Assessing the Effects of Anaerobic Mass Fraction on Enhanced Biological Phosphorus Removal Process Health and Performance

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Authorization to Submit Thesis

This Thesis of Nicole J. T. Brower, submitted for the degree of Master of Science with a Major in Civil Engineering and titled "**Assessing the Effects of Anaerobic Mass Fraction on Enhanced Biological Phosphorus Removal Process Health and Performance,**" 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.

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Abstract

Enhanced Biological Phosphorus Removal (EBPR) is a complex process with many different configurations, operational strategies, and difficulties. The advantages and disadvantages between two different operational schemes, one reactor was fed a higher volume of a lower concentration substrate and the other was fed a lower volume of a higher concentration substrate, were tested and compared under variable anaerobic mass fractions to understand several of the factors that contribute to EBPR process success. The reactor fed a higher volume of substrate at the start of the anaerobic phase yielded better Polyhydroxyalkanoate (PHA) production and consumption, glycogen consumption and production, and better phosphorus release based on the carbon consumed anaerobically. The reactor that was fed a smaller volume of higher concentration substrate yielded higher rates of phosphorus release and uptake and higher anaerobic carbon recovery ratios. Utilizing oxidation-reduction potential (ORP) in the anaerobic and aerobic zones indicated relationships between aerobic ORP and effluent phosphorus, anaerobic ORP and the rate of phosphorus uptake, and anaerobic ORP and PHA consumption. When applied to EBPR process control, ORP could be used to better characterize the process health and stability.

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Lastly, I want to thank Cynthia Brinkman for maintaining the laboratory space, and her patience in teaching procedures, problem solving, and providing a laugh whenever the situation calls for it.

Dedication

For my husband, Jacob Brower, who never let me doubt myself.

For my parents, Jeff and Jona Tompkins, who saw the engineer in me before I ever did.

For the ladies, Cheyenne, Anne, and Mimi, who held me together and lifted me up.

For The One who created me with the ability and the passion to help people as a Civil Engineer.

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List of Abbreviations

AE ..... Aerobic

AN ..... Anaerobic

ANOVA ..... Analysis of Variance

AO ..... Anaerobic-Aerobic

A^2O ..... Anaerobic-Anoxic-Aerobic

ATP ..... Adenosine Triphosphate

COD ..... Chemical Oxygen Demand

DO ..... Dissolved Oxygen

EBPR ..... Enhanced Biological Phosphorus Removal

GAO ..... Glycogen Accumulating Organism

GC ..... Gas Chromatography

HRT ..... Hydraulic Residence Time

MBR ..... Membrane Bioreactor

MFL ..... Municipal Fermenter Liquor

MLSS ..... Mixed Liquor Suspended Solids

NH_4 ..... Ammonia

NO_2 ..... Nitrite

NO_3 ..... Nitrate

NPDES ..... National Pollutant Discharge Elimination System

OHO ..... Ordinary Heterotrophic Organism

OLR ..... Organic Loading Rate

ORP, redox ..... Oxidation Reduction Potential

PAO ..... Phosphorus Accumulating Organisms

PHA ..... Polyhydroxyalkanoate

PHB ..... Polyhydroxybutyrate

PHV ..... Polyhydroxyvalerate

PLC ..... Programmable Logic Controller

PO_4 ..... Phosphorus, Phosphate

RAS ..... Return Activated Sludge

SBR ..... Sequencing Batch Reactor

SRT ..... Solids Retention Time

TCMP ..... 2-chloro-6(trichloromethyl) pyridine

TSS ..... Total Suspended Solids

VFA ..... Volatile Fatty Acid

VSS ..... Volatile Suspended Soilds

WAS ..... Waste Activated Sludge

WB ..... Westbank

WRRF ..... Water Resource Recovery Facility

WW ..... Wastewater

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Chapter One: Introduction

Phosphorus is an essential life nutrient that is key in cell development and maintenance for many living organisms (plants, animals, bacteria, etc.) including humans. Phosphorus is also used in the manufacturing of several different products such as glass, detergents, fireworks, and metals. Even more prevalent is the use of phosphorus in fertilizers, particularly in the agricultural industry. While phosphorus is a critical macronutrient, unlike other nutrients, phosphorus is finite in supply. To obtain phosphorus in its natural form, it must be mined, and the natural cycle for phosphorus is extremely slow, making the supply limited. Ultimately excess mining of phosphorus has been occurring for years, and readily available supplies are dwindling as phosphorus is being used and disposed of [1]. For these reasons, post-consumer phosphorus recovery is becomingly increasingly important. Without recovering phosphorus, future generations will continue to struggle with phosphorus sourcing and shortages.

Coupled with phosphorus supply concerns are environmental impact concerns. Following the usage of phosphorus, much of the nutrient enters liquid waste streams and eventually bodies of water. This discharge of excess phosphorus into natural bodies of water can cause water quality issues that adversely impact biological species, including the excessive growth of algae populations. Algae consume oxygen and other essential nutrients, depriving other living organisms, which can ultimately become detrimental to the water body; algal growth is particularly difficult to control in small and stagnant bodies of water. Excess phosphorus is a growing concern for most bodies of water which receive treated municipal wastewater effluent, particularly during the warmer periods of the year. Considering the negative impacts of phosphorus in wastewater, many municipal Water Resource Recovery Facilities (WRRFs) have or expect to receive a phosphorus limit in their National Pollutant Discharge Elimination System (NPDES) permits. In addition to permit demands, removal of phosphorus at the wastewater treatment level provides a unique opportunity to recover some of the soluble phosphorus for reuse, thereby reducing the need for phosphorus mining. Thus, it is important to understand and optimize phosphorus removal at wastewater treatment plants.

Treatment plants operating under a phosphorus limitation in their permit have a few options for removing phosphorus from the waste stream. The first treatment method is chemical phosphorus removal, in which chemical coagulation and filtration bind and remove the

phosphorus from solution. The most common coagulants are metal salts, specifically those derived from iron, aluminum, and calcium. A chemical phosphorus removal system typically consists of a small volume rapid mixing basin, which contains a mixing blade powerful enough to mix the chemical and liquid stream within seconds. The mixing basin is immediately followed by a settling basin, or a filtration system designed to remove the flocs formed in the mixing basin and separate the chemical sludge from the liquid stream as it continues through the WRRF.

Advantages of chemical phosphorus removal include the ability for it to be integrated into any existing WRRF, the relative ease of operation, the dosing flexibility to meet a variety of removal needs, and the ability to alter system operation depending on the specific permit requirements. Additionally, a chemical phosphorus removal system is relatively simple to operate and generally quite reliable; however, the addition of chemical phosphorus removal can introduce hazardous chemicals, increase the operational cost of the system by requiring chemical storage accommodations and chemical sludge disposal, and ultimately binds the phosphorus into a chemical sludge such that the phosphorus is more difficult, or practically impossible, to recover for reuse. While chemical phosphorus removal is currently the most widely used method for municipal phosphorus capture, more sustainable methods are needed.

The second method for phosphorus removal is a microbiological process known as Enhanced Biological Phosphorus Removal (EBPR), which utilizes microorganisms to uptake and store excess phosphorus from solution. Conventional EBPR can accomplish much more than just phosphorus removal, which makes it an advantageous process for municipalities with nutrient limits in their NPDES permit. Operating at its full potential, an EBPR system can remove Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), ammonia (NH_4), nitrate (NO_3), nitrite (NO_2), and phosphate (PO_4).

In addition to the broader treatment benefits, EBPR for phosphorus removal has several advantages, such as the ability to achieve extremely low effluent PO_4 values in a relatively reliable way. EBPR also offers the ability to recover usable phosphorus, and the organisms themselves can be repurposed after being wasted from the system. On the other hand, biological systems are more expensive to construct, require more physical space, and can be more difficult to incorporate into existing WRRFs. Also, because EBPR utilizes living

organisms, the process can become upset and potentially lead to permit violations. This vulnerability ultimately leads to the process requiring diligent monitoring of several known operational conditions, which increases the operational complexity of the WRRF.

There are several other technologies for phosphorus removal which are used less frequently than chemical or biological phosphorus removal including algal phosphorus removal systems, active filter systems, and ion exchange systems. Algal phosphorus removal typically occurs in one of two ways: either in a pre-manufactured photo-bioreactor unit or with a significant amount of fixed algal growth. Overall algal phosphorus removal can produce excellent effluent values and can help reoxygenate the treated water; however, algal phosphorus removal is still in preliminary proprietary stages of development and has very few full-scale systems in operation. Active filter systems consist of a filter made with an active medium that removes the phosphorus via sorption. These systems can also produce excellent effluent values but can be expensive to maintain depending on the media used. Additionally, the filter systems have yet to be successful at a full-scale level for a sustained period. Lastly, ion exchange systems have been shown to be useful for phosphorus removal, though they have only been tested in a laboratory setting. This technology operates on the same principle as desalination and deionization systems but targets the anionic phosphorus particles. One primary advantage of an ion exchange system is the removal and recovery of phosphorus all in one system. Recent research has shown reliable effluent quality, but the system upkeep can be expensive and has yet to be proven at a full-scale level.

Ultimately, most WRRFs accomplishing phosphorus removal are doing so by chemical and/or biological phosphorus removal methods. As noted, chemical phosphorus removal as a process is generally well understood and relatively simple in operational parameters and outcome. Conversely, EBPR is understood to the degree of successful wide-spread implementation, but many of the biological mechanisms, operational parameters, and treatment kinetics are lacking specific quantification [2]. This lack of operational clarity, in addition to the overall treatment benefits, the potential for recovering the removed phosphorus, and the sustainable nature of EBPR are the reasons that biological phosphorus removal was chosen as the focus of this thesis. A better understanding of the metabolisms involved in EBPR could increase the

success of existing systems, and well as provide specific operational metric recommendations for the design and upgrade of new EBPR systems.

Overall, EBPR metabolisms are quite complicated and remain poorly understood [1]. It is characterized by several interrelated metabolic pathways, which are exploited for the sake of phosphorus removal from liquid waste streams [3]. The first step in EBPR involves exposing bacteria to an anaerobic zone which is characterized by the near absence of nitrate and oxygen, ultimately starving the organisms of an external electron acceptor. Without an external electron acceptor, the organisms are unable to completely metabolize the raw wastewater COD - specifically the carboxylic acids - that are present in the waste stream. Instead of utilizing the carboxylic acids for cell growth and maintenance, the bacteria store it in a polymer called Polyhydroxyalkanoate (PHA) [4]. This carboxylic acid uptake and storage process requires energy; hydrolyzation of phosphates from a poly-phosphate chain produces required energy in the form of Adenosine Triphosphate (ATP). This phenomenon is referred to as phosphorus release and results in a higher concentration of soluble phosphorus in the anaerobic zone [5].

In design of the anaerobic zone, there is a critical uncertainty about sizing the basin and whether this is best quantified/controlled by hydraulic residence time (HRT; also expressed as the anaerobic mass fraction) or through an operational parameter such as Oxidation Reduction Potential (ORP). It is generally accepted that too little anaerobic exposure will not result in the desired phosphorus release, which can lead to a lower value of phosphorus removed from the system – although anaerobic phosphorus release may not be the most critical metabolic response, and instead may simply be an indicator of a successful anaerobic environment for Phosphorus Accumulating Organisms (PAOs) [4-6]. While there is evidence that a longer anaerobic retention time (achieving “Deep Anaerobic” conditions) can be beneficial to the overall system, there has not been a data-based recommendation for the optimal “Deep Anaerobic” retention time or what is considered to be “Deep Anaerobic” in terms of a measurable parameter (ORP, PO_4, time, etc.)[7].

To achieve excess phosphorus removal, the next critical step is the reintroduction of oxygen to the PAOs in the system. Oxygen in introduced to the system in the aerobic zone, typically through diffused air. In the presence of oxygen, the PAOs metabolize the PHA stored in the

anaerobic zone and use it for phosphorus storage/uptake, cell growth, and maintenance. This metabolic process, in the presence of external electron acceptors, is energy producing which allows the cells to uptake soluble phosphorus and store it in polyphosphate chains. In a healthy EBPR system, more phosphorus will be consumed in the aerobic zone than is released in the anaerobic zone, resulting in a net removal of phosphorus. When this is not the case, there remains excess phosphorus in the effluent, and the process is considered upset as this condition continues.

Beyond metabolic uncertainties and associated operational parameters to achieve ideal EBPR, there is also a need to quantify real-time parameters that may be indicative of process health. In the case of a process upset, which can take days to weeks to recover, the best approach may be prevention. However, this cannot be achieved without a clear understanding of the mechanisms which can cause process upsets (e.g., insufficient amounts of carboxylic acid in the waste stream) and a real-time parameter that can indicate when a potential upset is imminent. By tracking process health with parameters other than effluent PO_4, a process issue may be able to be identified and addressed before the effluent value is affected.

This thesis aims to address relevant concerns in the biological wastewater treatment community and to further inform municipalities on how to achieve improved operation of their EBPR systems. One focus of this research is to develop an enhanced quantitative understanding of the anaerobic zone and more specifically understand how the anaerobic zone affects the rest of the treatment process. The second focus of this research is to gain new knowledge on the mechanisms that cause EBPR system failure, and how to induce process recovery.

Research conducted in this thesis was driven by the following Research Questions:

1. It has been suggested that the concept of a “Deep Anaerobic” state will enable more stable and efficient EBPR processes. What is not clearly articulated, however, is the quantitative values that qualify as Deep Anaerobic or what benefits can be gained from operating within - or potentially negative outcomes from avoiding – such operational parameters. Research Question 1: How does the anaerobic mass fraction and associated anaerobic state in the EBPR process, monitored through ORP, affect overall process performance in maximizing phosphorus removal?

   a. Hypothesis 1: EBPR performance can be predicted by anaerobic ORP.
2. The existence of the anaerobic zone is critical to the success of EBPR systems; PAOs are exposed to a stressful environment, which causes a release of intra-cellular phosphorus and the accumulation of PHA. Though we know that this anaerobic zone is important, there is no data-based guidance on an optimal range for operational success or how it could be measured. Research Question 2: Is there a minimum anaerobic ORP value necessary to sustain stable EBPR performance?
   a. Hypothesis 2: Under optimal EBPR operating conditions, there exists a minimum ORP value in the anaerobic zone necessary to achieve stable and resilient EBPR performance.
3. In many cases the cause of an EBPR process upset is unknown, which makes it difficult to remedy. ORP measurements in an EBPR anaerobic zone correlate with the synthesis of PHA and release of phosphorus that is critical to EBPR stability and success; ORP may also be indicative of fermentation activities, which can enhance PHA synthesis. Research Question 3: Under process upset conditions, can ORP be used as a real-time metric to indicate sufficient PHA synthesis and re-establish process performance and stability?
   a. Hypothesis 3: Anaerobic PHA synthesis and ORP are directly correlated in a manner which can indicate the potential for EBPR recovery from a process upset.

1.1 Methods Overview

Research was executed by operating two bench scale sequencing batch reactors (SRBs), each equipped with dissolved oxygen (DO) probes for process control and redox probes for ORP monitoring; ORP was recorded continuously, with SBRs subjected to anaerobic HRTs of 1-4 hours in a factorial experimental design. ORP and DO were monitored continuously in both reactors, as well as effluent values recorded several times a week and regular batch testing to characterize the nutrient profile throughout the process. This evaluation was performed at 4 different anaerobic zone lengths to compare values and build relationships between parameters to inform the results and discussion of this thesis. Further description of methods can be found in Chapter 3.

Chapter Two: Literature Review

2.1 Overview of EBPR

Enhanced Biological Phosphorus Removal began as a treatment method to remove soluble phosphorus from liquid waste streams in the late 1970s and early 1980s [8]. Since then, the process has been studied extensively, especially as phosphorus limits on treated wastewater become more common [1, 9]. The focus on EBPR came from the discovery that anaerobic conditions were fundamental to the uptake of phosphates [5]. Furthermore, the research that Barnard performed in South Africa revealed the phenomenon of phosphorus being released in the anaerobic zone, before being taken up in the aerobic and anoxic zones [5]. Since then, the accepted understanding of the microbiological processes occurring throughout these treatment trains has expanded.

The EBPR process is based on the metabolisms of microorganisms generally known as Phosphorus Accumulating Organisms (PAOs) [3]. The PAOs have the ability to store phosphorus intracellularly, and this is the advantage that allows them to thrive in an anaerobic environment. The overall removal of phosphorus from wastewater involves a complex system of metabolic responses, which all begin with an influent stream rich in carboxylic acids flowing into an anaerobic environment. For the sake of wastewater treatment an anaerobic environment is defined as an environment lacking in external terminal electron acceptors, namely nitrate, nitrite, and oxygen [9]. Several processes begin to occur in the PAOs due to this lack of terminal electron acceptors, but in the presence of substrate (primarily carboxylic acids) which serve as “food” for the microorganisms. Firstly, the PAOs utilize their stored phosphorus reserves to generate energy. The phosphorus is stored in polyphosphate chains and when these chains are broken, energy is released in the form of Adenosine Triphosphate (ATP) [10]. ATP is used to uptake the substrate in the water, while the hydrolyzed phosphates are released into solution. This anaerobic zone thus serves as a “selector” for PAOs because organisms that do not have this ability to generate energy by hydrolyzing phosphates also do not have the ability to uptake the substrate and utilize it for cell growth and maintenance without a terminal electron acceptor [1].

Following the consumption of the substrate, through several metabolic pathways, the PAOs store the substrate primarily as a carbon polymer. This is done by utilizing the excess energy from the hydrolyzation of phosphates, as well as the breakdown of a substance called glycogen to produce metabolic intermediates and reducing equivalents [10, 11]. The carbon is stored as a polymer called Polyhydroxyalkanoate (PHA), and for PAOs these are most commonly Polyhydroxybutyrate (PHB) and Polyhydroxyvalerate (PHV) [4]. Within the anaerobic zone, the expected cellular response from PAOs is a decrease in stored cellular glycogen and phosphates, and an increase in stored cellular carbon in the form of PHA. In the wastewater, the expected observations during the anaerobic period are an increase in soluble phosphorus and a decrease in carboxylic acids (typically measured as volatile fatty acids or VFAs).

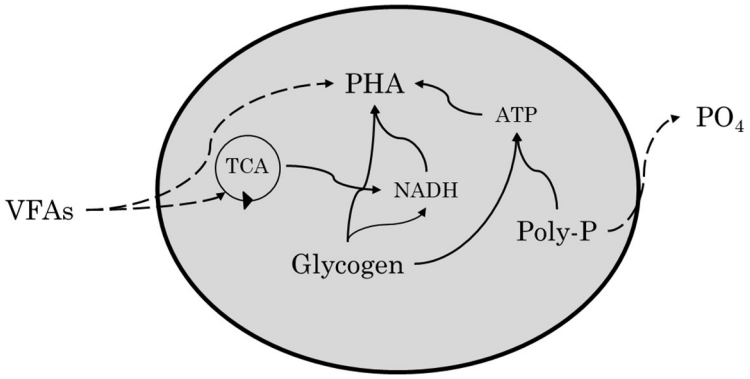

Figure 2.1.1: Known PAO Metabolisms in the Anaerobic Phase

Metabolically, the next significant state is the aerobic zone where oxygen is reintroduced to the system. This causes several different reactions to take place, many of which are the reverse of processes that took place in the anaerobic zone. These processes begin with the stored PHA; these molecules begin to be broken down to fuel other processes [4]. The carbon and electrons generated by the breakdown of PHA are used to fuel the tricarboxylic acid (TCA) cycle and the proton motive force, which generates energy and allows for cell maintenance, restoration of poly-phosphate stores, glycogen synthesis, and growth [9]. Any excess energy that is not used for growth or maintenance is then stored within poly-phosphate chains, as soluble phosphorus is taken up from solution, and the excess metabolic intermediates are utilized to replenish the glycogen [2]. The expected metabolic response in the aerobic zone is a decrease in stored PHA, an increase in intracellular phosphates, and an increase in intracellular glycogen stores. The expected response in the wastewater would then

be an increase in dissolved oxygen concentration, a decrease in soluble phosphorus, and an increase in suspended solids concentration.

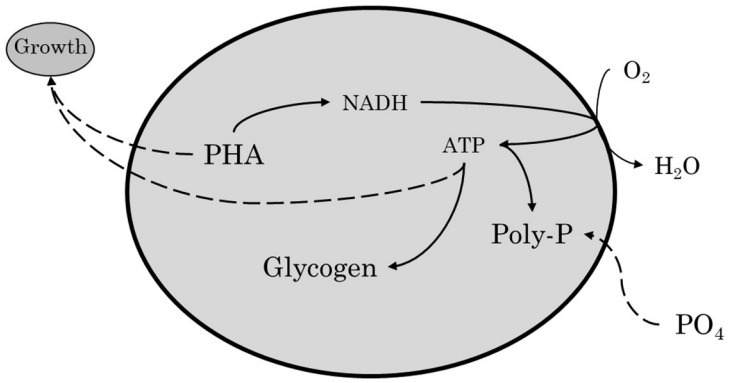

Figure 2.1.2: Known PAO Metabolisms in the Aerobic Phase

The EBPR process takes place entirely in secondary treatment at a WRRF, with the mixed liquor suspended solids (MLSS) acting as the method for treatment. These organisms are pumped from the secondary clarifier, where they are settled out back to the anaerobic basin in a process called Return Activated Sludge (RAS) [9]. The average amount of time that these organisms spend within the treatment system depends on the specific process, what other nutrients are being removed, and the loading concentrations of each constituent. This sludge age (or solids retention time, SRT) is controlled by the fraction of sludge that is not returned to the anaerobic basin after setting but is instead wasted (WAS) [9].

The recycle of activated sludge (RAS) can introduce oxygen, or nitrate, into the anaerobic environment, which would turn the anaerobic zone either anoxic or aerobic, depending on the concentration and significance. For EBPR to work properly, in addition to monitoring and maintaining the anaerobic and aerobic zones, the RAS must also be monitored to minimize these unwanted compounds in the anaerobic zone [9].

2.2 EBPR: The AO and Westbank Processes

Process flow for an EBPR system can differ depending on several factors including any other nutrients being removed, the size of the WRRF, existing facilities, budget, and any future considerations. The simplest process configuration would be the AO process, which stands for Anaerobic/Oxic (also known as aerobic), which primarily targets the removal of PO_4 and

COD [5, 9]. The AO process consists of two primary zones, with all influent wastewater and RAS entering the anaerobic zone. The anaerobic zone is immediately followed by the aerobic zone, before separating the effluent by settling/clarification. Several other process configurations also include the removal of ammonia and nitrogen have also been developed, such as the A^2O process (Anaerobic-Anoxic-Aerobic), the modified Bardenpho process (addition of an anaerobic zone, in addition to pre- and post-anoxic tanks), University of Cape Town (similar to A^2O but RAS enters the anoxic tank as opposed to the anaerobic tank), and variations of these processes using a membrane bioreactor (MBR) to separate the effluent from the solids [9]. This research focused on the study of two process configurations, the first of which is the standard AO process modified to a bench-scale SBR. Figure 2.2.1 shows a simple process flow diagram of the AO process used to operate the lab SBR.

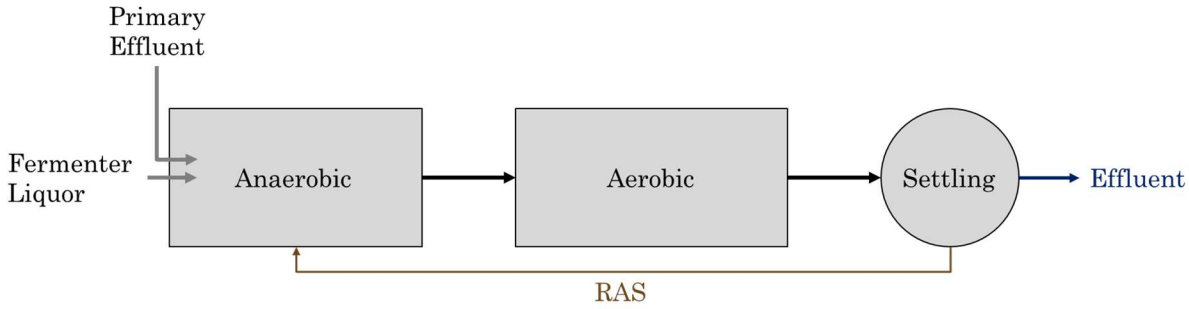

Figure 2.2.1: Flow Diagram for an AO Process

The second process configuration studied was the Westbank process (WB), a configuration discovered at the West Kelowna plant in the province of British Columbia, Canada [8]. This treatment configuration similarly consists of anaerobic and aerobic zones, but the feeding of these zones differs from that of the AO process. The original Westbank plant also includes anoxic zones on either side of the anaerobic basin for denitrification. In the Westbank process, the fermenter liquor and primary effluent are fed separately, with the fermenter liquor being fed to the anaerobic basin along with the RAS and primary effluent being fed to the anoxic zones basins [9]. Figure 2.2.2 shows the process flow used to operate the SBR (nitrification was controlled in both reactors, so there was no need for the anoxic zones) and highlights the differences between the operation of the two reactors.

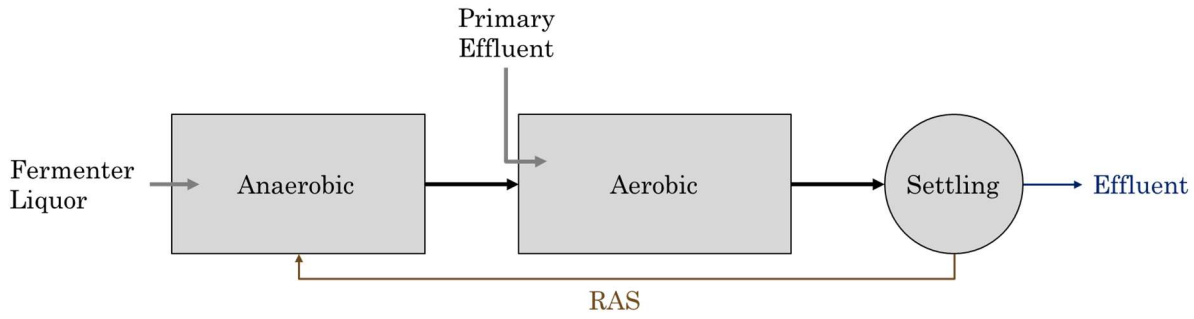

Figure 2.2.2: Flow Diagram for a Westbank Process

2.3 Current EBPR Anaerobic Zone Design Approach

Although the understanding of EBPR has significantly improved since its discovery and implementation in the 1980s, the complex chemical and biological nature of these systems creates many factors in determining how and what is occurring during each treatment phase [2]. Additionally, uncertainty about the specifics of what organisms are PAOs within a wastewater treatment system [12] and the inability to study these organisms as a pure culture creates difficulty in understanding the ideal conditions to sustain their growth and metabolism [1, 3]. The design of an EBPR system largely targets creating and maintaining environments where PAOs will flourish and will outcompete other organisms, such as Glycogen Accumulating Organisms (GAOs), which will hinder the system by consuming substrate without removing any additional nutrients [3]. Considering the fundamentals of EBPR metabolisms, it is critical to design the anaerobic zone to advantage PAOs in their uptake and storage of carbon [7, 13]. While the importance of anaerobic conditions has been understood for some time, the recommendations for specific design and monitoring parameters are limited and occasionally conflicting [13, 14].

In recent years, new recommendations to potentially enhance enrichment of PAOs have evolved to include a process known as “RAS fermentation”, which includes an additional basin for the RAS before entering the anaerobic zone [7]. This configuration is purported to benefit the EBPR process by increasing the anaerobic contact time for the organisms specifically and therefore providing better PAO enrichment as they consume the carbon from other organisms, which cannot survive under anaerobic conditions for an extended amount of time [7]. Additionally, this process claims to enrich for a greater variety of PAOs by enriching

for organisms with a greater affinity for fermentation, thereby increasing phosphorus removal capability and performance [7]. The RAS fermentation process has been studied for the past 10 years; evidence supporting process claims for improved EBPR performance are limited and in some cases unreliable [13]. Additionally, research into this process has not always indicated that it would be beneficial to phosphorus removal overall, and in one case, there were negative consequences shown [15]. Overall, while RAS fermentation may provide some benefit to EBPR, it is not likely to provide any benefits that cannot also be realized by an adequately design anaerobic basin [13].

If the key role of phosphorus removal systems lies in achieving the anaerobic state, then the question remains about what specific parameters create an optimal anaerobic environment for PAO growth and phosphorus removal. There appears to be an agreement within EBPR literature that the most important factor is the anaerobic hydraulic retention time (HRT) or, alternatively, the anaerobic mass fraction; one considers the contact time within the anaerobic basin and the other considers the fraction of activated sludge that is under anaerobic conditions [7, 13, 14]. The recommendations for a sufficient anaerobic HRT range from 0.5-4 hours [9, 14] and for an anaerobic mass fraction of 0.1-0.25 based on influent wastewater strength [13]. While these guidelines provide an excellent starting point, the reality at most EBPR treatment facilities is that everything from influent concentration and flow to MLSS concentrations and settling can impact the treatment process and final effluent quality [1]. Recognizing the lack of specific process knowledge on requisite anaerobic conditions, this research aimed to generate further knowledge on parameters that can be monitored in real time to assess the health of the anaerobic zone despite fluctuations in external factors.

2.4 EBPR Process Control: Redox and Dissolved Oxygen

One potential enhancement to EBPR anaerobic design and operation is the use of on-line measurement devices to provide more avenues to maintain effluent quality and prevent or warn of potential process issues [16]. The use of dissolved oxygen (DO) probes is fairly common, and can result in lower energy costs for the facility to prevent over-aerating the aerobic basin beyond what is required for nutrient uptake [16]. While monitoring DO can maintain control over the aeration tank and save on operational costs, controlling the level of

aeration does not significantly impact the EBPR process beyond ensuring that aeration is occurring in the aeration basin and that negligible oxygen is present in the anaerobic basin [17].

Oxidation-Reduction Potential (ORP or redox) on the other hand, may indicate the health of processes occurring within the anaerobic basin as well as within the aerobic basin [17]. The ORP probe measures the activity of electrons within the solution and calculates the ORP value, in millivolts, based on the potential created between the solution and the reference "node" within the probe itself [17]. This node is typically comprised of an inert metal, such as platinum, which creates the differential potential between the solution and the node to produce a reading. If there is a high number of electrons being donated to the probe, indicating that the solution is in a reducing state, the ORP value will be negative due to the negative potential between the probe and the solution. Conversely, a positive ORP value occurs when the potential becomes positive as the probes donates electrons to the solution.

Historically, ORP monitoring has been used to successfully control nitrification processes, and also ensure nitrification is not inhibiting phosphorus removal [18]. Several relationships between changes in ORP as well as pH have been linked to metabolic changes in nitrification and denitrification. Moreover, the efficient removal of ammonia and the ability to detect nitrate using ORP can be advantageous for maintaining a truly anaerobic zone, which in turn increases control over the EBPR system and may increase phosphorus removal capabilities [18, 19]; However, specific relationships related to phosphorus removal have not been clearly established [20, 21].

A primary disadvantage of using ORP and pH probes for process control is the requirement for precise calibration to return accurate measurements, which may increase complication significantly when applied to EBPR processes with less sensitive on-line monitoring. Challenges with ORP control can be addressed by regular maintenance, but the required workload for upkeep of the probes may be a limiting factor for some facilities [22].

Despite the potential application of ORP to EBPR processes, specific process implementation suggestions have never been made. There is some speculation about the advantages of a "deep anaerobic" state for enhanced phosphorus removal and has been suggested that an ORP value of -300 mV could enhance process function, but this was recommended in the case of RAS

fermentation, and not necessarily applied to the standard anaerobic basin [7, 13]. Furthermore, though the application of ORP in the aerobic basin has been demonstrated, there remains a lack of specific guidance for operation as it relates to nutrient removal [17]. There is, therefore, a need to better understand ORP as it applies to EBPR, and whether this can be used as a surrogate measure of process health.

Chapter Three: Methods and Materials

3.1 Experimental Design

Research was executed using two 2 L bench scale SBRs, each fitted with on-line monitoring of DO and ORP. Each SBR was fed raw influent wastewater (WW) harvested (post-screening and grit removal) from the Moscow, Idaho Water Reuse and Reclamation Facility; the wastewater was collected from the facility on a weekly basis and stored at 4 ° C until fed to the reactors. Additionally, municipal fermenter liquor (MFL) was added to comprise 10% of the total feed volume per cycle. The MFL was extracted from a 25-liter fermenter operated at a 5-day solids retention time (SRT) and an organic loading rate (OLR) of 2.25 grams of volatile solids per liter per day, with operations consistent with Romenesko and Coats [23]. The solids were separated out via centrifugation, and the supernatant was used to supplement the wastewater feed to both reactors. Nitrification of both reactors was controlled by adding thiourea to the feed mixture for the AO reactor, and the raw WW for the WB reactor, and occasionally 2-chloro-6(trichloromethyl) pyridine (TCMP) if nitrification control was lost.

Two EBPR operating schemes were studied, with one reactor operating under an Anaerobic-Aerobic (AO) scheme and the other operating under the Westbank scheme (see Figures 2.2.1 and 2.2.2) [9]. The operational differences between these two schemes for the purposes of this research is that the F Reactor (AO process) was fed at the start of each cycle a uniform volume of 10% MFL and 90% raw WW at 0.33 L total. Conversely, the WB reactor (Westbank process) was fed only MFL at the start of each cycle followed by raw WW at the start of the aerobic phase, still maintaining 10% of the total volume from MFL and 90% of the total volume from raw WW; 0.033 L of MFL was fed at the start of the anaerobic zone, and the remaining 0.3 L of WW was fed at the start of the aerobic zone.

Both reactors were operated on six-hour cycles consisting of five-minutes of feeding, an anaerobic period (variable), five-minutes of additional feeding (Westbank reactor only), an aerobic period (remaining cycle time), five-minutes of wasting, 20-minutes of settling, and ten-minutes of decanting. Both reactors were mixed using magnetic stir-plate and a stir bar, and the mixing occurred the entire cycle outside of the settling and decanting period. Wasting for each reactor was achieved via the Garrett wasting mode by removing 200 mL, and all

pumping for the reactors was achieved using peristaltic pumps (Watson Marlow, Wilmington, MA, USA). The reactors were aerated using aquarium pumps outfitted with stone diffusers, and dissolved oxygen was continuously monitored and maintained using a Hach LDO probe. The probe signals were sent to a Hach SC200 controller, which also received signals from the Hach pHD probe ORP measurements; the SC200 controller maintained the target DO by powering the aquarium pump on and off via a programmed relay. Once the aerobic fraction has begun, the DO probe controlled when the air is on or off based on the target DO setpoint (2 mg/L). When the DO is below 2 mg/L the aeration would come on, and once it reached a certain level above 2 mg/L it turned off. Reactor identification was according to the following notation: "reactor-identifying-code.SRT.HRT.AN.AE" where SRT is measured in days, HRT is measured in hours, AN indicates the amount of time (hours) spent anaerobically each 6-hour cycle, and AE indicates the amount of time (hours) spent aerobically each 6-hour cycle. Thus, the AO reactor ID would be F.10.18.AN.AE, and the Westbank reactor ID would be WB.10.18.AN.AE.

The early stages of this research intended to use continuous DO and ORP measurements to control the length of the anaerobic portion while still maintaining a consistent 6-hour cycle. The intent was to set a specific ORP value as the target, such as -250 mV, and program the SC200 to begin aeration after this value had been reached. Several sampling collection runs occurred under this operational scheme before it became apparent that it was not a sustainable method of operation to ensure the health of the reactors for sample comparison, due to inconsistent ORP realization (the reactors would go several consecutive cycles without any aeration). Ultimately ORP proved to be a poor operational parameter on which to base automated operations.

Building from this control strategy, the SC200 controls were simplified to designate a specific anaerobic length while still continuously monitoring ORP and allowing the DO setpoint to control the air within the aerobic portion. The length of the anaerobic zone was controlled by a programmable logic controller (PLC). This PLC is also what controlled feeding of both reactors, as well as decanting, wasting, aeration, and mixing. The wasting for each reactor maintained an average SRT of 10 days for both and was checked occasionally to ensure

accuracy by measuring the volume wasted and the mixed liquor suspended solids (MLSS). The decanting was used to maintain a 3 cycle HRT, or 18 hours for both reactors.

The experimental design included comprehensive sampling for performance analysis over the entirety of one 6-hour cycle, repeated multiple times for each operational state. The overall number of sampling runs performed, and their distribution can be seen in Table 3.1.1. Prior to the collection of samples, a 30-day (three times SRT) stabilization period was used to ensure steady-state conditions. These samples were taken at varying frequencies depending on the anaerobic length, with greater sample frequency at the start of the anaerobic and aerobic portions. For each sampling run, samples were collected at approximately 20 different time stamps across the 6-hour cycle for each reactor. When samples were collected, at least 2 samples were collected from each reactor at each time mark. Each sample was centrifuged for 5 minutes at 4500 rpm to separate the solids and liquid. The supernatant was analyzed for soluble nutrients and carboxylic acids, while the solids were dried at 104 °C and subsequently digested to analyze for PHA and glycogen.

Table 3.1.1: Number of Sampling Runs per Operational Change

	Reactor ID and Number of Sampling Events	
Anaerobic HRT (hrs)	F.10.18.AN.AE	WB.10.18.AN.AE
1	5	5
2	4	4
3	4	4
4	4	4

3.2 Analytical Techniques

Samples were collected in duplicate to monitor PO_4, NO_3, volatile fatty acids (VFAs), TSS, volatile suspended solids (VSS), and PHA. For 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. Soluble NO_3 was determined in accordance with

Hach method 10020 (consistent with Standard Methods). A Spectronic® 20 Genesys™ spectrophotometer (Thermo-Fisher Scientific Corp, Waltham, MA, USA) was utilized to measure the absorbance of the reacted sample at a wavelength of 410 nm for NO_3. NO_3 concentrations were determined utilizing a standard curve (R^2>0.99). PO_4 was determined in accordance with Hach (Loveland, CO, USA) method 8048 (method is equivalent to Standard Methods 4500-PE). TSS and VSS were measured in accordance with Standard Methods 2540 D and 2540 E [24].

Carboxylic acids as volatile fatty acids (VFAs or acetic, propionic, butyric, isobutyric, valeric, isovaleric, and caproic acids) and methanol were quantified using a Hewlett-Packard 6890 series gas chromatograph (GC) (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a flame-ionization detector (FID) 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 (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 nitric acid. 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 (R2>0.99).

Biomass PHA content was determined by gas chromatography/mass spectrometry (GC-MS) as described in Braunegg, et al.[25]. 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 ThermoScientific ISQ7000-Trace1300 GC-MS instrument. 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) 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 optimal molecular weight for PHA quantification was determined to be 103 g mol-1. 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 percent dry weight basis (mass PHA per mass TSS, w/w) and a percent cell weight basis (mass PHA per mass VSS, w/w).

Glycogen was determined with dried biomass samples as described by Parrou and Francois [26].

Chapter Four: Results and Discussion

4.1 EBPR Reactor Performance Results

As described, two EBPR reactors were operated and studied in conducting this research. The experimental criteria were i) conventional AO operation vs. a Westbank style configuration, and ii) variable length of the anaerobic period, or the anaerobic mass fraction. Performance of each reactor under difference anaerobic conditions was assessed at a minimum in triplicate, with assessments conducted 3-10 days apart unless otherwise indicated. The overall performance of the experimental reactors is presented and discussed in this section. The data presented is given as a statistical average with standard deviation bars for all data shown in the "Carbon Profile", "VFA Profile", and "PO_4 Profile" figures, and as individual data sets from each sampling event for the ORP figures.

4.1.1 One-hour Anaerobic State

As noted, during the one-hour anaerobic operational state samples were collected under two different process control strategies. Three sampling runs were conducted under the ORP controlled process state, which ultimately, and consistently, operated at exactly one hour anaerobic. However, ORP proved to yield poor process control, and operations were pivoted to ORP monitoring only. Subsequently, two sampling days occurred under PLC controlled operations, which specifically limited the anaerobic period to one hour.

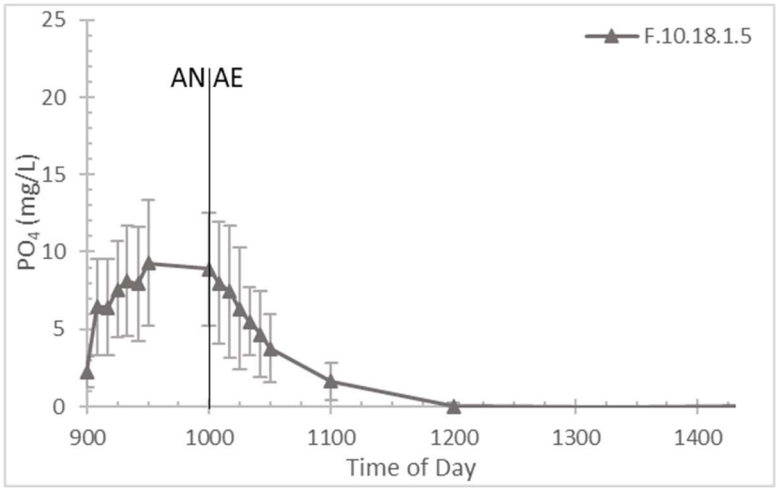

Figure 4.1.1.1: Average PO_4 concentrations in solution for F.10.18.1.5 with standard deviation bars for each value.

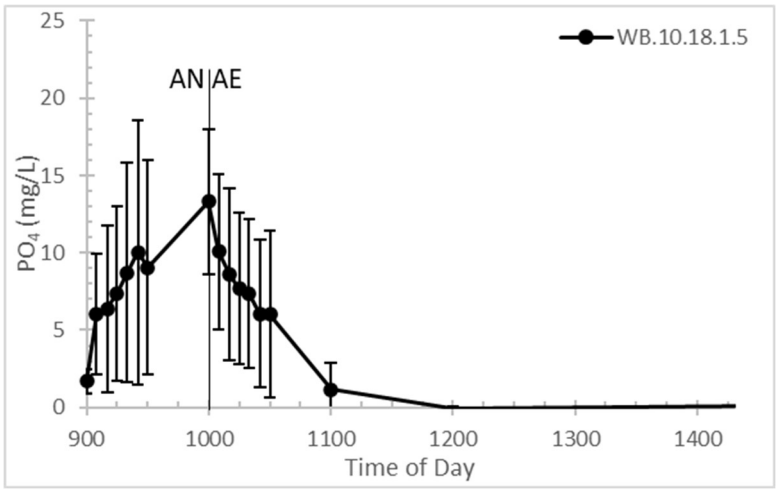

Figure 4.1.1.2: Average PO_4 concentrations in solution for WB.10.18.1.5 with standard deviation bars for each value.

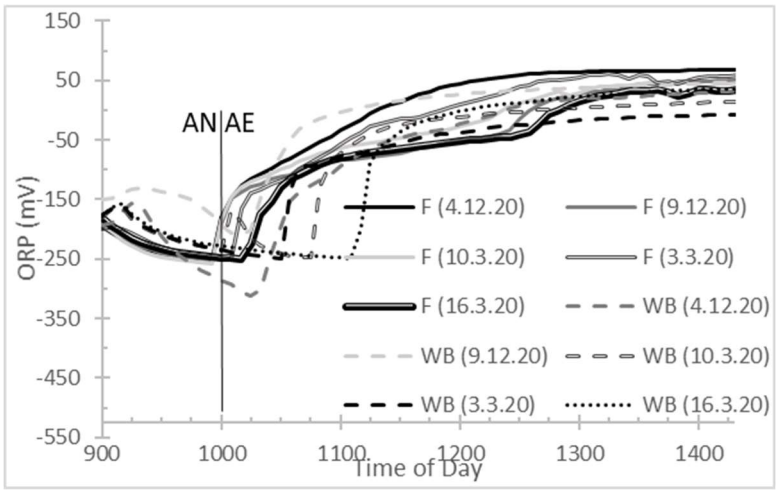

Figure 4.1.1.3: ORP values as measured continuously in solution for F.10.18.1.5 and WB.10.18.1.5 on several dates on which sampling events took place.

Performance data for the respective reactors is shown in Figures 4.1.1.1-7. Overall, the reactors exhibited consistent phosphorus release and uptake, the ORP was lowest within the anaerobic zone and increased throughout the aerobic zone, and the carbon profiles appeared as expected based on understood EBPR processes of PHA synthesis, storage, and usage as it relates to VFA uptake and glycogen stores[9]. Despite the phosphorus cycling variability shown in Figures 4.1.1.1 and 4.1.1.2, average effluent PO_4 was 0.03 +/- 0.05 mg/L (n=5) for F.10.18.1.5 and 0.12 +/- 0.21 mg/L (n=5) for WB.10.18.1.5; the average influent PO_4 (i.e., t=0 concentration) was 2.24 +/- 1.04

mg/L (n=5) for F.10.18.1.5 and 1.68 +/- 0.79 mg/L (n=5) for WB.10.18.1.5. The PO_4 profiles exhibit theoretically typical EBPR behavior with an average phosphorus release of 7.8 +/- 3.1 mg/L (n=5) for F.10.18.1.5 and 11.2 +/- 5.2 mg/L (n=5) for WB.10.18.1.5 and an average phosphorus uptake of 9.3 +/- 3.5 mg/L (n=5) for F.10.18.1.5 and 12.6 +/- 4.6 mg/L (n=5) for WB.10.18.1.5. The anaerobic ORP reached an average of -252 +/- 3.4 mV (n=5) for F.10.18.1.5 and an average of -253 +/- 37 mV (n=5) for WB.10.18.1.5. Additionally, the ORP never reached a level below -350 mV for either reactor and the redox conditions did not realize the minimum value until the end of the anaerobic period.

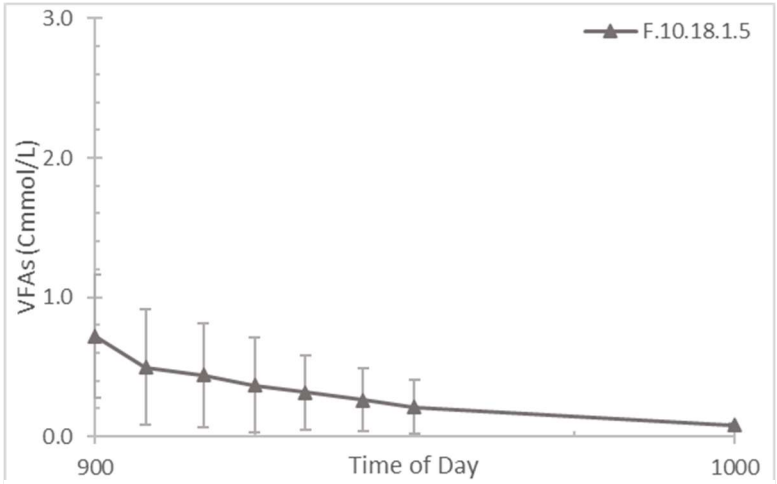

Figure 4.1.1.4: Average VFA concentrations in solution for F.10.18.1.5 within the first hour of the cycle, including standard deviation bars for each value.

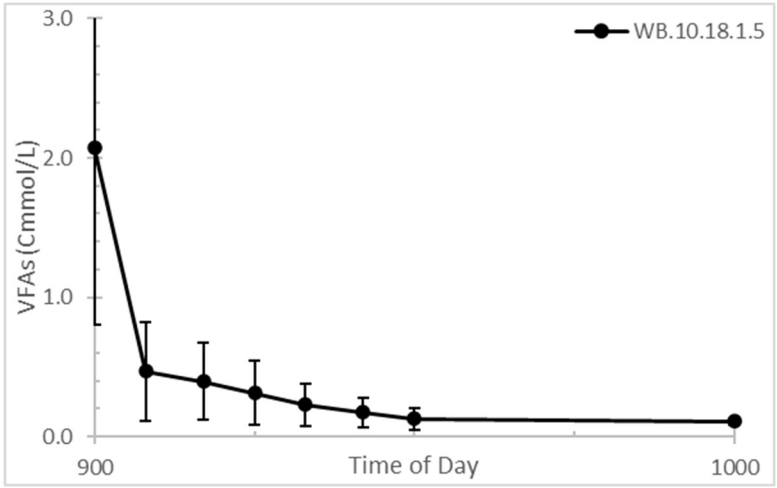

Figure 4.1.1.5: Average VFA concentrations in solution for WB.10.18.1.5 within the first hour of the cycle, including standard deviation bars for each value.

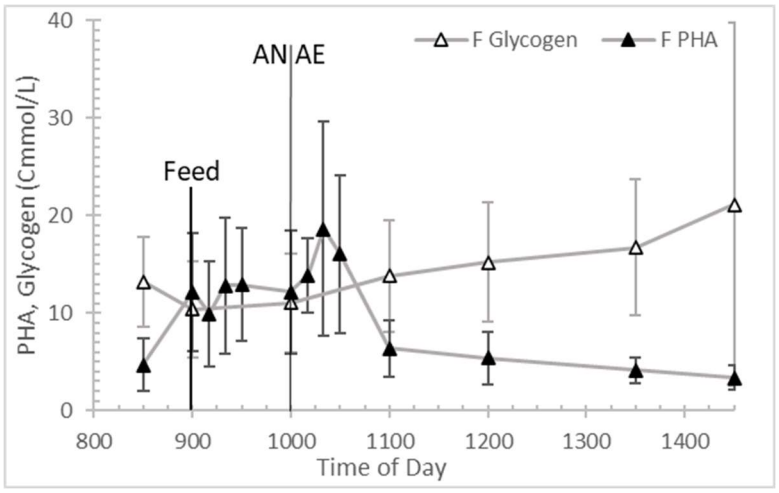

Figure 4.1.1.6: Average Glycogen and PHA concentrations for F.10.18.1.5 with standard deviation bars for each value.

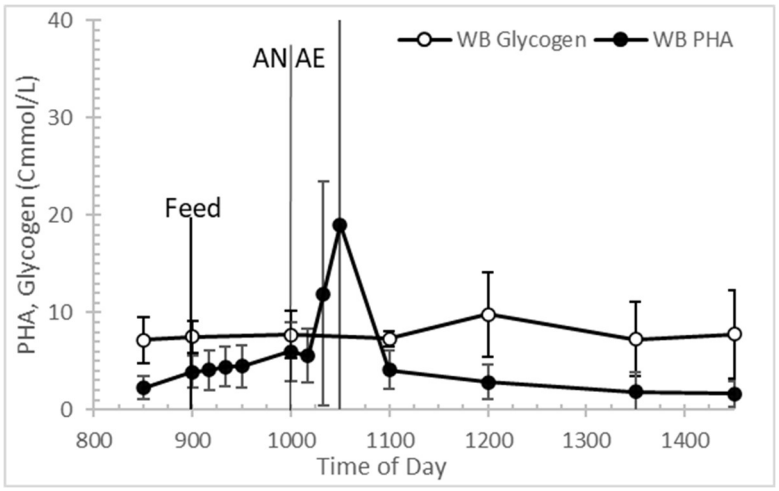

Figure 4.1.1.7: Average Glycogen and PHA concentrations for WB.10.18.1.5 with standard deviation bars for each value.

The carbon relationships followed theoretically expected EBPR behavior beginning with the VFAs. As would be expected when using real wastewater, influent VFA concentration was variable, with an average of 2.16 +/- 1.3 Cmmol/L (n=5) for the F.10.18.1.5 combined feed and 34.8 +/- 7.8 Cmmol/L (n=5) for the WB.10.18.1.5 MFL feed. The VFAs were depleted in about 30 minutes anaerobically in F.10.18.1.5 with a specific rate of 0.0032 +/- 0.0014 Cmmol/L+min+gVSS (n=5) and about 15 minutes in WB.10.18.1.5 with a specific rate of 0.0084 +/- 0.0052 Cmmol/L+min+gVSS (n=5); such rapid uptake is typical of EBPR systems [27].

Glycogen consumption within the anaerobic period was 3.7 +/- 2.3 Cmmol/L (n=5) for F.10.18.1.5 and 1.9 +/- 1.3 Cmmol/L (n=5) for WB.10.18.1.5. Conversely, glycogen production in the aerobic phase was 12.6 +/- 13.3 Cmmol/L (n=5) for F.10.18.1.5 and 6.3 +/- 6.2 Cmmol/L (n=5) for WB.10.18.1.5. PHA production during the anaerobic phase, as VFAs and glycogen are consumed, was 3.9 +/- 3.1 Cmmol/L (n=5) for F.10.18.1.5 and 2.4 +/- 1.0 Cmmol/L (n=5) for WB.10.18.1.5. Similarly, PHA consumption during the aerobic phase was 6.8 +/- 5.4 Cmmol/L (n=5) for F.10.18.1.5 and 4.5 +/- 2.4 Cmmol/L (n=5) for WB.10.18.1.5. Lastly, the effluent PHA averaged 3.4 +/- 1.2 Cmmol/L (n=5) for F.10.18.1.5 and 1.62 +/- 1.26 Cmmol/L (n=5) for WB.10.18.1.5.

4.1.2 Two-hour Anaerobic State

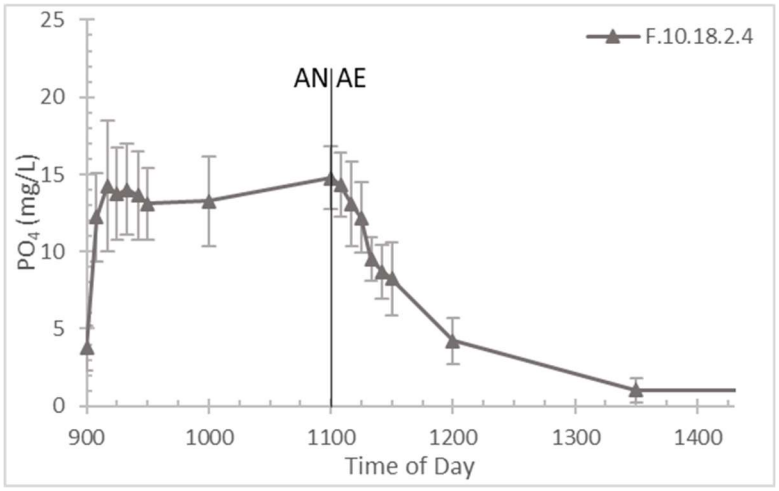

Figure 4.1.2.1: Average PO_4 concentrations in solution for F.10.18.2.4 with standard deviation bars for each value.

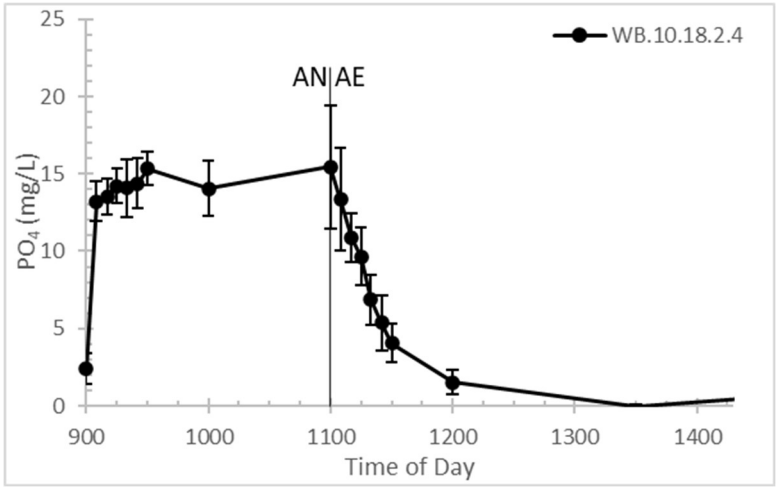

Figure 4.1.2.2: Average PO_4 concentrations in solution for WB.10.18.2.4 with standard deviation bars for each value.

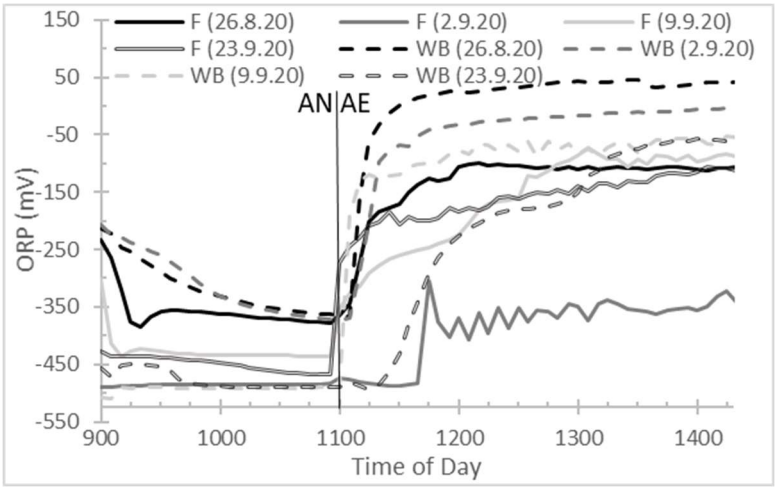

Figure 4.1.2.3: ORP values as measured continuously in solution for F.10.18.2.4 and WB.10.18.2.4 on several dates on which sampling events took place.

Four sampling events occurred during the two-hour anaerobic operational state, and results are shown in Figures 4.1.2.1-7. As indicated by Figures 4.1.2.1 and 4.1.2.2, the PO_4 profiles showed less variability than the one-hour anaerobic operational state, specifically lower standard deviation on phosphorus release, uptake, and effluent averages. Moreover, effluent phosphorus was higher; average effluent PO_4 was 0.98

+/- 0.50 mg/L (n=4) for F.10.18.2.4 and 0.54 +/- 0.43 mg/L (n=4) for WB.10.18.2.4; average influent PO_4 (i.e., t=0 concentration) was 3.76 +/- 1.45 mg/L (n=4) for F.10.18.2.4 and 2.40 +/- 0.97 mg/L (n=4) for WB.10.18.2.4. The PO_4 profiles exhibit theoretically typical EBPR behavior with an average phosphorus release of 11.4 +/- 4.6 mg/L (n=4) for F.10.18.2.4 and 13.6 +/- 0.6 mg/L (n=4) for WB.10.18.2.4 and an average phosphorus uptake of 14.1 +/- 1.9 mg/L (n=4) for F.10.18.2.4 and 15.5 +/- 3.8 mg/L (n=4) for WB.10.18.2.4. The anaerobic ORP reached an average of -445 +/- 45.3 mV (n=4) for F.10.18.2.4 and an average of -436 +/- 75.9 mV (n=4) for WB.10.18.2.4. Additionally, the anaerobic and aerobic ORP values for each cycle were realized and maintained longer than what was observed in the one-hour anaerobic ORP profiles.

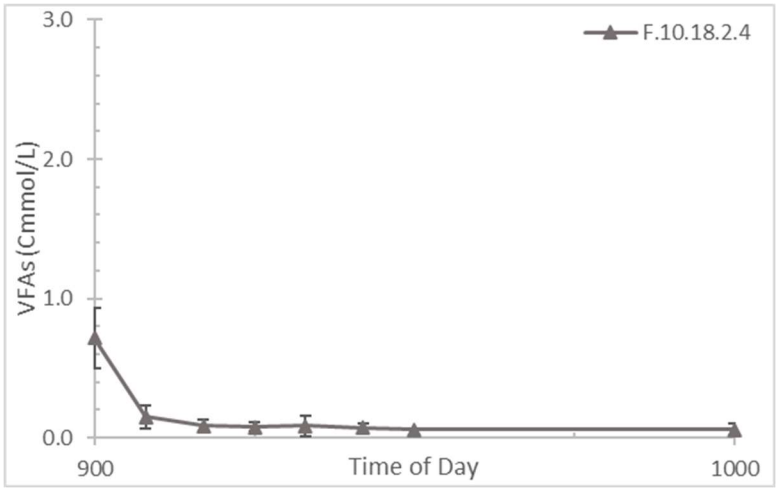

Figure 4.1.2.4: Average VFA concentrations in solution for F.10.18.2.4 within the first hour of the cycle, including standard deviation bars for each value.

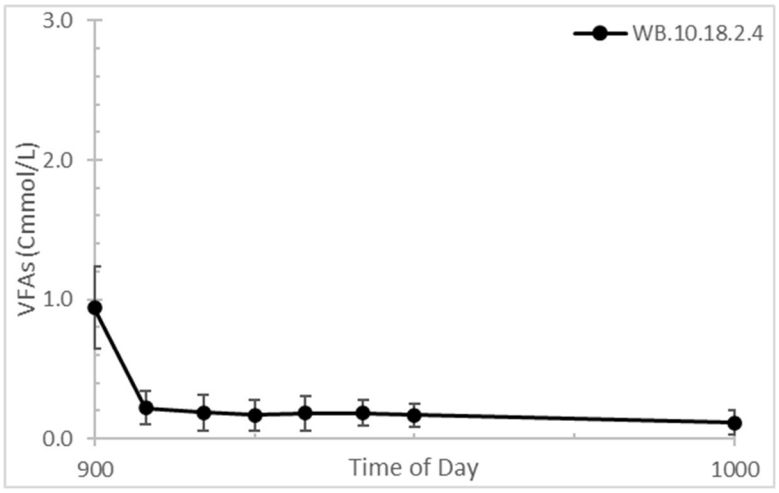

Figure 4.1.2.5: Average VFA concentrations in solution for WB.10.18.2.4 within the first hour of the cycle, including standard deviation bars for each value.

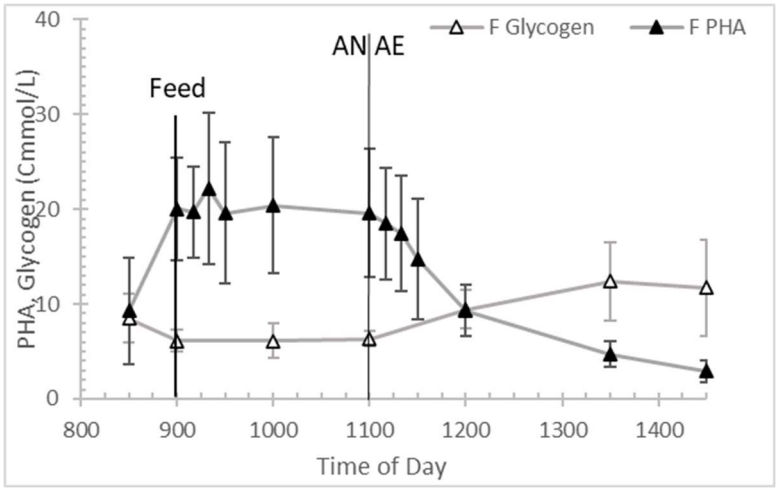

Figure 4.1.2.7: Average Glycogen and PHA concentrations for WB.10.18.2.4 with standard deviation bars for each value.

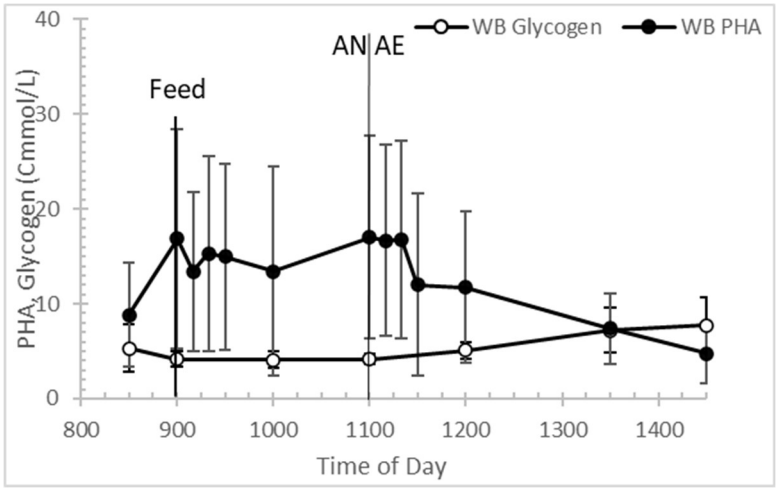

Figure 4.1.2.6: Average Glycogen and PHA concentrations for F.10.18.2.4 with standard deviation bars for each value.

The carbon profiles indicate a healthy EBPR system where VFAs are consumed quickly, PHA increases while glycogen decreases in the anaerobic zone, and glycogen increases while PHA decreases in the aerobic zone [9]. The influent VFA concentration was an average of 2.15 +/- 0.6 Cmmol/L (n=4) for the F.10.18.2.4 combined feed and 19.7 +/- 6.2 Cmmol/L (n=4) for the WB.10.18.2.4 MFL feed. Contrasted with the one-hour anaerobic HRT, VFA consumption was more rapid; VFAs were depleted in about 10 minutes in F.10.18.2.4 with a specific rate of 0.0038 +/- 0.0012 Cmmol/L+min+gVSS (n=4) and about 5 minutes in WB.10.18.2.4 with a specific rate of 0.005 +/- 0.0016 Cmmol/L+min+gVSS (n=4). Glycogen consumption within the anaerobic period was 3.5 +/- 2.2 Cmmol/L (n=4) for F.10.18.2.4 and 1.4 +/- 2.6 Cmmol/L (n=4) for WB.10.18.2.4. Conversely, glycogen production in the aerobic phase was 7.5 +/- 3.5 Cmmol/L (n=4) for F.10.18.2.4 and 3.8 +/- 2.7 Cmmol/L (n=4) for WB.10.18.2.4. PHA production during the anaerobic phase, as VFAs and glycogen were consumed, was 1.8 +/- 1.1 Cmmol/L (n=4) for F.10.18.2.4 and 4.1 +/- 5.6 Cmmol/L (n=4) for WB.10.18.2.4. Similarly, PHA consumption during the aerobic phase was 16.7 +/- 6.4 Cmmol/L (n=4) for F.10.18.2.4 and 12.3 +/- 7.6 Cmmol/L (n=4) for WB.10.18.2.4. Lastly, effluent PHA averaged 2.9 +/- 1.1 Cmmol/L (n=4) for F.10.18.2.4 and 4.8 +/- 3.1 Cmmol/L (n=4) for WB.10.18.2.4.

4.1.3 Three-hour Anaerobic State

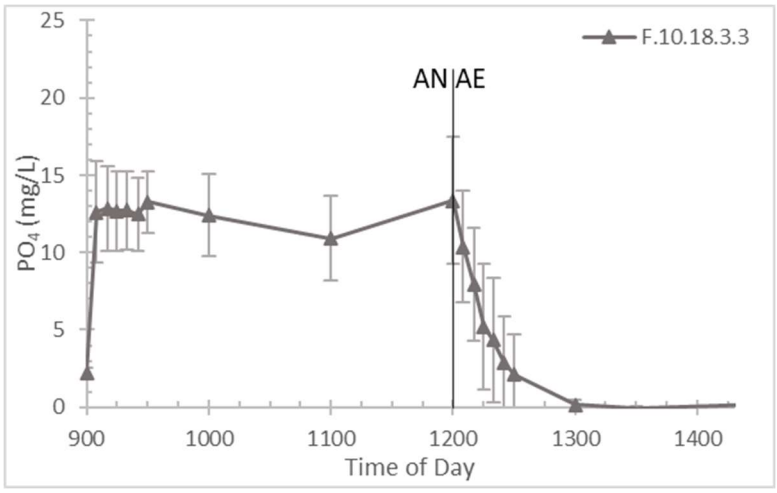

Figure 4.1.3.1: Average PO_4 concentrations in solution for F.10.18.3.3 with standard deviation bars for each value.

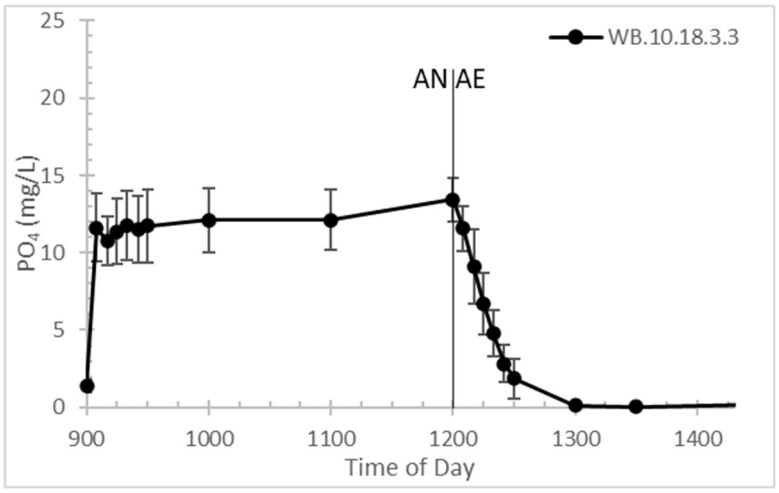

Figure 4.1.3.2: Average PO_4 concentrations in solution for WB.10.18.3.3 with standard deviation bars for each value.

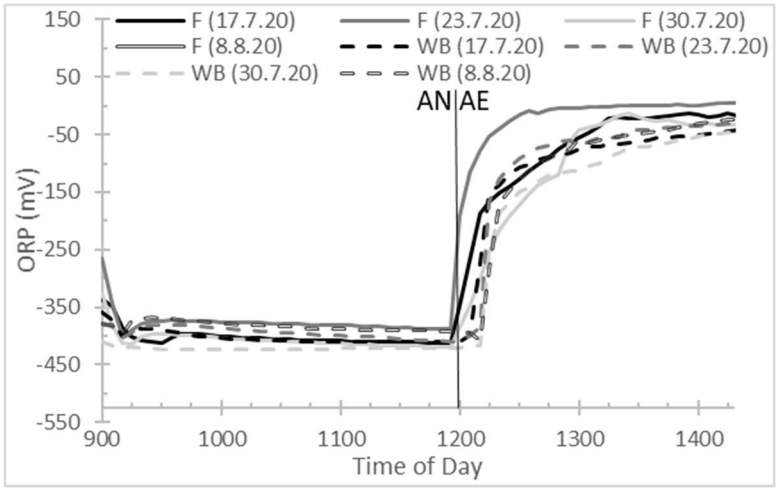

Figure 4.1.3.3: ORP values as measured continuously in solution for F.10.18.3.3 and WB.10.18.3.3 on several dates on which sampling events took place.

Four sampling events occurred during the three-hour anaerobic operational state, and results are shown in figures 4.1.3.1-7. The average effluent PO_4 was 0.16 +/- 0.26 mg/L (n=4) for F.10.18.3.3 and 0.15 +/- 0.18 mg/L (n=4) for WB.10.18.3.3; average influent PO_4 (i.e., t=0 concentration) was 2.18 +/- 0.41 mg/L (n=5) for F.10.18.1.5 and 1.40 +/- 0.38 mg/L (n=5) for WB.10.18.1.5. The PO_4 profiles exhibit theoretically typical EBPR behavior with an average phosphorus release of 12.4 +/- 2.7 mg/L (n=4) for F.10.18.3.3 and 12.5 +/- 1.0 mg/L (n=4) for WB.10.18.3.3 and an average phosphorus uptake of 13.4 +/- 4.1 mg/L (n=4) for F.10.18.3.3 and 13.5 +/- 1.3 mg/L

(n=4) for WB.10.18.3.3. The anaerobic ORP reached an average of -413 +/- 9.2 mV (n=4) for F.10.18.3.3 and an average of -413 +/- 7.6 mV (n=4) for WB.10.18.3.3. As seen in Figure 4.1.3.3, the ORP profiles show the least variability of any other samples taken, with clear and consistent profiles shown for both reactors over all sampling days.

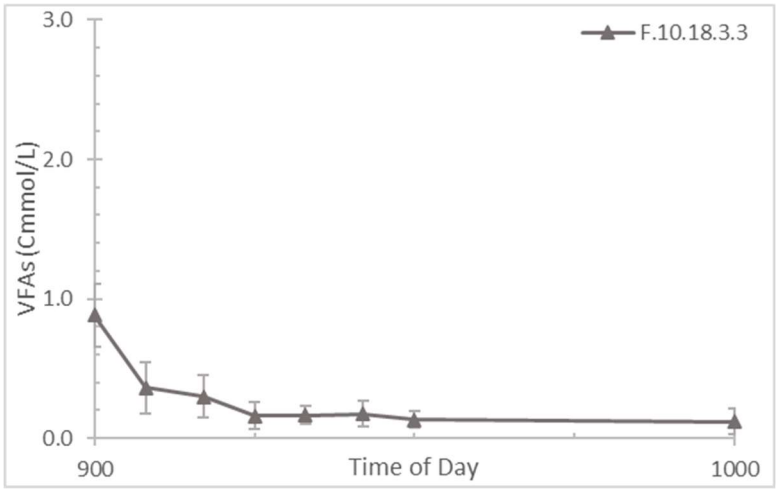

Figure 4.1.3.4: Average VFA concentrations in solution for F.10.18.3.3 within the first hour of the cycle, including standard deviation bars for each value.

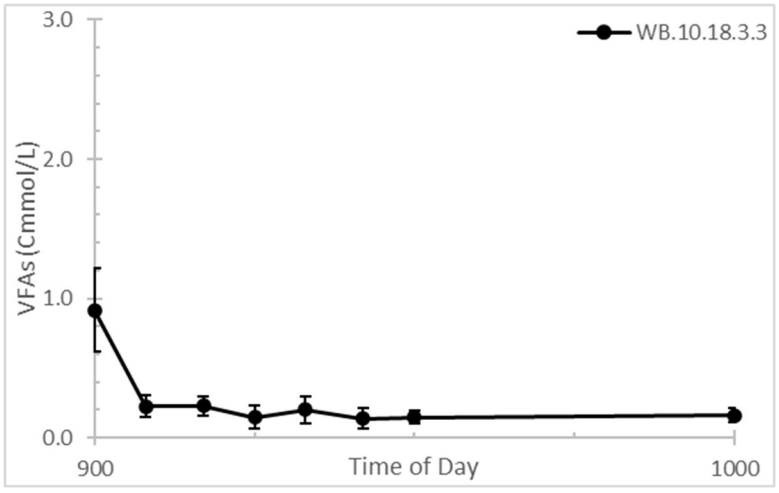

Figure 4.1.3.5: Average VFA concentrations in solution for WB.10.18.3.3 within the first hour of the cycle, including standard deviation bars for each value.

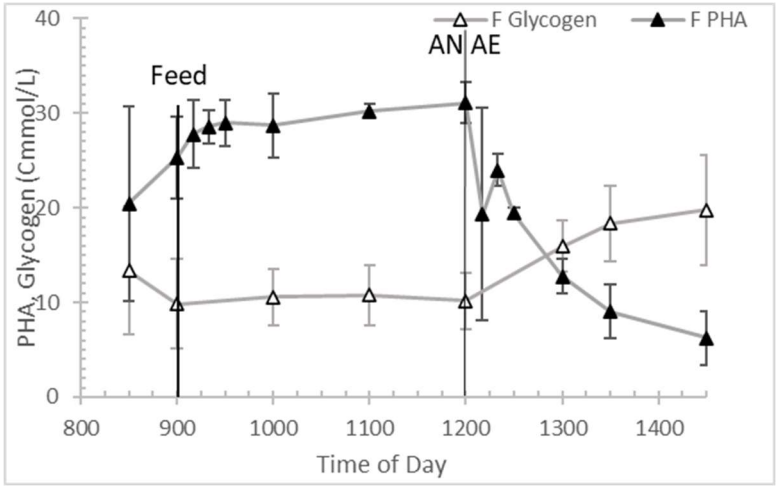

Figure 4.1.3.6: Average Glycogen and PHA concentrations for F.10.18.3.3 with standard deviation bars for each value.

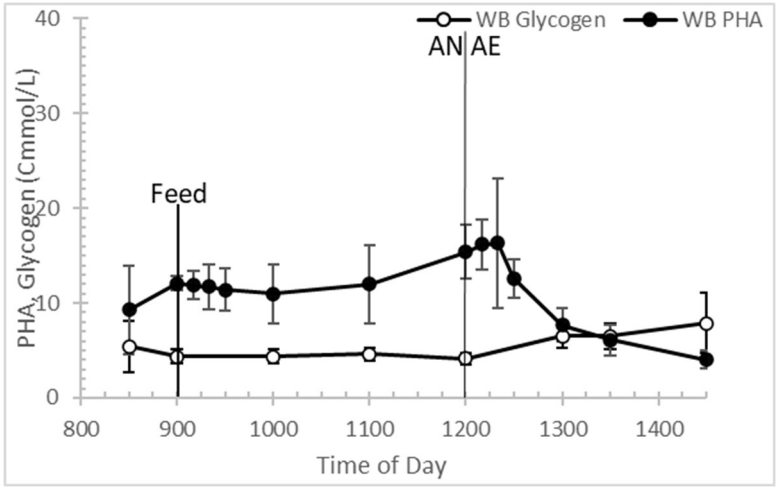

Figure 4.1.3.7: Average Glycogen and PHA concentrations for WB.10.18.3.3 with standard deviation bars for each value.

Lastly, the carbon profiles exhibit the expected response during the anaerobic and aerobic periods associated with overall process health; moreover, the carbon profiles show an overall higher level of PHA for both reactors than in previous operational states. The influent VFA concentration was an average of 2.65 +/- 0.7 Cmmol/L (n=4) for the F.10.18.3.3 combined feed and 19.3 +/- 6.2 Cmmol/L (n=4) for the WB.10.18.2.4 MFL feed. VFAs were depleted in about 15 minutes in F.10.18.3.3 and in WB.10.18.3.3, with specific rates of 0.0057 +/- 0.0014 Cmmol/L+min+gVSS (n=4)

and 0.0059 +/- 0.0019 Cmmol/L+min+gVSS (n=4) respectively. Glycogen consumption within the anaerobic period was 3.3 +/- 1.5 Cmmol/L (n=4) for F.10.18.3.3 and 2.5 +/- 2.6 Cmmol/L (n=4) for WB.10.18.3.3. Conversely, glycogen production in the aerobic phase was 12.7 +/- 4.7 Cmmol/L (n=4) for F.10.18.3.3 and 4.8 +/- 2.5 Cmmol/L (n=4) for WB.10.18.3.3. PHA production during the anaerobic phase was 3.9 +/- 2.1 Cmmol/L (n=4) for F.10.18.3.3 and 3.2 +/- 1.5 Cmmol/L (n=4) for WB.10.18.3.3. Similarly, PHA consumption during the aerobic phase was 25.3 +/- 2.9 Cmmol/L (n=4) for F.10.18.3.3 and 11.3 +/- 2.1 Cmmol/L (n=4) for WB.10.18.3.3. Lastly, the effluent PHA averaged 6.23 +/- 2.8 Cmmol/L (n=4) for F.10.18.3.3 and 4.1 +/- 0.9 Cmmol/L (n=4) for WB.10.18.3.3.

4.1.4 Four-hour Anaerobic State

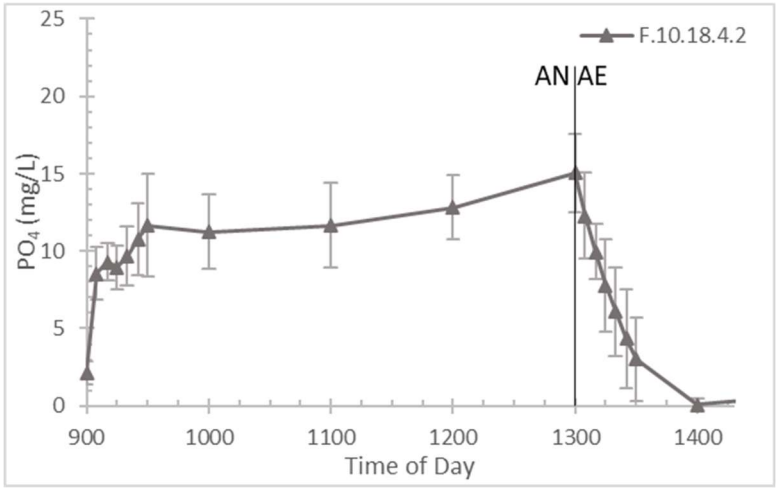

Figure 4.1.4.1: Average PO_4 concentrations in solution for F.10.18.4.2 with standard deviation bars for each value.

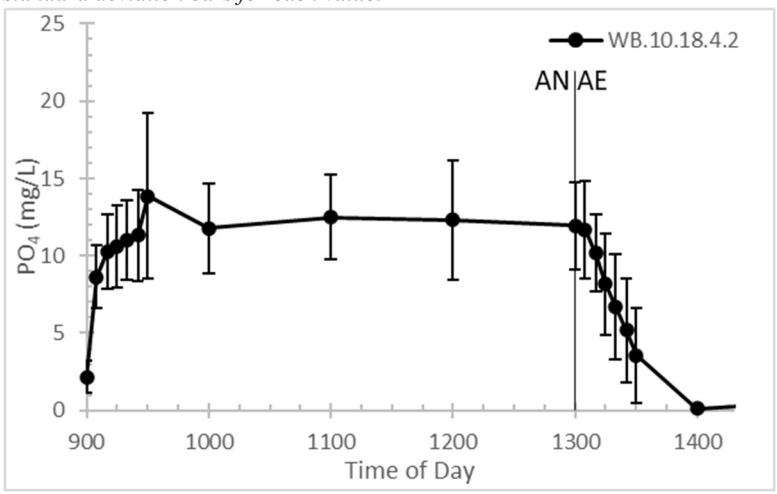

Figure 4.1.4.2: Average PO_4 concentrations in solution for WB.10.18.4.2 with standard deviation bars for each value.

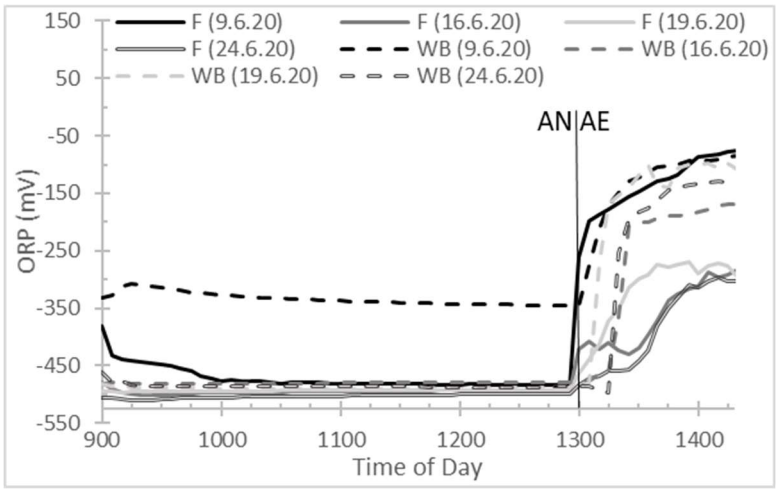

Figure 4.1.4.3: ORP values as measured continuously in solution for F.10.18.4.2 and WB.10.18.4.2 on several dates on which sampling events took place.

Four sampling events occurred during the four-hour anaerobic operational state, and results are shown in figures 4.1.4.1-7. The average effluent PO_4 was 0.45 +/- 0.47 mg/L (n=4) for F.10.18.4.2 and 0.33 +/- 0.23 mg/L (n=4) for WB.10.18.4.2; average influent PO_4 (i.e., at t=0) was 2.17 +/- 0.74 mg/L (n=5) for F.10.18.1.5 and 2.16 +/- 1.02 mg/L (n=5) for WB.10.18.1.5. The PO_4 profiles exhibit theoretically typical EBPR behavior with an average phosphorus release of 12.9 +/- 2.2 mg/L (n=4) for F.10.18.4.2 and 12.1 +/- 3.9 mg/L (n=4) for WB.10.18.4.2 and an average phosphorus uptake of 15.1 +/- 2.8 mg/L (n=4) for F.10.18.4.2 and 11.9 +/- 3.1 mg/L (n=4) for WB.10.18.4.2. The anaerobic ORP reached an average of -498 +/- 11.4 mV (n=4) for F.10.18.4.2 and an average of -454 +/- 72.1 mV (n=4) for WB.10.18.4.2. Additionally, the ORP reached its minimum value quickly into each cycle and maintained the relative minima until aeration began.

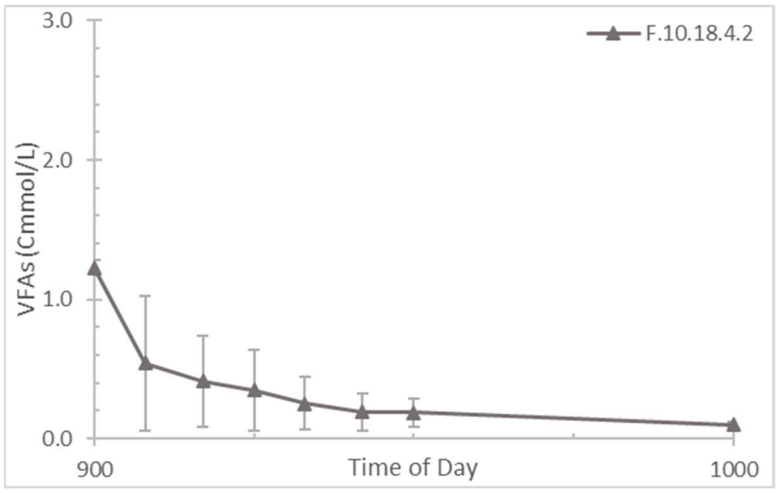

Figure 4.1.4.4: Average VFA concentrations in solution for F.10.18.4.2 within the first hour of the cycle, including standard deviation bars for each value.

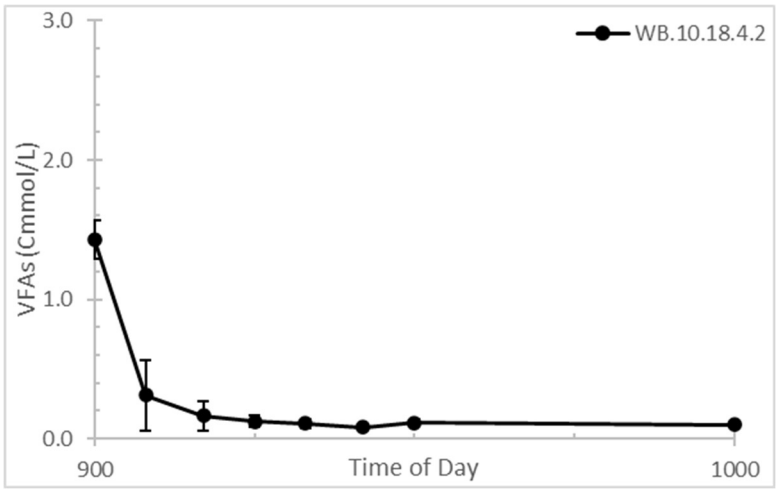

Figure 4.1.4.5: Average VFA concentrations in solution for WB.10.18.4.2 within the first hour of the cycle, including standard deviation bars for each value.

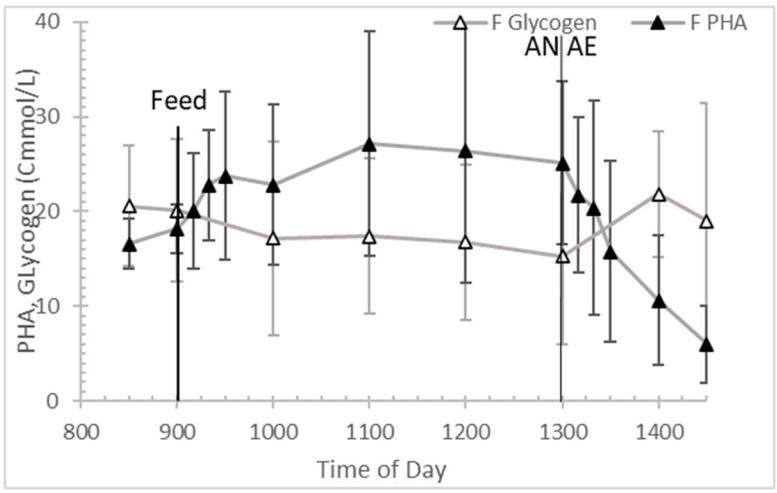

Figure 4.1.4.6: Average Glycogen and PHA concentrations for F.10.18.4.2 with standard deviation bars for each value.

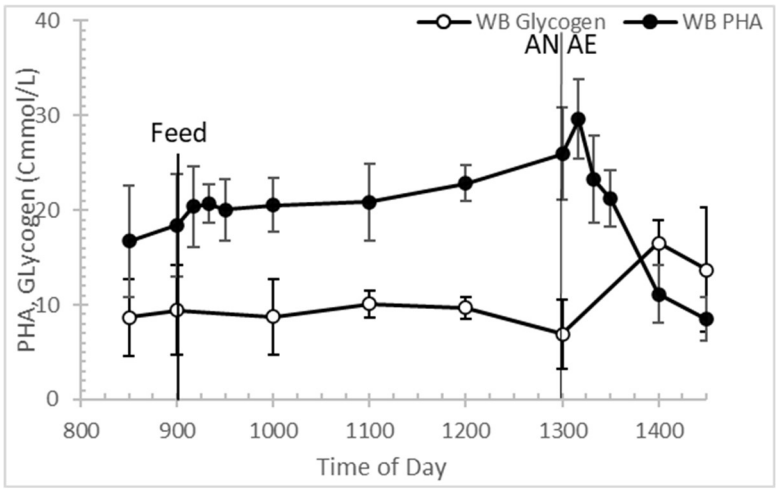

Figure 4.1.4.7: Average Glycogen and PHA concentrations for WB.10.18.4.2 with standard deviation bars for each value.

Lastly, the carbon profiles continued to exhibit the expected EBRP response during the anaerobic and aerobic periods associated with overall good process health. The influent VFA concentration was an average of 3.7 +/- 0.2 Cmmol/L (n=4) for the F.10.18.4.2 combined feed and 30.1 +/- 2.9 Cmmol/L (n=4) for the WB.10.18.4.2 MFL feed. VFAs were depleted in about 25 minutes in F.10.18.4.2 with a specific rate of 0.0052 +/- 0.0003 Cmmol/L+min+gVSS (n=4) and about 20 minutes in WB.10.18.4.2 with a specific rate of 0.006 +/- 0.0005 Cmmol/L+min+gVSS (n=4). Glycogen consumption within the anaerobic period was 8.1 +/- 4.5 Cmmol/L (n=4) for F.10.18.4.2 and 2.5 +/- 1.8 Cmmol/L (n=4) for WB.10.18.4.2. Conversely, glycogen production in the aerobic phase was 7.9 +/- 2.4 Cmmol/L (n=4) for F.10.18.4.2 and 9.6 +/- 1.8 Cmmol/L (n=4) for WB.10.18.4.2. PHA production during the anaerobic phase was 5.4 +/- 3.4 Cmmol/L (n=4) for F.10.18.4.2 and 5.1 +/- 2.4 Cmmol/L (n=4) for WB.10.18.4.2. Similarly, PHA consumption during the aerobic phase was 16.4 +/- 3.1 Cmmol/L (n=4) for F.10.18.4.2 and 17.5 +/- 6.3 Cmmol/L (n=4) for WB.10.18.4.2. Lastly, effluent PHA averaged 5.9 +/- 4.1 Cmmol/L (n=4) for F.10.18.4.2 and 8.5 +/- 2.3 Cmmol/L (n=4) for WB.10.18.2.4.

4.2 EBPR Reactor Performance Metrics

Complementing the EBPR performance results presented in Section 4.1, several other performance metrics can be used to characterize EBPR performance and health. Metrics beyond effluent phosphorus, such as anaerobic P:C (phosphorus release to carbon uptake)[27] and influent VFA:P (VFA to phosphorus) [9], can also be used to predict and asses performance of the system. Pulling in carbon usage metrics such as anaerobic PHA production to VFA uptake [27], PHA production to glycogen consumption [27, 28], and the overall carbon recovery ratio of PHA to glycogen plus VFAs [29] provide further characterization of the system. Tables 4.2.1 and 4.2.2 present these additional process health metrics, summarized by anaerobic HRT for each reactor.

Table 4.2.1: EBPR phosphorus removal metrics at different anaerobic HRTs

Metric	Units	Reactor	AN HRT	Average	SD	n
P:C	molP/Cmmol	AO	1	0.56	0.44	5
			2	0.58	0.32	4
			3	0.49	0.22	4
			4	0.34	0.06	4
			All	0.49	0.11	17
		WB	1	0.25	0.14	5
			2	0.51	0.18	4
			3	0.47	0.11	4
			4	0.27	0.09	4
			All	0.38	0.13	17
VFA:P	mgCOD/mgP	AO	1	7.45	3.66	5
			2	5.08	1.49	4
			3	13.99	9.06	4
			4	14.48	2.74	4
			All	10.25	4.71	17
		WB	1	56.54	55.18	5
			2	20.39	5.36	4
			3	19.91	6.11	4
			4	31.47	2.67	4
			All	32.08	17.16	17
Effluent P	mgP/L	AO	1	0.03	0.05	5
			2	0.98	0.50	4
			3	0.16	0.26	4
			4	0.45	0.47	4
			All	0.40	0.42	17
		WB	1	0.12	0.21	5
			2	0.54	0.43	4
			3	0.15	0.18	4
			4	0.33	0.23	4
			All	0.29	0.19	17
Influent P	mgP/L	AO	1	2.24	1.04	5
			2	3.76	1.45	4
			3	2.18	0.41	4
			4	2.17	0.74	4
			All	2.59	0.78	17
		WB	1	1.68	0.79	5
			2	2.40	0.97	4
			3	1.40	0.38	4
			4	2.16	1.02	4
			All	1.91	0.45	17

Table 4.2.2: EBPR Carbon performance metrics at different anaerobic HRTs

Metric	Units	Reactor	AN HRT	Average	SD	n
PHA:VFA	Cmmol/Cmmol	AO	1	5.12	3.12	5
			2	3.02	3.08	4
			3	4.52	2.19	4
			4	4.47	3.00	4
			All	4.28	0.89	17
		WB	1	1.34	0.71	5
			2	1.61	0.96	4
			3	3.72	1.93	4
			4	3.66	2.00	4
			All	2.58	1.29	17
PHA:Glycogen	Cmmol/Cmmol	AO	1	1.49	1.37	5
			2	0.60	0.33	4
			3	1.35	0.68	4
			4	0.71	0.32	4
			All	1.04	0.45	17
		WB	1	4.82	7.50	5
			2	4.27	2.48	4
			3	4.65	4.61	4
			4	2.65	2.11	4
			All	4.10	0.99	17
PHA:(Glycogen+VFA)	Cmmol/Cmmol	AO	1	0.94	0.68	5
			2	0.47	0.29	4
			3	0.98	0.47	4
			4	0.57	0.22	4
			All	0.74	0.26	17
		WB	1	0.86	0.74	5
			2	0.88	0.61	4
			3	1.22	0.83	4
			4	1.28	0.41	4
			All	1.06	0.22	17

The observed averages for anaerobic P:C lie within previously observed ranges of 0.16 to 0.75 [27, 30]. The AO reactor realized a higher P:C ratio on average for all anaerobic HRTs, possibly due to a higher volume of VFAs in the AO reactor feed. The influent VFA:P also averaged above the recommended 8 mgCOD/mgP [9]. The two-hour anaerobic state had the lowest VFA:P ratio for both reactors; this is likely the cause of the highest average effluent PO4 for the two-hour anaerobic state as well [31]. The carbon recovery ratio, defined as PHA produced over the sum of influent VFAs and glycogen consumed, is also an interesting metric to compare; the WB reactor averaging above 1.0 may indicate fermentation taking place during the anaerobic period, particularly as the anaerobic mass fraction increases [13, 32].

Further application and discussion of these performance metrics is continued in Sections 4.3 and 4.4.

Table 4.2.3: Average rate and specific rate of phosphorus removal and uptake in each reactor summarized by anaerobic retention time.

Metric	Units	Reactor	AN HRT	Average	SD	n
r_{Pr}	mgP/L-min	AO	1	0.13	0.05	5
			2	0.19	0.07	4
			3	0.19	0.03	4
			4	0.17	0.04	4
			All	0.17	0.03	17
		WB	1	0.22	0.09	5
			2	0.23	0.01	4
			3	0.19	0.02	4
			4	0.20	0.06	4
			All	0.21	0.02	17
q_{Pr}	mgP/L-min-gVSS	AO	1	0.03	0.01	5
			2	0.06	0.02	4
			3	0.07	0.01	4
			4	0.04	0.01	4
			All	0.05	0.02	17
		WB	1	0.05	0.02	5
			2	0.07	0.00	4
			3	0.07	0.01	4
			4	0.05	0.02	4
			All	0.06	0.02	17
r_{Pu}	mgP/L-min	AO	1	0.13	0.06	5
			2	0.18	0.02	4
			3	0.22	0.06	4
			4	0.25	0.04	4
			All	0.19	0.05	17
		WB	1	0.20	0.06	5
			2	0.23	0.06	4
			3	0.22	0.02	4
			4	0.20	0.04	4
			All	0.21	0.02	17
q_{Pu}	mgP/L-min-gVSS	AO	1	0.03	0.01	5
			2	0.06	0.01	4
			3	0.08	0.03	4
			4	0.06	0.01	4
			All	0.06	0.02	17
		WB	1	0.04	0.01	5
			2	0.07	0.02	4
			3	0.09	0.01	4
			4	0.05	0.01	4
			All	0.06	0.02	17

Table 4.2.4: Average rate and specific rate of PHA production and consumption for each reactor summarized by anaerobic retention time.

Metric	Units	Reactor	AN HRT	Average	SD	n
r_{PHAp}	Cmmol/L-min	AO	1	0.0048	0.0051	5
			2	0.0012	0.0017	4
			3	0.0020	0.0021	4
			4	0.0007	0.0008	4
			All	0.0022	0.0018	17
		WB	1	0.0036	0.0021	5
			2	0.0037	0.0050	4
			3	0.0028	0.0022	4
			4	0.0006	0.0008	4
			All	0.0026	0.0015	17
q_{PHAp}	Cmmol/L-min-gVSS	AO	1	0.0338	0.0308	5
			2	0.0328	0.0212	4
			3	0.0235	0.0192	4
			4	0.0140	0.0143	4
			All	0.0260	0.0093	17
		WB	1	0.0164	0.0068	5
			2	0.0245	0.0231	4
			3	0.0131	0.0073	4
			4	0.0096	0.0014	4
			All	0.0159	0.0064	17
r_{PHAc}	Cmmol/L-min	AO	1	0.0009	0.0003	5
			2	0.0020	0.0007	4
			3	0.0060	0.0007	4
			4	0.0046	0.0019	4
			All	0.0034	0.0023	17
		WB	1	0.0009	0.0004	5
			2	0.0024	0.0008	4
			3	0.0040	0.0007	4
			4	0.0037	0.0003	4
			All	0.0027	0.0014	17
q_{PHAc}	Cmmol/L-min-gVSS	AO	1	0.0067	0.0038	5
			2	0.0224	0.0086	4
			3	0.0541	0.0061	4
			4	0.0351	0.0066	4
			All	0.0296	0.0200	17
		WB	1	0.0037	0.0020	5
			2	0.0165	0.0102	4
			3	0.0242	0.0045	4
			4	0.0366	0.0132	4
			All	0.0202	0.0138	17

Tables 4.2.3 and 4.2.4 summarize pertinent rate data, organized into respective anaerobic operational states for each reactor. Lowercase ‘r’ denotes a rate presented as a concentration divided by time, as opposed to a specific rate (‘q’) which presents a concentration divided by

time as well as the weight of MLSS in the system (see appendix B for MLSS data). Average rates and specific rates for the anaerobic release and aerobic uptake of phosphorus, as well as the anaerobic production and aerobic consumption of PHA are presented alongside their respective standard deviations. On average, the AO reactor has higher rates and specific rates for PHA production and consumption while the WB reactor has higher rates and specific rates of PO_4 release and uptake. Further discussion can be found in Sections 4.3 and 4.4.

4.3 EBPR Reactor Performance Discussion

Considering the results presented in Sections 4.1 and 4.2, several observations across all anaerobic HRTs stand out. One surprising observation was the lowest average effluent PO_4, along with a low standard deviation, occurred during the one-hour anaerobic HRT for both reactors. On average, the AO reactor realized a higher effluent PO_4 than WB, but the AO reactor also realized the lowest average effluent PO_4 for any anaerobic HRT in the one-hour anaerobic operational state (0.03 +/- 0.05 mgP/L n=5). The expected outcome would be that longer anaerobic HRTs, and consequently lower anaerobic ORP values, would correlate to lower effluent PO_4 [7], but both reactors realized their lowest average effluent phosphorus under the one-hour anaerobic state.

While the lowest average effluent phosphorus appears to indicate that the one-hour anaerobic HRT was the optimal operational state, factors beyond effluent quality must be considered in assessing EBPR resiliency. In addition to the lowest effluent PO_4, the one-hour anaerobic HRT data also averaged the lowest PO_4 release for both reactors, and the lowest net PO_4 uptake for the AO reactor. Strong phosphorus release and uptake is considered a key component to successful EBPR [5, 9, 33], particularly in the case of sustained operation [28]. The low averages of release and uptake also resulted in the lowest specific rates of PO_4 release and uptake for both reactors, as indicated by Table 4.2.3; the production and consumption of PHA and the rate of PHA consumed during the aerobic phase were also lowest during the one-hour anaerobic HRT. Thus, despite the excellent effluent quality, the lower rates of PO4 release and uptake, lower rates of PHA consumption and production, and low average effluent PHA indicate that the one-hour anaerobic HRT may not be the most resilient and sustainable operational state [34]. The data in conjunction with previously

published work suggests that in the presence of an extended aerobic contact time, intracellular stores can be depleted which may disadvantage PAOs in a competitive anaerobic environment in the future [35, 36]. Others have recommended to reduce the SRT, if possible, under these conditions to increase the process sustainability [33, 35].

Another interesting observation was the two-hour anaerobic operational state compared to the one-hour anaerobic HRT. The data showed higher average values for effluent phosphorus, which could be expected given that the one-hour anaerobic state had the lowest average effluent PO_4 of any operational state. The two-hour anaerobic state exhibited higher PHA consumption as well as higher PO_4 release and uptake than the one-hour anaerobic state; the higher PHA consumption and PO_4 uptake make sense metabolically, as more PO_4 uptake requires the breakdown of PHA to generate ATP and store PO_4 as a poly-phosphate chain [33, 37]. Phosphorus release is also commonly considered an indicator for overall phosphorus removal; high PO_4 release is followed by high PO_4 uptake, indicating a healthy system [6, 38].

The last notable observation was a consistent discrepancy between the amount of PHA produced and the amount of PHA consumed. Across all anaerobic HRTs and in both reactors, PHA consumed aerobically on average was significantly greater than PHA produced anaerobically on average. This resulted in a lower effluent PHA value than what was recorded in the RAS prior to the sampled cycle. This is interesting given that very few sampling events ended with PHA below 2 Cmmol/L (the lowest average influent PHA in the RAS), despite consuming 8-20 Cmmol/L more PHA than was produced anaerobically. It has been suggested that anaerobic PHA storage has a significant impact on aerobic PO_4 uptake [4, 39], but this inverse relationship between anaerobic PHA production and aerobic PHA consumption may not be detrimental to the performance and health of an EBPR process long term. In these reactors, PHA production in the anaerobic phase was low compared to PHA consumption but the end anaerobic PHA value was sufficient to support aerobic PO_4 uptake; Each anaerobic HRT indicated these reactors maintained a deficit between PHA produced and consumed, and still operated at an average effluent phosphorus under 0.5 mg/L overall and ended with effluent PHA above 2 Cmmol/L in the RAS. This supports the theory that an ideal aerobic zone will convert much of the stored PHA to glycogen and poly-P in preparation for future

anaerobic conditions; the storage of glycogen and poly-P advantage PAOs in an anaerobic environment, so they might prioritize rebuilding those stores over cellular growth or PHA retention [40].

4.4 AO vs WB Process

While the discussion of reactor performance showed that both reactors behaved in similar ways when subjected to the same anaerobic-aerobic conditions, there were subtle differences between the performance of the two reactors, both overall as well as within specific sampling parameters. Two potential concerns with adding VFAs outside the anaerobic zone are i) the potential for aerobic P release, and ii) enrichment of Ordinary Heterotrophic Organisms (OHOs) over PAOs. On the former point, Pijuan et al. has shown that PAOs can release phosphorus aerobically when provided VFAs [30]; similar results have been observed in batch tests conducted in the Coats Environmental Engineering laboratory (data not shown), and in experiments conducted by Brdjanovic [36]. In contrast, there was no substantial PO_4 release (as measured in the reactor at the start of the aerobic period) observed in the WB reactor despite being fed VFAs in the raw wastewater under aerobic conditions. Regarding the latter point, the additional carbon substrate did not appear to have any adverse impact on the uptake of phosphorus in the WB aerobic period, with a similar response to the introduction of an external electron acceptor as what was expected and was observed in the AO reactor [4, 9]; moreover, effluent PO_4 in WB was 0.29 +/- 0.19 mgP/L (n=17), compared with the AO reactor which averaged 0.40 +/- 0.42 mgP/L (n=17). Under greater anaerobic mass fractions, the PO_4 release was comparable between the two reactors; conversely, with a smaller anaerobic mass fraction the WB reactor exhibited greater PO_4 release and PO_4 uptake on average. The WB reactor was fed more concentrated VFAs, in a smaller volume, which may have further advantaged PAOs under smaller anaerobic mass fractions [3].

One consistent difference between the two reactors was the intracellular carbon stores. The WB biomass had lower average glycogen and PHA (Cmmol/L and mgCOD) throughout the cycle for all anaerobic HRTs. Additionally, as seen in Table 4.2.2, the PHA yield on VFAs was much higher in the AO reactor, whereas the PHA yield on glycogen was substantially higher in the WB reactor. Overall, the carbon recovery ratio, as defined in Section 4.2, was

significantly higher on average for the WB reactor. The carbon recovery ratio also increases as the anaerobic HRT increases, which indicates potential fermentation of slowly biodegradable COD either from the feed, decaying biomass, or any residual COD from the wastewater fed during the previous aerobic phase and carried in through the RAS [13]. Fermentation of organic matter in the anaerobic basin has been shown to occur simultaneously with phosphorus release and PHA production [32], and the amount of fermentation increases with the anaerobic mass fraction [13, 41]. The higher PHA:VFA ratio corresponded with a higher average P:C ratio in the AO reactor, which makes theoretical sense; a larger release of polyphosphates will generate a larger amount of ATP which can then be utilized to uptake the VFAs and store them as PHA [37]. Indeed, a higher release of phosphorus should correlate to a greater yield of PHA [33]. Collectively, the PHA:VFA and P:C data in the AO reactor suggests fermentation under greater anaerobic mass fraction. In contrast to reactor AO, the elevated PHA:Glycogen ratio corresponded with a higher average VFA:P ratio in the WB reactor, which could be explained by the lower mass of carbon fed to WB and lower value of PHA and glycogen in WB overall. The WB anaerobic feed consisted only of municipal fermenter liquor, which had a relatively high concentration of VFAs when compared to the 10% MFL 90% raw WW feed for the AO reactor, but was fed at a much smaller volume. The higher concentration of VFAs increased the influent VFA:P ratio, but the mass of carbon fed (as VFAs) to the WB reactor was lower; with less carbon available from the influent, the PHA production may have been subsidized by fermentation of additional organic matter, such as biomass and slowly biodegradable substrate from the MFL or previous cycles, as indicated by the carbon recovery ratio and elevated PO_4 release [13]. This comparatively lower value of PHA production seen in the WB reactor was accompanied by lower glycogen consumption, which is likely why the PHA yield on glycogen was greater in the WB reactor.

Despite similar specific rates of PO_4 release and uptake, compared to the WB reactor the AO reactor exhibited slightly higher PHA production and consumption specific rates (Tables 4.2.3 and 4.2.4), which may be why the WB biomass retained more PHA intracellularly at the end of a cycle than the AO biomass despite lower RAS PHA concentrations at the beginning of the cycle. Another reason for this discrepancy could be the higher carbon recovery ratio, which along with fermentation potential, indicated that WB was able to produce more PHA

with lower influent carbon in the anaerobic zone. Despite the lower values of carbon accumulation and usage, the WB reactor had lower effluent PO_4 and similar values of PO_4 uptake aerobically, though theoretically we would expect that lower anaerobic PHA accumulation would lead to diminished PO_4 uptake [33, 39]. Overall, this leads to the conclusion that while carbon storage has impact on the efficiency and success of an EBPR process, the individual effect is likely biomass specific rather than universal; a comparative analysis of PHA production as compared to a baseline established under consistent, stable operation might be a more accurate indicator of process health [4, 39].

4.5 ORP, Anaerobic HRT, and EBPR Performance: Revisiting Research Questions

The purpose of this thesis was to answer three central research questions, using the results of these experiments, to further inform the design and operation of EBPR systems. All three of these questions, which have been stated in Chapter One, revolve around the use of ORP to characterize process health. Complementing the data and discussion already presented in Sections 4.1 and 4.2, an analysis of variance (ANOVA) on EBPR process metrics based on anaerobic or aerobic ORP. These ANOVA were performed at a significance level of 95% ($\alpha = 0.05$), and a resulting p-value less than 0.05 indicated that the relationship was considered statistically significant. The tables containing the results of each ANOVA can be found in Appendix A.

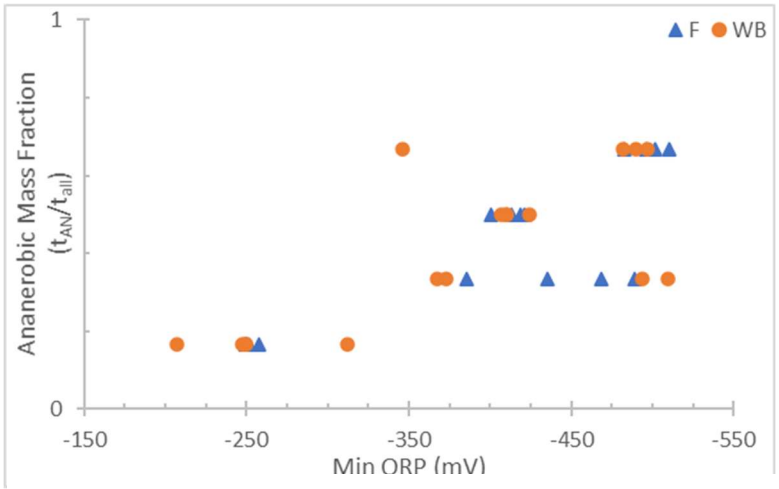

Figure 4.5.1: Minimum anaerobic ORP versus anaerobic mass fraction

Prior to revisiting the research questions, the relationship between anaerobic ORP and anaerobic mass fraction must be determined. The anaerobic mass fraction is a more standardized way of expressing the size of the anaerobic zone relative to the overall treatment volume, and is applicable to a broad scope of EBPR treatment applications [33]; exploring the correlation between anaerobic ORP and anaerobic mass fraction allows for a broader application of the following results as they relate to ORP. Visually (Figure 4.5.1) as well as the supporting ANOVA (Table A21, p=0.0000013), there is a strong relationship between anaerobic ORP and anaerobic mass fraction; as expected, a higher anaerobic mass fraction corresponds to a lower anaerobic ORP.

4.5.1 Can stable EBPR Performance be Predicted by Anaerobic ORP?

Research question one focused on the use of anaerobic ORP exclusively to characterize EBPR process health. The characterization of a healthy EBPR process can be subjective to several things such the process configuration, size of treatment plant, desired effluent quality, simultaneous nutrient removal processes, and several conflicting opinions in the literature. However, one required outcome of a healthy EBPR process is an overall removal of PO_4 from the system, indicated by good effluent phosphorus concentrations.

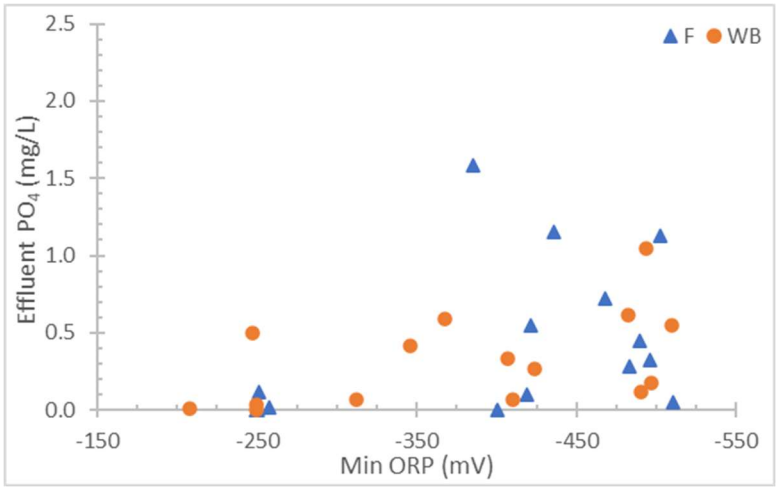

Figure 4.5.1.1: Minimum anaerobic ORP vs effluent PO_4

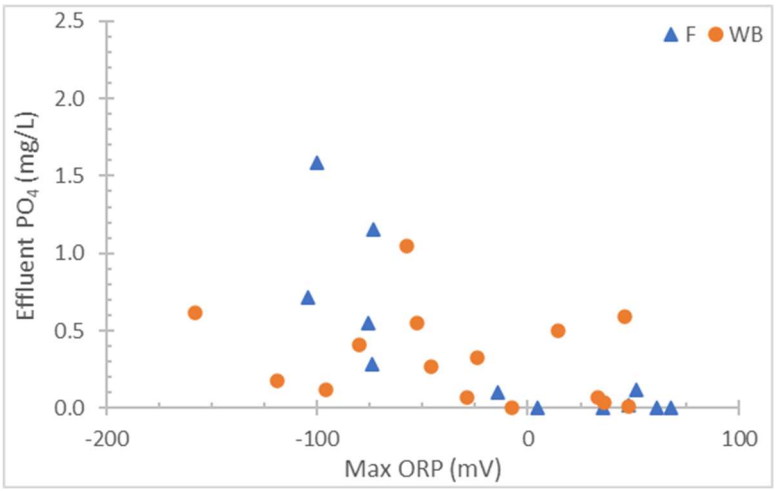

Figure 4.5.1.2: Maximum aerobic ORP vs effluent PO_4

Beyond the kinetics of phosphorus release and uptake, arguably the most important EBPR process health metric is the quality of effluent that the process produces. While not an all-encompassing metric due to the limited ability for diagnosing issues or troubleshooting preemptively, a relationship between anaerobic ORP and effluent phosphorus could mitigate some of those short comings. A plot of effluent PO_4 and the anaerobic ORP minimum value did not suggest any significant relationship (Figure 4.5.1.1), though it appear there could be a relationship where higher (negative) anaerobic ORP values correlated to lower effluent phosphorus. ANOVA (Table A1) confirmed that minimum ORP has no statistically significant impact on effluent PO_4. However, there are many factors which influence the effluent PO_4, and this value alone is not a reliable indicator of overall process health. One aim of this research was to identify, using ORP, metrics to identify process health outside of effluent values and within upstream processes to address issues before process upset occurs.

In contrast to the anaerobic redox value, the aerobic ORP value does correlate with lower PO_4 effluent values (Figure 4.5.1.2), with lower effluent PO_4 aligning with higher aerobic redox values (verified by Table A11 in Appendix A, $p=0.02$); this result could be theoretically predicted, based on what is known about PAO metabolisms and the need for an external electron acceptor to take phosphorus out of solution [5, 9]. Theoretically we could assume that a higher ORP value indicates more

complete aeration, as the oxidation potential increase is reflected by the ORP measurement. The reactors were controlled to 2 mg/L of DO during the aerobic phase of each cycle, and a greater anaerobic mass fraction (and consequently lower anaerobic ORP) resulted in a shorter aerobic mass fraction; a shorter aerobic period, despite the same DO concentration, introduces an additional challenge in reaching higher aerobic ORP values. Even so, this data implies that ORP could be a helpful addition in monitoring the aerobic basin to prevent external electron acceptor limitations on the phosphorus removal ability.

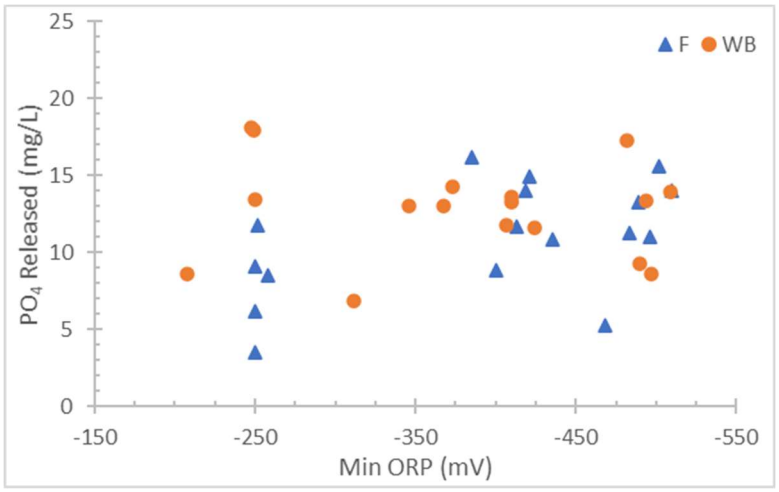

Figure 4.5.1.3: Minimum anaerobic ORP vs total anaerobic phosphorus released

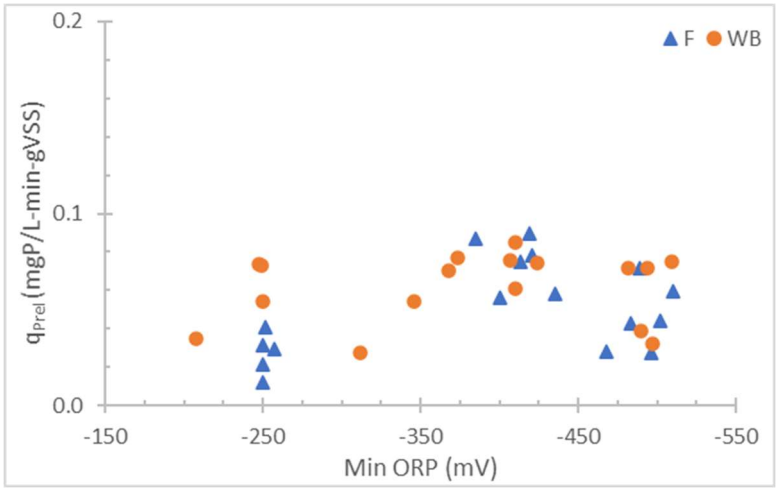

Figure 4.5.1.4: Minimum anaerobic ORP vs specific rate of anaerobic PO_4 release

Additional metrics which are said to be vital to EBPR process success, are the kinetics and stoichiometry of anaerobic phosphorus release and aerobic uptake of phosphorus [8, 28, 33]. However, the amount of phosphorus released into solution during the anaerobic phase does not visually or statistically have any significant correlation to the value of ORP in the anaerobic zone (Figure 4.5.1.3). The variability of phosphorus release continually decreases as the ORP decreases, but the most notable difference occurs between ORP above -300 mV and ORP below -300 mV (Table A2). Similarly, there was no correlation between the anaerobic ORP value and the specific rate at which phosphorus is released into solution (Figure 4.5.1.4). The rate at which phosphorus is released into solution, as well as the total quantity of phosphorus released, is affected by several factors such as MLSS, anaerobic mass fraction, and intracellular PO4 stores [33]; while the anaerobic ORP is a surrogate measure that correlates with anaerobic mass fraction, this data indicated ORP is not singularly able to influence the PO_4 release. Most importantly, the release of phosphorus is impacted by the availability of carbon at the beginning of the anaerobic phase, and the rate at which that carbon is consumed [31, 33].

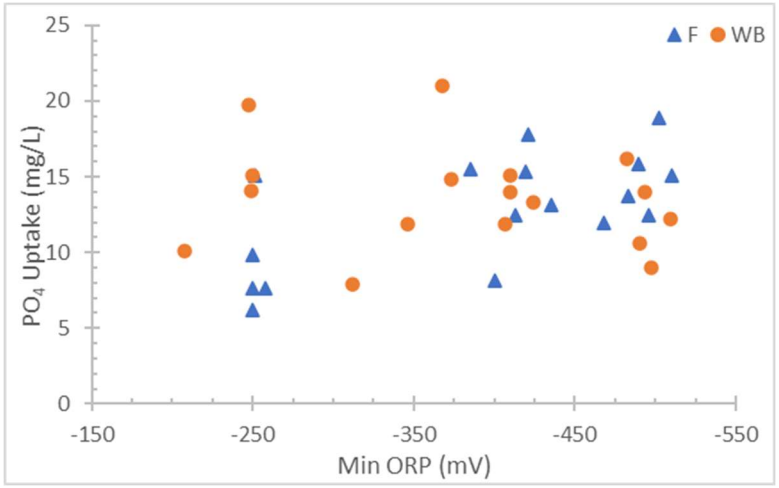

Figure 4.5.1.5: Minimum anaerobic ORP vs total aerobic phosphorus uptake

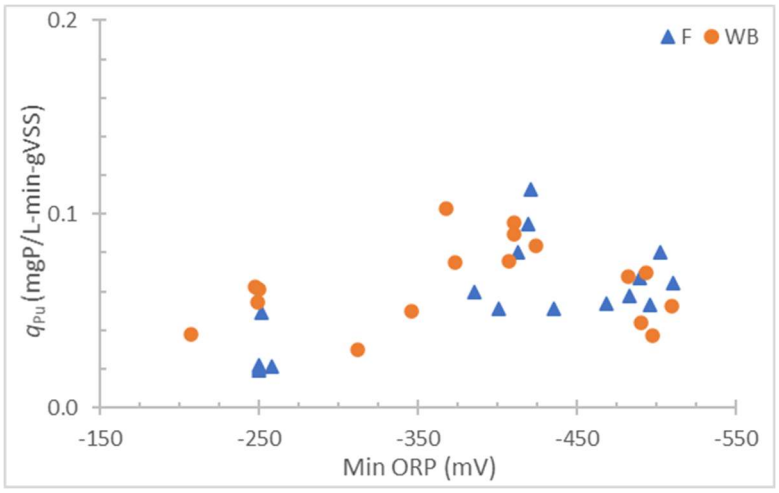

Figure 4.5.1.6: Minimum anaerobic ORP vs specific rate of aerobic PO_4 uptake

The last phosphorus metric which can be indicative of process health is phosphorus uptake. Again, visually (Figure 4.5.1.5) and statistically, the impact of anaerobic ORP on phosphorus uptake in the aerobic zone is not significant (Table A4). Phosphorus uptake was less variable at anaerobic ORP values below -400 mV, similar to the decrease in variability for the phosphorus release data below -300 mV. However, like effluent phosphorus and phosphorus release, the value of phosphorus taken up in solution is impacted by many factors such as PO_4 release, MLSS concentration, and intracellular carbon storage [4, 33].

The specific rate at which phosphorus is aerobically consumed, while still impacted by several of the aforementioned factors, did exhibit a statistically significant correlation to the anaerobic ORP value (see Table A5, p=0.01). This could be due to a higher value of PHA storage in the anaerobic zone [4], increased fermentation at lower ORP values [7], and/or the PHA that is consumed in the aerobic zone following the period of stress reaching low anaerobic ORP values [33].

Though many EBPR operational health parameters were unimpacted by the value of ORP in the anaerobic period, the relationship between anaerobic ORP and aerobic phosphorus uptake implied that ORP could be used to inform process health. The usage of anaerobic ORP, while limited, could provide an additional monitoring metric to maintain the health of an EBPR system; However, anaerobic ORP cannot inform

whether an EBPR system is fully healthy, and should not be used to wholly define the process in any way.

4.5.2 Is there an Anaerobic ORP Threshold for Optimal EBPR Performance?

The second research question driving this thesis has to do with utilizing ORP as a metric to determine whether a minimum anaerobic ORP value is needed for stable EBPR. To assess the threshold for process stability, a clear understanding of what constitutes a stable EBPR process in needed. As mentioned in Section 4.5.1, effluent phosphorus and rates of phosphorus release and uptake are commonly used metrics for assessing EBPR process health. Additional metrics commonly used to characterize process health also include the anaerobic carbon recovery ratio, as defined in Section 4.2, the anaerobic P release to influent carbon ratio; with a higher carbon recovery ratio indicating a more robust system and a P:C ratio between 0.3 to 0.7 is similarly considered ideal [27, 29].

On average, as seen in Appendix A and Section 4.1, the variability of phosphorus metrics decreases below anaerobic ORP values of -300 mV for phosphorus release and uptake. Similarly, as discussed in Section 4.5.1, lower anaerobic ORP values corresponded to greater specific rates of PO_4 uptake aerobically. The optimization of aerobic PO_4 uptake is a key factor in successful EBPR, and is influenced by the conditions of the anaerobic basin which precedes it [33, 36]. The influence of lower anaerobic ORP may be increasing the conditions which are optimal for fermentation, and increased fermentation is tied to greater anaerobic carbon storage; the increase of the carbon recovery ratio with anaerobic HRT, particularly in the WB reactor biomass, also supports the conclusion that greater anaerobic mass fraction, and consequently lower ORP, leads to optimal end anaerobic conditions to sustain aerobic phosphorus uptake.

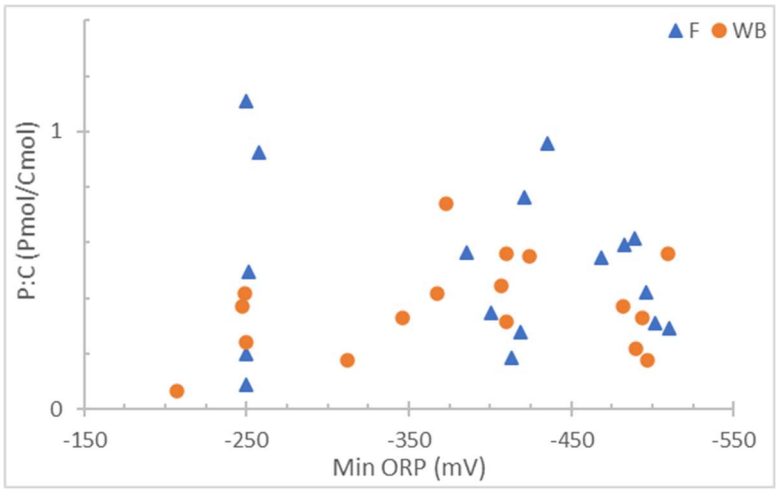

Figure 4.5.2.2: Minimum anaerobic ORP versus anaerobic P:C ratio

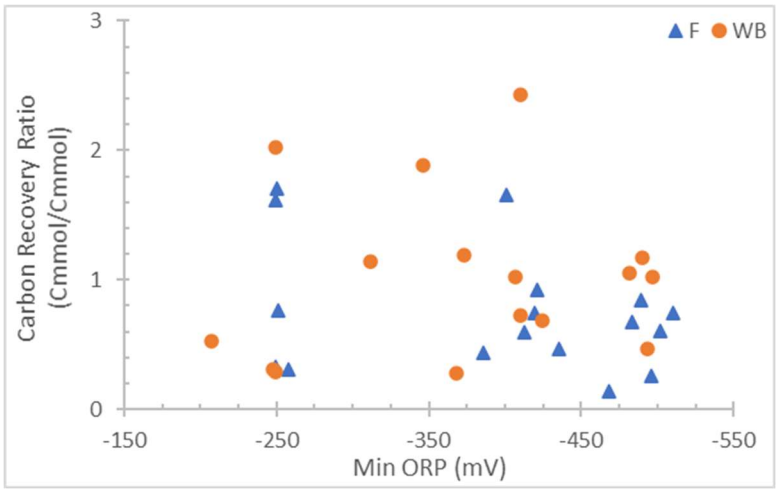

Figure 4.5.2.1: Minimum anaerobic ORP versus anaerobic carbon recovery ratio

However, ANOVA on the carbon recovery ratio and anaerobic ORP indicated there was no statistically significant correlation (Table A22). Furthermore, ANOVA on P:C versus the anaerobic ORP also indicated there was no statistically significant relationship between the two. The variability for both the carbon recovery ratio and the anaerobic P:C did decrease on average at anaerobic ORP below -300 mV. Despite not being able to use the anaerobic ORP to enhance these two metrics, maintaining an anaerobic ORP of -300 mV or less could increase the accuracy of these metrics for characterizing performance within the anaerobic basin.

While ORP proved to be a poor metric for operational control, the use of ORP both anaerobically and aerobically could provide further clarification on the health of the process overall. The operational states which exhibited the least variability – and the best EBPR health - across all metrics and values were the three- and four-hour anaerobic operational states, with average ORP values between -415 to -500 mV. Based on the results presented in Appendix A, a decrease in variation of common EBPR process health metrics such as specific rates of PO_4 release and uptake, carbon recovery ratio, and anaerobic P:C occurred at anaerobic ORP values below -300 mV. The recommendation, based upon the results of this research, is to aim for an anaerobic redox value below -300 mV to increase the reliability of the EBPR process.

4.5.3 Is There a Correlation Between ORP and PHA, and does it have Implications for EBPR Process Recovery?

The third research question addressed in this thesis, was intended to target investigations into the recovery of a failed EBPR system. While these research investigations did not take place as a part of this study, the data that was collected could still be used to theoretically assess the stability and recoverability of an EBPR process. This research question aims to use PHA as a surrogate measure for predicting process recovery in the case of failure and aims to establish a relationship between ORP and PHA that could be used to facilitate PHA production and consumption in an EBPR system that is underperforming.

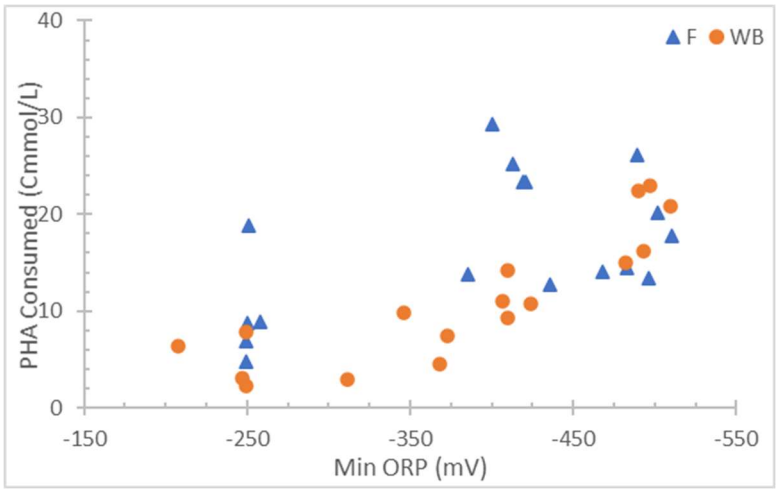

Figure 4.5.3.1: Minimum ORP vs. aerobic PHA consumed

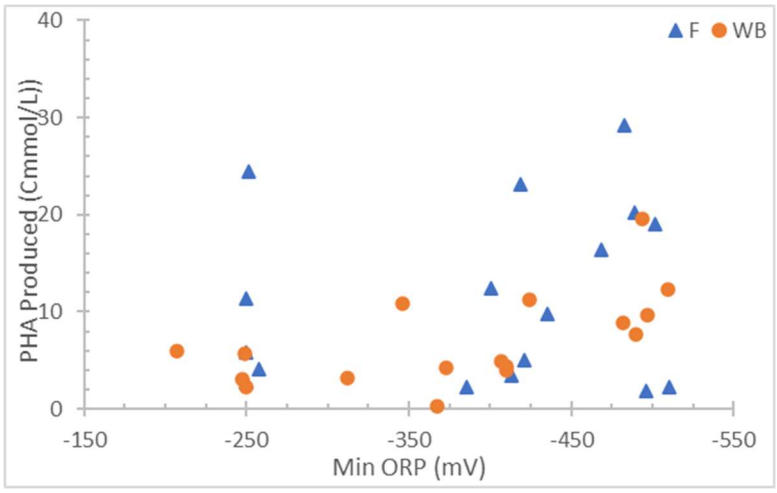

Figure 4.5.3.2: Minimum ORP vs. anaerobic PHA produced

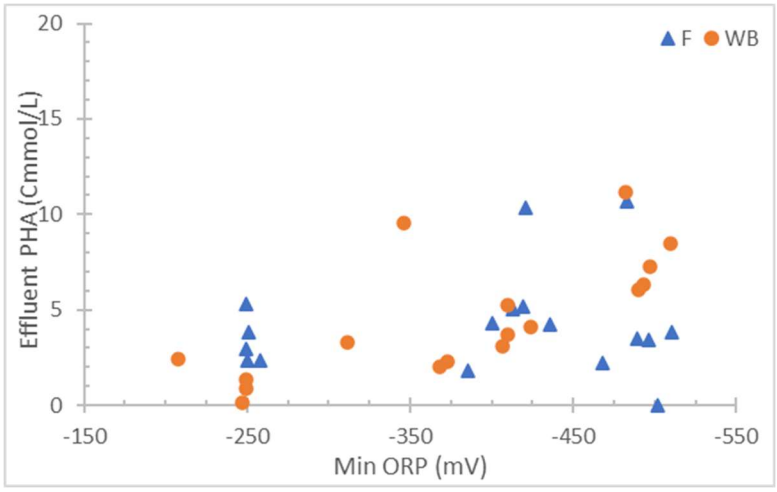

Figure 4.5.3.3: Minimum ORP vs. effluent PHA

Intracellular carbon storage can be tracked by quantifying the intracellular PHA and glycogen stores. Interestingly, the relationship between anaerobic ORP and several PHA metrics were statistically significant. In the anaerobic phase, there appears to be a loosely negative relationship between ORP and PHA production, where lower anaerobic ORP correlates to higher PHA production, though this relationship proved to not be statistically significant (Table A6). Conversely, a statistically significant relationship between aerobic PHA consumption and the minimum anaerobic ORP value, meaning that lower anaerobic ORP values corresponded to higher PHA consumption aerobically, is verified by ANOVA (Table A8, p=0.0001). PHA production and consumption are metabolically linked in EBPR; moreover, a higher quantity of stored PHA allows for a higher quantity of PO_4 to be consumed. This relationship supports a critical relationship between PO_4 uptake and anaerobic ORP, as shown in Section 4.5.1; greater amounts of stored carbon allow for a greater uptake of phosphorus in the aerobic zone [42]. The data indicated that lower anaerobic ORP corresponds to greater levels of carbon usage aerobically, which requires sufficient carbon storage to support that consumption [33].

Another interesting relationship occurred between the remaining PHA at the end of a cycle (or in the RAS in the case of a traditional treatment plant) and anaerobic ORP. It has been suggested that higher levels of PHA, as well as polyphosphates, remaining in the biomass at the end of the treatment train are associated with a greater level of process stability and resiliency [35, 36]. The data indicates a negative relationship between lower values of ORP and the amount of PHA remaining in the cell at the end of a cycle (Figure 4.5.3.3 and Table A10, p=0.006); in other words, a lower value of anaerobic ORP indicates a higher value of PHA remaining in the RAS. This implies increased fermentation at lower anaerobic ORP values, which in turn increases the PHA production and allows for a higher effluent PHA [32]. It is counterintuitive that the anaerobic ORP, which relates to higher levels of PHA consumption, would similarly relate to higher values of effluent PHA without also relating to higher values of PHA produced, yet that is what this data is indicates. This relationship supports the hypothesis that lower ORP values correspond to higher levels of anaerobic PHA storage overall. Lower ORP values correspond not only to greater values of PHA

consumption and PO_4 uptake, but also greater values of effluent PHA, none of which would be possible without sufficient intracellular carbon storage. Therefore, anaerobic ORP could potentially be used to stimulate PHA storage to buffer against or recover from a process upset; more research into the application of this on failed EBPR process states is needed support this theory.

Chapter Five: Conclusions

Research efforts conducted as part of this thesis sought to understand the relationship between ORP and EBPR systems by operating and monitoring performance of two bench-scale reactors under contrasting criteria, at variable anaerobic retention times (i.e., anaerobic mass fraction) with continuous ORP and DO monitoring. Performance was monitored by soluble reactive phosphorus, anaerobic VFAs, glycogen, and PHA throughout the cycle; the data was used to generate several EBPR metrics indicative of process health.

The AO reactor operation consisted of a feeding period at the beginning of the anaerobic period, with a 0.33 L feed mixture containing 10% municipal fermenter liquor and 90% municipal wastewater, and an aerobic period with DO maintained at 2 mg/L. Alternatively, the Westbank reactor was fed only the fermenter liquor, the same volume the AO reactor received (0.03 L), at the start of the anerobic period and the wastewater (0.3 L) was fed at the start of the subsequent aerobic period. Each reactor was subjected to the same anaerobic mass fraction for each 6-hour cycle, resulting in the same aerobic mass fraction as well.

The comparison between the A/O SBR and Westbank SBR yielded several interesting results. No secondary phosphorus release or inhibited phosphorus uptake was observed in the WB reactor as a result of feeding VFAs (in the form of raw wastewater) at the start of the aerobic period, as was expected [30]. However, the AO reactor realized higher PHA anaerobic production and aerobic consumption, higher glycogen anaerobic consumption and aerobic production, and higher anaerobic phosphorus release to carbon consumed (P:C) ratios compared to the WB reactor. This implies that the AO reactor was advantaged for anaerobic fermentation, possibly due to the higher mass of carbon fed at the start of the anaerobic period. On the other hand, the WB reactor realized higher carbon recovery ratios (i.e., more efficient use of carbon), lower effluent PO_4, higher specific rates of phosphorus release and uptake, and higher PHA in the RAS. This implied that the WB reactor may have advantaged PAOs with a smaller mass of highly concentrated VFAs that the start of the anaerobic zone, resulting in increased phosphorus kinetics and stoichiometry.

Overall, results from this research suggest that ORP is a metric which can be used to provide further clarification on the health of an EBPR system when used both anaerobically and

aerobically. ORP used in the aerobic basin can ensure that sufficient oxidation is occurring to prevent the limitation of PO_4 uptake, and ultimately assist phosphorus removal from the system. Alternatively, anaerobic ORP could be used to ensure maximum aerobic PO_4 uptake and PHA consumption; anaerobic ORP could also be monitored to ensure fermentation occurs, which could help stabilize the EBPR process. By targeting lower ORP values, the anaerobic basin can be used to maximize PO_4 uptake and consequently overall PO_4 removal. A significant relationship where lower anaerobic ORP values corresponded to higher aerobic specific rates of PO_4 uptake was observed in both reactors. Additionally, the anaerobic ORP value could also be used a surrogate measure of carbon storage; increased PHA consumption aerobically and higher effluent PHA values both correlated to lower anaerobic ORP. This has the potential to not only affect the current treatment cycle, but also has implications into the stability of the system in the long run.

Therefore, several recommendations for the design and operation of an EBPR system can be made from the conclusions of this research. First, and most important, is the design of the anaerobic basin to contribute significant anaerobic mass fraction. This research indicated that longer anaerobic mass fractions correlated strongly with lower anaerobic ORP values, which in turn increased several key EBPR performance metrics. It is recommended to design the anaerobic basin to result in a mass fraction of at least 0.3, or alternatively to consistently reach an anaerobic ORP below -300 mV. An anaerobic basin with these specifications, based on the presented results, should exhibit higher specific rates of aerobic PO_4 uptake, higher aerobic PHA consumption, and higher values of PHA in the RAS. This should increase not only the performance of the system, but the stability of the process over time as well.

Second, and lastly, while both reactors performed well and achieved average effluent phosphorus below 0.5 mg/L, the WB reactor on average exhibited lower effluent PO_4 and higher PHA in the RAS. While the Westbank configuration is what led to these results, it is likely due to the highly concentrated VFAs that were added to the anaerobic basin because of the split feed, rather than the specific process configuration. Though the design of an EBPR system rarely allows for control over the VFAs fed to the system, this research, as many others have found in the past, indicates that the influent VFA concentration does have an impact on the process kinetics and the overall process health. With an increased concentration

of VFAs, the system should see an increase in PO_4 release and uptake kinetics, increased accompanying PHA consumption, and potentially increased values of PHA in the RAS.

Overall, the most influential factor, and the focus of this research, was the impact of the anaerobic basin. Sufficient anaerobic contact time, as measured either by anaerobic mass fraction or ORP, can have a significant impact on the effluent quality and the stability of an EBPR system. To further explore these impacts, additional research is recommended, including:

- Exploring the effects of feed strength and volume on phosphorus and PHA kinetics
- Investigating the relationships between aerobic ORP and EBPR process health and stability
- Studying the impact of the total change in ORP from the anaerobic minimum to the aerobic maximum, and
- Quantifying the effects of anaerobic ORP on fermentation during the anaerobic period.

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Appendix A: ANOVA Tables

All ANOVA analysis were performed in Microsoft Word, at a significance level of 95% (α=0.05). The null hypothesis of each test is that there is no difference between the means of each group. With a p-value below α we reject the null hypothesis and conclude that there is a statistically significant difference between the means of each group.

Table A1: ANOVA of effluent PO_4 (mgP/L) versus anaerobic ORP minimum value (mV)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
-200 to -300 mV	9	0.69	0.08	0.03
-300 to -400 mV	5	2.65	0.53	0.40
-400 to -500 mV	17	6.22	0.37	0.13
< -500 mV	3	1.73	0.58	0.29

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.982	3.0	0.327	2.224	0.106	2.922
Within Groups	4.415	30.0	0.147			
Total	5.40	33				

Table A2: ANOVA on anaerobic PO_4 release (mgP/L) versus anaerobic ORP minimum value (mV)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
-200 to -300 mV	9	97.09	10.79	25.04
-300 to -400 mV	5	63.37	12.67	12.23
-400 to -500 mV	17	200.02	11.77	7.78
< -500 mV	3	43.57	14.52	0.92

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	35.071	3.0	11.690	0.934	0.437	2.922
Within Groups	375.628	30.0	12.521			
Total	410.70	33				

Table A3: ANOVA on the specific rate of anaerobic PO_4 release (mgP/L+min+gVSS) versus anaerobic ORP minimum value (mV)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
-200 to -300 mV	9	0.37	0.04	0.00
-300 to -400 mV	5	0.32	0.06	0.00
-400 to -500 mV	17	1.04	0.06	0.00
< -500 mV	3	0.18	0.06	0.00

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.003	3.0	0.001	2.089	0.123	2.922
Within Groups	0.013	30.0	0.000			
Total	0.02	33				

Table A4: ANOVA on aerobic PO_4 uptake (mgP/L) versus anaerobic ORP minimum value (mV)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
-200 to -300 mV	9	105.33	11.70	20.22
-300 to -400 mV	5	71.21	14.24	23.36
-400 to -500 mV	17	225.03	13.24	6.32
< -500 mV	3	46.15	15.38	11.25

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	39.879	3.0	13.293	1.053	0.384	2.922
Within Groups	378.776	30.0	12.626			
Total	418.66	33				

Table A5: ANOVA on the specific rate of aerobic PO_4 uptake (mgP/L+min+gVSS) versus anaerobic ORP minimum value (mV)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
-200 to -300 mV	9	0.35	0.04	0.00
-300 to -400 mV	5	0.32	0.06	0.00
-400 to -500 mV	17	1.19	0.07	0.00
< -500 mV	3	0.20	0.07	0.00

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.006	3.0	0.002	4.471	0.010	2.922
Within Groups	0.013	30.0	0.000			
Total	0.02	33				

Table A6: ANOVA on PHA produced anaerobically (Cmmol/L) versus anaerobic ORP minimum value (mV)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
-200 to -300 mV	9	62.99	7.00	50.88
-300 to -400 mV	5	23.58	4.72	16.06
-400 to -500 mV	17	191.76	11.28	62.13
< -500 mV	3	33.75	11.25	71.18

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	230.267	3	76.756	1.432	0.253	2.922
Within Groups	1607.763	30	53.592			
Total	1838.03	33				

Table A7: ANOVA on the rate of PHA produced anaerobically (Cmmol/L+min) versus anaerobic ORP minimum value (mV)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
-200 to -300 mV	9	1.05	0.12	0.01
-300 to -400 mV	5	0.20	0.04	0.00
-400 to -500 mV	17	1.17	0.07	0.00
< -500 mV	3	0.19	0.06	0.00

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.023	3	0.008	1.324	0.285	2.922
Within Groups	0.170	30	0.006			
Total	0.19	33				

Table A8: ANOVA on PHA consumed aerobically (Cmmol/L) versus anaerobic ORP minimum value (mV)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
-200 to -300 mV	9	64.42	7.16	25.81
-300 to -400 mV	5	41.85	8.37	12.70
-400 to -500 mV	17	303.95	17.88	39.34
< -500 mV	3	58.79	19.60	2.65

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	928.292	3	309.431	10.407	0.000	2.922
Within Groups	892.019	30	29.734			
Total	1820.31	33				

Table A9: ANOVA on the rate of PHA consumption aerobically (Cmmol/L+min) versus anaerobic ORP minimum value (mV)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
-200 to -300 mV	9	0.21	0.02	0.00
-300 to -400 mV	5	0.21	0.04	0.00
-400 to -500 mV	17	1.84	0.11	0.00
< -500 mV	3	0.40	0.13	0.00

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.058	3	0.019	13.431	0.000	2.922
Within Groups	0.043	30	0.001			
Total	0.10	33				

Table A10: ANOVA on effluent PHA (Cmmol/L) versus anaerobic ORP minimum value (mV)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
-200 to -300 mV	9	22.55	2.51	2.54
-300 to -400 mV	5	18.11	3.62	11.12
-400 to -500 mV	17	95.91	5.64	7.48
< -500 mV	3	29.07	9.69	43.16

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	137.950	3	45.983	5.096	0.006	2.922
Within Groups	270.720	30	9.024			
Total	408.67	33				

Table A11: ANOVA on effluent phosphorus (mgP/L) versus aerobic ORP maximum value (mV)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
> 0 mV	11	1.35	0.12	0.05
-100 to 0 mV	15	4.89	0.33	0.14
< -100 mV	8	5.05	0.63	0.26

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.199	2	0.599	4.426	0.020	3.305
Within Groups	4.198	31	0.135			
Total	5.40	33				

Table A12: ANOVA on anaerobic phosphorus release (mgP/L) versus aerobic ORP maximum (mV)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
> 0 mV	11	112.38	10.22	21.39
-100 to 0 mV	15	190.35	12.69	2.40
< -100 mV	8	101.32	12.67	16.86

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	45.215	2	22.608	1.918	0.164	3.305
Within Groups	365.484	31	11.790			
Total	410.70	33				

Table A13: ANOVA of the specific rate of anaerobic phosphorus release (mgP/L+min+gVSS) versus aerobic ORP maximum (mV)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
> 0 mV	11	0.47	0.04	0.00
-100 to 0 mV	15	1.01	0.07	0.00
< -100 mV	8	0.42	0.05	0.00

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.004	2	0.002	5.255	0.011	3.305
Within Groups	0.012	31	0.000			
Total	0.02	33				

Table A14: ANOVA on aerobic phosphorus uptake (mgP/L) versus aerobic ORP maximum value (mV)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
> 0 mV	11	127.33	11.58	26.39
-100 to 0 mV	15	205.38	13.69	3.27
< -100 mV	8	115.01	14.38	9.34

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	43.696	2	21.848	1.806	0.181	3.305
Within Groups	374.960	31	12.095			
Total	418.66	33				

Table A15: ANOVA on the specific rate of aerobic phosphorus uptake (mgP/L+min+gVSS) versus aerobic ORP maximum value (mV)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
> 0 mV	11	0.47	0.04	0.00
-100 to 0 mV	15	1.09	0.07	0.00
< -100 mV	8	0.48	0.06	0.00

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.006	2	0.003	6.785	0.004	3.305
Within Groups	0.013	31	0.000			
Total	0.02	33				

Table A16: ANOVA on PHA produced anaerobically (Cmmol/L) versus the aerobic ORP maximum (mV)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
> 0 mV	11	79.35	7.21	45.98
-100 to 0 mV	15	151.88	10.13	64.37
< -100 mV	8	80.86	10.11	59.17

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	62.827	2	31.414	0.549	0.583	3.305
Within Groups	1775.203	31	57.265			
Total	1838.03	33				

Table A17: ANOVA on the rate of PHA produced anaerobically (Cmmol/L+min) versus the aerobic ORP maximum (mV)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
> 0 mV	11	0.81	0.07	0.00
-100 to 0 mV	15	0.93	0.06	0.00
< -100 mV	8	0.50	0.06	0.00

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.001	2	0.001	0.205	0.816	3.305
Within Groups	0.079	31	0.003			
Total	0.08	33				

Table A18: ANOVA on PHA consumed aerobically (Cmmol/L) versus the aerobic ORP maximum (mV)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
> 0 mV	11	102.29	9.30	63.39
-100 to 0 mV	15	223.52	14.90	46.45
< -100 mV	8	143.24	17.91	22.91

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	375.828	2	187.914	4.033	0.028	3.305
Within Groups	1444.476	31	46.596			
Total	1820.30	33				

Table A19: ANOVA on the rate of PHA consumed aerobically (Cmmol/L+min) versus the aerobic ORP maximum (mV)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
> 0 mV	11	0.41	0.04	0.00
-100 to 0 mV	15	1.29	0.09	0.00
< -100 mV	8	0.97	0.12	0.00

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.034	2	0.017	7.885	0.002	3.305
Within Groups	0.067	31	0.002			
Total	0.10	33				

Table A20: ANOVA on effluent PHA (Cmmol/L) versus the aerobic ORP maximum (mV)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
> 0 mV	11	30.41	2.76	2.04
-100 to 0 mV	15	88.29	5.89	11.25
< -100 mV	8	47.93	5.99	22.11

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	74.267	2	37.134	3.461	0.044	3.305
Within Groups	332.640	31	10.730			
Total	406.91	33				

Table A21: ANOVA on the anaerobic mass fraction versus the anaerobic ORP minimum (mV)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
-200 to -300 mV	9	1.503	0.167	0
-300 to -400 mV	5	1.833	0.3666	0.033367
-400 to -500 mV	17	8.667	0.509824	0.015585
< -500 mV	3	1.667	0.555667	0.037185

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.771772	3	0.257257	16.88045	1.31E-06	2.922277
Within Groups	0.457198	30	0.01524			
Total	1.22897	33				

Table A22: ANOVA on the carbon recovery ratio (Cmmol/Cmmol) versus anaerobic ORP minimum (mV)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
-200 to -300 mV	9	7.88	0.875556	0.497678
-300 to -400 mV	5	4.93	0.986	0.42333
-400 to -500 mV	17	14.91	0.877059	0.289147
< -500 mV	3	4.9	1.633333	2.731433

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.544626	3	0.514875	0.979847	0.415331	2.922277
Within Groups	15.76396	30	0.525465			
Total	17.30859	33				

Table A23: ANOVA on the anaerobic P:C ratio (Pmol/Cmol) versus anaerobic ORP minimum (mV)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
-200 to -300 mV	9	3.9089	0.434322	0.131726
-300 to -400 mV	5	2.2837	0.45674	0.05112
-400 to -500 mV	16	7.0606	0.441288	0.043843
< -500 mV	3	1.3259	0.441967	0.011679

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.001624	3	0.000541	0.008093	0.998976	2.93403
Within Groups	1.939293	29	0.066872			
Total	1.940916	32				

Appendix B: MLSS and MLVSS Data

Table B1: MLSS and MLVSS concentrations for each reactor at each anaerobic retention time

Metric	Units	Reactor	AN HRT	Average	SD	n
MLSS	mg/L	AO	1	1295	78	2
			2	1195	21	2
			3	1215	49	2
			4	1230	28	2
			All	1234	43	8
		WB	1	1590	71	2
			2	1465	64	2
			3	1465	21	2
			4	1705	92	2
			All	1556	115	8
MLVSS	mg/L	AO	1	1090	113	2
			2	1005	7	2
			3	1035	21	2
			4	1085	35	2
			All	1054	41	8
		WB	1	1365	64	2
			2	1265	35	2
			3	1315	35	2
			4	1365	78	2
			All	1328	48	8