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AN INVESTIGATION OF PHOSPHORUS REMOVAL BY PURPLE BACTERIA IN DAIRY WASTEWATER LAGOONS

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by

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ABSTRACT

The objective of this study was to determine whether the polyphosphate metabolism in purple photosynthetic bacteria (PPB) is similar to that of wastewater polyphosphate-accumulating organisms (PAOs) such as Accumulibacter. Dairy lagoon samples were studied in continuous and batch laboratory tests to evaluate whether cyclic light and dark conditions for PPB are similar to aerobic and anaerobic conditions for PAOs in domestic wastewater treatment systems. 16s rRNA gene amplicon sequencing indicated the presence of the purple sulfur bacterium Thiolamprovum (53.92%) in one lagoon source, and Thiodictyon (3.50%) in a second lagoon. Phosphorus contents were at least 0.7% higher than the typical 2% for microbial biomass, suggesting polyphosphate accumulation. Experimental measurements indicated phosphorus uptake and release under various conditions, although a link between phosphorus release and acetate uptake was not established. This work lays the groundwork for future research to determine specific conditions for PPB to improve phosphorus removal in lagoon systems.

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Chapter 1: Introduction

Phosphorus (P) contamination is identified as a substantial amount of the nutrient phosphorus within an environmentally sensitive receiving water leading to environmental disruption (Bunce et al., 2018). High amounts of phosphorus and other nutrients can lead to anoxia and eutrophication (Bunce et al., 2018). Purple photosynthetic bacteria (PPB) are anoxygenic, phototrophic bacteria that can utilize sunlight for growth (Madigan & Jung, 2009). This group of bacteria is composed of different strains that can live in varied conditions and some PPB are known to take up phosphorus and store it as polyphosphate (Madigan & Jung, 2009). These bacteria are commonly found in dairy lagoons, which are used to treat the high nutrient wastes arising from dairy operations (Dungan & Leytem, 2015).

An enhanced biological phosphorus removal system (EBPR) is a wastewater treatment configuration applied to activated sludge systems for the removal of phosphorus using phosphorus-accumulating organisms (PAOs) (Metcalf & Eddy, 2014). These PAOs uptake phosphorus by storing it as polyphosphate (Metcalf & Eddy, 2014). Removal of phosphorus in an EBPR system is driven by health and safety of the environment. It was hypothesized that PPB may utilize polyphosphate utilizing a metabolism similar to that found in EBPR systems. If true, this finding could provide insights to improve the operation of lagoon systems for phosphorus removal.

1.1 Research Objective

Previous research has evaluated PPB and polyphosphate accumulation and explored PPB and light and dark phases (Lai et al., 2017), but analysis of the effects of light and dark phases specific to polyphosphate accumulation and metabolism for improved phosphorus removal have not yet been performed. It was hypothesized that the naturally occurring

light/dark cycles inherent in dairy lagoon daily operation may be analogous to the aerobic/anaerobic conditions necessary for EBPR with polyphosphate providing an energy source in the light and anaerobic phases for carbon uptake and storage, and with growth of the stored carbon occurring in the dark and aerobic phases. The objective of the research presented in this thesis is to identify whether the polyphosphate metabolism in PPBs is similar to that of wastewater PAOs with respect to polyphosphate storage for carbon substrate uptake and cyclic characteristics, and if this can provide insight as to how to improve phosphorus removal in lagoon systems.

Chapter 2: Background and Literature Review

Purple photosynthetic bacteria (PPB) are a group of phototrophic bacteria consisting of two groups: the purple sulfur bacteria (PSB) and the purple non-sulfur bacteria (PNSB) (Madigan & Jung, 2009). PNSB and PSB are different in several aspects including that PNSB are mainly photoheterotrophs whereas PSB are mainly photoautotrophs but can be chemoautotrophs and are capable of limited photoheterotrophy depending on their adaptation to environmental conditions (Madigan & Jung, 2009). These taxonomic designations are discussed in more detail below. It was originally thought all bacteria within the PSB taxonomic group could utilize sulfide and the PNSB could not, but this was disproven in 1972 when a study found that some PNSB strains could use the dissimilatory sulfur cycle (Hansen & Gemerden et al., 1972). Although shown to be inaccurate, these taxonomic labels have persisted. More studies into the use of sulfide by both PNSB and PSB found that the difference between the two was that PSB converted sulfide into elemental sulfur that was stored intracellularly whereas any elemental sulfur PNSB produced remained outside the cell (Madigan & Jung, 2009).

2.1 History and Taxonomy

PPB have been studied for over a century, with one of the oldest publications on purple bacteria dating back to 1897 (Ewart, 1897). Around this time, research was beginning on the relations of bacteria to oxygen and purple and green anaerobic bacteria that can thrive without the existence of oxygen. Over time, photosynthetic apparatuses were developed and further aided in the research on the evolution of bacteria which also included further research on PPB.

PSB are a large group of gram negative, phototrophic bacteria within the class Gammaproteobacteria (Imhoff, 2017). Most strains of PNSB are Alphaproteobacteria but a

few strains of PNSB are Betaproteobacteria (Imhoff, 2017). PPB require anoxic conditions for competitive success in phototrophic growth and are therefore found in anaerobic bodies of water such as stratified lakes, ponds, estuaries, dairy lagoons, swine lagoons, and other H_2S containing aquatic environments (Dungan & Leytem, 2015; Madigan and Jung, 2009). However, some PSB have been found to survive and grow in the presence of molecular oxygen (Diao et al., 2018). A typical indication of PPB in these locations is pigmentation of the waters. PPB are pigmented with bacteriochlorophyll (BChl) *a* or *b* (Madigan and Jung, 2009). BChl *a*-containing species are typically pigmented purple, purple-red, purple-violet, red, orange, or yellow brown (Figure 1). BChl *b*-containing species are commonly green or yellow in pigment (Madigan and Jung, 2009).

Figure 1. Dairy lagoon sample containing purple pigmentation. Image by author.

2.2 PPB Metabolism

PPB are thought to operate using a diverse set of metabolisms depending on the environmental conditions such as oxygen levels, available substrates, and light (Madigan & Jung, 2009). Autotrophic PPB microorganisms can use substances such as H_2, H_2S, S_0, $S_2O_3^{2-}$, Fe^{2+}, and NO^{2-} (Ehrenreich & Widdel, 1994; Griffin et al., 2007; Madigan et al., 2017; Koku

et al., 2002) as electron donors. Heterotrophic PPB can utilize a variety of organic compounds as electron donors such as ethanol as well as organic acids including acetate (Ac), malate, pyruvate, and succinate (Madigan & Jung, 2009; Nairn et al., 2020). These bacteria also offer a plentitude of molecular diversity because of their variability of metabolism.

PSB are strong photoautotrophs but are limited in photoheterotrophy and are limited for dark growth and metabolism. In contrast to the related PSB, PNSB are strong photoheterotrophs limited in photoautotrophy but can facilitate growth and dark metabolism in which these organisms can survive and grow during dark or night conditions (Madigan & Jung, 2009). PNSB are therefore able to uptake nutrients in dark conditions and use light for energy during the light cycle. Although most PPB that utilize dark metabolism are PNSB, it is possible for some PSB to also utilize dark metabolism (Madigan & Jung, 2009). PPB require anoxic conditions for photoheterotrophy because pigment synthesis in these organisms is repressed by molecular oxygen (Cohen-Bazire et al., 1957). The anoxic and light conditions therefore permit the bacteria to synthesize and continue in metabolic growth. Using this information, scientists often visit aquatic ecosystems that provide anoxic light conditions to cultivate and gather samples of PPB. Yeast extract has been found to be a common addition to PPB media to encourage growth of PPB (specifically but not limited to PNSB) because it is a source of B-vitamins for the bacteria. Yeast extract also stimulates the growth of PPB because of the assortment of organic compounds that fuel photoheterotrophic growth (Biebl & Pfennig, 1981; Madigan & Jung, 2009). Yeast extract has been used in PPB media in various studies and experiments and thus has been proven to encourage the metabolism and growth of PNSB and potential growth of PSB.

The metabolism of PNSB also differs from that of PSB in that PSB utilizes and oxidizes sulfide whereas PNSB does not need the sulfur for cell growth or metabolic change (Frigaard, 2016). Elemental sulfur produced by PSB is stored intracellularly whereas elemental sulfur formed by PNSB is stored extracellularly (Frigaard, 2016). This is a vital distinction in the metabolism of PNSB and PSB. The differentiation of PSB and PNSB is noticeable because of the location of the visible microscopic globules of elemental sulfur. PSB and PNSB can be differentiated by the location of the visible microscopic globules of elemental sulfur produced. Intracellular sulfur globules can be seen in PSB photomicrographs (Madigan & Jung, 2009). If sulfide is oxidized by PNSB, any of the produced elemental sulfur remains outside of the cell (Madigan & Jung, 2009). The metabolic differences between PSB and PNSB are highlighted in Table 1 below.

Table 1. *Purple Sulfur Bacteria and Purple Non-Sulfur Bacteria Metabolisms*

Purple Sulfur Bacteria (PSB)	Purple Non-Sulfur Bacteria (PNSB)
Strong photoautotroph Limited photoheterotrophy	Limited photoautotrophy Strong photoheterotrophy
Limited dark metabolism	Strong dark metabolism
Anoxic, light conditions for optimal growth	Anoxic, light conditions for optimal growth
Oxidizes sulfide for growth	Sulfide is not necessary for growth
Elemental sulfur is produced	Elemental sulfur can potentially be produced
Elemental sulfur is stored intracellularly	If produced, elemental sulfur is stored extracellularly

2.3 PPB and Polyphosphate Metabolisms

Recent research concerning PPB metabolism has led to the speculation that polyphosphate accumulation may occur during both light and dark phases (Lai et al., 2017). Polyphosphate, a linear biopolymer, is composed of three to hundreds of phosphorus residues and its metabolism can be used by microorganisms to store excess energy from light using the key enzyme polyphosphate kinase (PPK) for the microbial synthesis of intracellular

polyphosphate (Lai et al., 2017). PPK transfers the terminal phosphorus of adenosine triphosphate (ATP) to polyphosphate (Cao et al., 2017). Intracellular polyphosphate within PPB cells store energy so that it may be utilized as an extra energy source (Lai et al., 2017). Polyphosphate has also been shown in recent experimental studies to add to bacterial durability with respect to environmental changes and its potential for polyphosphate in a bacterium to positively correlate with its environmental durability (Wang et al., 2018) which could lead to added reasoning on the resilience of PPB.

In one study of PPB, samples were retrieved from an activated sludge plant that treated domestic wastewater to analyze the polyphosphate metabolism during light and dark conditions (Lai et al., 2017). PPB (PNSB) were isolated accumulated polyphosphate during stationary growth. In this study, it was found that the microorganisms grew slower and produced less energy under a dark phase as opposed to light illumination, but the microorganisms utilized the previously stored polyphosphate for energy production during dark conditions. Intracellular polyphosphate accumulation appeared to function as energy storage in this study (Lai et al., 2017).

Most studies conducted on PPB have used samples from wastewater, lakes, or lagoons and isolated the mixed cultures for strains of PSB or PNSB, although pure cultures have also been used to further identify and analyze specific features as well as metabolic features of a certain strain. One study analyzed PNSB diversity and its potential for phosphorus accumulation under different cultivation conditions (Liang et al., 2010). This study focused on four pure cultures known as *Rhodopseudomonas palustris* CC1, CC7, G11, and GE1 which were based on their differences within the bacteria's *puf*M gene. This is the bacteria's structural gene coding for the photosynthetic reaction center which facilitates the initial electron transfer

process of photosynthesis. Of all the cultures tested, these four cultures illustrated higher internal phosphorus content. A variety of conditions were implemented for studying PAOs such as variability of illumination, aerobic versus anaerobic, and photoheterotrophic conditions versus chemoheterotrophic conditions (see discussion of PAOs in the next section). PAOs are capable of phosphorus uptake in their cells for storage by converting the phosphorus to intracellular polyphosphate (Oehmen et al., 2007; Vieira et al., 2018). For identification, DNA samples were extracted then polymerase chain reaction denaturing gradient gel electrophoresis and cloning methods were utilized based on the characteristic *puf*M genes (Liang et al., 2010). Further analysis was done using a fluorescent dye to note the intracellular polyphosphate granules and it was found that during illuminated anaerobic incubation, among all isolated strains, the maximum level of phosphorus accumulation was 13.8% by the isolated PNSB GE1 under photoheterotrophic growth conditions (Liang et al., 2010). These findings correlate with the study conducted by Lai in 2017 in that light encourages optimal growth for polyphosphate and phosphorus accumulation. Previous studies on PPB and polyphosphate accumulation have ranged in a variety of fields, however a common focus is wastewater treatment.

2.4 Enhanced Biological Phosphorus Removal from Wastewater and Polyphosphate Accumulating Organisms

EBPR is a wastewater treatment configuration applied to activated sludge for the removal of phosphorus (Metcalf & Eddy, 2014). EBPR in wastewater treatment aims to remove phosphorus from wastewater by utilizing a novel metabolism conducted by PAOs (Schuler & Jenkins, 2003). PAOs require anaerobic and aerobic conditions in which the PAOs anaerobically utilize energy during the aerobic phase. During the anaerobic phase, PAOs take up volatile fatty acids (VFAs), such as acetate, and store them as polyhydroxyalkanoates

(PHAs), such as polyhydroxybutyrate (PHB) (Oehmen et al., 2007). ATP required for this process is provided by stored polyphosphate and reducing equivalents are provided by stored glycogen (Oehmen et al., 2007). During the aerobic phase, stored PHA is metabolized, and some glycogen is produced. The energy from PHA oxidation is used to form polyphosphate bonds in cell storage so that soluble orthophosphate is utilized by the polyphosphates (Metcalf & Eddy, 2014). The new biomass with high polyphosphate storage accounts for phosphorus removal. Stored phosphorus is then removed from the biotreatment reactor for disposal with the waste sludge.

A common bacterium typically found in wastewater treatment plants that perform EBPR is *Candidatus Accumulibacter phosphatis* (*Accumulibacter*). *Accumulibacter* is part of an unclassified group of Betaproteobacteria in which it is the only member of the group. It is a well-known and vital PAO in EBPR systems because the bacterial group can accumulate large amounts of intracellular polyphosphate and thus contribute to phosphorus removal in wastewater treatment (He & McMahon, 2011). *Accumulibacter* utilizes a cyclic metabolism in which polyphosphate is used anaerobically as an energy source to take up and store acetate for later aerobic processing for growth. *Accumulibacter* is closely related to the PNSB group. Similarities can be drawn between *Accumulibacter* and PPB in that both contain polyphosphates and the potential for phosphorus uptake.

Various studies have focused on improved conditions for cultivation and growth of PAOs as opposed to glycogen-accumulating organisms (GAOs) (Tu & Schuler, 2013). GAOs have been suggested as a potential reason for EBPR failure because of competition for VFAs (Oehmen et al., 2007). PAOs utilize a similar metabolism to PAOs, but do not accumulate polyphosphate. Previous research has suggested the low pH may favor GAOs over PAOs (Tu

& Schuler, 2013), but low acetate concentrations (achieved through a slow rate of acetate addition) can counteract this phenomenon (Tu & Schuler, 2013).

Each of these findings add to the study of polyphosphate metabolism related to the PPB along with the close relation of PPBs and *Accumulibacter*. By utilizing previous research and findings, a more conclusive experimentation can be derived, and improved results can be achieved. Further analysis of PPB and polyphosphate accumulation to improve enhanced biological phosphorus removal can be accounted for in terms of wastewater treatment efficiency.

2.5 Hypothesized PPB Metabolism with Analogies to EBPR Metabolism

It was hypothesized that naturally occurring light and dark conditions experienced by PPB may provide analogous conditions to the aerobic and anaerobic conditions required for PAO cultivation in EBPR systems (Figure 2). Variations in light and dark conditions can be used to further analyze PPB and polyphosphate growth. When PPB (specifically PNSB) is incubated under photoheterotrophic growth conditions, the PNSB obtained energy (ATP) from photosynthesis by a light-driven photosystem (Madigan & Jung, 2009), which could be used to synthesize polyphosphate, similar to aerobic polyphosphate accumulation by wastewater PAOs.

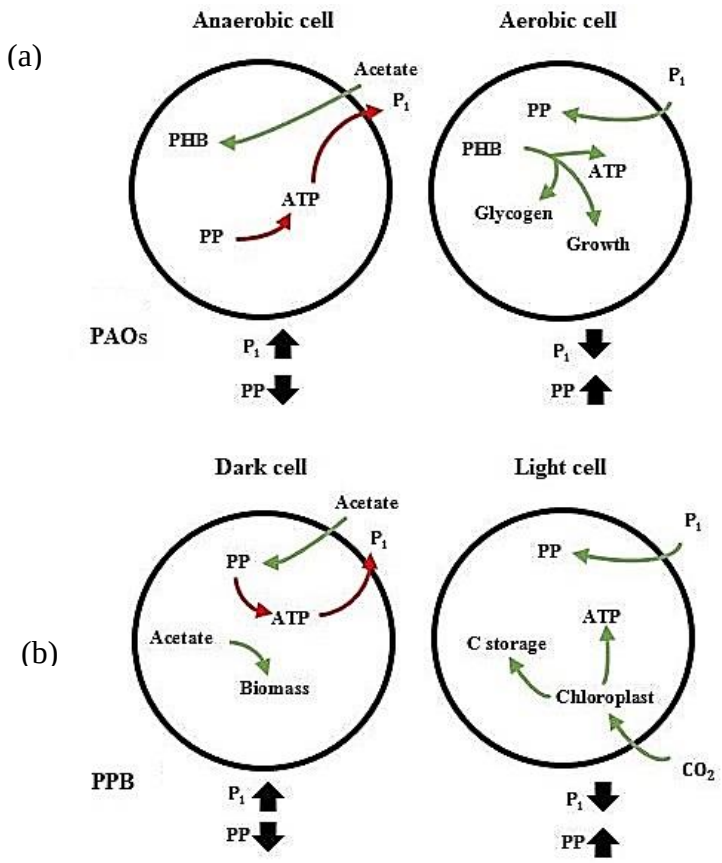

Figure 2. (a) EBPR metabolism with anaerobic and aerobic phases. (b) Hypothesized PPB metabolism showing analogies between anaerobic and dark phases, and aerobic and light phases. Image by author.

Compared to wastewater PAOs, PPB (specifically PNSB) are also known to uptake phosphorus in aerobic conditions and store glycogen and PHB under anaerobic conditions (Merugu et al., 2012). PPB take up acetate and store it as PHB and glycogen, while also synthesizing ATP (Merugu et al., 2012). This metabolism has been documented for PNSB; however, it is not verified for PSB although PSB are capable of this metabolism. Many of the same organic compounds assimilated by PNSB during the light phase, such as pyruvate, can also be used as electron donors and carbon sources for dark respiratory growth (Madigan & Jung, 2009). The hypothesis suggests phosphorus taken up during the aerobic conditions (light phase) can then be stored in the cell and utilized later during the anaerobic dark phase when light is no longer a source of energy. The dark phase of illumination for PPB is hypothesized

to be similar to the EBPR anaerobic phase with the same general system of acetate uptake and phosphorus release. The light phase of the PPB metabolism is speculated to be comparable to the aerobic phase of polyphosphate metabolism in which the phosphorus is being taken up and utilized for polyphosphate accumulation and growth.

Further investigation and analyses of the PPB can provide more insight into the polyphosphate metabolism, growth, and phosphorus uptake of these bacteria under varied conditions such as light and dark conditions. This study aims to examine some of those conditions as a related analogy to phosphorus uptake and removal in enhanced biological phosphorus removal in wastewater treatment systems.

2.6 Objective and Experimental Approach

It was hypothesized that cyclic light and dark conditions have similar roles for PPB in lagoon systems as aerobic and anaerobic conditions do for wastewater PAOs in domestic wastewater treatment systems. The objective of this study was to test this hypothesis using laboratory scale reactors operated under controlled conditions.

The experimental approach was to obtain samples from local dairy lagoons that had characteristics of PPB cultures and perform laboratory experiments under varied light and dark conditions and varied amounts of acetate addition. Along with the variation of conditions, measurements of VFAs, phosphorus, solids, and genetic characterization using 16s rRNA gene amplicon sequencing were conducted to assess whether polyphosphate may serve as an ATP source for acetate uptake and storage, analogous to its function in the anaerobic phase of EBPR systems. The potential utility of this research includes providing a better fundamental understanding of PPB metabolism and providing a basis for improved design and operation of dairy lagoons to better protect the environment.

Chapter 3: Methodology

One sequencing batch and multiple batch laboratory scale experiments were conducted over a 2-year period. Two locations of dairy lagoons were utilized for samples because of sampling constraints: Albuquerque, NM (Lagoon 1) and Clovis, NM (Lagoon 2). The sequencing batch experiment utilized sample from Lagoon 1 only. Samples were retrieved using a 1.0 L sample collecting device that was thoroughly cleaned before and after each sampling event. Samples were then transferred from the sample collecting device to the 1.0 L and 500 mL sample bottles. The sample bottles were then placed in a cooler and transferred to the lab where the sample bottles were refrigerated until the given experiment began.

3.1 Sequencing Batch Flow Reactor Experiment

The objective of the sequencing batch flow experiment was to analyze PPB growth and phosphorus removal over time in full light versus split light and dark conditions. The experiment included the operation of two 1.0 L bioreactors over 222 days.

3.1.1 Sequencing Batch Reactor Experimental System

Reactor 1 (R1) was continuously illuminated and was considered a control. Reactor 2 (R2) was operated on a cycle with 12-hour illumination followed by 12-hour darkness each day (Figure 3). The reactors were clear acrylic cylinders (15.0 inches in height and 12.5 inches in diameter) made with a square sheet of acrylic glued to the bottom (Port Plastics, Albuquerque, NM). Influent and effluent tubing (Masterflex Norprene L/S 15) was taped to the inside of the walls. Cole Parmer Masterflex pumps (Model 7520-25, Vernon Hills, IL) with Masterflex pump heads (Model 7016-16, Vernon Hills, IL) were used for influent and effluent and were controlled with a Chrontrol program controller (XT Table Top, Chrontrol Corporation, San Diego, CA). Air stones were placed at the bottom of each reactor for mixing.

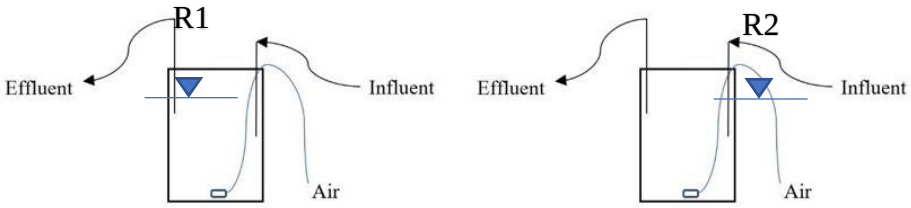

Figure 3. General reactor configurations for the photobioreactors R1 and R2. Image by author.

The liquid volume of each reactor was 1.0 L. The reactors were operating on 24-hour cycle as described below. The effluent line was secured to the wall of the reactor 250 mL below the liquid surface to provide 250 mL of effluent each cycle (Figure 4). The influent line outlet was placed 500 mL below the liquid surface to prevent feed from staying near the top of the reactor and to allow for the influent feed to be easily dispersed to bottom of the reactor where the biomass settled. The effluent line in each reactor was connected to its effluent pump and led to a 4.0 L effluent container. The influent line was set to the middle of the reactor to allow for a uniform distribution of feed within each reactor during the 12-hour feed time in which there was no mixing. Mixing by air stones was added twice per day to ensure a well-mixed reactor. The influent line was connected to the feed pump and to the feed container (10.0 L plastic Nalgene carboy). The reactors were placed in separate opaque plastic buckets that were lined with aluminum foil. The container lids were also lined with foil and had small holes for the tubing lines. A vertical 12.0-inch LED light strip (Build My LED, Austin, TX) was placed in each bucket. The LED light had a photon flux of 160 μmol/s. The light sources were placed vertically to be parallel to the reactors.

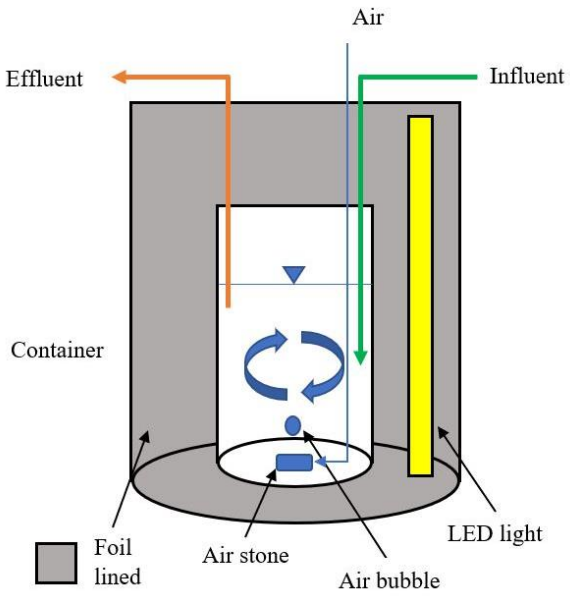

Figure 4. Continuous test system configuration, including the foil lined container, air stone, air bubble, LED light source, and the effluent, influent and air lines. Image by author.

3.1.2 Sequencing Batch Reactor Synthetic Feed

The synthetic feed was an adapted medium from Imhoff and Trüper. The original recipe was adapted to utilize the chemicals readily available in our lab. The original "AT" medium recipe called for KH_2PO_4, $MgCl_2*6H_2O$, $CaCl_2*2H_2O$, Na_2SO_4, NH_4Cl, $NaHCO_3$, NaCl, trace element solution "SLA", vitamin solution "VA", and sodium acetate or another carbon source (Dworkin et al., 2006; Imhoff & Trüper, 1976; Imhoff et al., 1982; Imhoff, 1988). Instead of $MgCl_2*6H_2O$, $MgSO_4$ was used, and the amount of this chemical was adjusted to provide equivalent magnesium. For acetate addition, 200 mg/L of acetate was used rather than 1.0 g/L and was made from $CH_3COONa*3H_2O$. The recipe listed 1.0 g/L of KH_2PO_4, however this was adapted to 0.25 g/L for a lower influent phosphorus concentration of 57.0 mg P/L. The recipe was also adapted to include 500 mg/L of yeast extract to the medium based on yeast extract historically encouraging PPB growth (Biebl & Pfennig, 1981; Madigan & Jung, 2009).

The yeast extract was autoclaved before use to prevent growth in the feed container. Table 2 includes the components of the media that were added to 1.0 L of distilled water.

Both the trace element solution and the vitamin solution were adapted as well. The original trace element solution "SLA" called for $FeCl_2*4H_2O$, $CoCl_2*6H_2O$, $NiCl_2*6H_2O$, $CuCl_2*2H_2O$, $MnCl_2*4H_2O$, $ZnCl_2$, H_3BO_3, and $Na_2MoO_4*2H_2O$. Of these, three were adapted because of availability of components: $FeCl_2*4H_2O$ was adapted to $FeSO_4*7H_2O$, $CuCl_2*2H_2O$ was adapted to $CuSO_4*5H_2O$, and $ZnCl_2$ was adapted to $ZnSO_4*7H_2O$. The original recipe for the vitamin solution "VA" listed biotin, niacin, thiamine dichloride, *p*-aminobenzoic acid, pyridoxolium hydrochloride, ca-panthothenate, and vitamin B12. For the experiment, only biotin, pyridoxolium hydrochloride, and vitamin B12 were utilized in the creation of the vitamin solution because of component availability. Tables 3 and 4 list the components of the adapted trace element solution and the adapted vitamin solution. All components were mixed in a 2.0 L beaker on a magnetic stirrer mixer with a stir bar. After being well mixed for 10 minutes, the feed mixture was transferred to a 10.0 L clear plastic Nalgene carboy. The feed was mixed and made every 3 days to ensure that the feed was relatively fresh. The pH was adjusted to 7.0 by dilution of hydrochloric acid after all components of the media recipe were added. The media recipe allowed for dilution of sodium hydroxide; however, hydrochloric acid was chosen because of availability in the lab. Over the course of a 12-hour dark phase, the feed was slowly added to the reactors to be aligned with the assumption that this will promote PAO growth over the GAO growth. Only 250 mL of feed was added to each of the reactors over the 12-hour dark phase. Feed pH was 6.9.

Table 2. *Components of Modified Imhoff and Trüper Medium*

Component	Concentration added in 1.0 L feed (g/L)
KH_2PO_4	0.250
$MgSO_4$	0.359
$CaCl_2*2H_2O$	0.100
Na_2SO_4	0.700
NH_4Cl	1.000
$NaHCO_3$	3.000
NaCl	1.000
$CH_3COONa*3H_2O$	0.461
Yeast extract	0.500
1 mL of trace element solution "SLA"	1.0 (mL)
1 mL of vitamin solution "VA"	1.0 (mL)

Table 3. *Components of Modified Imhoff and Trüper Vitamin Solution*

Component	Concentration added in 100 mL distilled water (g/L)
Biotin	0.010
Pyridoxine hydrochloride	0.010
Vitamin B12	0.005

Table 4. *Components of Modified Imhoff and Trüper Trace Element Solution*

Component	Concentration added in 1.0 L distilled water (g/L)
$FeSO_4*7H_2O$	1.800
$CoCl_2*6H_2O$	0.250
$NiCl_2*6H_2O$	0.010
CuSO4*5H_2O	0.010
$MnCl_2*4H_2O$	0.070
$ZnSO_4*7H_2O$	0.100
H_3BO_3	0.500
$Na_2MoO_4*2H_2O$	0.030

3.1.3 Sequencing Batch Reactor Inoculation

A total of 3.0 L of liquid inoculum was collected from a dairy lagoon in Albuquerque, New Mexico (Lagoon 1) and was placed in refrigeration until use for 2 days. The inoculum was stirred, and 500 mL was added to each reactor. Each reactor was placed in a bucket container and the lights were turned on. After 1 hour, 500 mL of feed was added to each reactor,

for a total volume of 1.0 L, and stirred for 5 minutes. Feed concentrations included 200 mg Ac/L and 57.0 mg P/L, respectively.

3.1.4 Sequencing Batch Reactor Operation

The schedule of operation for each reactor is shown in Table 5. The primary difference between the reactors was that the R1 light was on continuously, whereas the R2 light was on from 7:00 a.m. to 7:00 p.m. only. Aeration was turned on at 5:00 a.m. and 5:00 p.m. each for 30 minutes to provide intermittent mixing for the feed to be evenly distributed. The effluent pump removed 250 mL from each reactor each day from 6:00 p.m. to 6:30 p.m. to provide a hydraulic residence time 4 days. The influent feed pump was on from 7:00 p.m. to 7:00 a.m. for each reactor at a rate of 0.348 mL/minute. For R2, feed time was during the 12-hour dark phase, whereas R1 was continuously illuminated.

Table 5. *Sequencing Batch Reactor Operation*

Phase	Start Time	Air	Feed	Draw	R1 Light	R2 Light
Aeration on (mixing)	5:00 a.m.	ON	ON	OFF	ON	OFF
Aeration off	5:30 a.m.	OFF	ON	OFF	ON	OFF
Feed pump off	7:00 a.m.	OFF	OFF	OFF	ON	ON
Aeration on (mixing)	5:00 p.m.	ON	OFF	OFF	ON	ON
Aeration off	5:30 p.m.	OFF	OFF	OFF	ON	ON
Effluent pump on	6:00 p.m.	OFF	OFF	ON	ON	ON
Effluent pump off	6:30 p.m.	OFF	OFF	OFF	ON	ON
Feed pump on	7:00 p.m.	OFF	ON	OFF	ON	OFF

Samples were filtered to 0.45 μm (Pall Corporation Acrodisc Syringe Filter) and analyzed using ion chromatography for acetate and phosphorus Hach kits (described in section 3.3) for phosphorus. Triplicate samples were taken for total suspended solids (TSS) and volatile suspended solids (VSS) at the start of the experiment, as described in Section 3.3.

3.2 Batch Test Experiment

Multiple batch tests were conducted to evaluate phosphorus uptake or release with changes in acetate addition, light phases, and dark phases. These tests are described in more detail below.

3.2.1 Batch Test Experimental Systems

A total of four batch tests were conducted. All batch tests included in this thesis were performed in identical 500 mL Erlenmeyer flasks. A tube was inserted into each of the reactors for bubbling of nitrogen at 6.5 L/minute (monitored by VWR Acrylic Flowmeters) to provide anoxic conditions. A piece of Parafilm was placed over reactor to reduce oxygen mass transfer.

In Batch Tests 1, 2, and 3 reactors were placed in a dark box (30-inch by 12-inch) lined with aluminum foil inside which allowed for more uniform distribution of light within each reactor. The top of the box was removable for sampling. Small incisions at the top of the box were made for the nitrogen tubing lines. A 24-inch LED light strip was used as a light source and was placed horizontally along the lower middle height of the box (Figure 5). Batch Test 4 included three of these dark boxes. R1 was placed in one box, R2 in a second box, and Reactor 3 (R3) and Reactor 4 (R4) in a third box to account for one in full light conditions, one in full dark conditions, and one for light and dark conditions.

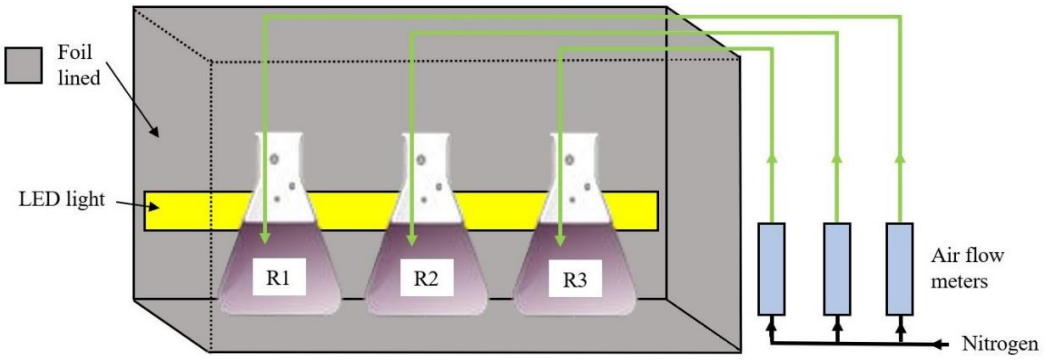

Figure 5. Batch test system configuration. Image by author.

3.2.2 Batch Test Source of Biomass

Batch Tests 1 and 2 utilized samples from Clovis, NM (Lagoon 2). Batch Tests 3 and 4 used samples from Albuquerque, NM (Lagoon 1) because of sampling constraints. For each test, a total 4.0 L of sample was collected and placed in 4°C refrigeration until use (2 days for Batch 1, 14 days for Batch 2, 2 days for Batch 3, and 2 days for Batch 4). At the start of the batch test, the sample was taken out of refrigeration and allowed 10 minutes to warm to room temperature. It was then stirred, and 400 mL of sample was added to each reactor.

Acetate was added as 0.001M stock solution (60 mg/L as CH_3COO). The stock solution was made by dissolving 13.6 g/L of EMD sodium acetate trihydrate ($CH_3COONa*3H_2O$) in 1.0 L of distilled water. For all tests, pH 5.2 acetate was added 1 hour after the experiment began (t = 0). All reactors in Batch Test 1 had an addition of 1.7 mL of stock acetate solution to equal 100 mg/L of acetate per reactor. R2 and R3 of Batch Test 3, all reactors of Batch Test 2, and R4 of Batch Test 4 had a 1.7 mL addition of 1:10 diluted acetate solution for a total of 10.0 mg/L of acetate per reactor. In both Batch Tests 2 and 3, R3 had a five-step addition of acetate addition every 15 minutes for 1 hour (t = 0, 15, 30, 45, and 60) to equal the same amount of acetate addition as R2 (10.0 mg/L of acetate). R1 in Batch Test 3 and R1, R2, and R3 in Batch Test 4 were used as controls with no added acetate. Acetate additions are summarized in Table 7 in Section 3.2.3.

3.2.3 Batch Test Reactor Operation

For each batch test, the dairy samples were stored at 4°C between sample collection and the experiment. The samples were taken out of refrigeration, stirred, and 500 mL of sample was added to each reactor. The reactors were then placed in the dark box and the lights were turned on. N_2 was added into each reactor throughout the experiment for Batch Tests 1 and 2.

Batch Tests 3 and 4 had initially low dissolved oxygen (DO) values (0.01 ± 0.02 mg/L) so N_2 was not added. Batch test parameters and operation are summarized in Table 6 and details for each batch test are described below.

Table 6. *Batch Test Operation*

Batch Test	Duration (hours)	N_2	Reactor	Acetate Addition (mg/L)	Light (time in minutes)				
					t= -60 to t=0	t=0 to t=60	t=60 to t=120	t=120 to t=180	t=180 to t=240
1	3	ON	R1	100.0	ON	ON	OFF	-	-
			R2	100.0	ON	ON	OFF	-	-
			R3	100.0	ON	ON	OFF	-	-
2	5	ON	R1	10.0	ON	ON	OFF	OFF	OFF
			R2	10.0	ON	ON	OFF	OFF	OFF
			R3	10.0	ON	ON	OFF	OFF	OFF
3	4	OFF	R1	0.00	ON	OFF	OFF	OFF	-
			R2	10.0	ON	OFF	OFF	OFF	-
			R3	10.0	ON	OFF	OFF	OFF	-
4	4	OFF	R1	0.00	ON	ON	ON	ON	-
			R2	0.00	OFF	OFF	OFF	OFF	-
			R3	0.00	ON	ON	OFF	OFF	-
			R4	10.0	ON	ON	OFF	OFF	-

Batch Test 1 of a test concerning the effects of light and dark conditions with addition of acetate on phosphorus uptake. The objective of this test was to determine whether acetate addition under anaerobic conditions to dairy lagoon samples led to phosphorus release, which could indicate degradation of polyphosphate for acetate uptake and storage in EBPR. Reactor containers were 500 mL Erlenmeyer flasks and were placed in a box container for 2 hours of light, proceeded by 1 hour of dark conditions. N_2 was added into the reactors. After 1 hour from the start of the experiment (t = 0), 100 mg/L of acetate was added to each reactor. The reactors were run as triplicates. The biomass was retrieved from a dairy lagoon in Clovis, NM (Lagoon 2) 2 days before the experiment began and was placed in 4°C refrigeration until use.

Batch Test 2 consisted of a test on the effects of dilution of acetate addition. The objective of this test was to identify potential phosphorus level changes with a lower acetate addition of 10.0 mg/L. After 1 hour from the start of the experiment (t = 0), 10.0 mg/L of

acetate was added into each reactor. The flasks, box containment, and N_2 addition were identical to Batch Test 1. The reactors were in light conditions for 2 hours and then 3 hours in dark conditions. The reactors were run as triplicates. The biomass used was retrieved from a dairy lagoon in Clovis, NM (Lagoon 2) 14 days before the experiment began and was placed in 4°C refrigeration until use.

Batch Test 3 consisted of testing the effect of slowing acetate addition for potential changes in phosphorus levels. The objective of this test was to continue to investigate whether PSB store and utilize polyphosphate for energy (ATP) to be used to take up and store acetate in dark, aerobic conditions, analogous to EBPR, with two reactors operated with a single large pulse addition of 10.0 mg/L acetate versus multiple step additions of the same total amount of acetate (2.0 mg/L added five times to equal 10.0 mg/L). The flasks used were identical to Batch Test 2. N_2 was not used during this test as DO levels were initially low and remained low. The light was on for 1 hour and then turned off for 3 hours of dark conditions. Reactor 1 (R1) had no added acetate. Reactor 2 (R2) had a pulse addition of 10.0 mg/L acetate 1 hour after the experiment began (t = 0). Acetate was added in Reactor 3 (R3) every 15 minutes in a five-step addition (t = 0, 15, 30, 45, and 60) to equal the same amount of acetate addition as R2 (10.0 mg/L of acetate). R3 acetate addition began 1 hour after the start of the experiment (t = 0). The biomass used was retrieved from a dairy lagoon in Albuquerque, NM (Lagoon 1) 2 days before the start of the experiment and was placed in 4°C refrigeration until use.

Batch Test 4 consisted of a test with the objective of continuing to investigate whether PSB store and utilize polyphosphate for energy (ATP) to be used to take up and store acetate in dark, aerobic conditions, analogous to EBPR, with differing light/dark conditions and multiple reactors with no acetate addition as experimental controls. N_2 was not added to the

reactors as DO levels were consistently low during the experiment. R1 was the light control with light throughout the experiment. R2 was the dark control with dark conditions during the entirety of the experiment. R3 was a light and dark control. The light in R3 was on for 2 hours and off for the last 2 hours. Reactor 4 (R4) had identical light and dark conditions to R3 but with the addition of acetate. An hour after the experiment began (t = 0), 10.0 mg/L of acetate was added to R4. The biomass was retrieved from a dairy lagoon in Albuquerque, NM (Lagoon 1) 2 days before the experiment started and was placed in 4°C refrigeration until use.

All reactors were stirred with their respective stir rod before sampling. Samples were taken at times t = -60, 0 (before acetate addition), 15, 30, 45, 60, 120, and 180. Batch Test 1 did not have a t = 180 sample and Batch Test 2 had an added t = 240 sample. Batch Test 3 had additional samples taken at t = -45 and t = -30. Triplicate samples were taken from each reactor at the start of the experiment t = -60, t = 0, and t = 120 minutes. Triplicate sampling was taken for all samples in Batch Test 4. R2 and R3 of Batch Test 3 and all reactors of Batch Test 1 had a 1.7 mL acetate addition for a total of 100 mg/L of acetate per reactor at t = 0. R2 and R3 of Batch Test 3, all reactors of Batch Test 2, and R4 of Batch Test 4 had a 1.7 mL addition of 1:10 diluted acetate solution for a total of 10.0 mg/L of acetate per reactor. R3 of Batch Test 3 had 0.34 mL (2.0 mg Ac/L) of acetate added at t = 0, 15, 30, 45, and 60. Sampling times and acetate additions are detailed in Table 7.

Table 7. *Batch Test Sampling and Acetate Addition*

Batch Test	Duration (hours)	Reactor	Sampling Time (minutes)	Acetate (mg/L)	Type of Addition	Time of Acetate Addition
1	3	R1	t= -60, 0, 15, 30, 45, 60, 120	100.0	Pulse	t=0
		R2	t= -60, 0, 15, 30, 45, 60, 120	100.0	Pulse	t=0
		R3	t= -60, 0, 15, 30, 45, 60, 120	100.0	Pulse	t=0
2	4	R1	t= -60, 0, 15, 30, 45, 60, 120, 180, 240	10.0	Pulse	t=0
		R2	t= -60, 0, 15, 30, 45, 60, 120, 180, 240	10.0	Pulse	t=0
		R3	t= -60, 0, 15, 30, 45, 60, 120, 180, 240	10.0	Pulse	t=0
3	4	R1	t= -60, 0, 15, 30, 45, 60, 120, 180, 240	0.00	None	-
		R2	t= -60, 0, 15, 30, 45, 60, 120, 180, 240	10.0	Pulse	t=0
		R3	t= -60, 0, 15, 30, 45, 60, 120, 180, 240	10.0	Step	2.0 mg Ac/L (at t=0, 15, 30, 45, 60)
4	4	R1	t= -60, 0, 15, 30, 45, 60, 120, 180	0.00	None	-
		R2	t= -60, 0, 15, 30, 45, 60, 120, 180	0.00	None	-
		R3	t= -60, 0, 15, 30, 45, 60, 120, 180	0.00	None	-
		R4	t= -60, 0, 15, 30, 45, 60, 120, 180	10.0	Pulse	t=0

After each sampling, the samples were filtered to 0.45 μm (Pall Corporation Acrodisc Syringe Filter) and analyzed using gas chromatography for acetate measurements and phosphorus Hach kits (described in section 3.3) for phosphorus levels and uptake/release. An initial 1.5 mL sample was taken in triplicate to be centrifuged and stored in the freezer for sequencing. Triplicate samples were also taken for TSS and VSS at the start of the experiment. The process is described in more detail in section 3.3. A few drops of the sample were placed onto two microscope slides in which one was to be for live examination and the other would be permitted to dry and would be utilized for Neisser staining (for polyphosphate indication).

3.3 Analytical Methods

pH and DO measurements were measured in the reactors for each time a sample was taken, and three analytical methods were used for testing measurements. The pH meter used

for the sequencing batch experiment was the Oakton DO6+ Dissolved Oxygen Meter and probe. The pH meter used for the batch tests was a Thermo Scientific STAR A111 pH Benchtop Meter and probe. The DO meter used was a Thermo Scientific Orion Star A323 Portable Dissolved Oxygen Meter and probe. Dissolved phosphorus was measured using Hach kits (Hach Kit 2767345 Reactive Phosphorus, High Range, Method 8114, wavelength of 420 nm) and a Hach DR 2700 spectrophotometer according to the manufacturer's instructions. Total phosphorus was measured using Hach Kit 2767345 Total Phosphorus (High Range), Method 10127, according to the manufacturer's instructions, with a Hach COD Reactor for heating the tubes during the digestion step.

TSS and VSS sampling occurred once every week. Triplicate 5.0 mL samples were taken from each reactor and were placed in a filter funnel on top of a Büchner flask for vacuum filtration following the standard method for TSS: "Standard Method 2540 D. Total Suspended Solids Dried at 103-105°C", (American Public Health Association, 2005). TSS was followed by the standard method for VSS: "Standard Method 2540 E. Fixed and Volatile Solids Ignited at 550°C", (American Public Health Association, 2005). The filters used were PALL Type A/E 47 mm Glass Fiber Filters. Only 5.0 mL of sample was used for each sample because of the viscosity of the sample.

Neisser staining was implemented for microscope visualization of polyphosphates stored in microbial cells following the Neisser staining procedure (Eikelboom, 2000). Stained samples were examined under oil immersion at 1000x magnification with direct illumination unless otherwise noted.

Triplicate samples were preserved for 16S rRNA Illumina gene sequencing of the bacterial community composition. The supernatant of the samples was removed after the

samples were centrifuged in 1.5 mL centrifuge tubes (Thomas Scientific). The samples were then preserved in a freezer with a temperature of -20°C until the samples were sent to be analyzed to MRDNA (Shallowater, TX, USA). DNA extraction, amplification, and sequencing were performed by MRDNA using the V4 variable region polymerase chain reaction (PCR) primers 515F and 806R. A thirty cycle PCR was used (five cycles for PCR products) with the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 95°C for 5 minutes, followed by thirty to thirty-five cycles of 95°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, followed by a final elongation step at 72°C for 10 minutes. After amplification, PCR products were then checked in 2% agarose gel to determine the success of amplification and the relative intensity of bands. Samples were then multiplexed and pooled together (e.g., 100 samples) in equal proportions based on their molecular weight and DNA concentrations to be purified using calibrated Ampure XP beads. Then the pooled and purified PCR product was used to prepare the Illumina DNA sequence library at MRDNA lab in Shallowater, TX, USA using a MiSeq and following the manufacturer's guidelines for use. The sequence data was processed using MRDNA analysis pipeline (MRDNA, Shallowater, TX, USA). The sequences were combined, sequences <150bp were removed, and sequences with ambiguous base calls were also removed. Quality filtering of the sequences was doing using a maximum expected error threshold of 1.0 and dereplicated. The dereplicated or unique sequences were denoised. The unique sequences were identified with sequencing and/or PCR point errors and then removed, followed by a chimera removal. This provided a denoised sequence of operational taxonomic unit (OTU). The OTUs were defined by clustering at 3% divergence (97% similarity). The final OTUs were taxonomically classified using BLASTn against a curated databased derived from the website NCBI (www.ncbi.nlm.nih.gov).

Acetate was measured using gas chromatography (GC) (Shimadzu GC-2010). The GC analyzed samples by flame ionization detector (FID) gas chromatography using a J&W Scientific DB-FFAP 0.53 mm capillary column with 2.0 μL injection volumes. After filtration, samples were placed into GC vials (Leap Pal Parts) and acidified with 10.0 μL of 85% phosphoric acid. The samples were stored at 4℃ prior to analysis. Following program parameters from Tu and Schuler (2013), nitrogen was used as the carrier gas with a flow rate of 15 mL/min, the hydrogen flow rate was 20 mL/min., and the air flow rate was 250 mL/min. to the FID. The oven temperature began at 90°C, ramped to 110°C at 50°C/m, remained at 110°C for 30 seconds, and then ramped up to 130°C at 50°C/min (Tu & Schuler, 2013). The injector temperature was at 250°C.

Chapter 4: Results and Discussion

For each of the continuous and batch experiments, results included measurements of some or all the parameters: pH, DO, phosphorus, absorbency (at 600 nm for solids determination), acetate, Neisser staining, and 16s rRNA gene amplicon sequencing. Acetate measurements were included in the batch tests but not the continuous test because the levels of acetate added were not high enough for acetate peaks to show nor to separate enough from other peaks for identification and verification as acetate levels within the sample. The acetate measurements were measured by GC for the batch tests. The sample for the sequencing batch experiment and Batch Tests 3 and 4 were from a dairy lagoon in Albuquerque, NM (Lagoon 1). The sample in Batch Tests 1 and 2 were from a dairy lagoon in Clovis, NM (Lagoon 2).

4.1 Sequencing Batch Experiment Results

The objective of the sequencing batch experiment was to analyze PPB growth and phosphorus removal over time in full light versus split light and dark conditions in a sequencing batch reactor. Two reactors with sample from Lagoon 1 were operated over 222 days with periodic measurements of pH, phosphorus, absorbency, and DO. Feed concentrations were 200 mg Ac/L and 57.0 mg P/L, respectively, and the hydraulic retention time was 4 days. R1 was operated with continuous lighting, and R2 was operated with alternating 12-hour light and dark cycles. After sampling on Day 138, an extra 0.25 L of each reactor was wasted to potentially improve biomass growth and phosphorus uptake.

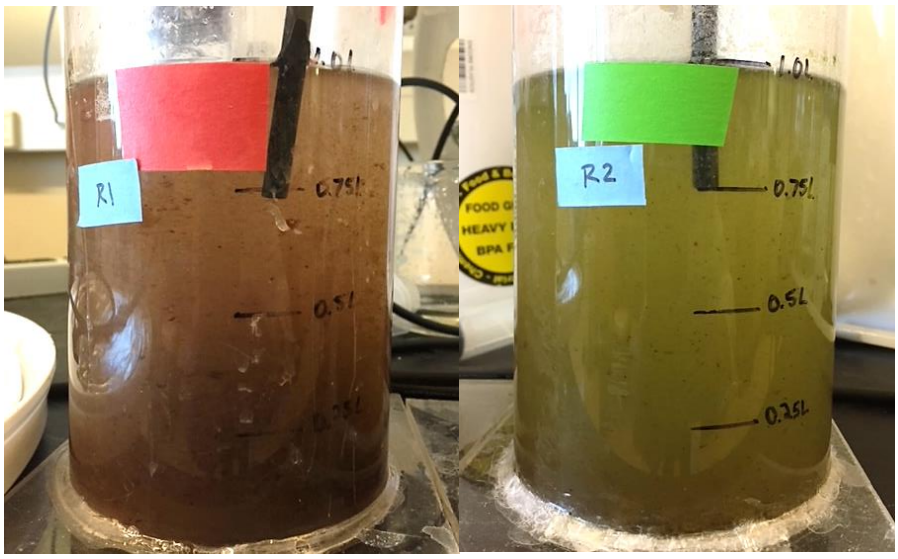

Figure 6. R1 and R2 on Day 19 of the sequencing batch experiment showing differences in pigmentation. R1 had a purple-brown hue and R2 had a green-yellow hue, indicating that the continuous lighting in R1 tended to enrich purple bacteria more than the cyclic lighting in R2 but this was not confirmed. R1 was operated with continuous lighting, and R2 was operated with alternating 12-hour light and dark cycles. Both reactors were inoculated from Lagoon 1. Image by author.

After 19 days of the experiment, the pigmentation of R1 and R2 differed in that R1 had a purple-brown hue and R2 had a green-yellow hue (Figure 6). This indicated that the continuous lighting in R1 tended to enrich purple bacteria more than the cyclic lighting in R2, but the reasoning for this result is not confirmed. pH fluctuated in both R1 and R2. pH decreased over the entirety of the experiment in both reactors from an initial pH of 9.32 to a pH of 8.22 for R1 and an initial pH of 9.24 to a pH of 8.29 for R2 (Figure 7). pH values were generally noisy and there were no consistent trends.

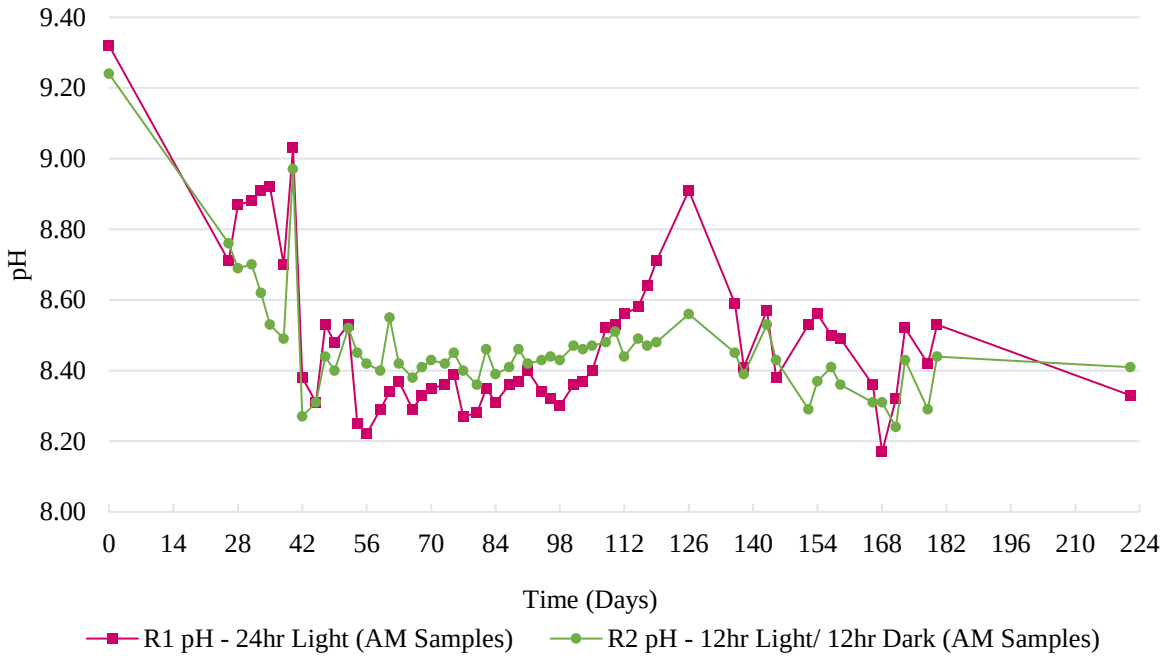

Figure 7. Sequencing batch experiment pH from day 0 to day 222. Sample from Lagoon 1. pH was measured at 7:00 a.m. and 12:30 p.m. after gentle mixing by stirring right before sampling. The 7:00 a.m. samples are shown in this figure for clarity as the p.m. samples varied more than the a.m. samples. Reactors were mixed by aeration from 5:00 to 5:30 a.m. and p.m. pH decreased over the entirety of the experiment in both reactors from an initial pH of 9.32 to a pH of 8.22 for R1 and an initial pH of 9.24 to a pH of 8.29 for R2. pH values were generally noisy and there were no consistent trends.

DO concentrations were measured later in the experiment starting at Day 119 because of delay in equipment. DO was measured but not regulated during this experiment so fluctuations in both R1 and R2 were high during the entirety of the experiment (Figure 8). This indicated that the conditions were oxygenic and non-ideal for most PPB. DO in R1 was at 3.01 mg/L by Day 119. R2 DO measurements were at 2.93 mg/L on Day 119. DO stayed relatively higher than ideal 0.00 to 0.03 mg/L throughout most of the experiment. DO in both R1 and R2 fluctuated and did not correlate to any trends.

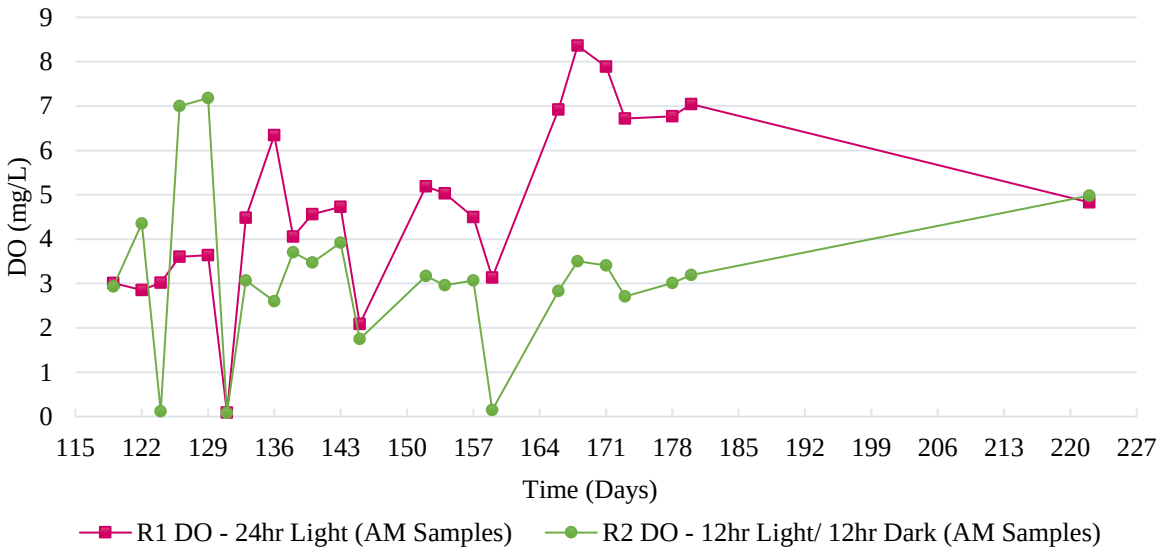

Figure 8. Sequencing batch experiment DO concentrations from days 119 to 222. Sample from Lagoon 1. DO was measured at 7:00 a.m. and 12:30 p.m. after gentle mixing by stirring right before sampling. The 7:00 a.m. samples are shown in this figure for clarity as the p.m. samples varied more than the a.m. samples. Reactors were mixed by aeration from 5:00 to 5:30 a.m. and p.m. DO was measured but not regulated during this experiment so fluctuations in both R1 and R2 were high during the entirety of the experiment. This indicated that the conditions were oxygenic and non-ideal for most PPB so no trends were found.

R1 had more biomass than R2 for most of the experiment. This is evident in Figure 9, which highlights the difference in samples from R1 and R2 on Day 82. The variation of light and dark phase could indicate biomass removal by the bacteria in R2 or improved biomass accumulation in R1, although no definite explanation was explored.

Figure 9. R1 and R2 afternoon samples showing the difference in settled biomass in the two reactors. Image by author.

Triplicate TSS and VSS samples were taken during each measurement. The average TSS and VSS values for R1 were 1,383 ± 602 mg/L (TSS) and 1000 ± 356 mg/L (VSS), and 692 ± 330 mg/L (TSS) and 545 ± 280 mg/L (VSS) for R2. Both R1 and R2 TSS and VSS values decreased by 43% in R1 and 68% in R2 over the course of the experiment (Figure 10). A two-sample *t*-test was used to determine if the TSS measurements were statistically significant from each other on each day TSS measurements were taken. The sample data supported the *t*-test hypothesis that TSS and VSS measurements in R1 as compared to R2 were significantly larger. R1 had twice as much light as R2 and almost twice as much biomass for majority of the experiment, suggesting that carbon dioxide fixation contributed much more than acetate for biomass production.

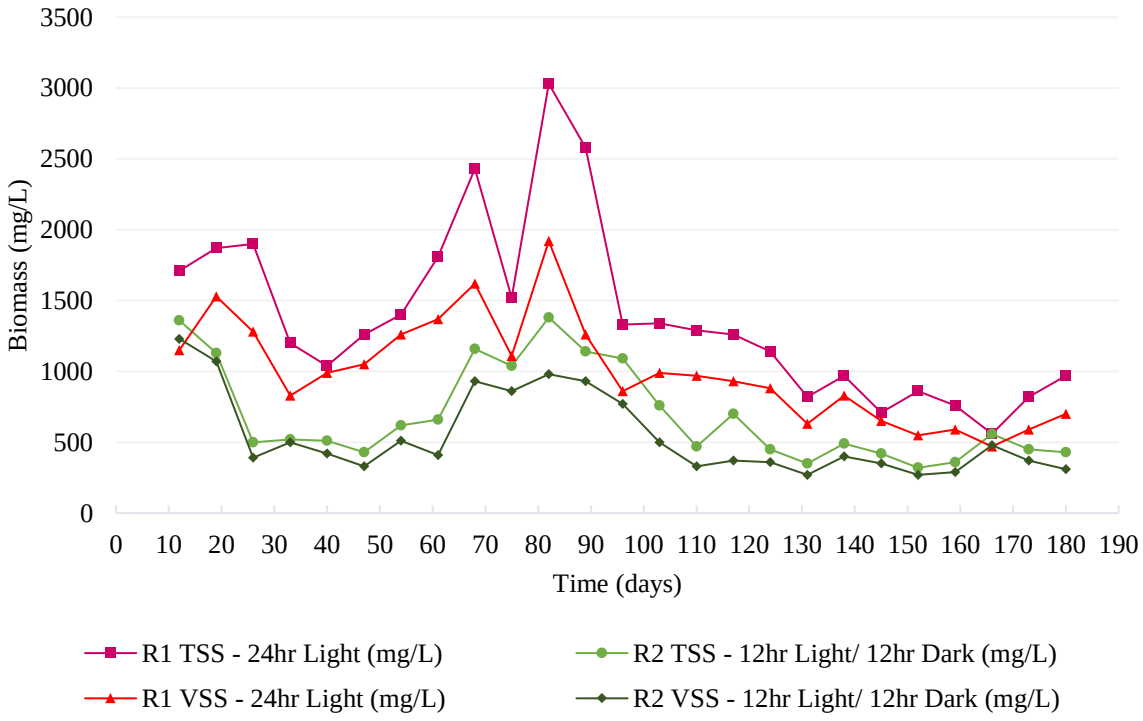

Figure 10. Sequencing batch experiment TSS and VSS measurements from days 12 to 180. Sample from Lagoon 1. Triplicate TSS samples were collected immediately after gentle mixing by stirring. Both R1 and R2 TSS and VSS values decreased by 43% in R1 and 68% in R2 over the course of the experiment. R1 had twice as much light as R2 and almost twice as much biomass for majority of the experiment, suggesting that carbon dioxide fixation contributed much more than acetate for biomass production.

Phosphorus concentrations in the sequencing batch experiment were measured between Day 124 and Day 222. This experiment initially was started as a preliminary test with extended incubation of the sample within the reactors. Phosphorus kits were not available during the start of the experiment and time was allotted for initial kinks to be adjusted so the phosphorus measurements began on Day 124. Two samples were taken each day with one around 7:00 a.m. and one midday around 12:30 p.m. The time 7:00 a.m. was chosen because it was the end time of feeding. A midday sample was chosen as a median time after feed processed throughout the reactor. R1 was continuously illuminated. The R2 schedule included the light turned on from 7:00 a.m. to 7:00 p.m. and turned off from 7:00 p.m. to 7:00 a.m. Wasting from each reactor occurred at 6:00 p.m. to 6:30 p.m., and feed occurred from 7:00 p.m. to 7:00 a.m. during the dark cycle. Feed concentrations were 200 mg Ac/L and 57.0 mg P/L, respectively. On days 124, 166, 173, 221, and 222, multiple samples were taken as part of batch tests during the sequencing batch experiment, but a consistent trend was not evident except for Day 222.

Generally, phosphorus levels were higher in R1 than in R2 during the start of the experiment and fluctuated to a decrease by the end of the experiment (Figure 11). Phosphorus levels in R2 generally followed the same trend as R1 over the entirety of the experiment. Both R1 and R2 had phosphorus concentrations that were close in value except for the start of phosphorus testing in which R1 was higher. A possible reasoning for this would be that R1 was not utilizing phosphorus as well as R2 because of light conditions, but over time R1 aligned with R2. R1 and R2 sampling and measurements were seemingly taken during a phosphorus uptake portion of the cycle since all measurements were less than the influent orthophosphate concentration of 57.0 mg P/L. This indicated that release was likely happening during another part of the cycle which could have been during the dark cycle.

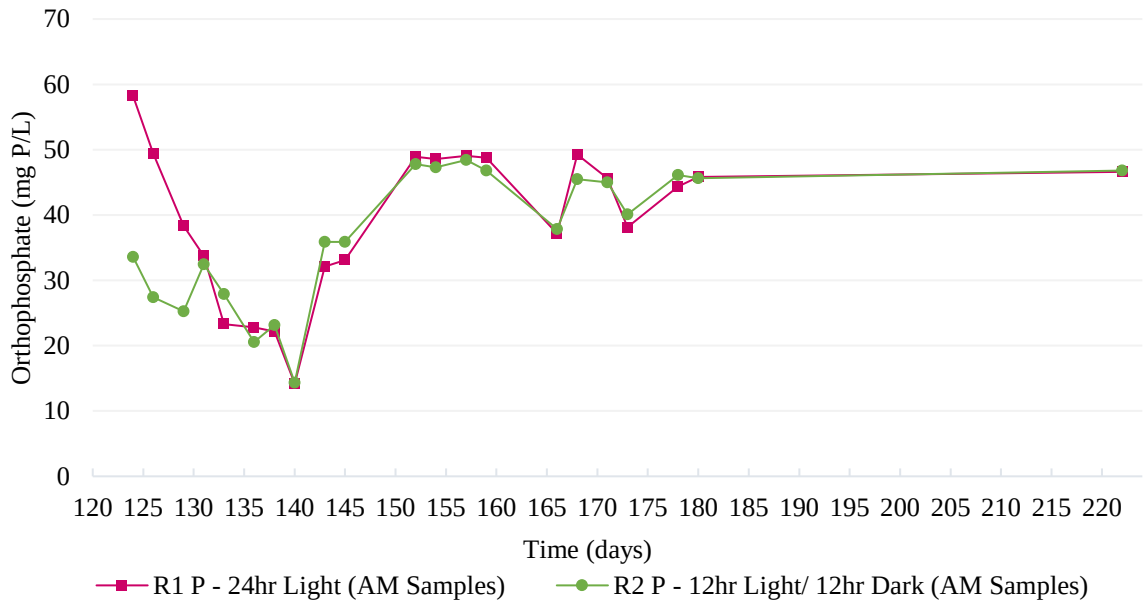

Figure 11. Sequencing batch experiment soluble phosphorus concentrations from days 124 to 222. Samples were taken at 7:00 a.m., which was immediately after the R2 light phase and immediately after feeding was complete in both reactors. Influent was 57.0 mg P/L (25% reactor volume per day), and so data less than 57.0 mg P/L indicates P uptake. R1 and R2 sampling and measurements were seemingly taken during a phosphorus uptake portion of the cycle since all measurements were less than the influent orthophosphate concentration of 57.0 mg P/L. This indicated that release was likely happening during another part of the cycle which could have been during the dark cycle.

Without triplicate measurements, it is difficult to determine whether the morning and midday samples in both R1 and R2 differed as there was no consistent trend throughout the study. After sampling on Day 138, an extra 0.25 L of each reactor was wasted to potentially improve biomass growth and phosphorus uptake. This corresponds to the increase of phosphorus on Day 140. Estimation of the phosphorus to TSS value for R1 was 2.7% P/TSS and 5.0% P/TSS for R2. The average ratio of morning to midday phosphorus concentrations were 0.955 ± 0.176 and 1.04 ± 0.341 in R1 and R2, respectively, suggesting no observable difference in R1 and a generally higher phosphorus morning sample than midday sample in R2. It can be generally inferred that phosphorus uptake occurred within R2 with a given day's light and dark cycle rather than over an extended time. This finding led to the decision to switch to batch testing. On Day 222, samples were taken throughout the day as a batch test (Figure

12). Phosphorus levels continually increased by 29% in R1 and 20% in R2 during the light phase before the mixing occurred in both reactors from 5:00 p.m. to 5:30 p.m. During this time, the phosphorus concentration increased by at least 14% in both reactors, indicating phosphorus release. This did not align with the hypothesis that phosphorus is taken up in light conditions.

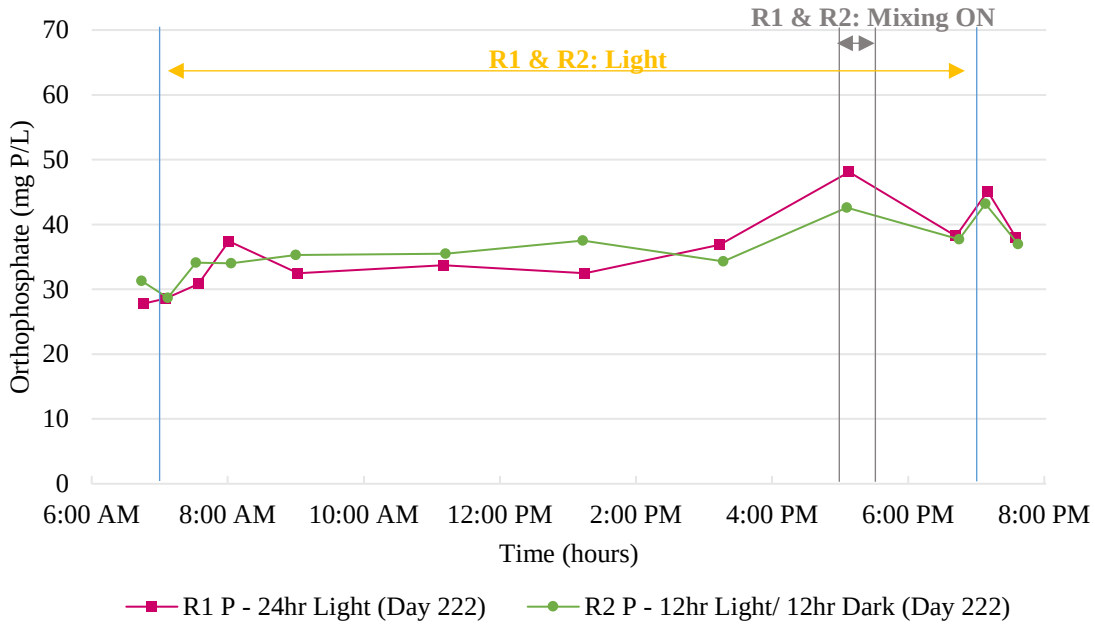

Figure 12. Sequencing batch experiment phosphorus concentrations collected throughout Day 222. Sample from Lagoon 1. Gentle mixing by stirring occurred right before each sampling. Phosphorus levels continually increased by 29% in R1 and 20% in R2 during the light phase before the mixing occurred in both reactors from 5:00 p.m. to 5:30 p.m., in which the phosphorus concentration increased by at least 14% in both reactors. This indicated phosphorus release during light conditions, which went against our hypothesis. Reactors were mixed by aeration from 5:00 to 5:30 a.m. and p.m.

4.2 Batch Test Experiment Results

The objective of the batch testing was to evaluate phosphorus uptake or release with variations of acetate addition and light and dark conditions. Four batch tests were conducted. All batch tests consisted of reactors that were covered 500 mL Erlenmeyer flasks, each containing 400 mL of biomass sample. Figure 13 shows the general three reactor configuration for Batch Tests 1, 2, and 3. Batch Test 4 consisted of four reactors.

Figure 13. Batch test setup for Batch Tests 1, 2, and 3. Batch Test 4 had R1 and R2 in separate boxes from R3 and R4 for varied light conditions. Image by author.

Batch Tests 1 and 2 were from Lagoon 2 and Batch Tests 3 and 4 were from Lagoon 1. N_2 was added into each reactor throughout the experiment for Batch Tests 1 and 2. Batch Tests 3 and 4 had initially low DO values (0.01 ± 0.02 mg/L) so N_2 was not added. The batch tests were operated with periodic measurements of pH, phosphorus, absorbency, and DO. The batch test reactor operation is summarized in Table 8.

Table 8. *Batch Test Reactor Operation Summary*

Batch Test	Duration (hours)	N_2	Volume of Sample per Reactor (mL)	Reactor	Acetate Addition (mg/L)	Time in Light (hours)	Time in Dark (hours)
1	3	ON	400	R1, R2, R3	100.0 (at t=0)	2	1
2	5	ON	400	R1, R2, R3	10.0 (at t=0)	2	3
3	4	OFF	400	R1	0.00	1	3
				R2	10.0 (at t=0)	1	3
				R3	10.0 (2.0 mg/L at t=0, 15, 30, 45, 60)	1	3
4	4	OFF	400	R1	0.00	4	0
				R2	0.00	0	4
				R3	0.00	2	2
				R4	10.0 (at t=0)	2	2

Batch Test 1, conducted on 9/9/2020, was performed with the objective of determining whether acetate addition under anaerobic conditions to dairy lagoon samples led to phosphorus release, which could indicate degradation of polyphosphate for acetate uptake and storage in EBPR. A total of 4.0 L of sample was collected from Lagoon 2 and was stored in 4°C for 2

days before the start of the experiment. The sample used for this test was from Lagoon 2 and was allowed 10 minutes to warm to room temperature after being stored in in 4°C for 2 days. It was then stirred and added to each reactor. An amount of 400 mL of sample from Lagoon 2 was added to each of the three 500 mL reactors. All three reactors were operated identically (experimental triplicates) and were set in a reflective box with one light source that was turned on for 2 hours (t = -60 to 60) and then turned off for 1 hour (t = 60 to 120). The reactors were stripped with N_2 and 100 mg/L of acetate was added to each reactor at t = 0. Samples were stirred before each sampling event and sampling at t = 0 occurred before acetate addition.

TSS and VSS values were 2,687 mg/L (TSS) and 2,260 mg/L (VSS) in the Lagoon 2 biomass before the beginning of the experiment. The total and soluble phosphorus concentrations were measured at 175 mg PO_4^{3-}/L and 56.0 mg P/L, respectively. The non-soluble phosphorus was therefore calculated to be 119.0 mg P/L and the non-soluble phosphorus to TSS ratio was 4.4%, which is higher than the 2% in typical microbial biomass, suggesting potential polyphosphate storage (Schuler & Jenkins, 2003). The average DO was 0.02 ± 0.01 mg/L and the average pH was 9.10 ± 0.03. Phosphorus concentrations during Batch Test 1 are shown in Figure 14. Phosphorus concentrations were highest during the first hour at approximately 119 mg P/L, dropped at t = 15, and rose again to 110 mg P/L at the end of the experiment. These findings suggested that acetate addition did not result in phosphorus release, and so did not support the hypothesis that stored polyphosphate serves as a source of ATP for acetate uptake and storage. However, the biomass was exposed to light until 60 minutes after acetate addition, and so the experiment was not well designed to test this hypothesis, as photosynthesis could provide an alternative means of producing ATP. It was interesting that phosphorus uptake appeared to only occur after acetate addition, suggesting

that acetate uptake was linked to phosphorus uptake because of increased growth after acetate addition.

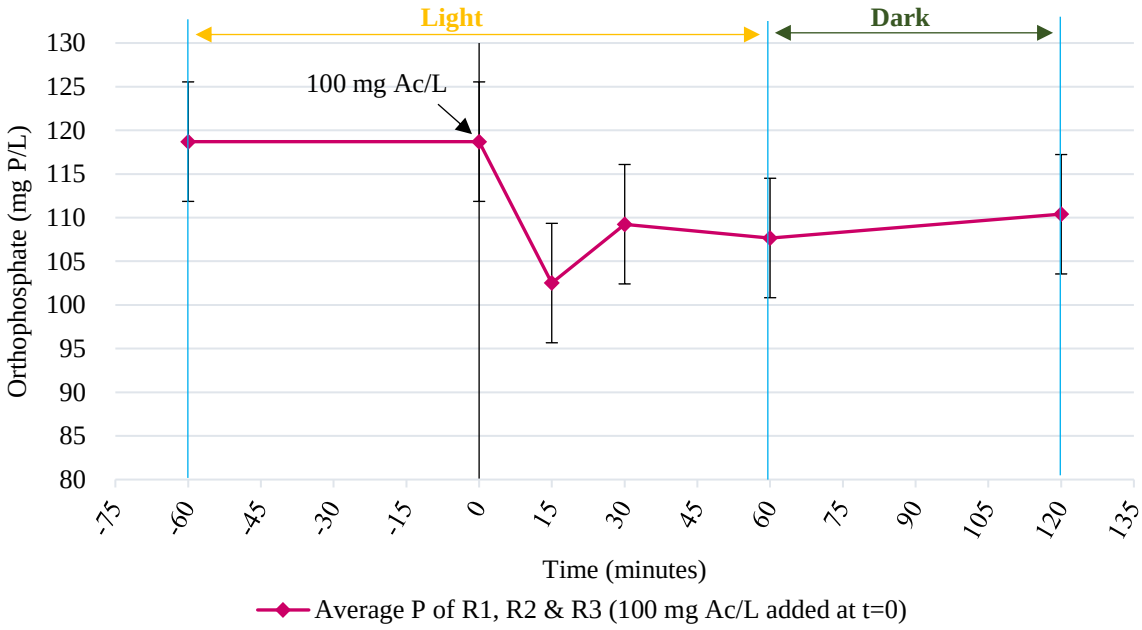

Figure 14. Batch Test 1 soluble phosphorus concentrations. Biomass sample was from Lagoon 2 and 100 mg Ac/L was added at t = 0. Data points are average values from triplicate run reactors. Phosphorus uptake occurred within the first hour after acetate addition, suggesting a link between acetate uptake and phosphorus uptake under light conditions.

Batch Test 2, conducted on 9/21/2020, was performed with the objective of determining phosphorus level changes with a lower acetate addition of 10.0 mg/L. The biomass sample for this test was collected from Lagoon 2 and was stored in 4°C for 14 days before the start of the experiment. At the start of the batch test, the sample was taken out of refrigeration and allowed 10 minutes to warm to room temperature. It was then stirred, and 400 mL of sample was added to each reactor. Three 500 mL reactors were set in a reflective box with one light source that was turned on for 2 hours (t = -60 to 60) and then turned off for 3 hours (t = 60 to 240). The reactors were stripped with N_2. Each reactor had an equal addition of 10.0

mg/L of acetate at t = 0 as triplicate reactors. Samples were stirred before each sampling and sampling at t = 0 occurred before acetate addition.

Batch Test 2 was operated with three identically operated batch reactors and an acetate addition of 10.0 mg/L. TSS and VSS values were 2,193 mg/L (TSS) and 2,007 mg/L (VSS) in the dairy lagoon sample used for the batch test. The total phosphorus and soluble phosphorus concentrations were 165 mg PO_4^{3-}/L and 56.0 mg P/L. The non-soluble phosphorus was calculated to be 109 mg P/L and the non-soluble phosphorus to TSS ratio was 5.0%, which was much higher than the 2% typically found in microbial biomass, suggesting polyphosphate storage. The average DO was 0.02 ± 0.01 mg/L and the average pH was 8.31 ± 0.04. Acetate concentrations were not measured in this test. Phosphorus concentrations are shown in Figure 15. Similar to Batch Test 1, the soluble phosphorus concentrations were highest during the first hour before acetate addition. By 30 minutes after acetate addition (t = 30), phosphorus concentrations had decreased to 55.0 mg P/L. After the dark phase start at t = 60 minutes, phosphorus concentrations were relatively constant until a decrease to 34.0 mg P/L during the last 60 minutes of the experiment. The total phosphorus uptake during the light phase was 30.0 mg P/L. As in Batch Test 1, the addition of acetate during the light phase made a correlation to the hypothesis that light conditions may be analogous to aerobic conditions in EBPR, however, this interpretation cannot be fully supported as photosynthesis could provide an alternative means of producing ATP. Similar to Batch Test 1, phosphorus uptake occurred after acetate addition, suggesting that acetate uptake was linked to phosphorus uptake, possibly because of increased growth, but the lack of acetate measurement made the results difficult to interpret.

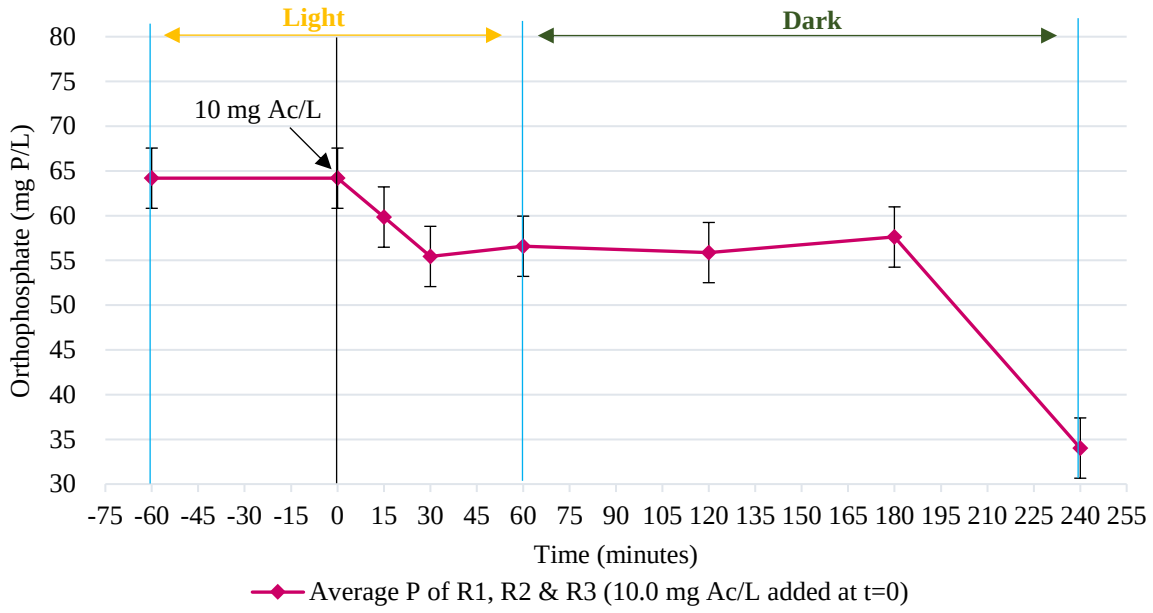

Figure 15. Batch Test 2 soluble phosphorus concentrations. Biomass sample was from Lagoon 2 and 10.0 mg Ac/L was added at t = 0. Data points are average values from triplicate run reactors. Phosphorus uptake occurred within the first hour after acetate addition under light conditions, suggesting acetate addition may have stimulated growth and possibly polyphosphate storage under light conditions.

Batch Test 3, conducted on 9/28/2020, was performed with the objective of continuing to investigate whether PSB store and utilize polyphosphate for energy (ATP) to be used to take up and store acetate in dark, aerobic conditions, analogous to EBPR, with two reactors operated with a single large pulse addition of 10.0 mg/L acetate versus multiple step additions of the same total amount of acetate (2.0 mg Ac/L added five times to equal 10.0 mg/L). A total of 4.0 L of sample was collected from Lagoon 1 and was stored in 4°C for 2 days before the start of the experiment. Tu and Schuler (2013) demonstrated that slow, continuous acetate addition (resulting on lower acetate concentrations) enriched for more PAOs than when acetate was as a single large pulse (resulting in higher short-term acetate concentrations) in lab-scale EBPR reactors. Three 500 mL reactors were filled with 400 mL of biomass from Lagoon 1 after being collected and stored in 4°C for 2 days before the start of the experiment. At the start of the

batch test, the sample was taken out of refrigeration and allowed 10 minutes to warm to room temperature. It was then stirred, and 400 mL of sample was added to each reactor. Three 500 mL reactors were set in a reflective box with one light source that was turned on for 1 hour (t = -60 to 0) and then turned off for 3 hours (t = 0 to 180). The reactors were not stripped with N_2 because of low DO levels. Mixing only occurred as gentle stirring before sampling events. No acetate was added to R1. A total of 10.0 mg/L of acetate was added to R2 at t = 0 and R3 received he same total amount of acetate with 2.0 mg/L of acetate added at t = 0, 15, 30, 45 and 60. Samples were stirred before each sampling event and when acetate was added but were otherwise unmixed. Sampling at t = 0 occurred before acetate addition.

Average total phosphorus for the biomass used in Batch Test 3 was 170 mg PO_4^{3-}/L, average soluble phosphorus was 58.4 mg P/L phosphorus, and the non-soluble phosphorus was 112 mg P/L. TSS and VSS values were 2,180 mg/L (TSS) and 1,967 mg/L (VSS), and the non-soluble P/TSS fraction was 5.1%, which was similar to the value of the Batch Test 2 biomass, and suggested polyphosphate storage. The average DO was 0.02 ± 0.01 mg/L and the average pH was 8.31 ± 0.02. The soluble phosphorus concentrations were highest at the beginning of Batch Test 3 (t = -60) with an average of 64.3 mg P/L in all reactors (Figure 16). Soluble phosphorus initially decreased and rose again at t = 0. Phosphorus concentrations were similar in all three reactors prior to t = 0, when acetate was added to R2 and R3. Phosphorus concentrations increased in all three reactors from t = 15 to 60 minutes, but were highest in R2, where the pulse of 10.0 mg/L acetate was added at t = 0. At t = 60, the phosphorus concentration in R2 as 59.4 mg P/L, 58.1 mg P/L in R3, and 56.1 mg P/L in R1, which was consistent with the hypothesis that some bacteria in the lagoon samples metabolized polyphosphate for acetate uptake. However, at later time points phosphorus uptake occurred

in R2 and a smaller amount in R3 despite continued anaerobic and dark conditions. In summary, less phosphorus uptake over the first 60 minutes after acetate was added to R2 and R3 in Batch Test 3 relative to the reactor with no acetate addition (R1) supported the hypothesis that stored polyphosphate may provide energy for acetate uptake and storage under dark conditions. However, phosphorus uptake occurred during the dark period and therefore contradicts the hypothesis that phosphorus was released during the dark phase. The later phosphorus uptake in these reactors was more difficult to explain. One possibility is that some oxygen and/or light was introduced during the later sampling events, which could have allowed aerobic metabolisms with phosphorus uptake and storage, but this explanation would require further experimental analysis for confirmation. Furthermore, acetate measurements would have facilitated the interpretation of results.

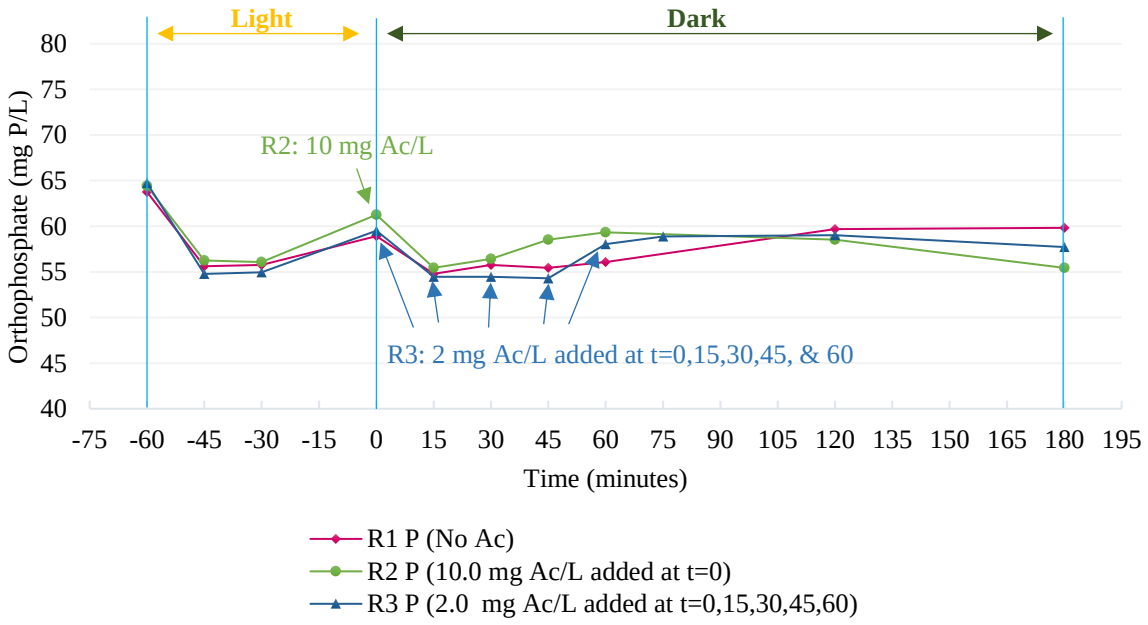

Figure 16. Batch Test 3 soluble phosphorus concentrations. Biomass sample was from Lagoon 2. In R2, 10.0 mg Ac/L was added at t = 0. In R3, 2.0 mg Ac/L was added at t = 0, 15, 30, 45, and 60. Less phosphorus uptake from minutes 15 to 60 in reactors R2 and R3 (with acetate addition) than in R1 (no acetate addition) supported the hypothesis that stored polyphosphate may provide energy for acetate uptake under dark conditions, analogous to the PAO anaerobic phase. However, phosphorus uptake occurred during the dark period and therefore contradicts the hypothesis that phosphorus was released during the dark phase.

Batch Test 4, conducted on 3/16/2021, was performed with the objective of continuing to investigate whether PSB store and utilize polyphosphate for energy (ATP) to be used to take up and store acetate in dark, anaerobic conditions, analogous to EBPR, with differing light/dark conditions and multiple reactors with no acetate addition as experimental controls. Acetate measurements were included in this batch test. The biomass sample was collected from Lagoon 1 and was stored at 4°C for 2 days before the start of the experiment. At the start of the batch test, the sample was taken out of refrigeration and allowed 10 minutes to warm to room temperature. It was then stirred, and 400 mL of sample was added to each of the four 500 mL reactors. R1 was set in its own foil-lined box with one light source that was kept on for the duration of the experiment (t = -60 to t = 180). R2 was placed in a separate box with no light source and kept in darkness for the duration of the experiment (t = -60 to 180). R3 and R4 were placed in the same foil-lined box with one light source that was turned on for 2 hours (t = -60 to 60) and then turned off for 2 hours (t = 60 to 180). The reactors were not stripped with N_2 as DO levels were consistently measured to be < 0.02 mg/L without N_2 stripping. No acetate was added to R1, R2, nor R3. R4 had an addition of 10.0 mg/L of acetate at t = 0. Samples were gently stirred before each sampling event and sampling at t = 0 occurred before acetate addition. Triplicate samples were taken from the reactor.

The average total phosphorus for the Lagoon 1 biomass used in Batch Test 4 was 242 mg PO_4^{3-}/L, the soluble phosphorus was 56.2 mg P/L, and the non-soluble P was 186 mg P/L. TSS and VSS values were 3,160 mg/L (TSS) and 1,800 mg/L (VSS). The non-soluble P/TSS fraction was therefore 5.9%, suggesting polyphosphate storage in the biomass. The average DO was 0.01 ± 0.01 mg/L and the average pH was 9.01 ± 0.02. The soluble phosphorus concentrations are shown in Figure 17. The large standard deviations shown in Figure 17 error

bars may have been related to the 1:20 dilution because of filtration issues with the samples. Each sample took three 0.45 μm syringe filter filtrations for 1.0 mL of filtered sample because of fine particles within the samples after initial filtering. After acetate was added at t = 0, soluble phosphorus continued to decrease in R4 throughout the rest of the light phase (t = 0 to 60). Surprisingly, it increased in R3, which was operated identically but did not receive additional acetate. However, acetate measurements indicated that acetate existed in all samples at the beginning of the test (Figure 18). Because acetic acid is a fermentation product, its presence suggests fermentation either in the dairy lagoon or in the samples after collection. By the end of the test, phosphorus decreased the most in R2 (no acetate added, full darkness) to 47.6 mg P/L with a total decrease of 12.4 mg P/L from the initial 60.0 mg P/L in R2, although most of this uptake occurred in the final hour and through t = 120 minutes. R2 had the highest soluble phosphorus concentrations. Final phosphorus concentrations were 55.4 mg P/L in R1, 57.8 mg P/L in R3, and 52.4 mg P/L in R4. R2 was not significantly less than the other reactors, however, compared to R3 it had a larger uptake by 10.2 mg P/L. R3 had the least amount of uptake of 2.20 mg P/L by the end of the test. Phosphorus concentrations in all reactors decreased by at least 7.56% at the end of the test.

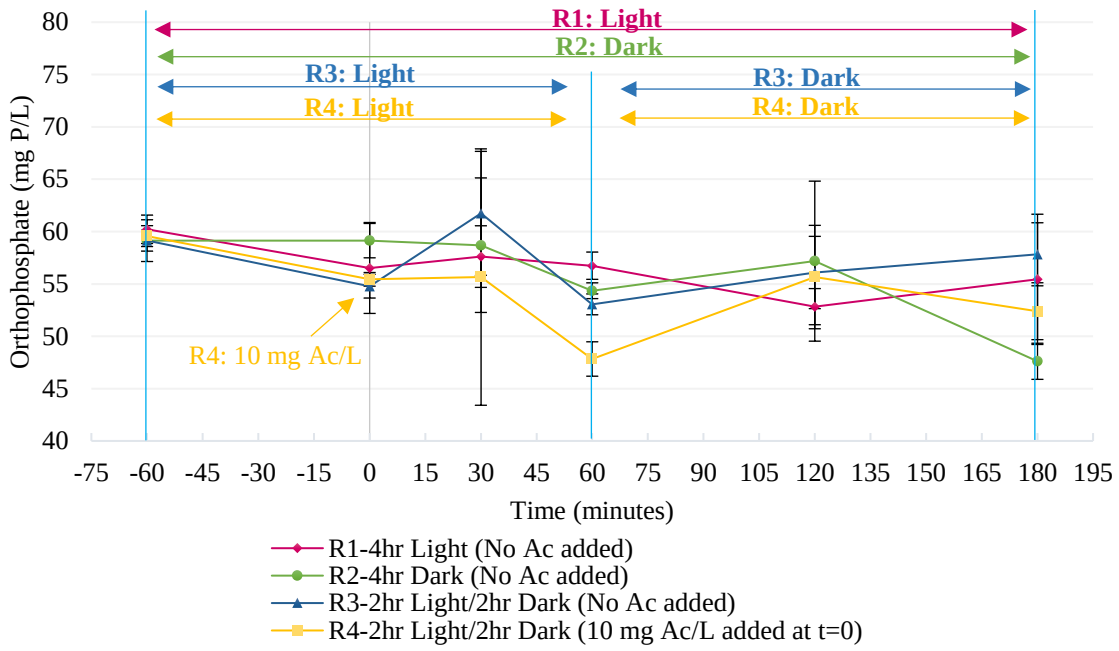

Figure 17. Batch Test 4 soluble phosphorus concentrations. Dairy Lagoon 1 was the source for all reactors. At t=0, 10 mg/L of acetate was added to R4. R4 had the most uptake at t=60. The lack of P increase during the dark phases did not support the hypothesis that the dark phase functions analogously to the anaerobic phase of PAOs.

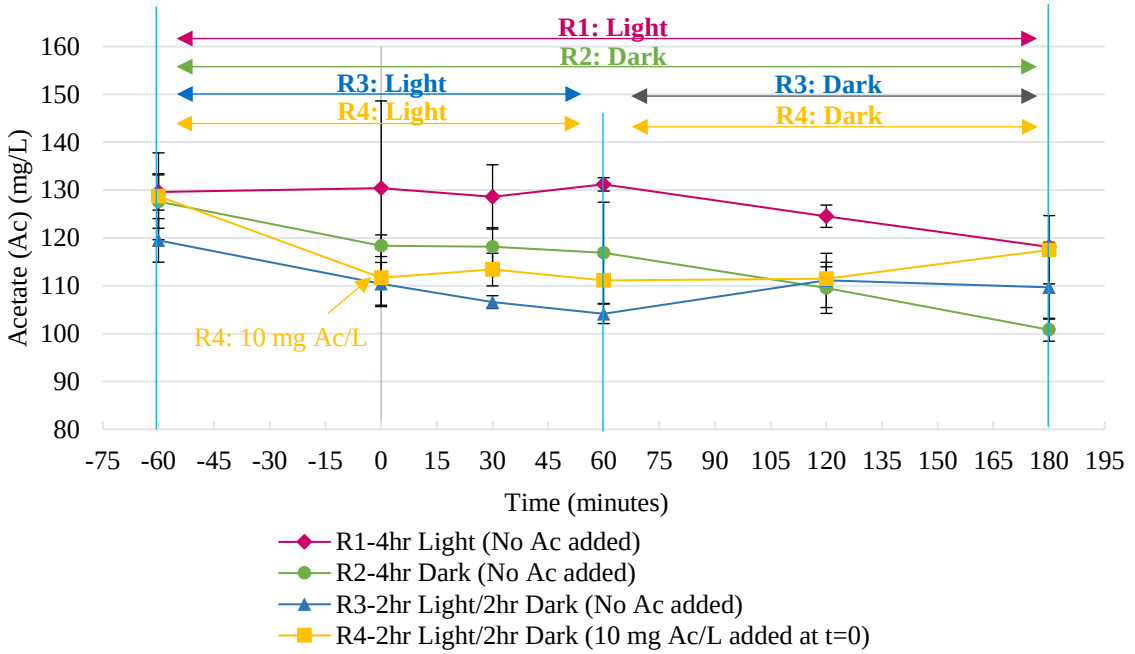

Figure 18. Batch Test 4 acetate concentrations. At t=0, 10 mg/L of acetate was added to R4. Acetate concentrations decreased in all reactors, with R2 having the largest decrease of 21% from the initial 128 mg Ac/L. There was therefore a consistent trend across all reactors of greater acetate uptake during dark conditions than in light conditions.

There was a general downward trend in acetate concentrations throughout Batch Test 4 with R1 having the highest values and R3 generally having the lowest values. The largest difference in acetate values was 27.1 mg/L of acetate which occurred at t = 60 between R1 (131 mg/L of acetate) and R3 (104 mg/L of acetate). Two-sample *t*-tests were used to determine which measurements were statistically significant from each other at each time point. The sample data supported the *t*-test hypothesis that acetate measurements in R1 as compared to R3 were statistically significant, indicating that all R3 acetate concentrations were significantly greater than those of R1 with the exception of the acetate concentrations at t = -60 and t = 180. By the end of the test, R2 had the lowest acetate concentration (101 mg/L of acetate) and R1 had the highest concentration (118 mg/L of acetate). R1 and R2 were operated light conditions, respectively. The absence of light could have induced the bacteria in R2 to uptake more acetate sooner than the bacteria of R1, which were in full light conditions. It is possible that light conditions favored CO2 fixation for carbon supply, while dark condition favored the uptake of organic carbon. Light conditions in reactors 1, 3, and 4 were identical from t = -60 to 0, but the reactors behaved differently, with greatest acetate uptake in R4 and R3 but none in R1, suggesting that there was some variation in the initial samples or unknown factors in how the reactors were operated. The *t*-test results for R1 and R4 showed acetate measurements in R1 were significantly greater than those of R4, which was to be expected as R1 had little need for acetate uptake because light was available as a continued source of energy. The R3 and R4 *t*-test comparison also showed that acetate measurements in R3 compared to R4 were statistically significant with R4 having greater acetate measurements, which was to be expected as additional acetate was added to R4. R4 had an addition of 10.0 mg/L of acetate at t = 0 minutes, but there was only a measured increase of 1.707 mg/L of acetate in R4 by t = 30 minutes. A

sample point at t = 15 would have clarified that acetate was being taking up during this time. R2 was kept in the dark throughout the experiment and yielded a seemingly constant rate of acetate uptake. R2 also exhibited the largest amount of acetate uptake (26.8 mg/L of acetate). Lights were turned off in R3 and R4 at t = 60 minutes, and acetate was produced in both reactors thereafter, suggesting that fermentation was occurring at a greater rate than uptake under dark conditions. There was therefore a consistent trend across all reactors of greater acetate uptake during dark conditions than in light conditions (R2 had more acetate uptake than R1, and dark phases in R1 and R4 had great acetate uptake than light phases in these reactors). From these findings, it can be inferred that PPB produce less energy under a dark phase but can utilize acetate during this time for energy production.

A general trend for all reactors in Batch Test 4 was not evident. For R1, phosphorus levels were inversely proportional to acetate levels. A decrease in acetate correlated to an increase in phosphorus. In R2, there was an initial decrease in acetate and no change in phosphorus. Acetate steadied and slowly decreased at which phosphorus decreased as well. R3 and R4 had similar trends of phosphorus levels compared to acetate levels in that a decrease in acetate corresponded to a decrease in phosphorus. Both R3 and R4 had identical light and dark phases, but the addition of acetate in R4 seemed to encourage more phosphorus uptake and more acetate uptake in R4 as opposed to R3 by the end of the experiment. These findings can be related by the general inference that the addition of acetate can improve phosphorus uptake, but other factors such as photosynthesis could have encouraged the phosphorus uptake during this time as it was part of the light phase.

4.3 DNA Sequencing and Microscopy Results

Triplicate samples were taken from both Lagoon 1 and Lagoon 2, as well as an activated sludge sample from the Albuquerque wastewater treatment facility (the Southside Water Reclamation Facility or SWRF) were analyzed to determine bacterial composition based on 16s rRNA gene amplicon sequencing. Lagoon 1 samples were from the biomass used in Batch Test 4 and Lagoon 2 samples were from the biomass used in Batch Test 2. The results are shown in Figure 19, which indicated that large differences existed between the three samples when analyzed at the phylum level. For example, Lagoon 1 consisted of mostly Proteobacteria (58.49%) with Firmicutes (24.59%) as the second largest group, whereas Lagoon 2 consisted of mostly Firmicutes (47.10%) with relatively little Proteobacteria (13.36%) and Synergistetes (21.44%) being the second largest group. The activated sludge sample from the wastewater treatment facility was compromised of mostly Proteobacteria (55.03%) and Synergistetes (19.16%). Firmicutes are known include many fermentative organisms (Zhao et al., 2012) and so this group could have been linked to the high acetate concentrations measured in the samples of the batch tests described above.

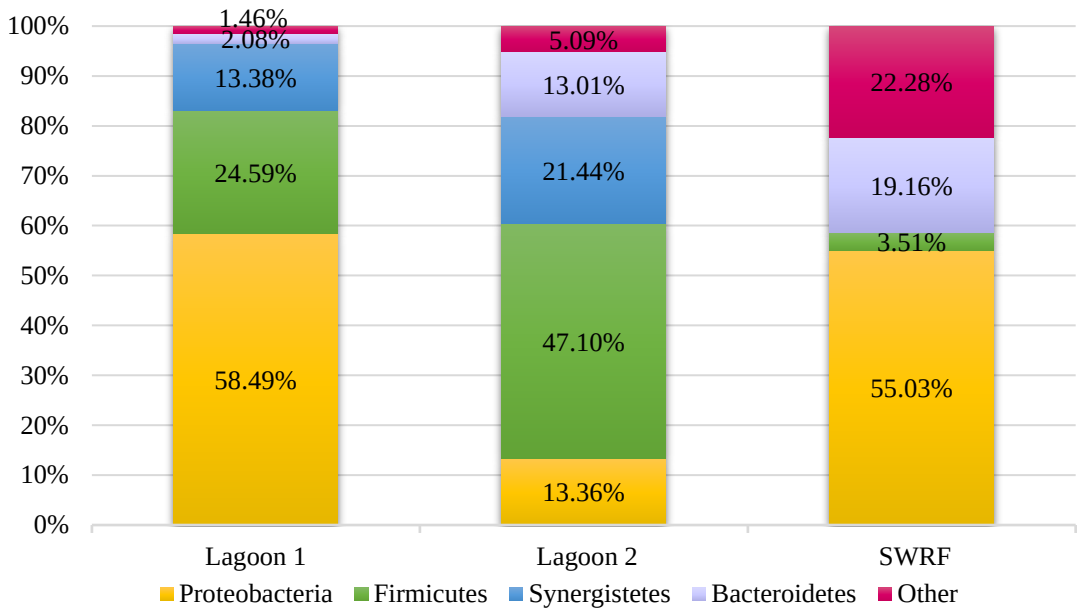

Figure 19. 16s rRNA gene amplicon sequencing phylum percentages for Dairy Lagoon 1 (Lagoon 1), Dairy Lagoon 2 (Lagoon 2), and the domestic activated sludge from the SWRF (SWRF). Known PSB are found in the phylum Proteobacteria. No known PNSB were found.

The DNA sequencing results with identification at the genus level indicated large differences in the three samples (Figure 20). Both lagoon samples contained relatively large amounts of *Clostridium* and *Synergistes* compared to other genera within the samples indicated common genera within the two lagoon samples. Lagoon 1 samples contained 11.40% *Synergistes* and 9.79% *Clostridium* among other genera. The Lagoon 2 samples contained 32.97% *Clostridium* and 12.17% *Synergistes* among other genera. Because Lagoon 1 and Lagoon 2 were gathered from different locations, the bacterial composition indicated the diversity between two similar ecosystems because of environmental conditions. The SWRF sample varied greatly from the lagoon samples in that there was a larger spectrum genus, each having minimal percentages. The largest genera in the SWRF sample was *Pedomicrobium* at 12.47%. This highlighted the difference in an activated sludge sample as compared to the lagoon samples. The known PAO genus *Dechloromonas* was found in Lagoon 2 (0.25%) and

in the SWRF sample (0.15%) (Stokholm-Bjerregaard et al., 2017). *Tetrasphaera*, another known PAO genus, was also found in the SWRF sample at 0.38% (Tu & Shculer, 2013). Of the known GAO genera, 0.5% *Defluviicoccus* and 0.01% *Propionivibrio* were found in the SWRF but no known GAOs were found in the lagoon samples (Stokholm-Bjerregaard et al., 2017; Tu & Schuler, 2013). Comparing this information to the genus in Lagoon 1 and Lagoon 2 suggests that lagoons offer better environmental conditions for PPB and PAO growth, but there is potential for PPB to grow in wastewater from a wastewater treatment plant to improve nutrient removal.

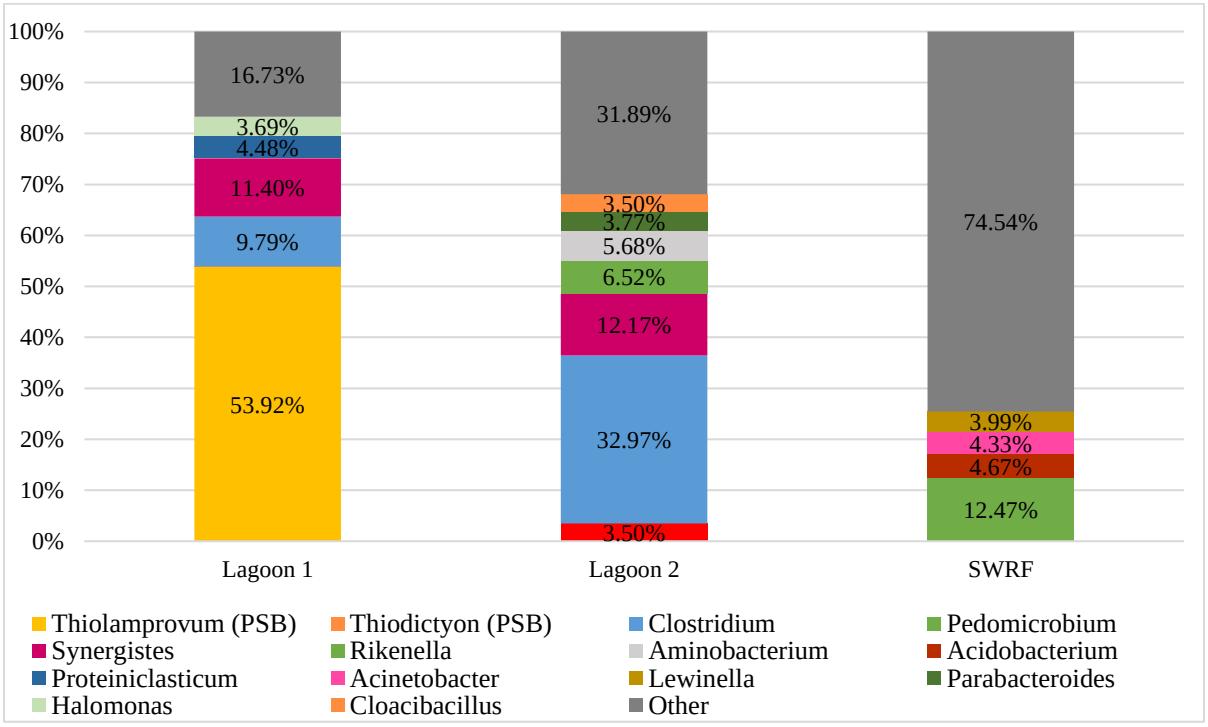

Figure 20. Most common genera percentages for Dairy Lagoon 1 (Lagoon 1), Dairy Lagoon 2 (Lagoon 2), and the domestic activated sludge from the SWRF (SWRF) as determined by 16s rRNA gene amplicon sequencing. All genera representing at least 3.5% of OTUs in at least one sample are shown. Known PSB genera include *Thiolamprovum* and *Thiodictyon*. No known PNSB genera were found.

Batch Tests 3 and 4 utilized biomass sample from Lagoon 1. Batch Tests 1 and 2 utilized biomass sample from Lagoon 2. Of the phylum representing at least 10% of the OTUs in at least one of the samples, known PSB exist in the genus *Thiolamprovum* (Caumette et al.,

2015), which comprised 53.92% of the Lagoon 1 sample. *Thiodictyon*, another known PSB, was also present in Lagoon 1 at 0.003% (Peduzzi et al., 2012). The Lagoon 2 sample contained a combined 4.29% of known PSB including 3.50% *Thiodictyon*, 0.52% *Thiocapsa*, 0.17% *Thioalkalibacter*, 0.05% *Thiocystis*, and 0.05% *Thiolamprovum* (Banciu et al., 2008; Caumette et al., 1991; Caumette et al., 2015; Imhoff, 2015; Peduzzi et al., 2012). Neither Lagoon 1 nor Lagoon 2 samples included known PNSB in the bacterial composition. Only a small amount of PPB (PSB) was found in the SWRF sample at an amount of 0.71% mostly comprised of the known PSB *Nitrosococcus* (0.36%) and *Lamprobacter* (0.11%) (Woese et al., 1985; Gorlenko et al., 2014). PSB composition of each sample is listed in Table 9. The PSB found in Lagoon 1 is almost 13 times that of the PSB amount in Lagoon 2 and almost 76 times that of the SWRF sample. The difference in PSB from Lagoon 1 and Lagoon 2 can be because of environmental conditions and ecological factors such as location, weather, and time of sample gathering. Common PPB organisms that have been found in dairy lagoon populations include *Thiocapsa roseopersicina* (McFarlane & Melcer, 1977), *Thiolamprovum pedioforme* (Goh et al., 2009), and *Thiopedia rosea* (Freedman et al., 1983; Wenke & Vogt, 1981).

Table 9. *Purple Sulfur Bacteria Genus Composition in Lagoon 1, Lagoon 2, and the SWRF Sample*

	Genus	Sample		
		Lagoon 1	Lagoon 2	SWRF
PSB	*Nitrosococcus*	0.000%	0.00%	0.365%
	Lamprobacter	0.000%	0.00%	0.112%
	Thiolamprovum	53.92%	0.05%	0.001%
	Thiodictyon	0.003%	3.50%	0.000%
	Thiocapsa	0.000%	0.52%	0.000%
	Thioalkalibacter	0.000%	0.17%	0.009%
	Thiocystis	0.000%	0.05%	0.000%
	Other combined PSB	0.000%	0.00%	0.226%
Total	Total PSB	53.923%	4.290%	0.713%

Neisser staining and analysis via microscopy was conducted on several days to note any potential polyphosphates. Clusters comprising polyphosphate containing coccobacilli were common in the Lagoon 1 samples (Figure 21). The non-soluble phosphorus to TSS ratios for Lagoon 1 and Lagoon 2 were higher than the 2% ratio typically found in microbial biomass, suggesting that polyphosphate storage was occurring within the biomass. The staining results showed that polyphosphates were evident in the biomasses and therefore polyphosphate storage was plausible within the biomass. Figure 22 shows one of the Neisser staining photos of Lagoon 2 before Batch Test 1. Tetrad-type cocci were observed by their arrangement of four or eight cells. GAOs have been shown to display tetrad-type morphology (Wong et al., 2004), thus alluding to the potential presence of GAOs as well as the PAOs within the biomass as related to the DNA sequencing. As related to the DNA sequencing, the genera *Dechloromonas* was present in the Lagoon 2 sample and the SWRF. Also present in the SWRF sample was the PAO *Tetrasphaera* and two known GAO genera (*Defluviicoccus* and *Propionivibrio*). No known GAOs were present within the lagoon samples, thus the presence of Neisser-positive bacteria indicates the existence of polyphosphate accumulation.

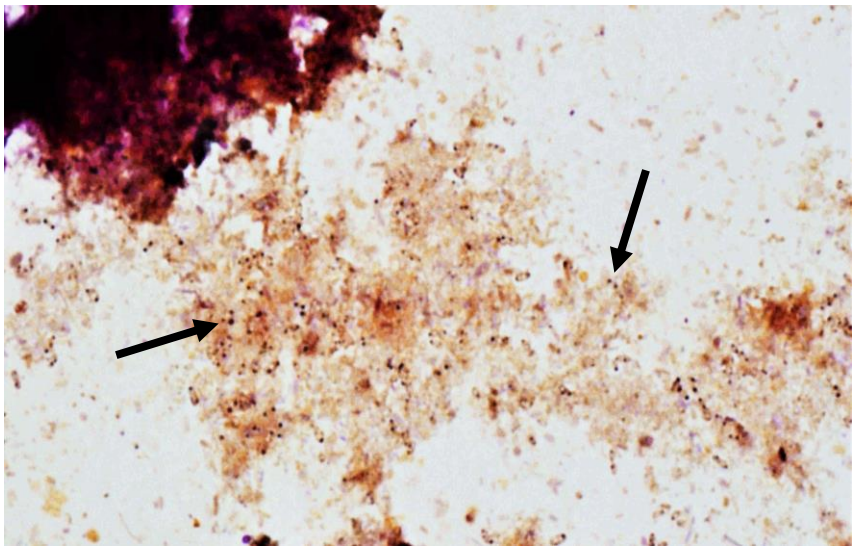

Figure 21. Neisser staining results of Lagoon 1 (Sequencing Batch Test, Day 49) at 1000x resolution. Purple dots (example denoted by arrows) are Neisser-positive, indicating likely polyphosphate-positive cells or granules. Image by author.

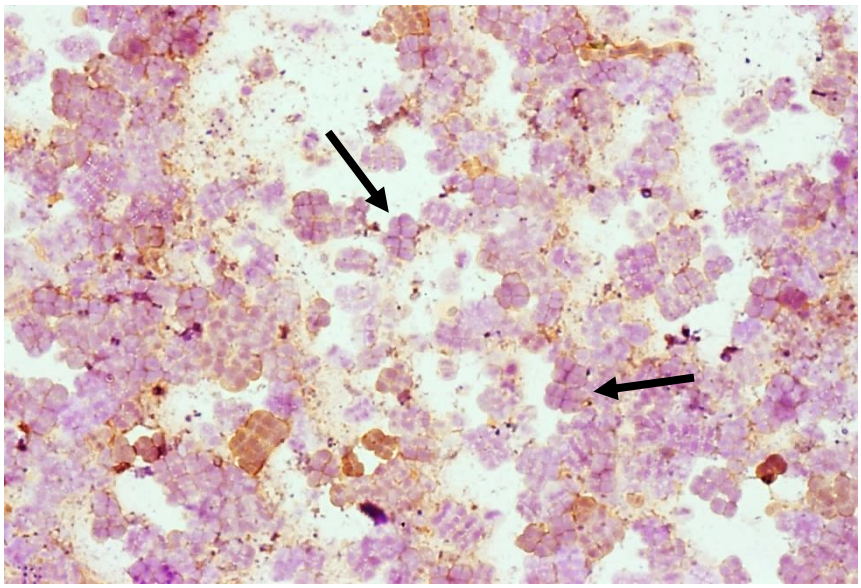

Figure 22. Neisser staining results of Lagoon 2 at 1000x resolution. Neisser positive tetrad forms were common (examples indicated by arrows). This showed evidence of polyphosphate accumulation. Image by author.

4.4 Analysis and Discussion

Genetic DNA results indicated that PSB was present within Lagoon 1 with majority of the PSB as *Thiolamprovum* (53.92%). There were no substantial trends that could be utilized to confidently confirm whether the PPB included in the biomass for the sequencing batch

reactor experiment had improved phosphorus removal with the addition of acetate. There were delays with equipment and issues with acetate measurements during the experiments, but it is possible that the sampling event times for acetate in the sequencing batch experiment (200 mg Ac/L in the feed) were not close enough together to show the impact on the metabolism of the PPB and polyphosphates. For the batch tests, the acetate concentrations added to the reactors (100 mg Ac/L in Batch Test 1 and 10.0 mg Ac/L in Batch Tests 2, 3, and 4) were too low for an effective impact on the metabolism of the PPB and polyphosphates as the batch test acetate measurements showed an initially large amount of acetate already within the lagoon samples. Because acetate was present in the biomass of Batch Test 4 without acetate addition, acetate or fermentation could have been present in all batch tests and the sequencing batch experiment.

Among the results, R1 and R2 did show differences in biomass, with R1 having more biomass within its reactor than R2. Greater autotrophic growth in R1 was obtainable because it received continuous light, while R2 received light only 12 hours per day. R1 had twice as much light as R2 and almost twice as much biomass for majority of the experiment, suggesting that carbon dioxide fixation contributed much more than acetate for biomass production. The phosphorus data for the sequencing batch experiment was highly variable and there were not any obvious trends corresponding to the 222 days of sampling. The average ratio of morning phosphorus sample to the midday phosphorus sample concentrations were 0.955 ± 0.176 and 1.04 ± 0.341 in R1 and R2, respectively, suggesting no observable difference in R1 and a generally higher morning sample than midday sample in R2. From this finding, it can be inferred that phosphorus uptake occurred within R2 with a given day's light and dark cycle. However, during a batch test of the sequencing batch experiment, phosphorus levels continually increased during the light phase in R1 and R2. This did not align with the

hypothesis that phosphorus is taken up in light conditions. R1 and R2 sampling and measurements were taken during a phosphorus uptake portion of the cycle since all measurements were less than the influent orthophosphate concentration of 57.0 mg P/L. This indicated that release was likely happening during another part of the cycle which could have been during the dark cycle. The generally inconclusive analysis of the data led to batch testing.

Batch Tests 1 and 2 utilized biomass from Lagoon 1. Batch Tests 3 and 4 utilized biomass samples from Lagoon 2. DNA results indicated that PSB was present in both Lagoon 1 and Lagoon 2. In the Lagoon 1 sample, 53.92% of the OTUs were known PSB genus *Thiolamprovum* while the Lagoon 2 OTUs consisted of only 3% PSB, with the most abundant PSB genus *Thiodictyon* at 3.5%. All the batch tests had a non-soluble phosphorus to TSS ratio higher than the typical 2% content for "normal" microbial bacteria which indicated evidence for polyphosphate accumulation and storage (Figure 23).

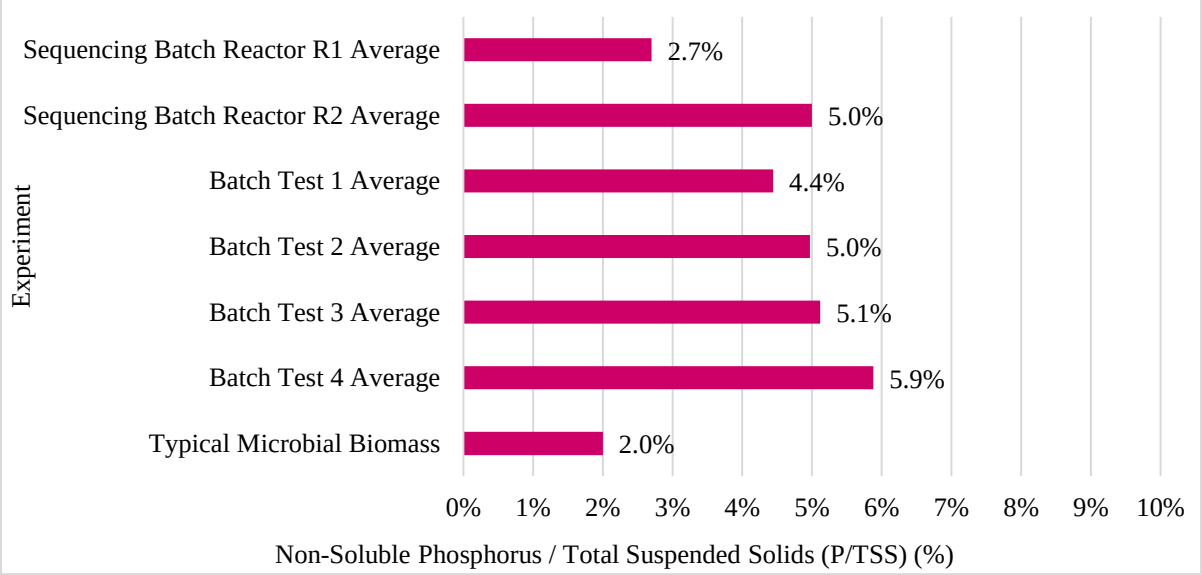

Figure 23. The average non-soluble phosphorus/TSS for all reactors in each batch test. Batch Tests 1 and 2 utilized Lagoon 2, while the Sequencing Batch Experiment, Batch Test 3, and Batch Test 4 utilized Lagoon 1. All the batch tests had non-soluble phosphorus/TSS values greater than the typical 2% content for typical microbial bacteria, suggesting polyphosphate accumulation and storage.

In Batch Tests 1, 2, and 3, phosphorus uptake occurred during the first hour after acetate addition (t = 0 to 60) with the most uptake occurring at t = 15. In both Batch Test 1 and 2, the addition of acetate during the light phase was aligned to phosphorus uptake (Figure 24). This correlated to the hypothesis that light conditions are analogous to anaerobic conditions in EBPR, however, this interpretation cannot be fully supported as photosynthesis could provide an alternative means of producing ATP. In Batch Test 3, the addition of acetate also seemingly correlated with phosphorus uptake similar to Batch Tests 1 and 2, although this occurred in the dark phase and therefore contradicted the hypothesis of phosphorus release during the dark phase (Figure 25). Acetate addition encouraged growth and phosphorus uptake within the first 15 minutes in Batch Tests 1, 2, and 3 and increased acetate in Batch Test 2 (100 mg Ac/L) showed almost twice as much phosphorus uptake (8.1 mg P/L) as an addition of 10 mg Ac/L (phosphorus uptake of 4.4 mg P/L). The uptake results of Batch Tests 1, 2, and 3 within the first hour after acetate addition (t = 0) were therefore consistent with the hypothesis that PPB utilize stored polyphosphate for acetate and phosphorus uptake, however, there is the possibility that acetate or other fermentation was already present within the biomass sample before acetate addition. The data for later time points were not consistent with this trend, and so clear conclusions cannot be drawn from this experiment. Less phosphorus uptake over the first 60 minutes after acetate was added to R2 and R3 in Batch Test 3 relative to the reactor with no acetate addition (R1) supported the hypothesis that stored polyphosphate provides energy for acetate uptake and storage under dark conditions. However, phosphorus uptake occurred during the dark period and therefore contradicts the hypothesis that phosphorus was released during the dark phase. By one hour after acetate addition, phosphorus was being released in Batch Tests 1, 2, and 3. This indicated that acetate no longer had an evident effect

after one hour from the initial acetate addition. Contrary to Batch Tests 1, 2, and 3, Batch Test 4 disproved phosphorus release after one hour from acetate addition with phosphorus uptake occurring at t=60 in R4 (Figure 26).

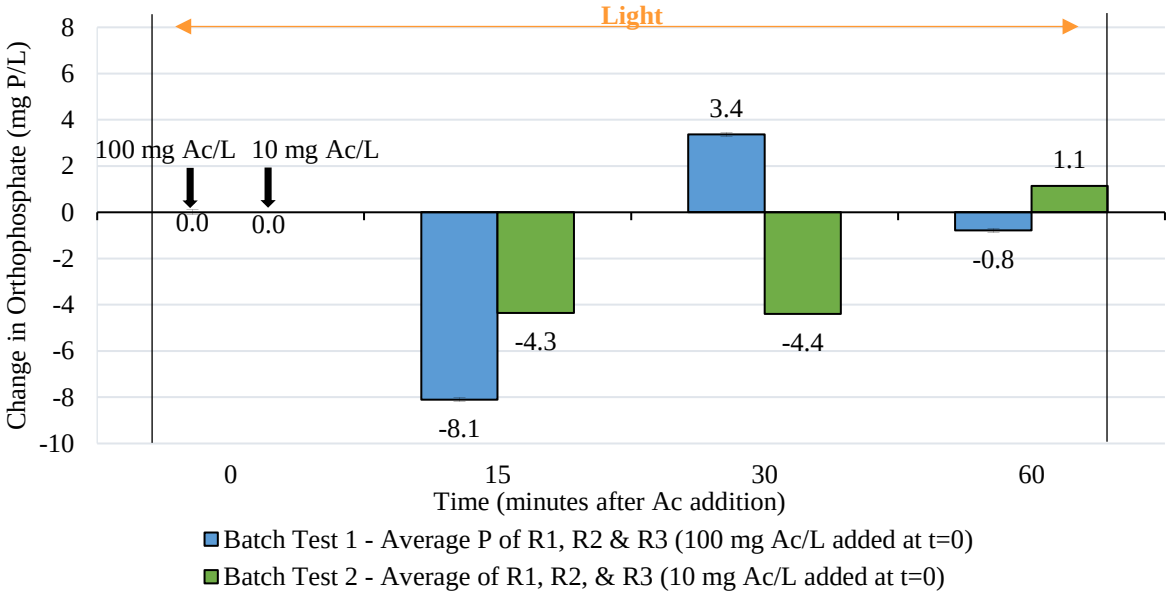

Figure 24. Batch Tests 1 and 2 average phosphorus from t = 0 to 60. Biomass from Lagoon 2. Negative values indicate phosphorus uptake and positive values indicate release. Acetate addition encouraged growth and phosphorus uptake within the first 15 minutes after acetate addition. Increasing acetate from 10 mg Ac/L to 100 mg Ac/L showed almost twice as much phosphorus uptake (8.1 mg Ac/L) as an addition of 10 mg Ac/L (phosphorus uptake of 4.4 mg Ac/L).

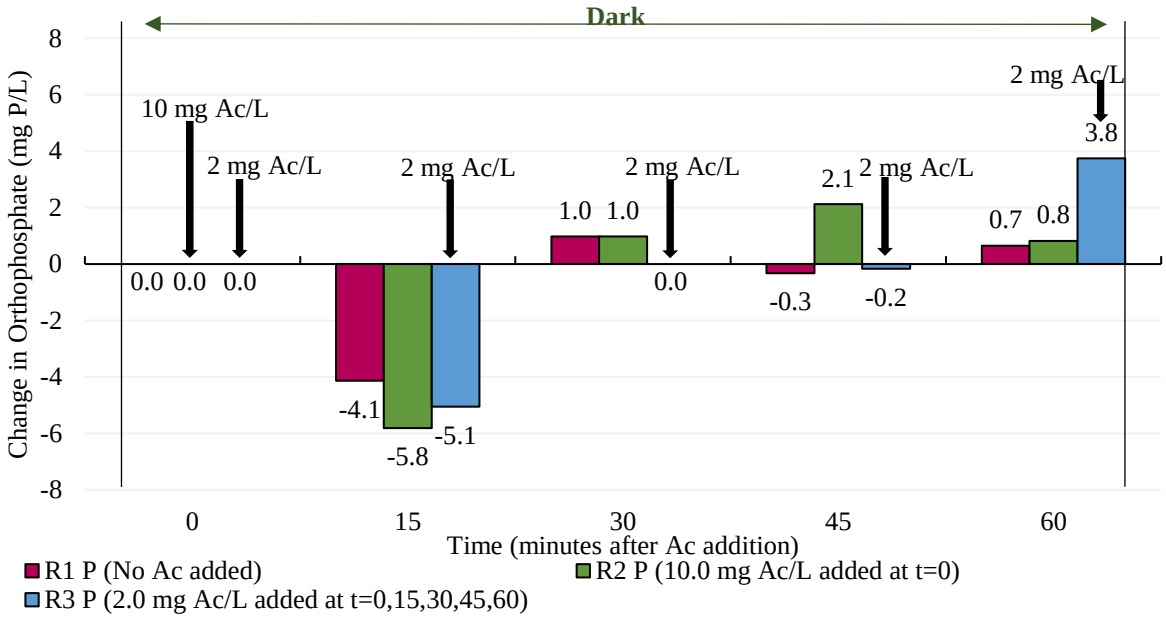

Figure 25. Batch Test 3 change in average phosphorus from t=0 to 60. Biomass from Lagoon 1. Negative values indicate phosphorus uptake and positive values indicate release. Phosphorus decreased after initial acetate addition (t=0). R2 had 10 mg Ac/L added at t=0 and R3 had 2.0 mg Ac/L added at t=0, 15, 30,45, and 60. Less phosphorus uptake after acetate was added to R2 and R3 in Batch Test 3 relative to the reactor with no acetate addition (R1) supported the hypothesis that stored polyphosphate provides energy for acetate uptake and storage under dark conditions. However, phosphorus uptake occurred during the dark period and therefore contradicts the hypothesis that phosphorus was released during the dark phase.

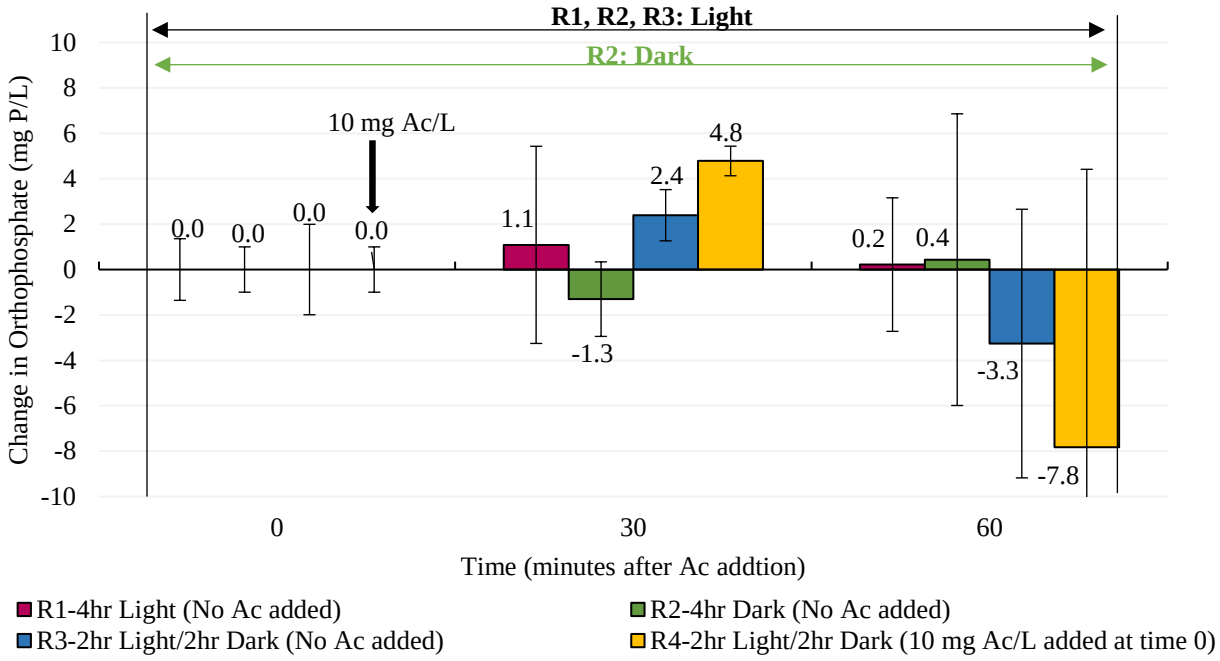

Figure 26. Batch Test 4 change in average phosphorus from t=0 to 60. Biomass from Lagoon 1. Negative values indicate phosphorus uptake and positive values indicate release. Acetate was added to R4 at t=0 (10 mg Ac/L). Phosphorus slightly increased after initial acetate addition in R4 (t=0). Phosphorus uptake occurred one hour after acetate addition in R4 and had the most uptake from t=0. Phosphorus decreased in all reactors of Batch Test 4 potentially because of acetate or fermentation already within the sample.

Summarizing the batch test data, phosphorus release and uptake were not shown to have been affected by changing light and dark conditions during a given test. These findings suggested that acetate addition did not result in phosphorus release and therefore did not support the hypothesis that stored polyphosphate serves as a source of ATP for acetate uptake and storage. The biomass was exposed to light until 60 minutes after acetate addition so photosynthesis could have provided an alternative means of producing ATP. However, phosphorus uptake occurred only after acetate addition, suggesting a link between acetate uptake and phosphorus uptake. Of the four reactors run in dark conditions, all had phosphorus uptake then release by t = 60. Of the nine reactors in light conditions after acetate addition (t = 0), six indicated phosphorus uptake. Comparing light and dark conditions from t = 0 to 60,

reactors in dark conditions seemed to generally have more probability of phosphorus uptake, however, the average phosphorus uptake values of the reactors in which uptake occurred in light conditions were higher than that of the reactors in dark conditions. It can be inferred that the PPB utilized stored polyphosphate for phosphorus uptake in the dark in which there were no other energy sources. The data for the rest of the tests were not consistent and therefore led to the assumption that photosynthesis could have encouraged phosphorus uptake more than acetate addition during the first hour of consumption (light phase) in the reactor, however, the possibility of initial acetate or other fermentation already present within the biomass sample before acetate addition should be considered. Acetate measurements indicated that all samples contained an initial average amount of 126 mg acetate/L in Batch Test 4 and likely similar values for Batch Tests 1, 2 and 3. From these findings, it can be inferred that PPB produce less energy under a dark phase but can produce acetate during this time for energy production. With all batch test results, phosphorus uptake occurred after acetate addition 75% of the time. In all batch tests, nine reactors had an addition of acetate and eight of the nine reactors showed phosphorus uptake after acetate addition. The detection of acetate in all samples in Batch 4, including the samples with no acetate addition, indicated that acetate may have existed in all of the source samples, which would affect interpretation of results. If acetate was already within the reactors of each test, small amounts of acetate addition (such as 10 mg Ac/L) may not have had an effect so majority of the phosphorus release or uptake could have been in reaction to the acetate already within the reactors. Without acetate measurements of each test, it is difficult to draw clear conclusions.

Chapter 5: Conclusions

This study included a sequencing batch test and four batch tests of dairy lagoon wastewater to assess whether PPBs in dairy lagoons may utilized polyphosphate for acetate uptake and storage in an analogous metabolism to EBPR systems. Dark phase phosphorus release coupled with acetate uptake was not detected, and so the hypothesis was not supported. Non-soluble phosphorus/TSS values indicated evidence for polyphosphate accumulation and storage as confirmed with microscopy results of polyphosphates. Phosphorus to TSS ratios were at least 0.7% higher than the typical 2% content for typical bacteria, which indicated evidence for polyphosphate accumulation. The staining results showed that polyphosphates were evident in the biomasses and therefore polyphosphate storage was plausible within the biomass. The phosphorus data for the entirety of the sequencing batch experiment was inconclusive as the levels of phosphorus were noisy and varied from uptake to release, thus indicating that there were no substantial trends that could indicate whether the PPB included in these samples had improved phosphorus removal with the addition of acetate. However, it was found that a.m. samples (taken immediately after the end of the dark phase) had generally higher phosphorus than p.m. samples (taken mid-day) in the reactor that was operated using cyclic 12-hour light and dark phases. This finding generally showed that phosphorus was released during the dark phase and utilized excess phosphorus during the light phase. This finding led to the decision to switch to batch testing.

With all batch test results, phosphorus uptake occurred after acetate addition 75% of the time. Acetate addition encouraged growth and phosphorus uptake within the first 15 minutes in Batch Tests 1, 2, and 3, suggesting that acetate uptake was linked to phosphorus uptake, possibly because of increased growth. This phosphorus uptake led to the assumption

that the PPB within the samples acted similar to PAOs of the aerobic phase of an EBPR system. However, photosynthesis could have allowed for an alternative means of ATP so these findings could not confirm the hypothesis that stored polyphosphate serves as a source of ATP for acetate uptake and storage although more uptake occurred in reactors with acetate addition. Increasing the amount of acetate added to 100 mg Ac/L showed almost twice as much uptake as an addition of 10 mg Ac/L. Comparing light and dark conditions from t = 0 to 60, reactors in dark conditions seemed to generally have more probability of phosphorus uptake, however, the average phosphorus uptake values of the reactors in which uptake occurred in light conditions were higher than that of the reactors in dark conditions.-From these findings, it can be inferred that PPB produce less energy under a dark phase but can produce acetate during this time for energy production.

These experiments were conducted with the assumption that majority of the bacterial community within the samples were heterotrophic phototrophs PNSB. 16s rRNA gene amplicon sequencing found a variety of genera within the samples and no PNSB genera. Although no PNSB genera was found, PSB genera were found in both Lagoon 1 and Lagoon 2 samples. PSB dominating approximately half of the Lagoon 1 sample, but Lagoon 2 had a minimal amount of PSB which could have added to the reasoning for inconclusive results. Findings of PSB as opposed to PNSB could have also played a role as to why the results were not as substantial because the relation to *Accumulibacter* included PNSB. However, PPB were proven to be similar to *Accumulibacter* in that both contain polyphosphates and are capable of phosphorus uptake although there were no trends that indicated PPB metabolism closely followed the *Accumulibacter* metabolism.

There were many variables within the given experiments that could have added to the nonconclusive results of majority of the experiments, but it can be inferred that controlling light and dark conditions can impact results for acetate utilization and phosphorus uptake in samples containing PPB. Further research on specific conditions to improve phosphorus removal using PPB can be implemented by meticulous controls, pure cultures, or by studying specific strains of PNSB as compared to a strain of PSB. Follow up work would include testing one variable at a time, such as light versus dark conditions, without any other variables and with a pure strain of PNSB or PSB. Specific light conditions that could be tested would include all light, all dark, half of the duration in light and then dark, and lastly half of the duration in dark and then light. A separate test can be conducted on the variable of acetate addition with a pure strain of PNSB or PSB. Variation of acetate amounts added would be best to test. After testing acetate addition and light and dark conditions separately, then there is the possibility of combining the two into an experiment. A pure strain of PNSB or PSB is also recommended for this experiment, but the next step would include an inoculated field sample containing known PNSB or PSB.

Appendices

A. Acronyms

Table A 1. *Acronyms in Order of Appearance*

Abbreviation	Meaning	Page
P	Phosphorus	1
PPB	Purple phototrophic bacteria	1
EBPR	Enhanced biological phosphorus removal	1
PAO	Polyphosphate-accumulating organism	1
PSB	Purple sulfur bacteria	3
PNSB	Purple non-sulfur bacteria	3
BChl	Bacteriochlorophyll	4
Ac	Acetate	5
PPK	Polyphosphate kinase	6
ATP	Adenosine triphosphate	7
VFAs	Volatile fatty acids	8
PHAS	Polyhydroxyalkanoates	8
PHB	Polyhydroxybutyrate	9
GAOs	Glycogen-accumulating organisms	9
R1	Reactor 1	13
R2	Reactor 2	13
TSS	Total suspended solids	18
VSS	Volatile suspended solids	18
R3	Reactor 3	19
R4	Reactor 4	19
DO	Dissolved Oxygen	20
PCR	Polymerase chain reaction	25
OTU	Operational taxonomic unit	26
GC	Gas Chromatography	26
FID	Flame ionization detector	26
SWRF	Southside Water Reclamation Facility	47

B. Raw data

Table B 1. *Sequencing Batch Reactor Experiment R1 Data Day - 124 to Day 171*

Reactor	Day	Time	DO	pH	Diluted P (mg PO4(3-)/L)	P (mg P/L)	Abs.	P change (mg P/L)	Uptake/Release
	124	10/2/19 7:06	3.02	8.78	35.70	58.21	0.436	n/a	release
	124	10/2/19 9:10	2.28	8.70	31.00	50.55	0.379	-4.70	uptake
	124	10/2/19 11:02	1.41	8.67	29.70	48.43	0.362	-1.30	uptake
	124	10/2/19 13:13	0.14	8.68	33.70	54.95	0.412	4.00	release
	124	10/2/19 15:15	0.13	8.69	31.20	50.87	0.381	-2.50	uptake
	124	10/2/19 17:08	0.11	8.69	29.50	48.10	0.360	-1.70	uptake
	124	10/2/19 19:04	0.19	8.72	32.70	53.32	0.399	3.20	release
	126	10/4/19 7:30	3.60	8.91	30.30	49.40	0.370	n/a	release
	126	10/4/19 12:08	3.54	8.88	27.90	45.49	0.340	-2.40	uptake
	129	10/7/19 7:30	3.64	8.71	23.50	38.32	0.287	n/a	release
	129	10/7/19 12:00	4.02	8.69	23.10	37.66	0.283	-0.40	uptake
	131	10/9/19 7:17	0.09	8.41	20.70	33.75	0.253	n/a	release
	131	10/9/19 13:40	0.42	8.46	21.80	35.55	0.266	1.10	release
	133	10/11/19 6:40	4.48	8.57	14.30	23.32	0.175	n/a	release
	133	10/11/19 12:08	6.78	8.53	12.90	21.03	0.158	-1.40	uptake
	136	10/14/19 7:30	6.34	8.59	14.00	22.83	0.171	n/a	release
	136	10/14/19 12:49	8.64	8.57	25.20	41.09	0.307	11.20	release
	138	10/16/19 7:30	4.06	8.41	13.60	22.18	0.166	n/a	release
	138	10/16/19 12:23	1.81	8.32	14.60	23.81	0.180	1.00	release
	140	10/18/19 7:02	4.56	8.54	8.70	14.19	0.107	n/a	release
	140	10/18/19 13:50	3.87	8.48	18.70	30.49	0.231	10.00	release
	143	10/21/19 7:03	4.73	8.57	19.70	32.12	0.245	n/a	release
	143	10/21/19 12:50	3.99	8.52	24.30	39.62	0.301	4.60	release
	145	10/23/19 7:04	2.09	8.38	20.30	33.10	0.251	n/a	release
	145	10/23/19 12:53	3.38	8.44	19.70	32.12	0.245	-0.60	uptake
	147	10/25/19 7:03	3.11	8.45	nd	n/a	nd	n/a	release
	147	10/25/19 12:27	4.51	8.52	nd	n/a	nd	nd	release
	150	10/28/19 7:05	3.45	8.55	nd	n/a	nd	n/a	release
R1 (24	150	10/28/19 12:58	4.58	8.49	nd	n/a	nd	nd	release
hr. light)	152	10/30/19 7:06	5.19	8.53	30.00	48.92	0.366	n/a	release
	152	10/30/19 23:50	5.01	8.42	28.10	45.82	0.343	-1.90	uptake
	154	11/1/19 7:07	5.03	8.56	29.80	48.59	0.364	n/a	release
	154	11/1/19 12:15	4.55	8.51	27.70	45.17	0.338	-2.10	uptake
	157	11/4/19 7:00	4.50	8.50	30.10	49.08	0.367	n/a	release
	157	11/4/19 13:30	3.96	8.45	28.20	45.98	0.344	-1.90	uptake
	159	11/6/19 7:16	3.13	8.49	29.90	48.75	0.365	n/a	release
	159	11/6/19 13:36	4.64	8.50	27.80	45.33	0.339	-2.10	uptake
	161	11/8/19 7:00	3.20	8.47	nd	nd	nd	n/a	release
	161	11/8/19 12:22	4.50	8.51	nd	nd	nd	nd	release
	164	11/11/19 7:00	7.05	8.56	nd	nd	nd	n/a	release
	164	11/11/19 12:20	8.98	8.52	nd	nd	nd	nd	release
	166	11/13/19 6:40	6.92	8.36	11.40	37.18	2.782	n/a	release
	166	11/13/19 7:07	6.87	8.44	11.00	35.87	2.685	-8.00	uptake
	166	11/13/19 7:32	7.02	8.40	12.00	39.13	2.929	20.00	release
	166	11/13/19 8:06	7.20	8.46	12.10	39.46	2.953	2.00	release
	166	11/13/19 10:22	8.01	8.41	12.20	39.78	2.977	2.00	release
	166	11/13/19 12:28	9.16	8.46	11.30	36.85	2.758	-18.00	uptake
	166	11/13/19 14:30	9.41	8.43	11.40	37.18	2.782	2.00	release
	166	11/13/19 16:30	9.57	8.50	11.70	38.15	2.855	6.00	release
	166	11/13/19 18:24	8.75	8.45	11.60	37.83	2.831	-2.00	uptake
	166	11/13/19 18:53	8.91	8.53	11.20	36.52	2.733	-8.00	uptake
	166	11/13/19 19:06	8.74	8.50	11.20	36.52	2.733	0.00	uptake
	166	11/13/19 20:10	8.45	8.55	11.40	37.18	2.782	4.00	release
	166	11/13/19 20:30	8.36	8.52	11.10	36.20	2.709	-6.00	uptake
	168	11/15/19 6:58	8.36	8.17	30.20	49.24	0.369	n/a	release
	168	11/15/19 12:01	9.22	8.40	26.50	43.21	0.323	-3.70	uptake
	171	11/18/19 6:52	7.89	8.32	28.00	45.65	0.342	n/a	release
	171	11/18/19 12:02	6.98	8.29	28.70	46.80	0.350	0.70	release

Table B 2. *Sequencing Batch Reactor Experiment R1 Data - Day 173 to Day 222*

Reactor	Day	Time	DO	pH	Diluted P (mg PO4(3-)/L)	P (mg P/L)	Abs.	P change (mg P/L)	Uptake/Release
R1 (24 hr. light)	173	11/20/19 6:55	6.72	8.52	11.70	38.15	2.855	n/a	release
	173	11/20/19 7:00	7.08	8.50	12.00	39.13	2.929	6.00	release
	173	11/20/19 7:05	8.01	8.42	12.20	39.78	2.977	4.00	release
	173	11/20/19 7:30	8.04	8.47	12.20	39.78	2.977	0.00	uptake
	173	11/20/19 8:00	9.12	8.45	11.80	38.48	2.880	-8.00	uptake
	173	11/20/19 10:25	9.07	8.47	11.40	37.18	2.782	-8.00	uptake
	173	11/20/19 12:28	9.13	8.51	11.50	37.50	2.807	2.00	release
	173	11/20/19 14:20	8.94	8.53	11.80	38.48	2.880	6.00	release
	173	11/20/19 16:30	8.02	8.51	11.50	37.50	2.807	-6.00	uptake
	173	11/20/19 18:35	8.90	8.46	11.60	37.83	2.831	2.00	release
	173	11/20/19 19:16	8.74	8.40	11.80	38.48	2.880	4.00	release
	173	11/20/19 19:50	8.41	8.45	11.50	37.50	2.807	-6.00	uptake
	178	11/25/19 7:30	6.77	8.42	16.68	27.20	0.332	n/a	release
	178	11/25/19 13:02	8.99	8.50	17.85	29.10	0.355	1.90	release
	180	11/27/19 7:04	7.04	8.53	17.23	28.09	0.343	n/a	release
	180	11/27/19 13:34	9.47	8.51	18.46	30.10	0.367	2.00	release
	221	1/7/20 18:40	4.45	8.25	16.44	26.81	0.328	n/a	release
	221	1/7/20 19:06	4.88	8.27	19.81	32.30	0.359	3.37	release
	221	1/7/20 19:30	3.84	8.27	15.33	25.00	0.306	-4.48	uptake
	221	1/7/20 21:08	4.96	8.30	19.01	31.00	0.379	3.68	release
	221	1/7/20 23:06	2.50	8.27	17.54	28.60	0.349	-1.47	uptake
	222	1/8/20 1:11	5.12	8.42	26.25	42.80	0.522	8.71	release
	222	1/8/20 3:03	4.80	8.29	19.63	32.01	0.391	-6.62	uptake
	222	1/8/20 6:46	5.06	8.38	17.05	27.80	0.339	-2.58	uptake
	222	1/8/20 7:05	4.83	8.33	17.54	28.60	0.349	0.49	release
	222	1/8/20 7:34	5.00	8.37	18.89	30.80	0.376	1.35	release
	222	1/8/20 8:01	4.89	8.30	22.94	37.40	0.457	4.05	release
	222	1/8/20 9:02	4.99	8.36	19.93	32.50	0.397	-3.01	uptake
	222	1/8/20 11:10	4.95	8.31	20.67	33.70	0.411	0.74	release
	222	1/8/20 13:15	4.96	8.34	19.93	32.50	0.396	-0.74	uptake
	222	1/8/20 15:14	4.91	8.38	22.63	36.90	0.451	2.70	release
	222	1/8/20 17:08	5.00	8.34	29.50	48.10	0.587	6.87	release
	222	1/8/20 18:42	4.98	8.34	23.43	38.20	0.466	-6.07	uptake
	222	1/8/20 19:10	5.08	8.35	27.66	45.10	0.551	4.23	release
	222	1/8/20 19:35	4.99	8.22	23.31	38.01	0.464	0.68	release

Table B 3. *Sequencing Batch Reactor Experiment R2 Data - Day 124 to Day 171*

Reactor	Day	Time	DO	pH	Diluted P (mg PO4(3-)/L)	P (mg P/L)	Abs.	P change (mg P/L)	Uptake/Release
	124	10/2/19 7:08	0.12	8.48	20.60	33.59	0.251	n/a	release
	124	10/2/19 9:12	0.26	8.53	17.70	28.86	0.216	-2.90	uptake
	124	10/2/19 11:05	0.12	8.46	19.20	31.31	0.234	1.50	release
	124	10/2/19 13:15	2.31	8.51	19.60	31.96	0.240	0.40	release
	124	10/2/19 15:17	4.54	8.47	19.70	32.12	0.241	0.10	release
	124	10/2/19 17:10	6.52	8.50	20.10	32.77	0.246	0.40	release
	124	10/2/19 19:06	7.48	8.49	19.20	31.31	0.234	-0.90	uptake
	126	10/4/19 7:32	7.00	8.56	16.80	27.39	0.206	n/a	release
	126	10/4/19 12:10	6.02	8.61	18.10	29.51	0.221	1.30	release
	129	10/7/19 7:32	7.18	8.59	15.50	25.27	0.190	n/a	release
	129	10/7/19 12:03	8.66	8.60	19.10	31.14	0.233	3.60	release
	131	10/9/19 7:20	0.08	8.55	19.90	32.45	0.243	n/a	release
	131	10/9/19 13:42	10.20	8.60	14.60	23.81	0.178	-5.30	uptake
	133	10/11/19 6:42	3.07	8.50	17.10	27.88	0.209	n/a	release
	133	10/11/19 12:02	4.02	8.42	9.50	15.49	0.116	-7.60	uptake
	136	10/14/19 7:33	2.60	8.45	12.60	20.54	0.154	n/a	release
	136	10/14/19 12:54	3.77	8.43	21.80	35.55	0.266	9.20	release
	138	10/16/19 7:28	3.70	8.39	14.20	23.15	0.173	n/a	release
	138	10/16/19 12:20	6.48	8.29	7.40	12.07	0.091	-6.80	uptake
	140	10/18/19 7:04	3.47	8.44	8.80	14.35	0.108	n/a	release
	140	10/18/19 13:53	3.02	8.39	17.30	28.21	0.215	8.50	release
	143	10/21/19 7:06	3.92	8.53	22.00	35.87	0.272	n/a	release
	143	10/21/19 12:53	2.91	8.46	21.90	35.71	0.270	-0.10	uptake
	145	10/23/19 7:00	1.75	8.43	22.00	35.87	0.272	n/a	release
	145	10/23/19 12:59	2.21	8.39	21.60	35.22	0.270	-0.40	uptake
	147	10/25/19 7:07	2.30	8.34	nd	n/a	nd	n/a	release
	147	10/25/19 12:31	3.01	8.32	nd	n/a	nd	nd	release
R2 (12	150	10/28/19 7:09	2.79	8.36	nd	n/a	nd	n/a	release
hr.	150	10/28/19 12:50	3.29	8.36	nd	n/a	nd	nd	release
light/12	152	10/30/19 7:08	3.17	8.29	29.30	47.77	0.358	n/a	release
hr. dark)	152	10/30/19 12:00	4.08	8.33	25.90	42.23	0.316	-3.40	uptake
	154	11/1/19 7:10	2.96	8.37	29.00	47.28	0.354	n/a	release
	154	11/1/19 12:17	3.95	8.32	26.40	43.05	0.322	-2.60	uptake
	157	11/4/19 7:02	3.07	8.41	29.70	48.43	0.362	n/a	release
	157	11/4/19 13:35	4.13	8.44	26.80	43.70	0.327	-2.90	uptake
	159	11/6/19 7:18	0.15	8.36	28.70	46.80	0.350	n/a	release
	159	11/6/19 13:40	2.11	8.43	26.80	43.70	0.327	-1.90	uptake
	161	11/8/19 7:04	8.17	8.33	nd	nd	nd	n/a	release
	161	11/8/19 12:26	2.10	8.44	nd	nd	nd	nd	release
	164	11/11/19 7:05	2.97	8.34	nd	nd	nd	n/a	release
	164	11/11/19 12:30	4.12	8.40	nd	nd	nd	nd	release
	166	11/13/19 6:42	2.83	8.31	11.60	37.83	2.831	n/a	release
	166	11/13/19 7:05	2.78	8.33	11.70	38.15	2.855	2.00	release
	166	11/13/19 7:35	2.88	8.34	11.80	38.48	2.880	2.00	release
	166	11/13/19 8:04	2.91	8.37	11.60	37.83	2.831	-4.00	uptake
	166	11/13/19 10:25	3.38	8.30	11.60	37.83	2.831	0.00	uptake
	166	11/13/19 12:25	3.57	8.35	11.50	37.50	2.807	-2.00	uptake
	166	11/13/19 14:33	4.09	8.30	11.20	36.52	2.733	-6.00	uptake
	166	11/13/19 16:28	4.06	8.37	11.40	37.18	2.782	4.00	release
	166	11/13/19 18:27	4.22	8.32	11.00	35.87	2.685	-8.00	uptake
	166	11/13/19 18:50	4.45	8.35	11.90	38.81	2.904	18.00	release
	166	11/13/19 19:08	4.44	8.39	11.60	37.83	2.831	-6.00	uptake
	166	11/13/19 20:07	4.44	8.41	11.60	37.83	2.831	0.00	uptake
	166	11/13/19 20:33	4.54	8.40	11.50	37.50	2.807	-2.00	uptake
	168	11/15/19 7:01	3.50	8.31	27.90	45.49	0.340	n/a	release
	168	11/15/19 12:05	4.01	8.32	28.60	46.63	0.349	0.70	release
	171	11/18/19 6:55	3.41	8.24	27.60	45.00	0.337	n/a	release
	171	11/18/19 12:06	4.09	8.31	26.40	43.05	0.322	-1.20	uptake

Table B 4. *Sequencing Batch Reactor Experiment R2 Data - Day 173 to Day 222*

Reactor	Day	Time	DO	pH	Diluted P (mg PO4(3-)/L)	P (mg P/L)	Abs.	P change (mg P/L)	Uptake/Release
R2 (12 hr. light/12 hr. dark)	173	11/20/19 6:58	2.71	8.43	12.30	40.11	3.002	n/a	release
	173	11/20/19 7:02	3.03	8.45	11.70	38.15	2.855	-12.00	uptake
	173	11/20/19 7:07	2.94	8.39	11.60	37.83	2.831	-2.00	uptake
	173	11/20/19 7:32	4.05	8.29	11.50	37.50	2.807	-2.00	uptake
	173	11/20/19 8:02	3.50	8.32	11.60	37.83	2.831	2.00	release
	173	11/20/19 10:28	3.99	8.35	11.60	37.83	2.831	0.00	uptake
	173	11/20/19 12:30	4.01	8.32	11.45	37.34	2.794	-3.00	uptake
	173	11/20/19 14:22	4.03	8.31	11.45	37.34	2.794	0.00	uptake
	173	11/20/19 16:33	4.19	8.33	11.55	37.66	2.819	2.00	release
	173	11/20/19 18:39	4.23	8.30	11.75	38.32	2.868	4.00	release
	173	11/20/19 19:18	4.24	8.36	11.90	38.81	2.904	3.00	release
	173	11/20/19 19:54	4.57	8.35	12.00	39.13	2.929	2.00	release
	178	11/25/19 7:40	3.01	8.29	17.36	28.31	0.345	n/a	release
	178	11/25/19 13:05	3.97	8.34	17.54	28.60	0.349	0.30	release
	180	11/27/19 7:06	3.19	8.44	17.17	28.00	0.342	n/a	release
	180	11/27/19 13:39	4.10	8.38	18.22	29.71	0.362	1.70	release
	221	1/7/20 18:42	4.95	8.26	17.97	29.30	0.358	n/a	release
	221	1/7/20 19:04	4.97	8.30	22.63	36.90	0.451	4.66	release
	221	1/7/20 19:33	5.03	8.32	19.81	32.30	0.395	-2.82	uptake
	221	1/7/20 21:06	4.89	8.34	23.92	39.00	0.476	4.11	release
	221	1/7/20 23:08	4.90	8.36	17.91	29.20	0.356	-6.01	uptake
	222	1/8/20 1:09	4.93	8.36	27.05	44.11	0.539	9.14	release
	222	1/8/20 3:06	5.07	8.38	21.34	34.80	0.425	-5.71	uptake
	222	1/8/20 6:44	5.02	8.43	19.20	31.31	0.383	-2.14	uptake
	222	1/8/20 7:07	4.98	8.41	17.60	28.70	0.351	-1.60	uptake
	222	1/8/20 7:32	4.97	8.36	20.91	34.09	0.416	3.31	release
	222	1/8/20 8:03	4.96	8.37	20.85	34.00	0.415	-0.06	uptake
	222	1/8/20 9:00	5.11	8.39	21.65	35.30	0.431	0.80	release
	222	1/8/20 11:12	5.13	8.36	21.77	35.50	0.433	0.12	release
	222	1/8/20 13:13	5.15	8.32	23.00	37.50	0.457	1.23	release
	222	1/8/20 15:17	5.11	8.36	21.04	34.31	0.419	-1.96	uptake
	222	1/8/20 17:06	5.12	8.35	26.13	42.61	0.520	5.09	release
	222	1/8/20 18:45	5.27	8.38	23.12	37.70	0.461	-3.01	uptake
	222	1/8/20 19:08	5.18	8.37	26.49	43.19	0.527	3.37	release
	222	1/8/20 19:37	5.16	8.29	22.69	37.00	0.452	1.65	release

Table B 5. *Batch Test 1 Data*

Reactor	Time After Ac (min.)	Time	DO	pH	P (mg PO4(3-)/L) (diluted 1:5)	P (mg P/L) (diluted 1:5)	P (mg P/L)	Abs	P change (mg P/L)	Uptake/ Release
R1 (Ac added at time 0)	(60)	9/9/2020 15:02	0.03	9.12	36.4	11.87	59.35	0.445	n/a	n/a
	0	9/9/2020 16:02	0.03	9.12	36.4	11.87	59.35	n/a	n/a	n/a
	15	9/9/2020 16:17	0.03	9.13	32.3	10.52	52.58	0.394	-2.7	uptake
	30	9/9/2020 16:32	0.02	9.13	33.7	10.97	54.87	0.411	0.9	release
	60	9/9/2020 17:02	0.02	9.09	34.2	11.15	55.76	0.418	0.4	release
	120	9/9/2020 18:02	0.02	9.08	34.6	11.28	56.42	0.423	0.3	release
R2 (Ac added at time 0)	(60)	9/9/2020 15:06	0.03	9.12	36.4	11.87	59.35	0.445	n/a	n/a
	0	9/9/2020 16:06	0.03	9.12	36.4	11.87	59.35	n/a	n/a	n/a
	15	9/9/2020 16:21	0.01	9.11	29.1	9.47	47.37	0.355	-4.8	uptake
	30	9/9/2020 16:36	0.01	9.1	33.9	11.05	55.27	0.414	3.2	release
	60	9/9/2020 17:06	0.01	9.09	32.7	10.65	53.24	0.399	-0.8	uptake
	120	9/9/2020 18:06	0.01	9.07	33.8	11.01	55.03	0.412	0.7	release
R3 (Ac added at time 0)	(60)	9/9/2020 15:10	0.03	9.12	36.4	11.87	59.35	0.445	n/a	n/a
	0	9/9/2020 16:10	0.03	9.12	36.4	11.87	59.35	n/a	n/a	n/a
	15	9/9/2020 16:25	0.02	9.1	33.0	10.76	53.81	0.403	-2.2	uptake
	30	9/9/2020 16:40	0.03	9.1	33.0	10.75	53.73	0.402	0.0	uptake
	60	9/9/2020 17:10	0.03	9.08	32.2	10.50	52.50	0.393	-0.5	uptake
	120	9/9/2020 18:10	0.01	9.08	33.2	10.83	54.13	0.405	0.7	release

Table B 6. *Batch Test 2 Data*

Reactor	Time After Ac (min.)	Time	DO	pH	P (mg PO4(3-)/L) (diluted 1:5)	P (mg P/L) (diluted 1:5)	P (mg P/L)	Abs	P change (mg P/L)	Uptake/ Release
R1 (Ac added at time 0)	(60)	9/21/2020 12:00	0.02	8.28	38.8	12.65	63.26	0.474	n/a	n/a
	-	9/21/2020 13:00	0.02	8.28	38.8	12.65	63.26	n/a	n/a	n/a
	15	9/21/2020 13:15	0.02	8.29	37.3	12.16	60.82	0.455	-0.5	uptake
	30	9/21/2020 13:30	0.03	8.35	33.2	10.83	54.13	0.405	-1.3	uptake
	60	9/21/2020 14:00	0.01	8.3	33.7	10.99	54.95	0.411	0.2	release
	120	9/21/2020 15:00	0.03	8.31	33.3	10.86	54.30	0.407	0.0	release
	180	9/21/2020 16:00	0.02	8.3	34.9	11.38	56.90	0.426	0.6	release
	240	9/21/2020 17:00	0.01	8.3	21.9	7.14	35.71	0.268	-3.8	uptake
R2 (Ac added at time 0)	(60)	9/21/2020 12:04	0.03	8.32	39.5	12.88	64.41	0.481	n/a	n/a
	-	9/21/2020 13:04	0.03	8.32	39.5	12.88	64.41	n/a	n/a	n/a
	15	9/21/2020 13:19	0.02	8.34	37.0	12.07	60.33	0.451	-0.8	uptake
	30	9/21/2020 13:34	0.02	8.35	34.4	11.22	56.09	0.420	-0.8	uptake
	60	9/21/2020 14:04	0.01	8.33	35.4	11.54	57.72	0.432	0.3	release
	120	9/21/2020 15:04	0.01	8.31	34.7	11.32	56.58	0.424	0.1	release
	180	9/21/2020 16:04	0.01	8.31	35.4	11.54	57.72	0.432	0.3	release
	240	9/21/2020 17:04	0.01	8.3	20.3	6.62	33.10	0.248	-4.9	uptake
R3 (Ac added at time 0)	(60)	9/21/2020 12:08	0.03	8.31	39.8	12.98	64.89	0.486	n/a	n/a
	-	9/21/2020 13:08	0.03	8.31	39.8	12.98	64.89	n/a	n/a	n/a
	15	9/21/2020 13:23	0.03	8.31	35.8	11.67	58.37	0.437	-1.3	uptake
	30	9/21/2020 13:38	0.01	8.35	34.4	11.22	56.09	0.420	-0.5	uptake
	60	9/21/2020 14:08	0.01	8.34	35.0	11.41	57.07	0.428	0.2	release
	120	9/21/2020 15:08	0.01	8.32	34.8	11.35	56.74	0.425	0.1	release
	180	9/21/2020 16:08	0.01	8.3	35.7	11.64	58.21	0.436	0.4	release
	240	9/21/2020 17:08	0.01	8.3	20.4	6.65	33.26	0.249	-4.8	uptake

Table B 7. *Batch Test 3 Data*

Reactor	Time After Ac (min.)	Time	DO	pH	P (mg PO4(3-) /L) (diluted 1:5)	P (mg P/L) (diluted 1:5)	P (mg P/L)	Abs	P change (mg P/L)	Uptake/ Release
R1 (No Ac)	(60)	9/28/2020 10:00	0.03	8.32	39.1	12.75	63.75	0.477	n/a	n/a
	(45)	9/28/2020 10:15	0.03	8.31	34.1	11.12	55.60	0.417	-1.6	uptake
	(30)	9/28/2020 10:30	0.03	8.31	34.2	11.15	55.76	0.418	0.0	release
	-	9/28/2020 11:00	0.03	8.32	36.5	11.90	59.51	0.446	0.8	release
	-	9/28/2020 11:00	0.03	8.32	36.0	11.74	58.70	0.440	-0.2	uptake
	-	9/28/2020 11:00	0.03	8.32	35.9	11.71	58.54	0.439	0.0	uptake
	-	9/28/2020 11:00	0.03	8.32	36.1	11.78	58.92	0.442	0.2	release
	-	9/28/2020 11:00	0.03	8.32	36.1	11.78	58.92	n/a	n/a	n/a
	15	9/28/2020 11:15	0.03	8.3	33.6	10.96	54.79	0.410	-0.8	uptake
	30	9/28/2020 11:30	0.03	8.31	34.2	11.15	55.76	0.418	0.2	release
	45	9/28/2020 11:45	0.02	8.3	34.0	11.09	55.44	0.415	-0.1	uptake
	60	9/28/2020 12:00	0.03	8.3	34.4	11.22	56.09	0.420	0.1	release
	120	9/28/2020 13:00	0.03	8.31	36.8	12.00	60.00	0.450	0.8	release
	120	9/28/2020 13:00	0.03	8.31	36.2	11.80	59.02	0.442	-0.2	uptake
	120	9/28/2020 13:00	0.03	8.31	36.8	12.00	60.00	0.450	0.2	release
	120	9/28/2020 13:00	0.03	8.31	36.6	11.94	59.68	0.447	0.3	release
	180	9/28/2020 14:00	0.02	8.31	36.7	11.97	59.84	0.448	0.0	uptake
	(60)	9/28/2020 10:04	0.02	8.32	39.5	12.88	64.41	0.483		n/a
R2 (Ac added at time 0)	-45	9/28/2020 10:19	0.02	8.31	34.5	11.25	56.25	0.421	-1.6	uptake
	-30	9/28/2020 10:34	0.03	8.31	34.4	11.22	56.09	0.419	0.0	uptake
	-	9/28/2020 11:04	0.03	8.31	37.9	12.36	61.80	0.463	1.1	release
	-	9/28/2020 11:04	0.03	8.31	37.5	12.23	61.14	0.458	-0.1	uptake
	-	9/28/2020 11:04	0.03	8.31	37.3	12.16	60.82	0.455	-0.1	uptake
	-	9/28/2020 11:04	0.03	8.31	37.6	12.25	61.25	0.459	0.3	release
	-	9/28/2020 11:04	0.03	8.31	37.6	12.25	61.25	n/a	n/a	n/a
	15	9/28/2020 11:19	0.03	8.33	34	11.09	55.44	0.415	-1.2	uptake
	30	9/28/2020 11:34	0.02	8.3	34.6	11.28	56.42	0.423	0.2	release
	45	9/28/2020 11:49	0.02	8.31	35.9	11.71	58.54	0.438	0.4	release
	60	9/28/2020 12:04	0.02	8.3	36.4	11.87	59.35	0.445	0.2	release
	120	9/28/2020 13:04	0.02	8.31	35.4	11.54	57.72	0.432	-0.3	uptake
	120	9/28/2020 13:04	0.02	8.31	36.1	11.77	58.86	0.441	0.2	release
	120	9/28/2020 13:04	0.02	8.31	36.2	11.80	59.02	0.442	0.0	release
	120	9/28/2020 13:04	0.02	8.31	35.9	11.71	58.54	0.438	0.0	uptake
	180	9/28/2020 14:04	0.02	8.3	34.0	11.09	55.44	0.415	-0.7	uptake
	(60)	9/28/2020 10:08	0.02	8.32	39.7	12.95	64.73	0.485	n/a	n/a
	(45)	9/28/2020 10:23	0.03	8.32	33.6	10.96	54.79	0.410	-2.0	uptake
R3 (Ac added at time=0, 15, 30, 45, 60)	(30)	9/28/2020 10:38	0.03	8.31	33.7	10.99	54.95	0.411	0.0	release
	-	9/28/2020 11:08	0.02	8.31	36.4	11.87	59.35	0.445	0.9	release
	-	9/28/2020 11:08	0.02	8.31	36.3	11.84	59.19	0.444	0.8	release
	-	9/28/2020 11:08	0.02	8.31	36.8	12.00	60.00	0.449	1.0	release
	-	9/28/2020 11:08	0.02	8.31	36.5	11.90	59.51	0.446	0.9	release
	-	9/28/2020 11:08	0.02	8.31	36.5	11.90	59.51	n/a	n/a	n/a
	15	9/28/2020 11:23	0.03	8.31	33.4	10.89	54.46	0.408	-1.0	uptake
	30	9/28/2020 11:38	0.02	8.33	33.4	10.89	54.46	0.408	0.0	uptake
	45	9/28/2020 11:53	0.02	8.31	33.3	10.86	54.30	0.406	0.0	uptake
	60	9/28/2020 12:08	0.03	8.32	35.6	11.61	58.05	0.435	0.8	release
	75	9/28/2020 12:23	0.03	8.3	36.1	11.77	58.86	0.441	0.2	release
	120	9/28/2020 13:08	0.03	8.3	35.9	11.71	58.54	0.439	-0.1	uptake
	120	9/28/2020 13:08	0.03	8.3	36.3	11.84	59.19	0.443	0.1	release
	120	9/28/2020 13:08	0.03	8.3	36.4	11.87	59.35	0.445	0.0	release
	120	9/28/2020 13:08	0.03	8.3	36.2	11.80	59.02	0.442	0.0	release
	180	9/28/2020 14:08	0.02	8.31	35.4	11.54	57.72	0.433	-0.3	uptake
	(60)	9/28/2020 10:00	0.03	8.32	39.1	12.75	63.75	0.477	n/a	n/a
	(45)	9/28/2020 10:15	0.03	8.31	34.1	11.12	55.60	0.417	-1.6	uptake

Table B 8. *Batch Test 4 - R1 and R2 Data*

Reactor	Time After Ac (min.)	Time	DO	pH	P (mg PO4(3-)/L) (diluted 1:20)	P (mg P/L) (diluted 1:20)	P (mg P/L)	Abs	P change (mg P/L)	Uptake/ Release	GC-Acetate (mg Ac/L)
R1 (No Ac, Light)	(60)	3/16/2021 4:55	0.00	9.00	9.0	2.93	58.70	0.110	n/a	n/a	128.068
	(60)	3/16/2021 4:55	0.00	9.00	9.4	3.07	61.31	0.115	0.1	release	126.843
	(60)	3/16/2021 4:55	0.00	9.00	9.3	3.03	60.66	0.114	0.0	uptake	133.945
	-	3/16/2021 5:55	0.01	9.01	9.1	2.97	59.35	0.111	-0.1	uptake	110.424
	-	3/16/2021 5:55	0.01	9.01	9.0	2.93	58.70	0.109	0.0	uptake	134.590
	-	3/16/2021 5:55	0.01	9.01	7.9	2.58	51.52	0.097	-0.4	uptake	146.163
	30	3/16/2021 6:25	0.02	9.03	9.3	3.03	60.66	0.113	0.5	release	120.864
	30	3/16/2021 6:25	0.02	9.03	8.8	2.87	57.39	0.108	-0.2	uptake	131.705
	30	3/16/2021 6:25	0.02	9.03	8.4	2.74	54.79	0.103	-0.1	uptake	133.141
	60	3/16/2021 6:55	0.00	9.01	8.7	2.84	56.74	0.106	0.1	release	132.223
	60	3/16/2021 6:55	0.00	9.01	8.9	2.90	58.05	0.109	0.1	release	131.753
	60	3/16/2021 6:55	0.00	9.01	8.5	2.77	55.44	0.104	-0.1	uptake	129.615
	120	3/16/2021 7:55	0.01	8.99	8.2	2.67	53.48	0.100	-0.1	uptake	121.842
	120	3/16/2021 7:55	0.01	8.99	8.3	2.71	54.13	0.101	0.0	release	125.748
	120	3/16/2021 7:55	0.01	8.99	7.8	2.54	50.87	0.096	-0.2	uptake	126.022
	180	3/16/2021 8:55	0.00	9.01	8.0	2.61	52.18	0.097	0.1	release	119.104
	180	3/16/2021 8:55	0.00	9.01	9.6	3.13	62.61	0.117	0.5	release	118.080
	180	3/16/2021 8:55	0.00	9.01	7.9	2.58	51.52	0.097	-0.6	uptake	117.149
R2 (No Ac, Dark)	(60)	3/16/2021 5:00	0.00	9.02	9.2	3.00	60.00	0.112	n/a	n/a	133.487
	(60)	3/16/2021 5:00	0.00	9.02	8.9	2.90	58.05	0.109	-0.1	uptake	126.887
	(60)	3/16/2021 5:00	0.00	9.02	9.1	2.97	59.35	0.111	0.1	release	122.408
	-	3/16/2021 6:00	0.00	9.01	9.1	2.97	59.35	0.111	0.1	release	120.227
	-	3/16/2021 6:00	0.00	9.01	9.3	3.03	60.66	0.113	0.1	release	119.014
	-	3/16/2021 6:00	0.00	9.01	8.8	2.87	57.39	0.108	-0.2	uptake	115.851
	30	3/16/2021 6:30	0.01	8.98	10.1	3.29	65.87	0.123	0.4	release	121.779
	30	3/16/2021 6:30	0.01	8.98	8.7	2.84	56.74	0.107	-0.5	uptake	118.797
	30	3/16/2021 6:30	0.01	8.98	8.2	2.67	53.48	0.100	-0.2	uptake	113.874
	60	3/16/2021 7:00	0.01	9.00	8.2	2.67	53.48	0.100	0.0	uptake	108.316
	60	3/16/2021 7:00	0.01	9.00	8.4	2.74	54.79	0.103	0.1	release	128.715
	60	3/16/2021 7:00	0.01	9.00	8.4	2.74	54.79	0.102	0.0	uptake	113.626
	120	3/16/2021 8:00	0.02	9.02	8.3	2.71	54.13	0.101	0.0	uptake	115.715
	120	3/16/2021 8:00	0.02	9.02	10.1	3.29	65.87	0.123	0.6	release	106.143
	120	3/16/2021 8:00	0.02	9.02	7.9	2.58	51.52	0.096	-0.7	uptake	106.830
	180	3/16/2021 9:00	0.00	9.01	7.1	2.32	46.31	0.087	-0.3	uptake	99.315
	180	3/16/2021 9:00	0.00	9.01	7.6	2.48	49.57	0.092	0.2	release	103.562
	180	3/16/2021 9:00	0.00	9.01	7.2	2.35	46.96	0.088	-0.1	uptake	99.552

Table B 9. *Batch Test 4 - R3 and R4 Data*

Reactor	Time After Ac (min.)	Time	DO	pH	P (mg PO4(3-)/L) (diluted 1:20)	P (mg P/L) (diluted 1:20)	P (mg P/L)	Abs	P change (mg P/L)	Uptake/ Release	GC-Acetate (mg Ac/L)
R1 (No Ac, 2 hr. Light/ 2 hr. Dark)	(60)	3/16/2021 5:04	0.01	9.01	9.4	3.07	61.31	0.115	n/a	n/a	124.111
	(60)	3/16/2021 5:04	0.01	9.01	9.0	2.93	58.70	0.110	-0.1	uptake	119.337
	(60)	3/16/2021 5:04	0.01	9.01	8.8	2.87	57.39	0.108	-0.1	uptake	115.006
	-	3/16/2021 6:04	0.00	9.01	8.5	2.77	55.44	0.104	-0.2	uptake	110.383
	-	3/16/2021 6:04	0.00	9.01	8.2	2.67	53.48	0.100	-0.1	uptake	105.952
	-	3/16/2021 6:04	0.00	9.01	8.5	2.77	55.44	0.104	0.1	release	114.886
	30	3/16/2021 6:34	0.01	9.01	8.5	2.77	55.44	0.104	0.0	uptake	107.828
	30	3/16/2021 6:34	0.01	9.01	10.3	3.36	67.18	0.125	0.6	release	105.176
	30	3/16/2021 6:34	0.01	9.01	9.6	3.13	62.61	0.117	-0.2	uptake	106.745
	60	3/16/2021 7:04	0.01	9.02	8.0	2.61	52.18	0.097	-0.5	uptake	104.652
	60	3/16/2021 7:04	0.01	9.02	8.3	2.71	54.13	0.102	0.1	release	105.888
	60	3/16/2021 7:04	0.01	9.02	8.1	2.64	52.83	0.099	-0.1	uptake	101.892
	120	3/16/2021 8:04	0.00	9.03	8.4	2.74	54.79	0.102	0.1	release	106.042
	120	3/16/2021 8:04	0.00	9.03	9.2	3.00	60.00	0.113	0.3	release	110.078
	120	3/16/2021 8:04	0.00	9.03	8.2	2.67	53.48	0.101	-0.3	uptake	117.231
	180	3/16/2021 9:04	0.01	9.02	8.6	2.80	56.09	0.104	0.1	release	115.888
	180	3/16/2021 9:04	0.01	9.02	9.4	3.07	61.31	0.115	0.3	release	110.409
	180	3/16/2021 9:04	0.01	9.02	8.6	2.80	56.09	0.105	-0.3	uptake	102.710
R2 (Ac added at time 0, 2 hr. Light/ 2 hr. Dark)	(60)	3/16/2021 5:08	0.00	9.00	9.3	3.03	60.66	0.114	n/a	n/a	139.127
	(60)	3/16/2021 5:08	0.00	9.00	9.1	2.97	59.35	0.111	-0.1	uptake	124.378
	(60)	3/16/2021 5:08	0.00	9.00	9.0	2.93	58.70	0.110	0.0	uptake	122.591
	-	3/16/2021 6:08	0.02	9.01	8.6	2.80	56.09	0.105	-0.2	uptake	118.650
	-	3/16/2021 6:08	0.02	9.01	8.4	2.74	54.79	0.102	-0.1	uptake	107.794
	-	3/16/2021 6:08	0.02	9.01	8.5	2.77	55.44	0.104	0.0	release	108.617
	30	3/16/2021 6:38	0.00	9.02	7.4	2.41	48.26	0.095	-0.4	uptake	117.282
	30	3/16/2021 6:38	0.00	9.02	7.5	2.45	48.92	0.092	0.0	release	110.840
	30	3/16/2021 6:38	0.00	9.02	10.7	3.49	69.79	0.131	1.0	release	112.059
	60	3/16/2021 7:08	0.02	9.01	7.3	2.38	47.61	0.089	-1.1	uptake	111.931
	60	3/16/2021 7:08	0.02	9.01	7.6	2.48	49.57	0.093	0.1	release	110.011
	60	3/16/2021 7:08	0.02	9.01	7.1	2.32	46.31	0.086	-0.2	uptake	111.408
	120	3/16/2021 8:08	0.01	8.99	9.4	3.07	61.31	0.115	0.8	release	114.331
	120	3/16/2021 8:08	0.01	8.99	8.2	2.67	53.48	0.100	-0.4	uptake	109.867
	120	3/16/2021 8:08	0.01	8.99	8.0	2.61	52.18	0.097	-0.1	uptake	110.311
	180	3/16/2021 9:08	0.01	9.02	7.9	2.58	51.52	0.097	0.0	uptake	111.506
	180	3/16/2021 9:08	0.01	9.02	8.5	2.77	55.44	0.104	0.2	release	115.658
	180	3/16/2021 9:08	0.01	9.02	7.7	2.51	50.22	0.094	-0.3	uptake	125.401

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