Evaluating the Impact of Algal Biomass Augmentation on Primary Solids Fermentation and Associated Impacts of Fermenter Liquor on a Novel Post-Anoxic Enhanced Biological Phosphorus Removal Process

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Taylor Mackey Romenesko

Major Professor: Erik R. Coats, P.E., Ph.D.
Committee Members: Patricia J. S. Colberg, Ph.D., P.E.; James Moberly, Ph.D.
Department Administrator: Patricia J. S. Colberg, Ph.D., P.E.

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Authorization to Submit Thesis

This thesis of Taylor Mackey Romenesko, submitted for the degree of Master of Science with a Major in Civil Engineering and titled "Evaluating the Impact of Algal Biomass Augmentation on Primary Solids Fermentation and Associated Impacts of Fermenter Liquor on a Novel Post-Anoxic Enhanced Biological Phosphorus Removal Process," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

Major Professor: _____ Date:_____
Erik R. Coats, P.E., Ph.D.

Committee Members: _____ Date:_____
Patricia J. S. Colberg, Ph.D., P.E.

_____ Date:_____
James Moberly, Ph.D.

Department Administrator: _____ Date:_____
Patricia J. S. Colberg, Ph.D., P.E.

Abstract

Nitrogen (N) and phosphorus (P) must be removed from wastewater to sustain the water quality of receiving bodies. In this regard, algae can be utilized to achieve tertiary wastewater treatment, removing residual N and P; moreover, algae production creates opportunities to enhance overall water resource recovery facility productivity. Research evaluated an integrated fermenter-biological nutrient removal (BNR) process, integrating algae cultured on secondary effluent. It was hypothesized that algae recycled to the fermenter would increase volatile fatty acid (VFA) production. VFAs are critical for BNR stability; however, concurrent addition of N and P (from the algal biomass) could stress the BNR system. Surprisingly, addition of algae decreased VFA production and consumed ammonia, seemingly due to heterotrophic algae growth in the fermenter. Conversely, the BNR system realized no effect from the algal biomass recycling; P removal was consistent with and without algae, while less efficient nitrification but more efficient denitrification was realized.

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Dedication

To my parents, Randy and Terry Romenesko, for their unwavering support and invaluable guidance in my pursuit of educational and personal advancement.

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List of Abbreviations

AA ..... Amino Acid

A_2O ..... Anaerobic, Anoxic, Oxic

ADF ..... Aerobic Dynamic Feeding

ADF ..... Anaerobic Digestion

ADF ..... Acid Detergent Fiber

ADL ..... Acid Detergent Lignins

ADM1 ..... Anaerobic Digestion Model 1

AMO ..... Ammonia Monooxygenase

AOB ..... Ammonia Oxidizing Bacteria

BIOPHO-PX ..... BIOlogical PHOsphorus removal with Post-anoXic Denitrification

BNR ..... Biological Nutrient Removal

COD ..... Chemical Oxygen Demand

DNA ..... Deoxyribonucleic acid

DO ..... Dissolved Oxygen

EBPR ..... Enhance Biological Phosphorus Removal

GAO ..... Glycogen Accumulating Organisms

GHG ..... Greenhouse Gas

HBu ..... Butyric Acid

HCa ..... Caproic Acid

HPr ..... Propionic Acid

HRT ..... Hydraulic Retention Time

HVa ..... Valeric Acid

LCA ..... Life Cycle Assessment

LCFA ..... Long Chain Fatty Acids

MF1 ..... Primary Solids Fermenter

MFA ..... Algal Biomass/Primary Solids Fermenter

MLR ..... Mixed Liquor Return

MLVSS ..... Mixed Liquor Volatile Suspended Solids

MMC ..... Mixed Microbial Consortia

MS ..... Monosaccharide

NDF ..... Neutral Detergent Fiber

NOB ..... Nitrate Oxidizing Bacteria

NO_x ..... Oxidize Nitrogen

OLR ..... Organic Loading Rate

P:C ..... Anaerobic P Release to VFA Uptake Ratio

PAO ..... Phosphorus Accumulating Organism

PHA ..... Polyhydroxyalkanoates

PHB ..... Polyhydroxybutyrate

PHV ..... Polyhydroxyvalerate

PMF ..... Proton Motive Force

Poly P ..... Polyphosphorus

PS ..... Primary Solids

qPCR ..... Quantitative Polymerase Chain Reaction

rbCOD ..... Readily Biodegradable Chemical Oxygen Demand

RT ..... Retention Time

SBR ..... Sequencing Batch Reactor

SRP ..... Soluble Reactive Phosphorus

SRT ..... Solids Retention Time

TP ..... Total Phosphorus

TS ..... Total Solids

TSS ..... Total Suspended Solids

VFA ..... Volatile Fatty Acids

VFA:P ..... Influent VFA to Phosphorus Ratio

VS ..... Volatile Solids

VSS ..... Volatile Suspended Solids

WRRF ..... Water Resource Recovery Facility

1 Introduction

Historically, the main goals of wastewater treatment have been the removal of suspended solids, biodegradable organics (which deplete dissolved oxygen), and nutrients such as ammonia and phosphorus. These constituents are removed in order to keep receiving water bodies healthy and improve overall local and downstream environmental quality. Excessive amounts of nutrients in wastewater effluent can lead to advanced eutrophication - the rapid growth of aquatic plants and algae, which reduces the biodiversity and overall health of lakes, rivers, and streams [1]. A secondary goal of wastewater treatment – although historically much less emphasized – is to capture the nutrients within the waste stream in a form that is reusable and sustainable. This secondary goal, referred to as resource recovery, has gained increased attention in recent years, in part, due to the limited quantity of such resources (e.g., nitrogen and phosphorus). The "mortality" of these resources is becoming increasingly apparent over time as evidenced by Hinsinger et al. [2], Rockstrom et al. [3], and Tilman et al. [4]. Specifically, Tilman et al. point out the agricultural sector is very inefficient when using nitrogen and phosphorus as fertilizer for crops. The carbon cycle is also very important in the wastewater world due to the end products of treatment; aerobic and anaerobic treatment of carbon exits the process as carbon dioxide and methane, respectively, both of which are greenhouse gases (GHGs).

In realizing enhanced resource recovery from wastewater, innovative technologies and processes are being implemented with increasing frequency [5]. For instance, the West Boise, Idaho wastewater treatment plant (WWTP) is conserving nutrients contained in wastewater in the form of struvite. The struvite, which is a naturally forming crystal containing equal parts magnesium, ammonium, and phosphate, is then sold as a fertilizer, which helps reduce agricultural dependency on virgin nutrient. The purposeful precipitation of struvite also alleviates maintenance issues associated with struvite forming in pipes or basins. Another example of a potentially valuable commodity being produced at a WWTP can be seen in Missoula, Montana, where algae are being cultivated to remove residual nutrients from secondary treatment effluent. This algal biomass has been shown to be useful in the production of biofuels and as a feedstock for livestock. More broadly, with the emphasized shift from "treatment" to "resource recovery," municipal WWTPs have been renamed water resource recovery facilities (WRRFs).

In seeking to further expand the WRRF concept and associated process options to commoditize wastewater, this thesis investigated an integrated system of biological processes to enhance wastewater carbon and nutrient capture while concurrently producing a novel, high-value bio-commodity. It was hypothesized that carbon capture could be achieved through algal production on biological nutrient removal (BNR) effluent, with the algal biomass being fermented and then re-used to enhance the BNR process performance and stability. Generally, BNR refers to an activated sludge WWTP process configuration that achieves removal of inorganic nutrients - nitrogen (in the form of ammonia, nitrate, and nitrite), and phosphorus - exclusively through the use of biological means. In this research, the BNR process of interest was a novel configuration that performs enhanced biological phosphorus removal (EBPR), nitrification, and post anoxic denitrification; the process is referred to as BIOPHO-PX (trademark in process). Investigations into the nutrient removal capabilities of two BIOPHO-PX sequencing batch reactors (SBR) were performed. Two BIOPHO-PX systems were operated at steady state, with a control system fed raw wastewater augmented with primary solids fermenter liquor and a second system fed raw wastewater augmented with algal/primary solids fermenter liquor. Secondarily, to generally achieve BNR, the research also investigated the potential to achieve nitritation.

1.1 Research Focus

Research was conducted to evaluate the integrated fermenter/BIOPHO-PX/ polyhydroxyalkanoates (PHA) production configuration at a "systems" level to:

i. Establish process performance potential (i.e., nutrient removal) of the entire system, and
ii. Assess the impact of internally recycling algal biomass to the fermenter. The algal biomass would theoretically be cultured on BIOPHO-PX effluent – thus loading the fermenter with quantities of algal biomass that could be produced on BIOPHO-PX effluent.

1.1.1 Specific research questions (RQs) that guided this thesis were as follows.

1. What is the nutrient removal potential of the integrated BIOPHO-PX and fermenter system?

2. What impacts can be observed on the entire system given an increased organic load to the primary sludge fermenter through algal biomass augmentation?
    a. How does the fermenter VFA speciation, yield, and concentration change with the retention time? Why?
    b. How might algal augmentation affect the influent BIOPHO-PX substrate stoichiometry?
    c. How might the algal augmentation affect BIOPHO-PX nutrient removal capabilities?
    d. How might nitritation potential be impacted in the BIOPHO-PX process?

2 Literature Review

In pursuit of answers to the research questions, a laboratory investigation was conducted on an integrated WRRF process configuration (Figure 2-1). The main processes that were investigated and that will be discussed in this thesis include: fermentation and biological nutrient removal (specifically the BIOPHO-PX process). There is some information on the capabilities of algal tertiary wastewater treatment; however, this was not a part of the research performed. The overall goal of this integrated suite of processes is to achieve effective wastewater treatment that is both energy efficient and capable of maximum removal/capture of nutrients from the waste stream. The fundamental concepts that underpin the treatment and resource recovery processes researched and discussed in this thesis are covered in this chapter.

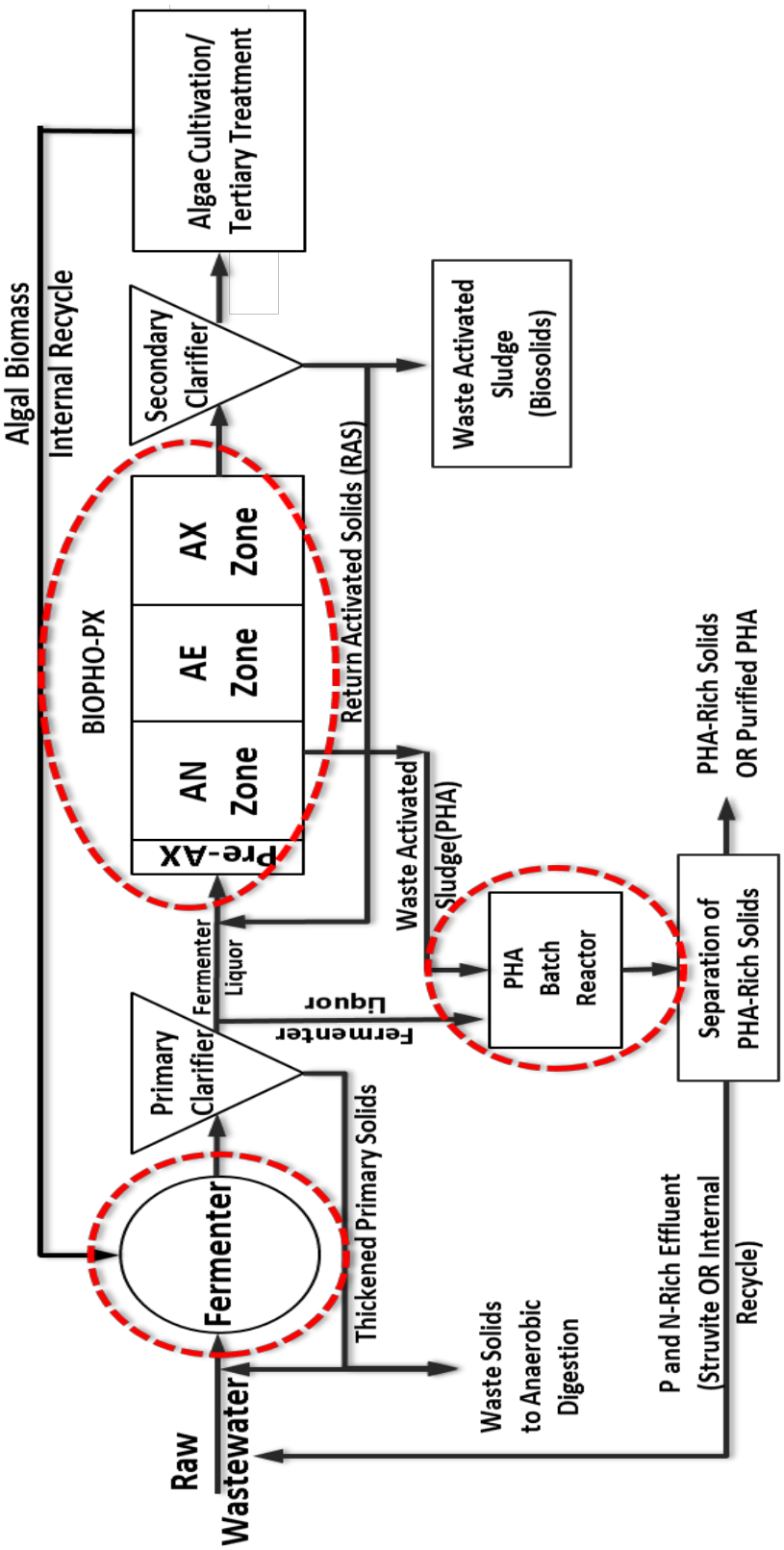

Figure 2-1. WRRF Process Diagram

2.1 Anaerobic Processes and Fermentation

2.1.1 Demand for Volatile Fatty Acids in WRRFs

Volatile fatty acids are both a critical product and a substrate in the proposed WRRF configuration – and for WRRFs in general – for multiple reasons. VFAs are used by mixed microbial consortia (MMC) to drive BNR (both for EBPR and denitrification) [6-8], can be used by MMC for PHA production [9], can be further anaerobically treated by Archaea in anaerobic digestion (AD) to generate methane [10], and can support heterotrophic algal growth [11]. Specifically, VFAs serve as a readily biodegradable carbon source (rbCOD) to MMC. VFAs are highly soluble, small (rapid diffusion through cell walls), and are easily activated to coenzyme A (CoA; i.e., acetyl-CoA and propionyl-CoA), which is a critical metabolic intermediate for both catabolic and anabolic processes. VFAs belong to two distinct groups: (1) acetic acid (2C); and (2) propionic acid (3C), butyric acid (4C), valeric acid (5C), and caproic acid (6C); however, in the context of this thesis, the term VFAs will reference 2C through 6C unless otherwise noted.

In considering the need for VFAs in WRRFs, not only does the quantity of VFAs impact biological processes, but the speciation of VFAs can have important impacts as well. First, considering enhanced biological phosphorus removal (EBPR; process description in Section 2.2.1), it has been suggested that a blend of VFAs (e.g., acetic and propionic acid) is the best substrate for maximal EBPR performance [6]. The bacteria that perform EBPR are referred to as phosphate-accumulating organisms (PAOs); PAOs store VFAs internally as polyhydroxyalkanoates (PHAs) and putatively have the ability to uptake acetate and propionate at about the same rate [12]. On the other hand, glycogen-accumulating organisms (GAOs), the main competition for PAOs and a group of microorganisms that can reduce EBPR performance, exhibit an insignificant uptake potential for propionate [6]. Moreover, propionate requires less energy to be metabolized (by the PAOs) for uptake [13], which conserves energy for the bacteria that can be used for other purposes. Thus, providing propionate in the substrate gives a competitive advantage to PAOs over GAOs. More broadly, research has been shown that providing a substrate containing full speciation of VFAs (that can be produced in fermenters) corresponds to enhanced kinetics and phosphorus removal efficiencies compared to acetate alone (e.g., 98.7 versus 71.1%, respectively) [7]. Tong and Chen suggest that increased phosphorus removal can be attributed to longer chain VFAs; the

hypothesis is that longer chain VFAs require less glycogen anaerobically (catabolism of which provides energy for VFA uptake), and subsequently less PHA is required for aerobic glycogen storage replenishment, which enables more energy to be used for phosphorus uptake (a more detailed description of EBPR metabolisms is discussed in Section 2.2.1) [7]. Along the same lines of thinking, it can be hypothesized that the longer chain VFAs would require less breakdown of internally stored phosphorus (poly phosphorus) to derive the energy for VFA uptake. Additionally, based on known VFA metabolic pathways (Figure 2-2), it can be seen that 1 mole of VFA requires 1 mole of ATP to activate to the CoA-form; however, the VFAs contain differing amounts of carbon (2 through 5 C). Thus the higher carbon VFAs would require less ATP when normalized to the number of carbons. For example, valeric acid (5C) requires 1 mole of ATP to activate; while acetic acid (2C) also requires 1 mole of ATP. In theory then, valeric acid could uptake 2.5 times more carbon for the same energy demand.

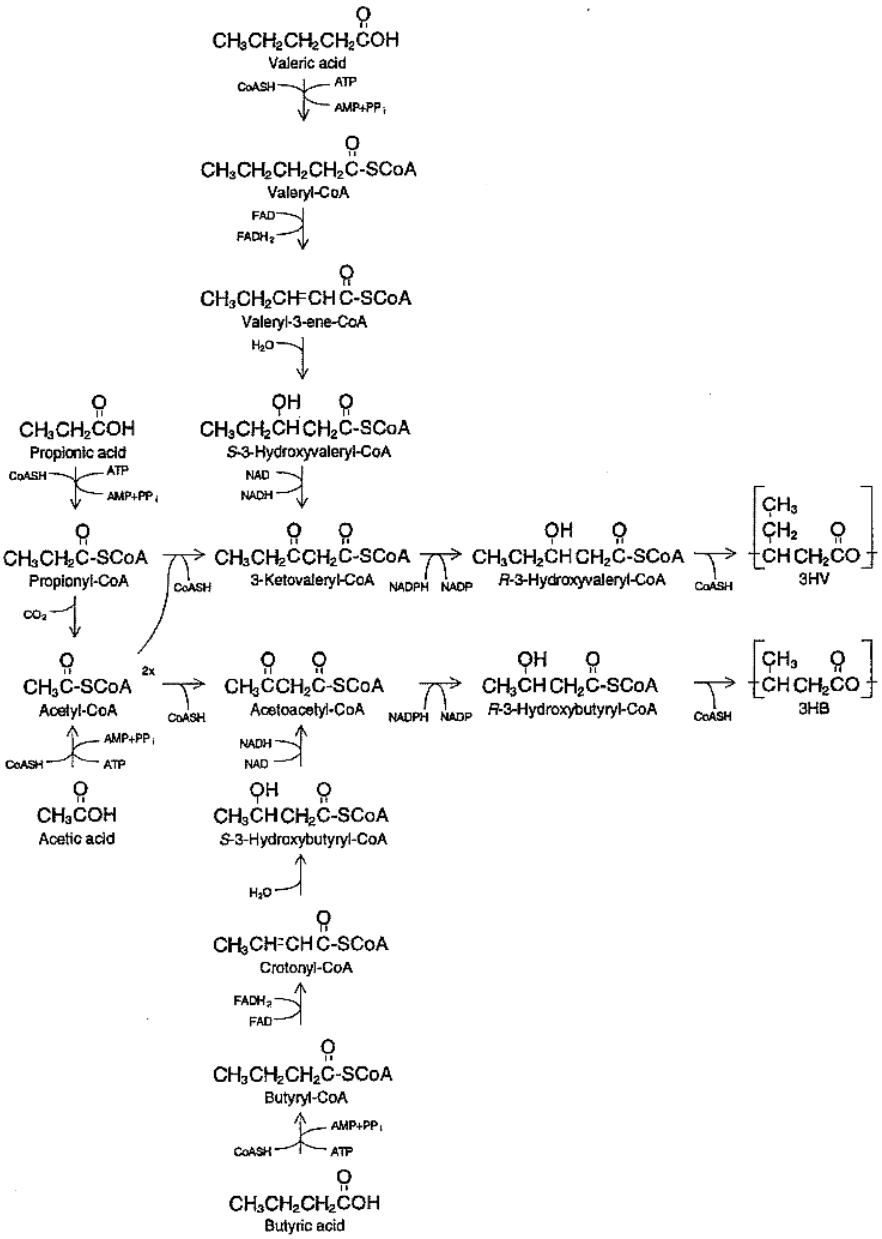

Figure 2-2. Metabolic pathways of VFAs to PHA [9]

Another potential value in producing VFAs (and acetic acid) in a WRRF is associated with the capture of wastewater carbon in the form of PHA (a biodegradable thermoplastic; process described in Section 2.2.4). The metabolic pathways for the conversion of VFAs to PHA were published by Braunegg et al. [9] and are illustrated in Figure 2-2. Two main forms of PHA are produced with the VFA speciation typically present in fermenter effluent, poly-3-hydroxybutyrate [P(3HB)] and poly-3-hydroxyvalerate [P(3HV)]. The two PHAs are often

polymerized into a copolymer of P(3HB) and P(3HV) called P(3HB-co-3HV). Polymers that contain high proportions of P(3HB) take a more crystalline form and are stiff and brittle [9]. Conversely, P3HV is a more amorphous polymer, due to the longer side chain. Therefore, PHAs with high content of P(3HV) will be more ductile (less crystalline), which expands potential commercial applications. The composition of P(3HB-co-3HV) is directly linked to the speciation of the VFAs within the PHA production substrate [14].

2.1.2 Fermentation Processes and Substrates

Fermentation, in the context of this thesis, is a biological process that uses organic compounds as both the electron acceptor (i.e., VFAs) and electron donor (i.e., proteins, carbohydrates, and lipids). While VFA production for other uses (as described above) is important in WRRFs, conventionally, fermentation is recognized as a critical and necessary preliminary stage associated with anaerobic digestion (AD; Figure 2-3). Anaerobic digestion is a complex biological process that generally consists of three main processes: hydrolysis, acidogenesis (also known as fermentation; includes anaerobic oxidation and acetogenesis), and methanogenesis [15]. Methane is the end product of AD; however, in regards to fermentation and the production of VFAs, methane formation is an indicator of process failure. Knowledge of AD mechanisms can be leveraged to produce the maximum amount of VFAs; specifically, the anaerobic process must be controlled to minimize or eliminate methanogenesis. In this regard, solids retention time (SRT) is a principal way to control methanogenesis [16], as is temperature and pH [17, 18] – (discussed more in Section 2.1.5).

Regarding fermentation substrates, most organic matter (OM), be it soluble or particulate, can be fermented to VFAs. Primary solids, which are the OM-rich solids removed from the primary clarifier in a municipal WRRF, are the most common form of OM used in fermentation for municipal processes; however, research has begun to investigate the viability of other substrates for fermentation, such as waste activated sludge (WAS) [19, 20] and algae [21]. WAS fermentation does not show much value or potential within an EBPR for a variety of reasons, including poor kinetics, the OM being partially recalcitrant (i.e., living biomass), and the potentially undesirable release of sequestered nutrients (most critically phosphorus). Conversely, algal fermentation shows some potential. Smith et al. [21] showed an 11% increase in VFA production by co-fermenting algae and dairy manure when compared to

dairy manure alone. Despite the potential of co-fermenting with this substrate, no research has been undertaken to evaluate co-fermentation of algae and primary solids.

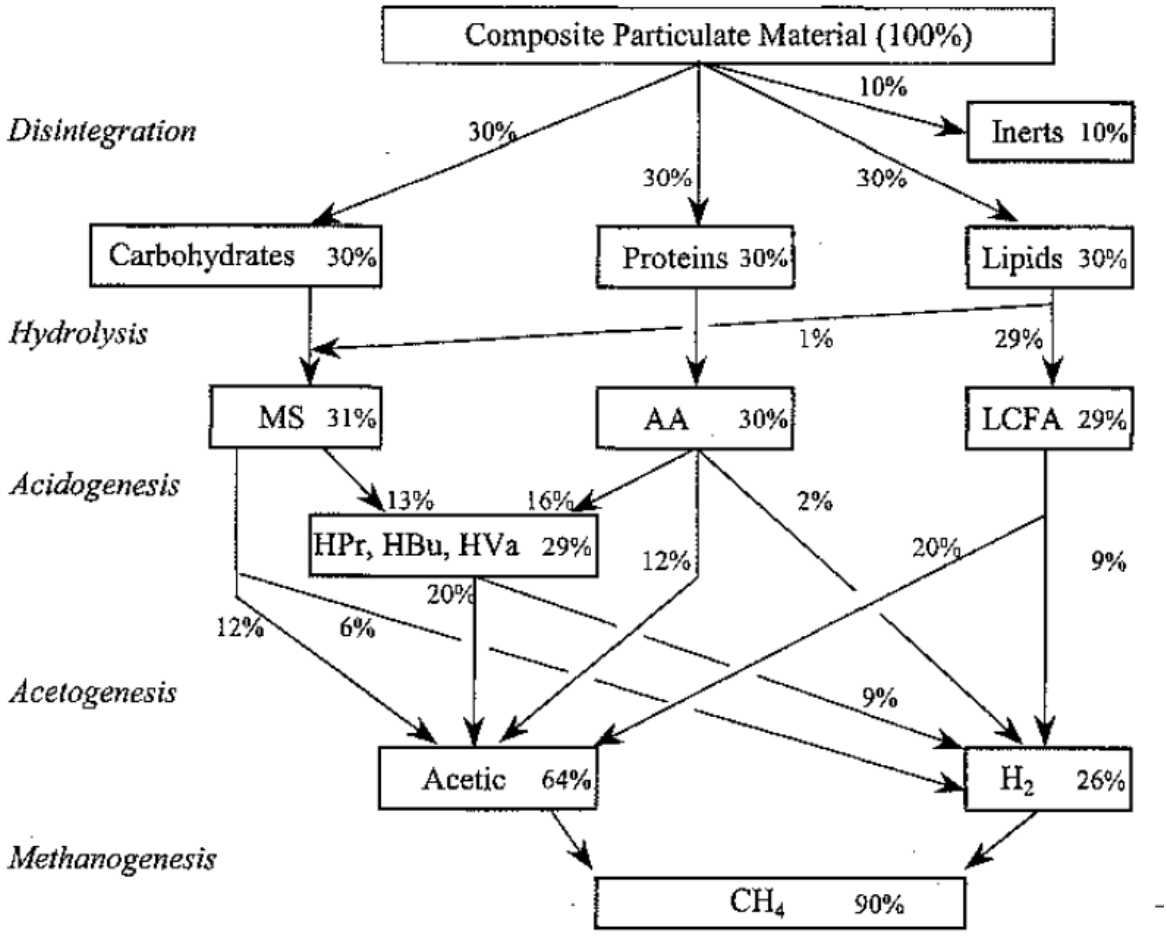

Figure 2-3. Anaerobic sludge treatment process diagram [10]

2.1.3 Fermentation Pathways and Important End Products

The anaerobic digestion process as a whole, which consists of multiple microbial-mediated pathways, is illustrated in Figure 2-3 [10]. As shown, the end products of fermentation (i.e., different species of VFAs) will differ based on the characteristics of the influent substrate. The proportions of the OM that are polysaccharides (complex carbohydrates), lipids, and proteins can be determined to help predict the pathways of fermentation and associated end products.

As illustrated, anaerobic metabolism occurs in a step-wise, synchronized manner. Bacteria excrete enzymes that drive the hydrolysis of OM and convert complex matter to

simple, fermentable molecules; carbohydrates are reduced to form simple sugars [monosaccharides (MS)], lipids are reduced into long chain fatty acids (LCFAs) with some carbohydrates (~1% [15]), and proteins are reduced into amino acids (AA). Sugars and amino acids then undergo fermentation to VFAs. Subsequently, the VFAs (3 through 6C) are oxidized to acetic acid and hydrogen gas; on the other hand, LCFAs are oxidized through anaerobic oxidation into predominantly acetic acid (propionic acid will be produced given an odd-carbon LCFA) and hydrogen gas (presuming no inhibition occurs; discussed in Section 4.2.1.2). A substrate that contains higher quantities of lipids will theoretically produce more acetic acid, the main fermentation end product of lipids. The thermodynamic favorability of LCFA oxidation also points to a potential increase in acetate concentration from lipid-rich substrates [15].

2.1.4 Assessing Fermentation Potential

Fermentation potential tests are an effective way to expedite analysis and to identify potential operating criteria to maximize VFA yield. In particular, analysis of VFA production as a function of retention time in a fermentation potential test – recognizing that SRT is a critical operating parameter [22] – will help determin optimal conditions for fermentation of a substrate of interest. The concept of fermentation potential tests was established by Lie and Welander [23]; however, the only substrate used in Lie and Welander's [23] study was municipal wastewater. Fermentation potential tests were later applied to a variety of substrates [dairy manure and primary solids (PS)] and normalized to the organic load (mg VFA_{COD}/g-VS) by Güngör et al. [24], which allowed comparisons between substrates to be made through normalization. Further exploration of the fermentation potential of substrates was undertaken by Coats et al. [22] on dairy manure only; this research showed fermentation potential tests were an effective way of estimating the VFA production potential of a substrate. Moreover, Coats et al. showed a correlation between fermentation potential test results and bench-scale fermentation performance, thereby demonstrating the value of performing fermentation potential tests as a rapid and efficient assessment toward process scale-up.

2.1.5 Effect of SRT and Temperature on Fermentation

In general, SRT is an operating parameter that is applied to achieve a specific outcome from a biological reactor, be it enrichment of target microorganisms for BNR and/or

realization of a specific suite of metabolisms. SRT is controlled by consistently wasting a fixed quantity of solids from a bioreactor system, with the goal to achieve system quasi-steady state in terms of the relative "age" of the solids in the system. With respect to fermentation and operation of fermenters, the SRT is controlled to maintain production of VFAs, while minimizing or preventing the formation of methane. Research has shown that at SRTs greater than 6 days, methanogens begin to accumulate in the fermenter, utilizing acetic acid and hydrogen to form methane and carbon dioxide [8, 15] and reducing VFAs. One effective method to limit methanogen growth is to maintain a low enough SRT to cause a washout of methanogens [8, 15]. Additionally, temperature plays a part in bacterial growth; for every 10 ˚C increase in temperature, the bacterial growth rate will double (and vice versa for a temperature decrease). Thus, at higher bioreactor temperatures, a shorter SRT may be required to inhibit methanogenesis; however, there are potential deleterious impacts of reducing SRT. Research suggests that SRTs below 6 days do not allow adequate time for maximum hydrolysis of lipids to LCFAs [15], which potentially limits VFA production from high-fat substrates (e.g., algae). Ultimately, controlling SRT for VFA production is a potential paradox: methanogenesis should be avoided, while anaerobic oxidation should be encouraged to maximize VFA production.

2.1.6 Nutrient Production and Cycling Within Fermenters

Organic matter subjected to fermentation can contain a relatively large fraction of nitrogen and phosphorus, in addition to the organic carbon. For example, dairy manure has been reported to contain about 5% nitrogen and 0.8% phosphorus (dry mass basis). In comparison, food waste contains 3% nitrogen and 0.5% phosphorus [25]. Additionally, algal biomass has been reported to contain between 2.5- and 3.9% nitrogen (phosphorus not reported) [26]. Thus, fermentation reactions typically result in an increase in soluble ammonia and phosphorus, in addition to the desired VFAs; moreover, the nutrient concentrations increase with SRT as more OM is hydrolyzed [27]. Ammonia originates principally from amino acids, which are hydrolyzed and released into the liquid phase [15]. While ammonia is a necessary nutrient for bacterial growth, slow anaerobic growth rates can yield a significant increase of ammonia in solution, as the bacteria cannot grow fast enough to uptake the ammonia released. For fermenters integrated with municipal WRRFs, increased concentrations of ammonia can place extra stress on the BNR process and increase the oxygen

demand (associated with nitrification; see section 2.2.2.1); aeration is the most energy intensive process at WRRFs [28]. Additionally, EBPR systems require an influent VFA to phosphorus (VFA:P) ratio of greater than 15 to ensure process performance [29]; therefore an increase in phosphorus from the fermenter (supplying the VFAs) would be detrimental to the process without a concurrent increase in VFAs.

While ammonia accumulation can occur during fermentation, removal can potentially occur as well. Ammonia can be off-gassed as NH_3 from solution given proper pH conditions; however, as long as the pH of the fermenter is below 8, there will be nominal off-gassing (Figure 2-4). Due to VFAs being weak acids, with pKa's around 4.8, it is unlikely ammonia off gassing in a municipal solids fermenter will occur, since system alkalinity will likely not be sufficient to prevent acidification.

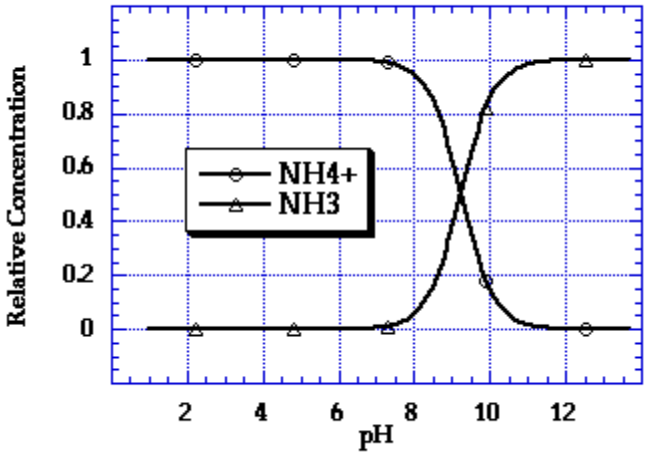

Figure 2-4. Distribution of ammonia/ammonium with pH

Ammonia can also be removed from bulk solution in an anaerobic system through struvite synthesis - either naturally or purposefully. Struvite is equal parts ammonium, magnesium, and phosphate, and it will precipitate from solution at high pH. Struvite will be naturally formed whenever there is sufficient magnesium, ammonium, and phosphate in solution; this also requires a high pH due to high dissociation constants of each chemical [1]. Additionally, struvite formation occurs in areas of low pressure (i.e., bends in pipes, pump inlets, surface of fluid) due to the release of carbon dioxide from solution and associated pH

increase [30]. On the other hand, one method employed to limit the recycle of nutrients from solids processing systems (e.g., fermentation; AD) into the WRRF liquid stream is through forced struvite precipitation. Considering that most WRRF liquid streams exhibit a pH of approximately 7, purposeful struvite formation would require chemical addition of a base (i.e., NaOH or lime); additionally, magnesium addition is likely required, which is not present in high quantities in fermentation effluent [30, 31]. Although struvite formation is not uncommon in anaerobic digestion (associated with high pH and low pressure), it is unlikely during a fermenter because the low pH and lack of magnesium.

Finally, research has been performed utilizing heterotrophic algae to remove both ammonia and phosphorus prior to BNR [32, 33]. The rapid growth rates of heterotrophic algae (0.95 day^{-1}) [34] provide a new and interesting way to reduce the nutrient recycle caused by fermentation and/or AD; however, it has been reported that algae consumed acetic and propionic acids in the process [11], which is detrimental to the overall fermentation goal (e.g., VFA production). Ultimately, special attention should be paid to the quantity of nutrients recycled versus the VFAs produced. McIntosh and Oleszkiewicz [35] showed the recycle of nutrients produced in the fermenter may actually offset the benefits of VFA production and be detrimental to BNR.

2.2 EBPR and the BIOPHO-PX Process

The removal of both nitrogen and phosphorus from municipal WRRFs has gained importance in recent years due to increasingly strict effluent regulations and overall consciousness of environmental quality [36].Wastewater nutrient removal is conventionally achieved through biological means, with WRRFs designed to remove ammonia, nitrate, and/or phosphorus. Nitrogen and phosphorus removal processes are commonly referred to as Biological Nutrient Removal (BNR), while Enhanced Biological Phosphorus Removal (EBPR) is a unique process configuration employed to remove soluble phosphorus. In this thesis, the treatment process investigated (referred to as BIOPHO-PX, which means BIOlogical PHOsphorus removal, Post anoXically) is a form of BNR that performs EBPR, nitrification, and post-anoxic denitrification (Figure 2-5). The BIOPHO-PX process has been developed at the University of Idaho by the Coats Environmental Engineering Laboratory [37-39]. BIOPHO-PX has the potential to reduce costs and energy demand compared to

conventional BNR processes [40]. In addition to efficient removal of potentially detrimental nutrients (i.e., nitrogen and phosphorus), the biomass within the BIOPHO-PX process can be leveraged to store carbon (i.e., VFAs) as a valuable commodity, PHA [40].

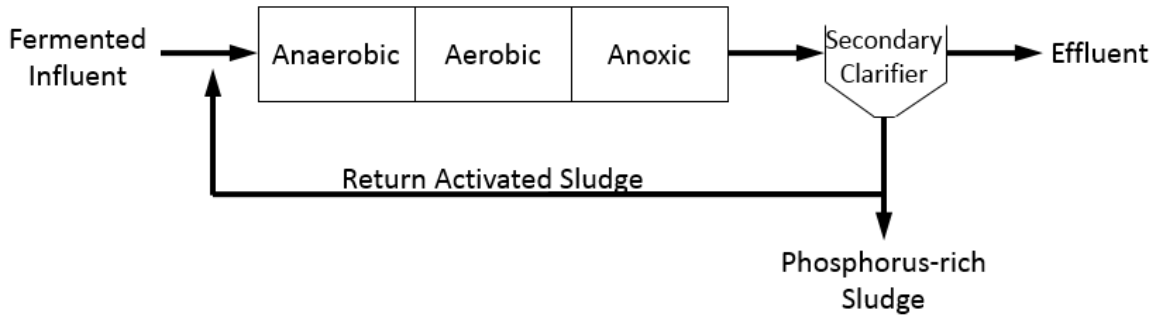

Figure 2-5. The BIOPHO-PX Process

2.2.1 Enhanced Biological Phosphorus Removal

Advanced eutrophication in freshwater bodies is caused primarily by soluble phosphorus that enhances algal growth; conversely, nitrogen is the problem nutrient in saltwater bodies [1]. To address the concern of excess phosphorus loading and to achieve sustainable phosphorus removal from wastewater [41], the BIOPHO-PX process is built on the EBPR nutrient removal scheme. Generally, wastewater phosphorus removal is performed either using EBPR or chemical phosphorus removal; however, a life cycle assessment (LCA) performed by Coats et al. [42] demonstrated that biological removal is more environmentally sustainable vs. chemical treatment. Indeed, LCA showed that EBPR would decrease the global warming potential by 5.2% and 13.2% compared to chemical treatment, when targeting 0.5 and 0.1 mg/L effluent phosphorus, respectively.

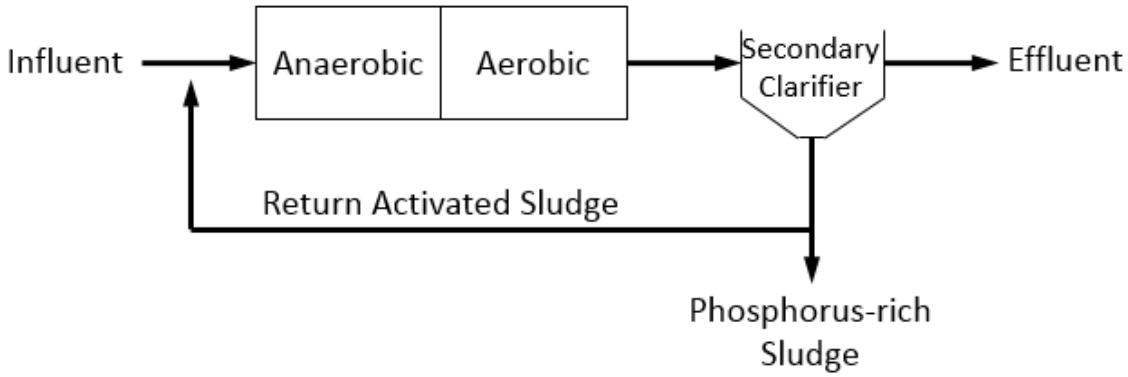

Figure 2-6. Phoredox process configuration

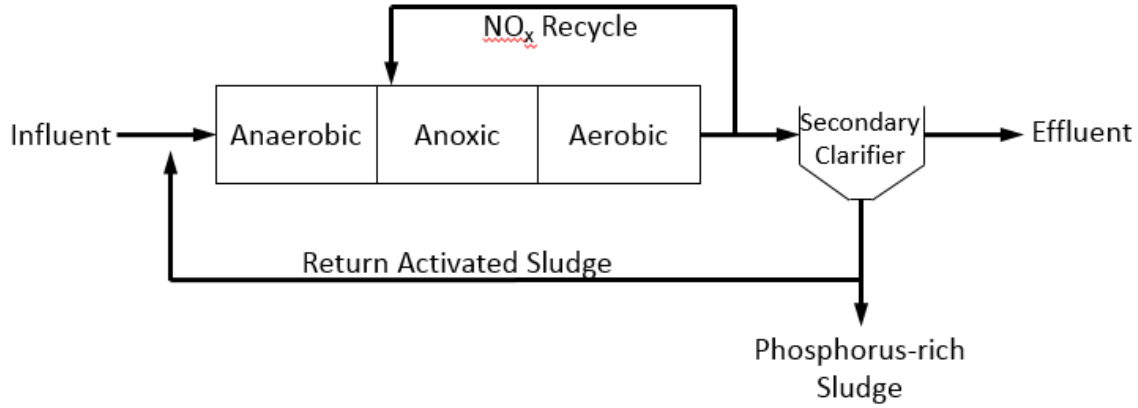

Figure 2-7. A_2O process configuration

Phosphorus removal in the BIOPHO-PX system occurs similarly to more conventional EBPR configurations, such as Phoredox and Anaerobic/Aerobic/Oxic (A_2O) (Figure 2-6 and Figure 2-7, respectively [1]); for all EBPR processes, removal is accomplished by phosphorus accumulating organisms (PAOs; unique heterotrophic bacteria shown in Figure 2-9). The unique metabolism of PAOs allow them to store excess phosphates within their cells; the PAOs are then wasted from the system, thereby accomplishing phosphorus removal. Applying prescribed environmental conditions enriches the MMC for PAOs by giving them a competitive advantage over other bacteria. Specifically, alternating anaerobic and anoxic/aerobic conditions must be imposed (known as electron acceptor cycling [40]) to enrich for PAOs. During the anaerobic phase, wherein there are no external electron acceptors

(i.e., O_2, NO_2^-, and NO_3^-) available in bulk solution, PAOs consume rbCOD in the form of VFAs. PAOs generate the energy required to uptake the VFAs from the hydrolysis of polyphosphate (Poly P) and glycogen (another internal carbon storage molecule) contained within the cells; the resulting phosphate [PO_4^{3-}; also known as soluble reactive phosphorus (SRP)] is released from the cell into bulk solution. Subsequently, the VFAs are converted into PHA, which is a carbon storage molecule vital to BIOPHO-PX process performance (i.e., in the denitrification process; more on this later). The importance of VFAs in the EBPR process is well established [29, 37, 43, 44]. Empirical data collected by Coats et al. [29] established that an influent ratio of VFA:P (mg VFA_{COD}/mg P) of 15 resulted in effluent phosphorus concentrations below 0.5 mg/L (Figure 2-8). Design guides typically recommend a VFA:P of more than 8 to ensure EBPR plants operational stability [1, 45].

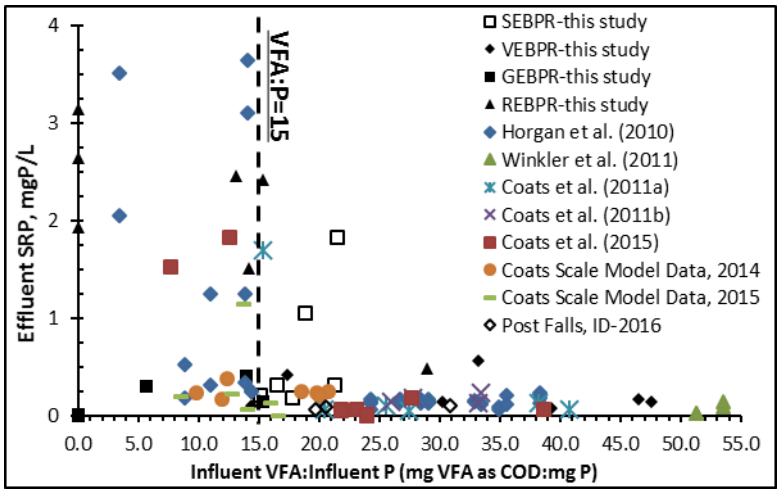

Figure 2-8. Effect of influent VFA:P ratio on effluent SRP [29]

After the PAOs have consumed the VFAs [and transformed to PHA (Figure 2-2)] at the cost of internal Poly P stores, the MMC enters an environment that supplies the PAOs with an external electron acceptor (O_2 for aerobic; NO_x for anoxic). In conventional processes, an aerobic environment is established, as oxygen is a more thermodynamically favorable electron acceptor than either nitrate or nitrite [1, 46]. Murnleitner et al. [46] estimates that PAOs produce double the energy when using oxygen as an electron acceptor compared to nitrate. In the electron acceptor environment, the PAOs use the internally stored PHA as a carbon source for growth and glycogen storage; oxidative phosphorylation produces an excess amount of energy that is used to uptake the phosphate released in the anaerobic

zone. Moreover, the PAOs uptake additional phosphate that was present in the influent associated with growth, effectively removing significant quantities of soluble phosphorus from the wastewater.

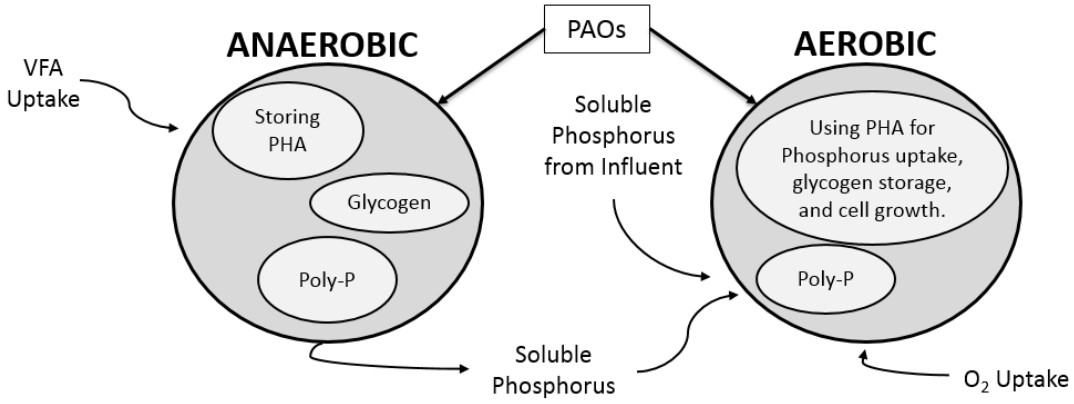

Figure 2-9. Simplified PAO metabolism

In addition to the recommended influent wastewater VFA:P ratio discussed above, Coats et al. [29] suggested the anaerobic P released-to-VFA uptake (P:C) ratio as a metric that could be used to assess EBPR metabolism induction and associated successful process performance. Specifically, the P:C ratio (mole P:mole C) sheds light on the anaerobic PAO metabolic response in that it suggests the amount of Poly P that is being hydrolyzed and released per mole VFA consumed. The P:C ratio was postulated by Filipe et al. [47] and Smolders et al. [46] to be a function of the extracellular pH; the relationship between pH and P:C ratio is empirically linked through the proton motive force (PMF). This prior work was conducted using synthetic wastewater that ultimately generated a MMC very highly enriched with PAOs - conditions that are not well representative of real wastewater systems [48]. The equations reported by Filipe et al. and Smolders et al., however, do not agree absolutely, in that they will calculate different P:C ratios from the same external pH (Equation 2-1 and Equation 2-2, respectively). Filipe et al. suggested the difference was due to the differing quantities and species of PAOs present in the MMC, and thus one data point should be assessed for each MMC to establish the intercept that corresponds to the actual microbes in the systems [47]. While Coats et al. did not include the variation of P:C ratio with pH values, an apparent trend was realized; as the P:C ratio increases, the effluent SRP also decreases (Figure 2-10).

Equation 2-1. Filipe et al. P:C ratio

$$P:C_{Filipe} = 0.16 * pH_{out} - 0.55$$

Equation 2-2. Smolders et al. P:C ratio

$$P:C_{Smolders} = 0.19 * pH_{out} - 0.85$$

Ensuring stable EBPR performance relies heavily on applying selective pressure in the WRRF in order to give a competitive advantage to PAOs over GAOs. Of principal concern, the GAOs compete in the anaerobic zone for the available VFAs. As discussed previously (Section 2.1.1), a blend of VFAs can help select for PAOs over GAOs; this is due to the GAOs putative inability to uptake propionate. Lopez-Vasquez et al. [12] show that PAOs are enriched using three main mechanisms: (1) temperature; PAOs grow faster than GAOs in lower temperatures, (2) VFA speciation; PAOs favor a more diverse VFA speciation, and (3) pH; PAOs can generate more energy from hydrolysis of Poly P with a higher external pH, thereby gaining a metabolic advantage over GAOs. A combination of these three mechanisms is typically applied to ensure a highly PAO dominant MMC.

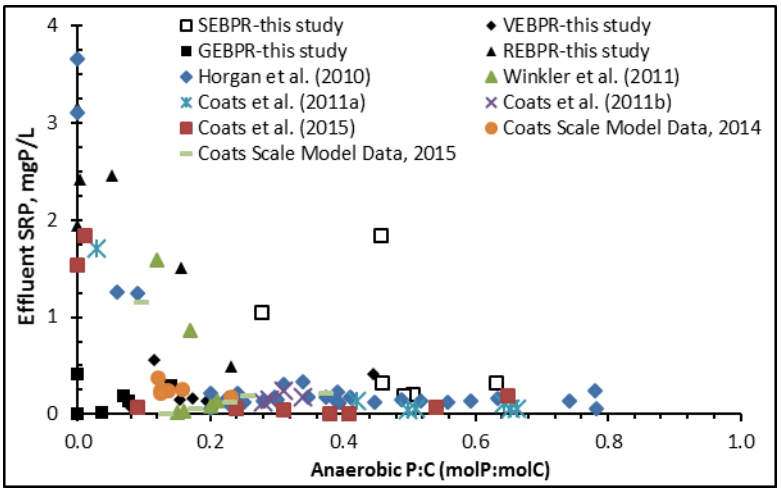

Figure 2-10. Relationship between anaerobic P:C ratio and effluent SRP [29]

2.2.2 Nitrogen Removal and BIOPHO-PX

Another focus of the BIOPHO-PX system involves nitrogen removal, achieved biologically through nitrification (ammonia oxidation) and subsequent denitrification (nitrite/nitrate reduction). In municipal WRRFs, effluent permits typically only require the first part of nitrogen removal, nitrification; however, the inherent nature of PAOs to

preferentially utilize external electron acceptors (specifically NO_x) for growth rather than cycling Poly P (metabolically induced through exposure to anaerobic conditions) causes a need for EBPR and the BIOPHO-PX system to perform denitrification in addition to nitrification (i.e., to rid the system of external electron acceptors and create truly anaerobic conditions).

Currently, most BNR configurations (e.g., A_2O) target the more traditional nitrification approach - the two-step oxidation of inorganic nitrogen to nitrate; however, the process can theoretically be stopped at nitrite (known as nitritation or partial nitrification; Equation 2-3. Nitritation stoichiometry). Nitritation is becoming more widely researched for two reasons: (1) nitritation reduces the oxygen requirement of the WRRF, and (2) denitritation requires less carbon than denitrification.

2.2.2.1 Nitrification and Nitritation

While nitrification is usually represented as a single step biological process (Equation 2-5), it actually involves two metabolic processes (ammonia oxidation and nitrite oxidation) mediated by different bacterial groups. Ammonia oxidation (Equation 2-3) is performed by ammonia-oxidizing bacteria (AOB), while nitrite oxidation (Equation 2-4) is performed by nitrite-oxidizing bacteria (NOB). Combined, AOBs and NOBs perform the complete nitrification process (Equation 2-5); both groups of bacteria use oxygen (O_2) as the terminal electron acceptor and carbon dioxide as the carbon source.

Equation 2-3. Nitritation stoichiometry

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O$$

Equation 2-4. Nitrite oxidation

$$2NO_2^- + O_2 \rightarrow 2NO_3^-$$

Equation 2-5. Nitrification stoichiometry

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$$

It is commonly thought that nitrification is an “all or nothing” process; in this regard the AOB population is assumed to be the only design parameter needed to model nitrification. The justification for utilizing the all or nothing approach is twofold. First, AOBs exhibit a low half-saturation coefficient for ammonia (K_{NH}=1.0 mg/L); a low half-saturation coefficient

indicates the bacteria will grow rapidly even at low concentrations of ammonia. Additionally, NOBs exhibit a higher maximum growth rate than AOBs (Table 2-1), which indicates that typically if there is any nitrite produced by AOBs, the NOBs will rapidly oxidize it; however, the all or nothing model begins to fall apart when temperatures rise above 29° C or when bulk solution DO concentrations drop below 0.50 mg/L [1].

Equation 2-6. Monod kinetics of AOBs

$$\mu_{AOB} = \mu_{max,AOB}\left(\frac{S_{NH}}{S_{NH} + K_{NH}}\right)\left(\frac{S_O}{S_O + K_{O,AOB}}\right) - b_{AOB}$$

Equation 2-7. Monod kinetics of NOBs

$$\mu_{NOB} = \mu_{max,NOB}\left(\frac{S_{NO}}{S_{NO} + K_{NO}}\right)\left(\frac{S_O}{S_O + K_{O,NOB}}\right) - b_{NOB}$$

Monod kinetic models are used to represent the growth of both AOBs and NOBs in WRRF systems (Equations 2-6 and 2-7, respectively); integrating NOB growth rates in addition to AOBs will increase the accuracy of the model (expands the nitrification model from a one-step to a two-step kinetic model). Indeed, it has been reported that the two-step model is highly favored for transient conditions [1, 15] when compared to the one-step model. Monod kinetics model the specific growth rate (μ_i) as a function of the maximum specific growth rate ($\mu_{max,i}$), substrate concentration (S_i; ammonia, nitrite, and DO are substrates of interest for nitrification), the half-saturation coefficient (K_i; same substrates), and specific endogenous decay rate (b). Beyond the modeling advantage, examination of the growth rates informs the ability to halt complete nitrification at nitrite; purposefully imposing conditions that kinetically favor AOBs over NOBs will result in nitritation. In this regard, methods have been proposed to enhance nitritation potential. Specifically, research has suggested: (1) limiting the DO available for oxidation reactions [1, 49-51], (2) raising the temperature [49], or (3) maintaining shorter SRTs [52].

Table 2-1. Nitrification kinetics [1]

	AOB	NOB
μ_{max} (day^{-1})	0.33-1.0	0.70-1.8
$K_{substrate}$ (mg/L)	0.30-0.70	0.05-0.30
K_{oxygen} (mg/L)	0.50	0.90

Restricting the residual DO concentration in the aerobic zone of the WRRF is one method that has been employed to favor AOBs over NOBs. The half-saturation coefficient on DO (Table 2-1) shows that NOBs cannot grow as fast compared to AOBs at low DO concentrations. AOBs utilize the ammonia monooxygenase (AMO) enzyme to oxidize ammonia to nitrite (with oxygen as the electron acceptor); this process leverages ATP synthase and acts in coordination with the PMF [53]. Moreover, AOBs utilize the cytochrome c enzyme to reduce oxygen and provide electrons to AMO. In contrast, NOBs do not utilize the AMO enzyme; however, they do need to reduce oxygen in cytochrome c. The affinity of AOBs for oxygen is higher than NOBs due to the AOB requirement for oxygen in two distinct reactions within their metabolism (AMO and cytochrome c), while NOBs only need oxygen for cytochrome c [54]. Figure 2-11 illustrates the affinity for oxygen of both AOBs and NOBs; the steeper slope of the curve for AOBs at low DO concentrations over NOBs indicates the preferential use of oxygen by AOBs. In addition to an increased oxygen affinity, AOBs exhibit a higher affinity for ammonia than NOBs do for nitrite; again this points to a MMC that can more strongly favor AOBs [55] and thus achieve nitritation.

Equation 2-8. Reaction at AMO enzyme in AOBs

$$\mathrm{NH_3} + O_2 + 2\mathrm{e^-} + 2H^+ \rightarrow \mathrm{NH_2OH} + H_2O$$

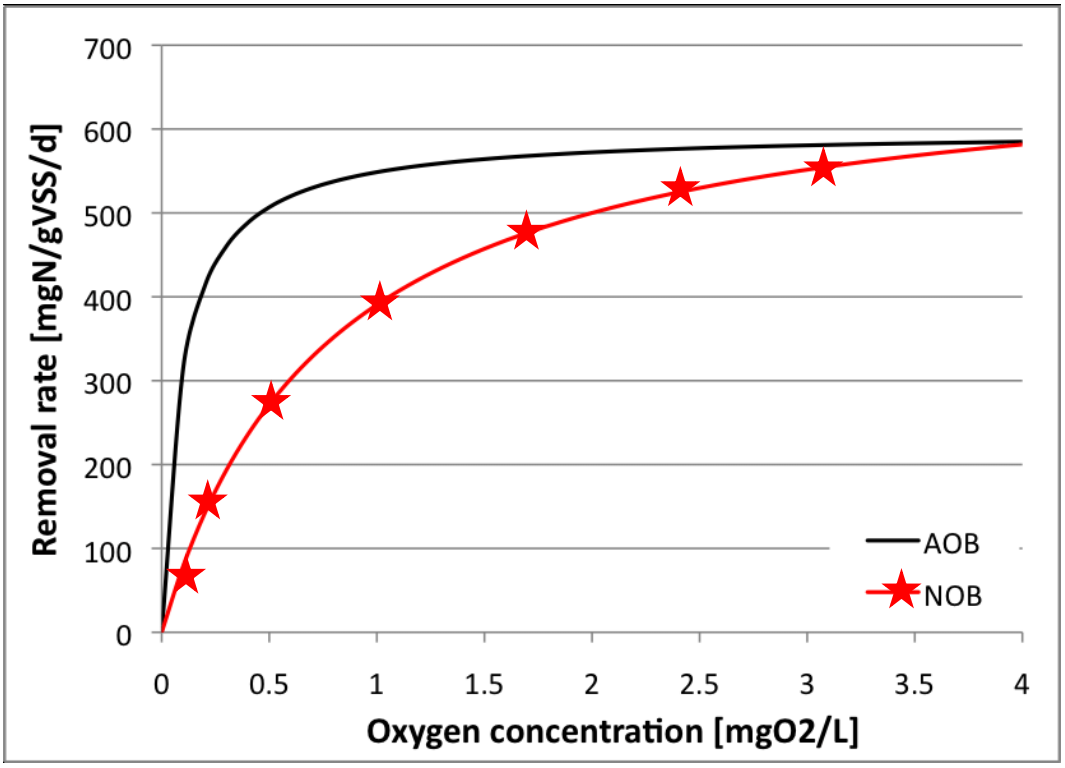

Figure 2-11. AOB vs NOB activity with DO concentration [56]

Increasing the temperature of the process also helps to enrich for AOBs instead of NOBs [49, 52]. As temperature increases, the amount of ammonia oxidation increases compared to nitrite oxidation (Figure 2-12) [57]; however, municipal WRRFs typically operate at ambient temperatures to minimize the heating costs associated with treatment.

Finally, maintaining shorter SRTs is another mechanism proposed to promote nitritation over nitrification; however, the slower growth rate of AOBs compared to NOBs dictates that a longer SRT must be maintained to ensure a stable population of AOBs. Using a short SRT is not, in and of itself, a viable method to enrich for AOBs and hinder growth of NOBs. Typically, the shorter SRT is used in combination with low DO or high temperatures to drive nitritation.

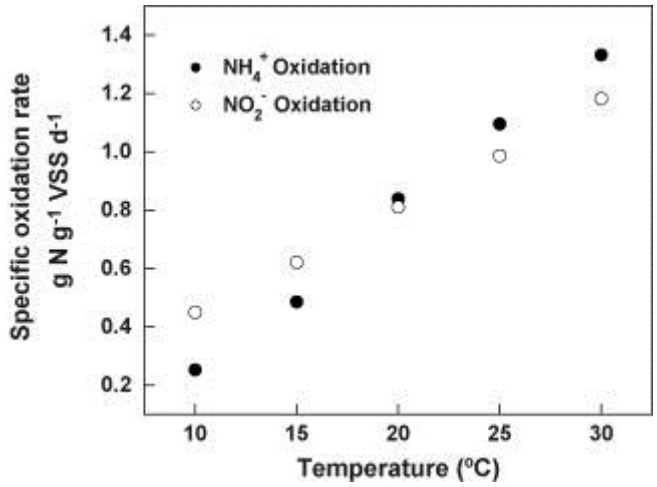

Figure 2-12. Effect of temperature on ammonia and nitrite oxidation [57]

2.2.2.2 Denitrification

Ensuring a truly anaerobic zone for the EBPR process requires the addition of a denitrification zone to the WRRF. In addition to facilitating anaerobic conditions, denitrification recovers alkalinity, which helps maintain an adequate pH for biological treatment. Denitrification is the biological reduction of nitrate, achieved through a series of intermediate products, to nitrogen gas (Figure 2-15); this series of reductions is performed by heterotrophic bacteria using organic carbon as the electron donor. Near-complete denitrification must be ensured to help keep the environmental impact of WRRFs low; incomplete denitrification will result in either nitric oxide or nitrous oxide, both potent GHGs.

Two different configurations to achieve denitrification are utilized at WRRFs: pre-anoxic denitrification (e.g., A_2O, Figure 2-7) and post-anoxic denitrification (Figure 2-13). The pre-anoxic configuration is more widely adopted in full-scale WRRFs; this is due to the requirement for organic carbon to drive the reaction. Specifically, pre-anoxic denitrification utilizes the soluble carbon in the influent for growth (or PHA, when integrated with EBPR). Ammonia is produced downstream of the anoxic basin in the pre-anoxic configuration, thus introducing a need to return the NO_x upgradient for denitrification. Not only is this an energy intensive process (increased pumping demands), but there is also a limit to the amount of denitrification. The internally recycled NO_x is known as the mixed liquor return (MLR), which dictates the degree of NO_x removal. The percent removal of NO_x is a function of the MLR, in that at higher MLR ratios the percent NO_x removal will increase as shown in Figure

2-14; however, there are diminishing returns on the percent NO_x removal above an MLR of 400% (relative to influent flow), indicating nominal increases in effluent quality at the expense of increased pumping. An MLR of 300-400% of influent flow is typically recommended to maximize denitrification and nitrogen removal within a pre-anoxic BNR/EBPR configuration [1, 15]. The reason full denitrification cannot be achieved in the pre-anoxic condition is because ultimately a portion of the NO_x will not be recycled for treatment (Figure 2-14).

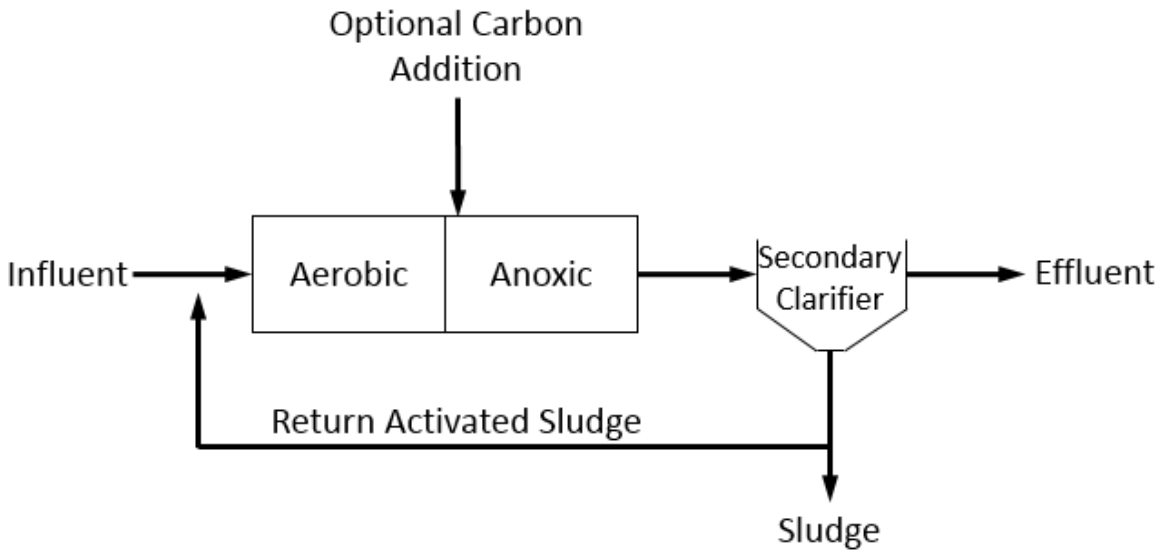

Figure 2-13. Post-anoxic denitrification configuration

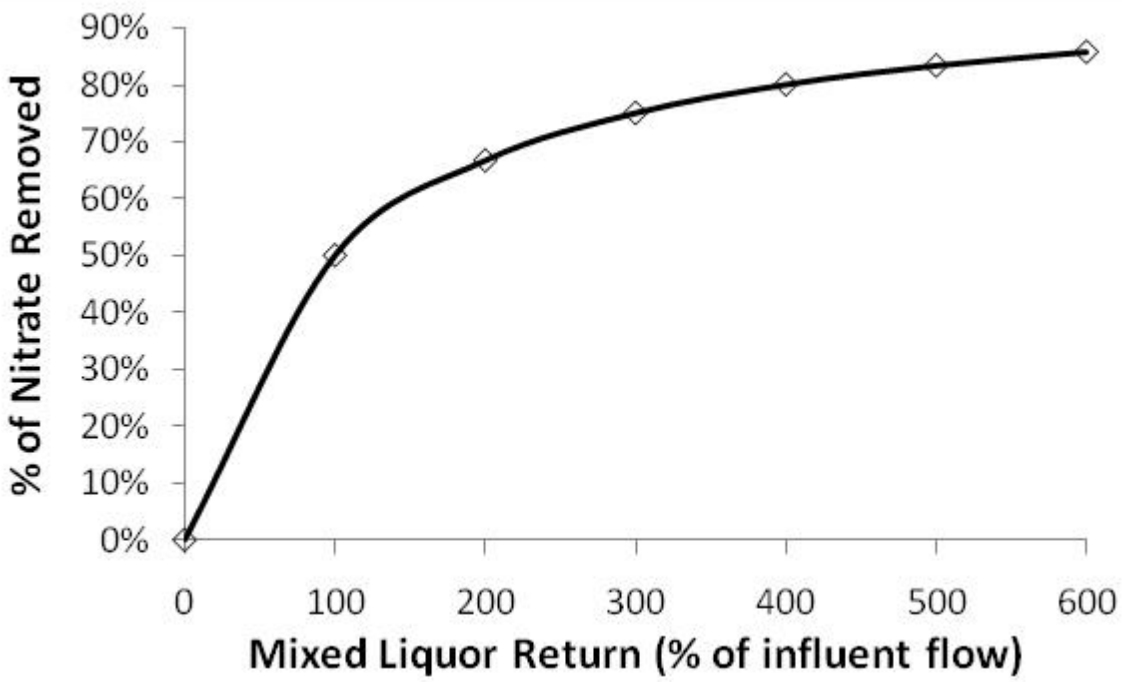

Figure 2-14. Nitrate removal with pre-anoxic configuration [39]

In contrast to pre-anoxic denitrification, post-anoxic denitrification is not limited by the recycle ratio, but instead by the availability of organic carbon. In this configuration, the anoxic basin is downstream (post) from the aerobic zone. Since oxidation of influent carbon takes place concurrently to the ammonia oxidation in the aerobic zone, there will be minimal-to-no organic carbon in the downstream anoxic basin that can be used for denitrification. In order to drive complete denitrification and avoid GHG emissions (incomplete denitrification produces nitric and nitrous oxides, potent GHGs; Figure 2-15), carbon must be added. Conventionally, endogenous decay of the bacteria in the waste stream provides the electron donor (carbon) for denitrification reactions; however, the denitrification rate when utilizing endogenous decay for a carbon source is reduced 3- to 6-fold over pre-anoxic denitrification that uses influent carbon [1]. To sustain and increase the denitrification rate, an external carbon source can be applied to the post-anoxic basin. Research suggests methanol, acetate, and even glucose can serve as substrates for denitrification [37, 58-61]; however, addition of external carbon is another operating cost of WRRFs that can be detrimental to overall economics.

To eliminate the need for providing external carbon to achieve post-anoxic denitrification, while concurrently realizing enhanced denitrification kinetics, the BIOPHO-PX process leverages PAOs and associated EBPR metabolism. Specifically, PAOs have the ability to utilize NO_x as a terminal electron acceptor without an externally supplied electron donor; this is accomplished though the utilization of internal carbon storage molecules (i.e., PHA and glycogen) [37, 38, 62, 63]. The PAOs will oxidize PHA or glycogen (more likely glycogen, since PHA is typically depleted in the aerobic zone) to provide the carbon for growth and as an electron donor. Thus, no external carbon source is needed for the PAOs, and the other heterotrophs will continue to operate in a conventional post-anoxic mode, utilizing endogenous decay for carbon.

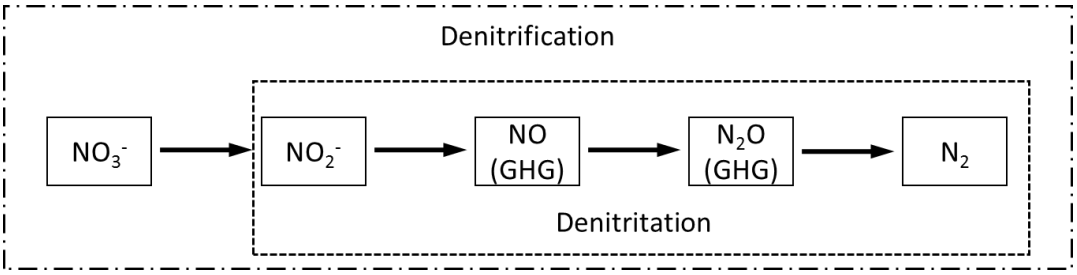

Figure 2-15. Denitrification pathway

2.2.3 BIOPHO-PX vs. Conventional BNR

In comparing BIOPHO-PX with conventional BNR, a primary difference lies in the BIOPHO-PX process ability to utilize the metabolisms of PAOs to achieve denitritation/ denitrification post-anoxically without external carbon addition. Specifically, the carbon storage achieved by PAOs is used to reduce NO_x. Further process value is gained by achieving short-cut nitrogen removal (i.e., nitritation-denitritation). Halting the nitrification process at nitrite, instead of going fully to nitrate, saves an estimated 25% in oxygen demand; additionally, by avoiding reduction of nitrate, an estimated 40% of the carbon can be conserved [64, 65]. The nitrification/denitrification and nitritation/denitritation sequences are illustrated in Figure 2-16 and Figure 2-17, respectively. Finally, carbon savings (i.e., conserving influent carbon for other purposes) is of paramount importance in the BIOPHO-PX system. Specifically, additional carbon, not used for nutrient removal (e.g., EBPR, nitritation, and denitritation), can be repurposed in a sidestream PHA production reactor. The

carbon savings from BIOPHO-PX would be used to generate a valuable commodity internally at the WRRF (i.e., PHA – discussed in the next section).

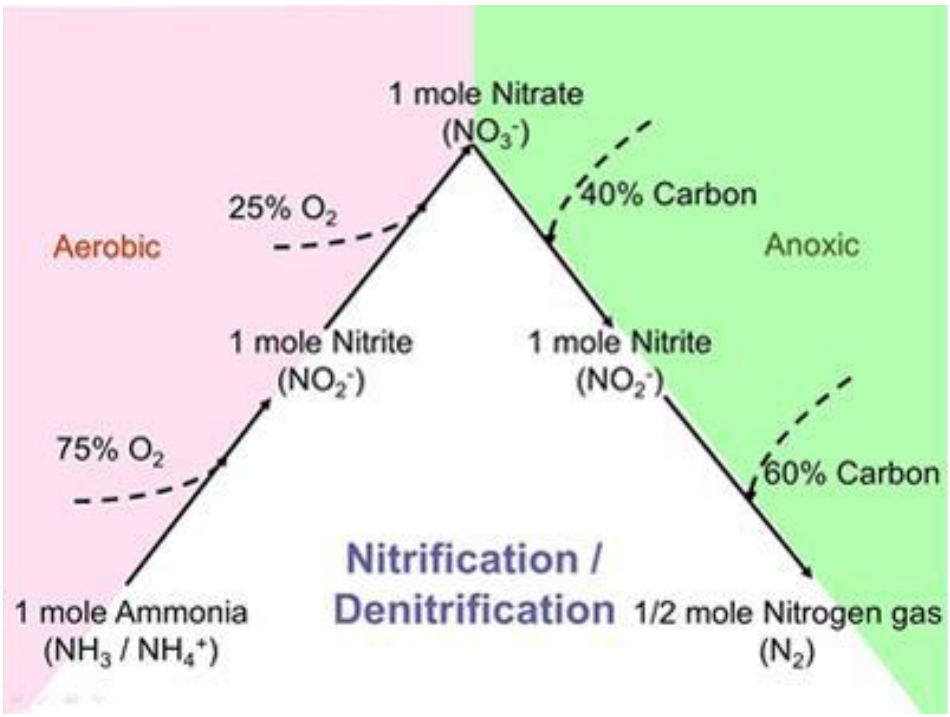

Figure 2-16. Nitrification/denitrification [65]

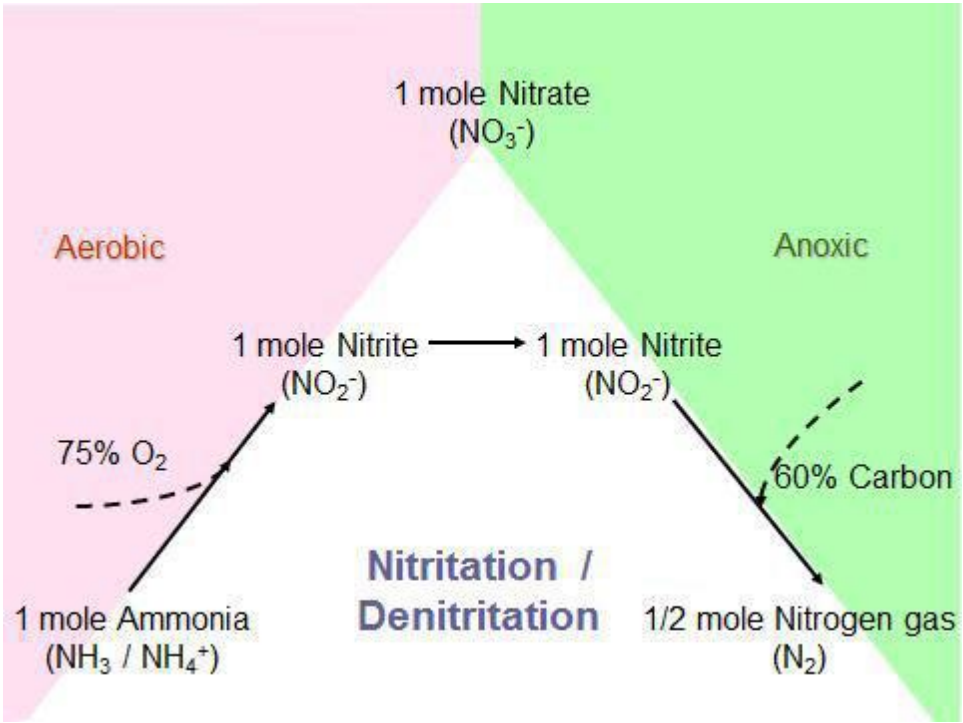

Figure 2-17. Nitritation/denitritation [65]

2.2.4 PHA Synthesis within Wastewater Treatment

Wastewater resource recovery is of increasing importance, given that sustainability is a goal within WRRFs. In this regard, biomass from the BIOPHO-PX process has shown potential to be used as inocula for a side-stream PHA reactor [39, 40, 66]; the goal is to capture influent wastewater carbon as a high-value (economically) product. PHAs are biodegradable thermoplastics that have multiple commercial uses [67]. There are two main methods employed to enrich for a PHA-producing biomass using MMC and wastewater-based VFAs: (1) electron acceptor cycling (as described in Section 2.2.1), and (2) aerobic dynamic feeding (ADF). Electron acceptor cycling and PHA synthesis is principally associated with facilitating nutrient removal (i.e., EBPR); this method has been shown to generate PHA concentrations of 0.5-5% (on a dry weight basis) [37, 38, 60, 63], concentrations that are not economically viable. The second PHA production strategy, ADF, shows much greater potential for commercial PHA production applications. Employing ADF involves VFAs being fed in excess to a MMC under aerobic conditions; this induces a so-called "feast" phase, where there is excessive carbon (VFAs) available to the PHA bacteria. In the subsequent "famine" phase, in which the carbon is bulk solution is depleted, microorganisms must survive on internal carbon storage (PHA). This metabolic response is known as feast/famine PHA synthesis, and applied ADF conditions select for PHA producers. Laycock et al. [68] showed the PHA production potential of PAOs to be between 80-90% by weight when utilizing an ADF strategy. PHA synthesis within the context of the integrated WRRF (Figure 2-1) combines both electron acceptor cycling and ADF; electron acceptor cycling occurs within BIOPHO-PX (Figure 2-5), while ADF would occur in a side-stream PHA production reactor.

2.3 Tertiary Treatment with Algae

There is a growing interest in the capabilities of algae to perform phytoremediation on wastewater sources; this phytoremediation will result in both nutrient removal and carbon capture, which are integral to sustainable wastewater treatment. As described in section 2.2, removal of nutrients from WRRFs can be both carbon and energy intensive; however, research has shown the potential of algae to remedy carbon limitation and decrease energy demand for a WRRF [26, 69]. Multiple strains of algae (e.g., *Scenedesmus obliquus* and *Chlorella vulgaris*) have been investigated for their nutrient removal potential on secondary

effluent. In these investigations, removal rates have been reported to be greater than 90- and 98-% for nitrogen (NH_4^++NO_x^-) and phosphorus, respectively [26]. Additionally, algae can operate in either an autotrophic or heterotrophic mode, known as mixotrophic [11, 32, 70, 71]; thus, in the absence of organic carbon (i.e., in secondary effluent), algae are able to fix carbon dioxide from the atmosphere (or scrub CO_2 from AD biogas [26]) and still remove residual nutrients (ammonia/phosphorus). Given that the BIOPHO-PX process relies on carbon savings for the post-anoxic denitrification, algae represent a potential to improve nutrient removal, while boosting carbon within system by recycling to fermenter.

3 Materials and Methods

3.1 Experimental Setup

3.1.1 Fermentation Potential Tests

Fermentation potential tests were performed in 500 mL screw-top Wheaton glass bottles covered with aluminum foil to prevent light penetration (and minimize phototrophic algae growth). The beakers were capped, and an air lock was applied to maintain 1-2 inches of water pressure to prevent oxygen entrainment into the headspace of the fermenters. Each test was conducted with an organic load of 10 grams volatile solids (VS). The tests compared three substrates: a fresh (never frozen) algae and primary solids blend (44% algae, 56% primary solids; on a VS weight basis), a frozen algae and primary solids blend (same loading), and a control reactor of only primary solids; triplicate reactors were evaluated. Beakers were placed on a New Brunswick Scientific Co. (Edison, NJ, USA) model G-25 controlled environment incubator/shaker table, and the investigation was conducted at room temperature. Samples were collected daily for VFA and pH analysis. In total, the fermentation potential tests were conducted for a 10-day period.

3.1.2 Bench-scale Fermenters

Two bench-top fermenters were operated for 230 days and at room temperature (23.5 ± 1.1 °C). An algal reactor (OLR=2.50 gm-VS/L-day; 10%:90% algae and primary solids blend on VS weight basis, respectively; 6-L volume; designated MFA) was inoculated from the control reactor (receiving only primary solids; OLR=2.25 gm-VS/L-day; 15-L volume; designated MF1). The proportion of the OLR from algae was determined based on theoretical production values of algae and primary solids at a 1 MGD treatment plant. Additionally, the proportions of algae and primary solids are different from the fermentation potential tests due to a correction in algal growth yields. The OLR of MFA was purposefully 10% higher than fermenter MF1 to reflect the supplementation of VS from algal biomass. Each batch of new primary solids was tested in quadriplicate for TS and VS, with the mass of primary solids added to the fermenters adjusted accordingly to maintain the target OLR. Fermenter MFA was mixed with an axial flow impeller, while fermenter MF1 was mixed using a 3.75” diameter helical impeller; both impellers were driven by an Oriental Motor (San Jose, CA, USA) USM315-401W 15 W AC speed control motor connected to 3GN35SA reduction

gearbox operated at a speed sufficient to provide uniform mixing of the reactor contents. Three distinct SRTs were investigated: 5 (74 days), 6 (56 days), and 7 (56 days) days. Fermenter influent and effluent was monitored for NH_4, PO_4, VFAs, pH, and TS/VS content.

3.1.3 BIOPHO-PX SBR Setup

Two 2-L sequencing batch reactors were operated at room temperature without pH control as shown in Figure 3-2. Both SBRs (identified as BIOPHO-PX 3 and BIOPHO-PX 3*) received a substrate of raw wastewater augmented with a VFA-rich fermenter liquor (95% raw wastewater, 5% fermenter liquor; volumetrically); the resulting VFA concentration is typical of WRRFs performing EBPR. Raw wastewater was collected regularly from the Moscow, ID WRRF and store at 4 °C. The difference between the reactors was the source of the fermenter liquor; BIOPHO-PX 3 received fermenter liquor from the primary solids fermenter (MF1), while BIOPHO-PX 3* received fermenter liquor from the primary solids and algal biomass fermenter (MFA). The BIOPHO-PX reactors operate on a 6-hour total cycle of which there are seven steps for the process (Figure 3-1); (1) the SBRs are fed, (2) followed by a one hour completely mixed pre-anoxic/anaerobic period, (3) air is pumped in to induce a one hour completely mixed aerobic period with a target residual DO of 0.7 mg/L, (4) biomass is wasted at the end of the aerobic period in a Garrett mode [1], (5) the air is turned off to create a 3.5 hour completely mixed post-anoxic period, (6) the biomass is allowed to settle for 15 minutes, and (7) the effluent is decanted from the reactor and the cycle starts over.

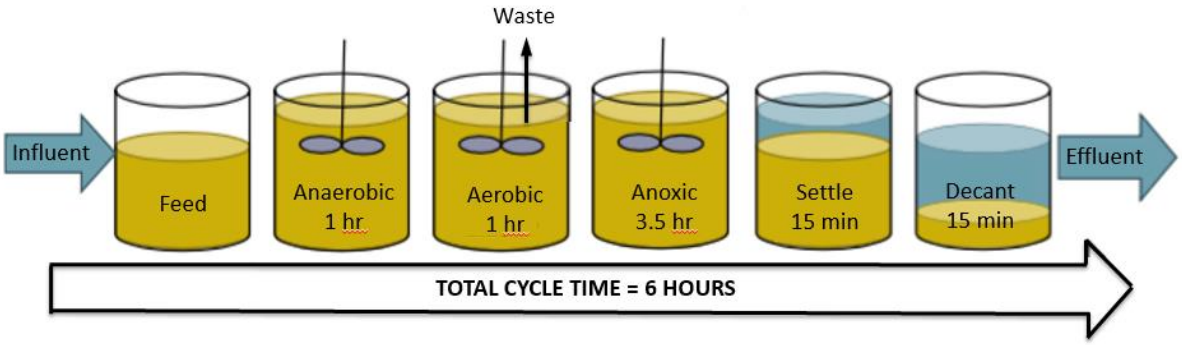

Figure 3-1. BIOPHO-PX cycle details

Figure 3-2. BIOPHO-PX operational setup

3.2 Analytical Techniques

Samples were collected from the BIOPHO-PX reactors to monitor pH, total solids (TS), VS, dissolved oxygen (DO), soluble nutrients (i.e., ammonia, nitrate, nitrite, and phosphorus), VFAs, intracellular glycogen and PHA. Samples were collected from the fermenters to monitor pH, TS, VS, nutrients (i.e., ammonia and phosphorus), solids characteristics, and VFAs. To analyze soluble constituents, samples were first centrifuged to remove biomass and then filtered through a 0.22 µm syringe filter (Millipore Corp., Billerica, MA, USA) prior to testing. Measurement of pH was accomplished with a Thermo-Fisher Scientific Corp (Waltham, MA, USA) Accumet AP85 Waterproof pH/Conductivity Meter. TS and VS were measured in accordance with Standard Methods 2540 D and 2540 E [72], respectively. DO measurements were collected using a Hach (Loveland, CO, USA) HQ30d Meter with a LDO101 DO Probe. Glycogen was determined as total glycogen with dried biomass samples according to Parrou and Francois [73].

3.2.1 Nutrient Analysis

A Thermo-Fisher Scientific Corp Spectronic® 20 Genesys™ spectrophotometer was utilized to measure the absorbance of the reacted sample at a wavelength of 655 nm for NH_4, 410 nm for NO_3, 507 nm for NO_2, and 890 nm for PO_4. Soluble NH_4, NO_3, and NO_2, and PO_4 concentrations were determined utilizing standard curves ($R^2 > 0.99$). Testing for soluble

NH_4, NO_3, and NO_2, and PO_4 testing followed Hach method 10031, 10020, 10019, and 8048 (method equivalent to Standard Methods 4500-PE [72]), respectively.

3.2.2 VFA Analysis

VFAs (acetic, propionic, butyric, isobutyric, valeric, isovaleric, and caproic acids) were quantified using a Hewlett-Packard 6890 series gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a flame-ionization detector and a Hewlett-Packard 7679 series injector. The system was interfaced with the Hewlett-Packard GC ChemStation software version A.06.01. VFA separation was achieved using a capillary column (Heliflex® AT™-AquaWax-DA , 30 m x 0.25 mm ID, W. R. Grace & Co., Deerfield, IL, USA) which was ramped from an initial 50°C to 200°C in three steps (following 2 min at 50°C, ramp to 95°C at 30°C min-1 then to 150°C at 10°C min-1 and hold for 3 min; finally, ramp to 200°C at 25°C min-1 and hold for 12 min) with helium as the carrier gas (1.2 mL min-1). The split/splitless injector and detector were operated isothermally at 210 and 300°C, respectively. Prior to analysis, samples were acidified to a pH of 2 using HCl. 0.5 µL of each sample was injected in 20:1 split mode. VFA concentrations were determined through retention time matching with known standards (Sigma-Aldrich Co., St. Louis, MO, USA; Thermo Fisher Scientific Inc., Waltham, MA, USA) and linear standard curves ($R^2 > 0.99$).

3.2.3 Intracellular PHA Analysis

Biomass PHA content was determined by gas chromatography/mass spectrometry (GC-MS) as described by Braunegg et al. [74]. Dried biomass samples were digested at 100°C in 2 mL of acidified methanol (3% v/v sulfuric acid) and chloroform. Benzoic acid was added as an internal standard to the chloroform at 0.25 mg/mL. After digestion, 2 mL of deionized water was added and vortexed to separate into chloroform and water phases. The chloroform phase was extracted and filtered through sodium sulfate anhydrous to remove excess moisture and particulates. GC-MS was performed on a Thermofinnigan PolarixQ iontrap GC-MS instrument (Thermo Electron Corporation). The sample was introduced using split injection. Separation was achieved on a ZB1 (15 m, 0.25 mm ID) capillary column (Phenomenex, Torrance, California, USA) with helium as the carrier gas (1.2 mL min^{-1}) and an initial temperature of 40°C (2 min) ramped to 200°C at 5°C min^{-1}. The compounds were confirmed by retention time and mass spectral matching with known PHA standards (PHB

and PHB-co-HV: Sigma Aldrich; NaHB: Alfa Aeser; Tianan) as methyl ester derivatives, and quantified based on the internal standard. The Xcalibur software program (Thermo Electron Corporation) was used to facilitate PHA quantification, and the m/z 103 ion was chosen for PHA quantification was relative to methyl benzoate. PHB eluted at approximately 5.4-5.6 min, and PHV eluted at approximately 7.9-8.4 min. The benzoic acid standard eluted at 11.9-12.1 min. Total intracellular PHA content was determined on a 42 percent dry weight basis (mass PHA per mass TSS, w/w) and a percent cell weight basis (mass PHA per mass VSS, w/w).

3.2.4 Solids Analysis

Samples of the influent and effluent of both fermenters were collected and shipped to the Dairy One Forage Laboratory (Ithaca, New York, USA) for quantification of acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignins (ADL), starch, crude fat, total phosphorus (TP), and crude protein. A summary of each method used is listed below. The results of the Dairy One analysis were used to estimate the total carbohydrate content (NDF + starch).

ADF: Solutions are as in AOAC 973.18 – Fiber (Acid Detergent) and Lignin (H2SO4) in Animal Feed [75] using ANKOM Technology Method 5 and an ANKOM A200 digestion unit. A solution of cationic detergent and 0.5 M H2SO4 is used to remove most carbohydrates, proteins, and fats, with the residue consisting of cellulose, lignin, and insoluble proteins.

NDF: Solutions are as in Van Soest [76] using ANKOM Technology Method 6 and an ANKOM A200 digestion unit. The sample is treated with a neutral detergent solution and α-amylase enzyme, leaving behind cellulose, hemicellulose, and lignin.

ADL: Solution as in AOAC 973.18 – Fiber (Acid Detergent) and Lignin (H2SO4) in Animal Feed [75]. Analysis is performed on residue of the ADF method using ANKOM Method 9 after digestion in 72% H2SO4 for three hours in a Daisy Incubator. ADL is defined as the residue remaining after removal of the acid-soluble material.

Starch: Analysis is performed using an YSI 2700 SELECT Biochemistry Analyzer. Starches present in samples are hydrolyzed to dextrose, which is quantified by the analyzer. Starch is calculated as 90% of the measured dextrose content.

Crude Fat: Determined in accordance with AOAC 2003.05 – Crude Fat in Feeds, Cereal Grains, and Forages [75]. The procedure uses anhydrous diethyl ether as the solvent in a Soxtec HT6 System, with crude fat residue determined gravimetrically after evaporation of the solvent.

TP: Determined using a Thermo ICAP 6300 Inductively Coupled Plasma (ICP) Radial Spectrometer after microwave accelerated digestion in HCl and H2O2.

Crude Protein: Determined in accordance with AOAC 990.03—Protein (Crude) in Animal Feed [75], using a Leco FP-528 Nitrogen/Protein Analyzer. The sample is combusted in pure oxygen, and total nitrogen is quantified in the gas produced using a thermal conductivity detector. Crude protein is calculated as % total nitrogen (w/w) * 6.25.

3.2.5 Microbial Population Analysis

Samples of biomass were collected from the BIOPHO-PX SBRs for quantitative polymerase chain reaction (qPCR) to evaluate the populations of AOBs, NOBs, PAOs, and GAOs. In qPCR analysis, a segment of 16S rDNA specific to the class of microorganism of interest is selected and then amplified using DNA polymerase enzymes and short lengths of single-stranded DNA called primers which are specific to the gene of interest. The nucleic acids for amplification are removed by disrupting the cells and genetic material is purified prior to qPCR. As the amplification process proceeds, dye is complexed with DNA that fluoresces when it binds to double-strand DNA. The intensity of fluorescence corresponds to the relative quantity of the bacteria of interest compared to the total microorganism community [77]. Quantitative real-time PCR (qPCR) was used to quantify 16S rDNA genes from total bacteria, *Accumulibacter* (the model PAO), GAOs, AOBs, *Nitrobacter* (r-strategists; low affinity for NO_2 and O_2), and *Nitrospira* (k-strategists; high affinity for NO_2 and O_2) to provide an estimation of relative abundance. qPCR was conducted on a StepOne Plus™ Real-Time PCR system (Applied Biosystems, Foster City, CA) using iTaq™ SYBR® Green Supermix w/ROX (Bio-Rad Laboratories, Inc., Hercules, CA, USA) with a total reaction volume of 25 ml. Total bacterial and total Accumulibacter 16S rDNA genes were

quantified with primer sets 341f/534r and 518f/846r, respectively. GAOs were quantified using primer set GAOQ431f/GAOQ989r (specifically designed to target *Candidatus* Competibacter phosphatis), which is a putative model GAO and the total bacteria primer set. Amplification of AOBs was based on a primer set for the gene ammonia monooxygenase. For NOBs, *Nitrobacter* and *Nitrospira* were amplified using 16S rDNA sequences. qPCR conditions were as follows: 3 min at 95°C, 45 cycles of 30 sec at 95°C, 45 sec at 60°C, and 30 sec at 72°C. All unknown samples were assessed in triplicate with 5 ng of total genomic DNA per reaction. Amplification efficiency was estimated for each primer set using baseline-corrected fluorescence data (from StepOne Software v2.0) with LinRegPCR. The cycle threshold was set at a constant value across all samples based on location within the log-linear region for determination of Cq values (cycle number at which the measured fluorescence exceeds the cycle threshold). Gel electrophoresis of qPCR products confirmed the presence of a single band for all GAO and PAO samples [37].

3.3 Calculations

Equation 3-1. Specific VFA Yield (Fermentation Potential)

$$Specific\ VFA\ Yield\ Fermenation - Potential = \frac{VFA_{Concentration}}{VS_{Loaded}} \quad \left[\frac{mg\ VFA_{COD}}{mg\ VS_{Loaded}}\right]$$

Equation 3-2. Specific VFA Yield (Bench-top)

$$Specific\ VFA\ Yield\ Bench - top = \frac{VFA_{Concentration}}{OLR * SRT} \quad \left[\frac{mg\ VFA_{COD}}{mg\ VS_{Loaded}}\right]$$

Equation 3-3. Proportion of VFAs

$$Proportion\ of\ VFA_{i=\#C}\ (Species) = \frac{VFA_i}{\sum_{i=2}^{6} VFA_i} \quad \left[\frac{Cmmol\ VFA}{Cmmol\ VFA}\right]$$

Equation 3-4. P:C ratio

$$P{:}C = \frac{(P_{release_{AN}} - P_{t=0})}{VFA_{Consumed}} \quad \left[\frac{Pmmol}{Cmmol}\right]$$

Equation 3-5. Influent VFA to P ratio

$$VFA{:}P = \frac{VFA_{Influent}}{P_{Influent}} \quad \left[\frac{mg\ VFA_{COD}}{mg\ P}\right]$$

Equation 3-6. Specific VFA uptake rate

$$q_{VFA} = \frac{VFA_{Consumed}}{MLVSS * Time} \quad \left[\frac{Cmmol\ \text{VFA}}{g\ \text{MLVSS} * \text{hr}}\right]$$

Equation 3-7. Phosphorus removal percent

$$TPR = \frac{(PO_4)_{Influent} - (PO_4)_{Effluent}}{(PO_4)_{Influent}} \quad \left[\frac{mg\ \text{P}}{mg\ P}, \%\right]$$

Equation 3-8. Specific phosphorus uptake rate

$$q_{PO_4} = \frac{PO_4}{MLVSS * Time} \quad \left[\frac{mg\ PO_4}{g\ \text{MLVSS} * \text{hr}}\right]$$

Equation 3-9. Growth rate from SRT

$$\mu = \frac{1}{SRT}$$

Equation 3-10. Total nitrogen removal percent

$$TNR = \frac{(NH_4 + NO_x)_{Influent} - (NH_4 + NO_x)_{Effluent}}{(NH_4 + NO_x)_{Influent}} \quad \left[\frac{mg\ \text{N}}{mg\ N}, \%\right]$$

Equation 3-11. Specific denitrification rate

$$SDNR = \frac{NO_{x_{Removed}}}{MLVSS * Time} \quad \left[\frac{mg\ NO_x}{g\ \text{MLVSS} * \text{hr}}\right]$$

Equation 3-12. Nitrite accumulation

$$\%\ Nitrite\ Accumulation_{End\ AE} = \frac{NO_2^-}{NO_x} \quad \left[\frac{mg\ \text{N}}{mg\ N}, \%\right]$$

4 Fermentation of Algal Biomass

4.1 Introduction

Nutrients (i.e., nitrogen and phosphorus) contained within municipal wastewater and processed through WRRFs must be removed to sustain the aquatic health of receiving bodies. WRRFs discharge treated effluent into either a freshwater body (i.e., river, lake, or stream) or the ocean. For freshwater bodies, phosphorus is the contaminant of concern [78], and excess P in treated effluent can lead to advanced eutrophication; this environmental detriment, along with increasingly stringent effluent quality regulations, have led to the need for BNR processes to be adapted by WRRFs. Indeed, the EBPR process is commonly utilized to achieve excess P removal from wastewater. Implementing EBPR requires readily biodegradable carbon, specifically volatile fatty acids (VFAs), to maintain high quality EBPR process performance [79].

Beyond the need to drive EBPR, VFAs can serve additional purposes in a WRRF. With the scarcity of virgin materials becoming more apparent every year [2], there is an increased drive to produce nutrients in new forms (i.e., recycling or resource recovery of nitrogen and phosphorus). In this regard, nutrients can be recovered as struvite, a naturally occurring crystal; however, the recovery of struvite is limited by the availability of VFAs, which helps drive the release of phosphorus from EBPR biomass [31]. The mixed microbial culture used to perform EBPR can also be used to store excess carbon in the form of polyhydroxyalkanoates (PHA), a biodegradable plastic and valuable commodity that can be produced onsite at WRRFs [66]. Here again, PHA production vies for the limited amount of VFAs contained within the influent wastewater. Considering the broad demand for VFAs associated with diversifying the WRRF footprint, alternative means to maximize onsite VFA production are needed.

Organic matter (OM) fermentation is the primary method utilized for producing VFAs. Historically, OM fermentation focused solely on primary solids (solids removed prior to biological treatment) as a substrate [80, 81]; however, more recently, in an effort to maximize VFA production, there has been a push to study the effects of different fermentation substrates that are internal at WRRFs; namely, activated sludge [19, 20, 82, 83]. Growing interest in the use of algae to polish secondary effluent as a form of tertiary treatment has led

to the creation of a new potential fermentation substrate [84, 85]. The algae serve multiple purposes: capturing nutrients contained within secondary effluent [86, 87], fixing carbon dioxide from the atmosphere to reduce the overall WRRF carbon footprint [88, 89], and providing an internal fermentation substrate. The growth of algae would provide carbon from an external source (i.e., CO_2 from the atmosphere or anaerobic digester biogas [88]) which could be blended with primary solids and fermented to potentially lead to an increased VFA production compared to primary solids alone.

Current investigations into uses for algal biomass focus almost exclusively on refinement of the conversion of lipid-rich algal biomass to biogas, with some research into the potential of the biomass to be used as a fertilizer or feedstock. There is a gap in knowledge as to the impacts an algal and primary solids blend could have on total VFA production within a WRRF. This research investigated how supplementing algal biomass to a primary solids fermenter would affect VFA production and speciation, soluble nutrient concentrations, and pH over a variety of solids retention times (SRT).

4.2 Results and Discussion

Investigations were undertaken to evaluate the potential additive value algal biomass would create (i.e., VFAs) in a WRRF. It was anticipated that an increase in VFA production would be realized due to increased carbon in the system from algal biomass being recycled to the fermenter. Additionally, the algal OM was hypothesized to increase both ammonia and phosphorus concentrations due to hydrolysis reactions. Fermentation potential tests were undertaken to rapidly assess VFA production potential and guide fed-batch design; subsequently, extended fed-batch studies were performed.

4.2.1 Establishing Algal Biomass Fermentation Potential

While it has been demonstrated that algal biomass exhibits the potential to enhance VFA production via fermentation when integrated with organic substrates [90], the potential in a municipal WRRF environment and commingled with municipal primary solids is undefined. Moreover, VFA production potential from algae cultured on WRRF effluent has not been considered. Fermentation potential tests were conducted to examine potential of algal biomass from Missoula, MT (USA), both to examine potential (relative to primary solids) as well as contrast fresh vs. frozen algae. Moreover, fermentation potential test data

was needed to inform bench-scale design and operational criteria. While it is not necessarily anticipated that frozen algae might be used in a full-scale WRRF application, the bench-scale investigations conducted in this study demanded algal supplies be frozen to preserve the viability of the algae over the length of the study [91]. Two metrics were used to evaluate fermentation potential: VFA yield (calculated as mg VFA (as COD) per g VS; Equation 3-1) and VFA speciation (Equation 3-3).

4.2.1.1 Effect of Retention Time on VFA Production Potential

First considering specific VFA yield, as shown in Figure 4-1, up to retention time (RT) of approximately 6 days, all substrates (fresh algae, frozen algae, and primary solids) exhibited similar fermentation potential. However, between a RT of 6 and 10 days, algal fermentation potential exceeded that of primary solids. Overall, for the 10 d RT, algae produced more VFAs than observed for the municipal primary solids; specifically, the fermentation potential for fresh algae, frozen algae, and primary solids was 0.28 ± 0.02 (n=3), 0.25 ± 0.02 (n=3), and 0.21 ± 0.01 (n=3) mg VFA_{COD}/mg VS, respectively. When comparing specific VFA production results for the duration of the assessment (10 days), there was no statistical difference in fermentation potential between fresh and frozen algae (t=2.06). Conversely, a statistical difference was realized between both the fresh algae and the primary solids (t=6.43), and the frozen algae and PS (t=3.31). Increased VFAs in the fresh algae reactors suggest using this substrate could be beneficial in a WRRF at longer SRTs.

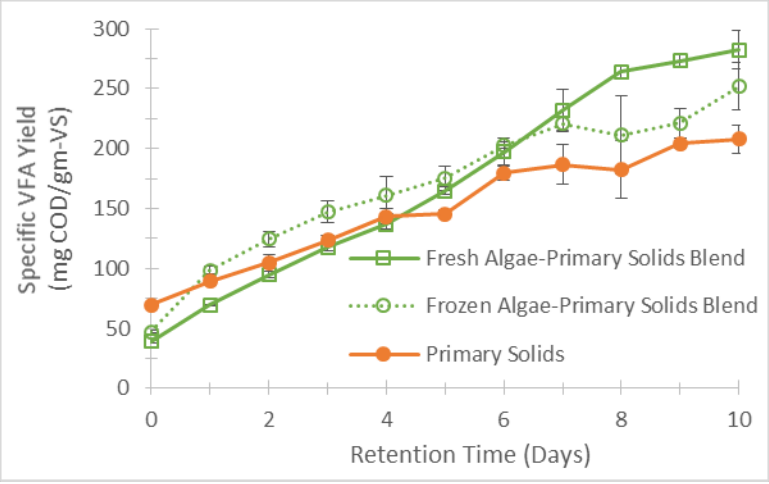

Figure 4-1. Specific VFA production of fermentation potential tests

4.2.1.2 Effect of RT on VFA Speciation

Beyond VFA specific yield, should algal fermentation be integrated into a WRRF, VFA speciation is equally important. Indeed, research has indicated that VFAs larger than acetic acid (e.g., propionic acid) can enhance EBPR [6, 13]. Moreover, should PHA production become a focus, a diverse array of VFAs will result in a better quality polymer [14]. Overall, VFA speciation was generally typical for fermentation of organic matter, in that acetic acid dominated in concentration, followed by C3-C6 VFAs (Figure 4-2). The algal-based fermenters [Figure 4-2 (A) and (B)] exhibited a more diverse distribution of VFAs, particularly at RTs exceeding 5 days. Of greater interest, however, the algal fermenters (fresh and frozen) both produced markedly higher concentrations of valeric (C5) and caproic (C6) acids when compared to the primary solids reactor (Figure 4-3). For valeric acid, the fresh algae reactor yielded an increase of 39% over primary solids, while the frozen algae yielded an increase of 28% at a RT of 10 days. Caproic acid was present at a concentration in the fresh algae reactor 977% and 164% greater than observed in the primary solids reactor and frozen algae reactor, respectively. Smith et al. [21] reported co-fermentation of algae and dairy manure produced more valeric and caproic acids (10% and 30%, respectively) than manure alone. The divergence in speciation occurs most dramatically after a 6-day RT (Figure 4-3). Comparatively, algal biomass is typically more enriched in lipids (vs. primary solids) and can contain upwards of 22% lipids (on dry weight basis) [89]. Theory dictates that lipids contained within the algae will begin to hydrolyze to LCFAs, and subsequently be anaerobically oxidized to acetate and hydrogen, around a 6-day SRT [15, 92]. While beta oxidation of LCFAs to acetic acid and hydrogen is thermodynamically favored over anaerobic oxidation of VFAs (3 through 6 C) [15], the ultimate build-up in hydrogen partial pressure can inhibit the fermentation of VFAs [15], resulting in larger VFAs. Indeed, as suggested by Bouzas et al. [81], hydrogen accumulation (associated with fermentation) induced inhibition of acetogenic bacteria, resulting in C3-C6 fatty acid accumulation. The higher lipid content in the algal biomass may account for the increased concentration of caproic and valeric acids (i.e., undigested VFAs) observed in the algal-based fermenters.

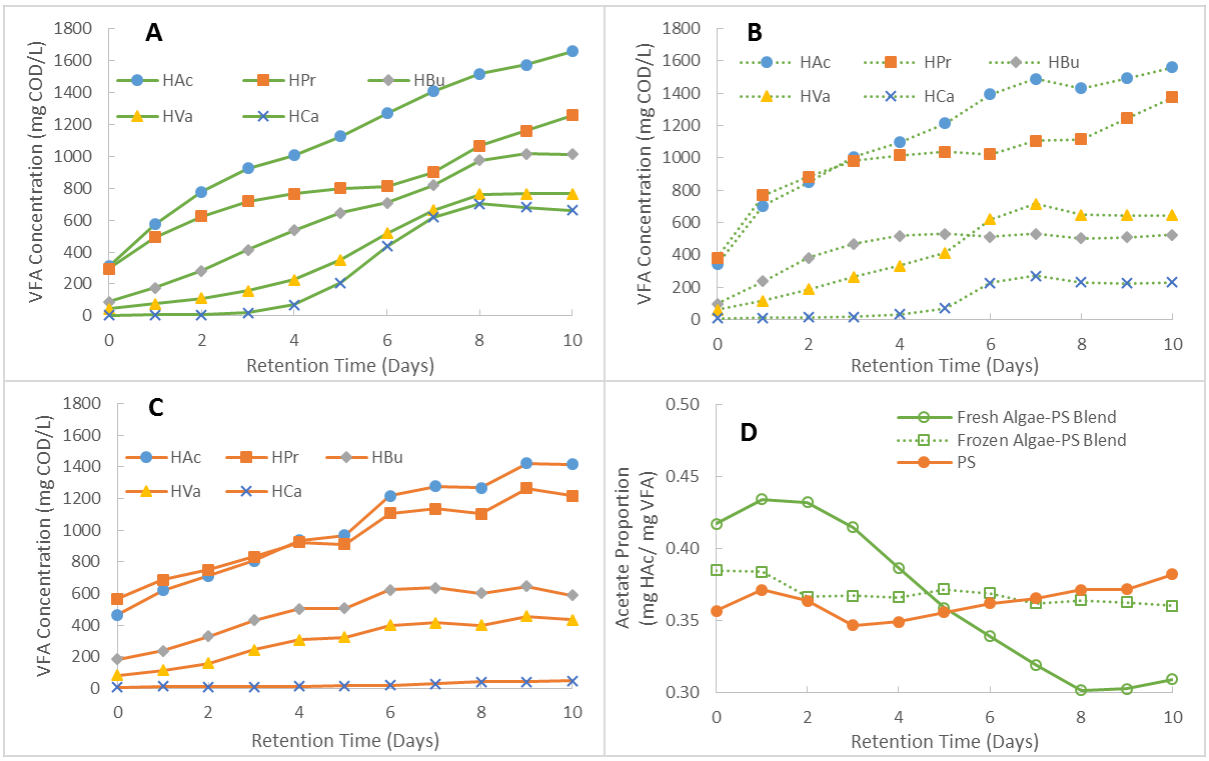

Figure 4-2. Speciation of VFAs in fermentation potential tests of: (A) fresh algae-PS blend, (B) frozen algae-PS blend, and (C) PS, and (D) acetate proportion of total VFAs

As a final point of interest, the proportion of acetate in the fermenters appeared to be affected by the substrate as well [Figure 4-2 (D)]. Both the frozen algae and primary solids fermenters maintained a relatively constant proportion of acetate over the course of the test. In contrast, the fresh algae reactor exhibited more variability, in that the acetic acid fraction decreased markedly beginning at a RT of approximately 3 days; ultimately, the acetic acid fraction decreased from over 40% of total VFAs to 30% at a RT of 8 days. One explanation for the variability in acetate proportion – and the observation that frozen algae did not exhibit a similar pattern – is the effect of freezing on the algae; freezing causes both intra- and extracellular ice crystals to potentially lyse the cells [93]. Therefore, compared to the primary solids and frozen algae, the fresh algae likely retained a larger fraction of intact lipids and LCFAs, which were fermented to yield a dramatic increase in valerate and caproate, effectively lowering the acetate proportion (not acetate concentration).

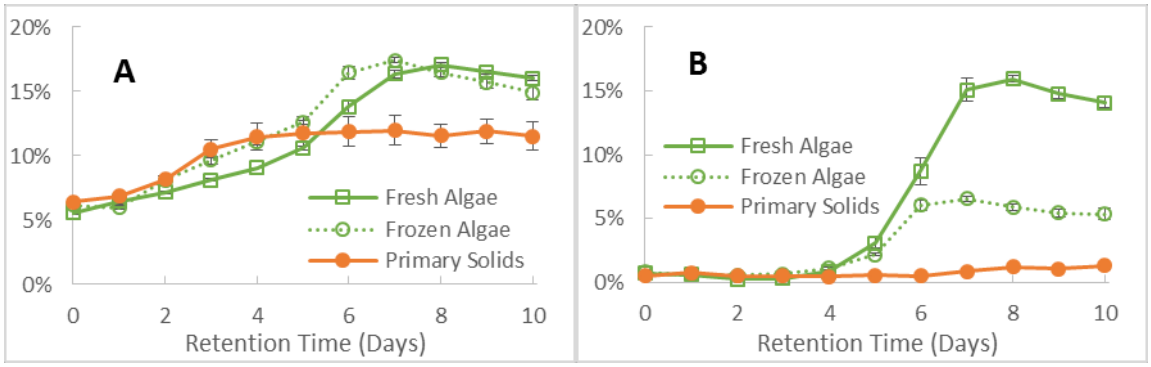

Figure 4-3. (A) Percent valeric acid, and (B) percent caproic acid in the fermentation potential tests

4.2.2 Fed-batch Fermentation and the Effects of Algal Biomass Augmentation

Fermentation at a full-scale WRRF would occur in a fed-batch or continuous flow configuration, and not as a batch operation. Moreover, algae-only fermentation is not envisioned; instead, blended PS-algae would be more likely. Thus, fed-batch algae/PS fermenters were designed, operated, and tested as a next-step in the research. As indicated by the fermentation potential test results, specific VFA yield increased with RT, while VFA speciation was also enhanced by the augmentation with algal biomass. SRT, which represents the RT of the solids, can impact VFA production and speciation associated with (1) realizing the onset of methanogenesis at longer SRTs (≥6 days, depending on temperature [15]); (2) increasing biomass (specifically fermenting bacteria) concentration, which can enhance disintegration, hydrolysis, and fermentation; (3) inhibiting anaerobic oxidation reactions associated with high hydrogen partial pressures; or (4) a combination of all the above. Considering the fermentation potential results and observed impact of RT, three SRTs were evaluated in this study (5, 6, and 7 days), applied to two bench-top fed-batch fermenters (labeled MF1, which received only primary solids, and MFA, which received primary solids augmented with algal biomass).

4.2.2.1 Effect of SRT on VFA Production

Maximizing total VFA production in a WRRF is significant due to the downstream implications discussed earlier (i.e., enhancing EBPR; potential enhanced resource recovery through PHA production). Theoretically, and empirically based on the fermentation potential data (Figure 4-1), the additional carbon supplied by the algal biomass will increase total VFA production; however, and unexpectedly, the addition of algal biomass did not increase VFA production (concentration or yield; Table 4-2) relative to primary solids alone. Specifically,

fermenter MFA yielded a decrease in VFA concentration of 30-, 34-, and 26% when compared to MF1 at 5-, 6-, and 7 days, respectively. First considering fermenter MFA, the VFA concentration increased both when SRT was increased from 5 to 6 days (49% increase) and again from 6 to 7 days (8% increase). The increase in VFA concentration was statistically significant between 5 and 6 days (t=10.80) and between 6 and 7 days (t=3.14). As suggested by the fermentation potential results and also by others [8, 17, 23, 24, 94, 95], longer SRTs will result in increased VFA production until the methanogenic population reaches a critical mass. On the other hand, in fermenter MF1, the VFA concentration increased when SRT was increased from 5 to 6 days (57% increase), but decreased slightly when the SRT was increased to 7 days (3% decrease); these latter results suggest potential onset of methanogenesis in MF1 at an SRT of 7 days. There was a statistical difference in VFA concentrations within MF1 between an SRT of 5 and 6 days (t=-20.5), but no statistical difference was realized between 6 and 7 days (t=1.29). While fermenter MFA realized an increase in VFA concentration at a 7 day SRT vs. 6 days, the increase was smaller than between 5 and 6 days; this also indicates MFA may have been close to establishing a methanogenic population. The increase in VFA concentrations in fermenter MFA, as compared to fermenter MF1, suggests enhanced lipid degradation associated with longer SRTs, a result observed by others [15, 96] and as indicated by the fermentation potential tests. In addition, the observation that a 6 day SRT is near-optimal for maximizing VFA production is consistent with other investigations [16, 81]. Some research has indicated there may be a relationship between VFA production and pH [17, 18]; however, such investigations involve active pH control through addition of base. Investigations of the pH within the bench-scale fermenters in this study showed variation between MF1 and MFA, and with SRT (Table 4-1). Statistically, there was a significant difference between the pH in MF1 and MFA across all SRTs. As expected (based on theory presented in ADM1 [10]), the pH will decrease in fermenters as more VFAs are produced (with increased SRT); thus, the pH is determined by the amount and species of VFAs, and not vice-versa.

Table 4-1. pH of bench-scale fermenters

SRT (Days)	MF1	MFA
5	4.83 ± 0.12 (83)	5.27 ± 0.42 (20)
6	4.65 ± 0.07 (44)	4.89 ± 0.07 (44)
7	4.58 ± 0.05 (49)	4.80 ± 0.07 (50)

Analysis of the specific VFA yields of the fermenters (normalized on a VS loading basis) allows for better comparison between substrates across SRTs. While VFA production (concentration) in the fermenters is an important metric, specific VFA yields shed light on the efficiency of the biomass to ferment the substrate. Results from this study (0.11-0.23 mg_{COD}/mg_{VS}) align well with reported specific VFA yields, which ranged from 0.125-0.20 mg_{COD}/mg_{VS} [1, 15, 78, 97, 98]. Similar to the differential VFA concentrations between the fermenters, MF1 realized the largest specific VFA yields. Regarding SRT, in both fermenters the specific VFA yields increased when SRT was increased from 5 to 6 days (MF1, 35% increase; MFA, 27% increase); however, when the SRT was increased to 7 days, there was an apparent loss in fermentation efficiency corresponding with specific VFA yields (MF1, 17% decrease; MFA, 14% decrease) which could be due to (1) onset of methanogenesis and/or (2) increased VS loading. Statistical analysis showed there was a significant difference between specific VFA yields for MF1 and MFA at all SRTs, as well as between MF1 and MFA at all SRTs tested.

Table 4-2. Total VFA production and specific VFA yield versus SRT [Avg. ± SD (number of samples)]

	MF1		MFA	
SRT (Days)	**mg_{COD}/L**	**mg_{COD}/mg_{VS}**	**mg_{COD}/L**	**mg_{COD}/mg_{VS}**
5	1953 ± 195 (53)	0.17 ± 0.02 (53)	1367 ± 150 (11)	0.11 ± 0.01 (53)
6	3073 ± 229 (19)	0.23 ± 0.02 (19)	2036 ± 170 (19)	0.14 ± 0.01 (19)
7	2966 ± 323 (38)	0.19 ± 0.02 (38)	2193 ± 183 (38)	0.12 ± 0.01 (38)

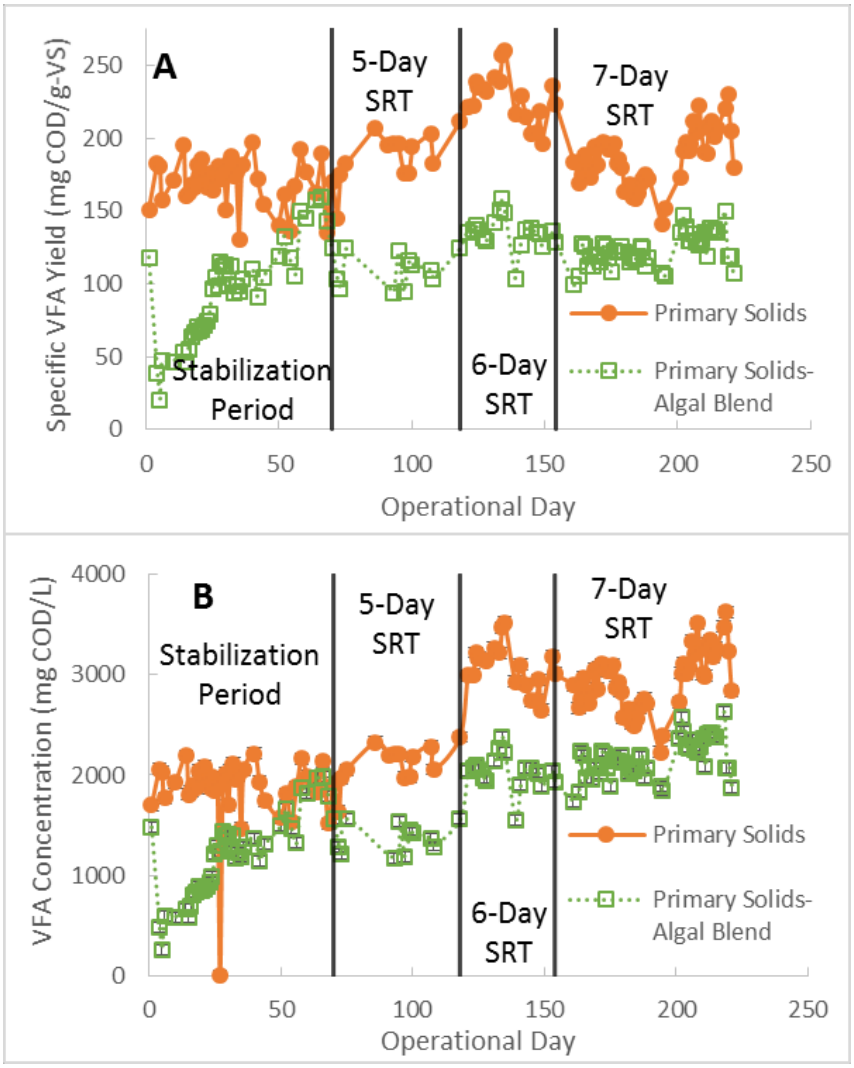

Figure 4-4. (A) Specific VFA production, and (B) Total VFA Production in Bench-Top Reactors

4.2.2.2 Effect of SRT on VFA Speciation

As evidenced by the fermentation potential results, and specifically during algal augmentation, there is potential to leverage the SRT to increase the variety of VFA species in a fermenter; as discussed, enhanced VFA speciation can enhance both EBPR and PHA production. While there was an unexpected decrease in VFA production for the algal-augmented system (relative to MF1), enhanced VFA speciation could nonetheless potentially increase the value of algal fermentation. First, considering primary solids fermentation, process stability was realized relatively quickly in MF1; moreover, VFA speciation of MF1 did not vary with the SRT, which mimics the results of the fermentation potential assessment [Figure 4-2 vs. Figure 4-5 (A)]; however, MF1 did show interesting speciation behavior.

Specifically, the dominant VFA alternated between acetic and propionic acid concentrations [Figure 4-5 (B)]; this is in contrast with the literature which commonly shows acetic acid as the predominant species [8, 16, 19, 21, 23, 24, 61, 80, 81, 99-101], although such a result was suggested by the fermentation potential data [Figure 4-2 (C)] and other sources [18, 98]. As mentioned earlier, the fraction of OM (i.e., polysaccharides, proteins, and lipids) will influence the VFA speciation in fermenters. In this regard, the OM was not characterized for each batch of PS; thus, the acetic to propionic acids behavior could be due to variations in OM characteristics. Regarding fermenter MFA, the bacterial culture required a longer stabilization period, as indicated by the convergence of acetic and propionic acid proportions as the research progressed [Figure 4-5 (B)]; this phenomenon was also observed during startup of a fermenter by Wu et al. [18]. As described, fermenter MFA was initially operated at a 45:55% distribution of algal biomass and primary solids (VS basis), but updated theoretical algae yields demanded a change in loading to a 10:90% distribution. Another potential explanation for higher acetic acid proportions in MFA could be due to mixing; since the two fermenters had different mixing systems, it is possible that MFA realized lower mixing intensity. A lower mixing intensity will cause decreased local turbulence (i.e., smaller velocity gradient, G) and potentially reduce the release of gases from solution (including hydrogen); a larger partial pressure of hydrogen in bulk solution will inhibit anaerobic oxidation of LCFAs and VFAs to acetic acid [15]. Investigations into the mixing (by switching the reactors and associated mixing system) showed no statistical difference in acetic acid proportions between reactors, which suggests the mixing intensity was similar. Ultimately VFA speciation in fermenter MFA followed the same trends as observed in the algae-PS blend fermentation potential (more closely mimicking frozen algae-PS blend, as would be expected). There was an increase in valeric and caproic acids at SRTs above 6 days, which supports the hypothesis that lipids in the algae are being hydrolyzed to LCFAs and anaerobically oxidized to acetic acid, causing a buildup of VFAs (3-6C) due to high hydrogen partial pressures in solution.

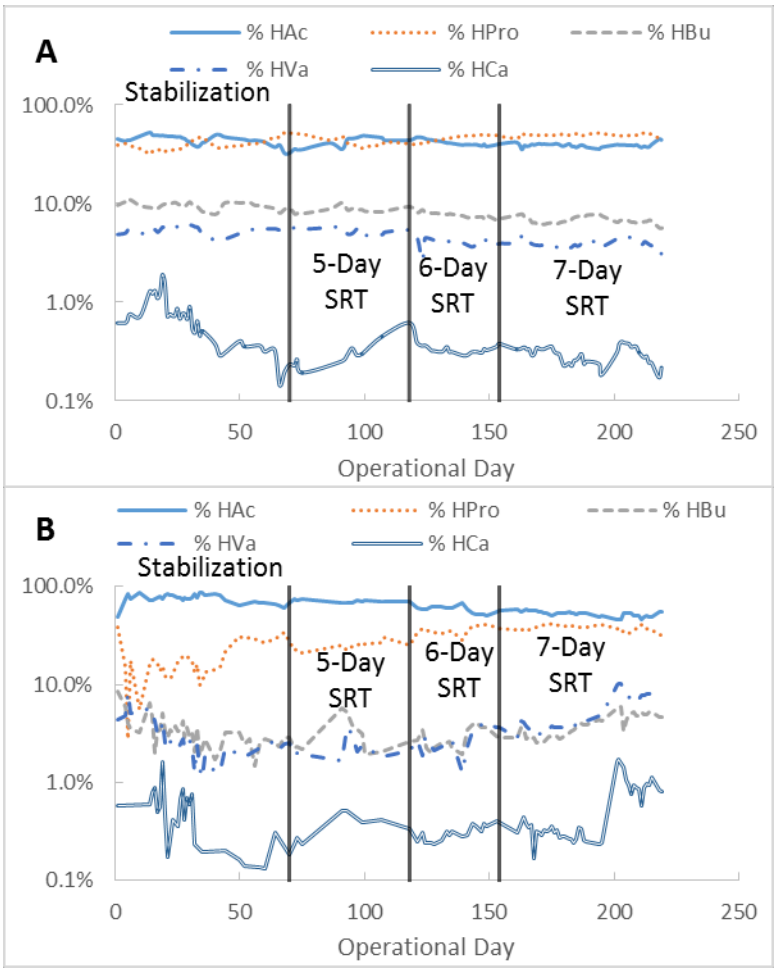

Figure 4-5. Speciation of VFAs within (A) MF1, and (B) MFA

4.2.2.3 Nutrient Concentrations

Fermentation of organic matter involves a complex array of microbially-mediated processes, including biomass disintegration and subsequent hydrolysis. The breakdown of complex organic matter to simple monomers not only yields VFA precursors, but also nitrogen (as ammonia) and phosphorus (as orthophosphate). Thus, fermentation of organic matter would be expected to result in an increase in bulk solution nutrient concentrations [27, 81, 102, 103]. While orthophosphate concentrations did increase in concentration for both fermenters and at all SRTs, an unexpected observation was the reduction in ammonia concentrations in both fermenters (Table 4-3). Relative to the influent ammonia-N concentrations, for MF1, ammonia-N decreased on average 15% and 61% for 5 and 7 day SRTs; no ammonia-N reduction was observed at the 6 day SRT. MFA realized even greater ammonia-N reduction, with 39%, 74%, and 96% for 5, 6, and 7 day SRTs. In full-scale

WRRFs, ammonia-N is commonly regulated in the effluent [104]. Thus, any additional generation of nitrogen from fermenting organic biomass only increases treatment requirements. Conversely, reducing the amount of ammonia could have a significant impact on the oxygen requirements of the WRRF, thereby reducing the operating costs associated with treatment.

In seeking alternative explanations for the observed decrease in ammonia-N, the potential for NH_3 off-gassing can be ruled out, since the pH in both fermenters was consistently below pH 5. Similarly, struvite production can be eliminated, since a minimum pH of 7 is required [30]; also, no struvite granules were observed, and the orthophosphate concentration increased. One potential explanation for the observed reduction in ammonia (without reduction in phosphorus) observed in the algal-fed fermenter is the presence of heterotrophic algae. These algae are capable of growing (and thus consuming ammonia) in the absence of sunlight and/or an inorganic carbon source (CO_2). Indeed, heterotrophic algae can use VFAs (most notably acetate and butyrate) as a carbon/energy source [11, 32, 70, 71, 105]. As discussed and noted (Table 4-2), fermenter MFA unexpectedly realized a markedly lower VFA production compared to MF1, despite being operated at a larger organic loading rate. The fermentation potential tests also suggested an increase in VFA production. Carbon utilized for algae growth could account for the lower than expected production of VFAs in MFA and the associated ammonia-N reductions. A theoretical stoichiometric assessment was performed to evaluate the potential of algae to reduce the ammonia and carbon (VFAs) in the fermenters. Assuming a molar ratio of 106:16:1 of carbon, nitrogen, and phosphorus, respectively [106], and further assuming that the difference in VFA production between MF1 and MFA was associated with heterotrophic algae growth, the resulting ammonia-N demand would be approximately 39 mg/L, 74 mg/L, and 60 mg/L at SRTs of 5, 6, and 7 days, respectively. Since this theoretically-based estimate exceeds the actual observed reduction in ammonia-N, it is feasible that heterotrophic algal growth was responsible. Solids analysis performed on the bench-scale fermenters additionally supports the hypothesis of heterotrophic algae growth in MFA. Specifically, the solids portion of MFA showed an uptake in crude protein, crude fats, and lignin, all of which indicates growth of a high fat/high nitrogen biomass consistent with algae. On the other hand, MF1 had no significant difference in any constituent besides fibers, which are easily hydrolyzed by the fermentative bacteria. While

heterotrophic algae growth potentially explains the significant ammonia-N reduction in fermenter MFA, this phenomenon does not explain the significant reduction in ammonia-N in fermenter MF1 at an SRT of 7 days.

Table 4-3. Influent and effluent nutrient concentrations in bench-scale fermenters for 5-, 6-, and 7-day SRTs

	Influent	5-Day SRT	6-Day SRT	7-Day SRT
	MFA Nutrients			
PO_4-P (mg/L)	38.83 ± 5.45 (6)	43.91 ± 12.03 (9)	47.31 ± 3.97 (12)	49.49 ± 6.41 (11)
NH_4-N (mg/L)	32.81 ± 4.58 (6)	20.10 ± 15.02 (9)	8.69 ± 5.30 (12)	1.22 ± 1.72 (11)
	MF1 Nutrients			
PO_4-P (mg/L)	24.29 ± 0.55 (6)	33.78 ± 6.22 (9)	45.43 ± 4.81 (12)	42.26 ± 4.93 (11)
NH_4-N (mg/L)	27.48 ± 2.91 (6)	23.37 ± 5.43 (9)	27.35 ± 4.02 (12)	10.78 ± 3.62 (11)

5 Effect of Algal Fermentation on BIOPHO-PX Performance

As discussed, two BIOPHO-PX SBRs were operated and evaluated for removal of nitrogen and phosphorus. The primary purpose of these investigations was to evaluate the potential effects on overall treatment performance associated with receiving fermenter liquor produced with and without algal augmentation. Comprehensive sampling (samples taken every 30 minutes over the course of the 6-hour cycle) was performed on operational days 108, 130, 155, 167, and 190; periodic monitoring was also performed between sampling runs (i.e., MLSS/ MLVSS, effluent nutrients). Each SBR was fed a different substrate (as indicated in Section 3.1.3), and the MMC response was evaluated through the measured EBPR/BNR operational parameters.

Results from the two BIOPHO-PX reactors (BIOPHO-PX 3 fed MF1 and wastewater; BIOPHO-PX 3* fed MFA and wastewater) were compared with the theory of EBPR, nitrification/nitritation, and denitrification/denitritation. A summary of influent and effluent characteristics are listed in Table 5-1. As discussed in Chapter 4, it was expected that the increased OLR to fermenter MFA would produce more VFAs that could be used to drive the BIOPHO-PX process; additionally, the increased OLR was expected to increase the concentrations of ammonia and phosphorus in the fermenter liquor. Contrary to expectations, however, fermenter MFA produced markedly less VFAs than fermenter MF1 (Table 5-1); the difference was statistically significant ($t=2.20$). In addition to fewer VFAs, fermenter MFA effluent produced less ammonia but with higher phosphorus concentrations. In contrast, the BIOPHO-PX reactors realized no significant differences between influent phosphorus and ammonia concentrations ($t=1.55$ and $t=0.30$, respectively; Table 5-1); the only statistical difference in substrates to the BIOPHO-PX reactors was VFA concentrations (BIOPHO-PX 3 greater than 3*).

The discussion that follows focuses on the effects that a decreased VFA load, associated with fermentation of algal biomass, will have on the BIOPHO-PX system in conjunction with slightly higher phosphorus and ammonia loading.

Table 5-1. Summary of BIOPHO-PX influent and effluent [Avg. ± SD (number of samples)]

	Influent NH_4 (mg N/L)	Effluent NH_4 (mg N/L)	Effluent NO_x (mg N/L)	Influent PO_4 (mg P/L)	Effluent PO_4 (mg P/L)	Influent VFA (Cmmol/L)
BIOPHO-PX 3	25.37 ± 9.67 (14)	4.70 ± 4.61 (16)	1.52 ± 2.55 (11)	4.65 ± 1.03 (10)	0.25 ± 0.26 (16)	3.55 ± 0.95 (12)
BIOPHO-PX 3*	26.54 ± 8.38 (14)	7.73 ± 7.92 (16)	0.28 ± 0.68 (12)	5.36 ± 1.02 (10)	0.59 ± 0.79 (16)	2.66 ± 1.03 (12)

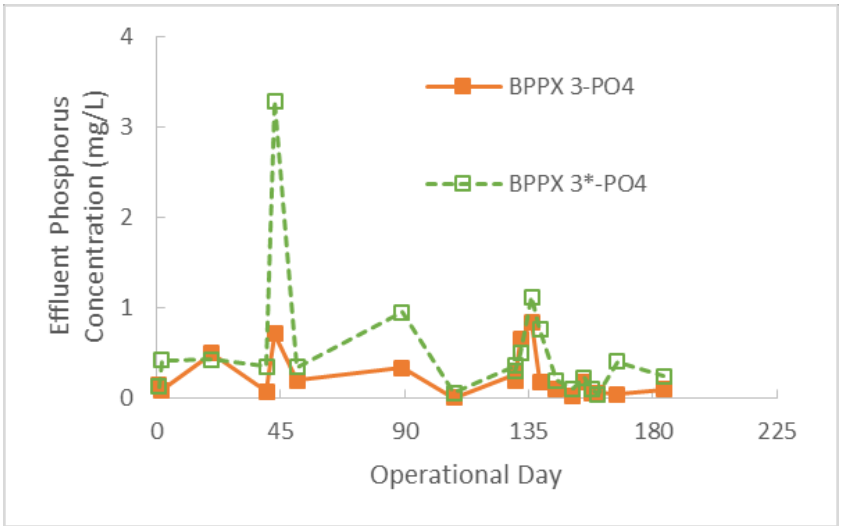

Figure 5-1. BIOPHO-PX effluent phosphorus concentrations

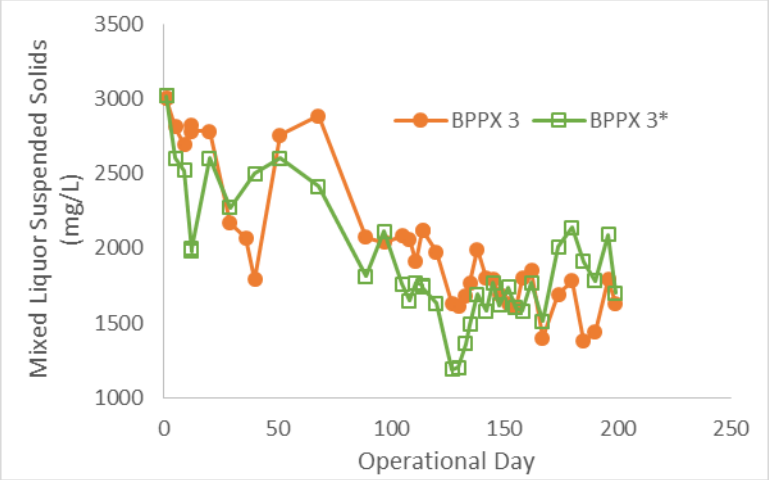

Figure 5-2. Mixed liquor suspended solids in BIOPHO-PX reactors over duration of project

5.1 Phosphorus Removal

5.1.1 Phosphorus Cycling and Removal

Phosphorus is the contaminant of concern in freshwaters; therefore, in most inland areas, phosphorus must be removed to protect the environmental quality of rivers, lakes, and streams. In achieving phosphorus removal, both BIOPHO-PX reactors generally performed in accordance with EBPR theory [1, 46, 107, 108] (

Figure 5-1; Table 5-1). The MMC cycled phosphorus and carbon (VFAs, PHA, and glycogen) in a manner consistent with EBPR; specifically, the MMC released phosphorus from Poly P reserves while consuming the VFAs in the anaerobic zone to store PHA. The PHA was then consumed in the aerobic zone to provide energy for phosphate uptake. Patterns for BIOPHO-PX 3 and 3* are shown in Figure 5-3 and Figure 5-4, respectively. The investigations performed by Winkler et al. [37] on the BIOPHO-PX process yielded similar patterns and effluent phosphorus concentrations (0.02 to 0.14 mg P/L), even though the operational parameters were different (1.5 & 2 hour aerobic period, vs. 1 hour applied in this study). Results suggest that the length of the aerobic period does not significantly affect phosphorus removal in the BIOPHO-PX systems, at least for the aeration period applied; > 97% of influent phosphorus was removed (Table 5-2 and [37]). Phosphorus removal was also consistent with literature values - 99% in an anaerobic-oxic-anoxic (AOA) configuration, similar to BIOPHO-PX [109], and 98% in a modified A2O process [110] (Figure 2-7). In the aerobic zone, the phosphorus was removed rapidly by both BIOPHO-PX 3 and 3*, at specific phosphorus uptake rates (mg P/gm VSS-hr; Equation 3-8) of 15.94 ± 5.24 and 15.70 ± 2.66, respectively. Winkler et al. reported similar results in their BIOPHO-PX studies (7.4 to 25.7; [37]).

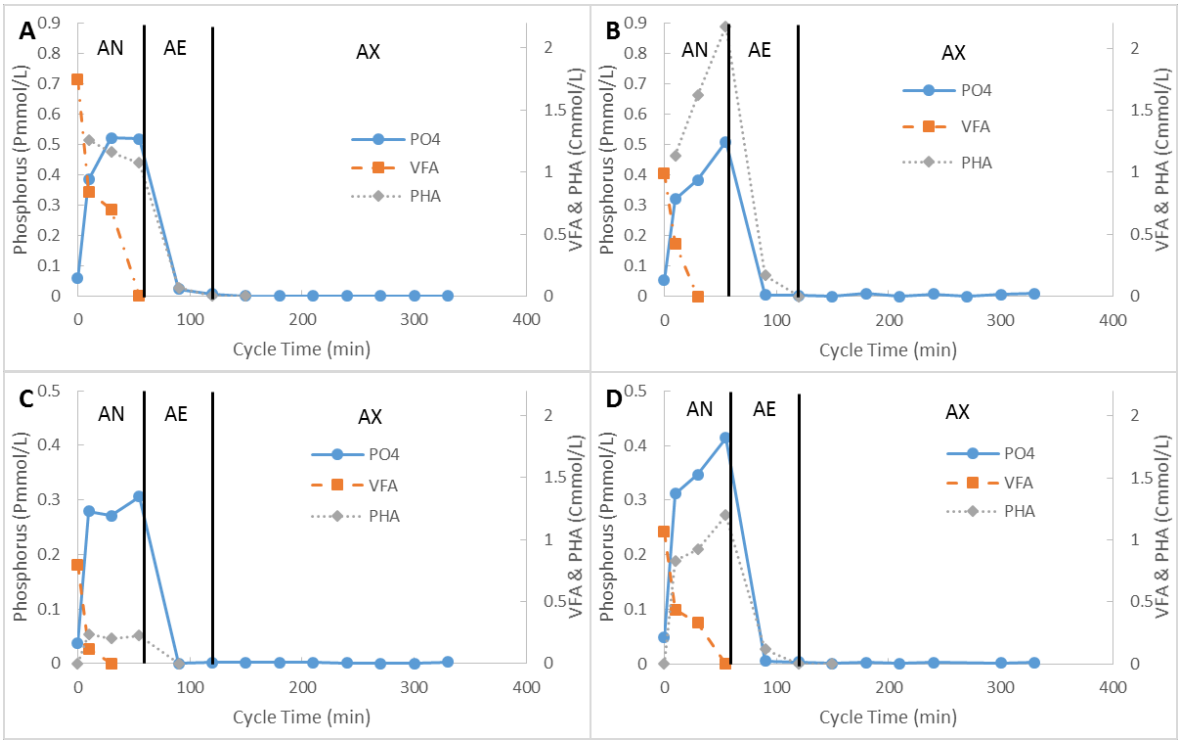

Figure 5-3. BIOPHO-PX 3 EBPR metabolism on operational day: (A) 108, (B) 130, (C) 190, and (D) average (n=5)

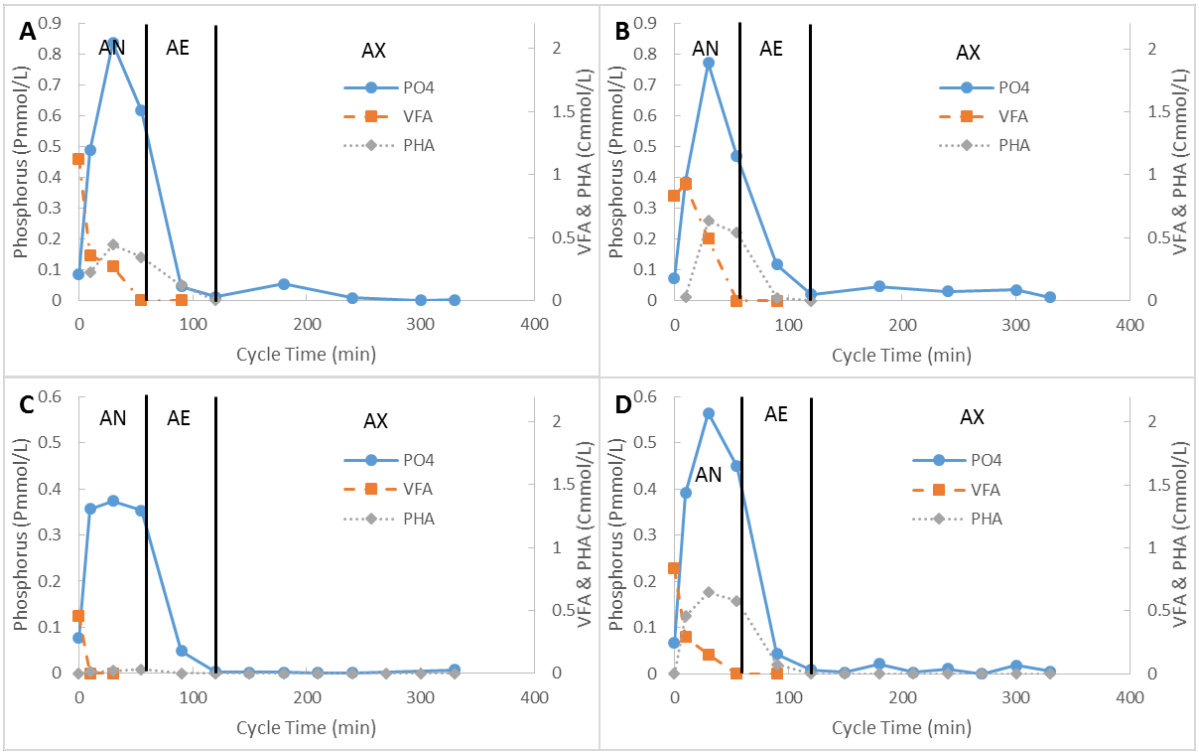

Figure 5-4. BIOPHO-PX 3 EBPR metabolism on operational day: (A) 108, (B) 130, (C) 190, and (D) average (n=5)*

5.1.2 EBPR Metrics

As discussed previously, EBPR performance can be both predicted and assessed based on the influent VFA:P and anaerobic P:C ratios; the former suggests EBPR potential, while the latter can describe induced metabolism. Interestingly, BIOPHO-PX 3 and 3* had statistically significantly different values for both EBPR metrics (P:C with t=3.22; VFA:P with t=3.31; Table 5-2). Filipe et al. [47] and Smolders et al. [108] suggested the P:C ratio varies with pH (Equation 2-1 and Equation 2-2); however, in this study the pH of the BIOPHO-PX reactors was not observed to be very different (7.18 and 7.43 for BIOPHO-PX 3 and 3*, respectively; only one pH value was taken); this pH would yield P:C ratios of 0.60 and 0.51 for BIOPHO-PX 3, and 0.64 and 0.56 for BIOPHO-PX 3*. While these predictions align well with results for BIOPHO-PX 3*, the theoretical P:C ratios for BIOPHO-PX 3 were markedly higher than observed (87- and 61% increase over the average observed P:C). Filipe et al. states that while there was a definite relationship between P:C and pH, it is not absolute, and that unique relationships may need to be established for individual MMCs; insufficient data was collected in this study to establish such empirical relationships; however, Coats et al. [38] reported similar P:C values as observed in this study for the BIOPHO-PX process (0.33-0.61).

Considering the higher VFA:P ratio in the BIOPHO-PX 3 substrate (Table 5-2), it could be argued that a higher P:C would result. Indeed, on average there was more rbCOD available per unit of phosphorus; however, BIOPHO-PX 3* exhibited a higher P:C, despite a lower VFA:P. Additional ATP would be required to catabolize VFAs, which could demand more Poly P. PAOs obtain energy for VFA catabolism both through poly P hydrolysis and glycogen utilization; thus, increased VFAs could have yielded more glycogen and a stronger reliance on this intracellular carbon substrate for VFA catabolism. On the other hand, this suggests that GAOs may have a greater prominence in BIOPHO-PX 3 by consuming the VFAs without the Poly P release. Indeed, qPCR data (Table 5-3) suggests there was a higher percentage of GAOs in BIOPHO-PX 3. Nevertheless, BIOPHO-PX 3 achieved excellent phosphorus removal.

In consideration of the VFA:P ratio alone, BIOPHO-PX 3 maintained a VFA:P ratio above the minimum suggested by Coats et al. (VFA:P > 15; [29]) and Tchobanoglous et al.

(VFA:P > 8; [1]); this was also correlated with excellent overall phosphorus removal. Conversely, BIOPHO-PX 3* substrate exhibited a lower VFA:P ratio, even at times below the suggested VFA:P. Nonetheless, BIOPHO-PX 3* achieved excellent overall phosphorus removal (Table 5-2). Coats et al. [29] reported some data that also showed good phosphorus removal despite a VFA:P below 15.

Table 5-2. EBPR metrics

	% P Removal		Influent VFA:P (mg COD/mg P)		P:C (Pmmol/Cmmol)		AN P Release (mg P/L)	
OP Day	BP PX 3	BP PX 3*	BP PX 3	BP PX 3*	BP PX 3	BP PX 3*	BP PX 3	BP PX 3*
108	100%	99%	35.0	19.4	0.27	0.67	14.37	23.27
130	94%	94%	22.3	13.5	0.46	0.75	14.15	21.65
155	99%	98%	37.9	23.7	0.33	0.44	14.78	13.58
167	99%	99%	26.5	16.3	0.19	0.40	6.79	9.34
190	97%	95%	24.8	11.0	0.34	0.64	8.30	9.18
Average	98%	97%	29.3	16.8	0.32	0.58	11.68	15.41
STDV	2%	2%	6.8	5.0	0.10	0.15	3.82	6.70

5.1.3 Carbon Cycling

As would be expected in EBPR, VFAs were always consumed rapidly in the anaerobic environment, most typically within the first 10 minutes after feeding (Figure 5-3 and Figure 5-4). The specific VFA uptake rate was calculated (Equation 3-6) to be 2.76 ± 0.71 and 2.53 ± 1.2 Cmmol/(hr-g-VSS) for BIOPHO-PX 3 and 3*, respectively (n=5). There was no statistical difference (t=0.37) between the specific VFA uptake rates of the two BIOPHO-PX reactors. The specific uptake rate of VFAs observed in BIOPHO-PX was markedly lower compared to the theoretical uptake rate of acetic acid by PAOs (11.54 Cmmol/gm VSS-hr) as hypothesized by Murnleitner et al. [107]. One likely reason for the lower observed values could be the presence of other AOBs and NOBs in the VSS that do not consume VFAs. This, in turn, would decrease the proportion of VSS that can uptake the VFAs, and decrease the VFA

uptake rate. In fact, the MMC contained a relatively large fraction of NOBs (Table 5-3). Additionally, the value reported by Murnleitner et al. only incorporates acetic acid and neglects the longer chain VFAs, which may also affect the specific uptake rate. Welles et al. [111] identified a relationship between the VFA uptake rate and the fraction of Poly P in the cell; specifically, as Poly P reserves are depleted, the specific VFA uptake rate is postulated to decrease, which agrees with EBPR theory that shows utilization of Poly P for production of ATP to uptake VFAs.

Table 5-3. qPCR results of BIOPHO-PX reactors

	%PAO		%GAO		%AOB		%NOB	
OP Day	BPPX 3	BPPX 3*	BPPX 3	BPPX 3*	BPPX 3	BPPX 3*	BPPX 3	BPPX 3*
108	6.1%	1.7%	2.0%	1.1%	0.11%	0.04%	15.5%	11.7%
130	5.6%	1.6%	6.0%	3.6%	0.06%	0.03%	3.7%	2.2%
155	3.3%	2.2%	5.0%	2.3%	0.24%	0.14%	10.4%	8.4%
167	2.4%	2.5%	5.0%	2.3%	0.00%	0.00%	9.0%	5.6%
190	12.3%	7.4%	7.1%	5.0%	0.03%	0.01%	26.4%	14.8%
Average	5.9%	3.1%	5.0%	2.9%	0.09%	0.05%	13.0%	8.5%
STDV	3.9%	2.4%	1.9%	1.5%	0.09%	0.06%	8.6%	4.9%

5.2 Nitrogen Removal

5.2.1 Nitrification and Nitritation

The amount and type of nitrogen removal is of significant concern in the BIOPHO-PX system; not only is it critical to remove NO_x in order to sustain EBPR, but the ability to achieve nitritation could yield a 40% reduction in carbon and a 25% reduction in oxygen demand for a WRRF – thus conserving VFAs for other purposes while reducing energy demands. Overall, the BIOPHO-PX reactors removed nitrogen less efficiently than expected; however, this was likely due to incomplete nitrification/nitritation resulting in ammonia in the effluent (Table 5-1; Figure 5-5). Total observed nitrogen removal (74 ± 16% (n=5); 70 ± 27% (n=5) for BIOPHO-PX 3 and 3*, respectively) was lower than expected for typical BNR

facilities (90-92% [109]); additionally, the BIOPHO-PX process has been shown to remove up to 100% of nitrogen at higher DO (>2.0 mg/L) and aerobic SRT (6.66 days) [37]. Both BIOPHO-PX reactors were operated with a relatively short aerobic period (1 hr), which likely contributed to the reduced ammonia removal. Indeed, it would appear that the imposed aerobic fraction of the SRT (3.33 days; AOBs are obligate aerobes, and thus can only grow in an aerobic environment) was near the critical SRT required to achieve nitrification. Figure 5-6 and Figure 5-7 show the trends of nitrogen species (i.e., NH_4^+, NO_3^-, and NO_2^-). Recall, as discussed earlier, that nitrification is commonly thought of as an "all or nothing process," based on Monod kinetics; this kinetic behavior potentially explains why nitrification was occurring completely on some days [e.g., operational day 108 in BIOPHO-PX 3; Figure 5-6 (A)] and not at all on others [e.g., operational day 130; Figure 5-7 (B)]. The aerobic SRT of 3.33 days would result in an average growth rate of AOBs of 0.3 day^{-1}, which aligns with the typical growth rates listed in Table 2-1; however the AOBs must survive for the remainder of the cycle (which is either anoxic or anaerobic) before realizing conditions that allow for growth again, which may slow the accumulation of AOBs. Research performed by Appel [39] on the BIOPHO-PX system similarly showed limited nitrification with an aerobic SRT of 3.33 days; however, Appel's setup was a 2 hr aerobic period (6 hr total cycle) of a 10-day SRT, whereas this research applied a 1 hr aerobic period (6 hr total cycle) at a 20 day SRT. The MMC in BIOPHO-PX 3 and 3* contained 0.09 ± 0.09% and 0.06 ± 0.05% AOBs, respectively. While these values are smaller than for other microbes (Table 5-3), it has been demonstrated that a very small AOB population can effectively oxidize ammonia at percentages as low as 0.003% [112]. Nevertheless, it appears that both BIOPHO-PX reactors would benefit from a slightly longer aeration period, which would theoretically increase the nitrification stability and improve overall nitrogen removal. Longer aerobic SRTs have been used to increase nitrogen removal [37-39].

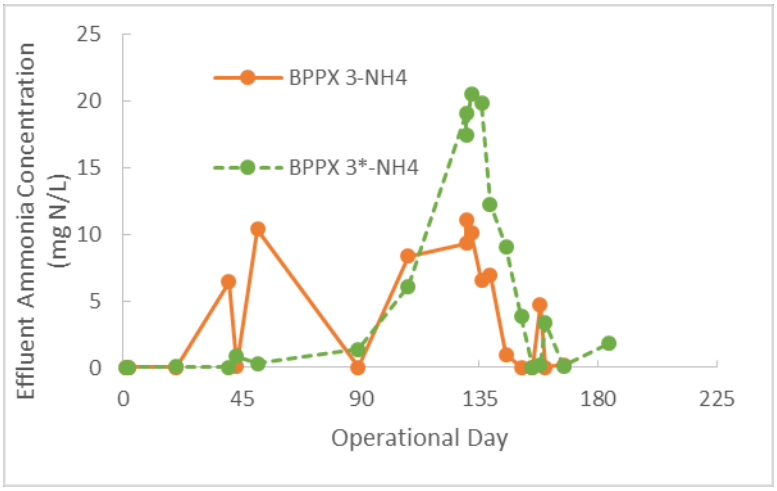

Figure 5-5. Effluent ammonia concentration of BIOPHO-PX

When considering the ability of the MMC to perform nitritation over nitrification, it can be seen that there was a larger proportion of nitrite in both BIOPHO-PX reactors (Figure 5-6 and Figure 5-7). BIOPHO-PX 3* was able to accumulate slightly more nitrite (calculated as in Equation 3-12; Table 5-4) when compared with BIOPHO-PX 3 (80 ± 6% and 71 ± 15% (n=5), respectively [not statistically significant (t=1.25)]. Interestingly, this result is supported by the microbial populations (Table 5-3), in which the BIOPHO-PX 3* MMC was enriched with fewer NOBs than BIOPHO-PX 3. A lower population of NOBs will, in theory, result in less nitrite oxidation to nitrate, and thus a higher proportion of nitrite. Additionally, as expected with the low residual DO concentrations, the NOBs were highly enriched for *Nitrobacter* over *Nitrospira* in both reactors; *Nitrobacter* accounted for 86- and 94% of total NOBs in BIOPHO-PX 3 and 3*, respectively. As mentioned in section 3.2.6, *Nitrobacter* has a lower affinity for oxygen than *Nitrospira*; thus a higher proportion of *Nitrobacter* will theoretically result in greater nitrite accumulation. Typical nitrite accumulation percentages are reported to be between 81 and 100% [55, 57] for MMCs that are enriched for nitritation. While the values observed in this research are lower than reported values, the BIOPHO-PX nitritation ability improved when compared to data from Appel [39]; however, in Appel's research, the MMC was able to more consistently oxidize all influent ammonia, a feat the MMC in this research could not accomplish (Figure 5-5; Table 5-4). Additional work is needed to understand how to achieve greater ammonia oxidation while maintaining nitritation.

5.2.2 Denitrification and Denitritation

Of particular interest in the BIOPHO-PX systems was the ability to denitrify in the anoxic period without external carbon addition. Both BIOPHO-PX 3 and 3* were able to, at a minimum, partially denitrify during the anoxic period (Figure 5-6 and Figure 5-7). BIOPHO-PX 3* performed denitrification much more rapidly and was able to fully denitrify; however, as already discussed BIOPHO-PX 3* also had less oxidized nitrogen to reduce than BIOPHO-PX 3. BIOPHO-PX 3 exhibited unusual behavior in the post-anoxic period on multiple occasions; specifically, nitrite was oxidized to nitrate without molecular oxygen available [Figure 5-6 (A) and (D)]. As far as the author is aware, there is no research that shows the anoxic oxidation of nitrite to nitrate. One possible explanation is the growth of anammox (anaerobic ammonia oxidation) bacteria that can utilize ammonia and nitrite to form nitrogen gas and water (as seen in Equation 5-1). There are, however, a multitude of reasons as to why it is unlikely anammox is occurring: (1) there was no reduction in ammonia concentration anoxically as would be seen with anammox, (2) the stoichiometry of Equation 5-1 shows about 20% of the nitrite going to nitrate, while this research showed a 90% conversion from nitrite to nitrate, and (3) anammox bacteria require extremely long SRTs in order to grow. Another possible explanation was carryover of oxygen into the anoxic period; however, DO was semi-continuously monitored over all operational cycles (measurements every 10 seconds) and showed nominal oxygen (0.04 ± 0.04 mg/L) in the anoxic period. More investigations into the mechanisms that would cause the oxidation from nitrite to nitrate in a post-anoxic mode must be performed.

Equation 5-1. Anammox stoichiometry [113]

$$NH_4^+ + 1.32NO_2^- \rightarrow 1.02N_2 + 0.26NO_3^- + 2H_2O$$

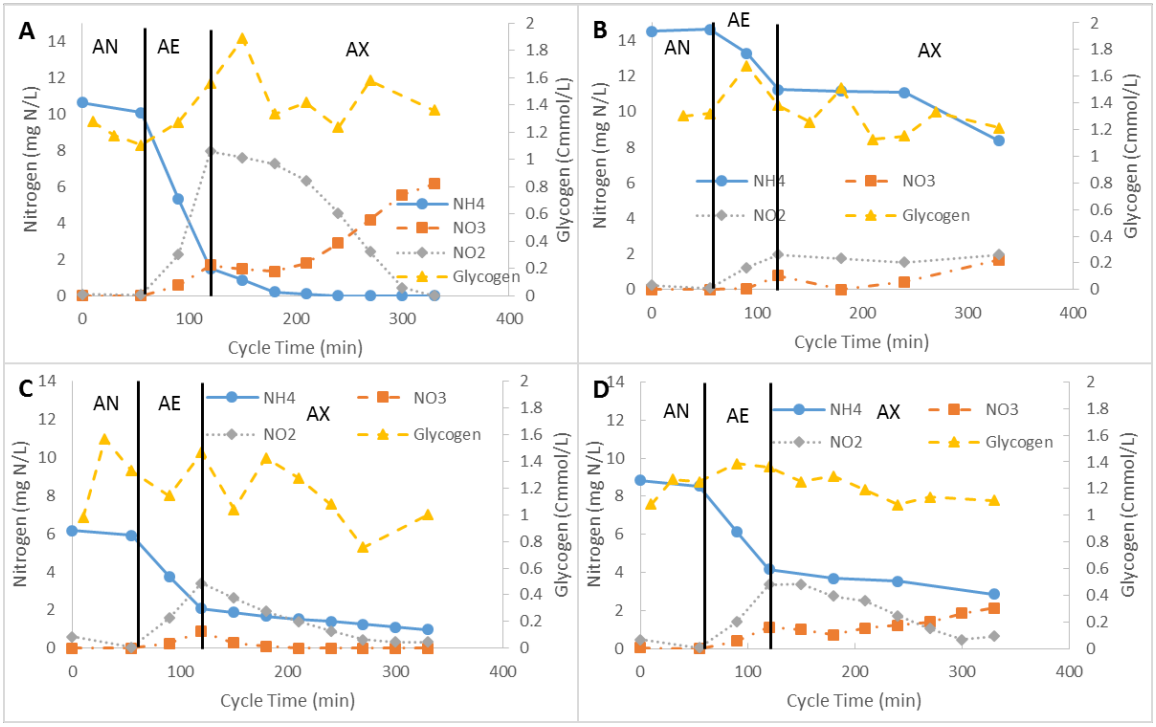

Figure 5-6. Nitrogen removal metabolisms of BIOPHO-PX 3 on operational day: (A) 108, (B) 130, (C) 155, and (D) Average (n=5)

Table 5-4. Summary of nitrogen removal of BIOPHO-PX reactors

	Percent N Removal		Percent NH_4 Removal		Average SDNR (mg N/gm-VS-hr)		Percent NO_x Accumulation	
OP Day	BPPX 3	BPPX 3*	BPPX 3	BPPX 3*	BPPX 3	BPPX 3*	BPPX 3	BPPX 3*
108	81%	80%	100.0%	42.2%	0.57	2.48	82%	72%
130	49%	22%	42.9%	6.2%	-0.07	0.55	72%	84%
155	93%	78%	83.6%	51.0%	0.87	1.78	79%	84%
167	72%	84%	40.1%	58.7%	0.43	1.51	78%	77%
190	74%	85%	94.8%	71.0%	0.06	1.69	46%	86%
Average	74%	70%	72%	46%	0.37	1.60	71%	80%
STDV	16%	27%	29%	25%	0.38	0.69	15%	6%

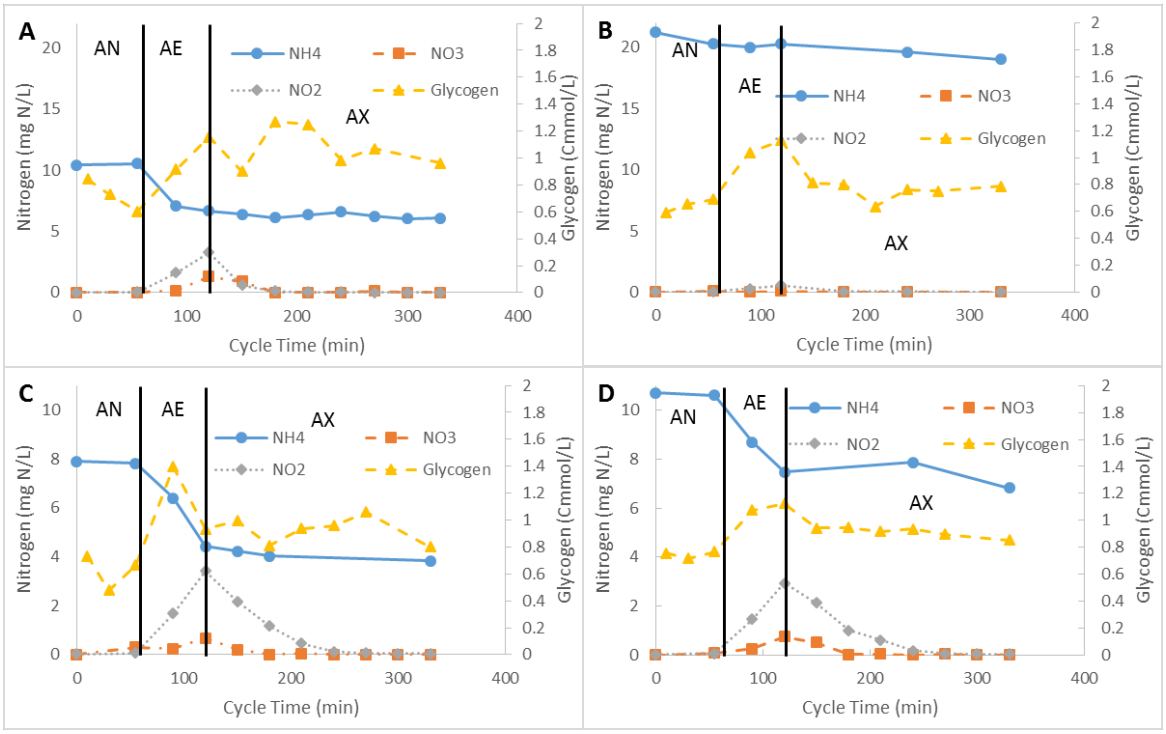

Figure 5-7. Nitrogen removal metabolisms of BIOPHO-PX 3 on operational day: (A) 108, (B) 130, (C) 155, and (D) Average (n=5)*

In investigating denitrification further, it was noticed that BIOPHO-PX 3 and 3* had significantly different specific denitrification rates (SDNR). BIOPHO-PX 3 exhibited denitrification at rates in agreement with post-anoxic SDNRs utilizing endogenous decay as a carbon source (0.2 to 0.6 mg N/g VSS-hr; [114]); however, there was an observed decrease in glycogen in BIOPHO-PX 3 during the anoxic phase. As described earlier, PAOs have the ability to use glycogen as a carbon source for denitrification; however, typically SDNRs are much higher (0.71-1.09 mg N/g VSS-hr) when the PAO's maintenance metabolism (i.e., glycolysis) are driving denitrification [37]. Moreover, the post-anoxic increase in nitrate (in BIOPHO-PX 3) actually caused a negative SDNR (-0.07 mg N/g VSS-hr) on operational day 130 [Figure 5-6 (B)]. Conversely, the SDNR of BIOPHO-PX 3* was observed to be affected by the oxidized nitrogen concentration in a Monod or saturation type of relationship (Figure 5-8; Equation 5-2) with the maximum SDNR being 2.8 mg N/g VSS-hr with the half saturation coefficient of 1.4 mg N/L. Again, there was an observed decrease in intracellular glycogen, which suggests the PAOs were actively denitrifying in BIOPHO-PX 3*; this supports the increased SDNR in BIOPHO-PX 3* over systems that are denitrifying with only endogenous decay as the carbon source.

*Equation 5-2. Monod equation for SDNR of BIOPHO-PX 3**

$$SDNR_{BIOPHO-PX\ 3*} = \frac{2.8 * NO_x}{1.4 + NO_x}$$

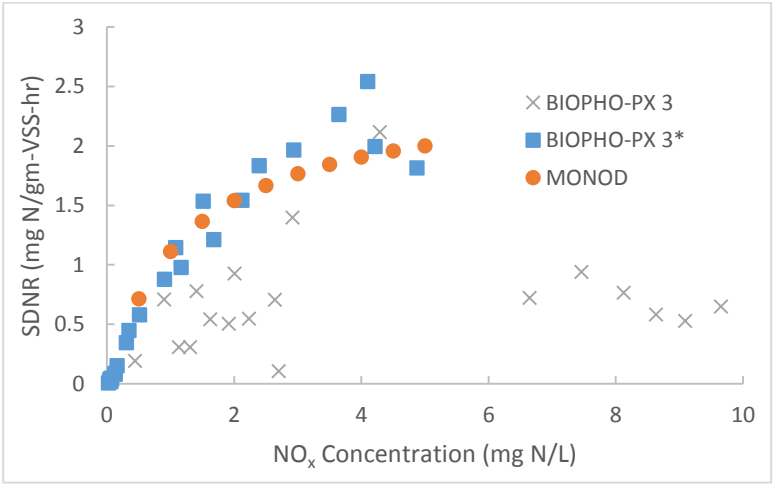

Figure 5-8. SDNR as a function of oxidized nitrogen concentration

While BIOPHO-PX 3* was able to efficiently denitrify the nitrogen that was oxidized in the aerobic basin, there was still some residual ammonia. A longer aeration period would thus be beneficial for both ammonia oxidation and potentially for total nitrogen removal (seeing as denitrification was not the limiting nitrogen removal process in BIOPHO-PX 3*; Figure 5-7). On the other hand, BIOPHO-PX 3 removed more ammonia than 3*, but was limited by the denitrification rate which inhibited total nitrogen removal.

6 Conclusions

6.1 Implications of Research

Research was conducted to evaluate the integrated fermenter/BIOPHO-PX suite of processes (Figure 2-1) with a specific focus on integrating algal biomass. It was hypothesized that cultivating algae on the BIOPHO-PX effluent would help to "polish" the wastewater by removing inorganic nutrients and also provide an additional, internally generated, carbon source to enhance the overall process. The algal biomass was added to a primary solids fermenter with the intention of increasing the organic matter available for fermentation, thereby increasing the production of VFAs (and ammonia and phosphorus, associated with hydrolysis of OM). Interestingly, algal addition did not increase the production of VFAs; however, the algal augmented fermenter (MFA) did realize ammonia removal, seemingly due to heterotrophic algal growth. As a whole, MFA showed a reduction in VFAs and ammonia; however, as the goal of fermentation is VFA production, it can be concluded that co-fermentation of algal biomass and PS does not result in a better substrate for BNR. Specifically, as the BIOPHO-PX reactors were operated (95:5% volumetric ratio of raw wastewater to fermenter liquor, respectively), the reduction in ammonia concentrations in MFA is diluted in the feed to the point where there is no statistical difference between substrates for BIOPHO-PX 3 and 3* for both ammonia and phosphorus, but there is a difference in VFA concentrations. Therefore, while a reduction in ammonia associated with MFA over MF1 would be beneficial to the BIOPHO-PX system, the commensurate reduction in VFAs is detrimental; thus it is not recommended to co-ferment algal biomass with PS, unless suppression of the hypothesized heterotrophic algae can be realized.

Evaluation of the BIOPHO-PX systems showed that the phosphorus removal capabilities of the MMC were not affected by the different substrates; however, a difference was realized in the nitrogen removal capabilities of the BIOPHO-PX. The BIOPHO-PX 3 MMC was able to oxidize the influent ammonia better than BIOPHO-PX 3*. Conversely, BIOPHO-PX 3* achieved a more rapid denitrification rate, which followed a Monod type of relationship with the oxidized nitrate concentration. Overall, it was observed that the aeration period for both BIOPHO-PX reactors was too short (1 hour at 20 day SRT; 3.33 day aerobic SRT) to achieve full ammonia oxidation; thus it is recommended that a longer aerobic period be imposed to ensure full nitrification. With regard to the ability of the MMC to perform

nitritation over nitrification, it was shown that both BIOPHO-PX 3 and 3* were able to accumulate nitrite to greater than 70% of the total oxidized nitrogen, which demonstrates that the BIOPHO-PX configuration has the potential to conserve both oxygen and carbon associated with nitritation vs. nitrification.

6.2 Future Work

Reflecting on the research performed, it was realized there were additional data that would have increased the understanding of the processes. With respect to the bench-scale fermenter, it is recommended that the algae present in MFA is characterized and classified. Additionally, it would be beneficial to monitor the nutrient and VFA concentrations in MFA following feeding; this would provide information on the rate of nutrient removal as well as the preferred VFA species for the heterotrophic algae. On the other hand, the BIOPHO-PX systems did not perform as well as previous operational states. Specifically, the BIOPHO-PX systems should be reverted back to previous operational states as in Appel and Winkler's works [37, 39], as both reported higher nitrogen removal percentages. The best removal (>99% phosphorus and nitrogen removal) occurred at a 20 day SRT and a 2 hour aeration period (6 hour total cycle length); however, with the goal of nitritation being paramount to conserving energy and carbon in the integrated suite of technologies (Figure 2-1), further control mechanisms should be investigated to maximize nitrite accumulation. Additionally, it would be of interest to further investigate the mechanisms that increased the SDNR of BIOPHO-PX 3* over BIOPHO-PX 3.

7 References

1. Tchobanaglous, G., et al., *Wastewater Engineering: Treatment and Reuse*. Fifth ed. 2011: McGraw Hill.

2. Hinsinger, P., et al., *P for Two, Sharing a Scarce Resource: Soil Phosphorus Acquisition in the Rhizosphere of Intercropped Species*. Plant Physiology, 2011. **156**(3): p. 1078-1086.

3. Rockstrom, J., et al., *A safe operating space for humanity*. Nature, 2009. **461**(7263): p. 472-475.

4. Tilman, D., et al., *Forecasting agriculturally driven global environmental change*. Science, 2001. **292**(5515): p. 281-284.

5. Coats, E.R. and P.I. Wilson, *Toward Nucleating the Concept of the Water Resource Recovery Facility (WRRF): Perspective from the Principal Actors*. Environmental Science and Technology, 2017.

6. Shen, N. and Y. Zhou, *Enhanced biological phosphorus removal with different carbon sources*. Applied Microbiology and Biotechnology, 2016. **100**(11): p. 4735-4745.

7. Tong, J.A. and Y.G. Chen, *Enhanced biological phosphorus removal driven by short-chain fatty acids produced from waste activated sludge alkaline fermentation*. Environmental Science & Technology, 2007. **41**(20): p. 7126-7130.

8. Banister, S.S. and W.A. Pretorius, *Optimisation of primary sludge acidogenic fermentation for biological nutrient removal*. Water SA, 1998. **24**(1): p. 35-41.

9. Braunegg, G., G. Lefebvre, and K.F. Genser, *Polyhydroxyalkanoates, biopolyesters from renewable resources: Physiological and engineering aspects*. Journal of Biotechnology, 1998. **65**(2-3): p. 127-161.

10. Batstone, D.J., et al., *The IWA Anaerobic Digestion Model No 1 (ADM1)*. Water Science and Technology, 2002. **45**(10): p. 65-73.

11. Lowrey, J., M.S. Brooks, and P.J. McGinn, *Heterotrophic and mixotrophic cultivation of microalgae for biodiesel production in agricultural wastewaters and associated challenges-a critical review*. Journal of Applied Phycology, 2015. **27**(4): p. 1485-1498.

12. Lopez-Vazquez, C.M., et al., *Modeling the PAO-GAO competition: Effects of carbon source, pH and temperature*. Water Research, 2009. **43**(2): p. 450-462.

13. Carvalheira, M., et al., *The effect of substrate competition on the metabolism of polyphosphate accumulating organisms (PAOs)*. Water Research, 2014. **64**: p. 149-159.

14. Anderson, A.J. and E.A. Dawes, *Occurrence, metabolism, metabolic role, and industrial uses of bacterial polyhydroxyalkanoates*. Microbiological Reviews, 1990. **54**(4): p. 450-472.

15. Grady, L.C.P.J., et al., *Biological Wastewater Treatment*. Third ed. 2011: IWA Publishing

16. Bouzas, A., et al., *Fermentation of municipal primary sludge: Effect of SRT and solids concentration on volatile fatty acid production*. Environmental Technology, 2002. **23**(8): p. 863-875.

17. Cokgor, E.U., et al., *Influence of pH and temperature on soluble substrate generation with primary sludge fermentation*. Bioresource Technology, 2009. **100**(1): p. 380-386.

18. Wu, H., et al., *The effect of pH on anaerobic fermentation of primary sludge at room temperature*. Journal of Hazardous Materials, 2009. **172**(1): p. 196-201.

19. Sosnowski, P., A. Wieczorek, and S. Ledakowicz, *Anaerobic co-digestion of sewage sludge and organic fraction of municipal solid wastes*. Advances in Environmental Research, 2003. **7**(3): p. 609-616.

20. Xie, S.H., R. Wickham, and L.D. Nghiem, *Synergistic effect from anaerobic co-digestion of sewage sludge and organic wastes*. International Biodeterioration & Biodegradation, 2017. **116**: p. 191-197.

21. Smith, S.A., et al., *Toward sustainable dairy waste utilization: enhanced VFA and biogas synthesis via upcycling algal biomass cultured on waste effluent*. Journal of Chemical Technology and Biotechnology, 2016. **91**(1): p. 113-121.

22. Coats, E.R., M. Gregg, and R.L. Crawford, *Effect of organic loading and retention time on dairy manure fermentation*. Bioresource Technology, 2011. **102**(3): p. 2572-2577.

23. Lie, E. and T. Welander, *A method for determination of the readily fermentable organic fraction in municipal wastewater*. Water Research, 1997. **31**(6): p. 1269-1274.

24. Gungor, K., et al., *Prefermentation of liquid dairy manure to support biological nutrient removal*. Bioresource Technology, 2009. **100**(7): p. 2124-2129.

25. El-Mashad, H.M. and R. Zhang, *Biogas production from co-digestion of dairy manure and food waste*. Bioresource Technology, 2010. **101**(11): p. 4021-4028.

26. Arbib, Z., et al., *Capability of different microalgae species for phytoremediation processes: Wastewater tertiary treatment, CO2 bio-fixation and low cost biofuels production*. Water Research, 2014. **49**: p. 465-474.

27. Banister, S.S., A.R. Pitman, and W.A. Pretorius, *The solubilisation of N and P during primary sludge acid fermentation and precipitation of the resultant P*. Water Sa, 1998. **24**(4): p. 337-342.

28. Cornel, P., M. Wagner, and S. Krause, *Investigation of oxygen transfer rates in full scale membrane bioreactors*. Water Science and Technology, 2003. **47**(11): p. 313-319.

29. Coats, E.R., C.K. Brinkman, and S. Lee, *Characterizing and contrasting the microbial ecology of laboratory and full-scale EBPR systems cultured on synthetic and real wastewaters*. Water Research, 2017. **108**: p. 124-136.

30. Doyle, J.D. and S.A. Parsons, *Struvite formation, control and recovery*. Water Research, 2002. **36**(16): p. 3925-3940.

31. Cullen, N., R. Baur, and P. Schauer, *Three years of operation of North America's first nutrient recovery facility*. Water Science and Technology, 2013. **68**(4): p. 763-768.

32. Dvorakov, J., *Utilization of organic substrates during mixotrophic and heterotrophic cultivation of algae*. Biologia Plantarum, 1966. **8**(5): p. 354-+.

33. Peng, S. and L.M. Colosi, *Anaerobic Digestion of Algae Biomass to Produce Energy during Wastewater Treatment*. Water Environment Research, 2016. **88**(1): p. 29-39.

34. Kong, Q.X., et al., *Culture of Microalgae Chlamydomonas reinhardtii in Wastewater for Biomass Feedstock Production*. Applied Biochemistry and Biotechnology, 2010. **160**(1): p. 9-18.

35. McIntosh, K.B. and J.A. Oleszkiewicz, *Volatile fatty acid production in aerobic thermophilic pre-treatment of primary sludge*. Water Science and Technology, 1997. **36**(11): p. 189-196.

36. EPA, U., *Biological Nutrient Removal Processes and Costs*. 2007, United States Environmental Protection Agency.

37. Winkler, M., E.R. Coats, and C.K. Brinkman, *Advancing post-anoxic denitrification for biological nutrient removal*. Water Research, 2011. **45**(18): p. 6119-6130.

38. Coats, E.R., A. Mockos, and F.J. Loge, *Post-anoxic denitrification driven by PHA and glycogen within enhanced biological phosphorus removal*. Bioresource Technology, 2011. **102**(2): p. 1019-1027.

39. Appel, F., *Advancing a Post-Anoxic Biological Nutrient Removal Process Selecting for Nitritation*, in *Civil and Environmental Engineering*. 2015, University of Idaho.

40. Probst, D., *Polyhydroxyalkanoate Production within a Novel WRRF Configuration*, in *Civil and Evironmental Engineering*. 2016, University of Idaho.

41. Coats, E.R., D.L. Watkins, and D. Kranenburg, *A comprative environmental life cycle analysis for removing phosphorus from wastewater: Biological versus physical/chemical processes*. Water Environ. Res., 2011. **83**(8): p. 750-760.

42. Coats, E.R., D.L. Watkins, and D. Kranenburg, *A Comparative Environmental Life-Cycle Analysis for Removing Phosphorus from Wastewater: Biological versus Physical/Chemical Processes*. Water Environment Research, 2011. **83**(8): p. 750-760.

43. Coats, E.R., et al., *Effect of Anaerobic HRT on Biological Phosphorus Removal and the Enrichment of Phosphorus Accumulating Organisms*. Water Environment Research, 2011. **83**(5): p. 461-469.

44. Coats, E.R., Z.T. Dobroth, and C.K. Brinkman, *EBPR Using Crude Glycerol: Assessing Process Resiliency and Exploring Metabolic Anomalies*. Water Environment Research, 2015. **87**(1): p. 68-79.

45. Wentzel, M.C., et al., *Enhanced polyphosphate organism cultures in activated-sludge systems III: Kinetic-model*. Water Sa, 1989. **15**(2): p. 89-102.

46. Smolders, G.J.F., et al., *Stoichiometric model of the aerobic metabolism of the biological phosphorus removal process*. Biotechnology and Bioengineering, 1994. **44**(7): p. 837-848.

47. Filipe, C.D.M., G.T. Daigger, and C.P.L. Grady, *Stoichiometry and kinetics of acetate uptake under anaerobic conditions by an enriched culture of phosphorus-accumulating organisms at different pHs*. Biotechnology and Bioengineering, 2001. **76**(1): p. 32-43.

48. Coats, E.R., C.K. Brinkman, and S. Lee, *Characterizing and contrasting the microbial ecology of laboratory and full-scale EBPR systems cultured on synthetic and real wastewaters*. Water Res., 2017. **108**: p. 124-136.

49. Zeng, W., et al., *Control and optimization of nitrifying communities for nitritation from domestic wastewater at room temperatures*. Enzyme and Microbial Technology, 2009. **45**(3): p. 226-232.

50. Bae, W., et al., *Optimal operational factors for nitrite accumulation in batch reactors*. Biodegradation, 2001. **12**(5): p. 359-366.

51. Kim, D.J., et al., *Nitrification of high strength ammonia wastewater and nitrite accumulation characteristics*. Water Science and Technology, 2003. **47**(11): p. 45-51.

52. Hellinga, C., et al., *The SHARON process: An innovative method for nitrogen removal from ammonium-rich waste water*. Water Science and Technology, 1998. **37**(9): p. 135-142.

53. Taher, E. and K. Chandran, *High-Rate, High-Yield Production of Methanol by Ammonia-Oxidizing Bacteria*. Environmental Science & Technology, 2013. **47**(7): p. 3167-3173.

54. Madigan, M.T. and J.M. Martinko, *Brock Biology of Microorganisms*. 2006.

55. Fang, F., et al., *Kinetic analysis on the two-step processes of AOB and NOB in aerobic nitrifying granules*. Applied Microbiology and Biotechnology, 2009. **83**(6): p. 1159-1169.

56. Mulder, C.D., *Impact of intrinsic and extrinsic parameters on the oxygen kinetic parameters of ammonia and nitrite oxidizing bacteria*. 2014.

57. Kim, J.H., X.J. Guo, and H.S. Park, *Comparison study of the effects of temperature and free ammonia concentration on nitrification and nitrite accumulation*. Process Biochemistry, 2008. **43**(2): p. 154-160.

58. Bolzonella, D., et al., *Denitrification potential enhancement by addition of anaerobic fermentation products from the organic fraction of municipal solid waste*. Water Science and Technology, 2001. **44**(1): p. 187-194.

59. Chen, H.B., et al., *Enhancement of post-anoxic denitrification for biological nutrient removal: effect of different carbon sources*. Environmental Science and Pollution Research, 2015. **22**(8): p. 5887-5894.

60. Liu, F., et al., *The use of fermentation liquid of wastewater primary sedimentation sludge as supplemental carbon source for denitrification based on enhanced anaerobic fermentation*. Bioresource Technology, 2016. **219**: p. 6-13.

61. Min, K.S., et al., *Acidogenic fermentation: Utilization of wasted sludge as a carbon source in the denitrification process*. Environmental Technology, 2002. **23**(3): p. 293-302.

62. Chen, H.-b., et al., *Post-anoxic denitrification via nitrite driven by PHB in feast-famine sequencing batch reactor*. Chemosphere, 2013. **92**(10): p. 1349-1355.

63. Li, Z.M., et al., *Nitrogen removal from medium-age landfill leachate via post-denitrification driven by PHAs and glycogen in a single sequencing batch reactor*. Bioresource Technology, 2014. **169**: p. 773-777.

64. Daigger, G.T., *Oxygen and carbon requirements for biological nitrogen removal processes accomplishing nitrification, nitritation, and anammox*. Water Environ. Res., 2014. **86**(3): p. 204-209.

65. Higgins, P., *Shortcut the nitrogen removal process in wastewater with UV sensors*. 2014, YSI Incorporated.

66. Coats, E.R., et al., *Synthesis of polyhydroxyalkanoates in municipal wastewater treatment*. Water Environment Research, 2007. **79**(12): p. 2396-2403.

67. Bugnicourt, E., et al., *Polyhydroxyalkanoate (PHA): Review of synthesis, characteristics, processing and potential applications in packaging*. Express Polymer Letters, 2014. **8**(11): p. 791-808.

68. Laycock, B., et al., *The chemomechanical properties of microbial polyhydroxyalkanoates*. Progress in Polymer Science, 2013. **38**(3-4): p. 536-583.

69. Ometto, F., et al., *Improving the Energy Balance of an Integrated Microalgal Wastewater Treatment Process*. Waste and Biomass Valorization, 2014. **5**(2): p. 245-253.

70. Liang, Y.N., N. Sarkany, and Y. Cui, *Biomass and lipid productivities of Chlorella vulgaris under autotrophic, heterotrophic and mixotrophic growth conditions*. Biotechnology Letters, 2009. **31**(7): p. 1043-1049.

71. Mitra, D., J. van Leeuwen, and B. Lamsal, *Heterotrophic/mixotrophic cultivation of oleaginous Chlorella vulgaris on industrial co-products*. Algal Research-Biomass Biofuels and Bioproducts, 2012. **1**(1): p. 40-48.

72. Clescerl, L.S., A.E. Greenberg, and A.D. Eaton, *Standard Methods for Examination of Water and Wastewater*. 1998: American Public Health Association.

73. Parrou, J.L. and J. Francois, *A simplified procedure for a rapid and reliable assay of both glycogen and trehalose in whole yeast cells*. Analytical Biochemistry, 1997. **248**(1): p. 186-188.

74. Braunegg, G., B. Sonnleitner, and R.M. Lafferty, *Rapid Gas-Chromatographic Method For Determination of Poly-Beta-Hydroxybutyric Acid in Microbial Biomass*. European Journal of Applied Microbiology and Biotechnology, 1978. **6**(1): p. 29-37.

75. Latimer, G.W., *Official methods of analysis of AOAC International*. 2012, AOAC International.

76. Vansoest, P.J., J.B. Robertson, and B.A. Lewis, *Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition*. Journal of Dairy Science, 1991. **74**(10): p. 3583-3597.

77. Im, J., et al., *Correlation between nitrite accumulation and the concentration of AOB in a nitritation reactor*. Environmental Earth Sciences, 2014. **72**(1): p. 289-297.

78. Yuan, Q. and J.A. Oleszkiewicz, *Biomass fermentation to augment biological phosphorus removal*. Chemosphere, 2010. **78**(1): p. 29-34.

79. Burow, L., et al., *Bioenergetic models for acetate and phosphate transport in bacteria important in enhanced biological phosphorus removal*. Environmental Microbiology, 2008. **10**(1): p. 87-98.

80. Chanona, J., et al., *Optimum design and operation of primary sludge fermentation schemes for volatile fatty acids production*. Water Research, 2006. **40**(1): p. 53-60.

81. Bouzas, A., et al., *Fermentation and elutriation of primary sludge: Effect of SRT on process performance*. Water Research, 2007. **41**(4): p. 747-756.

82. Ucisik, A.S. and M. Henze, *Biological hydrolysis and acidification of sludge under anaerobic conditions: The effect of sludge type and origin on the production and composition of volatile fatty acids*. Water Research, 2008. **42**(14): p. 3729-3738.

83. Yuan, Q., M. Baranowski, and J.A. Oleszkiewicz, *Effect of sludge type on the fermentation products*. Chemosphere, 2010. **80**(4): p. 445-449.

84. Li, Y., et al., *Volatile fatty acids distribution during acidogenesis of algal residues with pH control*. World Journal of Microbiology & Biotechnology, 2013. **29**(6): p. 1067-1073.

85. Thi Nhan, P., et al., *Volatile fatty acids production from marine macroalgae by anaerobic fermentation*. Bioresource Technology, 2012. **124**: p. 500-503.

86. Ji, M.K., et al., *Cultivation of microalgae species in tertiary municipal wastewater supplemented with CO2 for nutrient removal and biomass production*. Ecological Engineering, 2013. **58**: p. 142-148.

87. Markou, G. and E. Nerantzis, *Microalgae for high-value compounds and biofuels production: A review with focus on cultivation under stress conditions*. Biotechnology Advances, 2013. **31**(8): p. 1532-1542.

88. Cai, T., S.Y. Park, and Y.B. Li, *Nutrient recovery from wastewater streams by microalgae: Status and prospects*. Renewable & Sustainable Energy Reviews, 2013. **19**: p. 360-369.

89. Razzak, S.A., et al., *Integrated CO2 capture, wastewater treatment and biofuel production by microalgae culturing-A review*. Renewable & Sustainable Energy Reviews, 2013. **27**: p. 622-653.

90. Smith, S.A., et al., *Toward sustainable dairy waste utilization: Enhanced VFA and biogas synthesis via upcycling algal biomass cultured on waste effluent*. J. Chem. Technol. Biotechnol., 2016. **91**(1): p. 113-121.

91. Tsubu, S., *Preservation of marine and fresh-water algae by means of freezing and freeze-drying*. Cryobiology, 1973. **10**(5): p. 445-452.

92. Gujer, W. and A.J.B. Zehnder, *Conversion processes in anaerobic digestion*. Water Science and Technology, 1983. **15**(8-9): p. 127-167.

93. Taylor, R. and R.L. Fletcher, *Cryopreservation of eukaryotic algae - a review of methodologies*. Journal of Applied Phycology, 1999. **10**(5): p. 481-501.

94. Barajas, M.G., et al., *Solubilization and fermentation in a modified VFA-potential method*. Environmental Engineering Science, 2003. **20**(4): p. 329-336.

95. Zacharof, M.-P. and R.W. Lovitt, *Complex Effluent Streams as a Potential Source of Volatile Fatty Acids*. Waste and Biomass Valorization, 2013. **4**(3): p. 557-581.

96. Processes, I.T.G.F.M.M.o.A.D., *Anaerobic Digestion Model No. 1*. 2002.

97. Federation, W.E., *Nutrient Removal, WEF MOP 34*. 2010: McGraw Hill Professional.

98. Skalsky, D.S. and G.T. Daigger, *Waste-water Solids Fermentation for Volatile Acid Production and Enhanced Biological Phosphorus Removal*. Water Environment Research, 1995. **67**(2): p. 230-237.

99. Bolzonella, D., et al., *Anaerobic fermentation of organic municipal solid wastes for the production of soluble organic compounds*. Industrial & Engineering Chemistry Research, 2005. **44**(10): p. 3412-3418.

100. Danesh, S. and J.A. Oleszkiewicz, *Volatile fatty acid production and uptake in biological nutrient removal systems with process separation*. Water Environment Research, 1997. **69**(6): p. 1106-1111.

101. Longo, S., et al., *Recovery of volatile fatty acids from fermentation of sewage sludge in municipal wastewater treatment plants*. Bioresource Technology, 2015. **175**: p. 436-444.

102. Bai, J., et al., *Modified ADM1 for modeling free ammonia inhibition in anaerobic acidogenic fermentation with high-solid sludge*. Journal of Environmental Sciences, 2017. **52**: p. 58-65.

103. Wang, R., et al., *Phosphate release involving PAOs activity during anaerobic fermentation of ESPR sludge and the extension of ADM1*. Chemical Engineering Journal, 2016. **287**: p. 436-447.

104. EPA, U.S., *National Lakes Assessment Report*. 2010.

105. Zhang, T.Y., et al., *Isolation and heterotrophic cultivation of mixotrophic microalgae strains for domestic wastewater treatment and lipid production under dark condition*. Bioresource Technology, 2013. **149**: p. 586-589.

106. Pate, R., G. Klise, and B. Wu, *Resource demand implications for US algae biofuels production scale-up*. Applied Energy, 2011. **88**(10): p. 3377-3388.

107. Murnleitner, E., et al., *An integrated metabolic model for the aerobic and denitrifying biological phosphorus removal*. Biotechnology and Bioengineering, 1997. **54**(5): p. 434-450.

108. Smolders, G.J.F., et al., *Model of the anaerobic metabolism of the biological phosphorus removal process-stoichiometry and pH influence*. Biotechnology and Bioengineering, 1994. **43**(6): p. 461-470.

109. Liu, G., et al., *Biological nutrient removal in a continuous anaerobic-aerobic-anoxic process treating synthetic domestic wastewater*. Chemical Engineering Journal, 2013. **225**: p. 223-229.

110. Zeng, W., et al., *Denitrifying phosphorus removal and impact of nitrite accumulation on phosphorus removal in a continuous anaerobic-anoxic-aerobic (A(2)O) process treating domestic wastewater*. Enzyme and Microbial Technology, 2011. **48**(2): p. 134-142.

111. Welles, L., et al., *Accumulibacter clades Type I and II performing kinetically different glycogen-accumulating organisms metabolisms for anaerobic substrate uptake*. Water Research, 2015. **83**: p. 354-366.

112. Dionisi, H.M., et al., *Quantification of Nitrosomonas oligotropha-like ammonia-oxidizing bacteria and Nitrospira spp. from full-scale wastewater treatment plants by competitive PCR*. Applied and Environmental Microbiology, 2002. **68**(1): p. 245-253.

113. Sliekers, A.O., et al., *Completely autotrophic nitrogen removal over nitrite in one single reactor*. Water Research, 2002. **36**(10): p. 2475-2482.

114. Kujawa, K. and B. Klapwijk, *A method to estimate denitrification potential for predenitrification systems using NUR batch test*. Water Research, 1999. **33**(10): p. 2291-2300.