PRODUCTION OF VOLATILE FATTY ACIDS BY FERMENTATION OF ANAEROBIC MIXED LIQUOR FOR ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL

by

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ABSTRACT

SONIA TREMBLAY. Production of Volatile Fatty Acids by Fermentation of Anaerobic Mixed Liquor for Enhanced Biological Phosphorus Removal. (Under the direction of DR. HELENE A. HILGER)

Full-scale demonstration testing was conducted to evaluate a side-stream anaerobic mixed liquor fermentation process for internal generation of volatile fatty acids (VFAs) in a biological nutrient removal (BNR) process. The trials were performed at the McDowell Creek Wastewater Treatment Facility (WWTF) in Huntersville, NC, where acetic acid or waste sugar water is added to the BNR process influent to achieve biological phosphorus removal (BPR). A portion of the anaerobic mixed liquor was pumped to an upflow sludge blanket (USB) reactor to accomplish fermentation. A 2.5-day solids retention time (SRT) was tested, followed by trials at 7 and 12-day SRTs. A 13.6 hour hydraulic retention time (HRT) was maintained in all trials. The net VFA production was estimated to 149, 206, and 94 lb/d, for the 2.5-day, 7-day and 12-day SRT, respectively. Although the 2.5-day SRT did not generate the highest amount of VFAs, it was very manageable from a process point of view, and it is likely sufficient for the plant BPR process if VFA elutriation is efficient. At the 7-day and 12-day SRTs, a thick layer of floating solids formed at the surface of the fermentate tanks, which likely resulted because methane was being produced. The lower VFA levels at the 12-day SRT may have reflected the onset of methanogenesis. Phosphorus release batch tests conducted on the plant anaerobic mixed liquor suggest that the fermentate was a more efficient VFA source than the acetic acid or sugar water. Gas chromatography tests revealed that the fermentate contained a ratio of acetic to propionic acid of approximately 65:35, which is well-suited for BPR.

DEDICATION

This thesis is dedicated with deepest love to my husband and my best friend, Peter Schuler. His love, strength and understanding made it possible for me to complete my master's degree. His financial support also allowed me to be able to go back to school on a full-time basis.

Peter, I love you more every day.

SSM

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

μ	micron = 10^{-6}
β	beta (refers to the beta sub-class of *Proteobacteria* phylum)
γ	gamma (refers to the gamma sub-class of *Proteobacteria* phylum)
μL	micro liter = 10^{-6} liter
μm	micrometer = 10^{-6} meter
Δt	change in time
APT	activated primary tank
ARL	anoxic recycled liquor
BNN	biological nitrogen removal
BNR	biological nutrient removal
BOD	biological oxygen demand
BPR	biological phosphorus removal
C_1-C_5	one to five carbon acids
C_4	four carbon acid
CF	correction factor
CO_2	carbon dioxide
COD	chemical oxygen demand
DAPI	4’,6-diamidino-2-phenylindole
DI	deionized
DPAO	denitrifying phosphorus accumulating organism
DP/DS	phosphate released to substrate uptake ratio

EBPR	enhanced biological phosphorus removal
EPA	Environmental Protection Agency
FID	flame ionization detector
FZ1	fermentation zone No. 1
FZ2	fermentation zone No. 2
g	gram
gal	US gallon
GAO	glycogen accumulating organism
GC	gas chromatography
gpm	gallons per minute
h	hour
HAc	acetic acid
HCl	hydrochloric acid
HRT	hydraulic retention time
H_2S	hydrogen sulfide
H_2SO_4	sulfuric acid
IMUC	intermittently mixed upflow clarifier
kg	kilogram
L	liter
lb	pound
m	meter
m^3	cubic meter
mg	milligram

Mgal	million gallons
MGD	million gallons per day
min	minute
mL	milliliter
MLE	modified Ludzak-Ettinger
MLSS	mixed liquor suspended solids
mv	millivolt
m_w	mass/day of solids wasted in the supernatant in a given 24 hour period
N	nitrogen
NaOH	sodium hydroxyde
nm	nanometer
NO_3^-	nitrate ion
NPDE	national pollution discharge elimination
OP	orthophosphate
OP_1	orthophosphate at time 1
OP_2	orthophosphate at time 2
OP_{rr}	phosphorus release rate
ORL	oxic recycled liquor
ORP	oxidation reduction potential
P	phosphorus
PAO	phosphorus accumulating organism
PCE	primary clarifier effluent
PHA	polyhydroxyalkanoate

PHB	polyhydroxybutyrate
PHV	polyhydroxyvalerate
PO_4^{3-}	phosphate ion
Poly-P	polyphosphate
Q	supernatant flow
RAS	recycled activated sludge
SBR	sequencing batch reactor
SCOD	soluble chemical oxygen demand
$SCOD_1$	soluble chemical oxygen demand at time 1
$SCOD_2$	soluble chemical oxygen demand at time 2
$SCOD_c$	soluble chemical oxygen consumption
$SCOD_{cr}$	soluble chemical oxygen consumption rate
SCVFA	short chain volatile fatty acid
SER	sludge elutriation rate
SRT	solids retention time
SRT_d	daily solids retention time
SRR	sludge recirculation rate
TCA	tricarboxylic acid
TCOD	total chemical oxygen demand
t_m	mixing time
t_{off}	total time that pumps are off in one day
TP	total phosphate or total phosphorus
t_{pm}	time that pumps are on while mixing

t_{solset}	time while pumps are on and solids are still settling back
TSS	total suspended solids
UASB	upflow anaerobic sludge blanket
UCT	University of Cape Town
UNCC	University of North Carolina at Charlotte
USB	upflow sludge blanket
V	volume
V_{AN}	volume of anaerobic zone
VFA	volatile fatty acid
VSS	volatile suspended solids
WAS	waste activated sludge
WWTF	wastewater treatment facility
X	fermentation zone mixed liquor concentration
X_R	supernatant tested mixed liquor concentration
X_R(CALC)	supernatant calculated mixed liquor concentration

CHAPTER 1 : INTRODUCTION

1.1 Problem Statement

Excessive amounts of plant nutrients (phosphorus and nitrogen) in water bodies accelerate the natural eutrophication process by which they age and become more productive. Without human intervention, this process normally takes thousand of years to progress. Too much phosphorus (P) and nitrogen (N) in lakes and streams creates a fast and excessive growth of algae, which leads to dissolved oxygen depletion; light reduction, especially at the bottom level; and altered biodiversity.

1.1.1 A Case of Eutrophication

Lake Erie was the first important eutrophication case to get government attention in the late 1960's (USEPA, 2005a). The waters were green with algae, beaches were closed due to bacterial contamination, odiferous decaying masses of algae littered much of the shoreline, many fish kills were observed, and municipal water taken from Lake Erie suffered from taste and odor problems. Scientists from the United States and Canada conducted a series of investigations and reported that an oversupply of phosphorus was causing the huge excess of growth of algae. It is to be noted that dissolved phosphate occurs in small concentrations in water because of its low solubility and its ability to be readily taken up by biota. It also adsorbs to metal oxides in soils (Litke, 1999).

Therefore, large phosphorus concentrations in natural waters are abnormal and normally indicate that phosphorus has been discharged from human activities, such as farm run-off or sewage treatment plants.

The blue-green alga, which was not readily eaten by the organisms present in Lake Erie, had become abundant. It was growing during Spring and early Summer. When the algae died and sank to the bottom of the basin, microbial organisms decomposed the algae and used up all of the oxygen in the bottom water. Therefore, neither fish nor aquatic insects could survive in the lake.

In order to reclaim Lake Erie, the amount of phosphorus in the water had to be reduced. Since effluents from wastewater treatment plants were point sources and already regulated, an intensive and expensive campaign to reduce phosphorus loadings was initiated by the United States and Canada. Many billions of dollars were spent on new sewage treatment plant construction and on upgrading existing facilities to make them more efficient at removing phosphorus from domestic sewage (USEPA, 2005a).

1.1.2. Presence of Phosphorus in Water

Phosphorus is the limiting nutrient for plant growth because the ratio of its availability in the water to the amount found in plants is higher than for any other nutrients (Litke, 1999). Since 1950, phosphorus inputs to the environment in the United States have increased as the use of phosphate fertilizer, manure, and phosphate laundry detergent increased. Raw wastewaters contained about 3 mg/L of total phosphorus during the 1940's. This concentration increased to about 11 mg/L in the 1970’s and declined after the use of phosphate-based detergents was discontinued because of state bans or

changes in detergent formulations. Phosphate concentrations found in raw sewage are currently about 5 mg/L (Litke, 1999).

The National Pollutant Discharge Elimination System (NPDES) was created in 1972 under the Clean Water Act. This system was established to control the amount of pollutants that can be contained in point source discharges into a water body. The permitting system prohibits discharges of pollutants from any point source (operating and permitted facility) into the nation's waters except as allowed under an NPDES permit. The program gives the Environmental Protection Agency (EPA) the authority to regulate discharges into the nation's waters by setting limits on the effluent that can be introduced into a body of water from an operating and permitted facility (USEPA, 2005b)

Today, the EPA requires total phosphorus concentrations in wastewater treatment plant effluents (before discharging in a water body) to be between 0.5 and 1.5 mg/L (Litke, 1999). Several wastewater plants are presently being expanded in the United States to meet even stricter future limits in the range of 0.1-0.5 mg/L total phosphorus. Countries in South Africa, Australia and Canada are already requiring phosphorus concentrations in wastewater treatment plant effluents to be less than 0.5 mg/L (Münch and Koch, 1999; Rössle and Pretorius, 2001).

1.1.3. Biological Phosphorus Removal

Every wastewater treatment plant facility includes systems to remove coarse materials and suspended organic solids found in the raw sewage (preliminary and primary treatments). A secondary treatment system is required to remove soluble organic matter and nutrients. Research during the last two decades has focused on processes to remove P and N in a cost effective and efficient manner (Grady et al., 1999). Biological

processes have gained in popularity because they are environmentally sound and have lower operating costs compared to available chemical treatments. The biological methods are referred to as Biological Nutrient Removal (BNR) processes. BNR processes are a modification of the basic activated sludge process, which is designed mainly to remove soluble organic matter, except that BNR also exploits the capacity of certain bacteria to remove phosphorus and/or nitrogen.

Biological phosphorus removal (BPR) processes can be stand alone processes, but in most cases they are part of a complete BNR system. BPR processes take advantage of phosphorus accumulating organisms (PAOs) in wastewater that can take up phosphorus and store it in energy-rich polyphosphate chains. When these cells become part of the sludge that is wasted after final clarification, the phosphorus they contain is removed from the wastewater.

Barnard (1975) first proposed that wastewater influent and return activated sludge (RAS) be passed sequentially through an anaerobic zone and then an aerobic zone to induce PAO removal of phosphorus. The two sequential treatment steps are required to set the conditions for effective biotic phosphorus uptake. Under anaerobic conditions, a PAO will expend energy from the hydrolysis of stored polyphosphates to transport VFAs, such as acetic acid and propionic acid, across its cell membrane. As a result, phosphorus will be released into solution in the phosphate form (PO_4^{3-}). When subsequently passed through an aerobic zone, the PAO will take up soluble phosphorus for growth in excess of what is required for its metabolism (and in excess of what it released under anaerobic conditions. The net result is that the PAOs take up most of the phosphorus that was

originally present in the wastewater influent plus all of the PAO phosphorus released in the anaerobic zone.

1.1.4 Production of Volatile Fatty Acids

The wastewater must contain short carbon chain acids called volatile fatty acids (VFAs) such as, acetic and propionic acids (Fuhs and Chen, 1975) to achieve high PAO-mediated phosphorus removal. These acids are a source of both carbon and energy for the cell and are readily metabolized (Lilley et al., 1990). Several methods have been tested and utilized to ensure VFA availability. Acetic and propionic acids are the VFAs that typically predominate in raw wastewater. They are produced naturally when some of the organic solids contained in the sewage are fermented. If the travel time for sewage *en route* to the treatment plant is long enough, the wastewater will arrive with sufficient VFAs present to drive the BPR process. If this is not the case, supplemental VFAs are often added in the form of acetic acid or some type of glucose source.

An alternative to external VFA addition that is used by many plants in the United States and elsewhere is the fermentation of primary sludge (solids obtained from primary treatment). In some cases, the settled sludge in the primary clarifiers naturally ferments and produces acids that can be passed on to the anaerobic treatment zone. In other cases, the primary sludge is sent to a dedicated fermenter from which the VFAs are elutriated into the liquid phase to the anaerobic zone. While the soluble organics are readily available to the fermentative bacteria, primary sludge contains mainly particulates with a large portion of slowly biodegradable material that needs to be degraded into less complex chemical forms that can then be solubilized. The presence of soluble organics in primary sludge is limited.

Using anaerobic mixed liquor (from the BNR process) as a source of VFAs would have the advantage of including the soluble fraction of the influent wastewater (and the associated colloidal fraction) and not just the sludge solids. Therefore, in theory, fermentation of mixed liquor should be more efficient than fermentation of primary sludge for VFA production. Mixed liquor suspended solids (MLSS) fermentation was first documented to be a satisfactory VFA source when mixed liquor was exchanged between an anoxic zone and an accidental "dead zone" of activated sludge. Barnard observed that anaerobic mixed liquor fermentation and phosphorus removal (from 8 to <0.2 mg/L) resulted without the benefit of an anaerobic zone. When the dead zone was disconnected, effluent phosphorus concentrations rose to about 4 mg/L (Barnard, 1975; 1976). Subsequent tests conducted in Korea showed that a portion of the mixed liquor could be fermented with some success (Barnard and deBarbadillo, 2002).

Although there are some reports that mixed liquor fermentation can be a potential source of VFAs for BPR, the process has not been thoroughly investigated, and no wastewater treatment facility in or out of the United States has officially reported conducting full-scale trials to evaluate it as a viable means of VFA production. This study investigates further the potential for anaerobic mixed liquor fermentation to produce VFAs, and reports on full-scale demonstration testing of a side-stream system that was configured at the McDowell Creek Wastewater Treatment Facility (WWTF) in Huntersville, North Carolina.

1.2 Purpose

The research conducted as part of this study was initiated by Dr. James L. Barnard (senior process engineer) and Ms. Chris deBarbadillo (process engineer) from Black &

Veatch Corporation. The purpose of the study was to demonstrate that anaerobic mixed liquor fermentation can be a viable source of VFAs in a full-scale demonstration trial, and that the use of an upflow sludge blanket reactor (described in section 1.4) is the proper means by which to accomplish optimum fermentation.

1.3 Objective

The main objective of this study was to demonstrate that fermentation of the McDowell Creek Wastewater Treatment Facility anaerobic mixed liquor can produce enough VFAs to potentially replace the external VFA sources used by the plant with the internal VFAs produced from the fermentation process.

The specific objectives of this study were:

1. To determine the optimum solids retention time (SRT) for anaerobic mixed liquor fermentation to produce VFAs;
2. To determine the optimum hydraulic retention time (HRT) for anaerobic mixed liquor fermentation to produce VFAs;
3. To determine the type of VFAs produced and their proportion in the fermentate mix; and
4. To determine the operational feasibility of the fermentation process using a side-stream upflow anaerobic sludge blanket (UASB) reactor.

1.4 Scope

A fermentation process was configured as a side-stream system to the McDowell Creek WWTF BNR anaerobic zones. A UASB process was utilized for fermenting the mixed liquor. Upflow sludge blanket (USB) reactors are not mixed, which results in the accumulation of solids (sludge blanket) near the bottom with a liquid phase (supernatant)

at the top. A counter current mixed liquor flow is directed upward through the sludge blanket, which forms as the solids settle downward from the liquid phase. The advantage of operating the anaerobic mixed liquor fermentation process as a USB reactor is that a higher solids (biomass) concentration can be achieved relative to a complete mix regime, resulting in a higher VFA yield per unit of reactor volume.

Three trials were conducted. Sampling and testing were performed for soluble orthophosphate and total phosphorus (OP and TP), soluble and total chemical oxygen demand (COD) total suspended solids (TSS), volatile suspended solids (VSS) and total VFAs.

CHAPTER 2 : LITTERATURE REVIEW

2.1 Biological Nutrient Removal

This section provides an overview of biological nutrient removal (BNR), a process that has been well-reviewed by Grady et al. (1999). BNR processes are modifications of the basic activated sludge process. In addition to aerobic zones, they incorporate anaerobic zones, if designed to remove phosphorus and/or anoxic zones, if designed to remove nitrogen. BNR will usually refer to both P and N removal. Some people refer to biological nitrogen removal (BNN) for processes including only anoxic and aerobic zones to achieve nitrification/denitrification. It is very common to refer to BPR for biological phosphorus removal processes whether or not they are stand-alone processes or included within a BNR process that removes both N and P.

A typical BNR process flow diagram is shown in Figure 2.1. Primary clarifier effluent (PCE) and return activated sludge (RAS) enter the anaerobic zone where VFAs are taken up by PAOs, which in return, release phosphorus into the anaerobic mixed liquor (primary phosphorus release). Volatile fatty acids in wastewater are generally considered to include the short carbon chain acids (C_1 to C_5), such as acetic, propionic, and butyric acids that are readily biodegradable; they are also referred to as, short chain volatile fatty acids (SCVFAs). The role of PAOs is described in more detail in the next section.

From the anaerobic zone, the mixed liquor is transferred to the anoxic zone to be stirred with the nitrate-containing mixed liquor recirculated from the aerobic zone. In the

anoxic zone, in the absence of oxygen, nitrate (NO_3^-) serves as the electron acceptor for denitrifying bacteria such as *Achromobacter*, *Aerobacter*, *Bacillus* and others. Denitrification is the biochemical process by which nitrate is transformed into nitrogen gas (N_2).

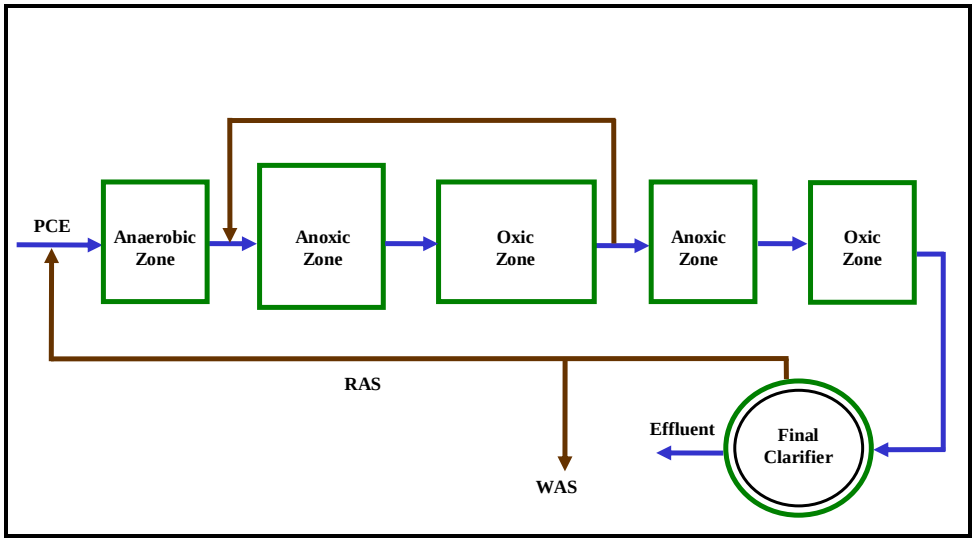

Figure 2.1: Schematic of a Typical BNR Process (adapted from Grady et al., 1999)

From the anoxic zone, the liquor is sent to an aerobic zone (oxygen and nitrate are present) where ammonia (NH_4^+) is transformed into nitrate by nitrifying bacteria such as *Nitrosomonas* and *Nitrobacter*. Nitrogen gas produced in the anoxic zone is stripped from the mixed liquor into the atmosphere, which completes the process of nitrogen removal from the wastewater. Also in the aerobic zone, PAOs carrying VFAs taken up in the anaerobic zone metabolize the VFAs. They also take up phosphorus to re-establish polyphosphate chains that were cleaved in the anaerobic zone for transferring VFAs into their cell. The virtue of PAOs in this process is that they are capable of "luxurious

phosphorus uptake", which means that they can uptake more than the amount of phosphorus they released in the anaerobic zone (primary phosphorus release), resulting in a net positive P removal from the mixed liquor. There is the potential for some phosphorus to be released after the VFAs have all been consumed (secondary phosphorus release), which is undesirable, because phosphorus released in this way will not be taken up again for net phosphorus removal (Barnard and Scruggs, 2003).

In many plants, a second anoxic zone is provided for additional denitrification, followed by a second aerobic zone, where more nitrogen gas is stripped and additional oxygen is added to the mixed liquor before it is sent to the clarifier. A minimum dissolved oxygen concentration of 1-3 mg/L promotes good solids settling in the final clarifier. Phosphorus removal is ultimately accomplished by wasting (via waste activated sludge (WAS)) the P-rich sludge from the bottom of the final clarifier, which is why good sludge settling is important.

Several different BNR processes are used in wastewater treatment plants. Some, like the Modified Ludzak-Ettinger (MLE) process and the four-stage Bardenpho process (patented by James L. Barnard in the late 60's), remove only nitrogen. The first commercial BPR process, Phostrip®, was developed by Levin et al. In this process, 30-40% of the RAS is sent through a stripper tank, which is similar to a gravity thickener. The sludge is maintained unaerated in a settled blanket for 8-12 hours (up to 30 hours in some cases (Barnard, 1994)), during which time, phosphorus is released; the stripper functions as an anaerobic selector. Treated effluent or raw wastewater is added to the stripper to remove the released phosphorus, while the sludge is taken from the bottom of

the stripper and returned to the activated sludge process. The rich phosphorus overflow is treated with lime to remove phosphorus.

The Phostrip® process is configured as a side-stream process, with the anaerobic zone (stripper) located outside of the main activated sludge process. It provides two means for phosphorus removal: first through wasting of a portion of the final clarifier sludge (waste activated sludge) and second, through the stripper overflow. For several years, the process was not widely used due to its operational complexity, but it has recently been revised in Germany as a process for VFA production (Barnard and deBarbadillo, 2002).

The Phoredox process (also developed by James L. Barnard in the 1970's), marketed under the name A/O™, is also a commercial process for removing phosphorus. A/O stands for anaerobic/oxic, where oxic describes aerobic conditions. It is a main-stream process where RAS is returned to the anaerobic zone, which is followed by an aerobic zone. Phosphorus removal is achieved through activated sludge wasting.

Several processes are currently being used for both nitrogen and phosphorus removal. Some of the more common ones include the University of Cape Town (UCT) process and adaptations of some of the single nutrient removal schemes. The UCT configuration eliminates the nitrate-N recycle to the anaerobic zone. This is accomplished by directing the RAS to the anoxic zone, where it is denitrified. Nitrified mixed liquor from the aerobic zone is also directed to the anoxic zone. Denitrified mixed liquor taken from the end of the anoxic zone is recirculated to the anaerobic zone to provide the microorganisms needed there. The A/O™ described above for phosphorus removal was adapted to remove both nitrogen and phosphorus by incorporating an anoxic zone to the

anaerobic and oxic zones (A^2/O™). The four-stage Bardenpho has also been adapted into the five-stage Bardenpho, which includes an anaerobic selector to remove phosphorus.

2.2. Biological Phosphorus Removal

The conventional activated sludge process does not remove more than 20% of the phosphorus present in the wastewater. By contrast, BPR processes, also called enhanced biological phosphorus removal (EBPR) processes, optimize the potential of PAOs to take up phosphorus. It was first noted to be occurring in an activated sludge process by Milbury et al. (1969). Eighty (80) to ninety (90) percent of the phosphorus present in the BNR influent can be removed when EBPR is integrated into conventional secondary treatments (Morse et al., 1998), and it offers an environmentally sustainable method for phosphorus removal from wastewater (Oehmen et al., 2005).

Barnard, along with others, was responsible for designing a series of process configurations for EBPR (Seviour et al., 2003). He first introduced the concept in 1975, (Barnard, 1975) when he proposed that wastewater influent and RAS be passed sequentially through an anaerobic zone and then an aerobic zone to induce phosphorus removal. He named this process scheme Phoredox, which can be considered the progenitor of most of the EBPR systems now used (Seviour et al., 2003). However, the organisms responsible for the phosphorus accumulation and the mechanism by which they accomplished it were not yet understood.

The same year, Fuhs and Chen (1975) explained that the presence of one specific obligate aerobic bacterium similar to *Acinetobacter Lwoffi* (member of the gamma (γ) subdivision of the *Proteobacteria* phylum) was responsible for the phosphorus uptake

observed in the aerobic zone of their laboratory-scale reactor. Acetic acid (or acetate), a common VFA in wastewater, was serving as the substrate for the bacteria to form poly-β-hydroxybuterate (PHB) granules. The authors postulated that high acetate concentrations in wastewater were supporting the growth of the same organisms in treatment plant aeration basins. Initially, acetate was thought to be the only VFA that could be used by *Acenitobacter*. Fuhs and Chen (1975) also noted that phosphorus release in the anaerobic zone was followed by phosphorus uptake in the aerobic zone. However, they did not link the two phenomena as the mechanism behind net removal of phosphorus from the treatment system. Nicholls and Osborn (1979) later showed that certain obligate heterotrophs (especially *Acinetobacter* species) could use energy expended from the hydrolysis of stored polyphosphate (poly-P) chains to transport acetate into their cell to be stored as PHB. Unused phosphate from the poly-P chains was released into the wastewater. When the PAOs subsequently passed through the aerobic zone, the stored PHB was metabolized and used as energy for growth and replenishment of poly-P.

Some researchers had postulated in the late 1960's that the phosphate uptake being observed was a form of the so-called "luxury uptake" (Yall et al, 1972), which is the tendency of certain microorganisms to accumulate a substrate in excess of their metabolic requirements. This hypothesis that "luxury uptake" was the mechanism by which PAOs were accomplishing a net phosphorus removal from wastewater was later confirmed by Comeau et al. (1986) and Wentzel et al. (1986). Barnard and Scruggs (2003) reported that uptake of acetate and other acids, provides PAOs with sufficient energy to accumulate 125% of the phosphorus released in the anaerobic zone.

In 1984, Barnard described a phenomenon called “secondary release,” which refers to the case when PAOs are retained under anaerobic conditions without VFAs present, and they release phosphorus that is not taken up later in the process. Secondary phosphorus release also can result when nitrate is absent in the anoxic zone or oxygen is absent in the aeration zone (Barnard and Scruggs, 2003).

Two models have been proposed to explain the biochemical pathways used by PAOs: the Comeau-Wentzel model (developed independently by Comeau and Wentzel in the late 80’s) and the Mino model (Grady et al., 1999), developed by V. Arun and his team in 1988. The models are in agreement that during the anaerobic phase, VFAs are consumed and subsequently stored as polyhydroxyalkanoates (PHAs). In the case of acetate, PHB is the main PHA stored in the PAO cell. In the case of propionate, the PHAs are polyhydroxyvalerates (PHVs). The models differ in their presumed source of reducing power for PHA storage. In the Comeau-Wentzel model, acetyl-CoA (acetate form once passed through the PAO cell) is utilized as the reducing power through the tricarboxylic acid (TCA) cycle, while in the Mino model, glycogen is the reducing power source.

The correct model remains unresolved, although the Mino model seems to have gained more acceptance than the Comeau-Wentzel model over the years (Grady et al., 1999; Pijuan et al., 2004a). Kong et al. (2004) found that the *Rhodocyclus* species found in the sludge of the three plants that they evaluated, used glycogen as the reducing power to accumulate the VFAs in the anaerobic zone (Mino Model). However, others assert that glycogen degradation alone would not satisfy the demands for reducing power for PHA synthesis, and that the TCA cycle may be the source of the additional reducing power needed (Seviour et al., 2003).

2.2.1. Bacterial Populations Present in EBPR Systems

In the 1980's, most investigations of the organisms responsible for phosphorus removal focused on isolates of the genus *Acinetobacter,* because members of this genus could be easily isolated (cultured) from BPR sludges. However, it is well known that many bacteria in microbial ecosystems are un-culturable, and it was suspected that some of the key organisms might yet be un-identified (Liu et al., 1998). With the use of non-culture-dependent molecular biology techniques (rRNA based methods) that became available in the early 1990's, such as Polymerase Chain Reaction (PCR) and Fluorescent *In Situ* Hybridization (FISH), other bacteria were identified as PAOs.

With these methods, it was found that *Acenitobacter* species did not prevail in most activated sludge systems (Wagner et al., 1994; Grady et al., 1999). However, Carr et al. (2002) warn that the *Acenitobacter* species found in activated sludge are not the same as *Acinetobacter* species previously reported in the literature, which are clinical strains different from those found in BPR systems. Most current DNA probes used to quantify the numbers of *Acinetobacter* in activated sludge were made from identified clinical strains and may not be useful for detecting the presence of these environmental strains. Therefore, numbers of *Acinetobacter* in activated sludge systems may have been underestimated in the past.

In laboratory study of anaerobic/aerobic sequencing batch reactors (SBRs) fed with synthetic wastewater and glucose, good phosphorus removal was observed over the 5.5 years of the trial, but no *Acinetobacter* species were found. Instead, some strains of

Pseudomonas and *Bacillus* were isolated in the sludge and suspected of accomplishing P removal. When the same reactors were fed starch instead of glucose, some *Acinetobacter* were isolated from the mix, but phosphorus removal capacity declined.

Research has proven that there is no single dominant PAO type, but that several different obligate aerobes can accumulate phosphorus under anaerobic conditions. Sudiana et al. (1998) found in bench-scale studies that members of the beta subclass of the *Proteobacteria* phylum (*β-Proteobacteria*) group dominated in activated sludge acclimated with either acetate or glucose as the major carbon source. They were judged responsible for EBPR when staining revealed they contained polyphosphate granules. Some *Acinetobacter* species were present, but in very small numbers.

Subsequently, Hesselmann et al. (1999) and Crocetti et al. (2000) identified *Rhodocyclus* species and *Propionibacter pelophilus*, members of the *β-Proteobacteria* group, as the dominant species performing phosphorus removal in laboratory mixed cultures of domestic sewage activated sludge. Zilles et al. (2002) confirmed that *Rhodocyclus* species were an important fraction of the PAOs found in the UCT activated sludge at Nine Springs WWTP in Madison, WI. Onuki et al. (2002) drew the same conclusion after using PCR and denaturing gradient gel electrophoresis (DGGE) on sludge from a WWTP in Tokyo. Zilles et al. (2002) noted that a significant portion of the PAOs identified in their study was not related to the *Rhodocyclus* genus.

Kong et al. (2004) examined sludge from three EBPR plants in Denmark that had been performing BPR well for several years. He found that *Rhodocyclus* species were present in all plants, and that they constituted 5-22% of the biomass community. Kong et al. suggested that *Rhodocyclus* species, such as *Candidatus Accumulibacter phosphotis*,

may be the dominant PAOs in EBPR plants. They also affirmed that other bacteria were involved in the BPR process and that further studies were needed to identify and quantify the role of these unknown bacteria. When Wong et al. (2005) examined 13 samples of sludge from 9 different wastewater plants in Japan, they found that 85% of the cells with poly-P granules in sludge from two of the plants were members of the *β-proteobacteria* phylum, but they were not related to *Rhodocyclus*.

Chua et al. (2004) reported that *Acinetobacter* and members of the alpha sub class of the *Proteobacteria* phylum (*α-Proteobacteria*) also tend to be numerically dominant in EBPR systems. Furthermore, the discovery of denitrifying PAOs (DPAOs) has been extensively reported and discussed (Kerrn-Jepersen and Henze, 1993; Rensink et al., 1997, Meinhold et al., 1999; Hu et al., 2002), although they are not considered to be of greatest importance in enhanced phosphorus removal.

It is evident that the ecosystems of EBPR sludges are very complex. Seviour et al. (2003) stressed the need to extend what has been done in lab-based studies to full-scale operating plants and to obtain in pure cultures as many PAOs as possible.

2.2.2. Glycogen Accumulating Organisms (GAOs)

Occasional failures in EBPR processes have been reported in both full-scale and laboratory-scale systems. In wastewater treatment plants, several events have been known to contribute to unstable phosphorus removal (and nitrogen removal): excessive rainfall, excessive nutrient and organic loading, excessive aeration, a shortage of potassium and high nitrate loading in the anaerobic zone (Mino et al., 1998). However, in well-designed laboratory-scale reactors, some researchers reported that BPR deteriorated without any obvious reasons (Matsuo, 1994; Liu et al., 1994).

Cech and Hartman (1990, 1993) first reported the involvement of a non-PAO bacterium that they thought was responsible for EBPR failure in their laboratory glucose-fed reactor. They noticed a large number of bacteria arranged in tetrads. Based on some biochemical analyses, they concluded that these bacteria (that they named G-bacteria) may have competed with the PAOs for VFAs. The result was removal of VFA substrate needed for luxury P uptake, which the G-bacteria could not accomplish. Cech and Hartman (1990) suggested that the G-bacteria assimilated glucose anaerobically better than PAOs and became dominant. Similar conclusions were reached by Liu et al. (1996, 1997).

The G-bacteria were later named glycogen accumulating organisms (GAOs), because of their capacity to use intracellular glycogen (and not poly-P) both as a source of reducing power (like some PAOs do) and as an energy source for VFA transport into the cell under anaerobic conditions (Randall et al., 1997). Satoh et al. (1992) suggested that the presence of high levels of glucose in mixed liquor, which can be used by GAOs to store intracellular glycogen, may allow GAOs to out-compete PAOs for available VFAs. However, the glycogen metabolism adopted by GAOs is much more complex and less efficient in terms of energy production than the poly-P metabolism of PAOs, which could be a metabolic disadvantage for GAOs when in competition with PAOs. In fact, PAO-enriched sludge takes up acetate faster than GAO-enriched sludge. Therefore, there are no possible biological reasons to explain why the GAOs should dominate the PAOs in a BNR system. It could be concluded that if uptake of substrates by PAOs is slowed down by one reason or another, the GAOs may have an advantage to grow, if part of the substrates remain in the anaerobic zone (Mino et al., 1998).

Several bacterial species have been identified as GAOs, but they are thought to be very different from the PAOs species identified so far (Wang et al., 2001). With GAOs, only the cell wall stains Neisser positive, whereas PAOs stain Neisser positive throughout the cell where poly-P granules are located (Mino et al., 1998; Jenkins et al., 2004). The dominant strain found by Cech and Hartman (1993) was gram-negative and appeared very similar in shape to species of the *Methanosarcina* genus (domain Archea). Maszenan et al. (1998) identified three new bacteria species from treatment plants in Austria, Italy and Macau. These species were gram-negative and members of a new genus called *Amaricoccus* (*α-Proteobacteria*). They concluded that the G-bacteria isolated by Cech and Hartman (1993) were not Archea, but belonged to the same phylum as the bacteria they identified. If true, it would mean that the G-bacteria were not from the Archea domain but from the Bacteria domain.

Maszenan et al. (2000) identified two new gram-positive GAO species that are members of a new genus named *Tetrasphaera*. Crocetti et al. (2002) found large coccoid/rod-shaped GAOs, *Competibacter phosphatis*, in laboratory-scale SBR and full-scale systems, and Saunders et al. (2003) reported finding the same organisms in sludge from six Australian wastewater treatment plants. The latter group also found other GAOs that could not be identified with the method used.

While researchers are still debating about the specific conditions that lead to GAO domination in certain EBPR systems, some conditions that may favor the growth of GAOs over PAOs have been discussed. When growth of GAOs and PAOs was compared in mixed liquor with varying acetate and glucose concentrations, the growth rate of PAOs was always greater (3 to 6 times) than that of GAOs (Wang et al., 2000),

suggesting that GAOs may be at a disadvantage when limited substrate is available. However, at long sludge ages (SRT ≥20 days) GAOs were able to remain competitive with PAOs. Barnard and Scruggs (2003) mentioned that long un-aerated times (under anaerobic and anoxic conditions) encourage the growth of GAOs. In addition, periods of low BOD in the plant influent favor GAOs because they can use stored carbohydrate reserves. Satoh et al. (1992) proposed that a protein or amino acid deficiency in the wastewater may also favor GAOs, because they can synthesize these substrates themselves faster than PAOs.

Whang and Park (2002) showed that the mixed liquor temperature affects the balance between the PAO and GAO populations in laboratory-scale SBR reactors, with GAOs becoming dominant over PAOs when the temperature increased from 20º C to 30ºC (at 10-day SRT). When the temperature was reduced back to 20º C, PAOs did not regain dominance until the SRT was reduced to 3 days at 30ºC. Recent findings suggest that propionate (propionic acid) in the anaerobic zone may favor the presence of PAOs over GAOs because GAOs are slower at consuming this VFA compared to acetate (Oehmen et al.,2004; Pijuan et al., 2004b). Oehmen et al. (2005) also found that GAOs are slower than PAOs in responding to a change in carbon source. Finally, as noted above, glucose fed to an anaerobic zone may favor GAOs. However, this is not always the case; glucose dosing has also been shown to enhance EBPR with no increased growth of GAOs (Mino et al., 1998; Lee et al., 2002).

2.3. Role and Type of Volatile Fatty Acids

Acetic and propionic acids are the VFAs that typically predominate in septic domestic wastewaters (Chen et al., 2002). VFAs are produced naturally when soluble and

particulate organics contained in the sewage (lipids, proteins, carbohydrates, amino acids, sugars or long carbon chain fatty acids) are hydrolyzed and fermented through the action of facultative and strict anaerobes (Grady et al., 1999).

The amount of VFAs required to remove 1 mg/L of phosphorus from raw sewage through luxury uptake is relatively consistent in the literature and ranges from 7-10 mg/L of acetate per mg of phosphorus (Manoharan,1988; Barnard, 1993; Grady, 1999). Münch and Koch (1999) reported the broadest range: 8-20 g VFA as COD per gram of P removed (or 7.4-18.5 mg/L HAc per mg of P). However, there is no consensus on which VFA or mixture of VFAs is the optimum substrate for PAOs to achieve high efficiency BPR (Barnard, 2005).

One of the first important studies of the effect of organic compounds on BPR was done by Abu-guararah and C.W. Randall (1991). They investigated the effect of separate additions of formic, acetic, propionic, butyric, iso-butyric, valeric and iso-valeric acids on phosphorus uptake using a pilot plant fed domestic sewage and operated as a UCT process. All substrates except formic acid caused significant increases in phosphorus removal efficiency over unamended controls. It was also found that the branched organic acids, iso-butyric and iso-valeric, caused better phosphorus removal efficiency than the non-branching forms of the same acids.

In studies aimed at discerning the effects of glucose on phosphorus removal on EPBR systems, Randall et al. (1994) found that EBPR proceeded well in anaerobic/aerobic SBRs when the reactors were fed synthetic wastewater with a glucose carbon source, but P removal was only marginal when starch was used in place of glucose. Subsequent tests indicated that success with glucose occurred because the glucose fermented in the

feedstock (made only once daily), while the starch did not. Further, the anaerobic zone did not act as a fermentation zone in either system. Biomass from the SBRs was also used in batch experiments, where they were dosed with one of several different carbon sources, including alcohols, fructose, glucose, starch, lactate, pyruvate as well as formic, acetic, propionic, butyric, iso-butyric, valeric and iso-valeric acids. The results indicated that all of the VFAs except propionate improved phosphorus removal. Also, when glucose and propionate were combined, the mix was detrimental to EBPR. All other substrates tested had no effect on EBPR.

The effects of propionic acid on phosphorus removal have not been consistently replicated. When Rustrian et al. (1997) tested propionic acid for its effect on phosphorus removal in batch experiments, P removal was enhanced, although at a lower rate than that observed with acetic and butyric acid carbon sources. Sludge from a wastewater treatment plant in Zurick was used in batch experiments where several VFAs were fed separately (Moser-Engeler et al, 1998). It was found that acetate and propionate were taken up much faster than the C_4-C_5 VFAs, even though n-butyrate and iso-butyrate (C_4 VFAs) showed the highest phosphate released (DP) to substrate uptake (DS) ratio. A higher DP/DS ratio means that PAO accumulation of phosphorus in the aerobic zone is high. However, the C_4 VFAs are not taken as readily as the shorter VFAs in the anaerobic zone. The authors fed one batch reactor with an equimolar mix of the C_1-C_5 VFAs (not a typical situation in real wastewaters) and found that n-butyrate was taken up as fast as acetate and propionate. It is to be noted that this VFA is a large share of the SCVFAs found in real wastewaters. Finally, contrary to other studies (Abu-guararah and C.W. Randall, 1991;A.A. Randall et al., 1994), the one conducted by Moser-Engeler et

al. (1998) found that the branched SCVFAs removed less phosphorus than their linear counterpart.

Similar experiments conducted by Randall and Khouri (1998) on biomass cultivated on a glucose/VFA mixture showed that acetic and iso-valeric acids (compared to proprionate and other VFAs) enhanced P-removal during short-term experiments and resulted in good BPR in a long-term cultivation. Liu et al. (1997) and Randall and Liu (2002) found that acetic and iso-valeric acids resulted in PHB storage, while propionic and valeric acids resulted in PHV storage, and suggested that PHB accumulation results in a higher P uptake than PHV accumulations. They questioned whether the uptakes observed were truly related to the PHA type formed (i.e. this would happen in other systems with real and different wastewaters) or to the type of bacteria that were "selected for" in the biomass by previous cultivation with acetic acid.

Hood and Randall (2001) compared the effects of acetic acid, iso-valeric acid, and propionic acid on P-removal, and also hypothesized that PHBs may lead to higher P-removal than PHVs. One SBR reactor was acclimated by supplementation with acetic acid while another was acclimated with propionic acid. After at least three SRTs (SRT = 6-6.5 days), the biomass populations developed in each conditions were tested in batch tests for P-removal under different VFA feed conditions. During acclimation, the propionic SBR was found to be more efficient at phosphorus removal than the acetic acid SBR. However, propionic acid added to the propionic-developed biomass during batch tests yielded the least efficient P-removal system compared to tests where the propionic-developed biomass received acetic acid or succinic acid or iso-valeric acid. When the concentration of propionate was increased from 0.133 mmol/L to 0.333 mmol/L, the

phosphorus release almost doubled. However, the phosphorus uptake could not compensate for the high phosphorus release and therefore could not create a net positive phosphorus removal. Succinic acid, a dicarboxylic acid, proved to be an excellent substrate as it was more efficient than iso-valeric acid and only slightly less efficient than acetic acid. It is to be noted that when biomass developed on acetic acid was batch tested, the results were almost identical. However, iso-valeric acid was slightly more efficient than acetic acid. These observations on the succinic acid were also reported by Randall et al. (1997).

Such differences between long term (SBR reactor) and short term (batch) trials had been previously reported (Manoharan, 1988; Abu-ghararah Randall, 1991), and the authors suggested that prefermentation may have occurred in the feed to the SBR reactors, resulting in a mix of proprionate and acetate rather than pure propionate substrate. This would not have happened in the batch experiments. They noted that only in past experiments where the presence of acetic acid was probable or possible was high efficiency with propionic acid observed. Others have proposed that the results observed could be due to substrate pressure, which would be observed in long term testing, but not in batch studies (Chen et al., 2002).

Biomass fed only acetate (Liu et al., 1997) achieved a much higher P uptake, but it deteriorated in the long run. Indeed, Randall and Chapin (1997) showed that feeding incremental amounts of acetic acid to the anaerobic zone led to ultimate failure of phosphorus removal. Futhermore, it was suggested that GAOs can out-compete PAOs for acetate, while the reverse is true for propionate and other higher molecular weight

fatty acids (Thomas et al., 2003). A similar hypothesis was made by Hood and Randall (2001).

More recent findings suggest that a mix of acetic and propionic acid optimizes EBPR (Liu et al., 2002; Barnard and Scruggs, 2003). Chen et al. (2002) evaluated the effect of propionic versus acetic acid content of domestic sewage on EBPR. Septic sludge from a Florida treatment plant, that routinely achieved EBPR, was used to seed laboratory reactors, and instead of synthetic feed, wastewater from the facility was used to feed the SBR reactors. The reactors were operated continuously for three 9-day SRTs, and the biomass was cultivated with externally added acetic or propionic acid. The reactors received acetic acid for several months and performed very similarly. Later, one reactor was switched to propionic acid. The wastewater contained a significant amount of acetate and propionate as well, so that after the switch, the acetic acid SBR had an influent propionate:acetate ratio of 0.145, and the propionic acid SBR had an average influent propionate:acetate ratio of 2.12. After one SRT, the effluent soluble phosphorus quality of the propionic SBR started improving rapidly. After two SRTs, the effluent quality had increased dramatically over that of the acetic acid SBR.

Interestingly, when the propionic-cultivated biomass from these experiments was tested in batch with acetic and propionic acid VFAs, those cultures fed acetic acid yielded superior phosphorus removal. The authors hypothesized that the differences in the batch and longer-term SBR studies could be explained by the fact that propionic acid is slightly less efficient than acetic acid as a substrate for PAOs, while it is a much less efficient substrate than acetic acid for non-PAOs. Therefore, in extended trials, the PAOs can out-compete non-PAOs (e.g. GAOs) when grown on propionic acid, while in short term batch

trials the non-PAOs can out-compete the PAOs. The authors also suggested that extrapolating from batch experiments to predict long-term performance may be misleading, because it does not take into account some effects of selective pressure that can be exerted by a substrate in long term cultivation.

Chen et al. (2002) further proposed that a mixture of propionic and acetic acid (with more propionic than acetic acid) may prove more effective than 100% acetic acid, because while acetic acid selected strongly for PHBs, propionic acid selected for PAOs. In terms of redox balance, the theoretically optimal propionic:acetic ratio would be 3:2, but because bacterial selection pressures may cause treatment systems to deviate from theoretical predications, it was recommended that a 2.12:1 ratio be used pending further studies.

2.4. Fermentation Processes for EBPR

Several methods have been tested and utilized to ensure the availability of VFAs for BPR. In some cases, fermentation of organics occurs during wastewater transit. When such "unintentional" fermentation does not occur, some organics will be fermented in the BNR anaerobic zone, but treatment facilities should not rely on this to provide the VFAs necessary for the EBPR process. When Barnard (1975) suggested that biomass be passed through an anaerobic zone first, it was generally believed that fermentation could take place in the anaerobic zone, which could then serve to produce VFAs as well as a contact zone for the PAOs. However, it was later determined that uptake of VFAs must happen at a limited HRT to minimize secondary release of phosphorus (Barnard, 1984) and the growth of GAOs (see section 2.2.2). Therefore, VFAs must already be available in the wastewater before it enters the BNR process. External addition of VFAs or a separate

fermentation process dedicated to VFA production must be implemented to compensate for the lack of VFAs contained in low septic wastewaters.

Recent findings have highlighted the fact that a wastewater composed mainly of propionic and acetic acid (like "unintentionally" fermented sewage) produced the optimum phosphorus removal. Although acetic acid can be affordable for certain plants, propionic acid produces an unpleasant odor and is very expensive (Chen et al., 2002). Waste sugar water from a soda bottling process has been used by McDowell Creek WWTF in Huntersville, NC, as a source of acetic and propionic acid, but it does not arrive fermented. Based on these facts, a VFA dedicated fermenter is the best solution from a process point of view for any wastewater facility that requires supplemental VFAs (Barnard, 1994). However, this will incur additional costs that will depend on the type of process used and also on the existing plant equipment that could be used as part of the new fermenter system (Grady et al., 1999).

VFA production from organics is part of a series of biochemical reactions that occur under anaerobic conditions (Table 2.1), and it occurs early in the reaction sequence. If allowed to go to completion, the VFAs will be subsequently consumed to yield methane. One important objective in designing a dedicated fermenter for BPR is to maximize the production of VFAs, while minimizing the formation of methane.

The suppression of methane production is achieved by maintaining a low SRT, which ensures that methanogenic bacteria are washed out of the fermenter (Münch, 1998). In on-site suspended growth fermenters, production of hydrogen sulfide (H_2S) is an indication of methane fermentation and a sign of potential process failure. A multi-tank plug flow fermenter design has been suggested to minimize methane formation (Barnard,

1994). It is interesting to note that this is not the case in the attached biomass in sewer pipelines, since methane formation only takes place in the deeper slime layers and does not affect VFA production. Although H_2S is a nuisance in pipelines (odors, corrosion), the VFAs associated with it are useful in BNR processes.

Table 2.1: Biological Pathways in Anaerobic Digestion Processes
(adapted from Rössle and Pretorius, 2001b)

BIOLOGICAL PATHWAYS IN ANAEROBIC DIGESTION PROCESSES	
Phase I: Fermentation (VFA generation processes)	
Hydrolysis	Extracellular enzyme-mediated transformations, where complex soluble and particulate (insoluble) organic material is transformed into simple soluble substrate, by incorporation into water molecules.
Acidogenesis	Acidogenic bacteria (faster growing when compared to methanogenic bacteria) ferment the hydrolysis products into long- and short-chain volatile acids, other acids, alcohols, etc.
Phase II: Methane generation (VFA consumption processes)	
Acetogenesis	High molecular fatty acids, as well as volatile acids (except for acetate), are decomposed into reaction intermediates: simple acids such as acetate, propionate and butyrate.
Methanogenesis	Methanogenic bacteria (slower growing) metabolise the VFA (decarboxylation of acetate), methane formation.
Phase III: Additional VFA consumption processes	
Aerobic respiration	DO present: bacteria (aerobic) consume VFAs
Sulphate reduction	SO_4^{2-} present: bacteria (sulphate reducing) consume VFAs
Denitrification	NO_3^- present: bacteria (heterotrophic) consume VFAs

It is also important to ensure that dissolved oxygen, nitrate and sulfate are not present in the fermenter, as organics, including any VFAs produced, would be consumed by

bacteria able to use these electron acceptors for metabolic processes (Rössle and Pretorius, 2001b). A VFA concentration of 100 mg/L of VFAs as acetic acid in the fermenter effluent is considered sufficient for good BPR in most cases. Below 50 mg/L of VFAs, special care must be given to BNR process design and optimization for the amount of VFAs to be sufficient in the anaerobic zone (Pitman 1992).

The second important objective of fermenter design is to ensure the recovery of the VFAs from the fermenter effluent in a stream that can be delivered to the BNR system (Grady et al, 1999). Separation of VFAs from the sludge, a process called elutriation, is a critical step in delivering VFAs produced to the BNR basins.

Full-scale implementation of fermenters has occurred only in the past 10 to 20 years (Rössle and Pretorius, 2001a). Although fermentation is a common practice associated with BNR facilities in many parts of the world, it has only been used in a few full-scale installations in the United States to date (McCue et al., 2003). The Canadian wastewater plant in Kelowna, British Columbia, was the first treatment facility in the world to be retrofitted with a fermentation system for the purpose of VFA production (process proposed by James L. Barnard) in 1982 (Barnard et al., 1995).

The most common fermenters found in WWTFs are the prefermenters. Prefermentation is the intentional anaerobic production of VFAs in primary treatment tanks; the substrate is suspended or settled solids (primary sludge) (Münch, 1998). Although this definition does not reference the soluble organics in the wastewater, fermentation systems that use the whole raw wastewater as substrate are also referred to as prefermenters. A list of the most prevalent prefermenter process configurations used worldwide is shown in Table 2.2. The listed configurations can be operated as either

batch or continuous flow processes. A schematic representation of these processes is included in Appendix B.

Table 2.2: List of Basic Process Configurations for Prefermenters
(adapted from Rössle and Pretorius, 2001b)

Basic Process Configurations for Prefermenters				
Configuration	**Equipment**	**Feed Inlet**	**Sludge Outlet**	**Supernatant Outlet**
In-Line Prefermenters				
APT	Primary Clarifier	Raw Wastewater	Recycle to inlet and waste	BNR
Side-Stream Single-Stage Prefermenters				
Static	Gravity settling tank	Primary sludge	Waste, no recycle	BNR
Complete-mix	Mixing tank	Primary sludge	None	BNR or return to primary clarifier
Side-Stream Two-Stage Prefermenters				
Complete-mix/thickener	Mixing tank and gravity settling tank	Primary sludge	Gravity settling tank sludge returned to mixing tank or waste	Gravity settling tank overflow to BNR

Fermenters can be in-line or side-stream systems. In-line, as the name indicates, means that the fermenter is configured to be included in the process main stream, while side-stream fermenters are situated outside of the main treatment process. Major advantages of an in-line fermenter are the lower capital cost and lower space requirement. A side-stream configuration allows for more operational and optimization flexibility (Rössle and Pretorius, 2001b). This can be beneficial in the absence of raw sewage hydraulic equalization (Banister, 1996).

In an activated primary tank (APT) system, a higher sludge blanket than normal is maintained in the primary clarifiers to allow for VFA generation in the primary sludge solids. A portion of the settled sludge is recycled to be combined with the raw sewage influent. The recycled stream serves the dual purpose of maintaining the sludge blanket and elutriating the VFAs (Barnard, 1984). VFAs are sent to the BNR system with the clarifier effluent. With this configuration, there is no need for new facilities, since most of the equipment will already be in place. However, control of the sludge blanket and the associated SRT is very difficult. If the plant was designed to conduct primary sludge fermentation, the clarifier sludge removal mechanisms will be able to handle the higher sludge load. In plants where fermentation was not accounted for during design, the mechanism will probably not be able to handle the high solids content of the sludge (Barnard, 1994).

The Bronkhorstspruit Plant in South Africa uses a different approach to the conventional APT in-line prefermenter. Screened raw wastewater is discharged into a high-rate, upflow acid-fermenter (Barnard and deBarbadillo, 2002) or UASB reactor. The sludge blanket fills the tank and is washed over to the anaerobic zone with the VFAs. Other similar configurations using the whole raw wastewater have been tested and are discussed in section 2.4.1.

A complete mix tank configuration (Table 2.2) enhances fermentation due to better contact between suspended solids and bacteria (Rössle and Pretorius, 2001b). Unlike APT or static prefermenters, the HRT equals SRT in this type of system. However, a higher effective volume is required for a complete mix system to treat the same amount of sludge to the same level as a static fermenter. In some cases, VFA elutriation is

achieved by returning a portion of the fermenter effluent to the primary clarifier feed. In other cases, all of the fermenter effluent is sent to the BNR process. A complete-mix prefermenter with a dedicated thickener (also called two-stage prefrementers) enables SRT control separately from HRT control, which allows for more flexibility to optimize the VFA concentration. A less costly variation of this design is a process configuration in which the primary clarifier is used as the thickener (Münch and Koch, 1999).

A static fermenter is a dedicated anaerobic sludge thickening tank with no mixing. It is a simple system, but the VFA production rate is lower than the production rate obtained in a mixed tank (Pitman, 1992). VFAs are elutriated into the supernatant (or overflow) by one method or another. As with the APT, control of the sludge blanket and the SRT is difficult. Other "home made" prefermentation systems exist in plants around the world, several of which have been described by Barnard (1994).

Prefermenters have become increasingly popular to improve the performance of existing BNR plants. However, once a prefermenter is built and operating, little attention is paid to its performance as long as the plant P removal efficiency is good (Münch and Koch, 1999). Randall et al. (1992) observed that prefermenter process monitoring is mostly done on a "this-may-work" basis.

More organics are available to produce VFAs at higher solids loading rates. However, inhibition, solids overloading and solids carry-over to the BNR plant can occur (Rössle and Pretorius, 2001b). Solids carry-over events may be an issue when the sludge recirculation rate of an APT system is too high or if a complete-mix fermenter has no thickener.

Skalsky and Daigger (1995) reported that higher VFA yields were obtained at solids content <1% rather than at 2.6% (at a SRT of 2 days). The authors stated that it may have been due to better mixing at 1% or to a reduction of inhibitory substance concentrations. Banister (1996) suggested a solids content of 2 to 3% maximum to prevent solids inhibition effects. However, this solids inhibitory substance effect was not observed by Lilley et al. (1990), who conducted VFA production laboratory trials where the VSS content varied between 11,000 to 42,000 mg/L (1.1-4.2%).

The most important fermenter operating parameters reported in the literature are the HRT and the SRT. The VFA yield (mg VFA produced / mg VSS in the influent or mg VFA produced/ mg Total COD in the influent) is mentioned by Münch and Koch (1999) as one other important parameter to evaluate fermentation performance between different systems. Values of 0.05 to 0.3 gram VFA as acetic acid per gram of VSS in the feed to the fermenter have been reported in the literature for prefermenters (Elefsiniotis and Oldham, 1993; Grady et al., 1999; Rössle and Pretorius, 2001b).

The sludge recirculation rate (SRR) and the sludge elutriation rate (SER) are rarely discussed but are equally important (Rössle and Pretorius, 2001b). The SER refers to the ratio of the mass of fermented solids recycled to the volumetric inflow rate of raw sewage to the fermenter. The SRR refers to the ratio of the volume of sludge recycled to the volumetric inflow rate of raw sewage. GonÇalves et al. (1994) noted that upflow velocity is also an important parameter to assess solids retention.

The literature is contradictory regarding an ideal SRT (Randall et al., 1992). Due to variable local conditions, there is no universal SRT suitable for all prefermenters. However, a range of 4 to 6 days is average (Rössle and Pretorius, 2001b). Rössle (1999)

also stated that the general guideline for the HRT in a single in-line APT system is between 2 and 4 hours. For side-stream fermenters (except for the complete-mix), Dawson et al. (1995) noted that the HRT should be around 15 hours.

Limited published data is available that correlates SER or SRR with the amount of VFAs produced. A recent full-scale evaluation (Hartley et al., 1999) indicated that a high SRR (0.5 m^3 sludge/m^3 raw sewage) increased the VFA outflow concentration in an APT. It was further reported that only 50% of the VFA production occurred in the sludge blanket. This can be attributed to the high SRR, leading to a high suspended solids inventory in the settled sewage layer above the sludge blanket. Another full-scale APT evaluation (Rössle and Pretorius, 2001c) indicated that a higher SRR (from 0.01 to 0.09 m^3 sludge/m^3 raw sewage) and a resulting higher SER (from 0.7 to 3.7 kg sludge/m^3 raw sewage) increased the VFA production rate (1.4 to 5.7 mg/ (L·h) of VFAs) in an APT.

McCue et al. (2003) stated that the benefits of primary clarification may be lost when using a prefermenter due to reduced BOD consumption and an increase of secondary sludge production that can occur if the system is not optimized. Lötter and Pitman (1986) also stated that the solids removal efficiency of primary clarifiers could decrease, because of the portion of sludge recirculated at the clarifier feed.

Other less common fermenting system configurations have also been employed at full-scale levels. Fermentation of RAS in a side-stream fermenter is being used at a few installations (Stroud and Martin, 2001; Narayanan et al., 2002). Auto-Thermophilic Aerobic Digestion (ATAD) of primary sludge and/or WAS has been evaluated as a means of VFA production for EBPR (McIntosh and Oleszkiewicz, 1997; Fothergill and Mavinic, 2000). Return of the high VFA liquid stream produced in the first stage of a

two-stage anaerobic digestion process could be utilized by the BNR process (Urbain et al., 1997; Rustrian et al., 1999).

Utilization of RAS or WAS for BPR enhancement is discussed in section 2.4.3. Side-stream or in-line mixed liquor fermentation, to the best of the author's knowledge, has not been officially reported as a means for VFA production at a full-scale level. The recent anaerobic mixed liquor fermentation pilot plant work of Barnard (Barnard and deBarbadillo, 2002) is discussed in section 2.4.4.

Finally, it should be noted that less conventional systems such as sludge liquor from heat treatment processes (e.g. centrate from Zimpco LPO system and Cambi process), have been tried occasionally as VFA sources (Barnard and deBarbadillo, 2002). However, the success of these systems was limited.

2.4.1. Primary Sludge and Raw Wastewater Fermentation (Prefermentation)

Most information on the operational performance of prefermenters is anecdotal. There is little quantitative information on the process and effluent changes resulting from prefermentation for a variety of wastewaters and climates (McCue et al., 2003). For that reason, only a few studies are described in the following paragraphs.

In 1983, Pitman et al. proposed that acid supernatant liquor from a high rate digester be added to the feed of an EBPR. He also proposed that primary sedimentation tanks be placed upstream of the EBPR process in order to capture the wastewater suspended solids for VFA production. Following the work by Pitman et al., Barnard (1984) reported on a new concept called activated primary tank (APT) as a way to produce VFAs for BPR. Pitman and Lötter (1986) compared VFA generation from high rate digesters and APTs and concluded that VFA concentrations in the acid supernatant were higher in the APTs.

Lilley et al. (1990) was the first to quantify the generation of VFAs from primary sludge fermentation. In laboratory-scale experiments, they found that VFA production was a first order reaction with a maximum VFA generation potential of 0.17 mg COD/ mg of primary sludge COD (0.158 mg HAc/mg COD in the feed). A compilation of VFA compositions in prefermenter effluents obtained from a series of studies performed in the 1990's (Table 2.3) show that prefermenter (side-stream type) effluents are composed mainly of acetic and propionic acid. Other acids produced are butyric and valeric acid (linear and branched forms). Only one study reported that other forms of acids were present in their prefermenter effluent. On average, the composition was 56% acetic acid, 32% propionic, with the remainder butyric and valeric acids. When considering only acetic and propionic acid, the average VFA composition is 64 and 36 % of acetic and propionic acid, respectively. This is very close to the acetic:propionic ratio suggested by Barnard and Scruggs (2003).

In the late 1980's, Johannesburg's Northern Works in South Africa utilized their flat bottom rectangular primary settling tanks for VFA production. Solids capture in the tanks was reduced from 50% to 20%, because these units were not designed for a high sludge blanket (Pitman et al., 1992). The increase in sludge solids content may have reduced the solids settleability. The presence of methane bubbles when the SRT was too high may have also caused some of the sludge to float and be carried over with the effluent.

In 1991, circular settling/fermentation tanks were added to the existing primary sludge treatment, which reduced the amount of solids carry-over to the BNR process. However, there was still 400 mg/L of suspended solids being carried over with the

fermenter effluent. Pitman et al. (1992) noted that this could either be a disadvantage or an advantage depending on the amount of COD present in the settled sewage. When the soluble COD is low, the extra solids sent to the BNR system could assist the nutrient removal process. However, if the soluble COD is high enough, the suspended solids sent to the BNR process may overload the BNR system with organics.

Table 2.3: Typical VFA Distributions for Prefermenters
(adapted from Rössle and Pretorius, 2001a)

Acetic Acid Weight (%)	**Propionic Acid Weight (%)**	**Butyric Acid Weight (%)**	**Valeric Acid Weight (%)**	**Other Acid Weight (%)**	**Reference**
38	36	16	10	-	Pitman et al., 1992
43	41	8	-	-	Randall et al., 1992
70	25	5	-	-	Carlsson et al., 1996
56	30	7	0	7	Rabinowitz et al., 1997
71	24	3	3	-	Münch, 1998
61	27	7	-	-	Münch, 1998
55	45	0	0	-	Münch, 1998
49	33	13	6	-	Münch, 1998
63	25	12	-	-	Rodriguez et al.,1998
AVERAGE					
56	32	8	2	-	-

Pitman et al. (1992) also noted that a large amount of solids carry over could be avoided by increasing the quantity of elutriant (i.e. reduce the sludge solids content). Furthermore, VFA production was affected by the increased nitrogen and phosphorus load to the BNR system. During the course of this literature review, it was noted that the nutrient overload aspect of prefermentation is not discussed very much in the literature. More attention is paid to sludge settling in the primary clarifier in APT systems and to solids carry-over to the BNR process.

Elefsiniotis and Oldham (1993) conducted a laboratory-scale study to evaluate the effect of HRT and SRT on the VFA production from primary sludge using two different process configurations. One fermenter was a completely mixed reactor followed by a thickener, and the second fermenter was a UASB reactor. The VFA production rate increased with increasing HRT (up to 12 hours) as well as increasing SRT. Both systems showed similar production rates at SRTs up to 10 days regardless of the HRT. At longer SRTs, the complete-mix fermenter became more efficient (by about 12%) than the UASB reactor. However, in both cases, the amount of VFAs produced was adequate to support EBPR under most conditions.

Following the work of Pitman et al. (1992), Banister and Pretorius (1998) conducted an optimization study on four Johannesburg fermenters. The main objective of their study was to determine whether supplementary addition of iron salts (for phosphorus precipitation), which was very costly, could be eliminated. Four batch reactors were fed primary sludge from a different plant. Acid fermentation occurred rapidly at SRTs of less than 6 days, and VFA yields reduced substantially, with only 10% of the influent COD converted to VFAs, after 6 days. Performance was found to improve with the addition of sludge (10 to 20% of total reactor volume) that had been allowed to ferment for a period of 1 to 3 days, perhaps because it reduced the SRT while VFA production remained sufficiently high. It was also determined that limiting the sludge solids content to 0.5-2% along with cessation of mixing, increased the VFA yield by at least 70%. Finally, Banister and Pretorius (1998) also experimented with a commonly used flocculation aid to dewater an 8 day old fermented sludge and evaluate both VFA recovery and solids retention. They concluded that mechanical dewatering was superior

to gravity thickening and sludge recirculation to optimize recovery of VFAs while minimizing solids carry-over. Barnard (1994) suggested a similar alternative to recover some of the VFAs that would otherwise stay in the sludge layer. He recommended using a dewatering step for the thickener underflow and mixing the liquid fraction from dewatering with the thickener supernatant being discharged to the BNR anaerobic basin.

A study by McCue et al. (2003) compared two UCT BNR processes with and without prefermentation. An initial bench-scale system treated septic, P-limited wastewater, and a larger pilot-scale plant treated a septic, COD-limited wastewater. For each system, two parallel treatment trains were operated identically, except one did not include a prefermenter. In the case of the P-limited influent, the prefermenter was an in-line intermittently mixed upflow clarifier (IMUC) with an HRT of 2.2-2.4 hours and an SRT of 4 days. The prefermenter used in the case of the COD-limited influent was a completely mixed side-stream tank. Prefermentation increased the soluble and readily biodegradable COD as well as VFA content for both types of influent. The COD-limited wastewater system with prefermentation achieved good P removal. The prefermentation system trial with P-limited influent did not yield a net P removal (and neither did the control) despite the fact that PHA levels in the anaerobic zone were elevated relative to those in the control system.

Raw wastewater prefermentation (in-line prefermentation) for VFA production has also been studied in bench-scale UASB reactors and bench-scale batch reactors. GonÇalves et al. (1994) fermented de-gritted raw wastewater in continuous bench-scale UASB reactors to evaluate the VFA production potential of both particulate and soluble fractions of the raw wastewater and also to assess whether a UASB reactor could replace

the primary clarifier. Two reactors of different height (to accommodate the range of HRTs evaluated) were tested in parallel for over a month with intermittent mixing. Six different HRT conditions, from 1.1 to 4.3 hours (upflow velocity of 0.6-3.2 m/h), were tested for each reactor. The SRT during these trials varied between 1.2 and 14.8 days.

Despite variations in influent suspended solids concentrations (95-248 mg/L), low supernatant suspended solids concentrations (<100 mg/L average) were observed under all operating conditions tested. The authors concluded that the UASB reactor may be able to replace the primary clarifiers in a new primary treatment system. The lowest supernatant suspended solids concentrations were obtained at 0.75 and 0.9 m/h upflow velocity (considered to be the main parameter that influences suspended solids retention). Retention of suspended solids decreased as the upflow velocity increased. However, at the lowest upflow velocity tested (0.6 m/h), a deterioration in effluent (supernatant) quality also occurred. Fine bubbles were observed at this low velocity, which indicated the start of methanization. Since no solid/gas separator was used, the fine bubbles most likely entrained some of the solids in the effluent.

The best VFA results were obtained at an HRT of 2.8 hours (0.17 mg HAc/mg of Total COD in the feed), with about 60% of the VFAs produced resulting from the fermentation of the soluble fraction. The effluent was composed of about 90% acetic acid with the remainder being proprionic acid. At an HRT of 2.3-3.3 hours, the VFA concentrations were nearly always in excess of 100 mg/L as acetic acid. This concentration of VFAs was considered sufficient to enhance P removal (Pitman et al., 1992). As the HRT increased from 3.3 hours (0.75 m/h) to 4.3 hours (0.60 m/h), the VFA production decreased significantly, from a mean value of 108 mg/L to 64 mg/L.

Bench-scale tests (SBR reactors) were also conducted on de-gritted raw sewage by Danesh and Oleszkiewicz (1995), who assessed the influence of SRT and pH on VFA production. The best fermentation performance was found at a relatively neutral wastewater pH (7.1-7.6) and a SRT of 13 days. The VFA concentrations in the reactor effluent were 34, 38 and 42 mg/L as acetic acid for a SRT of 4, 8 and 13 days, respectively. At a pH of 7.1-7.6, regardless of the SRT, acetic acid comprised 95 to 97% of the total acids, with the remainder being iso-valeric acid. In the pH range of 6.1-6.4, the acetic acid percentage fell to 86-88%, and valeric, butyric and propionic acid made up the balance. The amount of VFAs produced in the SBR reactor was much lower than the amount produced in the UASB reactor used by GonÇalves et al. (1994). However, the portion of acetic acid in the fermenter effluent was about the same, which is much higher than the values reported from side-stream prefermentation (see Table 2.3).

Danesh and Oleszkiewicz (1997) extended their research to demonstrate the side by side performance of a BNR-SBR system with a Fermenter-SBR/BNR-SBR system in a year long study. The fermenter was mixed only during the last 15 minutes of the reaction period. It was operated at an SRT of 12 days and a HRT of 12 hours. The two BNR-SBRs were operated similarly. The Fermenter-SBR /BNR-SBR system usually removed phosphorus to levels less than 0.5 mg/L total P (total phosphorus in the de-gritted raw wastewater was 6 mg/L on average). The BNR-SBR system reduced phosphorus to levels of greater than 1.5 mg/L most of the time.

Acetic acid made up 77% of the effluent VFAs in both systems. Prefermentation increased the seattleability of the sludge. The Fermenter-SBR/BNR-SBR system had a mean SVI value of 45 mg/g MLSS and did not experience any settling problems over the

course of the study. The mean SVI value for the BNR-SBR system was 60 mg/g MLSS, and it would sometimes reach 140 mg/g MLSS during bulking periods. More PAOs, which are good floc formers, were found in the sludge of the system with prefermentation. Finally, results indicated that the anoxic/anaerobic HRT of the BNR unit in the Fermenter-SBR/BNR-SBR system could be reduced to less than 50 minutes (operating anoxic/anaerobic HRT was 2 hours).

Rössle and Pretorius (2001c) used a full-scale APT system to evaluate the impact of elutriation rate on (a) the production of VFAs; (b) COD and solids removal; and (c) changes in TKN/COD ratios. The system was operated in each of three phases: continuous (60 days), semi-batch (90 days) and intermittent (30 days) sludge elutriation system. The continuous sludge recirculation mode (highest SRT = 7.5 days and SER = 0.09 m^3 sludge / m^3 raw sewage) lead to the highest amount of VFAs produced, as also found by Hartley et al. (1999). However, the lowest COD and solids removal occurred during that mode due to the continuous settled sludge recycling for elutriation purposes. This was also reported by Pitman et al. (1992). During the intermittent and semi-batch mode, the TKN/COD ratio increase across the clarifier was much higher than the expected 20-30% (46 and 64%, respectively). This could lead to denitrification and BPR failure for certain BNR processes. The TKN/COD ratio increase obtained under the continuous condition was 20%. The authors concluded that a compromise must be made between sufficient COD/solids removal at an acceptably small TKN/COD ratio, while maintaining a high enough VFA production.

A potential new generation of in-line short SRT fixed-film prefermenter designs was evaluated by Mavinic et al. (2001). The goal was to find a prefermenter design that was

more efficient (shorter SRT) and therefore less costly. Ringlace® rope type (Ringlace Products, Inc.,United States) and Kaldnes Miljøteknologi (Norway) polyethelene free floating media ("wheels") were independently used in pilot plant prefermentation trials. For each medium, a trial with raw sewage and a trial with primary effluent were conducted. The process scheme consisted of a coarse screened filter bucket (not required for the primary effluent run) followed by a storage bucket and three fixed-film down flow reactors in series. A filter mesh was installed in the bottom of the storage bucket for the primary effluent run.

The average VFA generation rate in the Kaldnes system treating effluent and raw wastewater was 3 and 20 mg/(L·h) as HAc, respectively. However, the authors suspected that the higher VFA generation in the raw wastewater run was mainly the result of the buildup of solids in the reactors resulting in a higher operating HRT than designed. In the Ringlace® rope type media, most of the VFA production was attributed to fermentation within the biofilm, with 5.5 and 11 mg/ (L·h) as HAc for the primary effluent and the raw wastewater runs, respectively. Although the two media types resulted in similar VFA generation rates, it was concluded that on the basis of biolfilm media plugging alone, the use of Kaldnes media, as utilized in this study (i.e. high rate, short SRT) could be ruled out for raw wastewater fermentation. Utilization of the Ringlace® media appeared feasible for EBPR and could translate into potential cost savings up to 25%. Only acetic and propionic acids were detected in these trials. In the raw wastewater trials, where VFA generation was significantly higher, the acetic: propionic ratio was 78: 22 and 74:26 for the Ringlace® and the Kaldnes medias, respectively.

2.4.2. Secondary Sludge (RAS or WAS) Fermentation

Fermenting part of the secondary sludge (RAS and/or WAS) has the added benefit of not adding additional carbon to the biological treatment process(Barnard and deBarbadillo, 2002). Fermentation of RAS is used in one patented side-stream biological phosphorus removal process, where a portion of the RAS is diverted to a fermentation zone. The VFA laden fermentate is then blended with the remaining RAS flow in an anaerobic zone and then flows on to the main biological treatment process (Lamb, 1994, US Patent No. 5,288,405).

This side-stream process has been successfully used at the South Cary Water Reclamation Facility in North Carolina (Stroud and Martin, 2001). As of July 2000, the South Cary plant was able to reduce total phosphorus in the plant effluent to 0.5 mg/L (influent P concentration not given) without any chemical phosphorus precipitation. This represented 80% more phosphorus removal then what could be achieved at this plant with chemical precipitation alone. The plant operates three parallel 4-stage Bardenpho basins each equipped with one side-stream system. The side-stream system includes an anoxic zone followed by an anaerobic zone and a fermenter. A portion of the RAS is sent to the side-stream anoxic zone and to the anaerobic zone. A portion of the side-stream anaerobic zone effluent is recirculated back to the mainstream anoxic zone, while the remaining part is sent to the fermenter. From there, the fermenter effluent is returned to the side-stream anaerobic zone.

Truckee Meadows Water Reclamation Facility in Reno, Nevada converted their existing Phostrip® process into a fermentation system for VFA generation (Narayanan et al., 2002). The goal was to eliminate the lime addition required for the Phostrip®

process, because it was very expensive. They conducted a desktop modeling evaluation followed by full-scale trials where they compared the existing system to a new system including RAS fermentation. One BNR train was operated as designed and the other train was modified to include RAS fermentation using the train's existing Phostrip® tank. A portion of the RAS was diverted to the fermentation zone for VFA production. The complete fermented stream was recirculated to be mixed with the primary effluent. No significant difference was observed in the amount of phosphorus removal between the two modes of operation. Therefore, the plant could be modified to replace the Phostrip® system with RAS fermentation, saving the plant $250,000 per year. The full conversion of the plant was initiated shortly after the tests ended.

Others have shown that waste activated sludge (WAS) subjected to an auto-thermal thermophilic aerobic digestion (ATAD) can yield higher VFA concentrations than primary sludge fermentation. The most recent experimental work on the subject was done by Fothergill and Mavinic (2000). Two stainless tanks were installed as control and test ATAD reactors on-line of the University of British Columbia modified UCT wastewater pilot plant. The reactors mimicked the first stage of a multistage process known to effectively treat primary sludge and WAS. They were operated in a semi-continuous complete mix mode with an average SRT (or HRT) of 3 days in an oxygen-restricted environment (-200 to -500 mV ORP). The temperature was maintained above 42ºC (thermophilic range) through mixing, aeration and microbial digestion without external heating. The control reactor was fed only primary sludge while the test reactor was fed with a combination of primary sludge and WAS. Four (4) trials, each at a different primary: WAS ratio, were conducted in parallel with the control reactor: 100:0,

65:35, 35:65 and 0:100. An acclimatization period was provided before each trial to ensure that the process was stable after the mix ratio change.

It was found that trials with both mixed sludge feed and secondary sludge (WAS) alone resulted in higher VFA production than was observed in the trial with the primary sludge control. The highest normalized net production of VFAs (about 757 mg/L as HAc) was obtained with 100% secondary sludge. Acetate predominated in the ATAD effluent in both the control and the test reactor for all the trials, representing over 80% of the total VFA concentration. At 100% WAS, the acetate concentration in the reactor effluent comprised 98% of all VFAs. Most of the bacterial stored phosphorus contained in the WAS was released, with a significant portion released before digestion when the primary and secondary sludge were mixed together. Nitrification did not occur in the reactors, so that ammonia was present in the aerobic digester and its effluent. While the evaluated system is promising as a VFA source for BNR plants, it would require nutrient removal from the first stage ATAD effluent before being sent to the BNR system. Careful considerations would be required before implementing such a system at wastewater treatment facility.

2.4.3. Anaerobic Mixed Liquor Fermentation

One advantage of using anaerobic mixed liquor as a source of VFAs is that it includes the soluble fraction of the influent wastewater (and the associated colloidal fraction). Soluble organics are readily available to the bacteria, while the particulates contain a large portion of slowly biodegradable material.

Although there is no mention of anaerobic mixed liquor fermentation for VFA generation in the literature, it occurred in several wastewater treatment plants around the

world before the use of prefermentation systems. Treatment facilities handling low septic wastewater unwittingly relied on unintentional anaerobic mixed liquor fermentation for VFA production, which yielded irregular P removal efficiencies in most plants. With the implementation of prefermenters in the 1980's and 1990's, anaerobic zone retention times were reduced (to avoid secondary phosphorus release), which also minimized the fermentation process in the BNR system, and the need for a separate fermentation step became evident. As discussed in the previous sections, primary sludge and raw wastewater became the normal substrates used for VFA production.

Anaerobic mixed liquor fermentation was first found to be a satisfactory means to generate VFAs in the mid 1970's (Barnard 1975; 1976). At the time, the mechanisms were not known, but it was clear that high phosphorus removal efficiencies (>90%) were obtained when anaerobic conditions were accidentally created in the second anoxic zone of a Bardenpho process pilot plant in Pretoria, South Africa. The aim of the study was to optimize nitrogen removal. Therefore, when the anoxic zone retention time was reduced to improve denitrification (less time under anaerobic conditions), the phosphorus removal efficiency decreased below 80%. Furthermore, when aerobic instead of anoxic conditions were intentionally created (no fermentation taking place), the phosphate removal efficiency dropped to 50%.

From June to September 2001, Barnard conducted anaerobic mixed liquor fermentation tests as part of a larger pilot plant study at a wastewater plant in Seoul, South Korea (Barnard and deBarbadillo, 2002). A USB fermenter (3 m^3 = 0.008 MGal) was positioned next to the anaerobic zone of the pilot plant BNR process. Mixed liquor from this zone was pumped to the fermenter at a rate of 1.5 m^3/h (0.3 gpm), and the

supernatant (with some biosolids) overflowed to the anaerobic zone. From the end of June to late July, low effluent phosphorus concentrations were achieved (Figure 2.2). When the rainy season started in late July, control of the fermentation process became extremely difficult, and problems persisted until the beginning of September.

Although there are some reports that mixed liquor fermentation can be a potential source of VFAs for BPR, the process has not been thoroughly investigated. There are no reports of full-scale mixed liquor fermentation trials for VFA production (Barnard; deBarbadillo, 2004). No wastewater treatment facility in the world has conducted full-scale trials to evaluate it as a viable means of VFA production. Of course, unintentional fermentation of mixed liquor may have occurred in some plants where "dead zones" exist within the BNR system, allowing mixed liquor solids to settle and ferment.

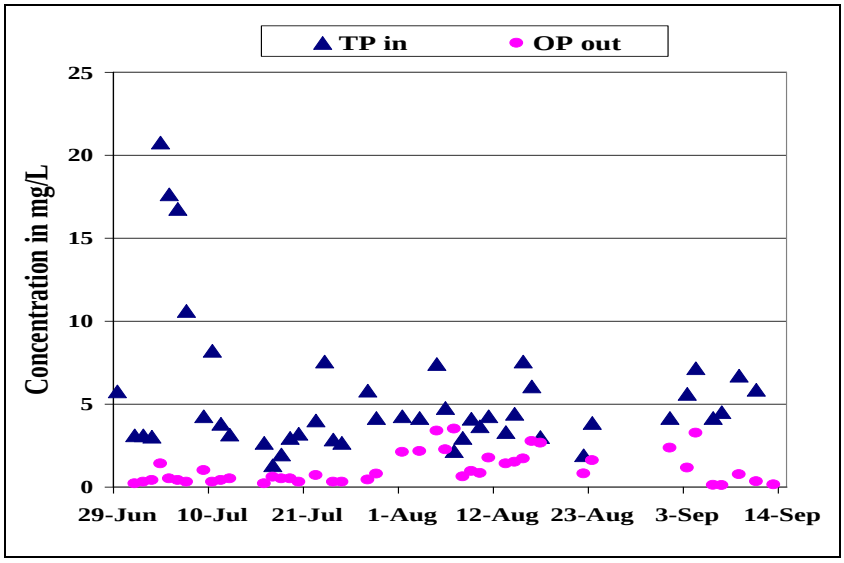

Figure 2.2: Phosphorus In an Out of the BNR Pilot Plant with Anaerobic Mixed Liquor Fermentation in Seoul, South Korea in 2001

CHAPTER 3 : MATERIALS AND METHODS

3.1 Treatment Facility Used for the Trials

The demonstration tests were performed at the McDowell Creek WWTF in Huntersville, North Carolina. The NPDES permit limits for this treatment facility are presented in Table 3.1. The plant effluent total phosphorus (TP) limit imposed by the EPA for discharging in the McDowell Creek is 1 mg/L on a monthly average.

Table 3.1: Summary of the McDowell Creek NPDES Permit Limits
(provided by Black and Veatch, Charlotte, NC)

Parameter	**Summer (April 1 through October 31) Monthly Average**	**Winter (November 1 through March 31) Monthly Average**
Flow MGD	6	6
BOD_5, mg/L	5	10
TSS, mg/L	30	30
NH_3-N, mg/L	2	2.5
DO min., mg/L	5	5
Fecal Coliform, number per mL	200/100	200/100
TN, mg/L	10	10
TP, mg/L	1	1

The plant receives a daily average of 5 million gallons of domestic wastewater and no industrial wastewater. Primary sedimentation is followed by two parallel UCT BNR treatment trains (Figure 3.1). Waste sugar water or acetic acid is added to the primary clarifier effluent to supplement the VFA content before the wastewater flow is split to the BNR trains. The travel time for sewage *en route* to the treatment plant is not long enough for the wastewater to arrive with sufficient VFAs to achieve good biological phosphorus removal. The UCT process is a modification of the typical BNR process presented in Chapter 2. In a UCT process, RAS is sent to an anoxic selector rather than to an anaerobic selector. Also, a portion of the anoxic liquor is recycled (ARL) to the anaerobic selector. The MLSS concentration in the anaerobic zone is about half of the concentration in the rest of the process due to the solids return being from the anoxic zone rather than directly from the RAS.

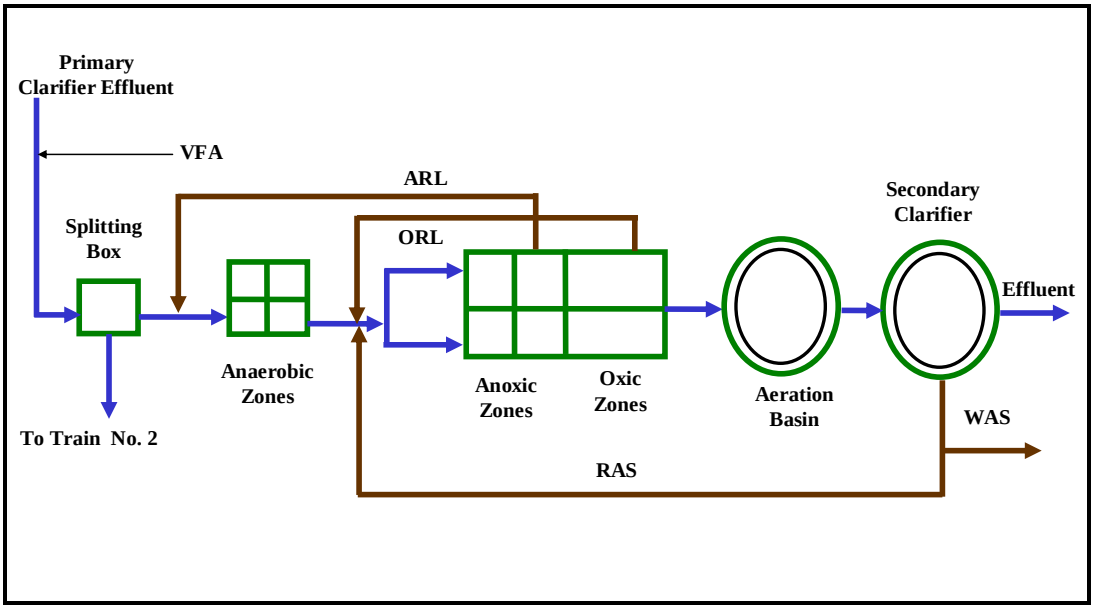

Figure 3.1**:** Schematic of the UCT BNR Treatment Trains at McDowell Creek WWTF

The anaerobic selector at the McDowell Creek facility is comprised of four zones. This is where the PAOs transfer the VFAs across their membrane and release phosphorus into the water. Only two of the zones were being used by the plant at the time of this research. The flow from the anaerobic zones is transferred to a distribution box where the flow is split in half and sent to two parallel anoxic/oxic basins. In these basins, the PAOs take up the phosphorus released in the anaerobic zones plus most of the phosphorus contained in the influent entering the BNR process. From the oxic zones, the flow is combined and sent to a circular aeration basin to increase the dissolved oxygen concentration (to achieve better sludge settling in the final clarifier) and allow more nitrogen gas to be sparged from the mixed liquor. The average total phosphorus content in the influent at the McDowell Creek WWTF is 5 mg/L. By the time the flow exits the final clarifier, the total phosphorus content averages about 0.3 mg/L, which is less than the permit limit (1 mg/L).

3.2 Side-Stream Anaerobic Mixed Liquor Fermentation Trials

The anaerobic mixed liquor fermentation demonstration test was accomplished by configuring a side-stream process side by side to the two BNR anaerobic zones of Train 1 (Figure 3.2). A portion of the anaerobic mixed liquor (6.5% of the Train 1 wastewater flow i.e. 2.5 MGD daily average) was pumped from the second anaerobic zone to a newly created Fermentation Zone No. 1 (FZ1). Flow exited FZ1 into the bottom of Fermentation Zone No. 2 (FZ2) through an opening in the wall, thereby creating an upflow stream in FZ2 and allowing the basin to function as a USB reactor. Supernatant from FZ2 was pumped back to the first anaerobic zone at the location where the primary clarifier effluent enters the basin. Sludge wasting from the fermentation process was

performed by completely mixing FZ2 for about one hour and allowing a portion of the fermentate mix to be pumped out of the tank. This was necessary to maintain a constant SRT in the fermentation zone. In a full-scale operation where a USB reactor would be designed to fit a particular side-stream fermentation process, the sludge wasting could be done from the side of the reactor at the sludge blanket level without having to mix the tank first.

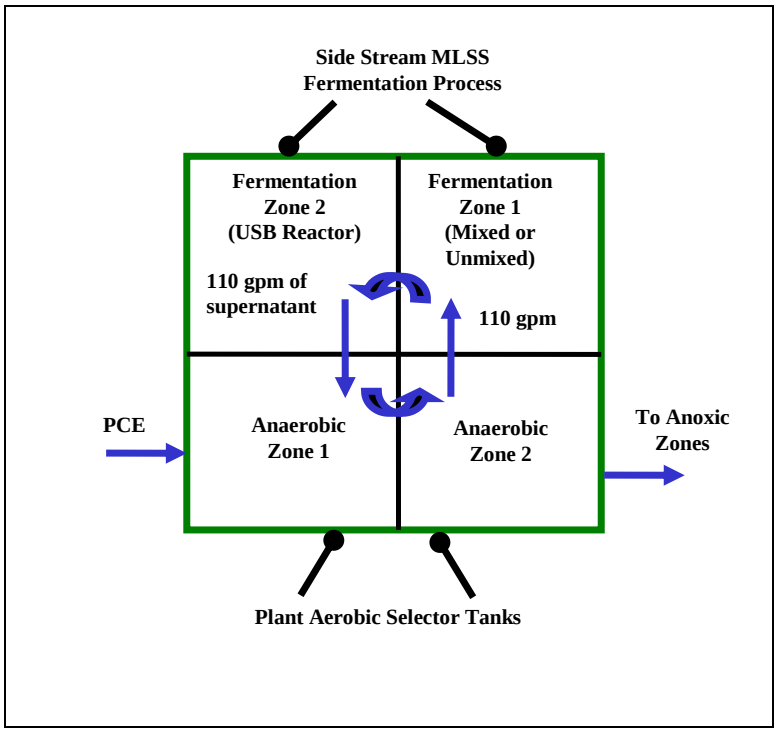

Figure 3.2: Schematic of the Side-Stream Mixed Liquor Fermentation Process

The mixed liquor fermentation process was operated continuously from May 2004 until April 2005. Intensive sampling was conducted during three separate trials: Trial 1 was conducted in August of 2004, Trial 2 in September-October, and Trial 3 in February-March of 2005 (Table 3.2). During Trial 1, the SRT was about 2.5 days, with an average

TSS content of 0.4%. During that period, FZ1 was completely mixed and FZ2 was operated as a USB reactor. However, it was noted during Trial 1 that the submersible mixer in FZ1 disturbed the sludge blanket in FZ2 and caused significant loss of solids in the supernatant. Under these conditions, it was not possible to increase the system SRT above 3 days. Therefore, to enable testing at higher SRTs, mixing of FZ1 was discontinued for the remainder of the trials, except during sampling events or sludge blanket elutriation.

Table 3.2: Operating Conditions for Demonstration Testing

Trial	**SRT (days)**	**Average TSS (% by weight) [1]**	**HRT (h)**	**Fermentation Zone pH**	**Fermentation Zone Mixing Regime**	
					Zone No. 1	**Zone No. 2**
1	2.5	0.40	13.6	6.8-7.0	Mixed	USB
2	7	0.88	13.6	6.6-6.8	Unmixed	USB
3	12	1.1	13.6	6.3-6.5	Unmixed	USB

[1]: Fermentation zones were completely mixed before sampling.

During Trial 2, the average TSS content of the fermentation zones was 0.88%. Trial 3 was operated at an SRT of 12 days, and the average TSS content was 1.1%. The HRT was adjusted by setting the feed and discharge flows to 110 gpm, which set the HRT at 13.6 hours in all trials. Although the pH usually remained between 6.4 and 7.6, occasionally it was slightly lower during the 12-day SRT trial. No chemicals were added for pH control. For reasons described in Chapter 4 under section 4.1, only one value of HRT (13.6 hours) was evaluated.

3.3 Sample Collection

Grab samples were collected from the fermentation side-stream process as well as from Trains 1 and 2 (Figure 3.3). Full-scale BNR Trains 1 and 2 were monitored to assess BNR process stability and to assist with interpretation of the fermentation results. Mixed liquor from the treatment train was sampled from Anaerobic Zone 2, and fermentation process samples were taken from FZ1 and FZ2. The supernatant samples were collected from a sampling valve located on the supernatant piping. Supernatant and anaerobic zone mixed liquor samples were collected first, after which the feed mixed liquor and the supernatant pumps were stopped. Following this, the submersible mixers in FZ1 (for Trials 2 and 3) and in FZ2 were started to completely mix the zones before collecting the fermenter samples. The pumps were re-started after the solids had settled back down in the fermentation tank.

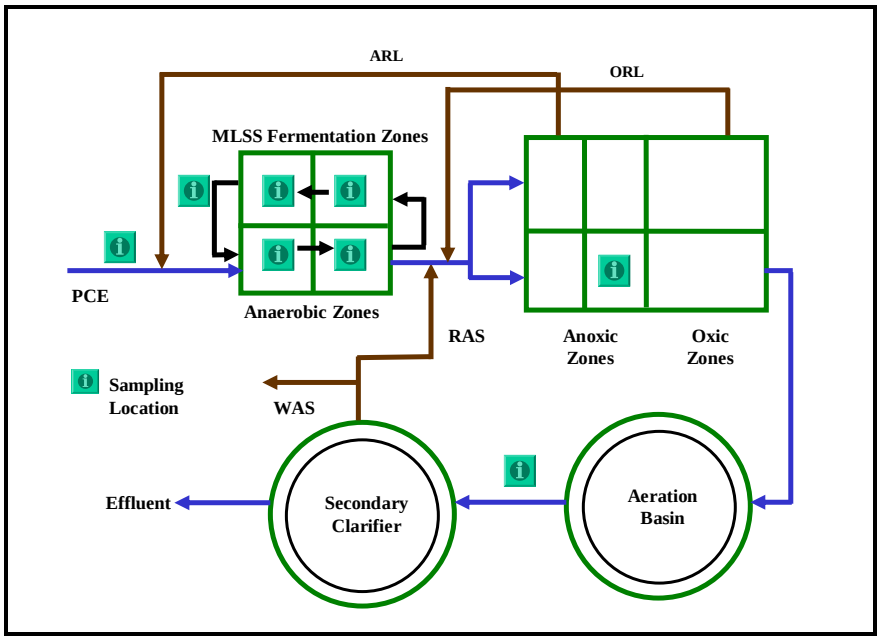

Figure 3.3: Schematic of the Side-Stream Process Showing the Sampling Locations

3.4 Sample Analysis

All samples were stored on ice for transport to The University of North Carolina at Charlotte (UNCC) Environmental Engineering Laboratories, where tests for TP, soluble OP (inorganic phosphate), total VFAs, soluble and total COD and TSS/VSS were conducted. It should be noted that the soluble COD represents the amount of COD related to the true soluble matter (without the suspended solids and colloidals). Mamais et al. (1993) developed a method to obtain the true soluble fraction of a wastewater sample. The method is briefly described in section 3.5.2. The analysis for TP, soluble OP and soluble and total COD was executed using the HACH methods listed in Tables 3.3 and 3.4. Most of the HACH methods used are EPA accepted for reporting wastewater analyses. There are two exceptions: Methods 10127 and 8114, which are adapted from Standard Methods for the Examination of Water and Wastewater, are not yet recognized by the EPA. All these tests are colorimetric tests, where the intensity of the color developed from the chemical reactions is correlated to the concentration of the chemical tested.

A HACH DR/2500 spectrophotometer was utilized for these colorimetric tests. The samples were tested for TSS/VSS according to Standard Methods Procedures No. 2540D and 2540E (1999). The method used for total VFA determinations is summarized in section 3.4.3.

Table 3.3: Methods used for TP and Soluble OP Analysis

<table>
<tr><th>Parameter</th><th>Sample</th><th>HACH Method
(HACH, 2004)</th></tr>
<tr><td rowspan="2">Total Phosphate</td><td>Fermentate,
Anaerobic liquor
Anoxic Liquor</td><td>• Method 8190 – Acid Persulfate Digestion with molybdate/ascorbic acid to produce blue color – Results measured at 880 nm</td></tr>
<tr><td>PCE
BNR Effluent</td><td>• Method 10127 – Acid Persulfate Digestion with molybdo-vanadate/vanadium to produce yellow color – Results measured at 420 nm</td></tr>
<tr><td rowspan="2">Soluble
Orthophosphate</td><td>PCE
BNR Effluent</td><td>• Method 8048 Ascorbic Acid/Molybdate to produce blue color – Results measured at 880 nm</td></tr>
<tr><td>Supernatant
Fermentate
Anaerobic liquor
Anoxic Liquor
PCE</td><td>• Method 8114 - Molybdovanadate/vanadium to produce yellow color – Results measured at 420 nm</td></tr>
</table>

In all cases, standard solutions were used for test calibration. Fermentation system samples were tested in triplicate and spiked samples (except for the VFA samples) were used to demonstrate accuracy. All other samples were spiked only (except for the VFA samples). All samples were brought to 20-25 ºC before testing. If required, ten-fold and twenty-fold dilutions of the samples were made to reduce the concentration of the analytes to a concentration level that could be detected by the methods used.

Table 3.4: Methods used for COD Analysis

Parameter	Sample	HACH Method (HACH, 2004)
Total Chemical Oxygen Demand	Supernatant Fermentate, Anaerobic liquor Anoxic Liquor	• Method 8000 – Potassium Dichromate Digestion to produce brown to green color – Results measured at 620 nm; used 0-1500 mg/L COD vials
	PCE	• Method 8000 – Potassium Dichromate Digestion to produce brown to yellow color – Results measured at 420 nm; used 0-150 mg/L COD vials
Soluble Chemical Oxygen Demand (using filtered and flocculated liquid phase of the sample)	Fermentate PCE	• Method 8000 – Potassium Dichromate Digestion to produce brown to green color – Results measured at 620 nm; used 0-1500 mg/L COD vials
	Supernatant Anaerobic liquor Anoxic liquor	• Method 8000 – Potassium Dichromate Digestion to produce brown to yellow color Results measured at 420 nm; used 0-150 mg/L COD vials

Spiking, also known as standard additions or known additions, is a common technique for checking the accuracy of the tests results. Triplicates are used to show the variability among the test replicates, which reflects the precision of the test. However, good precision does not tell whether or not the results are close to the true value, which is an indication of the accuracy of the results.

All samples in this study were spiked by adding a small amount of a standard solution (of a known concentration) to a given volume of the sample and testing it for a given parameter. If the analysis is accurate, the tested spiked sample concentration equals or is

close to (less than 10%) the theoretical value as calculated in Equation 3.1. Note that the square parentheses ([ ]) indicate concentration in moles/L.

$$[\text{Spiked Sample}] = \frac{([\text{Std Solution}]*\text{Vol. Added}) + ([\text{Un-spiked Sample}]*\text{Vol})}{\text{Total Volume}} \quad \text{(Equation 3.1)}$$

3.4.1. Total Phosphate and Soluble Orthophosphate Tests

Samples for TP and soluble OP determination were collected in 1 liter glass jars. Samples for soluble OP content were gravity filtered on site immediately after collection. The exceptions were the fermentate samples, which were not filtered on site because their high solids content would clog the filters. Instead, fermentate was filtered on an AP40 filter with a vacuum pump upon arrival at the UNCC laboratories. Also upon arrival at the laboratory, all the other OP samples were filtered on an AP40 filter with a hand pump. All soluble OP samples were kept at 4 $^{\circ}$C to be analyzed within 48 hours. Samples for TP were preserved at 4°C with sulfuric acid (pH < 2) to stop the bacterial activity until testing, at which time the pH was adjusted to between 6 and 9. It is to be noted that TP values were obtained for process information purpose and to compare with the soluble OP values. The TP values were not used in the results analysis.

The jars were washed with deionized water and phosphate free detergent followed by an acid-wash step before each collection. Acid washing consisted of a minimum of 20 minutes of soaking in 50% hydrochloric acid (HCl @ 32.7%) followed by a deionized water rinse in the dishwasher.

3.4.2. Chemical Oxygen Demand

Samples for soluble and total COD determination were also collected in 1 liter glass jars. The filtration protocol for samples for soluble COD content were performed as

described for the samples tested for soluble OP. All soluble COD samples were kept at 4 °C to be analyzed within 48 hours. The samples for total COD were preserved at 4°C with sulfuric acid (pH< 2), and no pH adjustment was required before COD testing started. It is to be noted that TCOD values were obtained for process information purpose and to compare with the TSS values. The TCOD values were not used in the results analysis.

Filtered soluble COD samples were filtered on a 0.45 micron membrane before testing (Mamais et al., 1993). Briefly, 1 mL of a 10% zinc sulfate solution was added to 100 mL of the filtered sample, and the solution was agitated for 1 minute. The pH was adjusted to 10.5 with a 5 Normal sodium hydroxide (NaOH) solution and then filtered through a 0.45 μm membrane, using a hand pump, to remove the flocculated colloids. During membrane filtration, samples were covered with a parafilm to minimize volatilization of volatile organics. The filtrate was tested for COD according to the HACH methods listed in Table 3.4.

3.4.3. Total Volatile Fatty Acids

Volatile fatty acid samples were collected in 125 ml polyethylene bottles. For each location, three different samples were collected, so that analyses were performed in triplicate. The mixed liquor, supernatant and fermentate were poured in the bottle until it overflowed in order to minimize the headspace air and the potential for volatilization or release of carbon dioxide, which would artificially increase the sample pH. The bottles were tightly capped and kept at room temperature until analysis, which was performed within 8 hours after collection.

Total VFAs were assayed titrimetrically according to the method developed by Anderson and Yang (1992). The method described in this article was evaluated on supernatant samples from anaerobic sludge digesters obtained from different wastewater plants. For this study, the sample was gently inverted a few times to mix it, and then 50 mL was poured gently into a 100 mL beaker (to minimize the headspace and volatilization). A sheet of parafilm covered the beaker, and small holes were cut to insert the pH and temperature probes and the digital titrator dispenser. The sample was stirred gently with a small magnetic stirring bar to ensure sample homogeneity during testing. The initial pH of the sample was measured, and then it was titrated with a 0.1 Normal sulfuric acid (H_2SO_4) solution in two stages: first to a pH of 5.1 and then to a pH of 3.5. Care was taken to add the titrant as quickly as possible to minimize VFA volatilization and pH increase (due to CO_2 volatilization) during testing.

Some fermentate samples were sent to Minnesota Valley Testing Laboratories, Inc., New Ulm, MN, for gas chromatography (GC) analysis. They used a GC method developed by Metropolitan Waste Commission in St-Paul, MN. The laboratory used a gas chromatograph with a flame ionization detector (FID) running at a temperature of 200 °C. The sample (1μL) was injected at 200 °C through a packed column with Helium as the carrier gas (Wierima, 2005).

3.4.4. Total Suspended Solids and Volatile Suspended Solids

The samples for TSS and VSS analysis were collected in 250 ml polyethylene bottles and kept at 4 ºC. They were analyzed within 7 days according to Standard Methods 2540D and 2540E for TSS and VSS, respectively (Standard Methods, (1999)).

3.5. Phosphorus Release Batch Tests

Two series of batch tests (two sets per series with a control for each test) were conducted to further examine the effectiveness of the fermentate as a VFA source for BPR. As noted in the literature review, the amount of phosphorus released (from VFA uptake) in the anaerobic zone is indicative of the amount of phosphorus taken up in the aerobic zone. The more phosphorus that is released through VFA consumption by the PAOs, the more phosphorus will be removed in the aerobic zone. Therefore, phosphorus release and COD uptake (as an indication of VFA uptake) were compared for the fermentate, waste sugar water and acetic acid.

These batch tests generally followed the methodology outlined for the "biological phosphorus removal potential" test (Park and Novotny, 1998). Fresh samples, collected the day of the tests, were used for each series of tests. Series No. 1 was performed in November 2004 and Series No. 2 was performed in January 2005. Primary clarifier effluent and anoxic liquor (Series No.1) or RAS (Series No.2) were mixed to create an anaerobic zone. A new sample of PCE was collected for each set of tests. Fermentate, anoxic liquor or RAS samples used were the same for each test within a series. Each sample was tested for OP, TP, TSS, VSS, soluble and total COD as well as total VFAs.

Three 4 L plastic jars were used as reactors. First, the reactors were filled with 3.5 L of the created anaerobic mixed liquor. The contents of the jars were mixed using magnetic stirrers to achieve complete mix conditions. A different VFA source was then added to two of the jars (10 minutes apart) and the third jar served as a control, containing only the created anaerobic liquor. As soon as the VFA source was added, a sample for OP and soluble COD was collected (Time 0). Each test was conducted for 45 minutes, during which samples for OP and soluble COD were taken from the jars every

15 minutes. Total COD and TSS samples were taken at the end of each test. Samples for soluble COD and soluble OP determination were filtered on an AP40 filter immediately after collection. All the samples were handled and analyzed as described in section 3.5.

Series No. 1

In the first series, a "UCT" anaerobic zone (TSS content of about 1500 mg/L) was created by mixing primary effluent and anoxic zone mixed liquor from the McDowell Creek Wastewater Plant. The first set of tests (Test A1) compared fermentate (from the side-stream fermentation process), acetic acid from the plant (@22%), and a control that received no external VFAs. The second set of tests (Test B1) included fermentate with the same VFA concentration as in Test A1, but of a volume reduced by half; waste sugar water from the plant (91,000 mg/L of BOD); and a control (Table 3.5).

Series No.2

In the second series of batch tests, an anaerobic zone was created from RAS and PCE (TSS content of about 1500 mg/L). The first set of tests, (Test A2) compared fermentate, acetic acid (@ 22%), and a control that received no external VFA. The second set of tests (Test B2) included fermentate with the same VFA concentration as in Test A2, but twice the volume; sugar water (90,000 mg/L of BOD); and a control (Table 3.5).

3.6. Microbial Analysis

Sludge samples were examined microscopically (oil immersion, 1000X) after Neisser staining to identify the PAOs and the GAOs. The stain adheres to the cell wall of PAOs and GAOs and to the internal polyphosphates of PAOs. When stained in this way, GAOs will appear in a distinctive tetrad cell formation (Jenkins et al, 2004). The microbial

analysis was performed at the University of Washington in Seattle under the supervision of Dr. H. David Stensel.

Table 3.5: Amount of VFAs Added for Each VFA Source Used in the Phosphorus Release Batch Tests

VFA Source	VFA Added in the Batch Test Reactors (as mg/L as HAc)			
	Series No. 1		Series No.2	
	Test A1	Test B1	Test A2	Test B2
Fermentate	20	11	13	24
Acetic Acid	58	N/A	66	N/A
Waste Sugar Water	N/A	44	N/A	38
Control	0	0	0	0

CHAPTER 4 : RESULTS AND DISCUSSION

4.1. Process Operation

The feasibility of using anaerobic mixed liquor fermentation to generate VFAs for BPR was tested at the McDowell Creek Wastewater Treatment Facility. A full-scale demonstration process of anaerobic mixed liquor fermentation was operated continuously from May 2004 until April 2005. Intensive sampling was conducted during three separate trials: Trial 1 (2.5-day SRT), Trial 2 (7-day SRT) and Trial 3 (12-day SRT)5. The author was at the plant daily during intensive sampling and every two or three days when not sampling.

Fermentation Zone No. 1 was originally mixed (Trial 1) to allow for a homogenous stream to enter the bottom of FZ2. However, it was noted during Trial 1 that the submersible mixer in FZ1 disturbed the sludge blanket in FZ2 and caused significant loss of solids in the supernatant. Although one of the advantages of mixed liquor fermentation over raw wastewater or primary solids fermentation is that solids carry over will not significantly impede overall plant performance (because the escaping solids are intact biomass that will not add a COD burden to the mixed liquor), the solids loss did preclude achieving an SRT above 3 days. Therefore, to enable testing at higher SRTs, mixing of FZ1 was discontinued for the remainder of the trials, except during sampling events or sludge blanket elutriation. At the 7-day and 12-day SRTs, a thick layer of floating solids formed at the surface of the fermentation zones, which likely resulted

because methane was being produced, and the rising gas bubbles were lifting the solids (Figure 4.1). Pitman et al. (1992) mentioned that floating sludge as a result of methane formation at high SRTs was observed in APT systems in South Africa. While the floating sludge layer was about 2 in thick at the 7-day SRT, it became about 2 ft thick at the 12-day SRT. At the 2.5-day SRT, it took 30 minutes to completely mix FZ2, while at the 7-day SRT, 1 hour was required. The rail-mounted submersible mixers (one for each fermentation zone) had to be raised in the fermentation tank to break the layer of sludge formed at the 12-day SRT, and 3 to 4 hours were required to achieve complete mixing in the fermentation zones.

Figure 4.1: Typical Layer of Floating Sludge at 12-Day SRT

As the wastewater temperature decreased (from November 2004 through February 2005) and the SRT was increased, more time was required for the solids in the

fermentation zone to settle back to the bottom of the tank after mixing. The anaerobic mixed liquor feed to FZ1 was temporarily discontinued during the settling period to ensure the return of FZ2 to the USB mode. From August to November, it took about 4 hours for the solids to settle after mixing. The settling time increased to about 8 hours at the end of November and was 12 hours by February. Therefore, sampling events could not be performed every day.

Although such mixing is not necessary to maintain a particular SRT in a UASB reactor, it is needed to obtain accurate TSS data to calculate or set a particular SRT. Once an optimum SRT is identified based on system performance, it can be sustained by pumping sludge from the side of the reactor directly from the sludge blanket, and monitoring sludge depth can be an indicator that the target SRT is being maintained (Barnard, 1994). Because mixing was necessary in these trials, and ambient temperatures led to long settling times, it took several weeks to increase the SRT from 7 days to 12 days. Furthermore, construction work performed at the plant from January through March resulted in the side-stream mixed liquor fermentation system to be down sporadically for period of 24 hours or less.

4.2. Calculations

4.2.1. Average Solids Retention Time

The average solids retention time, referred to as SRT, was determined for each trial period as shown below. The solids removed (wasted) from the fermentation zone were those contained in the supernatant flow from FZ2 to the anaerobic zone. The time per day that the supernatant was leaving the fermentation zone was partitioned into t_m, the time that the mixer operated; t_{solset}, the time when the inlet and outlet pumps operated, but

the solids were settling back down after mixing; and t_{pm}, time that pumps are on while mixing. During mixing and during settling after mixing, suspended solids in the effluent flow were calculated as the average of the MLSS for the basin during mixing and the supernatant TSS of the basin after settling. During the time the pumps were off, t_{off}, no solids were wasted.

Step 1: Calculate the solids wasted (lb/day) (Equation 4.1).

$$m_w = [1/2(X + X_R) * CF * (t_m + t_{solset})] + [X_R * CF * (24 - t_{off} - t_{solset} - t_{pm})]$$

Where: m_w = solids wasted (lb/d) (Equation 4.1)

X = mixed liquor suspended solids (MLSS) concentration in fermentation zones (average of Z_1 and Z_2 concentrations) = [mg/L]

X_R = Total suspended solids (TSS) concentration of supernatant, (mg/L based on one daily measurement)

CF = 8.34 * 110 gpm * 60 / 10^6

t_m = mixing time = t_{pm} when pumps are on = [h]

t_{solset} = time while pumps are on and solids are still settling back = [h]

t_{off} = total time that pumps are off in one day = [h]

t_{pm} = time that pumps are on while mixing =[h] (most of the time this variable was equal to zero)

The values used in these equations are shown in Appendix A under Section A.1.

Step 2: Use the calculated lb/day wasted to determine the theoretical supernatant TSS concentration that would yield this daily waste rate if the effluent flow was continuous over 24 hours (Equation 4.2):

$$X_{R(CALC)} = \frac{m_w}{24 * CF} \qquad \text{(Equation 4.2)}$$

Step 3: Calculate daily SRT (Equation 4.3).

$$SRTd = \frac{V * X}{Q * X_{R(CALC)}} \qquad \text{(Equation 4.3)}$$

Where: SRTd = daily solids retention time = [day]

V = volume of Fermentation Zones = 90,000 [gal]

Q = supernatant flow = 158,400 [gpd]

Step 4: Apply these equations to determine the operating SRT for each trial.

The calculated SRTs were plotted for each sampling date (Figures 4.2, 4.3 and 4.4). Except for August 9 data, there was a clear SRT trend over the last two weeks of sampling. Therefore, August 9 data was considered an anomaly, and the remaining data from the two last weeks of sampling were used to calculate the SRT for Trial 1.

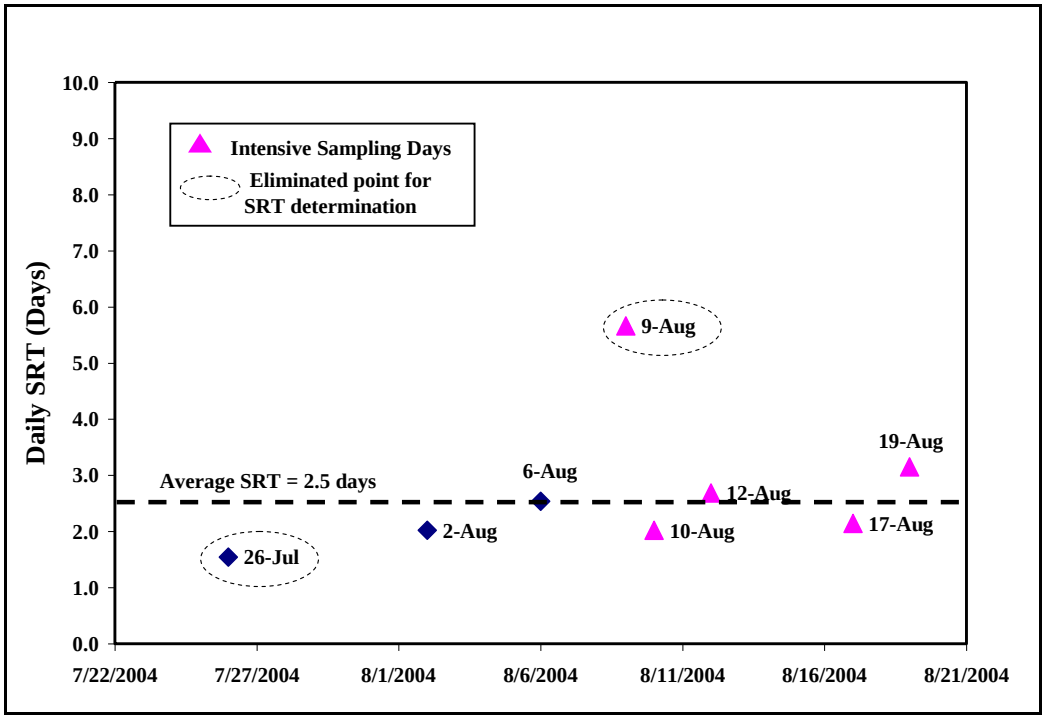

Figure 4.2: SRT Graph for the Trial 1 Period

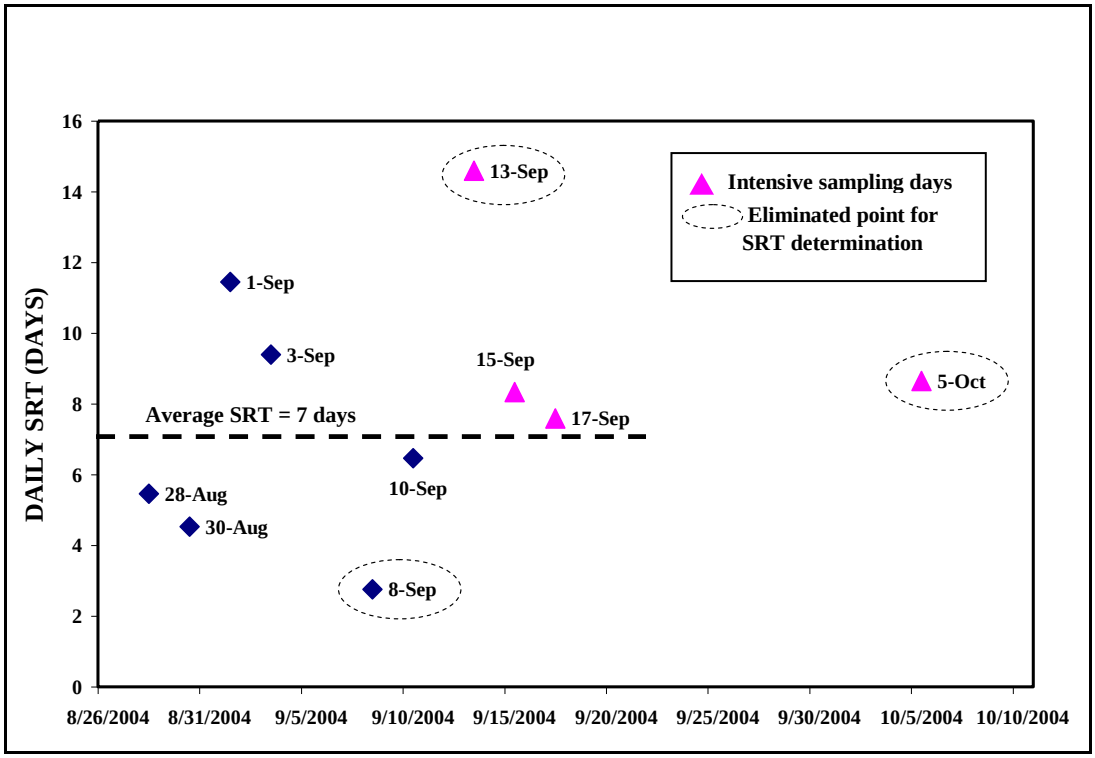

Figure 4.3: SRT Graph for the Trial 2 Period

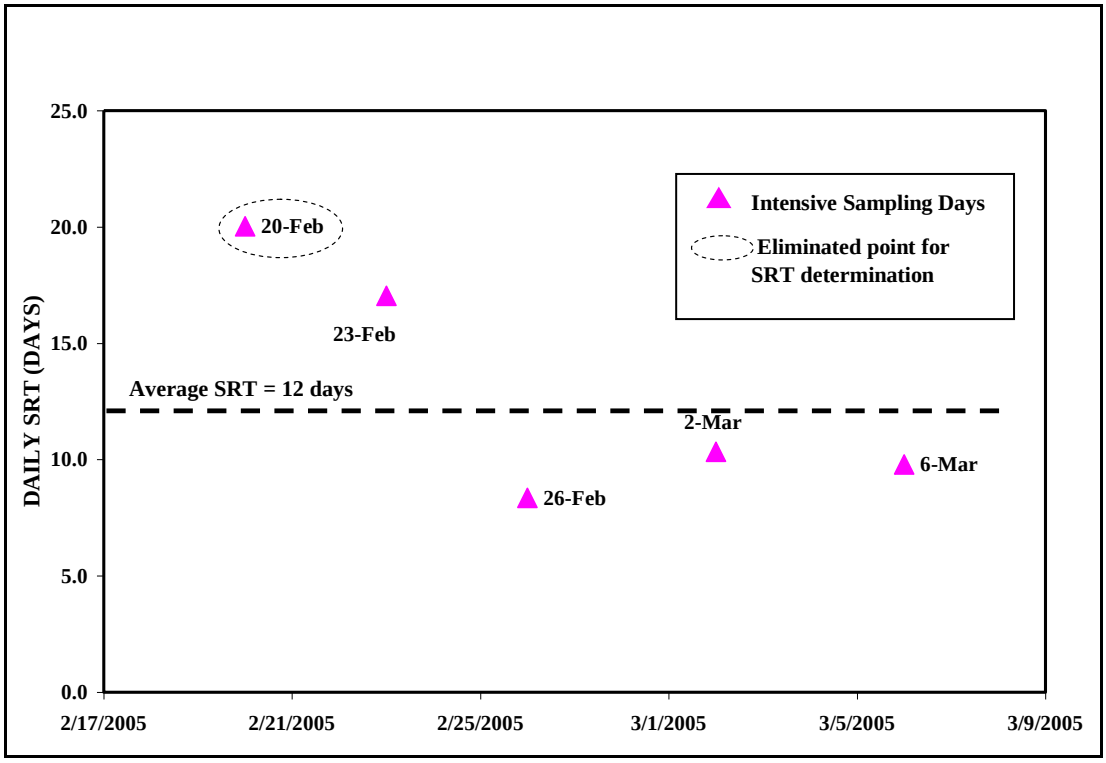

Figure 4.4: SRT Graph for the Trial 3 Period

4.2.2. Estimated VFA production

Step 1: The VFA concentrations measured on a given sampling date were averaged to yield a mean daily VFA concentration (Table 4.1). (Raw data is provided in Appendix A)

Example: Trial 1 data

Table 4.1**:** Mean ± Standard Error of VFA Concentrations for Trial 1

Date	Mixed Liquor Feed	FZ1	FZ2	Supernatant
	mg/L as HAc			
8/9/2004	47 ± 7	263 ± 15	432[1]	78 ± 4
8/10/2004	48 ± 2	255 ± 6	290 ± 2	61 ± 12
8/12/2004	32 ± 11	250 ± 8	287 ± 9	59 ± 2

[1] Unable to perform replicate tests for this sample.

Table 4.2: Daily VFA Loadings for Trial 1

Date	Mixed Liquor Feed[2]	FZ1[1]	FZ2[1]	Supernatant[2]
	lb as HAc			
8/9/2004	62.3	99.0	162.1	103.3
8/10/2004	63.4	96.0	108.8	80.8
8/11/2004[3]	52.5	-	-	79.3
8/12/2004	42.3	94.1	107.7	78.1

[1] mg/L * 3.785 L/gal * 0.045 Mgal * 1* 10^6*1g/1000 mg * 1kg/1000g * 2.21 lb/kg = lb in the fermenter zones

[2] mg/L * 3.785L/gal*110 gpm*1440 min/day*1g/1000 mg*1kg/1000g* 2.21 lb/kg = lb/d

[3]Values obtained from interpolation of the values from August 10 and August 12, 2004.

Step 2: Use the mean of VFA concentrations in Table 4.1 to calculate VFA loadings: (Table 4.2).

Step 3: Perform a mass balance for VFAs in the side-stream system. Values for VFA concentrations and loadings shown in the equations below are drawn from Tables 4.1 and 4.2. The lb of VFA /day added to the fermenter system are summed for the four sampling dates.

VFA (in) = 62.3 + 63.4 + 52.5 +41.6 (From Table 4.2).

VFA (in) = 219.8 lb

VFA (out)* = [0.5 (78) + 0.5 (432) + 0.5 (61) + 0.5 (290) + 1.0 (60)** + 0.5 (59) + 0.5 (287)] * 3.785 L/gal* 0.045 Mgal * 1 g/1000 mg * 1 kg /1000 g* 2.21 lb/kg (concentrations from Table 4.1 used for this calculation)

VFA (out) = 876.5 lb**

Notes: * Assumption : supernatant concentration is half of the time equaled to the FZ2 concentration for a given 24 hour period.
**For August 11, 2004, no sampling occurred, so it is assumed that the supernatant concentration did not vary for 24 hours: 79.3 lb = 60 mg/L.

VFA (consumed) = VFA in FZ1 + $FZ2_{Aug\ 9}$ - VFA in FZ1 + $FZ2_{Aug\ 12}$

= (107.7 + 93.6) – (162.1 + 100.5) (From Table 4.2)

VFA (consumed) = 61.3 lb

Step 4: Calculate the average amount of VFA produced (Equation 4.4)

$$\text{Average VFA production (lb/day)} = \frac{[\text{VFA (in)} - \text{VFA (consumed)} - \text{VFA (out)}]}{4 \text{ days}} \quad \text{(Equation 4.4)}$$

Average VFA production = (876.5 – 61.3 – 219.8) / 4

Average VFA production = 148.8 ≈ 149 lb/day (Table 4.3)

4.2.3. VFA Yield

The VFA yield is defined as the ratio of mass of VFAs produced/mass of VSS fed to the fermentation system. It is calculated as follows:

Step 1: calculate the mass of VSS fed to the system.

Example: Trial 1 data

The mixed liquor feed VSS masses for the period of August 10 to August 12

(Table 4.3) were averaged (refer to Appendix A, Section A.2 for the daily VSS values).

$VSS(in)_{avg}$ = (1326 + 1439 + 1283 +1126) / 4

$VSS(in)_{avg}$ = 1294 lb/day

Step 2: calculate the VFA yield (Equation 4.5).

$$\text{VFA Yield} = \frac{\text{Mass of VFAs produced (value calculated in section 4.2.1)}}{} \quad \text{(Equation 4.5)}$$

$$\text{VFA Yield} = \frac{\text{Mass of VSS in mixed liquor feed}}{\text{Mass of VSS in mixed liquor feed}} = \frac{149 \text{ lb/day}}{1294 \text{ lb/day}}$$

VFA Yield = 0.115

4.2.4. Batch Tests - Soluble COD uptake

The soluble COD uptake, which determines the efficiency of the different VFA sources used for the phosphorus release batch test, was calculated as shown below. The concentration values used in the calculations can be found in Appendix A, under Section A.5:

Step 1: calculate the OP release rate (equation 4.5):

$$OP_{rr} = \frac{([OP_2] - [OP_1]) * V_{AN}}{\Delta t} \quad \text{(Equation 4.6)}$$

Where: OP_{rr} = phosphorus release rate = [mg/min]

$[OP_2]$ = soluble concentration at time 2 = [mg/L]

$[OP_1]$ = soluble concentration at time 1 = [mg/L]

V_{AN} = volume of created anaerobic zone = [L]

Δt = time 2 – time 1 = 15 [minutes]

Example: Series No.1/Test A1/Fermentate/Time 15 minutes to Time 30 minutes

$OP_{rr} = (21.6 - 18.3) * (3.5 + (230/1000)) / 15$

OP_{rr} = 0.8 mg/min

Step 2: calculate the soluble COD uptake rate (Equation 4.6):

$$SCOD_{cr} = \frac{([SCOD_2] - [SCOD_1]) * V_{AN}}{\Delta t} \quad \text{(Equation 4.6)}$$

Where: $SCOD_{cr}$ = soluble COD consumption rate = [mg/min]

$[SCOD_2]$ = soluble concentration at time 2 = [mg/L]

$[SCOD_1]$ = soluble concentration at time 1 = [mg/L]

$SCOD_{cr}$ = (48 – 53) * (3.5 + (230/1000)) / 15

$SCOD_{cr}$ = - 1.2 mg/min

STEP 3: calculate the soluble COD consumption (Equation 4.7).

$$SCOD_c = \frac{SCOD_{cr}}{OP_{rr}} \quad \text{(Equation 4.7)}$$

Where: $SCOD_c$ = soluble COD consumption = [mg COD/mg P]

$SCOD_c$ = -1.2/0.8

$SCOD_c$ = - 1.5 mg COD/ mg P (in Table 4.9 the minus sign was changed for a positive sign and vice-versa).

4.3. Results Analysis

4.3.1. Volatile Fatty Acid Production

The estimated net VFA production at the 2.5-day SRT was 149 lb/d. This increased to 206 lb/day at the 7-day SRT and fell to 94 lb/day at 12-day SRT (Table 4.5). Theoretically, the 12-day SRT condition should have resulted in higher VFA production than the 7-day SRT. However, as mentioned in section 4.1, during the 7 and 12-day SRT trials, a thick layer of floating solids formed in the fermentation zones and numerous fine bubbles (presumably methane) were observed at the surface, particularly when the fermentation zones were mixed. The 7 and 12-day SRTs were well beyond the 3-day SRT recommended to prevent the growth of methanogens (Grady et al., 1999), bacteria that consume acetic acid and release methane and CO_2. It is likely that VFA production at the 12-day SRT was significant, but methanogen activity reduced the net VFA concentration available for BPR. The VFA yields expressed as g VFA/g VSS (Table 4.5)

are similar to those of primary sludge fermentation (0.05 to 0.3 g VFA as acetic acid per g VSS in the feed to the fermenter (Elefsiniotis and Oldham, 1993; Grady et al., 1999; Rössle and Pretorius, 2001b).

Table 4.3: Estimated Net VFA Production and VFA Yield for the Different SRTs Evaluated

SRT (days)	**Estimated Net VFA Production in Fermentation Zones[1] (lb/day as acetic acid)**	**VFA Yield (g of VFA as acetic acid per g of VSS fed to the fermentation zones)**
2.5	149	0.11
7	206	0.14
12	94	0.05

[1]Based on 4 or 5 days of data

The soluble COD values reported in Table 4.6 represent the truly soluble COD as determined by the Mamais et al. (1993) flocculation method. The truly soluble COD includes the readily biodegradable soluble COD and the non-readily biodegradable soluble COD. The VFAs present in a sample represent 80-90% of the truly soluble COD (deBarbadillo, 2005). Therefore, the soluble COD serves as an indicator of the amount of VFAs that could be produced in the fermentate.

Although the 7-day SRT produced the highest VFA concentration in the fermentation tank, the ratios of soluble COD in the fermentate to soluble COD in the anaerobic mixed liquor feed to the fermenter were comparable in Trials 2 (7-day SRT) and 3 (12-day SRT) at ratios of 4.8:1 and 5:0, respectively. In the anaerobic mixed liquor feed to the fermenter, some of the COD is soluble and some is particulate. In the fermenter, some of

the organic particulates and some of the soluble organics will be converted to VFAs, leading to a higher soluble COD in the fermenter than in the feed. Results in Table 4.6 suggest that more conversion of organics to VFAs occurred when the SRT was increased from 2.5 to 7 days, but no further gain in VFAs was achieved when the SRT was extended to 12 days. It is to be noted that standard deviations obtained for the fermentation zones soluble COD data are higher because of the small number of samples tested.

A concentration of 7-10 mg/L of VFA in the anaerobic zone is required to remove 1 mg/L of phosphorus (Barnard, 1993; Grady et al., 1999). The soluble phosphorus concentration in the BNR influent at the McDowell Creek WWTF averages about 5 mg/L. Therefore, the supplemental VFA dose required for the plant to discharge an effluent with less than 1 mg/L phosphorus would be 35 to 50 mg/L as acetic acid. The estimated net VFA production rate was 149 lb/day at the 2.5 day SRT. Assuming the elutriation process is efficient, and most of the VFAs produced are present in the supernatant stream, application of 149 lb/day to the average 2.5 MGD flow through McDowell Creek's BNR Train 1 translates to VFA supplementation of 7 mg/L.

Table 4.4: Mean ± Standard Error of Soluble COD in the Feed Sludge and in the Fermentation Zones for the Different SRTs Evaluated (Values are Means of Daily Averages)

SRT (days)	**Soluble COD Concentration in Anaerobic Mixed Liquor (mg/L as COD)**	**Soluble COD Concentration in Fermentation Zones (mg/L as COD)**	**Mean Ratio (Column 3/ Column 2)**

2.5	32 ± 6	75 ± 10	2.0
7	33 ± 6	158 ± 3	4.8
12	44 ± 2	220 ± 46	5.0

The demonstration anaerobic mixed liquor fermentation process was operated by diverting 6.5% of the UCT anaerobic mixed liquor from anaerobic zone No. 2 tank to the fermenter. The UCT anaerobic zone operates at a more dilute MLSS concentration than the aeration basins because solids are returned to the anaerobic basins from the anoxic zones rather than from the settled sludge of the secondary clarifiers (RAS). Typically, the biomass concentration in the anaerobic basins is about half of the concentration of the biomass in the aeration basins. Therefore, the flow to the side-stream fermentation system was equivalent to diverting just over 3% of the flow from an aeration basin in the plant.

Since some readily biodegradable material in the influent wastewater is quickly converted to VFAs in the anaerobic zones, it is not necessary to supplement with the entire VFA dose calculated for targeted P removal. It is estimated that sufficient VFAs to supplement the BPR process at this plant could be obtained if a flow equivalent to 5 to 15% of the aeration basin flow were diverted to the fermentation zone (deBarbadillo, 2005) and all the VFAs produced from anaerobic mixed liquor fermentation were captured in the supernatant. For a UCT anaerobic tank, this would equivalent to 10-30% of the anaerobic basin mixed liquor flow being diverted to the fermenter.

Comparing the VFA production at different SRTs to the dosing requirements for the plant, it appears that a 2.5 or 7-day SRT would likely yield satisfactory VFAs for good phosphorus removal. A 2.5-day SRT produced significant VFAs and was relatively manageable from an operational standpoint. Net VFA production was higher at the 7-day SRT, but in the system studied here, the sludge layer formed at the top of the fermentation zone was problematic.

4.3.2. Volatile Fatty Acid Composition

Gas chromatography tests were performed on samples of the fermentate obtained at the 12-day SRT to determine the VFA distribution. Acetic (55-57%) and propionic (26-28%) acids accounted for most of the acids present. Other VFAs found were Iso-, n-, and 2-methyl-butyric acids (10-12%) and valeric and iso-valeric acids (5%). This distribution is comparable to compositions found in several APT prefermenter systems (see Table 2.3). The average composition of the acids produced in systems shown in Table 2.3 was 56%, 32%, 8% and 2% of acetic acid, propionic acid, butyric acids and valeric acids respectively (Rössle and Pretorius 2001a)..

The acetic:propionic ratio was approximately 65:35 in all samples tested (refer to Appendix A, Section A.6) in March 2005 (at about 12-day SRT), which compares well with the optimum ratio of acetate to propionate suggested by Barnard and Scruggs (2003) for stable BPR performance (60:40 acetic to propionic acid). Raw wastewater fermentation has yielded acetic acid to propionic acid ratios more in the range of 90:10 in a UASB reactor (GonÇalves et al., 1994); an SBR reactor (Danesh and Oleszkiewicz, 1995); and a WAS ATAD system (Fothergill and Mavinic, 2000). This suggests that anaerobic mixed liquor fermentation is preferable to other fermentation schemes that do

not yield the optimum mix of VFAs for BPR. However, different SRTs may lead to different acetic to propionic ratios. Grady et al. (1999) stated that a significant reaction rate difference exists for the various acidogenic reactions that convert the hydrolysis products into acetic acid and hydrogen. For instance, the fermentation of amino acids and simple sugars occurs very rapidly while the anaerobic oxidation of fatty acids (propionic acid in particular) to acetic acid and hydrogen is much slower. At 5 to 6-day SRTs, significant amounts of propionic acid will accumulate (in comparison with acetic acid) in the fermentate, because the SRT is too short to allow for the growth of bacteria which oxidize propionic acid to acetic acid and hydrogen. At higher SRTs, oxidation of propionic acid will not be rate limiting.

However, it should be noted that a consensus has not been reached among those actively researching various VFA production schemes on the optimum VFA mix for good phosphorus removal (Barnard 2005). Chen et al. (2002) recommended a ratio of 47:53 acetic to propionic acid until the impacts of bacterial selection pressures in a full-scale EBPR process are better defined. There is some evidence that propionic acid is a slightly less efficient substrate for PAOs than acetic acid, but a much less efficient substrate for non-PAOS. This would suggest that having higher propionic than acetic acid concentrations in the anaerobic zone would help the PAOs out-compete the GAOs.

4.3.3. Volatile Fatty Acid Elutriation

In all trials, the VFA concentrations in the supernatant were low compared to those in the fermentate mixture (Table 4.7). The 2.5-day SRT condition did not yield the highest mixed fermentate VFA concentration, but it yielded the highest supernatant concentration. The influence of the FZ1 mixer on the FZ2 sludge blanket during this trial

(refer to section 4.1) likely resulted in better elutriation of VFAs from the sludge blanket. In samples from the 7-day SRT, the liquid phase VFA concentrations were lower along with the solids content. Results from the 12-day SRT trial were not consistent with this trend, because high solids but negligible VFAs were present in the supernatant. The likely explanation is that solids from the floating sludge layer were entrained in the supernatant because of the proximity of the pump suction. The floating sludge layer at the 12-day SRT was 2 ft thick and the pumps were installed about 2 ft below the surface. The biomass found in the top solids layer may have been less active for fermentation and therefore did not have significant VFAs associated with it. It is to be noted that standard deviations are high for the supernatant TSS content because of the variability of the elutriation process and also because of temperature variation and rain on some of the sampling days.

GonÇalves et al. (1994) fermented raw wastewater in a bench scale UASB reactor and reported the highest supernatant concentrations at an upflow velocity of 0.9 m/h. Lower supernatant VFA concentrations were obtained below or above 0.9 m/h. At 0.6 m/h, the supernatant VFA concentration declined substantially compared to 0.9 m/h. In the study described here, the upflow velocity was 0.3 m/h, which is well below the optimum value reported by GonÇalves et al.

One of the advantages of UASB reactors over static fermenters is that acid can not accumulate because it is continuously elutriated as the mixed liquor flows upward and the supernatant goes out of the basin. This continuous elutriation also regenerates the alkalinity and stabilizes the pH. (GonÇalves et al., 1994). Upflow velocity, and therefore HRT, can be optimized to ensure that optimum elutriation and pH control is achieved.

Table 4.5: Mean ± Standard Error of VFA Concentrations in the Fermentation Zones and Supernatant and TSS in the Supernatant for the Different SRTs Evaluated (Values are means of daily averages)

SRT (days)	**TSS in supernatant (mg/L)**	**VFA Concentration in Fermentation Zones (mg/L as acetic acid)**	**VFA Concentration in Supernatant (mg/L as acetic acid)**
2.5	1148 ± 212	281± 19	84 ± 12
7	43 ± 29	621 ± 25	17 ± 3
12	465 ± 118	386 ± 43	Below detection limit

In this study, pH was measured upon arrival of the samples at the laboratory. Fermentation zone sample pH ranged from 6.8-7.0; 6.6-6.8; and 6.3-6.5 at the 2.5-day, 7-day and 12-day SRTs respectively (Table 3.2). Although the poor VFA elutriation observed in these trials might be expected to result in lower supernatant pH values than reported, it is possible that by the time the pH was measured in the laboratory, carbon dioxide had escaped from the samples, artificially elevating the pH

Low VFA recovery in the supernatant fraction might also be due to uneven flow distribution into the sludge blanket (FZ2), which was due to the configuration of the basin (flat bottom rather than a conical shaped tank bottom normally included in a USB reactor design) and to the side location at which the flow from FZ1 entered.

4.3.4. Phosphorus Release in the Fermentation Zones

The PAOs released phosphorus in the fermentation tank during the three testing phases. The phosphorus mass was 68, 106, and 150% greater in the supernatant than in the anaerobic mixed liquor feed for the 2.5, 7 and 12-day SRT trials, respectively. The increase in phosphorus that occurred when the supernatant was returned to the McDowell Creek WWTF BNR train translated to 0.87 mg/L of additional phosphorus to be removed in the aerobic zone at the 2.5-day SRT; 0.89 mg/L at the 7-day SRT; and 1.3 mg/L at the 12-day SRT (Table 4.8). During the 7-day SRT trials, soluble phosphorus concentrations in the last aerobic basins of BNR Train No. 1 (with fermentation system) and Train No. 2 (without fermentation system) were compared (results not shown here; refer to Appendix A-Test and Process Raw Data). The few samples tested indicated that phosphorus levels were higher in Train No.1 than in Train No.2. Similar tests performed on oxic basin samples at 2.5-day and 12-day SRT were inconclusive.

Table 4.6: Mean ± Standard Error of the Mass Rate of Soluble Phosphorus in the Feed Sludge, in the Supernatant and mg/L of P added to BNR train for the Different SRTs Evaluated (Values are the Means of Daily Averages)

SRT (days)	**Soluble Phosphorus Mass in Anaerobic Mixed Liquor Feed**[1] **(lb/day as P)**	**Soluble Phosphorus Mass in Supernatant**[1] **(lb/day as P)**	**Extra P added to BNR Train**[2] **(mg/L as P)**
2.5	25 ± 3	43 ± 3	0.87 ± 0.18
7	18 ± 2	37 ± 2	0.89 ± 0.08
12	18 ± 2	45 ± 1	1.3 ± 0.05

[1] mg/L * 3.785L/gal*110 gpm*1440 min/day*1g/1000 mg*

1kg/1000g*2.21 lb/kg = lb/d
[2] (lb/day in supernatant – lb/day in feed) * 1 kg/2.21 lb
* 1000 g * 1000 mg/ 1g * 1/ 2.5 MGD* 1 gal/3.785L /1 *10^6

Phosphorus release in the side-stream fermentation zones increases with increasing SRT (Table 4.8). Larger retention time allowed more VSS solubilization, and some of the VSS were PAO biomass, from which accumulated P was released into the fermentate liquid. This type of phosphorus release is comparable to secondary release of phosphorus in the anaerobic zone i.e. that it was released without the energy intake by the PAOs. Phosphorus accumulating organisms present in the fermentation tank may have taken up some VFAs and released phosphorus. However, this type of phosphorus release would not be detrimental to the EBPR process. The SRT and HRT must be optimized to accomplish high VFA yields, but low phosphorus release from VSS destruction, if mixed liquor fermentation is to be practicable.

4.3.5. Presence of GAOs and PAOs in the Plant BNR System and in the Fermentate

Microscopic analysis of the plant BNR anaerobic mixed liquor showed that PAOs dominated in the mixed liquor (Figure 4.5). Some GAOs were observed, but in very small numbers. Microbial analysis of oxic zone samples showed abundance of PAOs, but also of an un-identified species with the same Neisser staining attributes as the GAOs, but without the tetrad-shaped formation (Figure 4.6). Some GAOs were also observed in the oxic samples, but in very small numbers.

Microscopic analysis of fermentate samples indicated that the sludge was dominated by tetrad-shaped GAOs (Figure 4.7) according to the classification by Jenkins et al. (2004). The fermentate had also a low count of typically-sized PAOs (1-1.5 μm), and a

higher frequency of smaller (0.2-0.4 µm) cocci-shaped strongly Neisser positive organisms that could not be identified (Figure 4.8). The latter were labeled "possible PAOs" because of their strong Neisser positive attributes. Typically PAOs are 0.8 – 1.5 µm (Sudiana et al., 1998).

The author has reviewed several articles published by groups of microbiologists that have studied intensively PAO populations (groups lead by Dan Noguera, Linda Blackall, Takashi Mino and Bob Seviour) and has found no report of PAOs in the 0.2-0.4 µm range. Simultaneous staining for poly-P (using 4',6-Diamidino-2-phenylindole (DAPI)) and PHAs (using Sudan black) would definitively show if these organisms are PAOs or not (Seviour et al, 2003).

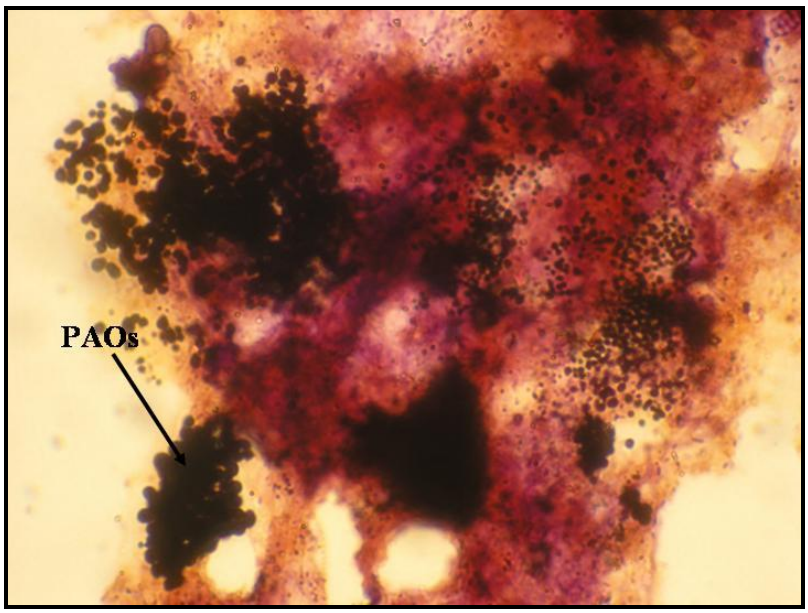

Figure 4.5: Microscopic Picture (1000X) – Mixed Liquor Sample Taken from BNR Anaerobic Zone During Trial 1. Floc was dominated by PAOs.

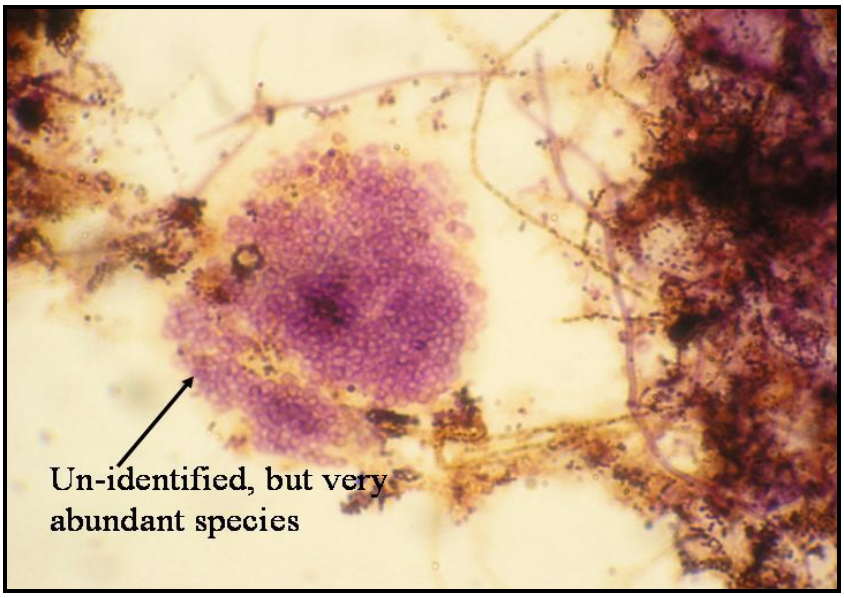

Figure 4.6: Microscopic Picture (1000X) – Mixed Liquor Sample Taken from BNR Oxic Zone in February 2005. Floc was dominated by abundant un-identified species showed in purple.

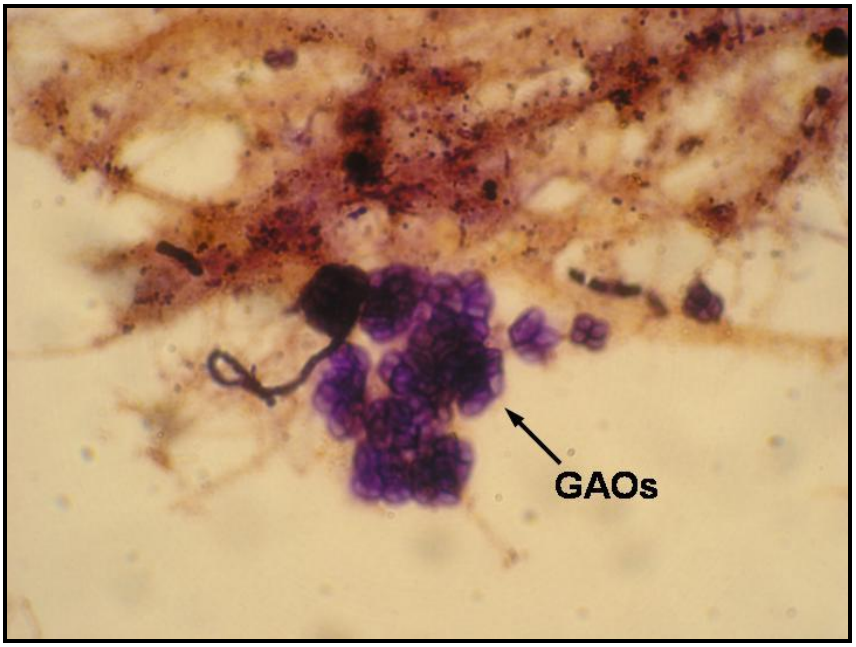

Figure 4.7: Microscopic Picture (1000X) – Mixed Liquor Sample Taken from Side-Stream Process Fermentation Zone at a SRT of about 10 days in January of 2005. Sample was dominated by GAOs.

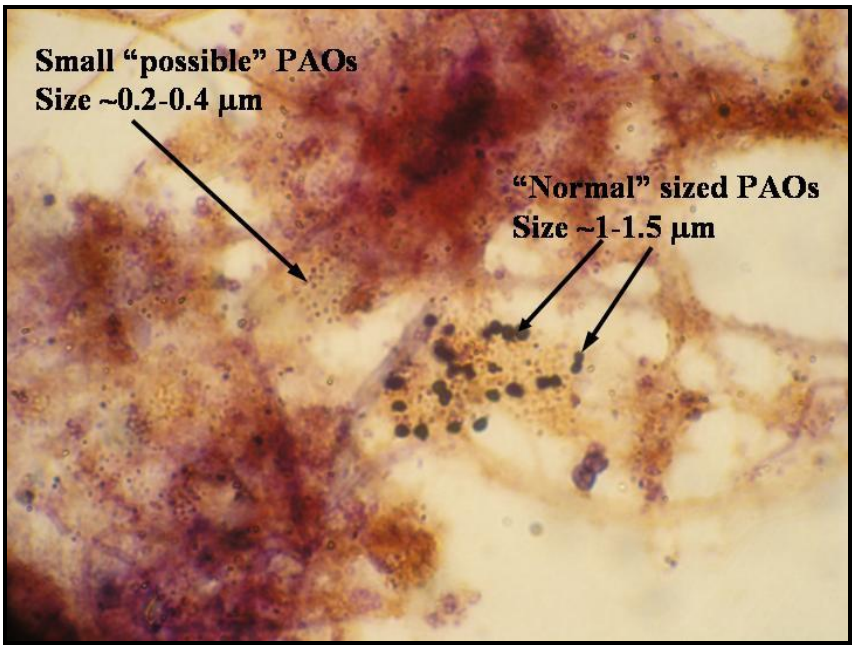

Figure 4.8: Microscopic Picture (1000X) – Mixed Liquor Sample Taken from Side-Stream Process Fermentation Zone at a SRT of about 10 days in January 2005. Small "possible" PAOs were observed in large numbers.

4.3.6. Phosphorus Release Batch Test Results

Series No.1 – Tests A1 and B1: anaerobic zone was created from the mix of plant anoxic liquor and primary effluent

The phosphorus release batch tests showed that most of the VFA uptake (measured as COD) occurred in the first 15-20 minutes of the runs (Figures 4.9 and 4.10). The final phosphorus concentrations were similar for all VFA sources in each trial. It is noted that the COD uptake per milligram of phosphorus released (Table 4.9) was significantly lower for the two fermentates and control tests than for acetic acid and waste sugar water. This suggests that although sufficient VFAs were present for phosphorus release in all trials, the release was more efficient with fermentate and in the controls. Subsequent analysis revealed that significant VFAs were present in the anoxic mixed liquor on the days it was

sampled for use in the batch tests, likely resulting in the good phosphorus release seen in the control samples.

Several months after the batch tests were conducted, the plant main operator reported discovering that the influent to the plant may have contained sufficient VFAs for EBPR at the time of the tests, while an external VFA source was still being added to the BNR influent. Therefore, the amount of VFAs found in the plant anaerobic zone may have been more than required for good BPR, which could explain why the anoxic liquor contained a significant amount of VFAs.

Although the batch test results were impacted by the VFA content of the control samples, the reduced COD uptake rates suggest that the fermentate would be a more efficient VFA supplement than either acetic acid or sugar water. It has been suggested that GAOs can out-compete PAOs for acetate while the reverse is true for propionate; and also that a mixture of propionic and acetic acid (with more of the propionic acid) may prove more efficient than 100% of acetic acid (Chen et al., 2002). Furthermore, glucose fed to an anaerobic zone may also favor GAOs (Oehmen et al., 2005).

Series No.2 – Tests A2 and B2: anaerobic zone was created from the mix of plant RAS and primary effluent

Series No. 2 release batch tests were conducted later in the project (Figures 4.11 and 4.12). The results were similar in terms of VFA uptake rate and phosphorus concentrations to the results obtained with Series No. 1 batch tests. They showed that most of the VFA uptake (measured as COD uptake rate) occurred in the first 20-25 minutes of the runs, except for the test with acetic acid. The final phosphorus concentrations were similar for all VFA sources in each trial.

The overall COD uptake per milligram of P released (Table 4.9) was significantly lower for Test B2 fermentate and control tests than for acetic acid and waste sugar water. However, the COD uptake per milligram of P released for Test A2 fermentate was higher (less efficient) than for all the other tests conducted in Series No. 2 and Series No. 1 test. The same fermentate sample was used for Test A2 and Test B2. Therefore, a similar COD uptake per mg of P would have been expected. It is to be noted that Test B2-fermentate was less efficient than Tests A1 and B1-fermentates. A large number of GAOs were observed in the fermentate used for Series No.2 tests (Figure 4.7). This could explain why the Series No.2 fermentate tests were less efficient in terms of mg COD consumed/mg P released than the Series No. 1 fermentate tests. However, the results obtained for Test A2 fermentate in comparison with Test B2 fermentate could not be explained except that is suggests that conditions favoring the growth of GAOs may have been present in the Series No. 2 fermentate.

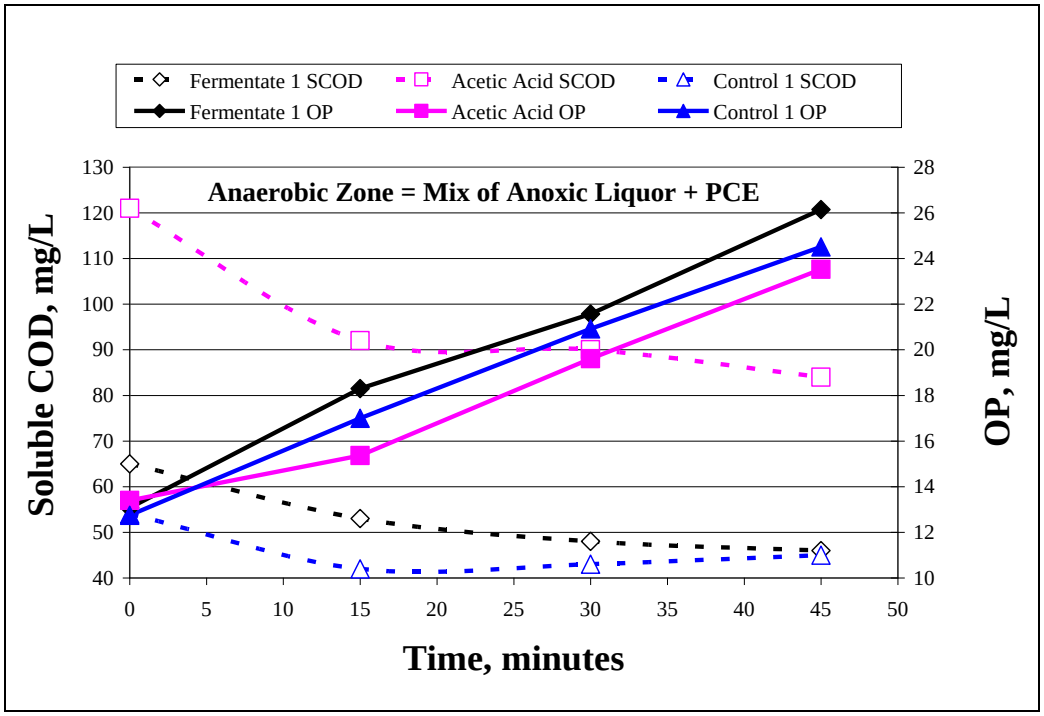

Figure 4.9**:** Batch Test Series No. 1 – Test A1: Soluble COD Consumption and Phosphorus Release versus Time

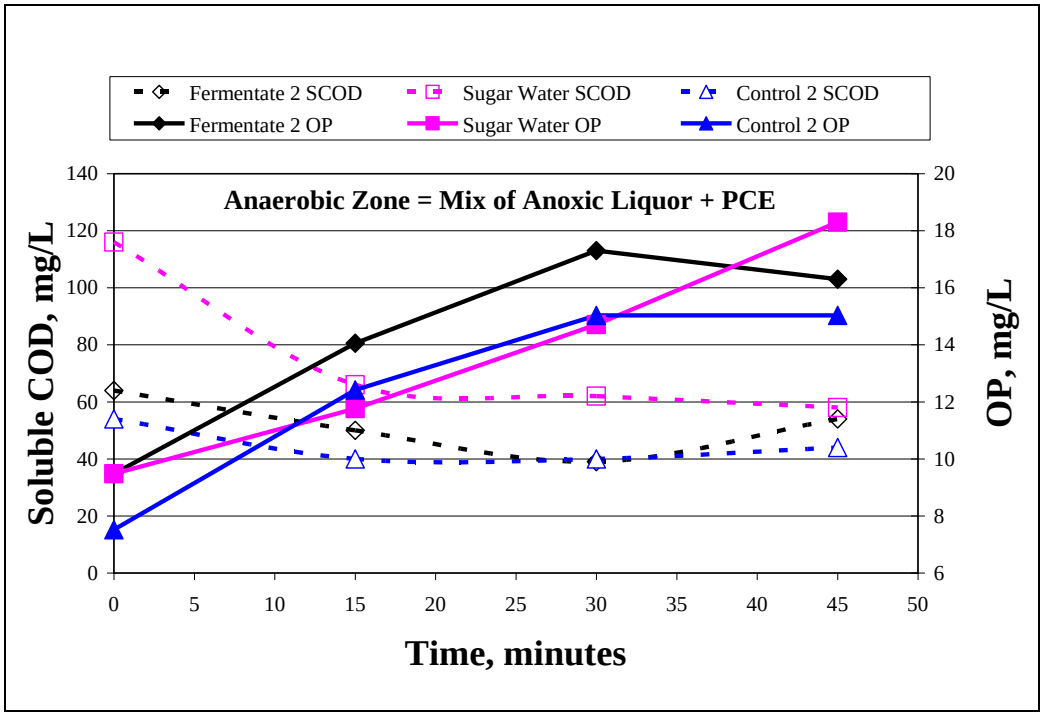

Figure 4.10: Batch Test Series No. 1- Test B1: Soluble COD Consumption and Phosphorus Release versus Time

Table 4.7: Soluble COD Uptake in Terms of mg COD/mg P
for Series No.1 and Series No. 2 Phosphorus Release Batch Tests

Time (Minutes)	**Soluble COD Uptake (mg of COD consumed / mg of P released)**			
	Series No. 1		**Series No. 2**	
	Test A1	Test B1	Test A2	Test B2
	Fermentate (20 mg/L as HAc)	**Fermentate** (11 mg/L as HAc)	**Fermentate** (13 mg/L as HAc)	**Fermentate** (24 mg/L as HAc)
0	-	-	-	-
15	2.3	3.1	27.5	2.4
30	1.5	3.4	1.1	-1.7
45	0.4	-	1.1	8.8
	Acetic Acid (58 mg/L as HAc)	**Sugar Water** (44 mg/L as HAc)	**Acetic Acid** (66 mg/L as HAc)	**Sugar Water** (38 mg/L as HAc)
0	-	-	-	-
15	14.8	21.9	4.2	10.2
30	0.5	1.4	7.7	1.2
45	1.5	1.1	13.3	3.6
	Control A	**Control B**	**Control A**	**Control B**
0	-	-	-	-
15	2.8	2.9	6.2	8.3
30	-0.3	0.0	2.0	1.8
45	-0.6	-	-1.5	-2.4

The use of RAS may have also contributed to more GAOs versus PAOs in the created anaerobic liquor at the beginning of the tests. The Series No. 2 control tests resulted in a less efficient phosphorus release compared to the Series No. 1 control tests. Furthermore, the SRT was approximately 7 days and 10 days for Series No.1 and Series No. 2

fermentate, respectively. The ratio of acetic to propionic acid may have been more favorable for GAOs for Series No. 2 fermentate tests than for Series No. 1 fermentate tests. Analysis revealed that significant VFAs were also present in the RAS on the days it was sampled for use in the batch tests, likely resulting in the good phosphorus release seen in the control samples. Based on the results of Series No. 2 tests alone, it could not be concluded that the fermentate from anaerobic mixed liquor was a better VFA source than acetic acid and waste sugar water.

4.3.7. Comparison of Soluble COD and VFA Concentration Results

It was previously mentioned that VFAs normally represent 80 to 90% of the truly soluble COD (section 4.3.1). However, although there were some exceptions, the results presented in Tables 4.6 and 4.7 show that the soluble COD was generally lower than the VFA concentration in the fermentate for all the trials (1 mg/L of HAc = 1.07 mg/L of COD). Since the soluble COD testing involves flocculation and filtration of the samples, while the titrimetric method for VFA analyses retains the solids in the sample, it was hypothesized that some of the VFAs may have been associated with the solids in the fermentation zones. To verify this hypothesis and further explore these results, additional tests, described here as "solids tests", were conducted. The "solids tests" were performed on anaerobic mixed liquor but not on the fermentate, because the tests were conducted after completion of the side-stream fermentation process trials.

Soluble COD and VFA tests were performed on anaerobic zone mixed liquor samples from the McDowell Creek WWTF. The solids obtained from the filtration and flocculation steps of the soluble COD analysis were retained and re-diluted in DI water. VFA and soluble COD tests were conducted on the re-suspended solids. Tests were done

in triplicates and the samples were spiked for accuracy verification. The results showed that truly soluble COD was present with the solid part of the anaerobic mixed liquor sample. Although VFAs were present in the mixed liquor, no VFAs were found with the re-suspended solids. These results indicate that the soluble matter found to be associated with the solids phase is not VFA. It was concluded that the association of VFAs with the solids likely did not occur in this study. Knappe (2005) and de los Reyes (2005) confirmed that acetate and other VFAs will follow the liquid phase and can not adsorb onto the biomass.

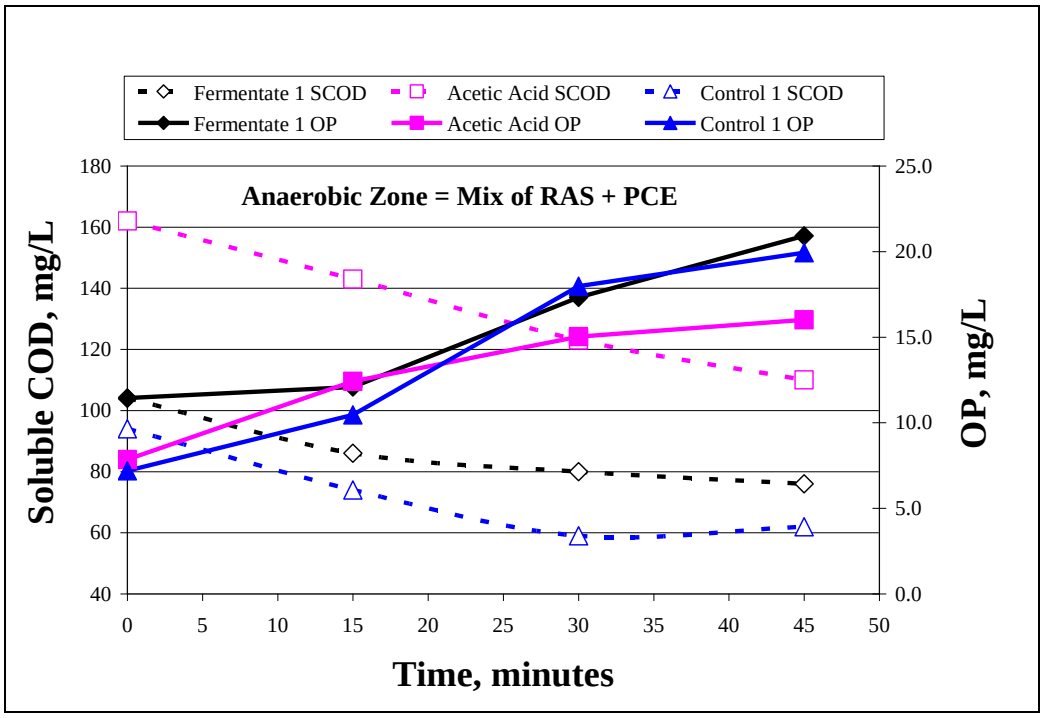

Figure 4.11: Batch Test Series No. 2 – Test A2: Soluble COD Consumption and Phosphorus Release versus Time

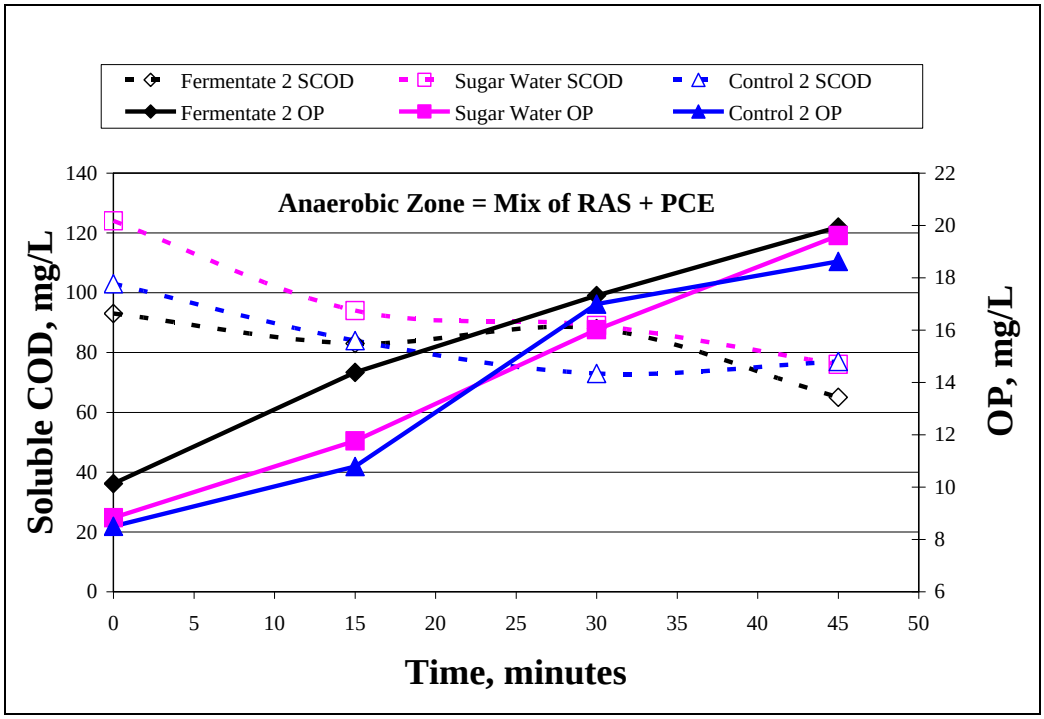

Figure 4.12: Batch Test Series No. 2 – Test B2: Soluble COD Consumption and Phosphorus Release versus Time

The "solids test" results suggest that the use of the titrimetric method for VFA determination could lead to an overestimation of the VFA concentration when used on high solids content samples. It is to be noted that the method used in this study (Anderson et al., 1992) was developed on digester supernatant samples, which contained low solids levels compared to most of the samples analyzed for this project. An alternative would be to use a GC method, which is also a well-used approach. However, gas chromatography involves ultra-centrifugation of the samples to separate the solids from the liquid phase prior to injecting the sample in the GC column, which could lead to VFA volatilization. This could also be true for the titrimetric method. However, this risk was noted and minimized in the tests described here. Hand pumps were employed to

filter samples, and parafilm sheets were used to cover samples during the titration. Furthermore, water contaminates GC columns, which further reduces detection accuracy (Carling, 2005).

CHAPTER 5 : CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The feasibility of using anaerobic mixed liquor fermentation to generate VFAs for biological phosphorus removal was tested at the McDowell Creek Wastewater Treatment Facility. A full-scale demonstration side-stream process was operated continuously from May 2004 until April 2005. During Trial 1 (2.5-day SRT), it was noted that the submersible mixer in FZ1 disturbed the sludge blanket in FZ2 and caused significant loss of solids in the supernatant. Although one of the advantages of mixed liquor fermentation over raw wastewater or primary solids fermentation is that solids carry over will not significantly impede overall plant performance, the solids loss did preclude achieving an SRT above 3 days. Therefore, to enable testing at higher SRTs, mixing of Fermentation Zone No.1 was discontinued for the remainder of the trials, except during sampling events or sludge blanket elutriation. At the 7-day (Trial 2) and 12-day SRTs (Trial 3), a thick layer of floating solids formed at the surface of the fermentation zones, which likely resulted because methane was being produced, and the rising gas bubbles were lifting the solids. This phenomenon was not observed at 2.5-day SRT (Trial 1).

The estimated net VFA production rates were 149, 206 and 94 lbs/day at 2.5, 7 and 12 day SRTs, respectively. The VFA yields expressed as g VFA/g VSS in feed were similar to the reported results for primary sludge fermentation. The reduced VFA production at the 12-day SRT may have reflected methanogen consumption of VFAs. More conversion of organics to VFAs occurred when the SRT was increased from 2.5 to 7 days, but no further gain in VFAs was achieved when the SRT was extended to 12 days.

Phosphorus release in the fermentation tank was expected and found to increase with increasing SRT due to VSS destruction. The SRT and HRT must be optimized to accomplish high VFA yield, but low phosphorus release from VSS destruction, if mixed liquor fermentation is to be practicable.

The acetic: propionic acid ratio in the fermentate was about 65:35 at 12-day SRT, which is well suited for PAOs. This result suggests that anaerobic mixed liquor fermentation is preferable to other fermentation schemes, such as raw wastewater fermentation, that do not yield the optimum mix of VFAs for EBPR. Furthermore, mixed liquor fermentation, unlike primary sludge and raw wastewater fermentation, has the added benefit of not adding additional carbon to the BNR process.

VFA recovery (elutriation) in the supernatant was low in all trials. The UASB reactor upflow velocity was 0.3 m/h, which is well below the optimum velocity reported in the literature, which could explain why VFA elutriation was not efficient. Trials to investigate the impact of HRT (upflow velocity) on the VFA recovery were not performed because of time constraints. Furthermore, low VFA recovery in the supernatant fraction might also be due to uneven flow distribution into the UASB reactor sludge blanket, which was due to the configuration of the basin (flat bottom rather than a conical shaped tank bottom normally included in a USB reactor design) and to the side location at which the flow from Fermentation Zone No. 1 entered.

Rough calculations showed that a 2.5-day SRT could produce significant VFAs (with a 10-30% anaerobic basin flow diverted to the fermentation process), if VFA elutriation is efficient, and be relatively manageable from an operational standpoint. VFA production would be higher at the 7-day SRT, but in the system studied here, a sludge

layer formed at the top of the fermentation zone, which was problematic. Therefore, implementation of a 3 to 4-day SRT will likely be optimum in a full-scale application.

Phosphorus release batch tests showed that lower mg of COD consumed per mg of phosphorus released was achieved in most tests for the fermentate and control trials than for the acetic acid and waste sugar water tests. GAOs have been observed at the plant in recent years and GAOs may have been present in the mixed liquor samples used in the batch tests. Since GAOS consume acetic acid and sugar, the mix of VFAs present in the fermentate may be a more efficient VFA source for BPR. However, the implications of the batch test results are ambiguous in light of the fact that the controls likely had a sufficient VFA concentration for EPBR and did not need supplemental VFAs, but this was not determined by the plant staff until several months after the tests were completed.

Microscopic analysis of the plant BNR anaerobic mixed liquor showed that PAOs dominated in the mixed liquor. Some GAOs were observed, but in very small numbers. Microbial analysis of oxic zone samples showed abundance of PAOs, but also of an un-identified species with the same Neisser staining attributes as the GAOs, but without the tetrad-shaped formation. Some GAOs were also observed in the oxic samples, but in very small numbers. Analysis of fermentate samples indicated that the sludge was dominated by tetrad-shaped GAOs. The fermentate had also a low count of typically-sized PAOs (1-1.5 µm), and a higher frequency of smaller (0.2-0.4 µm) cocci-shaped strongly Neisser positive organisms that could not be identified. The latter were labeled "possible PAOs" because of their strong Neisser positive attributes. Further testing is required to confirm that these "possible" PAOs are really PAOs.

5.2 Future Work and Recommendations

This study demonstrated that side-stream fermentation of anaerobic mixed liquor using a USB reactor can be a viable and feasible option for supplemental VFA production for EBPR. However, further investigations are required before a wastewater treatment plant, such as the McDowell Creek WWTF, could replace the external VFA sources with the internal VFAs produced from the fermentation process. First, more trials should be performed at 3-4-day SRT with 10-30% mixed liquor recirculated (in the case of a UCT mode BNR process) to the fermentation zone. The impact of HRT (upflow velocity) on VFA recovery, and other issues related to improving the efficiency of elutriating VFAs from the sludge blanket should be addressed in a demonstration testing set up before more trials are conducted on a full-scale basis. Operational trials using solely USB reactor supernatant from mixed liquor fermentation will be required to confirm the feasibility of using this VFA source for BPR enhancement. Comparison trials between RAS and mixed liquor fermentation could also be conducted.

The cost of implementing side-stream mixed liquor fermentation for VFA generation will need to be determined and compared with the cost of using external VFA sources before the process can be used on a full-scale basis. Although sugar water and acetic acid are not the optimum substrates for PAOs, they have proven to be effective for EBPR in the case of the McDowell Creek WWTF. Therefore, it would not probably be cost effective for this treatment facility to implement such a process as the sugar water and acetic acid supplements at this point are certainly less costly than a new fermentation process. This option, however, could be considered in treatment facilities where frequent

EBPR efficiency variations are observed and the use of phosphorus precipitation chemicals is often necessary.

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APPENDIX A: TEST AND PROCESS RAW DATA

A.1 SRT Data

It is to be noted that only the data used for Figures 4.2, 4.3 and 4.4 are shown in this section. The X_R , $X_{Z1,}$ X_{Z2} and X values were obtained from the TSS raw data found in the next sections. The SRT_d was calculated as per equation 4.3. Refer to Figures 4.2, 4.3 and 4.4.

	26-Jul	**28-Jul**	**31-Jul**	**2-Aug**	**6-Aug**	**9-Aug**
t_m (hour)	0.25	no mixing	0.01	0.28	0.25	1.00
Pump on while mixing (Y/N)	Y	-	Y	N	N	N
t_{solset} (hour)	4.0	-	4.0	0.0	0.0	0.0
t_{off} (hour)	0.0	0.0	0.0	4.3	4.3	6.0
m_w (lb)	2674	-	1283	1780	1248	495
$X_{R(CALC)}$ (mg/L)	2024	-	971	1348	945	375
X_R (mg/L)	1680	700	1060	1640	1150	500
X_{Z1} (mg/L)	3900	3867	3540	3800	2040	3167
X_{Z2} (mg/L)	7240	-	-	5900	6500	4400
X (mg/L)	5570			4850	4270	3784
SRT_d (days)	1.6	-	-	`	2.6	5.7

	10-Aug	**19-Aug**	**28-Aug**	**30-Aug**
t_m (hour)	1.00	2.00	1.00	1.00
Pump on while mixing (Y/N)	N	N	Y	N
t_{solset} (hour)	0.0	0.0	4.0	0.0
t_{off} (hour)	5.0	6.0	0.0	5.0
m_w (lb)	1516	921	1278	5
$X_{R(CALC)}$ (mg/L)	1148	698	968	968
X_R (mg/L)	1450	930	0	5
X_{Z1} (mg/L)	3950	3733	7740	6867
X_{Z2} (mg/L)	4317	4100	10840	8567
X (mg/L)	4134	3917	9290	7717
SRT_d (days)	2.0	3.2	5.5	4.5

SRT Data (cont'd)

	1-Sep	3-Sep	8-Sep	10-Sep	13-Sep	15-Sep
t_m (hour)	0.50	0.75	0.25	0.25	1.00	1.00
Pump on while mixing (Y/N)	N	N	N	N	N	N
t_{solset} (hour)	0.0	0.0	1.0	1.0	0.0	0.0
t_{off} (hour)	4.5	6.8	3.3	3.3	5.0	5.0
m_w (lb)	494	142	2046	760	21	146
$X_{R(CALC)}$ (mg/L)	374	374	1549	575	575	575
X_R (mg/L)	460	150	1650	520	20	140
X_{Z1} (mg/L)	5400	5533	5760	4775	9980	7540
X_{Z2} (mg/L)	9667	6833	9284	8314	19567	9340
X (mg/L)	7534	6183	7522	6545	14774	8440
SRT_d (days)	11.5	9.4	2.8	6.5	14.6	8.3

	17-Sep	5-Oct
t_m (hour)	1.00	1.00
Pump on while mixing (Y/N)	N	N
t_{solset} (hour)	0.0	0.0
t_{off} (hour)	5.0	24.0
m_w (lb)	10	-
$X_{R(CALC)}$ (mg/L)	575	575
X_R (mg/L)	10	-
X_{Z1} (mg/L)	6420	8880
X_{Z2} (mg/L)	8940	8620
X (mg/L)	7680	8750
SRT_d (days)	7.6	8.6

SRT Data (cont'd)

	20-Feb	23-Feb	26-Feb	2-Mar	6-Mar
t_m (hour)	5.42	4.50	4.17	1.00	2.50
Pump on while mixing (Y/N)	N	N	N	N	N
t_{solset} (hour)	8.00	0.00	0.00	0.00	0.00
t_{off} (hour)	12	12.00	10.17	9.00	5.25
m_w (lb)	176	297	533	586	733
$X_{R(CALC)}$ (mg/L)	133	225	403	444	555
X_R (mg/L)	400	450	700	710	710
X_{Z1} (mg/L)	14133	8867	7733	9100	10300
X_{Z2} (mg/L)	14067	8967	7933	12233	14967
X (mg/L)	14100	8917	7833	10667	12634
SRT_d (days)	20.0	17.0	8.3	10.3	9.8

A.2. Trial 1 Test Result Raw Data

Legend for the Tables included in Section A.2

T2P [=] Supernatant
T2M[=] FZ2
T3[=] FZ1
PCE [=] Primary clarifier effluent
T4-1[=] Anaerobic tank (fed to fermenter)
AX2-1[=] Anoxic tank – stage 2
T1-1[=] Anaerobic tank – stage 1
T4-2[=] Train No. 2 Anaerobic tank – stage 2
AX2-2 [=] Train No. 2 Anoxic tank – stage 2
T1-2 [=] Train No.2 Anaerobic tank – stage 1
Round OX-1 [=] Oxic Stage 2
Round OX-2 [=] Train No. 2 Oxic Stage 2

VFA Titration Results

			Burette Reading (ml)				
Date	**Sample**	**Initial pH**	**Initial**	**at pH 5.1**	**at pH 3.5**	**VFA mg/L as HAc[1]**	**VFA meq/L[2]**
8/9/2004	T2P	7.16	0	2.29	3.13	70	1.2
		7.15	0	2.29	3.18	79	1.3
		7.13	0	2.31	3.23	84	1.4
	T2M	6.86	0	1.83	4.64	432	7.2
	T3	6.98	0	3.18	5.26	278	4.6
	T3	6.96	0	3.18	5.1	248	4.1
	PCE	7.44	0	1.79	2.51	58	1.0
		7.38	0	1.74	2.47	60	1.0
		7.39	0	1.7	2.42	59	1.0
	T4-1	7.06	0	1.97	2.71	56	0.9
		7.06	0	2.07	2.7	34	0.6
		7.04	0	1.99	2.71	52	0.9
	Ax2-1	6.89	0	1.83	2.79	96	1.6
	AX2-1	6.89	0	1.81	2.76	95	1.6
	T1-1	7.16	0	2.31	3.01	44	0.7
8/10/2004	T2P	7.14	0	2.41	3.17	53	0.9
	T2P	7.11	0	2.4	3.12	46	0.8
	T2P	7.14	0	2.45	3.38	83	1.4
	T2M	6.98	0	3.17	5.31	289	4.8
	T2M	6.95	0	3.05	5.2	292	4.9
	T2M	6.97	0	3.23	5.36	286	4.8
	T3	7.01	0	2.95	4.91	260	4.3
	T3	7	0	2.95	4.85	249	4.2
	PCE	7.92	0	2.06	2.7	40	0.7
	PCE	7.87	0	2.05	2.65	33	0.5
	T4-1	7.14	0	2.4	3.12	46	0.8

Notes: (1) VFA as mg/L as HAc = meq/L * MW_{Hac} where MW_{Hac} = 60
(2) Calculated from equation provided in Anderson et al. (1992)

VFA Results – Trial 1 (cont'd)

			Burette Reading (ml)				
Date	**Sample**	**Initial pH**	**Initial**	**at pH 5.1**	**at pH 3.5**	**VFA mg/L as Hac[1]**	**VFA meq/L[2]**
8/10/04	T4-1	7.15	0	2.22	2.94	49	0.8
	T4-2	7.18	0	2.1	2.8	48	0.8
	T4-2	7.16	0	2.1	2.77	42	0.7
	AX2-1	6.94	0	1.8	2.79	103	1.7
	AX2-1	6.88	0	1.72	2.65	93	1.5
	AX2-2	6.93	0	1.85	2.76	87	1.5
	AX2-2	6.9	0	1.9	2.74	73	1.2
	T1-1	7.21	0	2.02	2.7	45	0.8
	T1-1	7.19	0	2.12	2.8	44	0.7
	T1-2	7.13	0	2.11	2.82	49	0.8
	T1-2	7.14	0	2.04	2.76	52	0.9
8/12/2004	T2P	7.08	0	2.35	3.15	61	1.0
	T2P	7.08	0.0	2.4	3.2	57	0.9
	T2M	6.83	0.0	3.0	5.1	278	4.6
	T2M	6.86	0.0	3.0	5.2	296	4.9
	T3	6.80	0.0	2.8	4.7	242	4.0
	T3	6.77	4.7	7.6	9.5	257	4.3
	PCE	7.27	0.0	1.7	2.5	76	1.3
	PCE	7.27	0.0	1.8	2.5	60	1.0
	T4-1	7.19	0.0	1.9	2.6	42	0.7
	T4-1	7.16	0.0	2.0	2.5	21	0.3
	T4-2	7.15	0.0	2.0	2.6	39	0.6
	T4-2	7.12	0.0	1.9	2.6	37	0.6
	AX2-1	6.93	0.0	1.7	2.5	62	1.0
	AX2-1	6.96	0.0	1.7	2.6	85	1.4
	AX2-2	6.95	0.0	1.8	2.5	68	1.1
	AX2-2	6.97	0.0	1.8	2.5	51	0.9
	T1-1	7.19	0.0	2.0	2.8	68	1.1
	T1-2	7.18	0.0	2.0	2.6	31	0.5
	T1-2	7.21	0.0	1.9	2.6	49	0.8
8/17/2004	T2P	7.11	0.0	2.4	3.5	120	2.0
	T2P	7.13	0.0	2.2	3.3	119	2.0
	T2P	7.10	0.0	2.2	3.3	112	1.9
	T2M	6.82	0.0	2.9	4.9	276	4.6
	T2M	6.86	0.0	2.7	4.9	306	5.1
	T3	6.93	0.0	2.4	4.2	237	4.0
	T3	6.97	0.0	2.5	4.2	213	3.5
	PCE	7.17	0.0	1.6	2.3	61	1.0

Notes: (1) VFA as mg/L as HAc = meq/L * MW_{Hac} where MW_{Hac} = 60

(2) Calculated from equation provided in Anderson et al. (1992)

VFA Results – Trial 1 (cont'd)

			Burette Reading (ml)				
Date	**Sample**	**Initial pH**	**Initial**	**at pH 5.1**	**at pH 3.5**	**VFA mg/L as Hac[1]**	**VFA meq/L[2]**
8/17/2004	PCE	7.16	0.0	1.6	2.3	59	1.0
	T4-1	7.11	0.0	1.8	2.4	42	0.7
	T4-1	7.14	0.0	1.8	2.4	38	0.6
	AX2	6.95	0.0	1.4	2.3	90	1.5
	AX2	6.93	0.0	1.5	2.4	90	1.5
	AX2	6.96	0.0	1.6	2.4	76	1.3
	T1-1	7.13	0.0	1.9	2.5	35	0.6
	T1-1	7.12	0.0	1.8	2.5	42	0.7
8/19/2004	T2P	7.01	0.0	2.0	3.0	106	1.8
	T2M	6.90	0.0	2.5	4.7	306	5.1
	T3	6.90	0.0	2.5	4.1	205	3.4

Notes: (1) VFA as mg/L as HAc = meq/L * MW_{Hac} where MW_{Hac} = 60

(2) Calculated from equation provided in Anderson et al. (1992)

Soluble COD Data – Trial 1

Date	**Sample**	**Sample #**	**Reading mg/L COD**	**Spike Theoretical Concentration mg/L COD**
8/9/2004	T2P	1	77	
		2	74	
		3	78	
	T3	1	77	
		2	80	
		3	77	
	T4-1	1	35	
		2	35	
		3	35	
		Spike	123	101
	PCE	1	97	
		Spike	151	148
	T1-1	1	negative	
		Spike	129	
	T2M	1	109	

Soluble COD Data – Trial 1 (cont'd)

Date	Sample	Sample #	Reading (mg/L as COD)	Spike Theoretical Concentration (mg/L as COD)
		2	113	
		3	110	
		Spike	OR	158
8/9/2004	Round OX - 1	1	negative	
		Spike	122	
	AX2-1	1	UR	
		Spike	112	
	Standard Sol.	100 ppm	100	
8/10/2004	T2P	1	126	
		Spike	84	103
	T3	1	54	
		Spike	46	67
	T4-1	1	26	
		Spike	35	53
	PCE	1	94	
		Spike	70	87
	T1-1	1	23	
		Spike	32	52
	T2M	1	63	
		Spike	62	72
	Round OX - 1	1	16	
		Spike	27	48
	AX2-1	1	19	
		Spike	24	50
	T4-2	1	UR	
		Spike	23	
	T1-2	1	UR	
		Spike	36	
	Ax2-2	1	15	

		Spike	33	48
	Round Ox-2	1	24	
		Spike	35	52

Soluble COD Data –Trial 1 (cont'd)

Date	Sample	Sample #	Reading (mg/L as COD)	Spike Theoretical Concentration (mg/L as COD)
8/12/2004	T2P	1	56	
		2	57	
		3	57	
8/12/2004		Spike	80	79
	T3	1	75	
		2	72	
		3	66	
		Spike	90	87
	T4-1	1	57	
		2	64	
		3	67	
		Spike	83	83
	PCE	1	106	
		Spike	113	103
	T1-1	1	48	
		Spike	78	74
	T2M	1	36	Too low
		2	48	Too low
		3	76	
		Spike	69	68
	Round OX - 1	1	21	
		Spike	54	61
	AX2-1	1	negative	
		Spike	60	
	T4-2	1	no sample was taken for this test	
		Spike		
	T1-2	1	20	
		Spike	65	60
	Ax2-2	1	16	
		Spike	63	58
	Round Ox-2	1	21	

		Spike	40	61
	Standard Sol.	100 ppm	102	

Soluble COD Data – Trial 1 (cont'd)

Date	Sample	Sample #	Reading (mg/L as COD)	Spike Theoretical Concentration (mg/L as COD)
8/17/2004	T2P	1	75	
		Spike	81	88
8/17/2004	T3	1	83	
		Spike	90	92
	T4-1	1	31	
		Spike	64	65.5
	PCE	1	106	
		Spike	103	103
	T1-1	1	38	
		Spike	68	69
	T2M	1	113	
		Spike	110	107
	Round OX - 1	1	14	
		Spike	59	57
	AX2-1	1	13	
		Spike	47	56.5
8/19/2004	T2P	1	63	
		Spike	81	82
	T3	1	70	
		Spike	90	85
	T4-1	1	36	
		Spike	69	68
	PCE	1	118	
		Spike	121	121
	T1-1	1	36	
		Spike	68	68

	T2M	1	86	
		Spike	101	105
	Round OX - 1	1		
		Spike	N/A	

Soluble COD Data – Trial 1 (cont'd)

Date	Sample	Sample #	Reading (mg/L as COD)	Spike Theoretical Concentration (mg/L as COD)
	AX2-1	1	21	
		Spike	72	73
	T4-2	1	17	
8/19/2004		Spike	84	71
	T1-2	1	33	
		Spike	81	79
	Ax2-2	1	21	
		Spike	73	73
	Round Ox-2	1		
		Spike	N/A	
	Standard Sol.	100 ppm	124	

Total COD Data – Trial

Date	Sample	Sample	Dil. Fact	Corr. Fact.	Reading * Dil. Fact*Corr. Fact	Spike Theo.
					mg/L as COD	mg/L as COD
8/9/2004	T2P	1	10	1.057	1046	
		Spike			210	212
	T3	1	10	1.027	5813	
		Spike			431	433
	T4-1	1	10	1.02	2815	
		Spike			258	288
	PCE	1	10	1.051	557	
		Spike 1			184	189
	T1-1	1	10	1.046	2155	
		Spike 1			245	253

	T2M	1	10	1.039	7356	
		Spike			496	504
	AX2-1	1	10	1.033	3151	
		Spike			306	303
	STD	300			325	

Total COD Data – Trial 1 (cont'd)

Date	**Sample**	**Sample #**	**Dil. Fact**	**Corr. Fact.**	**Reading * Dil. Fact*Corr. Fact mg/L as COD**	**Spike Theo. mg/L as COD**
8/10/2004	T2P	1	10	N/A	2190	
		Spike			615	610
	T3	1	10		5100	
		Spike			749	755
	T4-1	1	10		1930	
		Spike			628	597
	PCE	1	10		330	
		Spike 1			548	517
	T1-1	1	10		1790	
		Spike 1			593	590
	T2M	1	10		6540	
		Spike			859	827
	AX2-1	1	10		2820	
		Spike			657	641
8/12/2004	T2P	1	10.8		1613	
		2	10.8		1505	
		3	10.8		1591	
		Spike			356	360
	T3	1	10		5370	
		2	10		5250	
		3	10		5470	
		Spike			635	652
	T4-1	1	10		1480	
		2	10		1590	

		3	10		1620	
		Spike			423	367
	PCE	1	10		950	
		Spike			280	321
	T1-1	1	10.2		1653	
		Spike			375	372

Total COD Data – Trial 1 (cont'd)

Date	**Sample**	**Sample**	**Dil. Fact**	**Corr. Fact.**	**Reading * Dil. Fact*Corr. Fact mg/L as COD**	**Spike Theo. mg/L as COD**
	T2M	1	10		6200	
8/12/2004			10		6290	
			10		6190	
		Spike			635	717
	AX2-1	1	10		2710	
		Spike			439	453
	Std	300 ppm			316	
8/17/2004	T2P	1	10.0	N/A	2930	
		2	10.0		2640	
		3	10.0		2700	
	T3	1	10.0		5450	
		2	10.0		5400	
		3	10.0		5350	
	T4-1	1	10.0		1880	
		2	10.0		1910	
		3	10.0		1910	
	PCE	1	10.0		360	
		2	10.0		430	
		3	10.0		650	
	T1-1	1	10.0		1590	
		2	10.0		1650	

		3	10.0		1640	
	T2M	1	10.0		6860	
		2	10.0		6700	
		3	10.0		6760	
	AX2-1	1	10.0		2970	
		2	10.0		3010	
		3	10.0		2900	

Total COD Data – Trial 1 (cont'd)

Date	**Sample**	**Sample**	**Dil. Fact**	**Corr. Fact.**	**Reading * Dil. Fact*Corr. Fact mg/L as COD**	**Spike Theo. mg/L as COD**
	Standard Sol.	1000			not done	
8/19/2004	T2P	1	10	N/A	1380	
		Spike			395	354
	T3	1	10		6210	
		Spike			640	716
	T4-1	1	10		1840	
		Spike			390	388
	PCE	1	10		450	
		Spike			319	284
	T1-1	1	10		1600	
		Spike			391	370
	T2M	1	10		6210	
		Spike			683	716
	AX2-1	1	10		2800	
		Spike			478	460
	STD	1000			not done	

Soluble OP Data – Trial 1

Date	Sam	Samp #	Dil. Fact	Reading X Dil. Fact. mg/L as $PO4^{3-}$	Spike Theo. Conc. mg/L as $PO4^{3-}$	P Conc. (Column 5 /3.06) mg/L as P
8/9/2004	T2P	1	10	76		25
		2	10	75		25
		3	10	78		25
		Spike		15.7	16.1	5
	T3	1	10	81		26
		2	10	79		26
		3	10	83		27
		Spike		13.5	17.9	4
	T4-1	1	10	53		17
		2	10	52		17
		3	10	52		17
		Spike		10.4	14.7	3
	PCE	1	10	10		3
		Spike 1		10.8	10.8	4
		Spike 2		20.3	20.6	7
8/9/2004	T1-1	1	10	40		13
		Spike 1		12.3	13.2	4
		Spike 2		22.3	22.4	7
	T2M	1	10	95		31
		2	10	92		30
		3	10	89		29
		Spike		14.6	18.9	5
	Round OX - 1	1	10	negative		
		Spike		9.7		3

	AX1	1	10	23		8

Soluble OP Data – Trial 1 (cont'd)

Date	Sam	Sample #	Dil. Factor	Reading X Dilution Factor mg/L as $PO4^{3-}$	Spike Theoretical Conc. mg/L as $PO4^{3-}$	P Conc. (Column 5 /3.06) mg/L as P
8/9/2004		Spike		11.0	11.8	4
						0
						0
8/10/2004	T2P	1	5.56	105		34
		Spike		24.4	25.0	8
						0
	T3	1	5	101		33
		Spike		26.6	26.2	9
						0
	T4-1	1	5	46		15
		Spike		16.8	17.4	5
						0
	PCE	1	5	21.5		7
		Spike		12.4	13.4	4
	T1-1	1	5	34		11
		Spike		15.1	15.4	5
	T2M	1	12.5	271		89
8/10/2004	T2 M	Spike		27.8	27.4	9
	Round OX -1	1	5	2.0		1
		Spike		9.5	10.3	3
	AX2 -1	1	5.75	31.6		10
		Spike		14.9	14.4	5
	T4-2	1	5	44.0		14
		Spike		16.9	17.0	6

	T1-2	1	5	12.0		4

Soluble OP Data – Trial 1 (cont'd)

Date	Sam	Sample #	Dil. Factor	Reading X Dilution Factor mg/L as $PO4^{3-}$	Spike Theo. Conc. mg/L as $PO4^{3-}$	P Conc. (Column 5 /3.06) mg/L as P
8/10/2004	Ax2-2	1	5	16.0		5
		Spike		12.5	12.6	4
	Round Ox-2	1	5	negative		
		Spike		9.6		3
						0
						0
8/12/2004	T2P	1	5	100		33
		2	5	97		32
		3	5	99		32
		Spike		26.4	25.8	9
	T3	1	5	101		33
		2	5	103		34
		3	5	104		34
		Spike		27.4	26.4	9
	T4-1	1	7.7	65.4		21
		2	7.7	60.0		20
		3	7.7	60.0		20
		Spike		16.5	16.5	5

Soluble OP Data – Trial 1 (cont'd)

Date	Sam	Sample #	Dil. Factor	Reading X Dilution Factor mg/L as $PO4^{3-}$	Spike Theoretical Conc. mg/L as $PO4^{3-}$	P Conc. (Column 5 /3.06) mg/L as P
8/12/2004	PCE	1	6.0	29.2		10
		Spike		14.3	13.9	5
	T1-1	1	5	57.0		19
		Spike		19.3	19.1	6
	T2M	1	9.1	113		37
		2	9.1	115		37
		3	9.1	116		38
		Spike		20.7	20.1	7
	Round OX-1	1	12.8	3.33		1
		Spike		1.76	1.25	1
	AX2-1	1	5	23.5		8
		Spike		14.3	13.8	5
	T4-2	1	5	No sample taken for this test		
	T1-2	1	6.7	70.7		23
		Spike		20.1	18.5	7
	Ax2-2	1	5	27.0		9
		Spike		10.3	10.0	3
	Round Ox-2	1	5	5.25		2
		Spike		OR	2.00	
8/17/2004	T2P	1	5.3	105		34
		Spike		27.4	25.9	9

Soluble OP Data – Trial 1 (cont'd)

Date	Sam	Sample #	Dil. Factor	Reading X Dilution Factor mg/L as $PO4^{3-}$	Spike Theoretical Conc. mg/L as $PO4^{3-}$	P Conc. (Column 5 /3.06) mg/L as P
8/17/2004	T3	1	5	118		38
		Spike		29.6	28.8	10
	T4-1	1	5	78.0		25
		Spike		22.9	22.5	7
	PCE	1	5	28.0		9
		Spike		15.9	14.5	5
	T1-1	1	5	77.5		25
		Spike		23.3	22.4	8
	T2M	1	5	116		38
		Spike		30.6	28.5	10
	Round OX-1	1	5	6.05		2
		Spike		OR	11.0	
	AX2-1	1	5	26.5		9
		Spike		14.8	14.2	5
8/19/2004	T2P	1	6.02	113		37
		Spike		25.5	25.0	8
	T3	1	5.8	109.3		36
		Spike		25.2	25.0	8
	T4-1	1	5.2	48		16
		Spike		17.8	17.4	6
	PCE	1	5	24.5		8
		Spike		14.3	13.9	5
	T1-1	1	3.8	87		28
		Spike		28.4	28.1	9

Soluble OP Data – Trial 1 (cont'd)

Date	Sam	Sample #	Dil. Factor	Reading X Dilution Factor mg/L as $PO4^{3-}$	Spike Theoretical Conc. mg/L as $PO4^{3-}$	P Conc. (Column 5 /3.06) mg/L as P
8/19/2004	T2M	1	7.9	105.6		34
		Spike		20.9	20.6	7
	Round OX-1	1	5	7.5		2
		Spike		11.2	11.1	4
	AX2-1	1	4.5	21.4		7
		Spike		14.4	13.8	5
	T4-2	1	6.3	59.4		19
		Spike		18.3	17.6	6
	T1-2	1	3.8	34.5		11
		Spike		18.2	17.4	6
	Ax2-2	1	5	38.5		13
		Spike		17.6	16.2	6
	Round Ox-2	1	7.1	12.1		4
		Spike		11.0	11.4	4

Total P Data – Trial 1

Date	Sample	Sample #	Dil Fact	Corr Fact	Reading * Dil. Fact*Corr Fact mg/L as $PO4^{3-}$	Spike Conc. mg/L as $PO4^{3-}$	P Conc. mg/L as P
8/9/2004	T2P	1	5	1.055	132		43
		2	5	1.055	133		44
		3	5	1.055	140		46
		Spike			38.0	34.9	12
	T3	1	10	1.032	459		150
		2	10	1.032	506		165
		3	10	1.032	446		146
		Spike			60.0	54.5	20
	T4-1	1	5	1.037	189		62
		2	5	1.037	191		62
		3	5	1.037	212		69
		Spike			53.4	47.1	17
	PCE	1	5	1.032	15.5		5
		Spike			15.1	12.7	5
	T1-1	1	5	1.043	206		67
		Spike			48.7	48.5	16
	T2M	1	10	1.024	604		197
		2	10	1.024	641		209
		3	10	1.024	584		191
		Spike			74.1	68.2	24
	Round OX -1	1	5	The sample was not filtered so results included mixed liquor TP	-	-	-

Total P Data – Trial 1 (cont'd)

Date	Sample	Sample #	Dil Fact	Corr Fact	Reading * Dil. Fact*Corr Fact mg/L of PO_4^{3-}	Spike	Conc. mg/L of P
8/9/2004	AX2-1	1	5	1.034	385		126
		Spike			86.9	83.3	28
8/10/2004	T3	1	10.6	1.049	458		150
		Spike			47.8	45.5	16
	T4-1	1	10	1.06	210		69
		Spike			34.0	34.7	11
	PCE	1	10	1.063	19.1		6
		Spike			24.6	25.9	8
	T2M	1	10	1.065	563		184
		Spike			54.5	51.5	18
	Round OX -1	1	10.4	1.047	105		34
		Spike			28.6	29.8	9
	AX2-1	1	10	1.083	354		116
		Spike			41.6	41.4	14
	Round OX-2	1	31.3	1.049	105		34
		Spike			25.3	26.6	8
	STD	50 ppm			46.6		15
					45.0		15
					45.7		15
8/12/2004	T3	1	10	1.031	533		174
		2	10	1.031	508		166
		3	10	1.031	535		175
		Spike			26.6	30.3	9
	T4-1	1	10	1.033	161		53
		2	10	1.033	171		56
		3	10	1.033	165		54
		Spike			12.2	12.8	4
	PCE	1	10	1.071	13		4

Total P Data – Trial 1 (cont'd)

Date	Sample	Sample #	Dil Fact	Corr Fact	Reading * Dil. Fact*Corr Fact Conc. mg/L as $PO4^{3-}$	Spike Theo. Conc. mg/L as $PO4^{3-}$	P Conc. mg/L as P
8/12/2004		Spike			5.1	5.4	2
	T2M	1	10	1.033	583		190
		2	10	1.033	558		182
		3	10	1.033	616		201
		Spike			32.3	33.1	11
							0
	Round OX -1	1	10	1.042	148		48
		Spike			10.5	11.9	3
	AX2-1	1	10	1.043	345		113
		Spike			21.8	21.4	7
	Round OX -2	1	10		127		42
		Spike			9.6	11.1	3
	STD	10 ppm			9.6		3
8/17/2004	T3	1	10.0	1.036	500		164
		Spike			26.4	28.7	9
	T4-1	1	10	1.06	174		57
		Spike			12.4	13.1	4
	PCE	1	10	1.048	19.9		7
		Spike			10.9	5.5	4
	T2M	1	10	1.029	658		215
		Spike			54.5	51.5	18
	Round OX -1	1	10.0	1.037	137		45
		Spike			9.9	11.1	3
	AX2-1	1	10	1.031	348		114
		Spike			20.0	21.4	7
	STD	10 ppm			9.0		3

Total P Data – Trial 1 (cont'd)

Date	Sample	Sample #	Dil Fact	Corr Fact	Reading * Dil. Fact*Corr Fact	Spike Theo. Conc. mg/L as $PO4^{3-}$	P Conc. mg/L as P
8/19/2004	T3	1	10	1.020	469		153
		2	10	1.020	525		172
		3	10	1.020	530		173
		Spike			27.1	29.8	9
	T4-1	1	10	1.027	179		58
		2	10	1.027	163		53
		3	10	1.027	185		60
		Spike			11.8	13.2	4
	PCE	1	10	1.025	17		6
		Spike			4	5.7	1
	T2M	1	10	1.020	571		187
		2	10	1.020	500		163
		3	10	1.020	544		178
		Spike			28.7	31.3	9
	Round OX -1	1	10	1.010	99		32
		Spike			8.5	9.8	3
	AX2-1	1	10	1.021	302		99
		Spike			17.9	19.7	6
	Round OX -2	1	10	1.014	196		64
		Spike			9.9	14.6	3

TSS/VSS Data – Trial 1

W1	Paper only
W2	Paper with residue after one hour at 103 C
W3	Paper with residue after 15 minutes in furnace

Date	**Sam**	**Dil. Fact.**	**Samp Size ml**	**W1 g**	**W2 g**	**W3 g**	**TSS mg/L**	**VSS mg/L**
9-Aug	T2P	10	50	2.6912	2.6936	-	480	-
		10	50	2.6766	2.6792	-	520	-
	T3	20	30	2.6942	2.6993	0.1123	3400	-
		20	30	2.6716	2.676	0.1127	2933	-
	T4-1	10	50	2.6726	2.6776	2.6204	1000	-
		10	50	2.674	2.6796	2.6183	1120	-
	T2M	20	30	2.6707	2.6771	2.6701	4267	-
		20	30	2.6823	2.6891	0.1133	4533	-
	T1-1	10	50	2.6727	2.6772	-	900	-
		10	50	2.6734	2.679	-	1120	-
	Ax2-1	10	50	2.6851	2.6902		1020	-
		10	50	2.7043	2.7101		1160	-
10-Aug	T2P	10	50	2.718	2.725	-	1400	-
		10	50	2.6991	2.7066	-	1500	-
	T3	10	30	2.6948	2.7072	0.1100	4133	3367
		10	30	2.6973	2.7086	0.1101	3767	3300
	T4-1	-	-	LOST SAMPLE IN THE FRIDGE - HOLE IN THE BOTTLE				
		-	-	-	-	-		
	T2M	10	30	2.6816	2.6944	0.1148	4267	3800
		10	30	2.6981	2.7112	2.6997	4367	3833
	T1-1	10	50	2.6971	2.7027	-	1120	-
		10	50	2.6853	2.6912	-	1180	-
	T1-2	10	100	2.6817	2.6952	-	1350	-
		10	50	2.724	2.7307	-	1340	-

TSS/VSS Data – Trial 1 (cont'd)

Date	Sam	Dil. Fact.	Samp Size ml	W1 g	W2 g	W3 g	TSS mg/L	VSS mg/L
10-Aug	Ax2-1	10	50	2.6995	2.7105	-	2200	-
		10	50	2.7252	2.7365	-	2260	-
	Ax2-2	10	50	2.7245	2.733	-	1700	-
		10	50	2.7286	2.7388	-	2040	-
12-Aug	T2P	10	50	2.6875	2.6933	-	1160	-
		10	50	2.6906	2.6961	-	1100	-
	T3	10	30	2.672	2.6833	0.1092	3767	3367
		10	30	2.5912	2.6028	2.5924	3867	3467
	T4-1	10	50	2.6902	2.6947	2.6409	900	-
		10	50	2.703	2.7075	2.6528	900	-
	T2M	10	30	2.7112	2.7269	2.7129	5233	4667
		10	30	2.6865	2.6993	0.1077	4267	3800
	T1-1	10	50	2.6788	2.6831	-	860	-
		10	50	2.7075	2.7127	-	1040	-
	T1-2	10	50	2.7231	2.7285	-	1080	-
		10	50	2.7095	2.715	-	1100	-
	Ax2-1	10	50	2.7182	2.728	-	1960	-
		10	50	2.7045	2.7155	-	2200	-
	Ax2-2	10	50	2.6763	2.6855	-	1840	-
		10	50	2.6771	2.6874	-	2060	-
17-Aug	T2P	10	50	2.6619	2.6704	-	1700	-
		10	50	2.6701	2.6789	-	1760	-
	T3	10	50	2.5975	2.6143	2.6	3360	2860
		10	50	2.612	2.6294	2.6153	3480	2820
	T4-1	10	50	2.6762	2.6813	LOST	1020	-
		10	50	2.6383	2.6437	2.638	1080	-
	T2M	10	50	2.6041	2.6273	2.608	4640	3860
		10	50	2.6073	2.6324	2.6116	5020	4160

TSS/VSS Data – Trial 1 (cont'd)

Date	Sam	Dil.	Samp	W1	W2	W3	TSS	VSS
		Fact.	Size	g	g	g	mg/L	mg/L
			ml					
	T1-1	10	50	2.641	2.6457	-	940	-
		10	50	2.6428	2.648	-	1040	-
19-Aug	Ax2-1	10	50	2.6528	2.6626		1960	
		10	50	2.6393	2.6489		1920	
	T2P	10	50	2.6268	2.6308	-	800	-
		10	50	2.5898	2.5951	-	1060	-
	T3	10	30	2.6319	2.6439	2.6333	4000	3533
		10	30	2.5796	2.59	2.5869	3467	-
	T4-1	10	50	2.5958	2.6011	2.5961	1060	1000
		10	50	2.6013	2.6068	2.6016	1100	1040
	T2M	10	50	2.6147	2.6348	2.6178	4020	3400
		10	50	2.6179	2.6388	2.621	4180	3560
	T1-1	10	50	2.6788	2.6831	-	860	-
		10	50	2.6442	2.6495	-	1060	-
	T1-2	10	50	2.6112	2.6166	-	1080	-
		10	50	2.603	2.6082	-	1040	-
	Ax2-1	10	50	2.6404	2.6503	-	1980	-
		10	50	2.635	2.6449	-	1980	-
	Ax2-2	10	50	2.5987	2.6084	-	1940	-
		10	50	2.5997	2.6091	-	1880	-

A.3. Trial 2 Test Result Raw Data

Legend for the Tables Included in Section A.3

T2P [=] Supernatant
T2M[=] FZ2
T3[=] FZ1
PCE [=] Primary clarifier effluent
T4-1[=] Anaerobic tank (fed to fermenter)
AX2-1[=] Anoxic tank – stage 2
T1-1[=] Anaerobic tank – stage 1
T4-2[=] Train No. 2 Anaerobic tank – stage 2
AX2-2 [=] Train No. 2 Anoxic tank – stage 2
T1-2 [=] Train No.2 Anaerobic tank – stage 1
Round OX-1 [=] Oxic Stage 2
Round OX-2 [=] Train No. 2 Oxic Stage 2

VFA Titration Results

Date	Sample #	Initial pH	Burette Reading (ml)			VFA	VFA
			Initial	pH 5.1	pH 3.5	mg/L[1] as HAc	meq/L[2]
9/13/2004	T2P	7.08	0	1.86	2.32	7	0.1
	T2P	7.07	0	1.82	2.34	19	0.3
	T2P	7.08	0	1.87	2.36	12	0.2
	T2M	6.67	0	4.28	9.81	874	14.6
	T2M	6.63	0	4.23	9	735	12.2
	T2M	6.71	0	4.24	9.17	768	12.8
	T3	6.72	0	3.63	7.66	617	10.3
	T3	6.7	0	3.64	7.65	612	10.2
	PCE	8.27	0	2.12	2.62	14	0.2
	PCE	8.31	0	2.11	2.61	14	0.2
	PCE	8.23	0	2.12	2.65	19	0.3
	T4-1	7.32	0	2.02	2.59	26	0.4
	T4-1	7.3	0	2.01	2.68	44	0.7
	Ax2-1	7.07	0	1.8	2.72	92	1.5
	T1-1	7.29	0	2.05	2.61	24	0.4
	T1-1	7.3	0	2	2.64	39	0.7
9/15/2004	T2P	7.1	0	1.86	2.35	13	0.2
	T2P	7.1	0	2.02	2.5	8	0.1
	T2P	7.1	0	1.87	2.38	16	0.3

Notes: (1) VFA as mg/L as HAc = meq/L * MW_{Hac} where MW_{Hac} = 60
(2) Calculated from equation provided in Anderson et al. (1992)

VFA Results – Trial 2 (cont'd)

Date	Sample #	Initial pH	Burette Reading (ml)	VFA	VFA		
			Initial	pH 5.1	pH 3.5	mg/L[1] as HAc	meq/L[2]
	T2M	6.69	0	4.02	8.56	700	11.7
	T3	6.72	0	3.59	6.92	490	8.2
	T3	6.77	0	3.6	6.99	503	8.4
	PCE	8.05	0	1.68	2.3	42	0.7
	PCE	7.92	0	1.68	2.25	33	0.5
	T4-1	7.19	0	1.82	2.65	76	1.3
	T4-1	7.17	0	1.76	2.59	77	1.3
	T1-1	7.19	0	1.79	2.65	82	1.4
	T1-1	7.2	0	1.82	2.57	61	1.0
9/17/2004	T2P	7.15	0	1.79	2.35	27	0.4
	T2P	7.08	0.0	1.8	2.3	27	0.4
	T2M	6.77	0.0	4.2	8.5	658	11.0
	T2M	6.72	0.0	4.3	8.2	581	9.7
	T3	6.78	0.0	3.6	7.3	550	9.2
	T3	6.76	0.0	3.7	7.3	537	8.9
9/17/2004	PCE	7.18	0.0	1.5	2.2	62	1.0
	PCE	7.12	0.0	1.5	2.2	61	1.0
	T4-1	7.13	0.0	1.8	2.4	39	0.7
	T4-1	7.17	0.0	1.8	2.5	45	0.7
	T4-2	7.19	0.0	1.8	2.5	41	0.7
	AX2-1	7.04	0.0	1.8	2.7	91	1.5
	AX2-1	7.02	0.0	1.8	2.7	93	1.6
	AX2-2	7.05	0.0	1.8	2.7	83	1.4
	T1-1	7.13	0.0	1.8	2.4	47	0.8
	T1-1	7.15	0.0	1.8	2.4	47	0.8
	T1-2	7.14	0.0	1.8	2.8	95	1.6
	T1-2	7.15	0.0	2.0	2.8	58	1.0
10/5/2004	T2P	7.07	0	1.81	2.29	11	0.2
	T2P	7.10	0.0	1.8	2.3	20	0.3
	T2M	6.74	0.0	4.2	8.3	617	10.3
	T2M	6.74	0.0	4.0	8.0	601	10.0
	T2M	6.75	0.0	3.9	8.1	630	10.5

Notes: (1) VFA as mg/L as HAc = meq/L * MW_{Hac} where MW_{Hac} = 60
(2) Calculated from equation provided in Anderson et al. (1992)

VFA Results – Trial 2 (cont'd)

Date	Sample #	Initial pH	Burette Reading (ml)	VFA	VFA		
			Initial	pH 5.1	pH 3.5	mg/L[1] as HAc	meq/L[2]
	T3	6.76	0.0	3.5	7.3	581	9.7
	PCE	8.39	0.0	1.9	2.5	28	0.5
	PCE	8.17	0.0	1.9	2.4	32	0.5
	T4-1	7.36	0.0	1.9	2.5	43	0.7
	T4-2	7.27	0.0	1.9	2.6	57	0.9
	T4-2	7.26	0.0	1.9	2.6	59	1.0
	AX2-1	7.02	0.0	1.6	2.5	98	1.6
	AX2-1	7.01	0.0	1.7	2.6	92	1.5
	AX2-2	7.06	0.0	1.6	2.6	104	1.7
	AX2-2	7.03	0.0	1.6	2.6	102	1.7
	AX2-2	7.03	0.0	1.6	2.5	91	1.5
	T1-1	7.38	0.0	1.9	2.5	40	0.7
	T1-1	7.35	0.0	1.9	2.5	38	0.6
	T1-1	7.39	0.0	1.9	2.5	40	0.7
	T1-2	7.24	0.0	1.8	2.5	55	0.9
	T1-2	7.21	0.0	1.8	2.5	45	0.7
	T1-2	7.21	0.0	1.8	2.5	49	0.8

Notes: (1) VFA as mg/L as HAc = meq/L * MW_{Hac} where MW_{Hac} = 60
(2) Calculated from equation provided in Anderson et al. (1992)

Soluble COD – Trial 2

				Spike
		Sample	Result	Theoretical
Date	Sample	#	mg/L as COD	Concentration mg/L as COD
13-Sep				
	T2P	1	86	
		Spike	100	104
	T3	1	Sample was lost	
		Spike		
	T4-1	1	31	
		Spike	74	77
	PCE	1	147	

		Spike	136	135
	T1-1	1	35	
		Spike	78	79

Soluble COD Data – Trial 2 (cont'd)

Date	Sample	Sample #	Result mg/L as COD	Spike Theoretical Concentration mg/L as COD
	T2M	1	Sample was lost	
		Spike		
	AX2-1	1	26	
		Spike	71	74
	Standard Sol.	100 ppm	123	
15-Sep	T2P	1	74	
		Spike	98	97
	T3	1	Sample was lost	
		Spike		
	T4-1	1	33	
		Spike	81	76
	PCE	1	134	
		Spike	128	127
	T1-1	1	44	
		Spike	74	82
	T2M	1	153	
		Spike	134	136
	AX2-1	1	17	
		Spike	74	68
	STD	100	120	
17-Sep	T3	1	142	

Soluble COD Data – Trial 2 (cont'd)

Date	Sample	Sample #	Result mg/L as COD	Spike Theoretical Concentration mg/L as COD
17-Sep		Spike	138	124
	T4-1	1	44	
		2	43	
		3	No space left in reactor	
		Spike	75	85
	PCE	1	83	
		Spike	94	94
	T1-1	1	51	
		Spike	86	89
	T2M	1	165	
		2	160	
		3	163	
		Spike	158	145
	AX2-1	1	23	
		Spike	75	75
	T4-2	1	30	
		Spike	67	67
	T1-2	1	24	
		Spike	59	64
	Standard Sol.	100 ppm	113	
			127	
			105	
5-Oct	T2P	1	62	
		2	63	
		3	64	
		Spike	84	84
	T3	1	162	
		2	163	
		3	161	
		Spike	143	141
	T4-1	1	25	
		2	26	
		3	24	

		Spike	64	65

Soluble COD Data – Trial 2 (cont'd)

Date	Sample	Sample #	Result mg/L as COD	Spike Theoretical Concentration mg/L as COD
	PCE	1	129	
5-Oct		Spike	114	117
	T1-1	1	27	
		Spike	71	66
	T2M	1	193	
		2	179	
		3	177	
		Spike	150	152
	AX2-1	1	21	
		Spike	64	63
	T4-2	1	26	
		Spike	68	65
	T1-2	1	24	
		Spike	62	64
	Standard Sol.	100 ppm	121	
			105	

Total COD Data – Trial 2

					Spike
Date	**Sample**	**Sample #**	**Dil. Fact**	**Reading * Dil. Fact. mg/L as COD**	**Theoretical Concentration mg/L as COD**
13-Sep	T2P	1	10	260	
		Spike		301	290
	T3	1	10	Sample was lost while unfreezing - jar cracked	
		Spike			
	T4-1	1	10	1600	

		Spike		358	390

Total COD Data – Trial 2 (cont'd)

					Spike
Date	**Sample**	**Sample #**	**Dil. Fact**	**Reading * Dil. Fact. mg/L as COD**	**Theoretical Concentration mg/L as COD**
	PCE	1	10	320	
		Spike 1		304	294
	T1-1	1	10	Sample was lost while unfreezing - jar cracked	
		Spike 1			
	T2M	1	10	14430	
		Spike		1350	1352
	AX2-1	1	10	2670	
		Spike		496	470
	STD	1000 ppm		not done	Assumed 1080
15-Sep	T2P	1	10.0	120	
		2	10.0	140	
		3	10.0	120	
		Spike		307	281
	T3	1	10	10560	
		2	10	10380	
		3	10	10320	
		Spike		1064	1053
	T4-1	1	10	1700	
		2	10	1630	
		3	10	1770	
		Spike		420	399
	PCE	1	10	130	
		Spike		304	281
	T1-1	1	10.0	1060	
		Spike		358	351
	T2M	1	10	13100	
			10	12970	
			10	13330	
		Spike		1300	1256

Total COD Data – Trial 2 (cont'd)

					Spike
Date	**Sample**	**Sample #**	**Dil. Fact**	**Reading * Dil. Fact. mg/L as COD**	**Theoretical Concentration mg/L as COD**
	AX2-1	1	10	3000	
		Spike		489	496
15-Sep	STD	1000		1085	
17-Sep	T2P	1	10	160	
		Spike		391	285
	T3	1	10	11240	
		Spike		1084	1116
	T4-1	1	10	2240	???
		Spike		466	441
	PCE	1	10	jar cracked in freezer	
		Spike 1			
	T1-1	1	10	1280	
		Spike 1		389	369
	T2M	1	10	13080	
		Spike		1243	1254
	AX2-1	1	10	2990	
		Spike		511	497
	STD	1000 ppm		1092	
5-Oct	T2P	1	10.0	200	
		2	10.0	230	
		3	10.0	220	
		Spike		310	288
	T3	1	10	11750	
		2	10	11790	
		3	10	11760	
		Spike		1151	1154
	T4-1	1	10	1830	
		2	10	1770	

		3	10	1600	
		Spike		432	402

Total COD Data – Trial 2 (cont'd)

					Spike
Date	**Sample**	**Sample #**	**Dil. Fact**	**Reading * Dil. Fact. mg/L as COD**	**Theoretical Concentration mg/L as COD**
	PCE	1	10	190	
		Spike		295	286
	T1-1	1	10.0	1700	
		Spike		426	399
	T2M	1	10	14820	
			10	15100	
			10	15780	
		Spike		1368	1414
	AX2-1	1	10	5000	
		Spike		668	647
	STD	1000		1088	

Soluble OP Data – Trial 2

					Spike	
Date	**Sample**	**Sample #**	**Dil. Fact.**	**Reading * Dil Fact mg/L as $PO4^{3-}$**	**Theoretical Conc. mg/L as $PO4^{3-}$**	**P Conc. mg/L as P**
13-Sep	T2P	1	10	94		30.72
		Spike		18.3	17.5	5.98
	T3	1	10	Sample was lost		
		Spike				0.00
	T4-1	1	10	54		17.65

		Spike		14.6	14.3	4.77
	PCE	1	10	32		10.46
		Spike 1		13.3	12.6	4.35

Soluble OP Data – Trial 2 (cont'd)

					Spike	
Date	**Sample**	**Sample #**	**Dil. Fact.**	**Reading * Dil Fact**	**Theoretical**	
				mg/L as $PO4^{3-}$	**Conc. mg/L as $PO4^{3-}$**	**P Conc. mg/L as P**
	T1-1	1	10	43		14.05
		Spike 1		14.5	13.4	4.74
	T2M	1	10	Lost sample		
13-Sep		Spike		1.15	0.8	0.38
	AX2-1	1	10	20		6.54
		Spike		11.9	11.6	3.89
	STD	50 ppm		Not done		
15-Sep	T2P	1	11.1	92		30.14
		Spike		17.3	16.6	5.65
	T3	1		Lost sample		
		Spike				
	T4-1	1	10	52		16.99
		Spike		13.4	14.2	4.38
	PCE	1	10	18		5.88
		Spike		11.7	11.4	3.82
	T1-1	1	10	44		14.38
		Spike		13.4	13.5	4.38
	T2M	1	10	114		37.25
		Spike		20.3	19.1	6.63
	Round OX -1	1	10.9	1.6		0.53
		Spike		1.45	0.64	0.47
	AX2-1	1	10.9	10.9		3.55
		Spike		10.9	10.8	3.56

Soluble OP Data – Trial 2 (cont'd)

					Spike	
Date	**Sample**	**Sample #**	**Dil. Fact.**	**Reading * Dil Fact** mg/L as $PO4^{3-}$	**Theoretical** Conc. mg/L as $PO4^{3-}$	**P Conc. mg/L as P**
17-Sep	T2P	1	10	71		23.20
		2	10	70		22.88
		3	10	70		22.88
		Spike		16	15.2	5.23
		2	10	99		32.35
		3	10	96		31.37
		Spike		17.8	17.9	5.82
	T4-1	1	10.0	36		11.76
		2	10.0	29		9.48
		3	10.0	38		12.42
		Spike		13.2	12.3	4.31
	PCE	1	10.0	15		4.90
		Spike		11.7	11.2	3.82
	T1-1	1	10	38		12.42
		Spike		13.3	13	4.35
	T2M	1	10.0	100		32.68
		2	10.0	106		34.64
		3	10.0	105		34.31
		Spike		18.1	18.4	5.92
	Round OX -1	1	10.0	3		0.88
		Spike		1.44	0.76	0.47
	AX2-1	1	10	4		1.31
		Spike		10.8	9.9	3.53
	T4-2	1	10	10		3.27
		Spike		11.0	10.4	3.59
	T1-2	1	10.0	14		4.58
		Spike		10.8	10.7	3.53

	Ax2-2	1	10	1		0.33
		Spike		10.9	9.7	3.56
	Round Ox-2	1	10	1		0.26

Soluble OP Data – Trial 2 (cont'd)

					Spike	
Date	**Sample**	**Sample #**	**Dil. Fact.**	**Reading * Dil Fact** mg/L as $PO4^{3-}$	**Theoretical** Conc. mg/L as $PO4^{3-}$	**P Conc. mg/L as P**
		Spike		1.22	0.57	0.40
	STD	50 ppm		48.1		15.72
5-Oct	T2P	1	10	86		28.10
		Spike		9.3	8.5	3.04
	T3	1	10	109		35.62
		Spike		10	9.7	3.27
	T4-1	1	10.0	30		9.80
		Spike		4.2	5.7	1.37
	PCE	1	10.0	27		8.82
		Spike		4.0	5.6	1.31
	T1-1	1	10	3		0.92
		Spike		No room left in reactor		
	T2M	1	10.0	102		33.33
		Spike		9.9	9.3	3.24
	Round OX -1	1	10.0	4		1.21
		Spike		OR		
	AX2-1	1	25	10		3.27
		Spike		2.1	4.45	0.69
	T4-2	1	10	44		14.38
		Spike		6.8	6.4	2.22
	T1-2	1	10.0	2		0.65
		Spike		No room left in reactor		
	Ax2-2	1	20	10		3.27
		Spike		No room left in reactor		
	Round	1	10	1		0.36

	Ox-2					
		Spike		OR		
	STD	10 ppm		8.5	Was cold - out of the fridge	

Total P Data – Trial 2

Date	Sample	Sample #	Dil. Fact.	Reading * dil. fact. mg/L as $PO4^{3-}$	Spike Theo. Conc. mg/L as $PO4^{3-}$	P Conc. mg/L as P
13-Sep	T3	1	10	OR	1232	
		Spike		65.2		21.3
	T4-1	1	10	267		87.3
		Spike		15.7	16.9	5.1
	PCE	1	10	26		8.5
		Spike 1		7.1		2.3
	T2M	1	10	OR	1498	
		Spike		78.5		25.7
	Round OX -1	1	10	Was lost - was not refrigerated		
		Spike				
	AX2-1	1	10	358		117.0
		Spike		21.2	21.5	6.9
	STD	10 ppm		7.2		2.4
15-Sep	T3	1	10	OR	930	
		Spike		50.1		16.4
	T4-1	1	10	216		70.6
		Spike		14.4	14.4	4.7
	PCE	1	10	10		3.3
		Spike		3.6	4.1	1.2
	T2M	1	10	OR	1354	
		Spike		71.3		23.3
	Round OX -1	1	10	26.0		8.5
		Spike		5.8	4.9	1.9
	AX2-1	1	10	375.0		122.5
		Spike		22.3	22.3	7.3

Total P Data – Trial 2 (cont'd)

Date	Sample	Sample	Dil. Fact.	Reading * dil. fact. mg/L as $PO4^{3-}$	Spike Theo.	
15-Sep	STD	10 ppm		7.2		2.4
17-Sep	T3	1	20	972		317.6
		2	20	1040		339.9
		3	20	1052.0		343.8
		Spike		27.1	29.1	8.9
	T4-1	1	10.0	162		52.9
		2	10.0	170		55.6
		3	10.0	182		59.5
		Spike		11.8	12.2	3.9
	PCE	1	10.0	Lost in freezer		
		Spike				0.0
	T2M	1	20.0	1046		341.8
		2	20.0	1060		346.4
		3	20.0	1058		345.8
		Spike		31.3	30.0	10.2
	Round OX -1	1	10.0	<1		
		Spike		2.8		0.9
	AX2-1	1	10	339		110.8
		Spike		19.3	20.5	6.3
	Round Ox-2	1	10	UR		
		Spike		2.1		0.7
	STD	10 ppm		7.2		2.4
5-Oct	T3	1	10	OR		
		2	10	OR		
		3	10	1075.0	> 1000 (method limit)	351.3
		Spike		58.7	58.2	19.2
	T4-1	1	10.0	182		59.5
		2	10.0	209		68.3
		3	10.0	193		63.1
		Spike		12.7	14.2	4.2

	PCE	1	10.0	20		6.5

Total P Data – Trial 2 (cont'd)

Date	Sample	Sample #	Dil. Fact.	Reading * dil. fact. mg/L as $PO4^{3-}$	Spike Theo. Conc. mg/L as $PO4^{3-}$	P Conc. mg/L as P
		Spike		4.7	5.4	1.5
	T1-1	1	10	192		62.7
		Spike		12.9	14.0	4.2
	T2M	1	10	OR		
		2	10	OR		
		3	10	OR	1339	
		Spike		71.4	N/A	23.3
	Round OX -1	1	10	21		6.9
		Spike		4.8	5.5	1.6
	AX2-1	1	10	675		220.6
		Spike		36.0	38.2	11.8
	Round Ox-2	1	10	13		4.2
		Spike		3.7	5.10	1.2
	STD	10 ppm		8.9		2.9

TSS/VSS Data – Trial 2

W1Paper only
W2 Paper with residue after one hour at 103 C
W3Paper with residue after 15 minutes in furnace

Date	Sample	Dilution Factor	Sample Size ml	W1 g	W2 g	W3 g	TSS mg/L	VSS mg/L
13-Sep	T2P	10	150	2.651	2.651	-	40	-
		10	150	2.615	2.615	-	0	-
	T3	20	50	2.641	2.665	2.645	9760	7960
		20	50	2.638	2.663	2.642	10200	8360
	T4-1	10	50	2.61	2.617	2.611	1320	1200

		10	50	2.605	2.611	2.606	1100	1060
	T2M	20	30	2.619	2.648	2.624	19467	15933

TSS/VSS Data – Trial 2 (cont'd)

Date	**Sample**	**Dilution Factor**	**Sample Size ml**	**W1 g**	**W2 g**	**W3 g**	**TSS mg/L**	**VSS mg/L**
		20	30	2.614	2.643	2.619	19667	16133
	T1-1	10	50	2.593	2.598	-	1120	-
13-Sep	T1-1	10	50	2.626	2.632	-	1220	-
	Ax2-1	10	50	2.633	2.645	-	2380	-
		10	50	2.631	2.644	-	2460	-
15-Sep	T2P	10	150	2.613	2.615	-	140	-
		10	150	2.598	2.601	-	140	-
	T3	20	50	2.624	2.643	2.6270	7840	6440
		20	50	2.582	2.6	2.586	7240	5680
	T4-1	10	50	2.652	2.658	2.653	1260	1100
		10	50	2.591	2.597	2.592	1140	1020
	T2M	20	50	2.621	2.645	2.625	9480	7760
		20	50	2.614	2.637	2.618	9200	7520
	T1-1	10	50	2.596	2.602	-	1140	-
		10	50	2.588	2.594	-	1140	-
	Ax2-1	10	50	2.582	2.593	-	2160	-
		10	50	2.64	2.651	-	2220	-
	Ax2-2	10	50	2.681	2.691	-	2140	-
		10	50	2.712	2.723	-	2220	-
17-Sep	T2P	10	150	2.584	2.585	-	13	-
		10	150	2.624	2.624	-	7	-
	T3	20	50	2.62	2.641	2.6242	8280	6760
		20	50	2.611	2.623	2.613	4760	4040
	T4-1	10	50	2.701	2.706	2.701	1100	1040
		10	50	2.711	2.716	2.711	980	1060
	T2M	20	50	2.589	2.613	2.594	9440	7680
		20	50	2.636	2.657	2.64	8440	6840
	T1-1	10	50	2.577	2.583	-	1020	-
		10	50	2.617	2.622	-	1020	-
	Ax2-1	10	50	2.718	2.729	-	2200	-
		10	50	2.711	2.722	-	2260	-
5-Oct	T2P	10	150	2.7	2.7	-	-13	-
		10	150	2.7	2.7	-	-20	-

	T3	20	50	2.718	2.741	2.722	9120	7440
		20	50	2.693	2.715	2.697	8640	7080

TSS/VSS Data – Trial 2 (cont'd)

Date	**Sample**	**Dilution**	**Sample**	**W1**	**W2**	**W3**	**TSS**	**VSS**
		Factor	**Size**	**g**	**g**	**g**	**mg/L**	**mg/L**
			ml					
	T4-1	10	50	2.712	2.718	2.712	1220	1120
		10	50	2.676	2.682	2.676	1180	1160
	T2M	20	50	2.743	2.766	2.748	8960	7120
		20	50	2.701	2.721	2.704	8280	6800
	T1-1	10	50	2.686	2.693	-	1400	-
		10	50	2.711	2.718	-	1400	-
	T1-2	10	50	2.675	2.681	-	1300	-
		10	50	2.704	2.71	-	1240	-
	Ax2-1	10	50	2.707	2.72	-	2540	-
		10	50	2.717	2.73	-	2480	-
	Ax2-2	10	50	2.738	2.751	-	2440	-
		10	50	2.738	2.75	-	2320	-

A.3 Trial 3 Test Result Raw Data

Legend for the Tables Included in Section A.4

T2P [=] Supernatant
T2M[=] FZ2
T3[=] FZ1
PCE [=] Primary clarifier effluent
T4-1[=] Anaerobic tank (fed to fermenter)
AX2-1[=] Anoxic tank – stage 2
T1-1[=] Anaerobic tank – stage 1
T4-2[=] Train No. 2 Anaerobic tank – stage 2
AX2-2 [=] Train No. 2 Anoxic tank – stage 2
T1-2 [=] Train No.2 Anaerobic tank – stage 1
Round OX-1 [=] Oxic Stage 2
Round OX-2 [=] Train No. 2 Oxic Stage 2

VFA Results – Trial 3

Date	Sample #	Initial pH	Burette Reading (ml)			VFA	VFA
			Initial	pH 5.1	pH 3.5	mg/L[1] as HAc	meq/L[2]
2/20/2005	T2P	6.69	0	1.30	1.80	20	0.3
	T2P	6.94	0	1.30	1.80	23	0.4
	T2M	6.42	0	2.46	5.90	520	8.7
	T2M	6.52	0	2.46	5.82	511	8.5
	T2M	6.5	0	2.56	6.01	524	8.7
	T3M	6.35	0	2.46	5.84	505	8.4
	T3M	6.41	0	2.50	5.90	511	8.5
	PCE	7.87	0	1.11	1.51	11	0.2
	PCE	7.87	0	1.11	1.65	36	0.6
	T4-1	7.2	0	1.25	1.66	9	0.1
	T4-1	7.12	0	1.48	1.91	8	0.1
	Ax2-1	6.7	0	1.20	1.76	33	0.5
	Ax2-1	6.73	0	1.17	1.75	37	0.6
	Ax2-1	6.73	0	1.23	1.74	24	0.4
	T1-1	7.07	0	1.33	1.83	23	0.4
	T1-1	6.98	0	1.40	1.84	10	0.2
	T1-1	6.98	0	1.40	1.80	3	0.1
2/23/2005	T2P	7.07	0	1.29	1.65	-2	0.0
	T2P	7.03	0	1.25	1.60	-3	-0.1
	T2M	6.7	0	1.98	4.18	316	5.3
	T2M	6.73	0	2.02	4.20	312	5.2
	T2M	6.7	0	2.21	4.46	320	5.3
2/23/2005	T2M	6.52	0	2.00	4.30	328	5.5
	T3M	6.7	0	2.23	4.31	289	4.8
	T3M	6.78	0	2.02	4.35	340	5.7
	T3M	6.56	0	2.24	4.32	284	4.7
	PCE	7.63	0	1.18	1.57	7	0.1
	PCE	7.82	0	1.18	1.56	6	0.1
	T4-1	6.99	0	1.25	1.68	11	0.2
	T4-1	6.85	0	1.25	1.66	6	0.1
	T4-2	6.84	0	1.25	1.72	17	0.3
	T4-2	6.84	0	1.29	1.74	13	0.2
	Ax2-1	6.74	0	1.09	1.67	39	0.7

Notes: (1) VFA as mg/L as HAc = meq/L * MW_{Hac} where MW_{Hac} = 60
(2) Calculated from equation provided in Anderson et al. (1992)

VFA Results – Trial 3 (cont'd)

Date	Sample #	Initial pH	Burette Reading (ml)			VFA	VFA
			Initial	pH 5.1	pH 3.5	mg/L[1]	meq/L[2]
	Ax2-2	6.63	0	1.08	1.65	36	0.6
	T1-1	6.83	0	1.26	1.71	13	0.2
	T1-1	6.84	0	1.24	1.65	6	0.1
	T1-2	6.71	0	1.20	1.71	24	0.4
	T1-2	6.75	0	1.30	1.91	41	0.7
2/26/2005	T2P	6.75	0	1.25	1.66	5	0.1
	T2P	6.75	0.0	1.25	1.66	5	0.1
	T2P	6.86	0.0	1.30	1.70	4	0.1
	T2M	6.19	0.0	1.90	4.25	322	5.4
	T2M	6.22	0.0	1.86	4.15	315	5.2
	T2M	6.22	0.0	1.90	4.15	306	5.1
	T3M	6.25	0.0	1.80	4.15	329	5.5
	PCE	6.69	0.0	1.00	1.41	9	0.2
	PCE	6.69	0.0	0.99	1.54	35	0.6
	T4-1	6.71	0.0	1.15	1.61	16	0.3
	T4-1	6.66	0.0	1.17	1.60	9	0.2
	T4-1	6.64	0.0	1.14	1.60	15	0.2
	T4-2	6.64	0.0	1.16	1.61	13	0.2
	T4-2	6.65	0.0	1.13	1.60	17	0.3
	AX2-1	6.52	0.0	1.10	1.65	30	0.5
	AX2-1	6.47	0.0	1.10	1.66	31	0.5
	AX2-2	6.27	0.0	1.02	1.60	31	0.5
	T1-1	6.63	0.0	1.16	1.65	20	0.3
	T1-1	6.65	0.0	1.15	1.62	17	0.3
2/26/2005	T1-2	6.64	0.0	1.16	1.70	29	0.5
	T1-2	6.65	0.0	1.15	1.62	17	0.3

Notes: [1] VFA as mg/L as HAc = meq/L * MW_{Hac} where MW_{Hac} = 60
[2] Calculated from equation provided in Anderson et al. (1992)

VFA Results – Trial 3 (cont'd)

Date	Sample #	Initial pH	Burette Reading (ml)			VFA	VFA
			Initial	pH 5.1	pH 3.5	mg/L as HAc[1]	meq/L[2]
3/2/2005	T2P	6.75	0	1.10	1.47	1	0.0
	T2P	6.73	0.0	1.10	1.47	1	0.0
	T2P	6.71	0.0	1.13	1.52	3	0.1
	T2M	6.46	0.0	1.61	3.74	304	5.1
	T2M	6.46	0.0	1.60	3.72	303	5.0
	T2M	6.46	0.0	1.60	3.72	303	5.0
	T3	6.38	0.0	1.78	4.38	382	6.4
	T3	6.38	0.0	1.81	4.66	427	7.1
	T3	6.37	0.0	1.83	4.49	392	6.5
	PCE	7.10	0.0	1.03	1.39	3	0.0
	PCE	7.10	0.0	1.04	1.39	1	0.0
	T4-1	6.60	0.0	1.13	1.65	25	0.4
	T4-2	6.84	0.0	1.09	1.48	6	0.1
	T4-2	6.79	0.0	1.05	1.47	11	0.2
	AX2-1	6.46	0.0	0.88	1.46	39	0.7
	AX2-1	6.42	0.0	0.88	1.41	30	0.5
	AX2-2	6.52	0.0	0.90	1.46	36	0.6
	AX2-2	6.54	0.0	0.90	1.45	35	0.6
	T1-1	6.68	0.0	1.06	1.45	4	0.1
	T1-1	6.70	0.0	1.04	1.43	5	0.1
	T1-1	6.67	0.0	1.04	1.44	6	0.1
	T1-2	6.81	0.0	1.04	1.47	13	0.2
	T1-2	6.78	0.0	1.06	1.46	7	0.1
3/6/2005	T2P	6.81	0	1.16	1.49	-7	-0.1
	T2P	6.84	0	1.20	1.50	-13	-0.2
	T2P	6.79	0	1.15	1.48	-7	-0.1
	T2M	6.34	0	1.99	5.24	493	8.2

Notes: (1) VFA as mg/L as HAc = meq/L * MW_{Hac} where MW_{Hac} = 60
(2) Calculated from equation provided in Anderson et al. (1992)

VFA Results – Trial 3 (cont'd)

Date	Sample #	Initial pH	Burette Reading (ml)			VFA	VFA
			Initial	pH 5.1	pH 3.5	mg/L as HAc[1]	meq/L[2]
	T2M	6.28	0	2.00	5.24	487	8.1
	T2M	6.29	0	2.00	5.23	486	8.1
	PCE	7.37	0	1.05	1.48	16	0.3
	PCE	7.41	0	1.05	1.47	14	0.2
	PCE	7.33	0	1.05	1.48	16	0.3
	T4-1	6.69	0	1.12	1.50	2	0.0
	T4-1	6.7	0	1.10	1.55	15	0.2
	T4-1	6.67	0	1.10	1.50	5	0.1
	Ax2-1	6.61	0	1.03	1.59	35	0.6
	Ax2-1	6.53	0	1.04	1.59	32	0.5
	T1-1	6.65	0	1.11	1.53	8	0.1
	T1-1	6.7	0	1.11	1.52	7	0.1

Notes: (1) VFA as mg/L as HAc = meq/L * MW_{Hac} where MW_{Hac} = 60
(2) Calculated from equation provided in Anderson et al. (1992)

Soluble COD Data – Trial 3

				Spike
		Sample	Result	Theoretical
Date	Sample	#	mg/L as COD	Concentration mg/L as COD
02/20/05	T2P	1	66	
		2	67	
		3	65	
		Spike	89	
	T1-1	1	46	
		Spike	80	
	T4-1	1	49	
		2	44	
		3	45	
		Spike	82	
	STD	100 ppm	120	
	AX2-1	1	43	
		Spike	80	

Soluble COD Data – Trial 3 (cont'd)

				Spike
		Sample	**Result**	**Theoretical**
Date	**Sample**	**#**	**mg/L as COD**	**Concentration mg/L as COD**
		Spike	429	408
	T2M	1	403	
		2	408	
	T2M	3	392	
		Spike	581	570
	T3M	1	387	
		2	388	
		3	383	
		Spike	576	556
	STD	1000 ppm	1076	
02/23/05	T4	1	48	
		2	49	
		3	51	
		Spike	83	83
	T2P	1	40	
		2	48	
		3	41	
		Spike	76	78
	T4-2	1	26	
		Spike	69	71
	STD	100 ppm	115	
	T1-1	1	27	
		Spike	70	71
	T1-2	1	37	
		Spike	78	76

Soluble COD Data – Trial 3 (cont'd)

Date	Sample	Sample #	Result mg/L as COD	Spike Theoretical Concentration mg/L as COD
02/23/05	AX2-1	1	13	
		Spike	65	64
	AX2-2	1	21	
		Spike	69	68
	T2M	1	183	
		2	182	
		3	176	
		Spike	414	414
	T3M	1	202	
		2	200	
		3	189	
		Spike	448	429
	PCE	1	128	
	STD	1000 ppm	1113	
2/26/05	T2P	1	56	
		2	56	
		3	53	
		Spike	88	89
	T4-1	1	40	
		2	46	
		3	46	
		Spike	86	84
	T1-1	1	41	
		Spike	81	82
	STD	100 ppm	123	
	AX2-2	1	22	
		Spike	89	72
	AX2-1	1	33	
		Spike	79	78
	T4-2	1	42	
		Spike	80	
	T1-2	1	41	
		Spike	80	
	T3	1	149	
		2	148	
		3	140	
		Spike	399	373
	STD	1000 ppm	1052	

Soluble COD Data – Trial 3 (cont'd)

Date	Sample	Sample #	Result mg/L as COD	Spike Theoretical Concentration mg/L as COD
02/26/05	T2M	1	166	
		2	165	
		3	*146*	
		Spike	397	387
	PCE	1	208	
		Spike	415	419
03/02/05	T2P	1	51	
		2	47	
		3	47	
	T4-1	1	38	
		2	33	
		3	37	
	T1-1	1	46	
	STD	100 ppm	128	
	AX2-1	1	28	
		Spike	51	54
	T4-2	1	42	
	T1-2	1	52	
	AX2-2	1	30	
	PCE	1	205	
	STD	1000 ppm	1088	
	T2M	1	150	
		2	162	
		3	160	
	T3	1	123	
		2	128	
		3	122	
03/06/05	T2P	1	70	
		2	71	
		3	70	
		Spike	75	
	T4-1	1	46	
		2	46	
		3	46	
		Spike	61	
	AX2-1	1	30	
		Spike	55	

	STD	100 ppm	80	
	T1-1	1	42	

Soluble COD Data – Trial 3 (cont'd)

				Spike
		Sample	**Result**	**Theoretical**
Date	**Sample**	**#**	**mg/L as COD**	**Concentration mg/L as COD**
		Spike	59	
	T2M	1	242	
		2	273	
		3	249	
		Spike	357	
	T3M	1	198	
		2	178	
		3	190	
		Spike	322	
	STD	1000 ppm	711	
	PCE	1	174	
		Spike	309	

Total COD Data –Trial 3

						Spike
Date	**Sample**	**Sample #**	**Dil. Fact**	**Corr. Fact.**	**Reading * Dil. * Corr. Fact. mg/L as COD**	**Theoretical Concentration mg/L as COD**
02/20/05	T2P	1	10	1	1330	
		2	10	1	1160	
		3	10	1	1230	
		Spike	10	1	366	356
	T2M	1	20	1.019	23091	
		2	20	1.019	22744	
		3	20	1.019	23233	
		Spike			1108	1109
	T3M	1	20	1.02	20930	
		2	20	1.02	21338	

		3	20	1.02	20706	
		Spike			1039	1038

Total COD Data – Trial 3 (cont'd)

Date	**Sample**	**Sample #**	**Dil. Fact**		**Reading * Dil. Fact. mg/L as COD**	**Theoretical Concentration mg/L as COD**
02/20/05	STD	1000 ppm	-	-	1067	
	T4-1	1	10	1.05	2247	
		2	10	1.05	2279	
		3	10	1.05	2489	
		Spike			422	
	T1-1	1	10	1	2420	
		Spike			483	
	PCE	1	10	1.06	562	
		Spike			337	
	AX2-1	1	11.2	1.015	4252	
		Spike			564	
02/23/05	T2P	1	10	1	920	
		Spike			267	247
	T2M	1	20	1.01	15069	
		Spike			735	730
	T3M	1	20	1.01	15049	
		Spike			736	
	T4-1	1	10	1.01	2495	
		Spike			370	364
	PCE	1	10	1.03	649	
		Spike			225	226
	T1-1	1	10	1	2440	
		Spike			225	226
	STD	1000 ppm			713	
	AX2-1	1	10	1.01	4030	
		Spike			470	478
02/26/05	T2P	1	10	1	1070	
		2	10	1	1100	

		3	10	1	1040	
		Spike			210	
	T1-1	1	10	1	2530	

Total COD Data – Trial 3 (cont'd)

Date	**Sample**	**Sample #**	**Dil. Fact**	**Corr. Fact.**	**Reading * Dil.*Corr Fact. mg/L as COD**	**Theoretical Concentration mg/L as COD**
		Spike			285	
02/26/05	STD	300 ppm		1	304	
	T2M	1	20	1.01	12423	
		2	20	1.01	12383	
		3	20	1.01	12423	
		Spike			466	
	PCE	1	10	1.03	433	
		Spike			182	
	T4-1	1	10	1.01	2050	
		2	10	1.01	2202	
T4-1		3	10	1.01	2273	
		Spike			259	
	T3M	1	20	1.01	12282	
		2	20	1.01	11736	
		3	10	1.01	11756	
		Spike			449	
	AX2-1	1	10	1.03	3492	
		Spike			331	
03/02/05	T2P	1	10	1	1190	
		2	10	1	1060	
		3	10	1	1130	
		Spike			350	
	T1-1	1	10	1	-	
		Spike			-	
	STD	1000 ppm		1	1085	
	T2M	1	20	1.01	16968	
		2	20	1.01	17049	

		3	20	1.01	17029	
		Spike			907	
	PCE	1	10	1.03	450	

Total COD Data –Trial 3 (cont'd)

Date	**Sample**	**Sample #**	**Dil. Fact**	**Corr. Fact.**	**Reading * Dil.*Corr Fact. mg/L as COD**	**Theoretical Concentration mg/L as COD**
		Spike			310	
	T4-1	1	10	1.01	2242	
		2	10	1.01	2343	
T4-1		3	10	1.01	2273	
		Spike			465	
	T3M	1	20	1.01	-	
		2	20	1.01	-	
		3	10	1.01	-	
		Spike			-	
	AX2-1	1	10	1.03	3500	
		Spike			547	
03/06/05	T2P	1	10	1	130	
		Spike			324	
	T1-1	1	10	1	2050	
		Spike			447	
	STD	1000 ppm		1	1083	
	T2M	1	20	1.01	18746	
		Spike			969	
	PCE	1	10	1.02	530	
		Spike				
	T4-1	1	10	1.01	1930	
		Spike			425	
	T3M	1	20	1.01	13938	
		Spike			801	
	AX2-1	1	10	1.03	3676	
		Spike			536	

Soluble OP Data –Trial 3

					Spike	
Date	**Sample**	**Sample #**	**Dil. Fact.**	**Reading * Dil Fact** mg/L as $PO4^{3-}$	**Theoretical** Conc. mg/L as $PO4^{3-}$	**P Conc. mg/L as P**
02/20/05	T4-1	1	13.2	35.6		11.6
		2	13.2	35.6		11.6
		3	13.2	40.9		13.4
		Spike		4.5	4.3	
	T2P	1	10	108		35.3
		2	10	112		36.6
		3	10	110		35.9
		Spike		10.9	10.8	
	STD	10 ppm		10		
	T2M	1	10	172		56.2
		2	10	177		57.8
		3	10	174		56.9
		Spike		15.6	15.9	
02/20/05	T3M	1	10	193		63.1
		2	10	195		63.7
		3	10	194		63.4
		Spike		17.5		17.5
	T1-1	1	11.4	31.9		10.4
		Spike		4.2	4.2	
	AX2-1	1	10	13		4.2
		Spike		2.9	3.0	
	PCE	1	20	7.0		2.3
		Spike		0.44	0.43	
	STD	1 ppm		1.26		
02/23/05	T2P	1	10	98		32.0
		2	10	99		32.4
		3	10	95		31.0
		Spike		9.7	9.5	
	T3M	1	10	138		45.1

Soluble OP Data – Trial 3 (cont'd)

					Spike	
Date	**Sample**	**Sample #**	**Dil. Fact.**	**Reading * Dil Fact** mg/L as $PO4^{3-}$	**Theoretical** Conc. mg/L as $PO4^{3-}$	**P Conc. mg/L as P**
		2	10	139		45.4
		3	10	136		44.4
		Spike		12.5	12.8	
	STD	10 ppm		8.7		
	T2M	1	10	133		43.5
		2	10	134		43.8
		3	10	134		43.8
		Spike		12.5	12.5	
	T4	1	10	36		11.8
		2	10	32		10.5
		3	10	31		10.1
		Spike		4.3	4.4	
	T1-1	1	20.8	9		2.9
		Spike		2.4	2.5	
	T1-2	1	10	23		7.5
		Spike		3.5	3.6	
02/23/05	AX2-1	1	10	4		1.3
		Spike		2.2	2.1	
	AX2-2	1	10	12		3.9
		Spike		2.7	2.7	
	T4-2	1	10	20		6.5
		Spike		3.9	3.3	
	PCE	1	20	14.8		4.8
		Spike		0.76		
	OX1	1	20	0.4		0.13
		Spike		0.12	0.11	
	STD	1 ppm		1.2		
02/26/05	T4-1	1	10	51		16.7
		2	10	51		16.7
		3	10	51		16.7
		Spike		6.0	6.1	

Soluble OP Data –Trial 3 (cont'd)

Date	Sample	Sample #	Dil. Fact.	Reading * Dil Fact mg/L as $PO4^{3-}$	Theoretical Conc. mg/L as $PO4^{3-}$	P Conc. mg/L as P
	T2P	1	10	110		35.9
		2	10	109		35.6
		3	10	108		35.3
		Spike		10.9	10.7	
	T1-1	1	10	38		12.4
		Spike		5.2	5.0	
	T2M	1	10	163		53.3
		2	10	162		52.9
		3	10	161		52.6
		Spike		15.0	15.0	
	T3M	1	10	167		54.6
		2	10	172		56.2
		3	10	165		53.9
		Spike		15.6	15.4	
	STD	10 ppm		10		
	AX2-1	1	10	17		5.6
		Spike		3.6	3.4	
	AX2-2	1	10	17		5.6
		Spike		3.1	3.4	
	T4-2	1	10	43		14.1
		Spike		5.6	5.4	
	T1-2	1	10	38		12.4
		Spike		5.4	5.0	
02/26/05	PCE	1	20	14.8		4.8
		Spike		0.781	0.784	
	OX1	1	20	2		0.65
		Spike		0.191	0.202	
	OX2	1	20	1.4		0.46
		Spike		*0.221*	0.181	
	STD	1 ppm		1.22		
03/02/05	T2P	1	10	93		30.4

		Spike		9.4	9.1	

Soluble OP Data – Trial 3 (cont'd)

					Spike	
Date	**Sample**	**Sample #**	**Dil. Fact.**	**Reading * Dil Fact** mg/L as $PO4^{3-}$	**Theoretical** Conc. mg/L as $PO4^{3-}$	**P Conc. mg/L as P**
	T2M	1	10	137		44.8
		Spike		*12.3*	11.3	
	T3M	1	10	135		44.1
		Spike		*12.4*	11.3	
	T4-1	1	10	31		10.1
		Spike		5.2	6.0	
	T1-1	1	10	21		6.7
		Spike		*3.1*	5.2	???????
	STD	10 ppm		8.9		
	AX2-1	1	10	7		2.3
		Spike		*2.1*	4.8	???????
	AX2-2	1	10	8		2.6
		Spike		*2.4*		??????
	T4-2	1	10	39		12.7
		Spike		6.1	6.4	
	T1-2	1	10	38		12.4
		Spike		-		
	PCE	1	20	12.4		4.0
		Spike		0.65	0.68	
	OX2	1	10	0.9		0.30
		Spike		*0.16*	0.20	
	STD	1 ppm		1.33		
03/06/05	T2P	1	10	104		34.0
		Spike		9.9	10.1	
	T2M	1	10	146		47.7
		Spike		13.5	13.5	
	T3M	1	10	149		48.7
		Spike		13.4	13.7	
	T4-1	1	10	53		17.3

		Spike		6.8	6.0	
	T1-1	1	10	32		10.5

Soluble OP Data – Trial 3 (cont'd)

					Spike	
Date	**Sample**	**Sample #**	**Dil. Fact.**	**Reading * Dil Fact mg/L as $PO4^{3-}$**	**Theoretical Conc. mg/L as $PO4^{3-}$**	**P Conc. mg/L as P**
		Spike		4.3	4.3	
	AX2-1	1	10	89		29.1
		Spike		3.4	3.0	
	STD	10 ppm		8.9		
	PCE	1	20	15.6		5.1
	OX1	1	10	1.0		0.33
		Spike		0.18	0.20	
	STD	1 ppm		1.25		

Total P Data –Trial 3

Date	**Sample**	**Sample #**	**Dil. Fact.**	**Corr. Fact.**	**Reading * dil.*corr. fact. mg/L as $PO4^{3-}$**	**Spike Theo. Conc. mg/L as $PO4^{3-}$**	**P Conc. mg/L as P**
02/20/05	T2M	1	20	1.02	1520		497
		2	20	1.02	1632		533
		3	20	1.02	Lost Reagent		-
		Spike			Lost Reagent		
	T3M	1	20	1.02	Lost Reagent		-
		2	20	1.02	1542		504
		3	20	1.02	1489		487

		Spike			Lost Reagent		
	T4-1	1	10	1.05	160		52.2
		2	10	1.05	165		53.9

Total P Data –Trial 3 (cont'd)

Date	**Sample**	**Sample #**	**Dil. Fact.**	**Corr. Fact.**	**Reading * dil.*corr. fact. mg/L as $PO4^{3-}$**	**Spike Theo. Conc. mg/L as $PO4^{3-}$**	**P Conc. mg/L as P**
		3	10	1.05	161		52.5
		Spike			11.3		
	STD	10 ppm			Wrong vial used	7 - calculated	
	STD	1 ppm			1.29		
	OX1	1	11.2	1.2	*13.1*		*4.3*
		Spike			1.14		
	PCE	1	10	*1.02*	*5.1*		*1.7*
		Spike			3.6	3.6	
	AX2-1	1	10	1.02	337		110
		Spike			19.8	19.8	
02/23/05	T2M	1	20	1.01	1084		354
		Spike			28.0	31.6	
	T3M	1	20	1.01	1108		362
		Spike			28.5	32.2	
	T4-1	1	10	1.01	190		62.1
		Spike			12.8	14.0	
	AX2-1	1	10	1.01	360		118
	PCE	1	10	-	Used wrong vials		
	OX1	1	10	-	Used wrong vials		
	STD	10 ppm			8.9		

Total TP Data – Trial 3 (cont'd)

Date	**Sample**	**Sample**	**Dil. Fact.**	**Corr. Fact.**	**Reading * dil. *Corr. fact. mg/L as $PO4^{3-}$**	**Spike Theo.**	
02/26/05					Used wrong vials for whole batch		
03/02/05	T2M	1	20	1.01	1119		366
		2	20	1.01	1212		396
		3	20	1.01	1067		349
		Spike			32.0		
	T3M			1.01	Lost sample		
	T4-1	1	10	1.01	215		70.3
		2	10	1.01	204		66.7
		3	10	1.01	197		64.4
		Spike			12.9	14.9	
	STD	10 ppm			9.6		
	AX2-1	1	10	1.01	375		122
		Spike			20.4	23.6	
	PCE	1	20	1.01	25.8		8.5
		Spike			1.23	1.31	
	OX1	1	10	*1.05*	*32.7*		*10.7*
		Spike			*2.71*	*2.23*	
	OX2	1	10	1.03	*34.3*		*11.2*
		Spike			*1.87*	*2.33*	
	STD	1 ppm			1.34		
03/06/05	T2M	1	20	1.01	1824		596
		Spike			37.7		
	T3M	1	20	1.01	1050		343
		Spike			28.9		
	T4-1	1	10	1.01	199		65.0
		Spike			13.8		
	PCE	1	20	1.02	23.1		7.50
		Spike			0.82		
	AX2-1	1	10	1.01	387		126
		Spike			23.7		
	OX1	1	10	1.05	1.9		0.62
		Spike			1.39		

	STD	10 ppm			contaminated		

TSS/VSS Data –Trial 3

W1Paper only
W2 Paper with residue after one hour at 103 C
W3Paper with residue after 15 minutes in furnace

Date	**Sample**	**Dil.**	**Vol.**	**W1**	**W2**	**W3**	**TSS**	**VSS**
			Size	**g**	**g**	**g**	**mg/L**	**mg/L**
			ml					
20-Feb	T1-1	10	50	2.7	2.7185	2.7122	1260	1260
	T1-1	10	50	2.7	2.7319	2.7260	1160	1180
	T4-1	10	50	2.7	2.7258	2.7201	960	1140
	T4-1	10	50	2.7	2.7165	2.7107	1180	1160
	T4-1	10	50	2.7	2.7273	2.7220	1200	1060
	T2P	10	50	2.7	2.6850		400	
	T2P	10	50	2.7	2.7061		400	
	T2M	20	30	2.7	2.7315	2.7133	13733	12133
	T2M	20	30	2.7	2.7370	2.7175	14400	13000
	AX2-1	10	50	2.7	2.7218	2.7108	2520	2200
	AX2-1	10	50	2.7	2.7195	2.7094	2460	2020
	T3	20	30	2.7	2.7147	2.6960	14133	12467
	T3	20	30	2.7	2.7120	2.6933	14133	12467
21-Feb	T4	10	50	2.7	2.7059	2.6982	1640	1540
	T4	10	50	2.7	2.7074	2.7000	1780	1480
	T2P;9:15pm	20	30	2.7	2.7190	2.7024	12333	11067
	T2P	20	30	2.7	2.7176	2.7011	12533	11000
	T2P;12:30pm	20	30	2.7	2.7164	2.7039	9533	8333
	T2P	20	30	2.7	2.7160	2.7035	9267	8333
22-Feb	T4-1	10	50	2.7	2.7027	2.6960	1500	1340
	T4-1	10	50	2.7	2.7171	2.7104	1560	1340
	T2P	10	50	2.7	2.7098	2.7056	960	840
	T2P	10	50	2.7	2.7018	2.6976	980	840
23-Feb	T2P	10	50	2.7	2.6994	2.6968	500	520
	T2P	10	50	2.7	2.6848	2.6825	400	460
	AX2-2	10	50	2.7	2.7228	2.7107	2820	2420
	AX2-2	10	50	2.7	2.7083	2.6958	2940	2500
	AX2-1	10	50	2.7	2.7195	2.7083	2700	2240
	AX2-1	10	50	2.7	2.7142	2.7036	2800	2120
	T1-2	10	50	2.7	2.7148	2.7084	1420	1280
	T1-2	10	50	2.7	2.7152	2.7086	1500	1320
	T4	10	50	2.7	2.6865	2.6807	1320	1160
	T4	10	50	2.7	2.6748	2.6683	1420	1300

	T1-1	10	50	2.7	2.7205	2.7144	1180	1220
	T1-1	10	50	2.7	2.6834	2.6767	1220	1340

TSS/VSS Data – Trial 3 (cont'd)

Date	**Sample**	**Dil.**	**Vol.**	**W1**	**W2**	**W3**	**TSS**	**VSS**
			Size	**g**	**g**	**g**	**mg/L**	**mg/L**
			ml					
	T3	20	30	2.7	2.7445	2.7325	8867	8000
	T3	20	30	2.7	2.7262	2.7136	8867	8400
	T2M	20	30	2.7	2.7004	2.6879	8733	8333
	T2M	20	30	2.7	2.7201	2.7068	9200	8867
24-Feb	T2P	1	50	2.7	2.6774		34	
	T2P	1	50	2.7	2.7141		22	
	T4	10	50	2.7	2.7304	2.7252	1160	1040
	T4	10	50	2.7	2.7341	2.7288	1220	1060
25-Feb	T2P	10	50	2.7	2.7039	2.6986	1060	1060
	T2P	10	50	2.7	2.7401	2.7347	920	1080
	T4	10	50	2.7	2.7277	2.7214	1320	1260
26-Feb	T2M	20	30	2.6	2.6384	2.6277	8000	7133
	T2M	20	30	2.7	2.6781	2.6675	7867	7067
	T2P	10	50	2.7	2.6796	2.6762	660	680
	T2P	10	50	2.7	2.7231	2.7196	740	700
	AX2-1	10	50	2.7	2.7131	2.7026	2540	2100
	AX2-1	10	50	2.7	2.7078	2.6973	2500	2100
	T1-2	10	50	2.7	2.6979	2.6921	1340	1160
	T1-2	10	50	2.7	2.6886	2.6830	1320	1120
	T3M	20	30	2.7	2.6949	2.6845	7733	6933
	T3M	20	30	2.7	2.7150	2.7048	7733	6800
	T1-1	10	50	2.7	2.7317	2.7258	1360	1180
	T1-1	10	50	2.7	2.7297	2.7237	1340	1200
	AX2-2	10	50	2.7	2.7308	2.7204	2600	2080
	AX2-2	10	50	2.7	2.7442	2.7328	2720	2280
	T4-1	10	50	2.7	2.7229	2.7170	1320	1180
	T4-1	10	50	2.7	2.7189	2.7128	1380	1220
27-Feb	T2P	1	150	2.7	2.7266	2.7185	65	54
	T2P	1	150	2.7	2.7050	2.6980	57	47
	T4-1	10	50	2.7	2.7327	2.7271	1200	1120
	T4-1	10	50	2.7	2.6919	2.6862	1300	1140
28-Feb	T4-1	10	50	2.7	2.6726	2.6686	860	800
	T4-1	10	50	2.7	2.6862	2.6823	860	780
	T2P	10	50	2.7	2.6877		220	
	T2P	10	50	2.7	2.6914		240	
1-Mar	T4-1	10	50	2.7	2.6861	2.6799	1140	1240
	T4-1	10	50	2.6	2.6524	2.6460	1460	1280

	T2P	10	50	2.7	2.6790		280	
	T2P	10	50	2.7	2.6632		300	
2-Mar	T3M	20	30	2.6	2.6579	2.6462	9067	7800

TSS/VSS Data –Trial 3 (cont'd)

Date	**Sample**	**Dil.**	**Vol.**	**W1**	**W2**	**W3**	**TSS**	**VSS**
			Size	**g**	**g**	**g**	**mg/L**	**mg/L**
			ml					
	T3M	20	30	2.7	2.6712	2.6600	9133	7467
	T2P	10	50	2.7	2.6648	2.6616	720	640
	T2P	10	50	2.6	2.6522	2.6492	700	600
	Ax2-1	10	50	2.7	2.6974	2.6866	2920	2160
	Ax2-1	10	50	2.7	2.6730	2.6604	3140	2520
	T4-1	10	50	2.7	2.6751	2.6675	1700	1520
	T4-1	10	50	2.7	2.6874	2.6802	1640	1440
	T2M	20	30	2.7	2.6910	2.6749	12200	10733
	T2M	20	30	2.6	2.6517	2.6359	12267	10533
	T1-1	10	50	2.7	2.6662	2.6589	1740	1460
	T1-1	10	50	2.7	2.6716	2.6634	1780	1640
	Ax2-2	10	50	2.7	2.6872	2.6751	3100	2420
	Ax2-2	10	50	2.7	2.6848	2.6730	3020	2360
	T1-2	10	50	2.7	2.6967	2.6900	1780	1340
	T1-2	10	50	2.7	2.6771	2.6701	1760	1400
3-Mar	T2P	1	150	2.6	2.6574	2.6499	55	50
	T2P	1	150	2.7	2.6712	2.6646	55	44
	T4	10	50	2.7	2.6692	2.6624	1440	1360
	T4	10	50	2.7	2.6641	2.6577	1400	1280
4-Mar	T4-1	10	50	2.7	2.6659		1140	
	T4-1	10	50	2.7	2.6665		1220	
	T2P	10	100	2.7	2.6531		90	
	T2P	10	100	2.7	2.6656		0	
6-Mar	T4-1	10	50	2.7	2.6654	2.6585	1260	1380
	T4-1	10	50	2.7	2.6667	2.6599	1160	1360
	T2M	20	30	2.7	2.6787	2.6584	15133	13533
	T2M	20	30	2.7	2.6819	2.6622	14800	13133
	T3M	20	30	2.7	2.6696	2.6552	10400	9600
	T3M	20	30	2.6	2.6622	2.6482	10200	9333
	T1-1	10	50	2.7	2.6784	2.6713	1460	1420
	T1-1	10	50	2.7	2.6621	2.6554	1440	1340
	AX2-1	10	50	2.7	2.6761	2.6638	2400	2460
	AX2-1	10	50	2.7	2.6933	2.6809	2460	2480
	T2P	1	100	2.6	2.6400	2.6333	78	67
	T2P	1	100	2.7	2.6839	2.6772	78	67

A.5. Phosphorus Release Batch Test Results

Series No. 1 – Test A1

Sample ID	Sample	OP (mg/L as P)	TCOD (mg/L)	TSS (mg/L)	VSS (mg/L)	SCOD (mg/L)	TP (mg/L as P)	VFA (mg/L as AA)
1	PCE	5	343	80	66	115	7	16
2	AX	15	3172	*640*	*740*	43	115	76
3	Ferm	37	8900	6680	Availa	143	220	320
Fermentate 1 - 230 ml of Fermentate added								
4	0	13.1	2280	1540	1460	65	-	-
5	15	18.3	-	-	-	53	-	-
6	30	21.6	-	-	-	48	-	-
7	45	26.1	-	-	-	46	-	-
Acetic Acid - 0.7 ml added			**@ 22%**					
8	0	13.4	1930	1210	1190	121	-	-
9	15	15.4	-	-	-	92	-	-
10	30	19.6	-	-	-	90	-	-
11	45	23.5	-	-	-	84	-	-
Control 1								
12	0	12.7	1640	*250*	*290*	54	-	-
13	15	17.0	-	-	-	42	-	-
14	30	20.9	-	-	-	43	-	-
15	45	24.5	-	-	-	45	-	-

Acetic Acid (.2% solution) COD 628 mg/L COD

Series No. 1 – Test B1

Sample ID	Sample	Ortho-P (mg/L as P)	COD (mg/L)	TSS (mg/L)	VSS (mg/L)	SCOD (mg/L)	TP (mg/L as P)	VFA (mg/L as AA)
16	PCE	11	*503*	555	495	129	5	0
17	AX	8	2953	*500*	*680*	34	104	69
18	Ferm	Used same fermentate as the one for Trial #1						
Fermentate 2 - 125 ml a		**added**						
19	0	9.5	1790	*200*	*370*	64	-	-
20	15	14.1	-	-	-	50	-	-
21	30	17.3	-	-	-	39	-	-
22	45	16.3	-	-	-	54	-	-
Sugar Water - 1.8 ml added @ 9.0 Brix								
23	0	9.5	1840	*0*	*145*	116	-	-
24	15	11.8	-	-	-	66	-	-
25	30	14.7	-	-	-	62	-	-
26	45	18.3	-	-	-	58	-	-
Control 2								
27	0	7.5	1660	200	390	54	-	-
28	15	12.4	-	-	-	40	-	-
29	30	15.0	-	-	-	40	-	-
30	45	15.0	-	-	-	44	-	-

Sugar Water (.5% sol.) COD 470 mg/L COD

Series No. 2 – Test A2

Sample	Sample	OP (mg/L as P)	TCOD (mg/L)	TSS (mg/L)	VSS (mg/L)	SCOD (mg/L)	TP (mg/L as P)	VFA (mg/L as AA)
1	PCE	6	504	27	-	147	7	2
2	RAS	2	9239	6200	5880	42	149	157
3	Ferm	54	14841	8534	8266	179	292	362
Fermentate 1		**125 ml added**						
4	0	11.4	-	-	-	104	-	-
5	15	12.1	-	-	-	86	-	-
6	30	17.3	-	-	-	80	-	-
7	45	20.9	2933	1630	-	76	-	-
Acetic Acid		**0.75 ml added @ 22%**						
8	0	7.8	-	-	-	162	-	-
9	15	12.4	-	-	-	143	-	-
10	30	15.0	-	-	-	123	-	-
11	45	16.0	2330	1350	1320	110	-	-
Control 1								
12	0	7.2	-	-	-	94	-	-
13	15	10.5	-	-	-	74	-	-
14	30	18.0	-	-	-	59	-	-
15	45	19.9	2101	1320	1260	62	-	-

Acetic Acid @ 0.2% 663 mg/L COD

Series No.2 – Test B2

Sample ID	Sample	OP (mg/L as P)	TCOD (mg/L)	TSS (mg/L)	VSS (mg/L)	SCOD (mg/L)	TP (mg/L as P)	VFA (mg/L as AA)
16	PCE	NA	494	27	-	138	6	15
17	RAS	Used same RAS as the one for Trial #1						190
30	Ferm	Used same fermentate as the one for Trial #1						403
Fermentate 2 250 ml added								
18	0	10.1	-	-	-	93	-	-
19	15	14.4	-	-	-	83	-	-
20	30	17.3	-	-	-	88	-	-
21	45	19.9	3848	2010	1940	65	-	-
Sugar Water @ 9.1 Brix 1.74 ml added								
22	0	8.8	-	-	-	124	-	-
23	15	11.8	-	-	-	94	-	-
24	30	16.0	-	-	-	89	-	-
25	45	19.6	2392	1420	1330	76	-	-
Control 2								
26	0	8.5	-	-	-	103	-	-
27	15	10.8	-	-	-	84	-	-
28	30	17.0	-	-	-	73	-	-
29	45	18.6	2132	1380	1280	77	-	-

Sugar Water @ 0.5% 406 mg/L COD

A.6. Gas Chromatography Test Results

MVTL

MINNESOTA VALLEY TESTING LABORATORIES, INC.
1126 N. Front St. ~ New Ulm, MN 56073 ~ 800-782-3557 ~ Fax 507-359-2890
1411 S. 12th St. ~ Bismarck, ND 58502 ~ 800-279-6885 ~ Fax 701-258-9724
35 W. Lincoln Way ~ Nevada, IA 50201 ~ 800-362-0855 ~ Fax 515-382-3885

MEMBER
ACIL

Page: 1 of 1

CHRIS DEBARBADILLO
BLACK & VEATCH
8520 CLIFF CAMERON DR STE 350
CHARLOTTE NC 28269

Project Name: MLSS FERMENTATION
Sample Description: T2M #1
TANK 1.2 FERMENTER

Report Date: 28 Mar 05
Lab Number: 05-A7172
Work Order #:21-0315
Account #: 003272
Sample Matrix: WASTEWATER
Date Sampled: 23 Mar 05
Date Received: 24 Mar 05
Chain of Custody Number: 96837
Temp at Receipt: 4.0 C

	As Received Result		Method RL	Method Reference	Date Analyzed	Analyst
Acetic Acid	140	ppm	1.0	8015B	25 Mar 05	MDW
Propionic Acid	61.9	ppm	1.0	8015B	25 Mar 05	MDW
Isobutyric Acid	7.9	ppm	1.0	8015B	25 Mar 05	MDW
Butyric Acid	8.7	ppm	1.0	8015B	25 Mar 05	MDW
2-Methylbutyric Acid	9.0	ppm	1.0	8015B	25 Mar 05	MDW
Isovaleric Acid	9.4	ppm	1.0	8015B	25 Mar 05	MDW
Valeric Acid	2.2	ppm	1.0	8015B	25 Mar 05	MDW

Page: 1 of 1

CHRIS DEBARBADILLO
BLACK & VEATCH
8520 CLIFF CAMERON DR STE 350
CHARLOTTE NC 28269

Project Name: MLSS FERMENTATION
Sample Description: T2M #2
TANK 1.2 FERMENTER

Report Date: 28 Mar 05
Lab Number: 05-A7173
Work Order #:21-0315
Account #: 003272
Sample Matrix: WASTEWATER
Date Sampled: 23 Mar 05
Date Received: 24 Mar 05
Chain of Custody Number: 96837
Temp at Receipt: 4.0 C

	As Received Result		Method RL	Method Reference	Date Analyzed	Analyst
Acetic Acid	139	ppm	1.0	8015B	25 Mar 05	MDW
Propionic Acid	68.2	ppm	1.0	8015B	25 Mar 05	MDW
Isobutyric Acid	9.6	ppm	1.0	8015B	25 Mar 05	MDW
Butyric Acid	8.0	ppm	1.0	8015B	25 Mar 05	MDW
2-Methylbutyric Acid	7.6	ppm	1.0	8015B	25 Mar 05	MDW
Isovaleric Acid	8.7	ppm	1.0	8015B	25 Mar 05	MDW
Valeric Acid	2.3	ppm	1.0	8015B	25 Mar 05	MDW

Approved by: R. Dan O'Connell
Dan O'Connell, Organic
Laboratory Manager New Ulm, MN

Page: 1 of 1

PRELIMINARY REPORT

CHRIS DEBARBADILLO
BLACK & VEATCH
8520 CLIFF CAMERON DR STE 350
CHARLOTTE NC 28269

Project Name: MLSS FERMENTATION
Sample Description: T2M
FERMENTATION TANK

Report Date: 5 Apr 05
Lab Number: 05-A8298
Work Order #:21-0345
Account #: 003272
Sample Matrix: WASTEWATER
Date Sampled: 30 Mar 05 15:40
Date Received: 1 Apr 05 4:52
Chain of Custody Number: 096172

	As Received Result		Method RL	Method Reference	Date Analyzed	Analyst
Acetic Acid	166	ppm	1.0	8015B	4 Apr 05	MDW
Propionic Acid	83.1	ppm	1.0	8015B	4 Apr 05	MDW
Isobutyric Acid	11.9	ppm	1.0	8015B	4 Apr 05	MDW
Butyric Acid	12.4	ppm	1.0	8015B	4 Apr 05	MDW
2-Methylbutyric Acid	10.6	ppm	1.0	8015B	4 Apr 05	MDW
Isovaleric Acid	13.1	ppm	1.0	8015B	4 Apr 05	MDW
Valeric Acid	2.5	ppm	1.0	8015B	4 Apr 05	MDW

Page: 1 of 1

PRELIMINARY REPORT

CHRIS DEBARBADILLO
BLACK & VEATCH
8520 CLIFF CAMERON DR STE 350
CHARLOTTE NC 28269

Project Name: MLSS FERMENTATION
Sample Description: T2M
FERMENTATION TANK

Report Date: 5 Apr 05
Lab Number: 05-A8298
Work Order #:21-0345
Account #: 003272
Sample Matrix: WASTEWATER
Date Sampled: 30 Mar 05 15:40
Date Received: 1 Apr 05 4:52
Chain of Custody Number: 096172

	As Received Result		Method RL	Method Reference	Date Analyzed	Analyst
Acetic Acid	166	ppm	1.0	8015B	4 Apr 05	MDW
Propionic Acid	83.1	ppm	1.0	8015B	4 Apr 05	MDW
Isobutyric Acid	11.9	ppm	1.0	8015B	4 Apr 05	MDW
Butyric Acid	12.4	ppm	1.0	8015B	4 Apr 05	MDW
2-Methylbutyric Acid	10.6	ppm	1.0	8015B	4 Apr 05	MDW
Isovaleric Acid	13.1	ppm	1.0	8015B	4 Apr 05	MDW
Valeric Acid	2.5	ppm	1.0	8015B	4 Apr 05	MDW

APPENDIX B: PREFERMENTATION PROCESSES

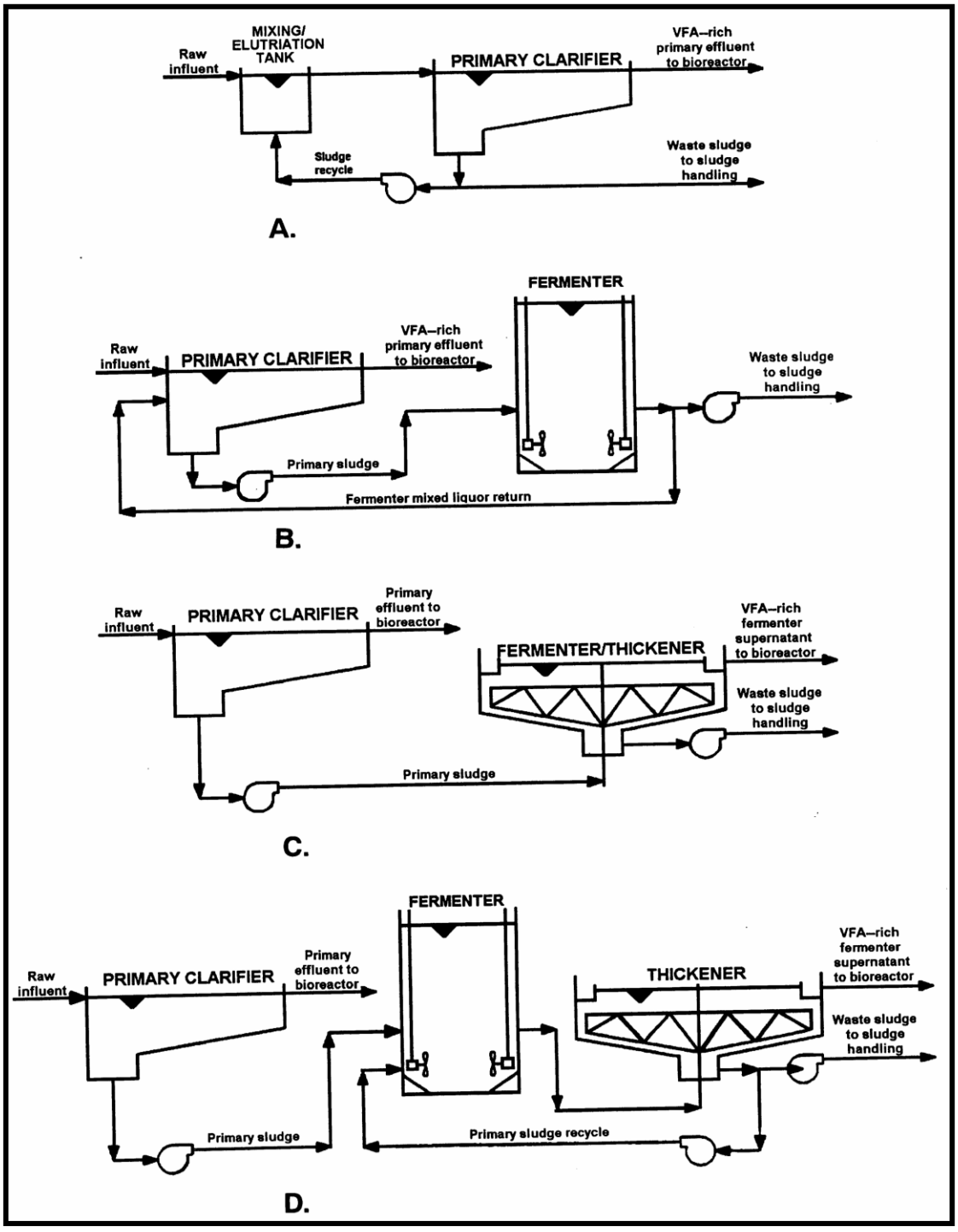

Figure B.1: Prefermentation Process Configurations (Grady et al., 1999)

A. Activated Primary Tanks (APTs) B. Complete Mix Fermenter C. Single-Stage Fermenter/Thickener D. 2-Stage Complete Mix/Thickener Fermenter