify the quality of the data collected, using the equation below:

$$\sum \text{TN}_{\text{influent}} = \sum \triangle \text{NO}_3 \text{ denitrified} + \text{N}_{\text{assimilated}} + \sum \text{SN}_{\text{effluent/WAS}}$$

(1)

where:

$\sum \text{TN}_{\text{influent}} = $ sum of total nitrogen in the influent, mg/d

$\sum \triangle \text{NO}_3 \text{ denitrified} = $ sum of nitrate denitrified in unaerated zones, mg/d

$\text{N}_{\text{assimilated}} = $ nitrogen assimilated into growth of new biomass, mg/d

$\sum \text{SN}_{\text{effluent/WAS}} = $ sum of soluble nitrogen in the effluent and waste activated sludge, mg/d

Table 7.2 shows the results of nitrogen mass balances conducted during this study: Of particular interest is the good agreement found in the nitrogen mass balances, with a 98.0% agreement in the PAS train and a 101.7% for the PCAS train, easily within the error of the measurements. Note that the nitrogen mass balances rely upon an assumed fraction of N in
biomass \( (f_N) \) of 0.1239 which is a common assumed value reflecting the average composition of activated sludge biomass used in the Environmental Engineering community (Metcalf and Eddy, Inc, 2003). A sensitivity analysis was also conducted over a broader range of possible values based on the literature, and mass balance agreements were still above 93.8% even with an N content of 0.10.

Also note that equation (1) assumed that all nitrate disappearance is attributed to \( N_2 \) formation, not nitrite formation, ammonia formation via dissimilatory reduction of nitrate to ammonia (DNRA), or biological assimilation of nitrate.

Table 7.2 Nitrogen Mass Balance

<table>
<thead>
<tr>
<th>Parameters (mg/day)</th>
<th>PAS</th>
<th>PCAS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TN influent</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assimilated N (^{1,2})</td>
<td>2270</td>
<td>2201</td>
</tr>
<tr>
<td>Nitrate Load to Unaerated Zones</td>
<td>10786</td>
<td>11888</td>
</tr>
<tr>
<td>Nitrate Load leaving Unaerated Zones</td>
<td>6036</td>
<td>7305</td>
</tr>
<tr>
<td>Unaerated Zones Denitrification</td>
<td>4749</td>
<td>4583</td>
</tr>
<tr>
<td>Soluble Nitrogen in Effluent and WAS</td>
<td>3360</td>
<td>3809</td>
</tr>
<tr>
<td>Secondary Clarifier Denitrification</td>
<td>16</td>
<td>24</td>
</tr>
<tr>
<td>% N Mass Balance agreement</td>
<td>98.0</td>
<td>101.7</td>
</tr>
<tr>
<td>Simultaneous Denitrification/Discrepancy (^3)</td>
<td>226</td>
<td>-213</td>
</tr>
</tbody>
</table>

\(^1\) Assumes \( f_N \) (nitrogen content of biomass) = 0.1239
\(^2\) Includes solids wasted, and in effluent
\(^3\) Calculated by difference

Evaluation of the effect of prefermentation upon denitrification when compared to a system that has primary clarification was one of the main objectives of this study. Table 7.3
compares specific anoxic denitrification rates measured in the both anoxic zones of the pilot systems. Actual denitrification rates could not be observed in Anoxic I zones since they were not fully loaded with NO\(_x\), as Figure 7.5 indicates that both trains had little measurable NOx. However in the Anoxic II zones actual denitrification capacities could be observed since the zones were overloaded with NOx. In this pilot study, the prefermented (PAS) train had a 13.3% greater specific denitrification rate in the second anoxic zone than the control train (PCAS) train. This difference between the average anoxic II specific denitrification rates had statistical significance, with a p-value of 0.0028. This result corresponded to an influent richer in VFAs and RBCOD resulting in higher specific rates in the zone where the bulk of the denitrification in the system occurs.

Table 7.3 Specific Anoxic Zone Denitrification Rates in the Pilot Scale Study (mg NO\(_x\)/g VSS*Day)

<table>
<thead>
<tr>
<th>Train</th>
<th>Anoxic I</th>
<th>Anoxic II</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS</td>
<td>&gt; 66.8</td>
<td>80.8</td>
</tr>
<tr>
<td>PCAS</td>
<td>&gt; 73.9</td>
<td>71.3</td>
</tr>
</tbody>
</table>

**Effects upon Oxygen Consumption, Sludge Production, and COD Mass Balance**

The previous two sections of this paper outline the benefits to BNR that prefermentation can have, when compared to an activated sludge system that has primary clarification. However, the superior BNR performance comes at the cost of increased oxygen consumption, sludge production, and increased capital costs (increased tankage volume, for example) due to extra COD loading found in an activated sludge train with a prefermenter, when compared to an
activated sludge train with primary clarification. A comparison between the train with a prefermenter (PAS) and the control train with a primary clarifier (PCAS) for various parameters that measure oxygen consumption and sludge production are shown in Table 7.4.

Table 7.4 Comparison Between the PAS and PCAS Trains Upon Parameters that Measure Oxygen Consumption and Sludge Production

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PAS</th>
<th>PCAS</th>
<th>% PAS larger than PCAS</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>OUR (mg/L/hr)</td>
<td>101.4</td>
<td>84.0</td>
<td>20.7</td>
<td>3.52 x 10^-4</td>
</tr>
<tr>
<td>SOUR (mg/g/hr)</td>
<td>19.9</td>
<td>18.0</td>
<td>10.6</td>
<td>0.027</td>
</tr>
<tr>
<td>WAS Production (mg/day)</td>
<td>18251</td>
<td>17704</td>
<td>3.1</td>
<td>0.145</td>
</tr>
<tr>
<td>MLSS Inventory (mg)</td>
<td>213571</td>
<td>201344</td>
<td>6.1</td>
<td>5.72 x 10^-5</td>
</tr>
<tr>
<td>MLVSS Inventory (mg)</td>
<td>163498</td>
<td>154225</td>
<td>6.0</td>
<td>1.48 x 10^-4</td>
</tr>
<tr>
<td>FSS Inventory (mg)</td>
<td>50073</td>
<td>47119</td>
<td>6.3</td>
<td>0.0110</td>
</tr>
<tr>
<td>Observed Yield (mg VSS/mg COD)</td>
<td>0.249</td>
<td>0.266</td>
<td>-6.4</td>
<td>0.0545</td>
</tr>
</tbody>
</table>

The P-value column in Table 7.4 refers to the results of a paired difference test in which the means are compared (Mendenhall and Sincich, 1995). Significant differences between the prefermented train (PAS) and the control train with primary clarification (PCAS) can be found in the oxygen uptake rate (OUR), specific oxygen uptake rate (SOUR), and the mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), and fixed suspended solids (FSS) inventories. All of these values indicate that increased oxygen costs can be expected while operating an activated sludge train with prefermentation, when compared to an activated sludge train that has primary clarification.

While the PAS train was found to have 3.1% more waste activated sludge (WAS) production, this difference was not found to be statistically significant, having a p-value of only
The PCAS train was actually found to have a 6.4% higher average observed yield than the PAS train, with a p-level of 0.0545. While the PAS train has slightly larger WAS production than the PCAS train, the PAS train also had much higher ΔCOD than the PCAS train, thus explaining the lower observed yields found in the PAS train. Additionally, acetic acid is highly oxidized and a low yield substrate, and its reduction to PHA comes at a glycogen cost (Yellore, et al, 1999). It may be that while fermentation of COD to acetic and propionic acid does not result in a COD loss, with respect to oxygen demand, it does result in a form of compound with lower yield characteristics since it has in fact been metabolized and resulted in anaerobic yield among the fermenters.

COD mass balances resulted in poor agreement, unlike the N mass balances. Percent agreement values for the COD mass balances were only 74.1% for the prefermented (PAS) train and 70.5% for the control train with primary clarification (PCAS) train. A profile of the soluble COD across each train is shown in Figure 7.6. Other researchers, including Barker and Dold (1995), have found similar poor COD mass balances agreement around activated sludge systems that include an anaerobic zone. In parallel anoxic/aerobic and aerobic activated sludge systems, Barker and Dold (1995) were able to achieve good COD mass balance agreement, but this agreement failed once an anaerobic zone was added. This identified a process occurring in the anaerobic zone as a potential cause of the poor COD mass balance agreements found in activated sludge systems with anaerobic zones. Given the quality of our N mass balances (again, assuming an f_N of 0.1239), analytical error of this magnitude seems unlikely. This COD mass balance discrepancy may have been due to the poorly understood and controversial phenomena of “anaerobic stabilization” (loss of COD in anaerobic zones; Randall, et al, 1992, and Barker and Dold, 1995).
Conclusions

The following bulleted list summarizes the important findings developed during the course of the comparison of an activated sludge system with prefermentation (PAS) to an activated sludge system with primary clarification (PCAS):

- Prefermentation was found to produce superior performance in regards to EBPR. A lower SOP effluent value (3.2 mg/L for the PAS train vs. 4.6 mg/L for the PCAS train) and a higher %P content of the biomass (9.0% for the PAS train vs. 7.8% for the PCAS train) was found to be statistically significant.
• The increased anaerobic P release and aerobic P uptakes due to prefermentation correlated with greater PHA formation and glycogen consumption during anaerobiosis of prefermented influent in the PAS train when compared to the PCAS train.

• Prefermentation increased RBCOD content by an average of 31.9% and VFA content by an average of 26.4% when compared to a septic system with primary clarification.

• Increasing the VFA:TP ratio from 5.7 to 7.1 at 28.0 oC improved EBPR, which was consistent with the design criteria published in the United States but not with the lower values from design experience in western Canada.

• Oxygen utilization rates and specific oxygen utilization rates were found to be 20.7% and 11.1% higher, respectively for the PAS train as compared to the PCAS train. These results were statistically significant, with p-values of 3.52 x 10^-4 and 0.0274, respectively.

• Statistically significant increases in MLSS (6.0%), MLVSS (6.1%), and FSS (6.3%) inventories were found in the PAS train as compared to the PCAS train.

• An increase (3.1%) in WAS production in the PAS train when compared to the PCAS train was not found to be statistically significant (p-value of 0.144).

• Observed Yields were larger (6.4%) in the PCAS train, as compared to the PAS train, with a p-value of 0.0545.

• The relative cost/benefit of improved effluent SOP and TN of prefermentation are partly offset by the increased oxygen demands of returned primary solids COD as SCVFAs. WAS however was not observed to increase in the same way, although future studies should be conducted for confirmation. This phenomena could be due to the energy poor nature of acetic acid (Yellore, et al, 1999).
Acknowledgements

Credits

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**References**


CHAPTER 8 CONCLUSIONS

Prefermenters as a separate unit process were developed by Dr. James Barnard in South Africa along with researchers at the University of Cape Town in the mid 1970s when BNR systems were first developed at full scale. In the United States, however, prefermenters have until recently rarely been considered even when they might arguably have been advantageous. Because of the very few quantitative comparisons of identical systems with and without prefermenters, design engineers often disagree on the necessity of a prefermenter and make decisions based on their prior experience.

The objective of this dissertation was to provide a controlled comparison of identical continuous flow BNR processes both with and without prefermentation in order to provide a stronger, more quantitative, technical basis for design engineers to evaluate the potential benefits of prefermentation to EBPR in treating domestic wastewater. In addition, the even less understood effect of prefermentation on denitrification kinetics and anoxic phosphorus (P) uptake was studied and quantified. Other aspects of BNR performance, which might change due to use of prefermentation, were also addressed, including anaerobic stabilization.

Important findings developed during the course of this dissertation regarding the impact of prefermentation upon the performance of activated sludge treatment systems are summarized below:

- For a septic COD-limited (TCOD:TP < 40:1) wastewater, prefermentation was found to enhance EPBR by 27.7% at a statistical significance level of $\alpha=0.05$ (95% confidence level).
• For septic P-limited (TCOD:TP > 40:1) wastewaters, prefermentation was not found to improve EBPR at a statistical significance level of $\alpha=0.05$ (95% confidence level).

• The increased anaerobic P release and aerobic P uptakes due to prefermentation correlated with greater PHA formation and glycogen consumption during anaerobiosis of prefermented influent.

• Improvements in biological P removal of septic, non-P limited wastewater occurred even when all additional VFA production exceeded VFA requirements using typical design criteria (e.g. 6 g VFA per 1 g P removal).

• Prefermentation increased RBCOD content by an average of 28.8% and VFA content by an average of 18.8%, even for a septic domestic wastewater.

• Prefermentation increased specific anoxic denitrification rates for both COD-limited (14.6%) and P-limited (5.4%) influent wastewaters. This increase was statistically significant at $\alpha=0.05$ for COD-limited wastewater, but not for P-limited wastewater.

• The data suggest that anaerobic stabilization is potentially significant when treating warm, septic influent wastewater.

A second focus of study throughout this project was to compare and contrast the impacts of prefermentation upon activated sludge performance to the more well-known impacts of primary clarification. The results of this comparison are bulleted below:

• Prefermentation was found to produce superior performance in regards to EBPR. A lower SOP effluent value (3.2 mg/L for the PAS train vs. 4.6 mg/L for the PCAS train)
and a higher %P content of the biomass (9.0% for the PAS train vs. 7.8% for the PCAS train) was found to be statistically significant.

- The increased anaerobic P release and aerobic P uptakes due to prefermentation correlated with greater PHA formation and glycogen consumption during anaerobiosis of prefermented influent in the PAS train when compared to the PCAS train.
- Prefermentation increased RBCOD content by an average of 31.9% and VFA content by an average of 26.4% when compared to a septic system with primary clarification.
- Increasing the VFA:TP ratio from 5.7 to 7.1 at 28.0 °C improved EBPR, which was consistent with the design criteria published in the United States but not with the lower values from design experience in western Canada.
- Oxygen utilization rates and specific oxygen utilization rates were found to be 20.7% and 11.1% higher, respectively for the PAS train as compared to the PCAS train. These results were statistically significant, with p-values of 3.52 x 10-4 and 0.0274, respectively.
- Statistically significant increases in MLSS (6.0%), MLVSS (6.1%), and FSS (6.3%) inventories were found in the PAS train as compared to the PCAS train.
- An increase (3.1%) in WAS production in the PAS train when compared to the PCAS train was not found to be statistically significant (p-value of 0.144).
- Observed Yields were larger (6.4%) in the PCAS train, as compared to the PAS train, with a p-value of 0.0545.
- The relative cost/benefit of improved effluent SOP and TN of prefermentation are partly offset by the increased oxygen demands of returned primary solids COD as SCVFAs. WAS however was not observed to increase in the same way, although future studies
should be conducted for confirmation. This phenomena could be due to the energy poor nature of acetic acid (Yellore, et al, 1999).

Finally, some of the biokinetic parameters necessary to successfully model activated sludge systems were measured for parallel activated sludge pilot systems both with and without prefermentation (see Appendix D). This aspect of the study focused upon conducting experiments to establish values for important domestic wastewater influent biokinetic parameters, including RBCOD, the maximum specific growth rate coefficient for autotrophic biomass ($\mu_{A_{\text{max}}}$), and inert COD fractionation. Determination of these biokinetic parameters provides information concerning the impact of prefermentation upon the biological treatability of wastewater. Additionally, using these experimentally determined values for influent biokinetic parameters, instead of standard default assumptions, should lead to superior performance of activated sludge modeling of BNR systems with prefermentation. Results from the determination of biokinetic parameters during this study are bulleted below:

- Prefermentation was found to increase the RBCOD in both COD-limited (from 121 to 149 mg/L) and P-limited (from 99 to 128 mg/L) wastewaters, with P-values of 0.0001 and 0.002 for COD-limited and P-limited wastewaters, respectively.

- Prefermentation was shown to increase the maximum specific growth rate coefficient for autotrophic biomass, $\mu_{A_{\text{max}}}$, by 9% (P-value of 0.23) for COD-limited wastewater and by 4% (P-value of 0.07) for P-limited wastewater. These values for prefermented influent (0.82 day$^{-1}$ for the COD-limited wastewater, and 0.79 day$^{-1}$ for the P-limited wastewater) are slightly higher than typical default values (0.77 day$^{-1}$) for temperatures around 20 deg C.
• The inert soluble COD fraction (sum of $S_I$ and $S_p$) was reduced from 11% of total COD ($CT_o$) to 7% (P-value of .08) for COD-limited wastewaters with prefermentation and from 12% to 8% (P-value of 0.08) for P-limited wastewaters with prefermentation.
Theory

In order to verify the accuracy of nitrogen data measured from the pilot activated sludge systems, nitrogen mass balances were conducted.

The daily mass of nitrogen that enters the system in the influent can leave the system in only three different ways:

1. Nitrogen that is denitrified
2. Nitrogen in the waste sludge
3. Nitrogen in the effluent

Note: The impact of nitrite is ignored in the following analysis, as no measurable quantity of nitrite was quantified during the course of this study. Additionally, assimilation of nitrate (i.e. nitrate being converted directly into biomass) is assumed to be negligible.

To determine the mass of nitrogen denitrified on a daily basis, a nitrate mass balance must be conducted around the unaerated zones of the system – namely the anaerobic (AN) and anoxic (AXI, AX II) zones. The sum of the mass of nitrate entering the unaerated systems minus the sum of the mass of nitrate leaving the unaerated zones equals the mass of nitrate denitrified. Expressing this statement in the form of an equation, and applying it to the flow schematic used in the pilot study, the mass of nitrate denitrified is calculated in Equation A.1:

\[ (Q_{\text{INF}} \cdot NO_3_{\text{INF}} + Q_{\text{NARCY}} \cdot NO_3_{\text{AE}} + Q_{\text{RAS}} \cdot NO_3_{\text{Eff}}) - (Q_{\text{INF}} + Q_{\text{NARCY}} + Q_{\text{RAS}}) \cdot NO_3_{\text{AXII}} \]  \hspace{1cm} (A.1)

Where,
Q_{INF} = Influent flow rate, (L/day)

NO3_{INF} = Influent nitrate concentration (mg/L)

Q_{NARCY} = Nitrate recycle flow rate, (L/day)

NO3_{AE} = Aerobic nitrate concentration (mg/L)

Q_{RAS} = Return activated sludge flow rate, (L/day)

NO3_{EFF} = Effluent nitrate concentration, (mg/L)

NO3_{AXII} = Anoxic II nitrate concentration, (mg/L)

The end result of this equation is the mass of nitrate denitrified in the unaerated zones of the system, measured in mg/day.

The mass of nitrogen in the waste sludge is determined by multiplying the mass of VSS wasted per day by the biomass nitrogen content \( f_n \), assumed to be 0.1239. The mass of nitrogen assimilated into new biomass is calculated below in Equation A.2, in mg/day:

\[
f_n \times MLVSS_{WAS} \times Q_{WAS}
\]  

(A.2)

Where,

\( f_n \) = Fraction of biomass that contains nitrogen

\( MLVSS_{WAS} \) = Mixed liquor volatile suspended solids in the waste activated sludge, mg/L

\( Q_{WAS} \) = Waste activated sludge flow rate, L/day
The mass of soluble nitrogen in the effluent is the product of the daily effluent flow rate and the sum of the effluent SKN and nitrate.

\[ Q_{\text{effluent}} \times (\text{SKN}_{\text{effluent}} + \text{NO}_3_{\text{effluent}}) \]  
(A.3)

Where,

\( Q_{\text{effluent}} = \) Effluent flow rate, L/day

\( \text{SKN}_{\text{effluent}} = \) Soluble Kjeldahl Nitrogen in the effluent, mg/L

\( \text{NO}_3_{\text{effluent}} = \) Nitrate in the effluent, mg/L NO\textsubscript{3}-N

The %N mass balance agreement can now be found dividing the sum of Equations A.1, A.2, and A.3, divided by the mass of nitrogen in the influent, and multiplied by 100.

**Sample Calculation**

To clarify the theory behind nitrogen mass balances, a sample calculation, taken from one of the results chapters, will be used as a case study. Table 6.3, which displays the nitrogen mass balances from Chapter 6 (Improved P Removal of Cod-Limited, Septic, Wastewater Via Prefermentation), is redisplayed here as Table A.1.
Table A.1  Nitrogen Mass Balance

<table>
<thead>
<tr>
<th>Parameters (mg/day)</th>
<th>COD-limited PAS</th>
<th>COD-limited CAS</th>
<th>P-limited PAS</th>
<th>P-limited CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TN influent</td>
<td>10303</td>
<td>10473</td>
<td>10370</td>
<td>10587</td>
</tr>
<tr>
<td>Assimilated N (^{1,2})</td>
<td>1967</td>
<td>1923</td>
<td>2179</td>
<td>2196</td>
</tr>
<tr>
<td>Nitrate Load to Unaerated Zones</td>
<td>11768</td>
<td>10260</td>
<td>11283</td>
<td>10767</td>
</tr>
<tr>
<td>Nitrate Load leaving Unaerated Zones</td>
<td>7156</td>
<td>6161</td>
<td>6558</td>
<td>6152</td>
</tr>
<tr>
<td>Unaerated Zones Denitrification</td>
<td>4612</td>
<td>4099</td>
<td>4725</td>
<td>4616</td>
</tr>
<tr>
<td>Soluble Nitrogen in Effluent</td>
<td>3533</td>
<td>3197</td>
<td>3496</td>
<td>3410</td>
</tr>
<tr>
<td>Secondary Clarifier Denitrification</td>
<td>49</td>
<td>86</td>
<td>31</td>
<td>12</td>
</tr>
<tr>
<td>% N Mass Balance agreement</td>
<td>99</td>
<td>89</td>
<td>101</td>
<td>97</td>
</tr>
<tr>
<td>Simultaneous Denitrification (^3)</td>
<td>142</td>
<td>1168</td>
<td>-61</td>
<td>354</td>
</tr>
</tbody>
</table>

\(^1\) Assumes \(f_n\) (nitrogen content of biomass) = 0.1239
\(^2\) Includes solids wasted, and in
\(^3\) Calculated by difference

Specifically, the nitrogen mass balance from the PAS train on COD-Limited wastewater will be used as a sample calculation. Phase averages for the raw data from this phase of this research can be found in Tables F.12 – F.16 of Appendix F.

To calculate the total nitrogen (TN) influent load, the first row in Table A.1, the sum of the influent TKN and nitrate is multiplied by the influent flow rate, according to the following equation:

\[
(Q_{\text{INF}}) \times (NO_3_{\text{INF}}) + (Q_{\text{INF}}) \times (TKN_{\text{INF}})
\]  \hspace{1cm} (A.4)

Where,

\(Q_{\text{INF}} = \) Influent flow rate, L/day
\[ \text{NO}_3_{\text{INF}} = \text{Nitrate concentration in the influent, mg/L NO}_3\text{-N} \]
\[ \text{TKN}_{\text{INF}} = \text{Total Kjeldahl Nitrogen in the influent, mg/L NH}_4\text{-N} \]

Plugging in the raw data from Tables F.12 – F.16 of Appendix F into Equation A.4 and solving for the TN influent load:

\[ (247.2 \text{ L/day}) \times (0.08 \text{ mg/L NO}_3\text{-N}) + (247.2 \text{ L/day}) \times (41.6 \text{ mg/L NH}_4\text{-N}) \]
\[ = 10303 \text{ mg N/day} \quad (A.5) \]

Row 2 in Table A.1 is Assimilated N. Assimilated N is nitrogen that is incorporated into the growth of new biomass in the treatment system. Assimilated N is calculated in a manner similar to that displayed in equation A.2. However, note that the impact of the N contained in the solids wasted in the effluent is also taken into account below in Equation A.6:

\[ (Q_{\text{WAS}}) \times (\text{TSS}_{\text{AE}}) \times (\text{VSS}_{\text{AE}}/\text{TSS}_{\text{AE}}) \times (f_n) + (Q_{\text{EFF}}) \times (\text{TSS}_{\text{EFF}}) \times (\text{VSS}_{\text{EFF}}/\text{TSS}_{\text{EFF}}) \times (f_n) \quad (A.6) \]

Where,
\[ Q_{\text{WAS}} = \text{Waste activated sludge flow rate, L/day} \]
\[ \text{TSS}_{\text{AE}} = \text{Total suspended solids in the aerobic zone, mg/L} \]
\[ \text{VSS}_{\text{AE}}/\text{TSS}_{\text{AE}} = \text{Ratio of volatile suspended solids to total suspended solids in the aerobic zone} \]
\[ f_n = \text{Fraction of biomass that is nitrogen, assumed to be 0.1239} \]
\[ Q_{\text{EFF}} = \text{Effluent flow rate, L/day} \]
\[ \text{TSS}_{\text{EFF}} = \text{Total suspended solids in the effluent, mg/L} \]
VSS\textsubscript{EFF}/TSS\textsubscript{EFF} = Ratio of volatile suspended solids to total suspended solids in the effluent

Plugging in the raw data from Tables F.12 – F.16 of Appendix F into Equation A.6, assuming \( f_n \) is 0.1239, and solving for N assimilated:

\[
(2.7 \text{ L/day}) \times (5892.5 \text{ mg/L}) \times (0.770) \times (0.1239) + (244.5 \text{ L/day}) \times (19.8 \text{ mg/L}) \times (0.750) \times (0.1239) = 1967 \text{ mg N/day} \tag{A.7}
\]

The next three rows (rows 3 through 5) in Table A.1 calculate the denitrification which occurs in the unaerated zones, as shown in Equation A.1. Row 3, which displays the nitrate load to the unaerated zones, is calculated as shown below:

\[
(Q_{\text{INF}}) \times (NO_{3\text{INF}}) + (Q_{\text{NARCY}}) \times (NO_{3\text{AE}}) + (Q_{\text{RAS}}) \times (NO_{3\text{EFF}}) \tag{A.8}
\]

Where,

\( Q_{\text{INF}} \) = Influent flow rate, L/day
\( NO_{3\text{INF}} \) = Nitrate concentration in the influent, mg/L NO\textsubscript{3}-N
\( Q_{\text{NARCY}} \) = Nitrate recycle flow rate, L/day
\( NO_{3\text{AE}} \) = Nitrate concentration in the aerobic zone, mg/L NO\textsubscript{3}-N
\( Q_{\text{RAS}} \) = Return activated sludge flow rate, L/day
\( NO_{3\text{EFF}} \) = Nitrate concentration in the effluent, mg/L NO\textsubscript{3}-N
Plugging in the raw data for the PAS train from Tables F.12 – F.16 of Appendix F into Equation A.8 and solving for the nitrate load to the unaerated zones (row 3 in Table A.1):

\[
(247.2 \text{ L/day}) \times (0.08 \text{ mg/L NO}_3^-\text{N}) + (767.8 \text{ L/day}) \times (12.49 \text{ mg/L NO}_3^-\text{N}) + (175.6 \text{ L/day}) \times (12.29 \text{ mg/L NO}_3^-\text{N}) = 11768 \text{ mg N/day} \tag{A.9}
\]

Row 4, which displays the nitrate load leaving the unaerated zones, is calculated as shown below:

\[
(Q_{\text{INF}} + Q_{\text{NARCY}} + Q_{\text{RAS}}) \times \text{NO}_3\text{AXII} \tag{A.10}
\]

Where

- \(Q_{\text{INF}}\) = Influent flow rate, L/day
- \(Q_{\text{NARCY}}\) = Nitrate recycle flow rate, L/day
- \(Q_{\text{RAS}}\) = Return activated sludge flow rate, L/day
- \(\text{NO}_3\text{AXII}\) = Nitrate concentration in the anoxic zone II, mg/L NO\(_3\)-N

Plugging in the raw data for the PAS train from Tables F.12 – F.16 of Appendix F into Equation A.10 and solving for the nitrate load leaving the unaerated zones (row 4 in Table A.1):

\[
(247.2 \text{ L/day} + 767.8 \text{ L/day} + 175.6 \text{ L/day}) \times (6.01 \text{ mg/L NO}_3^-\text{N}) = 7156 \text{ mg N/day} \tag{A.11}
\]
The difference between the nitrate load into the unaerated zones (row 3 in Table A.1, and calculated in Equation A.9) and the nitrate load leaving the unaerated zones (row 4 in Table A.1, and calculated in Equation A.11), is the unaerated zone denitrification (row 5 in Table A.1). Using the results of Equations A.9 and A.11 and finding the difference:

\[ 11768 \text{ mg N/day} - 7156 \text{ mg N/day} = 4612 \text{ mg N/day} \] \quad (A.12)

The soluble effluent nitrogen displayed in row 6 of Table A.1 is calculated according to Equation A.3. However, in addition to accounting for soluble nitrogen in the effluent, Table A.1 also includes soluble effluent in the waste activated sludge, as shown below in Equation A.13:

\[ Q_{\text{EFF}} \times (\text{NO}_3_{\text{EFF}} + \text{SKN}_{\text{EFF}}) + Q_{\text{WAS}} \times (\text{NO}_3_{\text{AE}} + \text{SKN}_{\text{AE}}) \] \quad (A.13)

Where,

\( Q_{\text{EFF}} \) = Effluent flow rate, L/day

\( \text{NO}_3_{\text{EFF}} \) = Nitrate concentration in the effluent, mg/L NO\(_3\)-N

\( \text{SKN}_{\text{EFF}} \) = Soluble Kjeldahl Nitrogen in the effluent, mg/L NH\(_4\)-N

\( Q_{\text{WAS}} \) = Waste activated sludge flow rate, L/day

\( \text{NO}_3_{\text{AE}} \) = Nitrate concentration in the aerobic zone, mg/L NO\(_3\)-N

\( \text{SKN}_{\text{AE}} \) = Soluble Kjeldahl Nitrogen in the aerobic zone, mg/L NH\(_4\)-N

Plugging in the raw data for the PAS train from Tables F.12 – F.16 of Appendix F into Equation A.13 and solving for the soluble nitrogen in the effluent (row 6 of Table A.1):
(244.5 L/day) * (12.29 mg/L NO$_3$-N + 2.0 mg/L NH$_4$-N) + (2.7 L/day) * (12.49 mg/L NO$_3$-N + 2.1 mg/L NH$_4$-N) = 3533 mg N/day \hspace{1cm} (A.14)

The secondary clarifier denitrification, displayed in row 7 of Table A.1, is calculated by measuring the nitrate depletion across the clarifier according to the equation shown below:

$$Q_{\text{EFF}} \times (\text{NO}_3_{\text{AE}} - \text{NO}_3_{\text{EFF}})$$ \hspace{1cm} (A.15)

Where,

\begin{align*}
Q_{\text{EFF}} &= \text{Effluent flow rate, L/day} \\
\text{NO}_3_{\text{AE}} &= \text{Nitrate concentration in the aerobic zone, mg/L NO}_3\text{-N} \\
\text{NO}_3_{\text{EFF}} &= \text{Nitrate concentration in the effluent, mg/L NO}_3\text{-N}
\end{align*}

Plugging in the raw data for the PAS train from Tables F.12 – F.16 of Appendix F into Equation A.15 and solving for the secondary clarification denitrification (row 7 in Table A.1):

\[(244.5 \text{ L/day}) \times (12.49 \text{ mg/L NO}_3\text{-N} - 12.29 \text{ mg/L NO}_3\text{-N}) = 49 \text{ mg N/day} \hspace{1cm} (A.16)\]

To solve for the % N mass balance agreement displayed in row 8 of Table A.1, the sum of N assimilated (Equation A.7), unaerated zone denitrification (Equation A.12), soluble effluent nitrogen (Equation A.14), and secondary clarifier denitrification (Equation A.16) is divided by
the total N in the influent (Equation A.5), and multiplied by 100 to be expressed as a percentage, as shown below:

\[
\frac{1967 \text{ mg N/day} + 4612 \text{ mg N/day} + 3533 \text{ mg N/day} + 49 \text{ mg N/day}}{10303 \text{ mg N/day}}
= \frac{10161 \text{ mg N/day}}{10303 \text{ mg N/day}} \times 100 = 98.6\% \quad (A.17)
\]

Simultaneous denitrification, which is the denitrification which occurs in the aerobic zone, was determined as the difference between the numerator and the denominator in Equation A.17, and shown in row 9 of Table A.1:

\[
(10303 \text{ mg N/day} - 10161 \text{ mg N/day}) = 142 \text{ mg N/day} \quad (A.18)
\]
APPENDIX B: COD MASS BALANCE
In order to further test the continuity of the data generated from the pilot system, a mass balance on chemical oxygen demand (COD) was conducted. The object of this mass balance was to verify that the mass of COD entering the system was accounted for, either through various biological activities of the microorganisms in the activated sludge system, or through leaving the system via the effluent and waste activated sludge (WAS). This particular COD mass balance was conducted on a system wide basis, with the boundary conditions encompassing the entire pilot plant. Equation B.1 provides the framework from which this COD mass balance was conducted:

\[ M_{\text{COD, influent}} = M_{\text{COD, effluent}} + M_{\text{COD, WAS}} + M_{\text{COD, oxidized}} \]  

(B.1)

Where,

\[ M_{\text{COD, influent}} = \text{mass of COD in the system influent, mg COD/d} \]
\[ M_{\text{COD, effluent}} = \text{mass of COD in the system effluent, mg COD/d} \]
\[ M_{\text{COD, WAS}} = \text{mass of COD in the waste sludge, mg COD/d} \]
\[ M_{\text{COD, oxidized}} = \text{mass of COD oxidized in the system, mg COD/d} \]

Further defining some of the above terms:

\[ M_{\text{COD, effluent}} = (\text{TCOD}_{\text{effluent}}) (Q_{\text{effluent}}) \]  

(B.2)
\[ M_{\text{COD, WAS}} = (Q_{\text{WAS}}) (\text{MLVSS}_{\text{WAS}}) (f_{CV}) \]  

(B.3)
Where,

- $\text{TCOD}_{\text{effluent}}$ = concentration of total COD in the effluent, mg COD/L
- $Q_{\text{effluent}}$ = flow rate of effluent, L/d
- $Q_{\text{WAS}}$ = flow rate of waste activated sludge, L/d
- $\text{MLVSS}_{\text{WAS}}$ = MLVSS of the waste activated sludge, mg VSS/L
- $f_{CV}$ = ratio of COD:VSS of waste activated sludge, 1.42 mg COD/mg VSS

In order to determine the mass of COD oxidized in the system, it must be recognized that the total quantity of oxygen consumed in the aerobic reactors consists of both carbonaceous and nitrogenous oxygen demand. The carbonaceous oxygen demand occurs as a result of the complete oxidation of reduced organics present in the pilot plant influent to $\text{CO}_2$ and $\text{H}_2\text{O}$, with $\text{O}_2$ serving as the terminal electron acceptor. The nitrogenous oxygen demand occurs as a result of nitrification, in which $\text{NH}_4^+$ is biologically transformed to $\text{NO}_3^-$ in an aerobic environment, thereby resulting in an oxygen demand. The nitrogenous oxygen demand is calculated by determining the mass of nitrate produced in the aerobic zone, and then multiplying the mass of nitrate produced by 4.57, which is the mass in $\text{O}_2$ (mg) required to produce each mg of nitrate via nitrification. The carbonaceous oxygen demand is then determined by subtracting the nitrogenous oxygen demand from the oxygen uptake rates measured in the aerobic zone. Note that equation B.4 assumes that simultaneous denitrification was negligible.

$$M_{\text{NO}_3^{-} \text{ produced}} = \Sigma M_{\text{NO}_3^{-} \text{ exiting aerobic zone}} - \Sigma M_{\text{NO}_3^{-} \text{ entering aerobic zone}} \quad \text{(B.4)}$$
\[ M_{\text{COD, aerobic}} = (OUR_{\text{aerobic}})(V_{\text{aerobic}}) - (M_{\text{NO}_3^{-}\text{produced}})(4.57) \]  \hspace{1cm} (B.5)

Where,

- \( M_{\text{COD, aerobic}} \) = carbonaceous oxygen demand, mg COD/d
- \( OUR_{\text{aerobic}} \) = oxygen uptake rate measured in the aerobic zone, mg O/L/d
- \( V_{\text{aerobic}} \) = volume of the aerobic reactor, L

Additionally, since the MUCT design of this pilot plant also allows for denitrification in the two separate anoxic zones, one must account for the oxygen equivalents of the amount of organic matter that would be oxidized during the denitrification process in which \( \text{NO}_3^- \) is used as the terminal electron acceptor. Quantitatively this is done through the use of the conversion factor 2.86 mg O\textsubscript{2} per mg \( \text{NO}_3^- \) denitrified (see Equation B.6).

\[ M_{\text{COD, denitrified}} = (M_{\text{NO}_3^{-}\text{denitrified}})(2.86) \]  \hspace{1cm} (B.6)

Where,

- \( M_{\text{COD, denitrified}} \) = mass of COD oxidized during denitrification, mg COD/d
- \( M_{\text{NO}_3^{-}\text{denitrified}} \) = mass of nitrate denitrified in anoxic zones, mg \( \text{NO}_3^- \)/d

Combining equations B.4, B.5, and B.6 to determine the total amount of COD oxidized:

\[ M_{\text{COD, oxidized}} = (OUR_{\text{aerobic}})(V_{\text{aerobic}}) - (M_{\text{NO}_3^{-}\text{produced}})(4.57) + (M_{\text{COD, denitrified}})(2.86) \]  \hspace{1cm} (B.7)
In order to calculate the % agreement of the COD mass balance, Equation B.8 can be utilized:

\[
% \text{ COD agreement} = \frac{\text{COD output}}{\text{COD input}} = \frac{M_{\text{COD, effluent}} + M_{\text{COD, WAS}} + M_{\text{COD, oxidized}}}{M_{\text{COD, influent}}} \quad (B.8)
\]

**Sample Calculation**

To clarify the theory behind nitrogen mass balances, a sample calculation, taken from one of the results chapters, will be used as a case study. Tables 6.5 and 6.6, which display the COD mass balances from Chapter 6 (Improved P Removal of Cod-Limited, Septic, Wastewater Via Prefermentation), are redisplayed here as Tables B.1 and B.2.
### Table B.1  COD Mass Balance

<table>
<thead>
<tr>
<th></th>
<th>COD-limited PAS</th>
<th>COD-limited CAS</th>
<th>P-limited PAS</th>
<th>P-limited CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>( M_{\text{COD, influent}} ) mg/d</td>
<td>87336</td>
<td>86458</td>
<td>82483</td>
<td>85472</td>
</tr>
<tr>
<td>( M_{\text{COD, effluent}} ) mg/d</td>
<td>13452</td>
<td>12178</td>
<td>14258</td>
<td>13893</td>
</tr>
<tr>
<td>( M_{\text{COD, WAS}} ) mg/d</td>
<td>17396</td>
<td>17179</td>
<td>19162</td>
<td>18691</td>
</tr>
<tr>
<td>( M_{\text{COD, oxidized}} ) mg/d</td>
<td>29747</td>
<td>26520</td>
<td>30554</td>
<td>29086</td>
</tr>
<tr>
<td>COD Loss mg/d</td>
<td>26741</td>
<td>30581</td>
<td>18509</td>
<td>23802</td>
</tr>
</tbody>
</table>

1\(^{\text{assumes } f_{CV} = 1.42}\)

2\(^{\text{includes oxygen inputs from recycle lines and diffusion from atmosphere}}\)

### Table B.2  COD Mass Balance % Agreement\(^{1}\)

<table>
<thead>
<tr>
<th></th>
<th>% COD Agreement</th>
<th>% COD Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PAS</td>
<td>CAS</td>
</tr>
<tr>
<td>Phase I (COD-limited)</td>
<td>69.4</td>
<td>64.6</td>
</tr>
<tr>
<td>Phase III (P-limited)</td>
<td>77.6</td>
<td>72.2</td>
</tr>
</tbody>
</table>

\(^{1}\text{assumes } f_{CV} = 1.42\)

Specifically, the COD mass balance from the PAS train on COD-Limited wastewater will be used as a sample calculation. Phase averages for the raw data from various phases of this research can be found in Tables F.12 – F.16 of Appendix F.
To calculate the total COD influent load ($M_{COD,\text{influent}}$), the first row in Table B.1, the sum of the influent total COD is multiplied by the influent flow rate, according to the following equation:

$$(Q_{INF}) \times (TCOD_{INF}) \quad (B.9)$$

Where,

$Q_{INF} = \text{Influent flow rate, L/day}$

$TCOD_{INF} = \text{Total COD concentration in the influent, mg/L}$

Note that TCOD implies a COD run on an unfiltered sample. The soluble COD for a given sample location is designated sCOD. Plugging in the raw data from Tables F.12 – F.16 of Appendix F into Equation B.9 and solving for the $M_{COD,\text{influent}}$:

$$(247.2 \text{ L/day}) \times (353.3 \text{ mg/L}) = 87336 \text{ mg/day} \quad (B.10)$$

To calculate the total COD effluent load ($M_{COD,\text{effluent}}$), row 2 in Table B.1, the sum of the influent total COD is multiplied by the influent flow rate, following Equation B.2. For completeness, the soluble COD in the WAS stream is added below in Equation B.11.

$$(Q_{EFF}) \times (TCOD_{EFF}) + (Q_{WAS}) \times (sCOD_{AE}) \quad (B.11)$$
Where,

\[ Q_{\text{EFF}} = \text{Effluent flow rate, L/day} \]

\[ \text{TCOD}_{\text{EFF}} = \text{Total COD concentration in the effluent, mg/L} \]

\[ Q_{\text{WAS}} = \text{Waste activated sludge flow rate, L/day} \]

\[ \text{sCOD}_{\text{AE}} = \text{Soluble COD concentration in the aerobic zone, mg/L} \]

Plugging in the raw data from Table XXX of Appendix F into Equation B.10 and solving for the 
\[ M_{\text{COD, effluent}}: \]

\[ (244.5 \text{ L/day}) \times (54.6 \text{ mg/L}) + (2.7 \text{ L/day}) \times (37.9 \text{ mg/L}) = 13452 \text{ mg/day} \]  \hspace{1cm} \text{(B.12)}

To calculate the daily mass loading of COD in the waste activated sludge (\( M_{\text{COD, WAS}} \)), row 3 in Table B.1, the waste activated sludge rate is multiplied by both the MLVSS of the WAS and the COD content of the biomass (\( f_{\text{CV}} \), assumed to be 1.42), following Equation sum of the influent total COD is multiplied by the influent flow rate, following Equation B.3, is shown below in Equation B.13:

\[ M_{\text{COD, WAS}} = (Q_{\text{WAS}}) \times (TSS_{\text{AE}}) \times (VSS_{\text{AE}}/TSS_{\text{AE}}) \times (f_{\text{CV}}) \]  \hspace{1cm} \text{(B.13)}

Where,

\[ Q_{\text{WAS}} = \text{Waste activated sludge flow rate, L/day} \]

\[ TSS_{\text{AE}} = \text{Total suspended solids of the aerobic zone, mg/L} \]

\[ \frac{VSS_{\text{AE}}}{TSS_{\text{AE}}} = \text{Ratio of volatile suspended solids to total suspended solids in the aerobic zone} \]
$f_{CV} =$ ratio of COD:VSS of waste activated sludge, assumed to be 1.42 mg COD/mg VSS

Note that since solids are wasted directly from the aerobic zone, values for various water parameters from the aerobic zone are used as the parameter values for the waste activated sludge.

Plugging in the raw data from Tables F.12 – F.16 of Appendix F into Equation B.13 and solving for the $M_{COD, WAS}$:

$$(2.7 \text{ L/day}) \times (5892.5 \text{ mg/L}) \times (0.770) \times (1.42) = 17396 \text{ mg/day} \quad (B.14)$$

To calculate the daily mass loading of COD oxidized in the system ($M_{COD, oxidized}$), row 4 in Table B.1, Equation B.7 is followed as modified below in Equation B.15

$$M_{COD, oxidized} = (OUR_{aerobic}) (V_{aerobic}) - (M_{NO3- \text{ produced}}) (4.57)$$

$$+ (M_{COD, \text{ denit}}) \times (2.86) \quad (B.15)$$

Where,

$M_{COD, oxidized} =$ COD oxidized in the system, mg COD/d

$OUR_{aerobic} =$ In-situ oxygen uptake rate measured in the aerobic zone, mg O/L/day

$V_{aerobic} =$ Volume of the aerobic reactor, L

$M_{NO3- \text{ produced}} =$ Mass of nitrate produced in the aerobic zone, mg/day

$M_{COD, \text{ denit}} =$ Mass of COD oxidized during denitrification, mg COD/d
Before Equation B.15 can be evaluated, the mass of nitrate produced in the aerobic zone ($M_{NO3\text{-}produced}$) and the mass of COD oxidized during denitrification ($M_{COD\text{,denit}}$) must be determined.

To calculate $M_{NO3\text{-}produced}$, the following equation can be used:

$$M_{NO3\text{-}produced} = \Sigma M_{NO3\text{-}exiting\ aerobic\ zone} - \Sigma M_{NO3\text{-}entering\ aerobic\ zone} \quad (B.16)$$

Equation B.16 can be further broken down into Equation B.17, shown below:

$$M_{NO3\text{-}produced} = (Q_{INF} + Q_{RAS} + Q_{NARCY}) \times (NO3_{AE} - NO3_{AXII}) \quad (B.17)$$

Where,

$Q_{INF}$ = Influent flow rate, L/day

$Q_{RAS}$ = Return activated sludge flow rate, L/day

$Q_{NARCY}$ = Nitrate recycle flow rate, L/day

$NO3_{AE}$ = Nitrate concentration in the aerobic zone, mg/L NO$_3$-N

$NO3_{AXII}$ = Nitrate concentration in anoxic II, mg/L NO$_3$-N

Plugging in the raw data from Table F.12 – F.16 of Appendix F into Equation B.17 and solving for the $M_{NO3\text{-}produced}$:

$$(247.2 \text{ L/day} + 175.6 \text{ L/day} + 767.8 \text{ L/day}) \times (12.49 \text{ mg/L NO}_3\text{-N} - 6.01 \text{ mg/L NO}_3\text{-N} )$$

$$= 7715 \text{ mg/day NO}_3\text{-N} \quad (B.18)$$
To calculate the mass of COD oxidized during denitrification ($M_{\text{COD, denit}}$), the mass of nitrate denitrified in the unaerated zones, and the secondary clarifier, must be determined. In Appendix A, Equations A.12 and A.16 calculate the nitrate denitrified in the unaerated zones (4612 mg/day) and in the secondary clarifier (49 mg/day).

Taking the value calculated for $M_{\text{NO}_3^-$ produced from Equation B.18, and the value for $M_{\text{COD, denit}}$ determined previously in Appendix A (4612 mg/day + 49 mg/day = 4661 mg/day), and using the in-situ OUR value found in Appendix F, Tables F.12 – F.16, the value for $M_{\text{COD, oxidized}}$ can be determined:

$$M_{\text{COD, oxidized}} = (111.8 \text{ mg/L/hr}) (18 \text{ L}) (24 \text{ hr/day}) - (7715) (4.57) + (4661) * (2.86) = 26371 \text{ mg/day} \quad \text{(B.19)}$$

As can be seen from Table 6.6, the COD mass balance % agreements do not approach 100% for either the COD-limited phase or the P-limited phase. The value shown in row 4 of Table B.1 also takes into account oxygen inputs from internal recycle lines, oxidation in the secondary clarifier, and from the liquid/atmosphere interface. Two recycle lines, the NARCY and the RAS, input oxygen into non-aerated zones. The third recycle line in the pilot plant, the ARCY, does not input any oxygen, as it draws from anoxic I (no measurable DO). The oxygen input from the recycle lines was calculated by multiplying the relevant flow rate by the average dissolved oxygen (DO) measured in the reactor from which the recycle line originated. For example, the NARCY flow rate (767.8 L/day) was multiplied by the average DO measured in the aerobic zone (3.1 mg/L) to determine the oxygen input from the NARCY into Anoxic II (2380
mg/day). The RAS flow rate (175.6 L/day) was multiplied by the average DO measured in the secondary clarifier (1.0 mg/L) to determine the oxygen input from the RAS into Anoxic I (176 mg/day). Summing the effects of the two recycle lines, a total of 2556 mg/day of DO was input into unaerated reactors via recycle lines.

The difference in dissolved oxygen concentrations between the aerobic zone (3.1 mg/L) and the secondary clarifier (1.0 mg/L) indicate that oxidation occurred within the secondary clarifier. Conducting a mass balance around the secondary clarifier, it can be shown that a total of 888 mg/day of oxygen is consumed in the secondary clarifier.

Oxygen input from the atmosphere/liquid interface was determined by a batch test in which the DO increase of non-chlorinated effluent within the reactors used in the pilot study was measured vs. time. The non-chlorinated effluent was spiked with sodium sulfite to drop the initial DO level, along with cobalt (a catalyst which facilitates the sulfite depletion the dissolved oxygen). Results from the atmosphere/liquid interface evaluation for the anaerobic reactor are displayed graphically in Figure B.1. The slope of the linear region of Figure B.1 was 0.0516 mg/L/min. Multiplying by the anaerobic reactor volume (3.5 L) and converting into days, oxygen input from the atmosphere/liquid interface in the anaerobic zone was determined to be 260 mg DO/day. A similar test run on the larger anoxic zones resulted in a value of 280 mg DO/day per reactor due to oxygen input from the atmosphere/liquid interface.
Summing the impact of oxygen inputs from both internal recycle lines and from the liquid/atmosphere interface, a total of 3376 mg/day of DO inputs (2556 from the recycle lines and 820 mg/day from the liquid/atmosphere interface of the anaerobic, anoxic I, and anoxic II zones) into the unaerated zones for the PAS train in the COD-limited phase can be calculated. This value (3376 mg/day) is added to the measured mass of COD oxidized (26371 mg/day) to result in a total mass of 29747 mg/day, which is the value shown in row 4 of Table B.1. While this additional calculation improves the COD mass balances, there is still significant COD loss, as shown in row 5 of Tables B.1.
APPENDIX C: PHOSPHORUS MASS BALANCE
Theory

In wastewater treatment systems, phosphorus is a conservative element, meaning that phosphorus cannot escape the system in a gaseous phase. The only way for phosphorus to leave a wastewater treatment system is either through the liquid effluent (either soluble, or in solid form), or through the waste activated sludge (in the form of biomass). Expressing this statement in the form of an equation:

\[
TP_{\text{influent}} \times Q_{\text{influent}} = TP_{\text{effluent}} \times Q_{\text{effluent}} + MLVSS_{\text{WAS}} \times \%P \times Q_{\text{WAS}}
\]  

(C.1)

Where,

\(TP_{\text{influent}}\) = Total phosphorus concentration in the influent, mg/L

\(Q_{\text{influent}}\) = Influent flow rate, L/day

\(TP_{\text{effluent}}\) = Total phosphorus concentration in the effluent, mg/L

\(Q_{\text{effluent}}\) = Effluent flow rate, L/day

\(MLVSS_{\text{WAS}}\) = Mixed liquor volatile suspended solids concentration of the waste activated sludge, mg/L

\(\%P\) = Percent phosphorus content of the biomass, %

\(Q_{\text{WAS}}\) = Waste activated sludge flow rate, L/day

The percent agreement of a phosphorus mass balance indicates the percentage of the influent mass of phosphorus you are able to measure in the effluent and waste activated sludge, according to equation C.1.
Sample Calculation

To clarify the theory behind a phosphorus mass balance, a sample calculation, taken from one of the results chapters, will be used as a case study. Table 6.2, which displays the phosphorus mass balances from Chapter 6 (Improved P Removal of Cod-Limited, Septic, Wastewater Via Prefermentation), is redisplayed here as Table C.1.

Table C.1 Phosphorus Mass Flux Values for the COD-Limited and P-Limited Phases

<table>
<thead>
<tr>
<th>Parameters (mg/day)</th>
<th>COD-limited PAS</th>
<th>COD-limited CAS</th>
<th>P-limited PAS</th>
<th>P-limited CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP influent</td>
<td>2917</td>
<td>2905.1</td>
<td>1680.8</td>
<td>1683.7</td>
</tr>
<tr>
<td>Anaerobic SOP Release</td>
<td>4746</td>
<td>2910.2</td>
<td>2801.4</td>
<td>1956.0</td>
</tr>
<tr>
<td>Anoxic I SOP Release</td>
<td>8567</td>
<td>7178.5</td>
<td>6359.7</td>
<td>6643.5</td>
</tr>
<tr>
<td>Anoxic II SOP Uptake</td>
<td>5481</td>
<td>3450.5</td>
<td>1355.4</td>
<td>1069.4</td>
</tr>
<tr>
<td>Net SOP Anoxic Release</td>
<td>3086</td>
<td>3728.0</td>
<td>5004.3</td>
<td>5574.1</td>
</tr>
<tr>
<td>Total SOP Release</td>
<td>13313</td>
<td>10088.7</td>
<td>9161.1</td>
<td>8599.5</td>
</tr>
<tr>
<td>Aerobic SOP Uptake</td>
<td>10120</td>
<td>8461.1</td>
<td>9250.0</td>
<td>8924.3</td>
</tr>
<tr>
<td>Clarifier SOP Uptake</td>
<td>-211</td>
<td>-84.8</td>
<td>41.6</td>
<td>42.0</td>
</tr>
<tr>
<td>Total SOP Uptake</td>
<td>15390</td>
<td>11826.8</td>
<td>10647.0</td>
<td>10035.7</td>
</tr>
<tr>
<td>SOP Uptake:SOP Release Ratio</td>
<td>1.16</td>
<td>1.17</td>
<td>1.16</td>
<td>1.17</td>
</tr>
<tr>
<td>Net SOP Uptake</td>
<td>2077</td>
<td>1738</td>
<td>1486</td>
<td>1436</td>
</tr>
<tr>
<td>%P in MLSS as calculated via MB</td>
<td>10.0</td>
<td>8.7</td>
<td>6.5</td>
<td>6.3</td>
</tr>
</tbody>
</table>

Specifically, the phosphorus mass balance from the PAS train on COD-Limited wastewater will be used as a sample calculation. Phase averages for the raw data from various phases of this research can be found in Tables F.12 – F.16 of Appendix F.

To calculate the total phosphorus (TP) influent load, the first row in Table C.1, the sum of the influent total phosphorus is multiplied by the influent flow rate, according to the following equation:
(Q_{INF} \times TP_{INF}) \quad \text{(C.2)}

Where,

Q_{INF} = \text{Influent flow rate, L/day}

TP_{INF} = \text{Total phosphorus concentration in the influent, mg/L PO}_4^{-}\text{P}

Plugging in the raw data from Table XXX of Appendix F into Equation C.2 and solving for the TP influent load:

(247.2 \text{ L/day}) \times (11.8 \text{ mg/L PO}_4^{-}\text{P}) = 2917 \text{ mg P/day} \quad \text{(C.3)}

Rows 2 through 9 in Table C.1 display soluble ortho-phosphorus (SOP) release or uptake calculated for various zones of the PAS train during the COD-limited phase. SOP release and uptake are phenomena associated with biological phosphorus removal. SOP release (i.e. more SOP leaves a treatment zone than enters, due to biological activity) generally takes place in the anaerobic zone of wastewater treatment systems. SOP uptake (i.e. more SOP enters than leaves a treatment zone) takes place within aerobic zones, as biomass incorporates P into new growth. Either SOP release or uptake could take place within anoxic zones, depending upon the various concentrations of SOP and upon flow rates. The SOP release found in the anaerobic zone of the PAS train during the COD-limited phase (row 2 of Table C.1) is calculated by conducting a mass balance around the anaerobic zone, according to the equation shown below:
\[(Q_{\text{INF}} + Q_{\text{ARCY}}) \times (SOP_{\text{AN}}) - (Q_{\text{INF}}) \times (TP_{\text{INF}}) - (Q_{\text{ARCY}}) \times (SOP_{\text{AXI}})\]  \hspace{1cm} (C.4)

Where,

\(Q_{\text{INF}}\) = Influent flow rate, L/day

\(Q_{\text{ARCY}}\) = Anaerobic recycle flow rate, L/day

\(SOP_{\text{AN}}\) = Soluble ortho-phosphorus in the anaerobic zone, mg/L PO\(_4\)-P

\(TP_{\text{INF}}\) = Total phosphorus in the influent, mg/L PO\(_4\)-P

\(SOP_{\text{AXI}}\) = Soluble ortho-phosphorus in anoxic zone I, mg/L PO\(_4\)-P

Note that total phosphorus, not soluble phosphorus, is used for the influent, as the particulate phosphorus in the influent can generally be readily converted biologically to ortho-phosphate.

Plugging in the raw data from Tables F.12 – F.16 of Appendix F into Equation C.4, and solving for the anaerobic SOP release:

\[(247.2 \text{ L/day} + 247.2 \text{ L/day}) \times (35.4 \text{ mg/L PO}_4\text{-P}) - (247.2 \text{ L/day}) \times (11.8 \text{ mg/L PO}_4\text{-P})\]

\[- (247.2 \text{ L/day}) \times (39.8 \text{ mg/L PO}_4\text{-P}) = 4746 \text{ mg P/day}\]  \hspace{1cm} (C.5)

The SOP release found in anoxic I of the PAS train during the COD-limited phase (row 3 of Table C.1) is calculated by conducting a mass balance around anoxic I, according to the equation shown below:

\[(Q_{\text{INF}} + Q_{\text{ARCY}} + Q_{\text{RAS}}) \times (SOP_{\text{AXI}}) - (Q_{\text{INF}} + Q_{\text{ARCY}}) \times (SOP_{\text{AN}}) - (Q_{\text{RAS}}) \times (SOP_{\text{EFF}})\]  \hspace{1cm} (C.6)

Where,
\[ Q_{\text{INF}} = \text{Influent flow rate, L/day} \]

\[ Q_{\text{ARCY}} = \text{Anaerobic recycle flow rate, L/day} \]

\[ Q_{\text{RAS}} = \text{Return activated sludge flow rate, L/day} \]

\[ \text{SOP}_{\text{AXI}} = \text{Soluble ortho-phosphorus in anoxic zone I, mg/L PO}_4\text{-P} \]

\[ \text{SOP}_{\text{AN}} = \text{Soluble ortho-phosphorus in the anaerobic zone, mg/L PO}_4\text{-P} \]

\[ \text{SOP}_{\text{EFF}} = \text{Soluble ortho-phosphorus in the effluent, mg/L PO}_4\text{-P} \]

Note that equation C.6 is set up assuming there is SOP release in anoxic zone I (i.e. more SOP leaving than entering anoxic zone I). If the calculated SOP release using Equation C.6 is negative, then SOP uptake actually occurred. Plugging in the raw data from Tables F.12 – F.16 of Appendix F into Equation C.6, and solving for the anoxic I SOP release:

\[
(247.2 \text{ L/day} + 247.2 \text{ L/day} + 175.6 \text{ L/day}) \times (39.8 \text{ mg/L PO}_4\text{-P}) - (247.2 \text{ L/day} + 247.2 \text{ L/day}) \times (35.4 \text{ mg/L PO}_4\text{-P}) - (175.6 \text{ L/day}) \times (3.4 \text{ mg/L PO}_4\text{-P}) = 8567 \text{ mg P/day} \] (C.7)

The SOP uptake found in anoxic II of the PAS train during the COD-limited phase (row 4 of Table C.1) is calculated by conducting a mass balance around anoxic II, according to the equation shown below:

\[
(Q_{\text{INF}} + Q_{\text{RAS}}) \times (\text{SOP}_{\text{AXI}}) + (Q_{\text{NARCY}}) \times (\text{SOP}_{\text{AE}}) - (Q_{\text{INF}} + Q_{\text{ARCY}} + Q_{\text{NARCY}}) \times (\text{SOP}_{\text{AXII}}) \] (C.8)

Where,

\[ Q_{\text{INF}} = \text{Influent flow rate, L/day} \]
Q_{RAS} = Return activated sludge flow rate, L/day

SOP_{AXI} = Soluble ortho-phosphorus in anoxic zone I, mg/L PO₄-P

Q_{NARCY} = Nitrate recycle flow rate, L/day

SOP_{AE} = Soluble ortho-phosphorus in the aerobic zone, mg/L PO₄-P

SOP_{AXII} = Soluble ortho-phosphorus in anoxic II, mg/L PO₄-P

Note that Equation C.8 is set up assuming there is SOP uptake in anoxic zone II (i.e. more SOP entering than leaving anoxic zone II). If the calculated SOP uptake using Equation C.8 is negative, then SOP release actually occurred. Plugging in the raw data from Tables F.12 – F.16 of Appendix F into Equation C.8, and solving for the anoxic II SOP uptake:

\[
\begin{align*}
(247.2 \text{ L/day} + 175.6 \text{ L/day}) & \times (39.8 \text{ mg/L PO}_4\text{-P}) + (767.8 \text{ L/day}) \times (2.9 \text{ mg/L PO}_4\text{-P}) \\
- (247.2 \text{ L/day} + 247.2 \text{ L/day} + 767.8 \text{ L/day}) & \times (11.4 \text{ mg/L PO}_4\text{-P}) \\
= 5481 \text{ mg P/day} 
\end{align*}
\] (C.9)

The net SOP anoxic release (row 5 of Table C.1) is calculated by summing the SOP release in the anoxic zones. Note that an SOP uptake implies a negative SOP release. Therefore, adding the SOP release in anoxic zone I (8567 mg P/day) to the SOP release in anoxic zone II (-5481 mg P/day) results in a net SOP anoxic release of 3086 mg P/day (row 5 of Table C.1).

Total SOP release (row 6 of Table C.1) is found by summing the SOP release from those zones which had SOP release – namely the anaerobic zone (row 2 of Table C.1) and anoxic I (row 3 of Table C.1). The sum of these values results in a total SOP release of 13313 mg P/day.
The SOP uptake found in the aerobic zone of the PAS train during the COD-limited phase (row 7 of Table C.1) is calculated by conducting a mass balance around the aerobic zone, according to the equation shown below:

\[(Q_{\text{INF}} + Q_{\text{RAS}} + Q_{\text{NARCY}}) \times (\text{SOP}_{\text{AXII}}) - (Q_{\text{INF}} + Q_{\text{RAS}} + Q_{\text{NARCY}}) \times (\text{SOP}_{\text{AE}})\]  \hspace{1cm} (C.10)

Where,

- \(Q_{\text{INF}}\) = Influent flow rate, L/day
- \(Q_{\text{RAS}}\) = Return activated sludge flow rate, L/day
- \(Q_{\text{NARCY}}\) = Nitrate recycle flow rate, L/day
- \(\text{SOP}_{\text{AXII}}\) = Soluble ortho-phosphorus in anoxic zone II, mg/L PO\(_4\)-P
- \(\text{SOP}_{\text{AE}}\) = Soluble ortho-phosphorus in the aerobic zone, mg/L PO\(_4\)-P

Note that Equation C.10 is set up assuming there is SOP uptake in the aerobic zone (i.e. more SOP entering than leaving anoxic zone II). If the calculated SOP uptake using Equation C.10 is negative, then SOP release actually occurred. Plugging in the raw data from Tables F.12 – F.16 of Appendix F into Equation C.10, and solving for the aerobic zone SOP uptake:

\[
(247.2 \text{ L/day} + 175.6 \text{ L/day} + 767.8 \text{ L/day}) \times (11.4 \text{ mg/L PO}_4\text{-P}) - (247.2 \text{ L/day} + 175.6 \text{ L/day} + 767.8 \text{ L/day}) \times (2.9 \text{ mg/L PO}_4\text{-P}) = 10120 \text{ mg P/day} \]  \hspace{1cm} (C.11)
The SOP uptake found in the secondary clarifier of the PAS train during the COD-limited phase (row 8 of Table C.1) is calculated by conducting a mass balance around the secondary clarifier, according to the equation shown below:

\[
(Q_{\text{EFF}} + Q_{\text{RAS}}) \times (SOP_{\text{AE}}) - (Q_{\text{EFF}} + Q_{\text{RAS}}) \times (SOP_{\text{EFF}}) = 0
\]  

(C.12)

Where,

\[Q_{\text{EFF}} = \text{Effluent flow rate, L/day}\]

\[Q_{\text{RAS}} = \text{Return activated sludge flow rate, L/day}\]

\[SOP_{\text{AE}} = \text{Soluble ortho-phosphorus in the aerobic zone, mg/L PO}_4\text{-P}\]

\[SOP_{\text{EFF}} = \text{Soluble ortho-phosphorus in the effluent, mg/L PO}_4\text{-P}\]

Note that Equation C.12 is set up assuming there is SOP uptake in the secondary clarifier (i.e. more SOP entering than leaving secondary clarifier). If the calculated SOP uptake using Equation C.12 is negative, then SOP release actually occurred. Plugging in the raw data from Tables F.12 – F.16 of Appendix F into Equation C.12, and solving for the secondary clarifier SOP uptake:

\[
(244.5 \text{ L/day} + 175.6 \text{ L/day}) \times (2.9 \text{ mg/L PO}_4\text{-P}) - (244.5 \text{ L/day} + 175.6 \text{ L/day}) \times (3.4 \text{ mg/L PO}_4\text{-P}) = -211 \text{ mg P/day}
\]

(C.13)

Total SOP uptake (row 9 of Table C.1) is found by summing the SOP uptake from those zones which would have been expected to have SOP uptake – namely anoxic zone II (row 4 of...
Table C.1), the aerobic zone (row 7 of Table C.1), and the secondary clarifier (row 8 of Table C.1). The sum of these values results in a total SOP uptake of 15390 mg P/day. Note that the “expected” uptake in the secondary clarifier was actually a release, but was considered in this calculation.

The SOP Uptake:SOP Release ratio is simply the ratio of the total SOP uptake (row 9 of Table C.1) to the total SOP release (row 6 of Table C.1). Solving, the ratio is 15390:13313, or 1.16:1.

The net SOP uptake is the total sum of SOP uptake, minus the total sum of SOP release. More specifically, the net SOP uptake is the aerobic uptake (10120 mg P/day), minus the sum of the anaerobic SOP release (4746 mg P/day), the net SOP anoxic release (3086 mg P/day), and the clarifier SOP release (211 mg P/day). This calculation results in a net SOP uptake of 2077 mg P/day. Note that without a net SOP uptake in the system, no biological phosphorus removal can occur.

The final calculation presented in Table C.1 (row 12) is the %P content of the MLSS as calculated via the mass balance. The %P content is calculated assuming that 100% of the influent phosphorus either goes out the effluent, or is incorporated into new biomass. The %P content can be directly measured using analytical methods, but the analytical results are generally poor, as digestion techniques (persulfate, in this case) typically are not sufficient for complete breakdown of organically-bound phosphorus. Other phosphorus digestion methods (nitric, perchloric) were explored, but discarded either due to budgetary or equipment constraints. The %P content was therefore calculated by assuming that all influent phosphorus not leaving via the effluent was incorporated into new biomass. This concept is expressed below in Equation C.14:
\[
\frac{(Q_{\text{INF}}) \times (T_P_{\text{INF}}) - (Q_{\text{EFF}}) \times (S_O_{\text{P}}_{\text{EFF}}) - (Q_{\text{WAS}}) \times (S_O_{\text{P}}_{\text{AE}}))}{(Q_{\text{WAS}}) \times (T_S S_{\text{AE}}) + (Q_{\text{EFF}}) \times (T_S S_{\text{EFF}})} \times 100
\]

(C.14)

Where,

\(Q_{\text{INF}}\) = Influent flow rate, L/day

\(T_P_{\text{INF}}\) = Total phosphorus in the influent, mg/L PO\(_4\)-P

\(Q_{\text{EFF}}\) = Return activated sludge flow rate, L/day

\(S_O_{\text{P}}_{\text{EFF}}\) = Soluble ortho-phosphorus in the effluent, mg/L PO\(_4\)-P

\(Q_{\text{WAS}}\) = Waste activated sludge flow rate, L/day

\(S_O_{\text{P}}_{\text{AE}}\) = Soluble ortho-phosphorus in the aerobic zone, mg/L PO\(_4\)-P

\(T_S S_{\text{AE}}\) = Total suspended solids in the aerobic zone, mg/L

\(T_S S_{\text{EFF}}\) = Total suspended solids in the effluent, mg/L
Plugging in the raw data from Tables F.12 – F.16 of Appendix F into Equation C.14, and solving for the %P content:

\[
\frac{(247.2 \text{ L/day}) \times (11.8 \text{ mg/L P}) - (244.5 \text{ L/day}) \times (3.4 \text{ mg/L P}) - (2.7 \text{ L/day}) \times (2.9 \text{ mg/L P})}{(2.7 \text{ L/day}) \times (5892.5 \text{ mg/L}) + (244.5 \text{ L/day}) \times (19.8 \text{ mg/L})} \times 100 = 10.0\% \quad (C.15)
\]
APPENDIX D: BIOKINETIC PARAMETERS
Introduction

The ability of a prefermenter to enhance EBPR performance in BNR systems through enhanced VFA production is well understood in the literature (McCue et al., 2003; McCue et al., 2004). Less well understood are other potential benefits of prefermentation upon activated sludge performance. This study focused upon conducting experiments to establish values for important domestic wastewater influent biokinetic parameters, including RBCOD, the maximum specific growth rate coefficient for autotrophic biomass ($\mu_{A_{max}}$), and inert COD fractionation. Determination of these biokinetic parameters provides information concerning the impact of prefermentation upon the biological treatability of wastewater. Additionally, using these experimentally determined values for influent biokinetic parameters, instead of standard default assumptions, can potentially lead to superior performance of activated sludge modeling of BNR systems with prefermentation.

Materials and Methods

A pilot-scale system consisting of two parallel 4 stage modified UCT systems (Figure D.1) was operated over a two year period to determine impacts of prefermentation upon activated sludge performance, especially BNR. The prefermented activated sludge system (PAS) influent was augmented with the effluent from a static prefermenter operated at a 10 day SRT, while the control activated sludge system (CAS) received an equal amount of fresh, unprefermented primary solids. Different phases of the study focused on the impacts of prefermentation on both COD-limited (TCOD:TP ratio less than 40:1) and P-limited (TCOD:TP ratio greater than 40:1) wastewaters (WEF, 1998). Readily biodegradable chemical oxygen
demand (RBCOD) was determined following techniques developed both by Ekama et al. (1986) and Wentzel et al. (1995). The determination of the maximum specific growth rate for autotrophic biomass was determined by using a batch technique similar to those outlined in the literature (Hall, 1974; Antoniou et al., 1990; Drtil et al., 1993). The COD fractions in wastewaters are defined as follows: initially inert particulate (X_I), initially inert soluble (S_I), particulate inert metabolic (X_P) and soluble inert metabolic (S_P). The fractionation procedure was done according to Germirli et al., (1993).

Figure D.1  Schematic of Pilot-Scale System

Results and Discussion

Prefermentation was found to significantly increase the RBCOD in both COD-limited and P-limited wastewaters, as shown in Table D.1. RBCOD values, including the initial values, were very high, showing the nature of highly septic Florida wastewaters. In spite of the septic
nature of the wastewater prefermentation increased the RBCOD content of both COD-limited and P-limited wastewaters by 23% and 29%, respectively. Using a paired difference test between two population means, it can be shown that the increased RBCOD in prefermented influent was highly significant, with P-values of 0.0001 and 0.002 for COD-limited and P-limited wastewaters, respectively (Mendenhall and Sincich, 1995). Increased RBCOD content in wastewater has numerous positive impacts upon activated sludge performance, including improved BNR.

Table D.1 RBCOD Values for COD-Limited and P-Limited Wastewaters

<table>
<thead>
<tr>
<th></th>
<th>RBCOD (mg/L)</th>
<th>RBCOD (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COD-limited</td>
<td>P-limited</td>
</tr>
<tr>
<td>With Prefermentation (PAS)</td>
<td>149</td>
<td>128</td>
</tr>
<tr>
<td>Without Prefermentation (CAS)</td>
<td>121</td>
<td>99</td>
</tr>
</tbody>
</table>

Table D.2 displays the values determined for the maximum specific growth rate for autotrophic biomass ($\mu_{A_{\text{max}}}$) for both COD-limited and P-limited wastewaters. Prefermentation was shown to increase $\mu_{A_{\text{max}}}$ by 9% (P-value of 0.23) for COD-limited wastewater and by 4% (P-value of 0.07) for P-limited wastewater. These values for prefermented influent are slightly higher than typical default values (0.77 day$^{-1}$) for temperatures around 20 deg C. These increases to $\mu_{A_{\text{max}}}$ caused by prefermentation are important, as $\mu_{A_{\text{max}}}$ is the most critical parameter for the design and control of nitrifying bioreactor systems (Grady et al., 1999).
### Table D.2 Maximum Specific Growth Rate for Autotrophic Biomass Values for COD-Limited and P-Limited Wastewaters

<table>
<thead>
<tr>
<th></th>
<th>μ&lt;sub&gt;Amax&lt;/sub&gt; (day&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>μ&lt;sub&gt;Amax&lt;/sub&gt; (day&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>COD-limited</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With Prefermentation (PAS)</td>
<td>0.82</td>
<td>0.79</td>
</tr>
<tr>
<td>Without Prefermentation (CAS)</td>
<td>0.75</td>
<td>0.76</td>
</tr>
<tr>
<td><strong>P-limited</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The inert COD fractionation of COD-limited and P-limited wastewaters impacted by prefermentation is shown in Tables D.3 and D.4. The inert soluble COD fraction (sum of S<sub>i</sub> and S<sub>p</sub>) was reduced from 11% of total COD (C<sub>T0</sub>) to 7% (P-value of .08) for COD-limited wastewaters with prefermentation and from 12% to 8% (P-value of 0.08) for P-limited wastewaters with prefermentation. A reduction in the inert soluble COD fraction can improve process performance by reducing soluble COD in the effluent.

### Table D.3 Inert COD Fractions for a COD-Limited Wastewater

<table>
<thead>
<tr>
<th></th>
<th>S&lt;sub&gt;i&lt;/sub&gt;/C&lt;sub&gt;T0&lt;/sub&gt;</th>
<th>X&lt;sub&gt;i&lt;/sub&gt;/C&lt;sub&gt;T0&lt;/sub&gt;</th>
<th>S&lt;sub&gt;p&lt;/sub&gt;/C&lt;sub&gt;T0&lt;/sub&gt;</th>
<th>X&lt;sub&gt;p&lt;/sub&gt;/C&lt;sub&gt;T0&lt;/sub&gt;</th>
<th>S&lt;sub&gt;i&lt;/sub&gt;+S&lt;sub&gt;p&lt;/sub&gt;/C&lt;sub&gt;T0&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>With Prefermentation (PAS)</td>
<td>0.02</td>
<td>0.04</td>
<td>0.05</td>
<td>0.11</td>
<td>0.07</td>
</tr>
<tr>
<td>Without Prefermentation (CAS)</td>
<td>0.05</td>
<td>0.05</td>
<td>0.06</td>
<td>0.12</td>
<td>0.11</td>
</tr>
</tbody>
</table>
Table D.4 Inert COD Fractions for a P-Limited Wastewater

<table>
<thead>
<tr>
<th></th>
<th>$S_i/C_{T0}$</th>
<th>$X_i/C_{T0}$</th>
<th>$Sp/C_{T0}$</th>
<th>$Xp/C_{T0}$</th>
<th>$S_i+Sp/C_{T0}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>With Prefermentation unit (PAS)</td>
<td>0.03</td>
<td>0.04</td>
<td>0.04</td>
<td>0.10</td>
<td>0.08</td>
</tr>
<tr>
<td>Without Prefermentation unit (CAS)</td>
<td>0.05</td>
<td>0.06</td>
<td>0.06</td>
<td>0.11</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Conclusions

To conclude, prefermentation had statistically measurable impacts upon the RBCOD, $\mu_{A_{\text{max}}}$, and inert COD fractionation of domestic wastewater. Additionally, it should be noted that the effect of prefermentation might be expected to be much greater in a fresh wastewater as this was an extremely septic Florida wastewater with a very high initial RBCOD content.

- Prefermentation was found to increase the RBCOD in both COD-limited (from 121 to 149 mg/L) and P-limited (from 99 to 128 mg/L) wastewaters, with $P$-values of 0.0001 and 0.002 for COD-limited and P-limited wastewaters, respectively.

- Prefermentation was shown to increase the maximum specific growth rate coefficient for autotrophic biomass, $\mu_{A_{\text{max}}}$, by 9% ($P$-value of 0.23) for COD-limited wastewater and by 4% ($P$-value of 0.07) for P-limited wastewater. These values for prefermented influent (0.82 day$^{-1}$ for the COD-limited wastewater, and 0.79 day$^{-1}$ for the P-limited wastewater) are slightly higher than typical default values (0.77 day$^{-1}$) for temperatures around 20 deg C.

- The inert soluble COD fraction (sum of $S_i$ and $Sp$) was reduced from 11% of total COD ($C_{T0}$) to 7% ($P$-value of .08) for COD-limited wastewaters with prefermentation and from 12% to 8% ($P$-value of 0.08) for P-limited wastewaters with prefermentation.
Sample Calculations

RBCOD

Rapidly biodegradable chemical oxygen demand (RBCOD) is an influent fraction important in the modeling of activated sludge systems. Techniques developed both by Ekama, et al (1986) and Wentzel, et al (1995) were used during this study. A BOD bottle probe and dissolved oxygen meter (YSI, Yellow Springs, Wyoming) were used in Ekama’s method, while an automatic OUR meter (High Tech Microsystems, Capetown, South Africa) was used for Wentzel’s method. In both cases, the tests revolve around OURs taken over time for a given sample. The changes in the slope of the OUR measurements assist in determining values for RBCOD. A 3L rectangular Plexiglas reactor was filled with influent wastewater, and some seed biomass from the activated sludge system in order to have a proper F/M ratio (see Ekema, et al, 1986). The reactor was kept continuously stirred by a magnetic stirrer. The OUR meter (following Wentzel’s method) consisted of a DO probe attached to a controlling mechanism, which cycled the air on and off while collecting data for the RBCOD calculation. A sample plot of OUR vs. time, as generated by the OUR meter, is shown below in Figure D.2.
The next step in determining RBCOD is to calculate $Z_{BH}$, according to the following equation:

$$Z_{BH} = \frac{e^{(Y_{int \text{ intercept}} - \text{slope} \cdot 24 \cdot b_H)} \cdot 24}{1 - \frac{Y_{ZH}}{Y_{ZH}} \cdot \text{slope} \cdot 24 \cdot b_H} \tag{D.1}$$

Where,

$Z_{BH} = \text{Heterotroph active biomass concentration (mg COD/L)}$

$Y_{ZH} = \text{Heterotroph yield, assumed to be 0.666 mg COD/mg COD}$
\( b_H = \) Heterotroph specific death rate, assumed to be 0.62/day

\( \text{y-intercept} = \) Y-intercept of linear region of a plot of ln OUR vs time

\( \text{slope} = \) Slope of linear region of a plot of ln OUR vs time

To complete the calculation, you must plot the ln OUR vs. time from the beginning of the test, though the peak value of ln OUR, and determine the slope and intercept of the line. Figure D.3, shown below, plots ln OUR vs. time for this sample calculation.

Figure D.3  Plot of ln OUR vs. Time, for RBCOD calculation
Using Excel’s slope and intercept commands for linear region of Figure D.3 (roughly from at the 
1.5 hr mark extending through the 6 hour mark), the slope is 0.426, and the intercept is 0.541.
Plugging in the values determined for the slope and intercept into Equation D.1, \( Z_{BH} = 7.58 \) mg
COD/L, in this example.

The next step in calculating RBCOD is to determine \( K_{MP} \), according the following

equation:

\[
K_{MP} = \frac{OUR_{SBCOD(t=s)} \cdot 24}{1 - \frac{Y_{ZH}}{Y_{ZH}} \cdot Z_{BH} \cdot e^{slope(t=s)}}
\]  

(D.2)

Where,

\( K_{MP} \) = Heterotroph max specific growth rate on a slowly biodegradable substrate, day\(^{-1}\)

\( OUR_{SBCOD(t=s)} \) = Observed OUR on OUR vs. time plot immediately following the precipitous
drop in OUR

Slope = Slope of ln OUR vs. time plot

\((t=s)\) = Time immediately following precipitous drop, hr

Plugging values from this sample problem into Equation D.2, noting that the time immediately
following the precipitous drop \((t=s)\) is 5.96 hrs and the OUR value at \( t = 5.96 \) hr is 7.307
mg/L/hr, \( K_{MP} = 3.64/\)day.

The next step in determining RBCOD is to calculate \( \mu_H \), the maximum heterotrophic
specific growth rate on readily biodegradable substrates, according to equation D.3:
\( \mu_H = \text{slope} \times 24 - K_{MP} + b_H \)  \hspace{1cm} (D.3)

As all of these terms have been previously calculated in this sample calculation, \( \mu_H \) is determined to be 7.21/day.

Now, one can calculate a value for RBCOD, according to the following equation:

\[
RBCOD = \frac{\mu_H \cdot Z_{HH}}{Y_{ZH} \cdot \text{slope} \cdot 24} \cdot (e^{\text{slope} \cdot t_d} - 1)
\]  \hspace{1cm} (D.4)

Where,

\( t_d = \text{Time of precipitous drop, Ideally in the middle of the steep decline} \)

In this sample problem, \( t_d = 6.96 \text{ hr} \), as this point is near the middle of the precipitous drop. As all other parameters necessary to calculate RBCOD were previously determined, applying Equation D.4 results in an RBCOD of 147.5 mg/L for this sample problem.

**Maximum Specific Growth Rate of Nitrifying Bacteria**

To determine the growth rate coefficient for autotrophic biomass, \( \mu_{A_{max}} \), a method modified by that used by Hall (1974) and Antoniou, et al (1990) was used during this study. A 3 L Plexiglas rectangular reactor was filled with approximately 100 mL of activated sludge, then filled to the 2 L mark with non-chlorinated effluent. The reactor was spiked with \( \text{NH}_4 \), to ensure
ammonia remains the preferred N source for biosynthesis. However, care was taken in selecting the concentration to be beneath toxicity levels, typically 15 mg/L NH₄-N. The Plexiglas reactor was stirred continuously, both with aeration and a magnetic stirrer. An automatic control device, such as used by Antoniou to control pH and alkalinity was not available, so alkalinity was manually between 150 and 400 mg/L through the manual addition of alkalinity every 4-8 hours during the duration of the test. The technique described by Hall (1974) is also a manual technique, with no automatic control, but the technique used in this study was closer to that of Antoniou. As mentioned, pH was also not controlled, but it was monitored. The typical test lasted for three days, taking samples for nitrate and alkalinity, to maintain sufficient buffering. Nitrite was not measured, as the peak for nitrite coincided with that of chloride on the ion chromatograph that was used for analysis (see Chapter 3). The slope of the line generated by plotting ln (mg/L NO₃-N + NO₂-N) has been shown in the literature to equal (µA_max – b_A). The decay coefficient, b_A, cannot be accurately determined in mixed cultures, but does vary between 0.05 and 0.15 d⁻¹ (Henze, et al, 1986). Therefore, assuming a value for b_A within this range can be satisfactorily assumes (Antoniou, 1990). For calculations during this study, a value of 0.10 was assumed for b_A.

Figure D.XXX displays plot of ln NO₃ versus time generated during this study as a sample calculation. Note that the slope of the line is actually (µA_max – b_A). An assumed value for bA (in this case, 0.10) must be subtracted from the value of the slope shown on Figure D.XXXX, resulting in a value for µA_max of 0.81 day⁻¹.
Figure D.4 Sample Calculation of a Determination of $\mu_{A_{max}}$
References


APPENDIX E: PAIRED DIFFERENCE TEST SAMPLE CALCULATION
In order to test the difference between two population means, a matched pairs difference test was used during this study for statistical evaluations (Mendenhall and Sincich, 1995). The test statistic for a small-sample test of hypothesis about \((\mu_1 - \mu_2)\) matched pairs is shown below in Equation E.1:

\[
t = \frac{(d_{\text{avg}} - D_0)}{\{s_d/\sqrt{n}\}}
\]

(E.1)

Where,

- \(t\) = t test statistic
- \(d_{\text{avg}}\) = average difference between pairs
- \(D_0\) = Specified difference between pairs (zero in all cases during this study)
- \(s_d\) = Standard deviation of the difference between pairs
- \(\sqrt{n}\) = Square root of n, the number of pairs

Illustrated below in Table E.1 is a sample calculation, in which the difference between the RBCOD values for the PAS train and the CAS train treating a COD-limited wastewater is tested. Plugging the appropriate values found in Table E.1 into Equation E.1:

\[
t = \frac{(27.3 - 0)}{\{13.3/\sqrt{9}\}} = 6.18
\]

(E.1)

In addition to calculating the t value, the P-value is also calculated. The P-value was calculated using an Excel command, TDIST, which returns the P-value for a specified t-value, degrees of
freedom, and “tailness” of the test (i.e. one-tailed or two-tailed test). In this study, all statistical tests were one-tailed.

Table E.1 Sample Statistical Calculation

<table>
<thead>
<tr>
<th></th>
<th>PAS RBCOD (mg/L)</th>
<th>CAS RBCOD (mg/L)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>163.2</td>
<td>141.6</td>
<td>21.6</td>
<td></td>
</tr>
<tr>
<td>136.0</td>
<td>115.0</td>
<td>21.0</td>
<td></td>
</tr>
<tr>
<td>143.8</td>
<td>115.6</td>
<td>28.2</td>
<td></td>
</tr>
<tr>
<td>124.4</td>
<td>126.2</td>
<td>-1.8</td>
<td></td>
</tr>
<tr>
<td>169.3</td>
<td>132.2</td>
<td>37.1</td>
<td></td>
</tr>
<tr>
<td>144.6</td>
<td>110.3</td>
<td>34.3</td>
<td></td>
</tr>
<tr>
<td>141.8</td>
<td>116.4</td>
<td>25.4</td>
<td></td>
</tr>
<tr>
<td>156.6</td>
<td>119.1</td>
<td>37.5</td>
<td></td>
</tr>
<tr>
<td>157.3</td>
<td>114.7</td>
<td>42.6</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>148.6</td>
<td>121.2</td>
<td>27.3</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>14.2</td>
<td>10.1</td>
<td>13.3</td>
</tr>
<tr>
<td>t value rejection region, a=0.05 (one tailed)</td>
<td>6.18</td>
<td>1.86</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.000132344</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX F: PHASE AVERAGE RAW DATA
### General Purpose Tables

#### Table F.1  Acronyms used in Appendix F, and units

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS</td>
<td>Total Suspended Solids (mg/L)</td>
</tr>
<tr>
<td></td>
<td>Ratio of Volatile Suspended Solids to Total Suspended Solids</td>
</tr>
<tr>
<td>VSS/TSS</td>
<td>Solids</td>
</tr>
<tr>
<td>TCOD</td>
<td>Total (unfiltered) Chemical Oxygen Demand (mg/L)</td>
</tr>
<tr>
<td>sCOD</td>
<td>soluble Chemical Oxygen Demand (mg/L)</td>
</tr>
<tr>
<td>TP</td>
<td>Total (unfiltered) Phosphorus (mg PO$_4$-P/L)</td>
</tr>
<tr>
<td>SOP</td>
<td>Soluble Ortho Phosphorus (mg PO$_4$-P/L)</td>
</tr>
<tr>
<td>TKN</td>
<td>Total Kjeldahl Nitrogen (mg NH$_4$-N/L)</td>
</tr>
<tr>
<td>SKN</td>
<td>Soluble Kjeldahl Nitrogen (mg NH$_4$-N/L)</td>
</tr>
<tr>
<td>NH4</td>
<td>Ammonium (mg NH$_4$-N/L)</td>
</tr>
<tr>
<td>NO3</td>
<td>Nitrate (mg NO$_3$-N/L)</td>
</tr>
<tr>
<td>OUR</td>
<td>Oxygen Uptake Rate (mg O$_2$ / L * hr)</td>
</tr>
<tr>
<td>INF</td>
<td>Influent</td>
</tr>
<tr>
<td>AN</td>
<td>Anaerobic</td>
</tr>
<tr>
<td>AX I</td>
<td>Anoxic I</td>
</tr>
<tr>
<td>AX II</td>
<td>Anoxic II</td>
</tr>
<tr>
<td>AE</td>
<td>Aerobic</td>
</tr>
<tr>
<td>EFF</td>
<td>Effluent</td>
</tr>
<tr>
<td>ARCY</td>
<td>Anaerobic Recycle</td>
</tr>
<tr>
<td>NARCY</td>
<td>Nitrate Recycle</td>
</tr>
<tr>
<td>RAS</td>
<td>Return Activated Sludge</td>
</tr>
<tr>
<td>WAS</td>
<td>Waste Activated Sludge</td>
</tr>
</tbody>
</table>
### Chapter 4 Data

### Phase 1 Data

Table F.2 Average Parameter Values for the PAS train, mg/L

<table>
<thead>
<tr>
<th></th>
<th>Influent (INF)</th>
<th>Anaerobic (AN)</th>
<th>Anoxic (AX I)</th>
<th>Aerobic (AE)</th>
<th>Effluent (EFF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS</td>
<td>120</td>
<td>1672</td>
<td>2629</td>
<td>2189</td>
<td>6</td>
</tr>
<tr>
<td>VSS/TSS¹</td>
<td>0.77</td>
<td>0.78</td>
<td>0.77</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>TCOD</td>
<td>425.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sCOD</td>
<td>230.37</td>
<td>120.60</td>
<td>89.63</td>
<td>59.40</td>
<td>45.24</td>
</tr>
<tr>
<td>TP</td>
<td>7.98</td>
<td></td>
<td></td>
<td></td>
<td>2.00</td>
</tr>
<tr>
<td>SOP</td>
<td>6.23</td>
<td>13.25</td>
<td>6.18</td>
<td>1.29</td>
<td>1.05</td>
</tr>
<tr>
<td>TKN</td>
<td>44.52</td>
<td></td>
<td></td>
<td></td>
<td>12.19</td>
</tr>
<tr>
<td>SKN</td>
<td>30.70</td>
<td></td>
<td>10.38</td>
<td>4.85</td>
<td>3.85</td>
</tr>
<tr>
<td>NO₃</td>
<td>0.06</td>
<td>0.33</td>
<td>1.61</td>
<td>6.33</td>
<td>5.55</td>
</tr>
</tbody>
</table>

¹ VSS/TSS ratio is dimensionless

Table F.3 Average Parameter Values for the CAS train, mg/L

<table>
<thead>
<tr>
<th></th>
<th>Influent (INF)</th>
<th>Anaerobic (AN)</th>
<th>Anoxic (AX I)</th>
<th>Aerobic (AE)</th>
<th>Effluent (EFF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS</td>
<td>120</td>
<td>1420</td>
<td>2396</td>
<td>2983</td>
<td>8</td>
</tr>
<tr>
<td>VSS/TSS¹</td>
<td>0.790</td>
<td>0.790</td>
<td>0.770</td>
<td>0.770</td>
<td></td>
</tr>
<tr>
<td>TCOD</td>
<td>425.08</td>
<td></td>
<td></td>
<td></td>
<td>92.44</td>
</tr>
<tr>
<td>sCOD</td>
<td>230.37</td>
<td>132.10</td>
<td>85.28</td>
<td>67.10</td>
<td>55.79</td>
</tr>
<tr>
<td>TP</td>
<td>7.98</td>
<td></td>
<td></td>
<td></td>
<td>3.29</td>
</tr>
<tr>
<td>SOP</td>
<td>6.23</td>
<td>11.24</td>
<td>6.03</td>
<td>1.60</td>
<td>1.52</td>
</tr>
<tr>
<td>TKN</td>
<td>43.55</td>
<td></td>
<td></td>
<td></td>
<td>12.48</td>
</tr>
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<td>28.65</td>
<td>7.18</td>
<td>5.10</td>
</tr>
<tr>
<td>NO₃</td>
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<td>0.29</td>
<td>1.17</td>
<td>5.30</td>
<td>4.32</td>
</tr>
</tbody>
</table>

¹ VSS/TSS ratio is dimensionless
Table F.4 Average Flow Rates, L/day

<table>
<thead>
<tr>
<th></th>
<th>INF</th>
<th>ARCY</th>
<th>NARCY</th>
<th>RAS</th>
<th>WAS</th>
<th>EFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS</td>
<td>40.7</td>
<td>59.6</td>
<td>54.0</td>
<td>63.9</td>
<td>1.0</td>
<td>39.7</td>
</tr>
<tr>
<td>CAS</td>
<td>40.1</td>
<td>57.7</td>
<td>54.6</td>
<td>66.8</td>
<td>1.0</td>
<td>39.1</td>
</tr>
</tbody>
</table>

Table F.5 Reactor Volumes, L

<table>
<thead>
<tr>
<th></th>
<th>AN</th>
<th>AX</th>
<th>AE</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS</td>
<td>2.5</td>
<td>5</td>
<td>7.5</td>
<td>15</td>
</tr>
<tr>
<td>CAS</td>
<td>2.5</td>
<td>5</td>
<td>7.5</td>
<td>15</td>
</tr>
</tbody>
</table>

Phase 2 Data

Table F.6 Average Parameter Values for the PAS train, mg/L

<table>
<thead>
<tr>
<th></th>
<th>Influent (INF)</th>
<th>Anaerobic (AN)</th>
<th>Anoxic (AX I)</th>
<th>Aerobic (AE)</th>
<th>Effluent (EFF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS</td>
<td>123</td>
<td>1577</td>
<td>3032</td>
<td>3389</td>
<td>5</td>
</tr>
<tr>
<td>VSS/TSS</td>
<td>0.46</td>
<td>0.88</td>
<td>0.71</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>TCOD</td>
<td>440.00</td>
<td>0.88</td>
<td>0.71</td>
<td>0.67</td>
<td>75.00</td>
</tr>
<tr>
<td>sCOD</td>
<td>205.40</td>
<td>77.06</td>
<td>57.70</td>
<td>45.80</td>
<td>31.84</td>
</tr>
<tr>
<td>TP</td>
<td>6.79</td>
<td>20.24</td>
<td>7.77</td>
<td>0.68</td>
<td>0.73</td>
</tr>
<tr>
<td>SOP</td>
<td>6.13</td>
<td>20.24</td>
<td>7.77</td>
<td>0.68</td>
<td>0.73</td>
</tr>
<tr>
<td>TKN</td>
<td>40.74</td>
<td></td>
<td></td>
<td></td>
<td>11.96</td>
</tr>
<tr>
<td>SKN</td>
<td>34.41</td>
<td>7.42</td>
<td>5.50</td>
<td>4.48</td>
<td></td>
</tr>
<tr>
<td>NO₃</td>
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<td>0.31</td>
<td>7.21</td>
<td>6.71</td>
</tr>
</tbody>
</table>

¹ VSS/TSS ratio is dimensionless
### Table F.7  Average Parameter Values for the CAS train, mg/L

<table>
<thead>
<tr>
<th></th>
<th>Influent (INF)</th>
<th>Anaerobic (AN)</th>
<th>Anoxic (AX I)</th>
<th>Aerobic (AE)</th>
<th>Effluent (EFF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS</td>
<td>123</td>
<td>1416</td>
<td>2960</td>
<td>3501</td>
<td>5</td>
</tr>
<tr>
<td>VSS/TSS(^1)</td>
<td>0.99</td>
<td>0.75</td>
<td>0.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCOD</td>
<td>440.20</td>
<td></td>
<td></td>
<td></td>
<td>70.66</td>
</tr>
<tr>
<td>sCOD</td>
<td>205.40</td>
<td>88.36</td>
<td>62.50</td>
<td>45.44</td>
<td>36.48</td>
</tr>
<tr>
<td>TP</td>
<td>6.79</td>
<td></td>
<td></td>
<td>143.84</td>
<td>1.44</td>
</tr>
<tr>
<td>SOP</td>
<td>6.13</td>
<td>15.24</td>
<td>7.94</td>
<td>0.84</td>
<td>1.00</td>
</tr>
<tr>
<td>TKN</td>
<td>40.74</td>
<td></td>
<td></td>
<td>10.92</td>
<td></td>
</tr>
<tr>
<td>SKN</td>
<td>34.41</td>
<td></td>
<td>12.07</td>
<td>6.46</td>
<td>5.04</td>
</tr>
<tr>
<td>NO(_3)</td>
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<td>0.25</td>
<td>6.44</td>
<td>4.71</td>
</tr>
</tbody>
</table>

\(^1\) VSS/TSS ratio is dimensionless

### Table F.8  Average Flow Rates, L/day

<table>
<thead>
<tr>
<th></th>
<th>INF</th>
<th>ARCY</th>
<th>NARCY</th>
<th>RAS</th>
<th>WAS</th>
<th>EFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS</td>
<td>48.0</td>
<td>54.0</td>
<td>54.0</td>
<td>63.9</td>
<td>1.0</td>
<td>47.0</td>
</tr>
<tr>
<td>CAS</td>
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<td>54.0</td>
<td>54.0</td>
<td>66.0</td>
<td>1.0</td>
<td>47.0</td>
</tr>
</tbody>
</table>

### Table F.9  Reactor Volumes, L

<table>
<thead>
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<th></th>
<th>AN</th>
<th>AX</th>
<th>AE</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS</td>
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<td>5</td>
<td>7.5</td>
<td>15</td>
</tr>
<tr>
<td>CAS</td>
<td>2.5</td>
<td>5</td>
<td>7.5</td>
<td>15</td>
</tr>
</tbody>
</table>
### Table F.10 Pilot-Scale Phosphorus Concentrations

<table>
<thead>
<tr>
<th>Parameters (mg/L)</th>
<th>PAS</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP influent</td>
<td>11.6</td>
<td>12.4</td>
</tr>
<tr>
<td>Anaerobic SOP</td>
<td>36.7</td>
<td>27.3</td>
</tr>
<tr>
<td>Anoxic I SOP</td>
<td>41.7</td>
<td>33.0</td>
</tr>
<tr>
<td>Anoxic II SOP</td>
<td>12.7</td>
<td>10.5</td>
</tr>
<tr>
<td>Aerobic SOP</td>
<td>4.2</td>
<td>6.3</td>
</tr>
<tr>
<td>Clarifier SOP</td>
<td>4.0</td>
<td>6.7</td>
</tr>
<tr>
<td>% P removal</td>
<td>64.2</td>
<td>49.3</td>
</tr>
<tr>
<td>Apparent Anaerobic P Release</td>
<td>25.1</td>
<td>14.9</td>
</tr>
<tr>
<td>Apparent Anoxic I P Release</td>
<td>5.0</td>
<td>5.7</td>
</tr>
<tr>
<td>Apparent Anoxic II P Uptake</td>
<td>29.0</td>
<td>22.5</td>
</tr>
<tr>
<td>Aerobic P Uptake</td>
<td>8.6</td>
<td>4.2</td>
</tr>
<tr>
<td>Net P Uptake (excluding clarifier)</td>
<td>7.4</td>
<td>6.1</td>
</tr>
</tbody>
</table>

### Table F.11 Pilot-Scale Nitrogen Concentrations

<table>
<thead>
<tr>
<th>Parameters (mg/L)</th>
<th>PAS</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TKN Influent</td>
<td>41.4</td>
<td>35.2</td>
</tr>
<tr>
<td>SKN Influent</td>
<td>34.0</td>
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</tr>
<tr>
<td>Ammonia Influent</td>
<td>30.8</td>
<td>28.7</td>
</tr>
<tr>
<td>Nitrate Influent</td>
<td>0.27</td>
<td>0.19</td>
</tr>
<tr>
<td>TKN Effluent</td>
<td>7.3</td>
<td>9.4</td>
</tr>
<tr>
<td>SKN Effluent</td>
<td>6.5</td>
<td>7.8</td>
</tr>
<tr>
<td>Ammonia Effluent</td>
<td>5.1</td>
<td>6.7</td>
</tr>
<tr>
<td>Nitrate Effluent</td>
<td>5.19</td>
<td>2.10</td>
</tr>
</tbody>
</table>
## Chapter 6 Data

### COD-Limited Phase

Table F.12  Average Parameter Values for the PAS train, mg/L

<table>
<thead>
<tr>
<th></th>
<th>Influent (INF)</th>
<th>Anaerobic (AN)</th>
<th>Anoxic I (AX I)</th>
<th>Anoxic II (AX II)</th>
<th>Aerobic (AE)</th>
<th>Effluent (EFF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS</td>
<td>109.8</td>
<td>2840.8</td>
<td>5145.0</td>
<td>5737.5</td>
<td>5892.5</td>
<td>19.8</td>
</tr>
<tr>
<td>VSS/TSS&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.742</td>
<td>0.754</td>
<td>0.760</td>
<td>0.755</td>
<td>0.770</td>
<td>0.750</td>
</tr>
<tr>
<td>TCOD</td>
<td>353.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>54.6</td>
</tr>
<tr>
<td>sCOD</td>
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<td>111.2</td>
<td>89.0</td>
<td>79.2</td>
<td>37.9</td>
<td>33.4</td>
</tr>
<tr>
<td>TP</td>
<td>11.8</td>
<td></td>
<td></td>
<td></td>
<td>298.3</td>
<td>3.7</td>
</tr>
<tr>
<td>SOP</td>
<td>9.9</td>
<td>35.4</td>
<td>39.8</td>
<td>11.4</td>
<td>2.9</td>
<td>3.4</td>
</tr>
<tr>
<td>TKN</td>
<td>41.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.3</td>
</tr>
<tr>
<td>SKN</td>
<td>35.8</td>
<td></td>
<td></td>
<td></td>
<td>7.8</td>
<td>2.1</td>
</tr>
<tr>
<td>NH&lt;sub&gt;4&lt;/sub&gt;</td>
<td>30.3</td>
<td></td>
<td></td>
<td></td>
<td>6.7</td>
<td>1.3</td>
</tr>
<tr>
<td>NO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.08</td>
<td>0.08</td>
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<td>6.01</td>
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<td>12.29</td>
</tr>
</tbody>
</table>

<sup>1</sup> VSS/TSS ratio is dimensionless

Table F.13  Average Parameter Values for the CAS train, mg/L

<table>
<thead>
<tr>
<th></th>
<th>Influent (INF)</th>
<th>Anaerobic (AN)</th>
<th>Anoxic I (AX I)</th>
<th>Anoxic II (AX II)</th>
<th>Aerobic (AE)</th>
<th>Effluent (EFF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS</td>
<td>110.0</td>
<td>2795.8</td>
<td>4937.5</td>
<td>5549.2</td>
<td>5759.2</td>
<td>18.1</td>
</tr>
<tr>
<td>VSS/TSS&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.733</td>
<td>0.750</td>
<td>0.762</td>
<td>0.770</td>
<td>0.778</td>
<td>0.769</td>
</tr>
<tr>
<td>TCOD</td>
<td>348.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>49.1</td>
</tr>
<tr>
<td>sCOD</td>
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<td>115.4</td>
<td>92.0</td>
<td>82.0</td>
<td>44.2</td>
<td>35.3</td>
</tr>
<tr>
<td>TP</td>
<td>11.7</td>
<td></td>
<td></td>
<td></td>
<td>244.0</td>
<td>6.2</td>
</tr>
<tr>
<td>SOP</td>
<td>9.9</td>
<td>28.0</td>
<td>32.6</td>
<td>11.6</td>
<td>4.5</td>
<td>4.7</td>
</tr>
<tr>
<td>TKN</td>
<td>42.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.3</td>
</tr>
<tr>
<td>SKN</td>
<td>36.7</td>
<td></td>
<td></td>
<td></td>
<td>9.0</td>
<td>2.4</td>
</tr>
<tr>
<td>NH&lt;sub&gt;4&lt;/sub&gt;</td>
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<td></td>
<td></td>
<td></td>
<td>8.1</td>
<td>1.4</td>
</tr>
<tr>
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<td>0.08</td>
<td>0.09</td>
<td>0.10</td>
<td>5.17</td>
<td>10.92</td>
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<sup>1</sup> VSS/TSS ratio is dimensionless
Table F.14 Average Flow Rates, L/day

<table>
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<th></th>
<th>INF</th>
<th>ARCY</th>
<th>NARCY</th>
<th>RAS</th>
<th>WAS</th>
<th>EFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS</td>
<td>247.2</td>
<td>247.2</td>
<td>767.8</td>
<td>175.6</td>
<td>2.7</td>
<td>244.5</td>
</tr>
<tr>
<td>CAS</td>
<td>248.3</td>
<td>247.2</td>
<td>767.8</td>
<td>175.6</td>
<td>2.7</td>
<td>245.6</td>
</tr>
</tbody>
</table>

Table F.15 Reactor Volumes, L

<table>
<thead>
<tr>
<th></th>
<th>AN</th>
<th>AX I</th>
<th>AX II</th>
<th>AE</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS</td>
<td>3.5</td>
<td>6.6</td>
<td>6.8</td>
<td>18</td>
<td>34.9</td>
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<tr>
<td>CAS</td>
<td>3.3</td>
<td>7.1</td>
<td>7.3</td>
<td>18</td>
<td>35.7</td>
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</table>

Table F.16 In-Situ OUR Values, mg/L/hr

<table>
<thead>
<tr>
<th>In-Situ OUR</th>
<th>PAS</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>111.8</td>
<td>97.3</td>
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</tbody>
</table>

P-Limited Phase

Table F.17 Average Parameter Values for the PAS train, mg/L

<table>
<thead>
<tr>
<th></th>
<th>Influent (INF)</th>
<th>Anaerobic (AN)</th>
<th>Anoxic I (AX I)</th>
<th>Anoxic II (AX II)</th>
<th>Aerobic (AE)</th>
<th>Effluent (EFF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS</td>
<td>98.0</td>
<td>3040.0</td>
<td>5480.0</td>
<td>6270.0</td>
<td>6340.0</td>
<td>22.6</td>
</tr>
<tr>
<td>VSS/TSS</td>
<td>0.743</td>
<td>0.755</td>
<td>0.759</td>
<td>0.761</td>
<td>0.774</td>
<td>0.751</td>
</tr>
<tr>
<td>TCOD</td>
<td>338.6</td>
<td>58.7</td>
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<td></td>
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<td>103.5</td>
<td>100.1</td>
<td>85.6</td>
<td>43.8</td>
<td>42.4</td>
</tr>
<tr>
<td>TP</td>
<td>6.9</td>
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<td>1.1</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>SOP</td>
<td>5.7</td>
<td>22.4</td>
<td>26.4</td>
<td>8.7</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>TKN</td>
<td>42.5</td>
<td>2.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SKN</td>
<td>36.8</td>
<td>7.9</td>
<td>2.5</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH₄</td>
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<td>7.0</td>
<td>1.2</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>0.07</td>
<td>0.06</td>
<td>0.11</td>
<td>5.53</td>
<td>11.98</td>
<td>11.85</td>
</tr>
</tbody>
</table>

¹ VSS/TSS ratio is dimensionless
Table F.18 Average Parameter Values for the CAS train, mg/L

<table>
<thead>
<tr>
<th></th>
<th>Influent (INF)</th>
<th>Anaerob In (AN)</th>
<th>Anoxic I (AX I)</th>
<th>Anoxic II (AX II)</th>
<th>Aerobic (AE)</th>
<th>Effluent (EFF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS</td>
<td>103.0</td>
<td>2900.0</td>
<td>5350.0</td>
<td>6180.0</td>
<td>6160.0</td>
<td>24.9</td>
</tr>
<tr>
<td>VSS/TSS&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.742</td>
<td>0.755</td>
<td>0.759</td>
<td>0.771</td>
<td>0.777</td>
<td>0.748</td>
</tr>
<tr>
<td>TCOD</td>
<td>345.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>56.2</td>
</tr>
<tr>
<td>sCOD</td>
<td>159.4</td>
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<td>87.6</td>
<td>76.4</td>
<td>47.9</td>
<td>46.2</td>
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<tr>
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<td>6.8</td>
<td></td>
<td>201.0</td>
<td>1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOP</td>
<td>5.8</td>
<td>19.8</td>
<td>24.9</td>
<td>8.6</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>TKN</td>
<td>42.7</td>
<td></td>
<td></td>
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<td>2.7</td>
</tr>
<tr>
<td>SKN</td>
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<td>8.1</td>
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<td></td>
</tr>
<tr>
<td>NH&lt;sub&gt;4&lt;/sub&gt;</td>
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<td>7.2</td>
<td>1.4</td>
<td>1.4</td>
<td></td>
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</tr>
<tr>
<td>NO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.06</td>
<td>0.07</td>
<td>0.09</td>
<td>5.17</td>
<td>11.42</td>
<td>11.37</td>
</tr>
</tbody>
</table>

<sup>1</sup> VSS/TSS ratio is dimensionless

Table F.19 Average Flow Rates, L/day

<table>
<thead>
<tr>
<th></th>
<th>INF</th>
<th>ARCY</th>
<th>NARCY</th>
<th>RAS</th>
<th>WAS</th>
<th>EFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS</td>
<td>243.6</td>
<td>243.6</td>
<td>770.0</td>
<td>172.3</td>
<td>2.8</td>
<td>240.9</td>
</tr>
<tr>
<td>CAS</td>
<td>247.6</td>
<td>247.6</td>
<td>770.0</td>
<td>172.3</td>
<td>2.8</td>
<td>244.9</td>
</tr>
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</table>

Table F.20 Reactor Volumes, L

<table>
<thead>
<tr>
<th></th>
<th>AN</th>
<th>AX I</th>
<th>AX II</th>
<th>AE</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS</td>
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<td>6.8</td>
<td>18</td>
<td>34.9</td>
</tr>
<tr>
<td>CAS</td>
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<td>7.1</td>
<td>7.3</td>
<td>18</td>
<td>35.7</td>
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Table F.21 In-Situ OUR Values, mg/L/hr

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<tbody>
<tr>
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</tr>
<tr>
<td>CAS</td>
<td>105.4</td>
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</table>
### Chapter 7 Data

Table F.22  Average Parameter Values for the PAS train, mg/L

<table>
<thead>
<tr>
<th></th>
<th>Influent (INF)</th>
<th>Anaerobic (AN)</th>
<th>Anoxic I (AX I)</th>
<th>Anoxic II (AX II)</th>
<th>Aerobic (AE)</th>
<th>Effluent (EFF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS</td>
<td>106.3</td>
<td>3280.0</td>
<td>5942.0</td>
<td>6499.0</td>
<td>6593.0</td>
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<tr>
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<td>0.740</td>
<td>0.750</td>
<td>0.757</td>
<td>0.761</td>
<td>0.770</td>
<td>0.770</td>
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<tr>
<td>TCOD</td>
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<td>79.1</td>
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<tr>
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<td>39.9</td>
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<td></td>
<td></td>
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<td>6.9</td>
</tr>
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<td>0.09</td>
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</table>

1 VSS/TSS ratio is dimensionless

Table F.23  Average Parameter Values for the PCAS train, mg/L

<table>
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<th>Influent (INF)</th>
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<th>Anoxic I (AX I)</th>
<th>Anoxic II (AX II)</th>
<th>Aerobic (AE)</th>
<th>Effluent (EFF)</th>
</tr>
</thead>
<tbody>
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<td>5519.0</td>
<td>5979.0</td>
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<tr>
<td>VSS/TSS</td>
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<td>0.758</td>
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</tr>
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1 VSS/TSS ratio is dimensionless
Table F.24  Average Flow Rates, L/day

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<th>NARCY</th>
<th>RAS</th>
<th>WAS</th>
<th>EFF</th>
</tr>
</thead>
<tbody>
<tr>
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<td>175.6</td>
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<td>CAS</td>
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<td>767.8</td>
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<td>245.6</td>
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</table>

Table F.25  Reactor Volumes, L

<table>
<thead>
<tr>
<th></th>
<th>AN</th>
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<th>AX II</th>
<th>AE</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
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<td>6.6</td>
<td>6.8</td>
<td>18</td>
<td>34.9</td>
</tr>
<tr>
<td>CAS</td>
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</table>

Table F.26  In-Situ OUR Values, mg/L/hr

<table>
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