NUTRIENT TRANSFORMATIONS IN ALGAE RACEWAY PONDS FED
MUNICIPAL WASTEWATER

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by

Carter Michael Reiff

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COMMITTEE MEMBERSHIP

TITLE: Nutrient Transformations in Algae Raceway Ponds Fed
Municipal Wastewater

AUTHOR: Carter Michael Reiff

DATE SUBMITTED: June 2015

COMMITTEE CHAIR: Dr. Tryg Lundquist, Ph.D.
Associate Professor of Civil & Environmental Engineering

COMMITTEE MEMBER: Dr. Rebekah Oulton, Ph.D.
Assistant Professor of Civil & Environmental Engineering

COMMITTEE MEMBER: Dr. Greg Schwartz, Ph.D.
Assistant Professor of BioResource and Agricultural
Engineering

ABSTRACT

Nutrient Transformations in Algae Raceway Ponds Fed Municipal Wastewater

Carter Michael Reiff

Microalgae can be useful in wastewater treatment processes by releasing dissolved oxygen into the water and assimilating nutrients. After treatment, the resulting algal-bacterial biomass can be harvested and used as a biofuel feedstock while the water is discharged or reused off-site. However, to sustainably produce nationally significant quantities of algae biofuel, the water and nutrients used in cultivation would need to be recycled on site for additional algae cultivation. In the present thesis research, several pilot and lab scale experiments were conducted on algae wastewater treatment and recycling of water and nutrients in wastewater-based algae cultivation. The main topics were (1) the treatment of primary clarifier effluent by algae raceways and the resulting transformations of nitrogen and phosphorus compounds, and (2) the potential of algae cell disruption techniques to increase nutrient solubilization from algal-bacterial biomass to support cultivation of additional algae from biomass.

The pilot-scale experiments were conducted using nine 33-m², 0.3-m deep, paddle wheel-mixed raceway ponds. These ponds were setup in triplicates and fed municipal wastewater (primary clarifier effluent) to grow algae polycultures. Three experiments conducted using these raceways are reported herein: a comparison of grab and 24-hour composite samples of influent, a study of the effect of hydraulic residence times (HRT) on nitrogen and phosphorus transformations, and a nitrogen and phosphorus depletion study with water recycle.

The grab and 24-hour composite comparison samples were collected weekly during the latter half of 2014. Dissolved reactive phosphorus (DRP) and total ammonia nitrogen (TAN) concentrations of the two sample types were compared and linear correlation equations were made to allow an approximate conversion of grab concentrations to 24-hour concentrations. The results from the sample types, for both DRP and TAN, were on average within 10% of each other.

A comparison was made of data from parallel raceway ponds operated at different HRTs, and linear correlations were determined for soluble nitrogen and phosphorus removals. From the 1.5-day HRT to the 6-day HRT the soluble nitrogen removal ranged from 23% to 61% and the DRP removal ranged from 17% to 53%.

The experiment on nutrient depletion (removal to low concentrations) was conducted in October, 2015. In this experiment, raceway effluent was passed through settling tanks for algae harvest, with the supernatant returned to the raceway. Soluble nitrogen removal was linear with a rate of 1.5 mg-N/L-day (14 days required to decrease from 24 mg-N/L to 2.6 mg-N/L). DRP removal was also linear with a rate of 0.18 mg-P/L-day.

For algae biofuel production, the rate and ultimate extent of nutrient resolubilization from biodegradation of biomass is important to estimating the amount of supplemental fertilizer to be provided, which is a factor in both cost and environmental sustainability. Mechanical or thermal cell disruption methods were evaluated for their ability to promote re-solubilization. The disruption were high-pressure drop orifice homogenization, sonication, autoclaving, and boiling, with the intention that the best technique would be used at pilot-scale. Algae harvested from the pilot ponds underwent

cell disruption treatment followed by anaerobic digestion and then aerobic digestion. This sequence was meant to simulate projected full-scale algae biofuel production, including long-term solubilization of biomass in the aerobic raceways. No significant difference between the pretreated and non-pretreated digested samples occurred. The results indicate that anaerobic digestion plus long-term aerobic degradation in ponds would allow 75-95% of nitrogen and 46-67% of phosphorus to be recycled in algae biofuel production facilities using the process demonstrated in this thesis research.

Keywords: algae, raceway pond, nutrient transformation, anaerobic digestion, aerobic digestion, assimilation, volatilization, nitrogen, phosphorus, pretreatment of algae, nutrient resolubilization, nutrient solubilization

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1. Introduction

Reduce. Reuse. Recycle. Applying these three ideas to limited resources allows for prolonged use and availability of resources such as water and energy supplies. Water supply is short given the current drought in California, and reducing water consumption will stretch the supply but also reduce energy use associated. The water-energy nexus is a relationship between water use and energy consumption and production. The California Energy Commission (CEC) found that 19% or approximately 48 terawatt-hours per year of the state's electricity is used on water related processes (CEC, 2005). Wastewater treatment accounts for 5% of the 19% of water related consumption, or 1% of the total electricity use in the state (CEC, 2005). One technological area directly linked to both water and energy is the integration of wastewater treatment and biofuel production to reduce overall water and energy demands. The most common existing example of this kind of joint-function facility is conventional bacteria-based treatment plants with anaerobic digesters and biogas utilization. This thesis will focus on an alternative: algae-based treatment including production of algae as a biofuel feedstock.

An integrated wastewater treatment and biofuel production plant uses wastewater to both grow algae and treat the wastewater entering the plant. According to the Renewable Fuel Standard (RFS) set by the United States Environmental Protection Agency (USEPA), by 2022 36 billion gallons of renewable fuel is required to be blended into transportation fuel (Agency, 2014). Corn and soybeans are terrestrial food crops that also have the potential to produce biofuel, but growing these crops for biofuels creates competition for them as a food crop. By using municipal wastewater as the growth media, it has the potential to reduce nitrogen demand for algae production by 20% and

phosphorus demand by 49% in order to meet this goal given current technologies (Venteris, Skaggs, Wigmosta, & Coleman, 2014). This nutrient demand can further be reduced by recycling nutrient from harvested algae. Without nutrient recycling, fertilizers would likely need to be used and this would increase operational costs and reduce the sustainability of the process.

Raceway ponds are a cost-effective and efficient upgrade to conventional wastewater treatment with the added bonus of possible biofuel production (Craggs, Sutherland, & Campbell, 2012). Algae are expected to be able to produce between 700-7,000 gallons of oil per acre per year in suitable climates in the US (Cushman, 2010). Compared to corn at 18 gallons per acre and soybeans at 48 gallons per acre, algae biofuel production has a much greater potential to expand while not competing with food/feed production (Cushman, 2010). Algae raceway ponds can remove 80-99% of ammonia/ammonium and phosphate in municipal wastewater (Cushman, 2010). They also remove biological oxygen, pathogens, trace metals, and other impurities in waters. The removal rate and extent of removal vary on the feed water composition and hydraulic residence time (HRT) in the algae raceway ponds. Without inhibitors, water treatment increases with HRT.

This thesis examined the difference between grab and composite sampling of the feed primary clarified wastewater entering algae raceway ponds, removal of nitrogen and phosphorus with algae raceway ponds operating at different HRTs, and the ability of algae to deplete nitrogen and phosphorus in primary clarified effluent wastewater while operating in batch mode. This thesis also looked into the results of the aerobic digestion of algae previously boiled prior to anaerobic digestion. The works of Hill (2014), Chang

(2014), and Kraetsch (2015) regarding other pretreated algae digestion experiments were summarized to compare against each other. These findings can assist with future modeling and the potential to further scale up and implement into a functioning wastewater treatment and biofuel production facility.

2. Background

The following section discusses the concepts related to the experiments performed and examined in later sections of this thesis.

2.1 Algae Raceway Pond Basics

Algae raceway ponds are designed to maximize algae growth and allow for harvesting (**Figure 2-1**). The ponds are shallow and constantly mixed to prevent light limitation and maximize contact with the nutrients in the water. Carbon Dioxide is injected into the ponds to regulate pH and provide a carbon source for the algae.

Figure 2-1: Diagram of a high rate algae pond system used to grow algae for collection (Craggs, Sutherland, & Campbell, 2012).

There is also a relationship between the algae and bacteria growing and living within the algae ponds (**Figure 2-2**).

Figure 2-2: A simplified diagram of the relationship between algae and bacteria growing in algae ponds fed wastewater (*Oswald, Gotass, Ludwig, & Lynch, 1953*).

Nitrogen and phosphorus are important nutrients required for algae growth and for wastewater treatment purposes, these nutrients need to be removed. The removal mechanisms for nitrogen in a suspended growth system are” assimilation, volatilization, sedimentation, nitrification and denitrification (Mayo & Mutamba, 2005). Nitrogen has many forms that is can be converted to throughout the treatment process in the raceway ponds (**Figure 2-3**). Phosphorus is removed through assimilation into biomass.

Figure 2-3: A general nitrogen cycle showing the changes in nitrogen forms (Finger, 2014).

The solar radiation available is one factor that influences algae growth and it is important to select a location with a sufficient amount (**Figure 2-4**).

Figure 2-4: Map of the United States and the solar radiation levels present in units of kWh/m²/day (NREL, 2008).

2.2 Primary Clarified Wastewater Sampling

Fluctuations and variability are typical with wastewater flows and loading, both by region but also throughout the day. As noted in Metcalf and Eddy, constituents like ammonia and inorganic phosphorus can typically vary between 14 to 41 mg/L-N and 1.6 to 4.7 mg/L-P, respectively (Metcalf and Eddy, Inc., 2003). The primary clarified effluent grab entering the algae raceway ponds in San Luis Obispo have an average ammonia concentration of 36.6 mg/L-N and inorganic phosphorus concentration of 3.7 mg/L-P (Kraetsch, 2015). These averages are from grab samples collected at the same time of the day on each individual sample day. These values will vary throughout the day and it is important to have a more representative sample, both for the influent and pond effluent.

2.3 Algae Raceway Pond Treatment at Varying HRTs

Increasing the HRT of the ponds should correlate to an increase in nutrient removal and biomass growth. The rate of consumption for nitrogen and phosphorus and the growth rate of biomass fluctuated over time, but as time continued more biomass was produced as nutrient concentrations decreased (Martinez, Sanchez, Jiminez, Yousfi, & Munoz, 2000). It is important to find if a correlation between varying HRTs is present for potential future modeling or scale-up purposes.

2.4 Nutrient Depletion

Operating algae raceway ponds in batch mode fed primary effluent wastewater with 100% water recycle and removal of biomass will provide removal rates, extent of removal, and the limiting growth factor. The removal rate of nitrogen and phosphorus increases with higher initial concentrations (Aslan & Kapdan, 2006). Increased concentrations of nutrients will result in greater uptake and growth due to the elimination of any possible limiter. Over a ten day batch experiment with algae fed synthetic wastewater, initial nitrogen concentrations of 13.2 and 21.2 mg-N/L were completely removed with some phosphorus remaining, but these conditions did not produce as much biomass as the apparatuses with concentrations exceeding 100 mg-N/L (Aslan & Kapdan, 2006). This information also indicates that nitrogen was the limiting growth factor for the synthetic wastewater media. Information from the batch experiment will assist with examining different growth media in future experiments.

2.5 Anaerobic followed by Aerobic Digestion with Pretreatment

Recycling nutrients is an important step in sustainably maintaining an integrated wastewater treatment and biofuel production facility. By recycling nutrients within the

plant, the need for additional nutrients, such as fertilizers, to supplement growth will be reduced or possibly eliminated. Recycling can be accomplished by anaerobically digesting algae and then reintroducing the digestate into the ponds to resolubilize the nutrients previously in the harvested algae. Adding a pretreatment process before anaerobic digestion can add to the breakdown of algae cells in order to release additional nutrients (**Figure 2-5**).

Figure 2-5: The basic process flow of the pilot scale raceway facility for nutrient and water recycling. Tube settler supernatant is recycled for algae growth, and the anaerobically digested algae slurry, also returned to the ponds, comprises the nutrient recycle component. This diagram shows the process flow if a pretreatment technology was used. Below the "Pretreatment Technology" box are the pretreatment methods examined in this thesis. Also included in the diagram are the energy input and output of the system and the sewage influent to the ponds (Hill, 2014).

The aerobic decay of mixed algae cultures with bacteria and zooplankton followed a first order decay rate (Jewell & McCarty, 1971). Of these cultures with ages varying between a few days to several months, the younger cultures decayed more rapidly than the older cultures (Jewell & McCarty, 1971). The algae raceway ponds are operated and harvested with short HRTs and this will yield more favorable aerobic digestion for biomass breakdown and subsequent nutrient resolubilization.

The aerobic digestion of the already anaerobically digested algae biomass will be accomplished through the movement of water through the raceway pond setup, the oxygen produced by the algae, and the sparging of carbon dioxide into the ponds. This aeration process will convert the organic nitrogen in the algae digestate into soluble nutrients to support additional algae growth in the ponds (**Figure 2-6**).

Figure 2-6: The decay of organic nitrogen into ammonia, nitrite, and nitrate when anaerobically digested biomass is introduced into an aerobic digestion process. These soluble forms of nitrogen are then available for uptake and continued algae growth (Finger, 2014).

If the effects of pretreatment technologies increase the decay of biomass and nutrient resolubilization with the bench scale test will be evaluated for potential scale-up to the pilot scale.

2.6 Research Goals

The research goals of this thesis were the following:

- (1) Determine a conversion factor to convert from grab to composite samples
- (2) Determine the relationship between HRT and nutrient removal
- (3) Determine the rate and extent of nutrient removal with 100% water recycle
- (4) Determine the ultimate extent of nutrient resolubilization from algae pretreated by boiling, anaerobically digested then aerobically digested

3. Methods and Materials

Two types of experiments were performed: laboratory- and pilot plant-based, both described in this chapter. A series of bench-scale anaerobic and aerobic digestion experiments were conducted jointly by several graduate students during 2013-2014. The present author participated in only the final boiled aerobic degradation experiment, but the whole series of experiments are summarized herein. In addition, the pilot raceway pond experiments are described including operation, experimental procedures, sampling, and water quality analytical methods.

3.1 Algae Field Station: Site Layout

The Cal Poly Algae Field Station (AFS) facility is located at 35 Prado Road at the City of San Luis Obispo Water Resource Recovery Facility (WRRF), which has a treatment capacity of 5.2 million gallons of wastewater per day (MGD) (Kraetsch, 2015). The AFS has nine 33-m² pilot raceway ponds located west of the primary clarifier (**Figure 3-1**). The primary clarifier effluent was the main water and nutrient source for the pond experiments. However, some experiments used reclaimed water that had been nitrified and filtered. The nine raceway ponds are arranged in three sets of three ponds each termed the Alpha (Ponds 1-3), Beta (Ponds 4-6), and Gamma (Pond 7-9) pond sets. The specific details of the ponds will be discussed later in Section 3.2.3.

Figure 3-1: An aerial view of the AFS showing the Alpha, Beta, and Gamma triplicate pond sets and the primary clarifier (*Kraetsch, 2015*).

3.2 Raceway Pond Experiments

A long-term study of pond performance was conducted to evaluate (1) transformation of nitrogen and phosphorus compounds in raceways operated at different hydraulic retention times (HRTs), and (2) the nitrogen and phosphorus depletion of batch-mode ponds with continuous algae harvesting. Refer to Section 2.1 for a conceptual model of nitrogen and phosphorus transformations and fates. These accomplish the experimental goals as outlined in Section 2.6. In addition, a side study was conducted to compare the water quality in primary clarifier effluent grab samples with 24-hour composite samples.

3.2.1 Experimental Concepts and Purposes

The pilot plant experiments were focused on algae productivity, wastewater treatment, and nutrient transformations, but this thesis focuses on the latter topic.

3.2.1.1 Influent Grab and 24-hour Composite Sampling Comparison

Both primary clarifier effluent quality and algae culture characteristics can vary widely over the course of a single day. However, much of the current and prior research

done at this facility depended on morning grab samples. During the present research, 24-hour composite sampling of pond influent (primary clarifier effluent) began on July 31, 2014 and continued until December 3, 2014. Comparisons were made between composite samples and grab samples taken on the same day to determine a correlation, if any, between the grab and 24-hour composite samples. Samples were drawn from the constant head tank of the influent distribution system for the ponds (Section 3.2.2). For the present thesis, the main focus on this comparison was the readily bioavailable forms of nitrogen and phosphorus in the pond influent water, in the form of total ammonia nitrogen (TAN) and dissolved reactive phosphorus (DRP).

3.2.1.2 Nitrogen and Phosphorus of Various HRTs

A major consideration in designing raceway ponds is the HRT needed to achieve treatment and algae productivity targets. The HRT comparison study compared water quality data from October 30 and November 6 of 2013 and October 29 and November 5 of 2014 when a wide range of HRTs were being tested (**Table 3-1**). These experiments compared the ability of the raceways, operating at different HRTs, to assimilate and convert nitrogen and phosphorus, as discussed in the Background chapter.

Table 3-1: The HRTs used for the comparison.

HRT	Year
1.5-day	2014
2-day	2013
3-day	2013
3-day	2014
6-day	2013

The 6-day HRT pond set was actually two 3-day HRT ponds connected in series, with algae sedimentation harvesting following the first pond (**Figure 3-2**). For the 2013

time period, the Beta ponds were referred to as “Round 1” and the Alpha ponds as “Round 2.”

Figure 3-2: On the left is the process flow diagram for the 1.5-day, 2-day, and 3-day HRT pond systems. On the right is the process flow diagram of the 6-day HRT system (Ripley, 2013).

3.2.1.3 Nitrogen and Phosphorus Nutrient Depletion with Water Recycle

The purpose of the nutrient depletion experiment was to monitor the transformation of nitrogen and phosphorus compounds in batch mode pond operation and determine the amount of time needed to achieve low nitrogen and phosphorus concentrations. The main route of nutrient removal was the biomass continuously removed in the settling tanks, while the supernatant was recycled back into the ponds. To start the experiment, in October 2014, the primary clarifier effluent supplied to the Beta ponds was discontinued, and the existing Beta pond water was continuously

recycled through the settling tanks back to the Beta ponds to achieve a 3-day HRT. In this experiment, settling occurred in tube settlers connected in series with cone bottom tanks (**Figure 3-3** and described in a later section). Settled solids were removed from the tube settlers and cone bottom tanks as need.

Figure 3-3: Process flow diagram of nutrient depletion experiment in the Beta pond set. The tube settler supernatants flowed into parallel cone-bottom tanks for additional settling.

3.2.2 Pond Process Flow

In this and the next several sections, details of the pilot plant setup and operation are provided. The ponds receiving primary clarifier effluent were supplied by a sump pump suspended between the scum baffle and overflow weir of the primary clarifier (**Figure 3-4**).

Figure 3-4: The influent line in the primary clarifier. A sump pump was suspended in the primary clarifier with the blue tubing leading to the pilot facility (*Chang, 2014*).

The primary effluent wastewater was pumped continuously 24 hours per day to the three constant head tanks associated with each of the pond sets. From the constant head tanks, the flow was distributed to the individual ponds via continuously-rotating scoop and gravity-flow pipe distribution systems (**Figure 3-5**).

The water level in these head tanks were kept constant with an overflow standpipe located in the head tank. The rotational speed and submersion depth of the scoops were adjusted to maintain the desired flow rate into the ponds. For the Round 2 and Beta nutrient depletion experiments, the head tank water was not allowed to overflow by setting the scoop rotational speed high enough to keep the water level below the overflow standpipe. The flow of water into the head tanks controlled the flow delivered to the ponds.

Figure 3-5: Head tank and distribution system with motor rotating the scoop arms. The 90° elbows scooped up water from the constant head tank and delivered it to the square containers. The water in each container then gravity-flowed to the three ponds. In experiments without water recycling, the amount of water delivered to the ponds was dependent on the water level, rotational speed, and length of the scoops. Excess water was pumped into the constant head tank and allowed to overflow to maintain the water level (*Chang, 2014*).

The Round 1 and Beta nutrient depletion ponds had water continually pumped from them into six tube settlers, using three peristaltic pumps (**Figure 3-6**).

Figure 3-6: The peristaltic pumps set up in the Beta pump house. Each pond had one pump and two tubing lines associated with it. In this photograph, water entered from the right and exited to the left into the tube settlers (*Chang, 2014*).

The suction tubing for tube settler influent pumping was placed in closed bottom standpipes which were used to maintain the 30-cm pond depth. The two types of closed bottom standpipes for the tube settlers were: 5-cm ramped for Ponds 4 and 5 (**Figure 3-7**) and 10-cm vertical for Pond 6 (**Figure 3-8**). These standpipes were kept in the same location in each of the ponds located next to the overflow standpipes (**Figure 3-9**).

Figure 3-7: Ramped standpipe used to collect pond effluent for pumping into tube settlers. The ramp was closed at the bottom and this standpipe controlled the heights in Ponds 4 and 5. The standpipe sits in concrete that was poured into a cut off bottom from a 20-L bucket (*Kraetsch, 2015*).

Figure 3-8: Vertical 10-cm standpipe used to collect pond effluent for pumping into tube settlers. The standpipe was closed at the bottom, and this standpipe controlled the height of Pond 6. The 5-cm diameter standpipe set in concrete that was poured into a cut-off bottom of a 20-L bucket. The black sleeve at the top was used to make small adjustments to water height.

Figure 3-9: Typical location of the moveable closed bottom standpipes (left) in the ponds relative to the fixed standpipe (right). In this configuration, the standpipe on the right served as an emergency overflow point. Pond sampling occurred near this location in all ponds.

The tube settlers were set at an angle of 60° (**Figure 3-10**). Refer to Chang 2014 thesis for a complete description of the tube settler process flow.

Figure 3-10: Side view of the tube settlers set up at a 60° angle to settle algae before the water flowed to the next process (Chang, 2014).

For the ponds-in-series experiment, the supernatant from the tube settlers flowed by gravity into the Round 2 head tank (**Figure 3-11**) where an aquarium mixer kept the water well mixed while it was distributed to the Round 2 ponds.

Figure 3-11: Round 2 head tank and distribution system. The top left photo is a down-facing view showing the rotating arms used to distribute the water to the ponds. The top right photo shows three of the six lines that distributed the tube settler effluent into the head tank. The bottom photo shows the influent piping into each of the Round 2 ponds (Chang, 2014).

During the summer of 2014, solids loading to the tube settlers appeared to be excessive, and daily algae slurry removal did not prevent formation of floating algae mats near the surface overflow pipes. In September 2014, cone bottom tanks were added to follow the tube settlers to provide a second opportunity for solids settling to thereby improve the clarity of the settled effluent being returned to the Round 2 ponds and, later, to the ponds of the nutrient depletion experiment (**Figure 3-12**). Because productivity was calculated using the difference of the influent and effluent pond concentrations,

better clarified water being fed to the ponds should have contributed to higher productivity results.

Figure 3-12: Cone-bottom settling tank covered in black plastic to minimize algae growth inside the tank. Influent (tube settler effluent) was supplied to the bottom of the cone-bottom tank.

Effluent water from Alpha and Gamma left the ponds by flowing into one of two types of standpipes: a 5-cm diameter ramped standpipe (**Figure 3-13**) or a 10-cm diameter vertical standpipe (**Figure 3-14**).

Figure 3-13: Ramped standpipe installed effluent collection point used in Ponds 1, 2, 4, 5, 8, and 9 (*Chang, 2014*).

Figure 3-14: Vertical standpipe (10-cm diameter) effluent collection point used in Ponds 3, 6, and 7 (*Chang, 2014*).

The effluent water from the ponds and overflow water was collected into a sump which was pumped back to the full scale treatment plant.

3.2.3 Pond Characteristics

The pond area, depth, channel water velocity, and tube settler flows remained constant throughout the experiments. Instead, the experiments tested the effects of influent flow (HRT) and system configuration (**Table 3-2** and **Table 3-3**).

Table 3-2: Pond characteristic for the HRT comparison for October 30 and November 6 of 2013 (*Kraetsch, 2015*).

Specification	Triplicate Set		
	Alpha	Beta	Gamma
Ponds in Set	1, 2, 3	4, 5, 6	7, 8, 9
Depth (m)	0.3	0.3	0.3
Channel Velocity (cm/sec)	19.8	19.2	19.5
Influent (L/min)	2.25	2.25	3.30
Tube Settler Flow (L/min)	2.25	2.25	N/A
HRT (days)	3.0	3.0	2.0

Table 3-3: Pond characteristics for the HRT comparison for October 29 and November 6 of 2014. The Beta pond characteristics apply to the nutrient depletion experiment.

Specification	Triplicate Set		
	Alpha	Beta	Gamma
Ponds in Set	1, 2, 3	4, 5, 6	7, 8, 9
Depth (m)	0.3	0.3	0.3
Channel Velocity (cm/sec)	19.8	19.2	19.5
Influent (L/min)	2.25	2.25	4.50
Tube Settler Flow (L/min)	2.25	2.25	N/A
HRT (days)	3.0	3.0	1.5

3.2.4 Pond Operations

The AFS pond operations included tasks such as: maintenance of paddle and water wheels, field data collection, recording pH, dissolved oxygen (DO), and temperature, and peristaltic pump repair and maintenance.

A supervisory control and data acquisition (SCADA) system was used to monitor dissolved oxygen (Neptune Systems, Morgan Hill, California), pH (Sensorex, Garden

Grove, California), and temperature (Neptune Systems) in each of the nine ponds at the AFS. Data-loggers (Apex Aqua Controllers, Neptune Systems) stored these data at designated intervals. pH was recorded every ten minutes from March 2013 to June 2014 when it was changed to hourly recordings in July 2014. The dissolved oxygen and temperature were recorded hourly throughout the duration of the AFS pond experiments. The SCADA system was controlled from a computer located in a shed onsite and connected to each Neptune unit for the ponds (**Figure 3-15**).

Figure 3-15: Schematic of the SCADA system that measured and recorded pH, DO, and temperature data. Carbon dioxide sparging was controlled by the pH signal from the ponds (Hutton, 2014).

The three pond sets each had a pump house containing temperature and dissolved oxygen recording and pH recording and control units (**Figure 3-16**) and two variable

frequency drives (VFD) that independently regulated the paddle wheel and water wheel rotational speeds (**Figure 3-17**).

Figure 3-16: The Neptune PM1 control units for pH and temperature data recording. This setup was the same for the Alpha, Beta, and Gamma pond sets (*Chang, 2014*).

Figure 3-17: VFD units for controlling the paddle wheel and the water wheel. This setup was the same for Alpha, Beta, and Gamma (*Chang, 2014*).

The DO reading modules were located in separate waterproof plastic containers next to the ponds (**Figure 3-18**).

Figure 3-18: One of the Neptune PM3 modules used to collect DO readings from the probes located in each pond (*Kraetsch, 2015*).

The pH in Alpha and Beta pond sets was controlled by the sparging of carbon dioxide (CO₂) into the water (**Figure 3-19**). CO₂ release was triggered by real-time pH readings to maintain the desired pH range. In 2013 the range was between 8.4 and 8.5 and on June 5, 2014 this range was changed to between 8.0 and 8.5. CO₂ release was triggered when the range was exceeded and stopped when the lower limit was achieved. This range was increased to provide less wear and tear on the triggering system. Gamma was not regulated with CO₂ during the experimental periods in this thesis. The CO₂ was stored in 23-kg (50-pound) cylinders with 99.5% purity and initial pressure of 51 atmospheres (750 pounds per square inch) from the vendor Airgas (**Figure 3-20**).

Figure 3-19: Carbon dioxide was diffused into the ponds through the black perforated tubing secured along the length of the PVC weight pipe. These diffusers were located about 90-cm downstream of the paddle wheel in each pond. The diffuser was removed from the pond and laid on the wall for the photograph.

Figure 3-20: The left photo shows the secured CO₂ cylinders, and the right photo shows the automated solenoids that distributed the CO₂ to the ponds (*Chang, 2014*).

The sensors for the temperature, pH, and DO were kept in the ponds using probe stands located on the east end of the ponds upstream of where the influent water entered the ponds (**Figure 3-21**).

Figure 3-21: A probe stand that held the pH, DO, and temperature probes in each pond (Chang, 2014).

The data from these probes were recorded hourly and downloaded weekly. The DO and pH probes were calibrated weekly. DO was calibrated using a two-point calibration at 0% and 100% saturation. pH was also calibrated using a two-point calibration at pH 7 and 10. On March 17, 2015, the temperature readings of the ponds were compared to a certified thermometer and less than a 0.1°C difference between the readings of the ponds and certified thermometer. The certified thermometer was an Ever-Safe with a certified accuracy of $\pm 1\%$ of the total scale. The thermometer ranged from -20 to 150 °C, which equates to an accuracy of $\pm 1.7^\circ\text{C}$.

Refer to Chang 2014 thesis for detailed descriptions of other AFS maintenance and tube settler operations.

3.2.5 Weekly Pond System Sampling Procedures

Pond system sampling was conducted weekly between 7 am and 8 am. Grab samples of the ponds were taken next to the standpipe locations in each of the ponds to imitate what was leaving the ponds. These grab samples were collected in 4-L small-mouthed plastic sample bottles by submerging them inverted into the ponds and then

turning them mouth up at half the depth of the ponds. The water was slowly collected from the half of the pond's depth. This procedure was followed until June 19, 2014 when sampling was changed to better mimic the collection of the standpipes in the ponds. A large-mouthed polypropylene pitcher, was submerged upside down into the ponds, 4-cm to the bottom, and then quickly inverted. It would quickly collect water and provide a more representative sample of the pond by sampling an entire water column and not one particular depth of the pond. The 24-hour pond influent samples were drawn from the constant head tank using an autosampler (Hach Sigma, Loveland, Colorado). The former method had the potential to exclude larger flocs of algae during times of increased growth due to the low flowrate into the small-mouthed containers. Influent grab samples for the ponds were collected from the discharge of the influent pipes as they entered the ponds. The influent samples were taken from one of the Gamma ponds due to the higher flowrate of the influent water.

All samples were stored in a dark cooler to prevent algae growth or other changes, like ammonia volatilization, in the samples during transportation. Samples were taken to the laboratory at Cal Poly immediately after collection to conduct analytical water quality testing (**Figure 3-22**). Section 3.4 describes these tests.

Figure 3-22: Sample testing flow diagram with approximate sample volumes used (Chang, 2014).

3.3 Digestion Experiments

Recycling nutrients is important to the sustainable of an algal biofuel production plant and would reduce the need for external nutrient sources, such as fertilizers. Following the extraction of fuel components from the algae (primarily carbon), the residual nitrogen, phosphorus, and other nutrients should be recycled for growth of additional crops of algae. Anaerobic digestion is one option for decomposing the biomass residuals back into readily bioavailable nutrients, and additional degradation is expected in the aerobic raceway ponds.

The rate and extent of nutrient resolubilization has been explored in previous work (Hill, 2014), (Foree & McCarty, 1970), and (Jewell & McCarty, 1971), but the present laboratory experiment extended the work by Hill (2014) by testing the aerobic degradation of waste-grown algae that had been pretreated by boiling and then anaerobically digested. The lab experiment estimated the rates and maximum amount of solubilization possible in an aerobic raceway pond. The aerobic degradation experiment was carried out in the dark.

In previous work, prior to anaerobic digestion, several cell-disrupting pretreatment methods were tested for increased methane yield and nutrient resolubilization. The pretreatment technologies were sonication, high-pressure homogenization, autoclaving, and boiling (Hill, 2014). The sonication did not have the non-pretreated sample aerobically digested due to lack of sample and the autoclaving samples were not aerated due to analytical errors (Hill, 2014).

If any pretreatments substantially improved nutrient resolubilization, then the cost merits of the pretreatment could be evaluated in future work. Refer to Section 2-5 for a conceptual process flow of the AFS raceway ponds including cultivation, digestion, and nutrient recycling with pretreatment.

This thesis will summarize the results from all the laboratory pretreatment experiments conducted by Hill (2014), Chang (2014), Kraetsch (2015), and the present author, giving the rates and ultimate fractions of organic nitrogen and particulate phosphorus resolubilized during long-term sequential anaerobic-aerobic digestion of pretreated and control algae slurries.

3.3.1 Anaerobic Digestion Experiments

This subsection describes the pretreatments and anaerobic digestion steps of the experiments, all of which were conducted by Alex Hill (2014). Refer to Hill (2014) for a complete description of the collection, preparation, theory, sampling, treatment technologies, experimental setup, results, conclusion, and other information related to the pretreatment anaerobic digestion experiments.

Four digesters were used for the following feeds: (1) digester seed (control), (2) raw untreated algae, (3) non-pretreated algae with seed (control), and (4) pretreated algae with seed. These controls were used to evaluate the effectiveness of the pretreatment during anaerobic digestion. The non-pretreated algae with seed (control) and the pretreated algae with seed were the samples aerobically digested.

The seed used for the lab digesters came from the full-scale WRRF wastewater sludge digester effluent. The lab digesters were incubated for 39 to 43 days (**Table 3-4**) at a temperature of 32°C.

Table 3-4: Anaerobic digestion incubation durations for the various anaerobic digestion experiments. The first three pretreatments listed all were tested by others (Hill, 2014; Chang, 2014; Kraetsch, 2015).

Pretreatment Technology	Start Date	End Date	Incubation Duration (Days)
Sonication	7/9/2013	8/20/2013	42
High-Pressure Homogenization	9/16/2013	10/25/2013	39
Autoclaving	11/14/2013	12/27/2013	43
Boiling	1/23/2014	3/7/2014	43

The digesters were serum bottles of either 125 mL or 1.2 L. These bottles were placed in a gravity convection incubator (Precision, Chicago, Illinois) at $35 \pm 2^\circ\text{C}$

throughout the experiment (Hill, 2014). Extra bottles of identical pretreated and non-pretreated algae were incubated to provide enough biomass for the aeration experiments (Section 3.3.2). Algae slurry for these experiments was harvested from the pond tube settlers (**Figure 3-23**).

Figure 3-23: The complete process flow diagram of the anaerobic digestion step of the experiment from the collection of algal sludge to the finished digesters (Hill, 2014). After the digestion process, the treated and untreated samples were used for the aeration experiments.

3.3.2 Aerobic Digestion Experiments

This subsection describes the aerobic digestion steps of the sonicated, homogenized, and boiled experiments. The sonicated and homogenized experiments were conducted by Mike Chang (2014) and the boiled experiment was performed in the same manner as the homogenized experiment. For a detailed and complete description of the

experimental setup of the sonicated and homogenized aerobic digestions refer to Chang (2014). A brief overview is provided here.

The purpose of these aeration experiments was to find the rate and ultimate biomass aerobic degradation that could occur after the pretreatments and anaerobic digestion. Laboratory anaerobic digestion was used to estimate the maximum degradation and release of nitrogen and phosphorus possible in the ponds (**Table 3-5**).

Table 3-5: Aerobic digestion incubation durations for the various pretreatments. “np”= not performed.

Pretreatment Technology	Start Date	End Date	Incubation Duration (Days)
Sonication	8/20/2013	9/20/2013	31
High-Pressure Homogenization	10/26/2013	2/7/2014	104
Autoclaving	np	np	np
Boiling	3/12/2014	5/22/2014	71

The water quality tests performed were the following: total suspended solids, volatile suspended solids, total ammonia nitrogen, nitrite, nitrate, total Kjeldahl nitrogen, dissolved reactive phosphorus, and total phosphorus (**Figure 3-24**). Nutrients were measured using methods outlined in Section 3.4.

Figure 3-24: Aeration sampling flow diagram with approximate sample volumes used (Chang, 2014).

Samples for these tests were taken daily during the first week and a half, and afterwards sampling occurred on a weekly basis. When not being analyzed, samples were stored in a refrigerator. Tests not run on the same day they were sampled were acidified and placed in the refrigerator for later testing. Temperature, pH, and DO were recorded daily.

3.3.2.1 Sonicated Pretreatment Aerobic Digestion Experiment

This experiment was conducted using triplicate 3.78-L polypropylene aeration reactors placed in a water bath (Figure 3-25 and Figure 3-26). The water bath was not temperature controlled but meant only to dampen daily temperature changes. The reactors were continually mixed using stir plates and continually aerated using two ceramic diffuser stones (3.5-cm long, 1-cm diameter, commonly used in aquaria) connected to the house air supply without air filtration. The aeration stones provided aeration and

complete stirring of the sample with a constant flowrate of approximately $2 \text{ L}_{\text{air}}/\text{L}_{\text{initial water-second}}$ (Chang, 2014). Water lost to evaporation was made-up with reverse osmosis water daily, but the volume in the reactors decreased over the course of the experiment due to sampling. The anaerobic digester biomass was diluted to 2,000 mg/L total solids content using dechlorinated tap water, and each reactor was seeded with 10 mL of return activated sludge (RAS) from the nitrifying aeration basins at the WRRF.

Figure 3-25: The triplicate 3.78-L polypropylene aeration reactors with the aeration lines leading to the two aeration stones located at the bottom of the reactors (Chang, 2014).

Figure 3-26: A photograph of the non-pretreated and pretreated triplicate 3.78-L polypropylene aeration reactors. The water bath was heated to $19.5^{\circ}\text{C} \pm 1.5^{\circ}\text{C}$ for all

experiments except the sonication one. The non-pretreated sample reactors are on the left and the pretreated ones on the right (*Chang, 2014*).

3.3.2.2 Homogenized and Boiled Pretreatment Aerobic Digestion Experiments

Several changes were made to the experimental setup of the homogenized and boiled aerations after the sonicated aeration experiment. Influent air was passed through an activated carbon filter to remove any oil vapor that was seen in the sonicated experiment. To better regulate temperature between the reactors in the triplicate sets, they were placed in a temperature controlled water bath. The stir plates and stir bars were removed and stirring was accomplished with only the airflow from the aeration stones.

3.4 Water Quality Analyses for Pond and Aeration Experiments

Samples from each of the nine ponds were subjected to weekly water quality tests. The following water quality tests were performed on the aeration samples as well. Except for subsamples to be used for total Kjeldahl nitrogen (TKN), total phosphorus (TP), and chemical oxygen demand (COD) analyses, which were acidified within two hours, all samples were stored in a cooler and tests typically were performed within ten hours of sampling, or if not, they were filtered as needed, acidified, and refrigerated. All tests and sample preservation techniques were performed in accordance to Standard Methods for the Examination of Water and Wastewater (1995) unless otherwise stated (**Table 3-6**). Refer to Kraetsch (2015) for detailed information about each testing method used, except for the Timberline Total Ammonia Nitrogen method (Section 3.4.1).

Table 3-6: The analytical methods used to run each constituent and the APHA Standard Methods title associated with each is listed. These methods were used for all pond and digestion experiments (*Kraetsch, 2015*).

Constituent	Analytical Method
Nutrients	

Total Ammonia Nitrogen	Ammonia Selective Electrode (APHA Method 4500-NH ₃ D)
Nitrite	Automated Selective Electrode (Based on APHA Method 4500-NH ₃ D) Colorimetric, Fisherbrand 0.45-μm Multiple Cellulose Ester filtration (APHA Method 4500-NO ₂ ⁻ B)
Nitrate	Nitrate Ion Selective Electrode with Interference Suppression Solution (APHA Method 4500-NO ₃ ⁻ D)
Total Kjeldahl Nitrogen	Macro-Kjeldahl and manual titration (APHA Method 4500-N _{org} B)
Dissolved Reactive Phosphorus	Ascorbic Acid, Fisherbrand 0.45-μm Multiple Cellulose Ester filtration (APHA Method 4500-P E)
Total Phosphorus	Vanadomolybdophosphoric Acid Colorimetric (APHA Method 4500-P C)
Organics	
Total and Volatile Suspended Solids	Gravimetric with 1.2-μm Fisherbrand G4 Glass Fiber filters filtration (APHA Method 2540 D and E)
Total and Soluble Carbonaceous Biochemical Oxygen Demand	5-day with 20°C incubation, 1.2-μm Fisherbrand G4 Glass Fiber filtration (APHA Method 5210 B)
Other	
Microscopy for Algae ID	Selected Taxonomic References, Optical Microscope (Method 10900 E. 2.)
Alkalinity	Sulfuric Acid Titration (APHA Method 2320 B)

3.4.1 Automated Total Ammonia Nitrogen Analysis

Total ammonia nitrogen (TAN) concentrations were obtained using an automated version of APHA Method 4500-NH₃ D using a Timberline Instruments Ammonia Analyzer (TL-2800, Boulder, Colorado). Standards were created for each testing run by

diluting a 2500 ppm as NH_3 stock ammonium chloride standard (Aqua Solutions). These standards were used to create a calibration curve with the standards of 0.1, 1, 10, and 50 mg/L- NH_3 concentrations.

Samples were filtered through glass fiber filters (1.2- μm pore size, Fisherbrand, Catalog No. 09-753-50) and 10 mL were collected in plastic Falcon tubes for testing/storage. Immediately after filtering, samples were acidified with H_2SO_4 to a pH <2 in order to prevent possible ammonia losses.

3.5 Weather Data

Weekly weather data from the California Irrigation Management Information System (CIMIS) were collected from a station located on the California Polytechnic State University, San Luis Obispo campus (Chang, 2014), which was located about six km from the pilot plant at a similar elevation.

4. Results and Discussion

The results for nitrogen and phosphorus grab and 24-hour composite sampling is presented first. Then the nitrogen and phosphorus removal from different HRTs is discussed followed by the nitrogen and phosphorus nutrient depletion experiment. Then the aerobic digestion of the boiled experiment will be covered. Finally, a summary of the anaerobic followed by aerobic digestion experiments with regards to organic nitrogen and particulate phosphorus results from pretreatment experiment will be presented.

4.1 Comparison of Grab and 24-hour Composite Sampling of Pond Influent

Grab sampling was the main sampling method used in the present project, which introduces potentially important errors in estimating mass flows of water quality constituents. Both influent wastewater and effluent pond water constituents are well-known to vary extensively over the course of the day. A special study was conducted on the influent wastewater to compare concentrations in the typical morning grab samples with 24-hour composite samples. Pond effluent composite samples were not collected for the present thesis research. In conjunction with grab sampling, composite sampling was conducted from July 31 until November 19, 2014 on the primary clarifier effluent water entering the Gamma pond set. This section will describe the total ammonia nitrogen (TAN) and dissolved reactive phosphorus (DRP) concentrations in the pond influent water. These constituents were selected because they are the readily available forms of nitrogen and phosphorus for algae growth.

The percent difference for the samples was calculated using **Equation 4-1**. Equations to convert grab samples into composite samples are in the following sections.

Equation 4-1 was used as an initial comparison between the two sample types to assist with determining if composite samples are necessary for the pond influent.

Equation 4-1: The percent difference was calculated using the average of the two values since neither sample method was assumed to be the correct sampling method.

$$\text{Percent Difference} = \frac{(\text{First Value} - \text{Second Value})}{\text{Average of both Values}} * 100\%$$

4.1.1 TAN Comparison

TAN concentrations varied more than the DRP both in terms of sample method and the percent difference between the samples (**Table 4-1**). Samples within 10% of each other were deemed identical in the following data analysis. The composite influent TAN concentrations averaged 9% greater than the grab sample concentrations, with a standard deviation of 23%.

Table 4-1: Comparison of TAN concentrations in grab and 24-hour composite samples. The number of samples matching the criterion per the total number of samples are shown. The “Higher TAN value” column shows the number of data points where that sample type was higher in concentration.

Influent Sample Type	Higher TAN value	Within $\pm 10\%$
24-Hour Composite	7 of 11	2 of 7
Grab	4 of 11	2 of 4

The data were graphed and a linear regression equation was determined to convert between the two sampling types (**Figure 4-1**).

Figure 4-1: Correlation of the grab and 24-hour composite sample TAN influent concentrations. The best-fit equation and R^2 value are shown.

The equation uses the data to provide a conversion from grab TAN concentration to 24-hour TAN concentration (**Equation 4-2**).

Equation 4-2: TAN grab concentration conversion to 24-hour composite concentration.

24 – Hour Composite TAN Concentration

$$= 0.87 * (\text{Grab TAN Concentration}) + 9.1$$

The TAN concentrations contained a range of values was highly variable. More data should be collected to make the conversion from grab to 24-hour TAN concentrations more reliable. The above Equation 4-2 should be used for an approximation of a 24-hour sample and not an exact value.

4.1.2 DRP Comparison

The DRP concentrations of the grab and 24-hour composite samples were closer in concentration and were more consistent when compared to the TAN concentrations discussed above (**Table 4-2**). The concentrations were consistently higher for the 24-hour composite sample and mostly within 10% of the grab sample value. On average, the

composite sample contained 3% more DRP than the grab sample with a standard deviation of 7%.

Table 4-2: Comparison of DRP concentrations in grab and 24-hour composite samples. The number of samples matching the criterion per the total number of samples are shown. The “Higher DRP value” column shows the number of data points where that sample type was higher in concentration.

Influent Sample Type	Higher DRP Value	Within $\pm 10\%$
24 Hour Composite	12 of 15	11 of 12
Grab	3 of 15	2 of 3

The data were graphed and a linear regression equation was determined to convert between the two sampling types (**Figure 4-2**).

Figure 4-2: The graphed data of the grab and 24-hour composite DRP influent concentrations. The equation and R^2 value for the trend line can be seen in the top left corner of the graph.

The equation uses the data to provide a conversion from grab DRP concentration to 24-hour DRP concentration (**Equation 4-3**). The R^2 values is much lower than the TAN conversion graph. However, the grouping is more consistent showing less variability in concentrations.

Equation 4-3: TAN grab concentration conversion to 24-hour composite concentration.

24 – Hour Composite DRP Concentration

$$= 0.71 * (\text{Grab DRP Concentration}) + 1.3$$

Because DRP concentrations from the 24-hour composite samples were close to identical to the grab samples of the same date and the variability of DRP concentrations of the sample set was low, composite sampling is not necessary. However, the low variability allows the above Equation 4-3 to be used to convert grab to 24-hour if desired.

4.2 Nitrogen and Phosphorus of Various HRTs

The HRT comparison study compared nitrogen and phosphorus data from ponds operated over a wide range of HRTs. Two periods for comparison were selected based on them having similar insolation (October 30- November 6, 2013 and October 29- November 5, 2014). The HRTs considered were 1.5-day, 2-day, 3-day (2013 and 2014) and 6-day. As described in Section 3.1.2.2, the 6-day HRT condition was actually two 3-day HRT ponds operated in series with algae sedimentation harvesting following the first pond set. Nutrient removal was expected to correlate with HRT (**Table 4-3**).

Table 4-3: Nutrient concentrations in the influent and effluent of ponds operated at a range of HRTs.

Constituent and HRT	Influent Concentration	Effluent Concentration
Ammonium + Ammonia (mg N/L)		
1.5-day (2014)	44.8	34.1
2-day (2013)	34.3	20.0
3-day (2014)	44.8	18.6
3-day (2013)	34.3	9.0
6-day (2013)	34.3	0.3
Nitrite (mg N/L)		
1.5-day (2014)	0.02	0.1
2-day (2013)	0.01	1.3
3-day (2014)	0.02	2.7

3-day (2013)	0.01	2.4
6-day (2013)	0.01	0.6
<hr/>		
Nitrate (mg N/L)		
1.5-day (2014)	1.1	0.7
2-day (2013)	0.5	2.0
3-day (2014)	1.1	6.5
3-day (2013)	0.5	9.6
6-day (2013)	0.5	12.8
<hr/>		
Soluble Nitrogen (mg N/L)		
1.5-day (2014)	46.0	34.9
2-day (2013)	34.8	23.4
3-day (2014)	46.0	27.8
3-day (2013)	34.8	21.0
6-day (2013)	34.8	13.7
<hr/>		
*Organic Nitrogen (mg N/L)		
1.5-day (2014)	5.6	14.4
2-day (2013)*	9.3	20.9
3-day (2014)	5.6	31.3
3-day (2013)*	9.3	26.0
6-day (2013)*	9.3	43.4
<hr/>		
Total Nitrogen (mg N/L)		
1.5-day (2014)	51.5	49.2
2-day (2013)	43.1	43.9
3-day (2014) ¹	51.5	59.0
3-day (2013)	43.1	30.7
6-day (2013)	43.1	30.7
<hr/>		
Dissolved Reactive Phosphorus (mg P/L)		
1.5-day (2014)	3.9	3.2
2-day (2013)	4.4	3.6
3-day (2014)	3.9	2.6
3-day (2013)	4.4	3.2
6-day (2013)	4.4	2.1

* Denotes that only one week of data was available for the comparison.

(1) The greater effluent total nitrogen concentration average was greater than the influent possibly due to the short 2-week sampling period, in conjunction with grab sampling instead of composite sampling.

4.2.1 Weather and Pond Conditions

The selected 2013 and 2014 time periods had comparable average insolation conditions but different air temperature (**Table 4-4**).

Table 4-4: The 24-hour mean air temperature and solar insolation during the HRT study. These data were collected from the California Irrigation Management Information System (CIMIS) weather station on the Cal Poly campus.

HRT	Air Temperature (°C)	Insolation (W/m ²)
1.5-day (2014)	17.5	165
2-day (2013)	14.3	156
3-day (2014)	17.5	165
3-day (2013)	14.3	156
6-day (2013)	14.3	156

Pond conditions were recorded using the SCADA system set up in each pond. The water temperatures differed by 2.8°C at most, and pH values were kept near 7 to prevent ammonia volatilization. Except for the 1.5-day and 2-day HRT ponds, dissolved oxygen (DO) readings correlated with HRT (**Table 4-5**).

Table 4-5: The average water temperature, pH, and DO readings for each pond over each of the two-week time periods. The 3-day (2013) data are from the Round 1 pond, and the 6-day data are from the Round 2 pond in series.

HRT	Water Temperature (°C)	pH	Dissolved Oxygen (mg/L)
1.5-day (2014)	19.3	7.62	4.2
2-day (2013)	17.9	7.32	3.0
3-day (2014)	18.5	7.84	5.1
3-day (2013)	17.5	7.57	5.3
6-day (2013)	16.5	7.12	15.1

Some of the pond temperature, pH, and DO values were not available or excluded due to the SCADA system malfunctions. See Appendix A for a complete time series of the data used to obtain the average values.

4.2.2 Nitrogen Constituents

A vast majority of the nitrogen that entered the ponds from the primary clarifier effluent water was in the form of total ammonia nitrogen (TAN). A pattern of increased

TAN removal with increased HRT can be seen in the sampled weeks (**Table 4-6**).

Despite the water temperature being 1°C warmer in 2014 than 2013, TAN removal was 15% less in 2014. In addition, if TAN is assumed to be first-order, the higher influent TAN in 2014 should have led to more rapid TAN removal.

Table 4-6: The removal of TAN at the different HRTs.

Constituent and HRT	Influent Concentration	Effluent Concentration	Percent Removed
Ammonium + Ammonia (mg N/L)			
1.5-day (2014)	44.8	34.1	24%
2-day (2013)	34.3	20.0	42%
3-day (2014)	44.8	18.6	59%
3-day (2013)	34.3	9.0	74%
6-day (2013)	34.3	0.3	99%

In nitrification, TAN is oxidized to nitrite which is then oxidized to nitrate. The 2- and 3-day HRTs were found to accumulate small concentrations of nitrite. Presumably the 1.5-day HRT ponds had insufficient dissolved oxygen and HRT for significant nitrification, and the 6-day HRT ponds had enough of both to complete conversion of TAN to nitrate (**Table 4-7**). The 12% difference between the two 3-day HRTs could be caused by the slightly different pond and weather conditions.

Table 4-7: The nitrite production of the ponds.

Constituent and HRT	Influent Concentration	Effluent Concentration	Percent Increase
Nitrite (mg N/L)			
1.5-day (2014)	0.02	0.1	400%
2-day (2013)	0.01	1.3	12900%
3-day (2014)	0.02	2.7	13400%
3-day (2013)	0.01	2.4	23900%
6-day (2013)	0.01	0.6	5900%

With longer HRTs, nitrate concentrations were higher (**Table 4-8**). However, the 1.5-day ponds removed nitrate due to assimilation by algae or denitrification. The two 3-day HRT pond sets displayed a large difference in nitrate concentration possibly due to environmental and pond conditions as stated earlier.

Table 4-8: The nitrate production of the ponds.

Constituent and HRT	Influent Concentration	Effluent Concentration	Percent Increase
Nitrate (mg N/L)			
1.5-day (2014)	1.1	0.7	-36%
2-day (2013)	0.5	2.0	300%
3-day (2014)	1.1	6.5	491%
3-day (2013)	0.5	9.6	1820%
6-day (2013)	0.5	12.8	2460%

Summing TAN, nitrite, and nitrate, the removal of soluble nitrogen by the ponds increased with longer HRTs (**Table 4-9**).

Table 4-9: The soluble nitrogen removal of the ponds.

Constituent and HRT	Influent Concentration	Effluent Concentration	Percent Removed
Soluble Nitrogen (mg N/L)			
1.5-day (2014)	46.0	34.9	24%
2-day (2013)	34.8	23.4	33%
3-day (2014)	46.0	27.8	40%
3-day (2013)	34.8	21.0	40%
6-day (2013)	34.8	13.7	61%

The soluble nitrogen data produced the most linear relationship, with a majority of the nitrogen as TAN (**Figure 4-3**). The correlation of HRT to TAN percent removal (not shown) produced a similar linear relationship with an R^2 value of 0.88.

Figure 4-3: The soluble nitrogen removal percentages graphed with the corresponding HRTs.

Organic nitrogen levels were highly variable (**Table 4-10**). In the 6-day HRT pond setup, the settleable solids from the 3-day HRT pond were removed by sedimentation before the supernatant entered the second 3-day HRT pond to remove potential light limitations on algae growth in the second pond.

Table 4-10: The organic nitrogen levels exiting the ponds.

Constituent and HRT	Influent Concentration	Effluent Concentration	Percent Increase
*Organic Nitrogen (mg N/L)			
1.5-day (2014)	5.6	14.4	157%
2-day (2013)*	9.3	20.9	125%
3-day (2013)*	9.3	26.0	180%
3-day (2014)	5.6	31.3	459%
6-day (2013)*	9.3	43.4	367%

* Denotes that only one week of data was available for the comparison.

The assimilation of nitrogen into algae was checked using the production of organic nitrogen and the decrease in soluble nitrogen for each HRT (**Table 4-11**).

Similarly to the soluble nitrogen, the assimilation produced a linear correlation with an R^2

value of 0.98. The largest difference between the organic nitrogen measured and the difference in influent and effluent soluble nitrogen was a 37% increase. This increase signifies that more nitrogen was present than what was added. It is likely that the increases in nitrogen exiting the ponds can be accounted for by the small sample set and by not having composite samples.

Table 4-11: The nitrogen assimilated due to algae growth. Negative percent values denote an increase in the total nitrogen that was present.

HRT	Nitrogen Assimilated	Losses or Error in Measurements
1.5-day (2014)	19%	5%
2-day (2013)*	33%	0%
3-day (2013)*	56%	-16%
3-day (2014)	48%	-8%
6-day (2013)*	98%	-37%

* Denotes that only one week of organic nitrogen data was available for the comparison.

4.2.3 Phosphorus Constituents

Dissolved reactive phosphorus (DRP) is a readily available form of phosphorus for algae to uptake for growth. As with nitrogen, DRP assimilation should correlate with volumetric algae productivity (mg/L-d). The removals measured for the various HRTs generally support this supposition; however, the removal for the 1.5-day HRT pond set was greater than the 2-day HRT pond set by a small amount (0.1 mg-P/L). Removal by the 2013 and 2014 3-day HRT pond sets was similar, differing by only 0.1 mg-P/L (Table 4-12).

Table 4-12: The DRP levels exiting the ponds.

Constituent and HRT	Influent Concentration	Effluent Concentration	Uptake	Percent Removal
Dissolved Reactive Phosphorus (mg P/L)				
1.5-day (2014)	3.9	3.2	0.7	18%

2-day (2013)	4.4	3.6	0.8	17%
3-day (2014)	3.9	2.6	1.3	33%
3-day (2013)	4.4	3.2	1.2	26%
6-day (2013)	4.4	2.1	2.3	53%

The DRP removal of the ponds at the different HRTs produced a linear relationship as did the soluble nitrogen will (**Figure 4-4**).

Figure 4-4: The DRP uptake percentages graphed with the corresponding HRTs of the ponds.

The assimilation of phosphorus into algae was checked using the production of particulate phosphorus and the decrease in DRP for each HRT (**Table 4-13**). Similarly to the DRP removal, the assimilation produced a linear correlation with an R^2 value of 0.89. The largest discrepancy between the particulate phosphorus measured and the difference in influent and effluent DRP was a 45% increase. This increase signifies that more phosphorus was present than what was added. As stated above, it is likely that the increases in phosphorus exiting the ponds can be accounted for by the small sample set and by not having composite samples.

Table 4-13: The nitrogen assimilated due to algae growth. Negative percent values denote an increase in the total nitrogen that was present.

HRT	Phosphorus Assimilated	Losses or Error in Measurements
1.5-day (2014)*	64%	-25%
2-day (2013)*	56%	-20%
3-day (2013)*	62%	-8%
3-day (2014)	86%	-33%
6-day (2013)*	140%	-45%

* Denotes that only one week of particulate phosphorus data was available for the comparison.

4.3 Nitrogen and Phosphorus Nutrient Depletion with Water Recycle

The purpose of the nutrient depletion experiment with water recycle was to determine the number of days of cultivation and harvesting needed to decrease soluble nitrogen and phosphorus concentrations levels low enough for strict wastewater discharge permits. For biofuel applications, the purpose of this experiment was to determine the time needed to reach low enough nutrient concentrations that lipid accumulation would occur (~1 mg-N/L or ~0.1 mg-P/L). (Note lipid measurements were made.) The experiment was also serve as confirmation that nitrogen, not phosphorus, was the limiting nutrient. This experiment was conducted over fourteen days with sampling on Day 0, Day 7, and Day 14. See Appendix B for time series graphs of total ammonia nitrogen, nitrate, nitrite, organic nitrogen, and dissolved reactive phosphorus.

4.3.1 Pond and Weather Conditions

The pond and weather conditions for this experiment were recorded using the onsite SCADA system and the downloaded CIMIS station weather data. Values for Day 0 were recorded at the time of the initial sample collection, and Day 7 and Day 14 are averaged values from the preceding week between sample grabs (**Table 4-14**). Some data

were excluded due to apparent calibration problems. See Appendix C for the time series graphs of the dissolved oxygen, pH, and temperature pond conditions.

Table 4-14: The pond and weather conditions of ponds during the nutrient depletion experiment (November 15-29, 2014).

	Dissolved Oxygen (mg/L)	pH	Pond Temperature (°C)	Solar Radiation (W/m ²)	Air Temperature (°C)
Day 0	2.3	7.63	19.5	205	20.7
Day 7	10.0	8.08	19.8	194	15.5
Day 14	12.9	8.09	18.2	137	12.7

4.3.2 Nitrogen Uptake and Conversion

Prior to switching to water recycling mode, the ponds were being operated with continuous flow of primary clarifier effluent. In recycling mode, the pond water was passed through settling tanks to remove algae and then returned to the ponds for additional growth. The readily available nitrogen was mainly in the form of ammonium. Oxidized nitrogen was also present in the form of nitrate and organic nitrogen was present at the start of the experiment due to the previous operation of the ponds. The total ammonia nitrogen (TAN) concentration in the ponds was reduced close to zero after seven days and converted into organic nitrogen and oxidized nitrogen. By Day 14, a majority of the nitrogen in the ponds had been consumed by the algae and converted into organic nitrogen (**Figure 4-5**). The water was recycled on a 3-day HRT and settleable algae was removed as the water was recycled. The pH of the system was regulated to minimize ammonia volatilization to ensure nitrogen only left the system via organic nitrogen in the settled algae. It was assumed in this short experiment, with paddlewheel mix ponds, that no net sedimentation occurred in the raceways.

Figure 4-5: The conversion of nitrogen over the course of the experiment. Oxidized nitrogen was mainly in the form of nitrate, although some nitrite was present in small amounts. Error bars denote the total nitrogen concentration standard deviation of the pond set.

The nitrogen transformation followed the expected trend of TAN being converted to oxidized nitrogen and organic nitrogen. By Day 7, TAN was converted into nitrate or it was consumed by the algae for growth. On the final day of the experiment, a small amount of oxidized nitrogen remained, no TAN was present, and mostly organic nitrogen remained. The equivalent of about 17 mg-N/L was removed, presumably mostly via harvest algae removed from the settling tanks.

Between Day 0 and Day 7, 11.5 mg-N/L of soluble nitrogen was removed from the ponds, and between Day 7 and Day 14 another 10 mg-N/L was removed. Less than 3 mg-N/L of soluble nitrogen remained in the ponds on Day 14. The removal rate was 1.5 mg-N/L-day for the entire length of the experiment.

4.3.3 Dissolved Reactive Phosphorus Removal

The dissolved reactive phosphorus (DRP) concentrations in each of the ponds decreased approximately 1 mg-P/L over the first seven days and about 2.3 mg/L over the full 14 days (**Figure 4-6**). The removal rate for the fourteen days was 0.18 mg-N/L-day. The total phosphorus removal over 14 days (~1.9 mg/L) was expected to have occurred entirely from removal of harvested algae from the settling tanks. This supposition cannot be confirmed due to insufficient collection of information on the harvested algae mass.

After 14 days, the residual DRP was 0.9 mg/L, giving an N:P ratio of 3:0.9 or 3.3:1. Green algae experiencing N limitation contained <5% N in their cell tissue. For P limitation, the cell tissue may contain <0.1% P. The ratio of the nutrient limited algae would be about 5:0.1 or 50:1. Thus, nitrogen is almost certainly the limiting nutrient to algae growth in the wastewater tested. Complete phosphorus removal by algae assimilation would actually require nitrogen addition or growth of algae with a more plastic N:P ratio than illustrated here.

Figure 4-6: The phosphorus concentrations in the ponds decreasing over time. The error bars denote the total phosphorus standard deviation of the pond set.

4.4 Results of Boiled Pretreatment Aerobic Digestion Experiment

In this section, the results of the boiled pretreatment aerobic digestion experiment will be discussed. These results will be compared with previous anaerobic-aerobic digestion pretreatment experiments to evaluate pretreatment methods in Section 4.6. See Chang (2014) for the aerobic digestion results for the sonicated and homogenized pretreatment experiments (discussed in Background Section 2-5) and Hill (2014) for all the anaerobic digestion results. **The standard deviations, $\pm$ values, throughout this section were calculated from triplicate reactors.**

4.4.1 Temperature, pH, and Dissolved Oxygen

As described in the Methods chapter, the aerobic digestion reactors were placed in heated water baths for a consistent temperature in the triplicate sets. The target temperature of 20°C was well maintained: Over the 71-day digestion period, the average

temperature for the non-boiled algae reactors was $19.7 \pm 0.1^\circ\text{C}$ and for the boiled algae was $19.1 \pm 0.1^\circ\text{C}$ (**Figure 4-7** and **Figure 4-8**).

Figure 4-7: Mean reactor water temperature for the non-boiled algae reactor triplicate set with error bars ($\pm$ one standard deviation) during aerobic digestion.

Figure 4-8: Mean reactor water temperature for the boiled algae reactor triplicate set with error bars ($\pm$ one standard deviation) during aerobic digestion.

Both reactor sets started at pH 8.5 and slowly decreased to a pH of 8.0 before rapidly dropping to a pH of around 5.5 over the course of 5 days. The boiled algae reactors continued to slowly drop to a pH of approximately 5 over the course of 11 days where it remained for the duration of the experiment (**Figure 4-9**). The non-boiled algae reactors dropped to a pH of roughly 4.5 over the course of 8 days and remained at this pH for the remainder of the experiment (**Figure 4-10**).

Figure 4-9: Mean reactor pH for the non-boiled algae reactor triplicate set with error bars ($\pm$ one standard deviation) during aerobic digestion.

Figure 4-10: Mean reactor pH for the boiled algae reactor triplicate set with error bars ($\pm$ one standard deviation) during aerobic digestion.

Dissolved oxygen (DO) levels in the pilot ponds often exceeded 8.5 mg/L during the day due to photosynthesis. To ensure similar conditions in the reactors, the DO levels were maintained close to or above 8.5 mg/L (**Figure 4-11** and **Figure 4-12**). The average DO for the non-boiled algae reactors was 8.97 ± 0.04 mg/L and for the boiled algae reactors 9.04 ± 0.04 mg/L.

Figure 4-11: Mean reactor DO for the non-boiled algae reactor triplicate set with error bars ($\pm$ one standard deviation) during aerobic digestion.

Figure 4-12: Mean reactor DO for the boiled algae reactor triplicate set with error bars ($\pm$ one standard deviation) during aerobic digestion.

4.4.2 Volatile Suspended Solids Degradation

Initially the volatile suspended solids (VSS) decayed quickly, but after about a month of aeration, the degradation rate slowed considerably, leaving approximately 60% of the VSS as non-biodegradable VSS (**Figure 4-13**).

Figure 4-13: VSS concentration for both the non-boiled and boiled algae reactor triplicate sets with error bars ($\pm$ one standard deviation) during aerobic digestion.

When the VSS concentration for both sets of reactors was normalized to 100%, the final solids degradation for the two conditions was found to be approximately proportional to each other (**Figure 4-14** and **Table 4-15**).

Figure 4-14: Normalized VSS concentration for both the non-boiled and boiled algae reactor triplicate sets with error bars ($\pm$ one standard deviation) during aerobic digestion.

Table 4-15: Summary of boiled aeration experiment results.

Pretreatment	Initial VSS	Final VSS	Inert Percentage
Type	(mg/L)	(mg/L)	(%)
Boiled	1306	765 $\pm$ 54	59 $\pm$ 4
Non-Boiled	1533	866 $\pm$ 109	56 $\pm$ 7

The non-boiled reactors followed a second-order VSS decay rate and the boiled reactors followed a first-order VSS decay rate.

4.4.3 Nitrogen Transformations

As would be expected based on the VSS degradation curve, organic nitrogen degraded in both reactor sets (**Figure 4-15** and **Figure 4-16**). It is assumed that organic nitrogen degrades into nitrogen forms that could support more algae growth. In the non-

boiled algae, organic nitrogen decayed from 130 mg/L (8% of initial VSS) to 19 mg/L (2% of residual VSS). For the boiled algae, organic nitrogen decayed from 107 mg/L (8% of initial VSS) to 43 mg/L (6% of residual VSS). The decrease in nitrogen content in the VSS suggests that proteins are solubilized and released from the algal cells during aerobic digestion. Overall, the non-boiled released more nitrogen mass than the boiled biomass.

Because the aerobic digestion were conducted in the dark, algae did not assimilate the soluble nitrogen initially present and that which subsequently solubilized from the biomass. Instead, under aeration, the soluble nitrogen underwent further transformations, which would not necessarily occur in a raceway pond. A large portion (approximately 72% non-boiled and 68% boiled) of the total ammonia nitrogen (TAN) was volatilized rapidly in the first nine days due to aeration leading to a decrease in the total nitrogen of the solutions. The remaining TAN was converted into nitrite, nitrate, or bacterial cell mass.

Peak nitrite concentrations occurred simultaneously on Day 14 in both sample sets. Peak nitrate concentrations occurred on Day 29 for the non-boiled algae and Day 71 for the boiled algae reactors. The non-boiled reactors had a higher nitrate concentration than organic nitrogen on the final day of the experiment, unlike the boiled reactors. The non-boiled reactors also had a greater degradation of organic nitrogen, with respect to both concentration and percentage. However, a greater solids content concentration was present at the start of aeration. In Section 4.6, the degradation of organic nitrogen was normalized with the VSS to better compare the aerobic-anaerobic digestion experiments.

Figure 4-15: The time-course concentrations of various nitrogen forms in the non-boiled algae aerobic reactor triplicate set with error bars ($\pm$ one standard deviation).

Figure 4-16: The time-course concentrations of various nitrogen forms in the boiled algae aerobic reactor triplicate set with error bars ($\pm$ one standard deviation).

4.4.4 Phosphorus Transformations

The release of dissolved reactive phosphorus (DRP) from both non-boiled and boiled algae followed similar trends (**Figure 4-17**). The final concentrations at the conclusion of the study were 26.6 mg-P/L in both aerobic reactors.

Figure 4-17: DRP concentrations for both the non-boiled and boiled algae reactor triplicate sets with error bars ($\pm$ one standard deviation).

The non-boiled reactor set released approximately 1.70 mg/L more phosphorus in the form of DRP than the boiled reactor set during the aeration process (**Table 4-16**).

Table 4-16: The change in DRP concentrations of the aerobic reactor sets. All values are in mg-P/L unless specified.

Pretreatment Type	Initial DRP	Final DRP	DRP Release
Non-Boiled	19.1	26.6 $\pm$ 2.4	7.42 $\pm$ 2.4
Boiled	20.9	26.6 $\pm$ 1.0	5.72 $\pm$ 1.0

Total phosphorus (TP) loss occurred in the both sets of reactors (**Table 4-17**), likely due to an analytical error. Careful cleaning measures were taken in the present aeration experiment to prevent solids adhesion, as described in Section 3.3.2. Visibly, little solid matter was present at the end of the experiment. The sampling method prevented solids from settling by keeping the sample well mixed, also described in Section 3.3.2.

Table 4-17: TP concentrations for the non-boiled and boiled aerobic reactor sets. All values are in mg-P/L unless specified.

Pretreatment Type	Initial TP	Final TP	TP Loss
Non-Boiled	108.3	99.8±62.5	8.53±62.5
Boiled	121.7	91.1±28.8	30.6±28.8

The typical phosphorus content of rapidly-growing algae is between 0.5% and 1.2%. The VSS decreased in the non-boiled reactors by approximately 670 mg/L. The expected DRP release, based on 0.5-1.2%, was between 3.35 mg-P/L and 8.04 mg-P/L, which brackets the measured change in DRP concentration of 7.42 mg-P/L (**Figure 4-18**). The VSS decreased in the boiled reactors by approximately 540 mg/L. The expected DRP release was between 2.70 mg-P/L and 6.48 mg-P/L, which again brackets the measured change in DRP concentration of 5.72 mg-P/L (**Figure 4-19**).

Figure 4-18: The time-course DRP release and VSS degradation for the non-boiled aerobic reactor triplicate set with error bars ($\pm$ one standard deviation).

Figure 4-19: The time-course DRP released and VSS degradation for the boiled aerobic reactor triplicate set with error bars ($\pm$ one standard deviation).

4.5 Anaerobic followed by Aerobic Digestion Experiments

This section covers the amount of degradation and the resulting solubilization of the total organic nitrogen and particulate phosphorus for the lab scale anaerobic and aerobic digestion experiments (Methods Section 3.3). The pretreatment technology results discussed will be sonicated, homogenized, autoclaved, and boiled and the non-pretreated controls run in parallel. The aerobic digestion of the boiled algae is the focus of the present thesis; refer to Hill (2014) for detailed results and information on the anaerobic digestion portion of these experiments and Chang (2014) for detailed results and information on the sonicated and homogenized aerobic digestion.

The purpose of these lab scale experiments was to simulate an algae biofuel production facility (Section 2-5) with internal nutrient recycling. In this flow schematic algae biomass is decomposed in anaerobic digesters, with the effluent digestate being metered into the aerobic raceway ponds for algae regrowth on the nutrients of the digestate. The aerobic lab experiments were meant to determine the extent of nitrogen

and phosphorus resolubilization during aerobic digestion, which was meant to simulate raceway pond degradation conditions. These lab scale experiment implemented several pretreatment methods in order to evaluate if there was a need to pretreat the algae before digestion. Knowing the approximate possible resolubilization of nutrients is important for the next set of pond experiments where the ponds will only be fed the digester effluent. It was assumed for this experiment that the difference between VS (volatile solids) and VSS values were negligible.

4.5.1 Effect of Pretreatment Methods on VSS Decay

The initial VSS levels differed between the digestion experiments. To compare these tests, the fraction of the initial VSS remaining after anaerobic and aerobic digestion was used (**Figure 4-20**).

Figure 4-20: Fraction of initial VSS remaining for each of the pretreated and the non-pretreated control groups. For each group, the bars represent “initial,” “post anaerobic,” and “post aerobic” digestions. The “initial” represents the sample before being introduced into the digesters. “Post anaerobic” is after anaerobic digestion is completed and before

aeration begins. “Post aerobic” is after the aeration process on the final day of aeration. Groups with only two bars do not have “post aerobic” data.

The decay of the VS (volatile solids) after anaerobic digestion is within the expected range of between 20-60% for each sample (Bohutskyi, 2014). Excluding the sonicated aeration sample, all reactors experienced an additional 10% decay of VSS and the pretreated samples decayed approximately 5% more than the non-pretreated samples. In most cases this additional breakdown exceeds the 20-60% expected range of decay. The additional aerobic decay of VS was within the expected range of 13-88% (Jewell & McCarty, 1971). With regards to VSS, the pretreatment technologies were successful in causing additional decay.

4.5.2 Effect of Pretreatment Methods on Organic Nitrogen Decay

The initial organic nitrogen levels differed between the digestion experiments. To compare these tests, the fraction of the initial organic nitrogen remaining after anaerobic and aerobic digestion was used (**Figure 4-21**).

Figure 4-21: Fraction of initial organic nitrogen remaining for each of the pretreated and the non-pretreated control groups. For each group, the bars represent “initial,” “post anaerobic,” and “post aerobic” digestions. The “initial” represents the sample before being introduced into the digesters. “Post anaerobic” is after anaerobic digestion is completed and before aeration begins. “Post aerobic” is after the aeration process on the final day of aeration. Groups with only two bars do not have “post aerobic” data.

Similarly to the VSS decay, the nitrogen decay after anaerobic digestion was greater for the pretreated samples, excluding the sonicated pretreated sample. However, after aerobic digestion the results are more varied (**Table 4-18**).

Table 4-18: Nitrogen released from the biomass and the VSS decayed.

Pretreatment	Nitrogen Released	VSS Decayed
Homogenized	87%	66%
Non-Homogenized	90%	59%
Sonicated	94%	40%
Non-Sonicated	*93%	*65%
Autoclaved	*57%	*45%
Non-Autoclaved	*49%	*39%
Boiled	76%	64%
Non-Boiled	75%	59%

*Denotes aerobic digestion was not performed on sample.

The organic nitrogen decay was unexpectedly not proportional to VSS, instead the fraction of organic nitrogen decayed and the subsequent nitrogen release was greater in all samples than the VSS decay. Organic nitrogen compounds (e.g. amino acids) decayed at a more accelerated pace than other cell components. Some nitrogen remained after both digestion processes and this nitrogen could be in non-biodegradable or slowly degrading biomass.

The nitrogen transformation graphs from the boiled aeration digestion experiment (Section 4.5.3) show a steady decline in organic nitrogen throughout aeration. As seen above in Table 4-18, the percentage of nitrogen released for the boiled pretreated and

non-pretreated samples were essentially the same and the boiled VSS decay was only 5% higher.

These results indicate that for nitrogen release from biomass pretreatment before anaerobic digestion does not have a significant impact. After the aeration process, the percent of nitrogen released was essentially the same for pretreated and non-pretreated samples.

4.5.3 Effect of Pretreatment Methods on Particulate Phosphorus Decay

As with the organic nitrogen analysis, initial particulate phosphorus levels differed between the experiments and so were normalized. **Figure 4-22** shows the particulate phosphorus remaining after each step as a fraction of the initial raw biomass for each pretreatment method.

Figure 4-22: Fraction of initial particulate phosphorus remaining for each of the pretreated and the non-pretreated control groups. For each group, the bars represent “initial,” “post anaerobic,” and “post aerobic” digestions. The “initial” represents the sample before being introduced into the digesters. “Post anaerobic” is after anaerobic

digestion is completed and before aeration begins. “Post aerobic” is after the aeration process on the final day of aeration. Groups with only two bars do not have “post aerobic” data.

The particulate phosphorus decay was more proportional to the VSS decay than the organic nitrogen with only the pretreated homogenized and non-pretreated samples not within 10% (**Table 4-19**). However, the non-pretreated samples did not go through aerobic digestion.

Table 4-19: Phosphorus released from the biomass and the VSS decayed.

Pretreatment	Phosphorus Released	VSS Decayed
Homogenized	53%	66%
Non-Homogenized	49%	59%
Sonicated	*47%	40%
Non-Sonicated	*48%	*65%
Autoclaved	*38%	*45%
Non-Autoclaved	*41%	*39%
Boiled	71%	64%
Non-Boiled	67%	59%

*Denotes aerobic digestion was not performed on sample.

Particulate phosphorus compounds (e.g. phospholipids) decayed at a similar rate to the other cell components. The slower release of phosphorus compared to nitrogen indicates that the phosphorus is more tightly bound within the cells and nitrogen is more available for release. A large portion of phosphorus remained after both digestion processes and this phosphorus is likely associated to the VSS that did not continue to decay.

These results indicate that for phosphorus release from biomass pretreatment before anaerobic digestion does not have a significant impact. After the aeration process, the percent of phosphorus released was essentially the same for pretreated and non-pretreated samples.

5. Conclusions

The conclusions, limitations, and recommended future research based on the information previously discussed in Section 4 will be presented in the following sections.

As stated in Section 2, the goals of this research were to determine:

- (1) A conversion factor to convert from grab to composite samples
- (2) The relationship between HRT and nutrient removal
- (3) The rate and extent of nutrient removal with 100% water recycle
- (4) The ultimate extent of nutrient resolubilization from algae pretreated by boiling, anaerobically digested then aerobically digested

5.1 Experimental Conclusions

Three pilot scale and one lab scale experiments will be presented. The pilot scale experiments include grab and 24-hour composite influent comparison, HRT comparison, and nutrient depletion experiments. The information obtained is important for scale up purposes and future experiments of a wastewater treatment and biofuel production facility. The pilot scale experiment conducted was the aerobic digestion of boiled pretreated algae.

5.1.1 Comparison of Grab and 24-Hour Composite Influent

Equations to convert TAN and DRP concentrations from grab to composite concentrations were made for the pond influent. On average the TAN composite concentration was 9% greater than the grab and for DRP the composite was 3% greater. With values on average being essentially the same, there is no difference between grab and composite sampling.

5.1.2 Nitrogen and Phosphorus of Various HRTs

Both the soluble nitrogen and DRP removal were linearly proportional. The soluble nitrogen developed a linear relation with an R^2 of 0.98 composed mostly of TAN,

which had an R^2 of 0.88. The DRP removal had a linear relation with an R^2 of 0.95. Both were able to develop equations to approximate the percent removal of different HRT for each nutrient.

5.1.3 Nitrogen and Phosphorus Nutrient Depletion with Water Recycle

Several conclusions were formed from the nutrient depletion experiment.

Nitrogen was the limiting nutrient and required fourteen days for concentrations to reach below 5 mg-N/L during the Fall. The removal rate for nitrogen was 1.5 mg-N/L-day and for DRP the rate was 0.18 mg-P/L-day.

5.1.4 Boiled Pretreatment Aerobic Digestion Experiment

Boiling pretreatment had no significant effect on the decay of organic matter and resolubilization of nutrients after aerobic digestion. The VSS decay for non-boiled was 56% and for boiled it was 59%. The organic nitrogen decay and resultant resolubilization for the non-boiled was 65% and the boiled was 61%. DRP release for the non-boiled was 7.42 mg-P/L and boiled was 5.72 mg-P/L. The results from the boiling aeration process does not show a significant change with the use of pretreatment.

5.1.5 Anaerobic followed by Aerobic Digestion Experiments

Pretreatment technologies did not have a significant effect to the decay of organic matter or the resolubilization of nutrients after both anaerobic and aerobic digestions. Without the need for pretreatment, this saves on overall costs of creating a full scale wastewater treatment and biofuel production facility. The VSS did decay 5% more for pretreated samples. There was 75-95% nitrogen and 46-67% phosphorus solubilization after both digestion processes (**Table 5-1**). It was also interesting to note that relative to

the controls for each experiment, the solubilization differed between each experiment. This difference results from the use of different source harvested algae.

Table 5-1: The decay of VSS and the release of nitrogen and phosphorus for the digestion experiments.

Pretreatment	VSS Decayed	Nitrogen Released	Phosphorus Released
Homogenized	66%	87%	53%
Non-Homogenized	59%	90%	49%
Sonicated	40%	94%	*47%
Non-Sonicated	*65%	*93%	*48%
Autoclaved	*45%	*57%	*38%
Non-Autoclaved	*39%	*49%	*41%
Boiled	64%	76%	71%
Non-Boiled	59%	75%	67%

*Denotes aerobic digestion was not performed on sample or no aeration data available.

5.2 Research Limitations

The limitations of the studies were:

- (1) The pH, DO, and temperature probes malfunctioned periodically and produced unreliable data
- (2) Pond grab samples were not representative of the entire day
- (3) The exact amount of organic nitrogen and particulate phosphorus leaving the tube settlers was not measured
- (4) The pH in the boiling experiment dropped to 4.5 during the aeration process

5.3 Future Research

To better understand and fully develop the conclusions made here, the following future research should be performed:

- (1) Composite pond effluent samples
- (2) Take hourly influent grab samples to find optimum sample time
- (3) Pilot scale nutrient and water recycling
- (4) Repeat the HRT comparison in different seasons to develop modeling parameters
- (5) Repeat nutrient depletion until concentrations are less than 1 mg/L
- (6) Monitor nitrogen rate during the anaerobic digestion process

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APPENDICES

Appendix A: Time series graphs of the DO, pH, and temperature of the raceway ponds operating at different HRTs

Appendix B: Time series graphs of TAN, nitrite, nitrate, organic nitrogen, and DRP from the nutrient depletion experiment

Appendix C: Time series graphs of the DO, pH, and temperature of the raceway ponds during the nutrient depletion experiment

Appendix A: Time series graphs of the DO, pH, and temperature of the raceway ponds operating at different HRTs

Figure A-1: Dissolved oxygen time series graph of the 1.5-day HRT ponds.

Figure A-2: Dissolved oxygen time series graph of the 2-day HRT ponds.

Figure A-3: Dissolved oxygen time series graph of the 3-day (2014) HRT ponds.

Figure A-4: Dissolved oxygen time series graph of the 3-day (2013) HRT ponds.

Figure A-5: Dissolved oxygen time series graph of the 6-day HRT ponds. Note that the ponds shown were the second 3-day HRT ponds and the above 3-day (2013) HRT ponds were the first set of ponds.

Figure A-6: pH time series graph of the 1.5-day HRT ponds.

Figure A-7: pH time series graph of the 2-day HRT ponds.

Figure A-8: pH time series graph of the 3-day (2014) HRT ponds.

Figure A-9: pH time series graph of the 3-day (2013) HRT ponds.

Figure A-10: pH time series graph of the 6-day HRT ponds. Note that the ponds shown were the second 3-day HRT ponds and the above 3-day (2013) HRT ponds were the first set of ponds.

Figure A-11: Temperature time series graph of the 1.5-day HRT ponds.

Figure A-12: Temperature time series graph of the 2-day HRT ponds.

Figure A-13: Temperature time series graph of the 3-day (2014) HRT ponds.

Figure A-14: Temperature time series graph of the 3-day (2013) HRT ponds.

Figure A-15: pH time series graph of the 6-day HRT ponds. Note that the ponds shown were the second 3-day HRT ponds and the above 3-day (2013) HRT ponds were the first set of ponds.

**Appendix B: Time series graphs of TAN, nitrite, nitrate, organic nitrogen, and
DRP from the nutrient depletion experiment**

Figure B-16: Time series graph of the TAN concentrations of the nutrient depletion ponds.

Figure B-17: Time series graph of the nitrite concentrations of the nutrient depletion ponds.

Figure B-18: Time series graph of the nitrate concentrations of the nutrient depletion ponds.

Figure B-19: Time series graph of the organic nitrogen concentrations of the nutrient depletion ponds.

Figure B-20: Time series graph of the DRP concentrations of the nutrient depletion ponds.

Appendix C: Organic nitrogen and particulate phosphorus data from the sequential Anaerobic and Aerobic Digestion Experiments

Figure C-21: Dissolved oxygen time series graph for the nutrient depletion experiment.

Figure C-22: pH time series graph for the nutrient depletion experiment.

Figure C-23: Temperature time series graph for the nutrient depletion experiment.