ANAEROBIC DIGESTION OF WASTEWATER-GROWN ALGAE BIOMASS WITH OPTIMIZED BIOGAS YIELDS AND NUTRIENT SOLUBILZATION

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ABSTRACT

Anaerobic Digestion of Wastewater-Grown Algae Biomass With Optimized Biogas Yields And Nutrient Solubilization

Tiffany Racz

Whole-cell algae biomass grown on nutrient-rich wastewater can be anaerobically digested to produce renewable heat and power, and to solubilize nutrients to grow additional algae biomass in a biorefinery system. In this study, algae biomass was grown on clarified primary wastewater in 33-m^2 ponds at a 4-day residence time with sedimentation harvesting of the biomass. 1.2-L lab digesters were used to test biogas yields and nutrient solubilization from the anaerobic digestion of algal biomass with and without mixing and with sonication as a pretreatment. Additionally, algae were fed to unheated and unmixed 1135-L pilot digesters to determine the effects of seasonal temperatures and organic loading rates on biogas yields and nutrient solubilization. Finally, a scalability experiment was conducted to determine how well lab digesters replicated the nutrient solubilization and biogas yields of pilot digesters when they were operated at the same average daily temperature, and organic load. Overall, the tested conditions included mixing, temperature, feed pretreatment by sonication, scale, and organic loading. It was determined that unmixed, 20˚C digesters fed an average variable organic loading of 0.12 g VS/L-day had the highest yield of 0.3 L CH_4/g VS fed. Compared to similarly operated digesters (30˚C, constant organic load 0.25 g VS/L-day) with a mass yield of 0.24 L CH_4/g VS fed, sonicated feed increased the mass yield of methane by 18% (0.28 L CH_4/g $VS_{introduced}$), and mixing increased the mass yield of methane by 4% (0.25 L CH_4/g VS). For the same digesters, sonicated feed increased the average nitrogen and phosphorus solubilization 10% and 11% with 36% N and 28% particulate P remaining, respectively. Eliminating mixing increased the average nitrogen and phosphorus solubilization by 13% and 27%, with 40% and 31% remaining as particulates,

respectively. The pilot digesters produced an overall average mass yield of 0.19 L CH_4/g VS, with a summer average of 0.46 L CH_4/g VS and a winter average of 0.15 L CH_4/g VS. For the pilot digesters, the average amount of remaining particulate nitrogen and phosphorus was 36% and 39%, respectively, with an average of 57% volatile solids destruction. Finally for the scale experiment, the pilot digesters exhibited mass and volumetric yields of 47% and 28% (0.19 L CH_4/g VS; 0.011 L CH_4/L-d) greater than the lab digesters. Additionally, the pilot digesters had 2% greater nitrogen solubilization and 29% less phosphorus solubilization with 23% N and 15% P, than the lab digesters. Based on these results, for a low organic load (0.01 – 0.65 g VS/L-day), it is recommended that digesters be unmixed and heated which, and have a longer winter residence time. In addition to benefiting methane yield and nutrient solubilization, these digester operating conditions would allow increased supply of nutrients to ponds during the most productive months when nutrients are being consumed at faster rates in the algae ponds.

Keywords: Anaerobic digestion, algae digestion, digester feed pretreatment, digester mixing, biogas yields

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1 Introduction

1.1 The global perspective

An increasing global population and standard of living has led to a growing demand for renewable energy. Energy consumption around the world is expected to rise 28% by 2040, with most of the increase occurring in countries with strong economic growth, growing access to energy, and rapidly growing populations. Fossil fuels, such as petroleum, natural gas, and coal still meet 86% of the world's energy demands, with only 14% met by renewables such as ethanol and biodiesel, hydroelectric, nuclear, and other sources (Bowman, 2017; Heinberg, 2009). Despite their continuing prevalence, net energy return on energy investment (EROEI) for liquid fossil fuels has decreased from over 100:1 to an average of 1:1 in the U.S, due to limited sources and increased costs of extraction. For biofuels, research and development (R&D) has focused on the production and conversion of biomass (plant material) to biodiesel, bioethanol, biohydrogen and biogas (Uggetti et al., 2014). Due largely to R&D the EROEI of renewable liquid fuel such as biodiesel has recently increased from 1.9:1 to 9:1 improving the business case for renewable fuels. This coupled with the reduced greenhouse gas (GHG) emissions of renewable energy points to a promising future (Heinberg, 2009).

Global climate change is widely acknowledged to be a result of increased GHG in the atmosphere (The "Greenhouse" effect, 1989). The primary GHGs is carbon dioxide (CO_2) from the combustion of fossil fuels, which accounts for 75% of the net emissions globally (Climate, 2017). Carbon neutral or negative sources of energy offset CO_2

emissions and decrease GHGs, slowing down climate change. In recent years, the carbon intensity of fuels (MT CO_2/ Bbtu) has decreased due to the transition to more efficient and/or renewable energy sources, but overall CO_2 emissions have increased due to increased energy demand (Bowman, 2017). By increasing the production of renewable fuels and further decreasing fuel carbon intensity, it may be possible to limit additional GHG emissions and slow climate change.

1.2 The California perspective

State environmental policies have driven R&D to slow GHG emissions. In 2015, California re-adopted policies that promoted clean energy and reduced GHG emissions and created a market for carbon emissions through cap-and-trade (Low Carbon Fuel Standard, 2018). Additionally, numerous State programs and agencies such as the California Energy Commission, and the Public Interest Energy Research (PIER) Program provide funding for R&D for biomass power generation. The Low Carbon Fuel Standard (LCFS) (Executive order S-1-07 effective as of January 1, 2016, amended in 2018) was one of nine measures in California Assembly Bill (AB 32) to reduce GHG emissions to 1990 levels by 2020. The LCFS encourages the use and production of low-carbon transportation fuels such as renewable compressed natural gas (RCNG) in California to reduce GHG emissions (Biomass Energy in California, n.d.).

In California, development of biomass energy was identified as important for reaching the policy goal of 75% in-state production of renewable fuels by 2050 (Youngs, 2011). The LCFS uses a life-cycle assessment to evaluate the potential GHG emissions from renewables that includes water and/or land used for the production of biomass (Low

Carbon Fuel Standard, 2018). This limits the land used for biomass production to unusable or abandoned agricultural land, or unused timber land and eliminates most biomass crops (Youngs, 2011). However, algae can be produced on otherwise non-arable land, using non-potable water, making it a promising source of biomass (Dunlap, 2009).

1.3 Algal biomass used as a source of biofuels

Microalgae (algae) are a promising source of biomass for biofuels because they are highly productive (up to 70 dry ton per ha per year) compared to conventional land-based plants such as corn (7 dry ton per ha per year), non-food crop that can be grown on non-potable water sources and non-arable land (Dunlap, 2009; Ugetti, 2014). Microalgae grow rapidly and in a variety of waters unsuitable for other uses including alkaline lakes, municipal and industrial wastewaters, and seawater (Gouleke et al., 1960; Oswald, 1995; Hunter-Cueva et al., 2012). The major growth requirements for algae are sunlight, water, nutrients such as nitrogen and phosphorus (N & P), and carbon such as CO_2. The major limit to widespread production of algae biomass is the availability of suitable land co-located with a CO_2 source and recycled nutrients (Venteris et al., 2014; Hunter-Cevera, 2012). Currently, recycling CO_2 in flue gas is the leading method of CO_2 provision in algae biofuel studies but in reality co-location is difficult (Sialve, 2009; Lundquist, 2010). Recycling nutrients from wastewater, anaerobic digester effluent, or agricultural runoffs reduces chemical fertilizer inputs and is also critical to sustainability (Sialve, 2009).

The processes used for converting algal biomass to fuel are numerous and include thermochemical or biochemical processes to produce both liquid and gaseous fuels (Brennan, 2010). Algal biomass conversion processes such as lipid extraction,

hydrothermal liquefaction (HTL), anaerobic digestion, and fermentation have been widely studied as effective methods for producing fuels such as bio-oil, renewable compressed natural gas (RCNG) and bio-ethanol (Brennan, 2009). Of the fuel conversion processes, anaerobic digestion is a well-established biomass-to-biogas conversion process that can be used alone or in conjunction with other processes, that also conserves and solubilizes nutrients that can then be used again as algae fertilizer (Uggetti, 2014). Digestion also produces CO_2 gas in the biogas and from on-site combustion of methane, which can supply carbon for the algae (Woertz, 2009). Digestion for biofuel production has the additional advantage of not needing the algae to be dried or greatly thickened, as required for the lipid extraction and HTL processes (Lundquist et al., 2010; Sialve, 2009).

For algae, success of the anaerobic digestion process is dependent on the breakdown of the recalcitrant algal cell walls during hydrolysis (Sialve, 2009). Adding difficulty, algal cell walls and biochemical makeup are species dependent but both effect biogas yields and the biochemical composition ranges widely from 40-60% proteins, 5-60% lipids, and 8-30% carbohydrates (Ugetti, 2014, Buswell, 1952). In the literature, typical methane yields from algae range from 0.15 L CH_4/g VS -0.80 L CH_4/g VS (Sialve, 2009; Montingelli, 2015). Yields are generally improved with digester heating, mixing and/or pretreatment, but these improvements also use energy and thus require careful evaluation in energy balances (Montingelli, 2015).

2 Background

2.1 Anaerobic digestion

Anaerobic digestion is a mature process for the stabilization municipal or industrial wastewater solids or sludge (Ward, 2014). Stabilization is the controlled degradation of the waste to decrease odor and to decrease sludge mass by converting organics to biogas (CH_4 and CO_2). Anaerobic digestion occurs in four major steps: (1) hydrolysis where complex macromolecules are solubilized to simple sugars (2) fermentation and (3) acetogenesis where the simple sugars are converted to hydrogen and acetate and (4) methanogenesis where hydrogen and CO_2, and acetate are converted to methane (McCarty, 1964). The fermentation and acetogenesis steps are typically the fastest steps and must be balanced with methanogenesis to prevent acid accumulation and low pH which is toxic to methanogenic bacteria (McCarty, 1964). For municipal wastewater sludge, the typical biogas composition is about 65% CH_4 and 35% CO_2 (McCarty, 1964).

2.2 Algae biofuels

In the 1970s, the US energy crisis led to increased research into alternative liquid fuels including algae (Ward, 2014). During the Regan administration and later, algae biofuel research funding by the US government was minimal until dependence on “foreign oil” again became an issue. The 2007 Energy Independence and Security Act was initiated by the G.W. Bush administration to increase the production of renewable fuels and develop renewable fuel production. Currently, algae are federally recognized in the U.S. as a source of biomass for biofuels and research funding for advancing the implementation of

large-scale operations is available through several federal programs inside the Department of Energy (DOE) (Algal, n.d.).

Algae biofuels include biodiesel, bioethanol, biocrude, and biogas (Uggetti, 2014). Biodiesel is made by extracting triacylglycerides from the algae and can surpass the oil productivity yield from the best oilseed crop of rapeseed because it can be grown year-round (Brennan, 2010). Bioethanol is produced by the fermentation of algal carbohydrates and has promise due to the low lignin and hemicellulose of algae biomass, compared to other feedstocks (Ugetti, 2014). Bio-oil production using hydrothermal liquefaction (HTL) of algal biomass is promising because it eliminates the need to dry the biomass, and it rapidly converts algae into energy dense bio-oil (Guo, 2015). HTL, lipid extraction and carbohydrate fermentations all produce residuals that can then be digested. Anaerobic digestion of algae biomass is promising because it is an established and well understood process that can be used to produce biogas from whole-cell algae, and residuals from other fuel processes and/or co-digested with other wastes (Sialve, 2009). Challenges for algae biofuels systems include fertilizer demands, high energy needed to harvest and dewater algae, and inefficient fuel conversion practices (Ward, 2014).

2.3 Algae for wastewater treatment and biomass production

Early research on algae-based wastewater treatment in California investigated shallow aerobic ponds for the removal of BOD and nutrients and found 85% BOD removal (Gouleke,Oswald & Gotaas, 1957). In algae-based wastewater treatment, mechanical aeration is completely or partially replaced with oxygenation via algal photosynthesis (Gouleke et al., 1960; Oswald, 1995). Gotaas et. al. (1957) gave six justifications for

algae–based wastewater treatment including that algae-base wastewater treatment is a low-cost option in remote regions, the process is stable, and the process results in a valuable biomass co-product (Gotaas, et al, 1957).

Early research also established that algae could be used to oxygenate the surface of deep ponds, reducing odors (Gouleke,Oswald & Gotaas, 1957). In conventional wastewater treatment facultative ponds, algae grow near the surface where light penetrates, and wastewater solids and microbial biomass settle to the pond floor. There the settled biomass is anaerobically digested by bacteria, which resolubilizes some nutrients. Observation of this naturally occurring process led to the development of methods to harvest and digest the excess algal biomass (Gouleke et al., 1956).

Versions of the early process flow diagrams by Oswald and others show the cultivation of algae biomass in shallow “high-rate” or raceway ponds on municipal wastewater treatment plant primary clarifier effluent. The algae are harvested by gravity and digested for biogas production and nutrient solubilization (Figure 1). These steps are discussed below in four main topics: (1) the production of algae in raceway ponds, (2) harvesting and thickening of the collected algal biomass, (3) direct anaerobic digestion or indirect digestion of HTL or lipid residuals, and (4) nutrient recycling and CO_2 fixation.

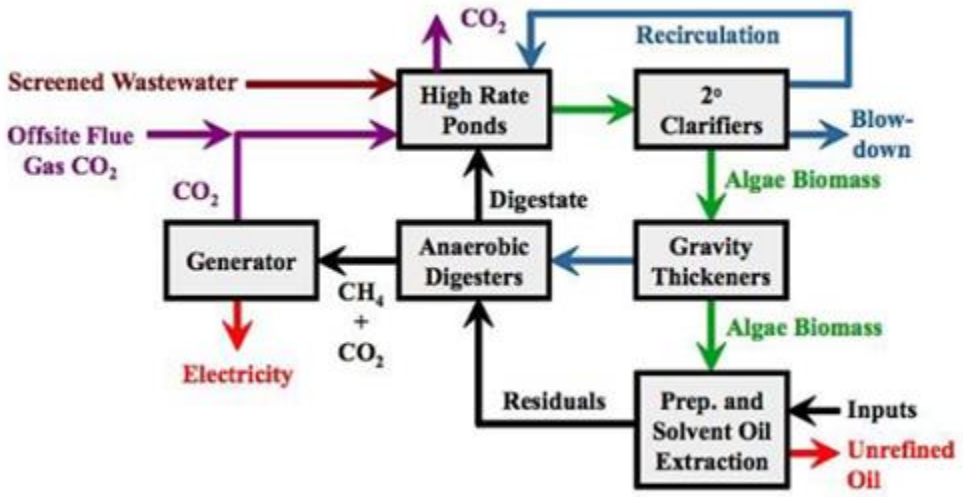

Figure 1*: One possible design of algae biofuel system. Algae, grown in high rate raceway ponds using primary clarifier effluent as a nutrient source, is gravity thickened for anaerobic digestion. The biogas and nutrients solubilized from the digestion process are used for energy production and nutrient recycling. (Lundquist et al., 2010).*

2.4 Algae production on wastewater

The first step of an algae-based wastewater treatment is the treatment of clarified primary wastewater in 30 to 90-cm deep raceway ponds. In this step, algae assimilate wastewater nutrients and produce oxygen through photosynthesis (Oswald, 1995; Lundquist et al., 2010). The oxygen is used by bacteria to oxidize organics and, to a limited extent, ammonia to nitrate. Bacterial respiration provides CO_2 for the algae, but flue gas can also be added to supplement the bacterial CO_2 demand (Park, 2011; Huang, 2016). Pond HRT is changed depending on the season, treatment target, and biomass production target.

2.5 Algae harvesting and thickening

Harvesting and thickening of algal biomass is required for biofuel production. Unicellular algae are often colloidal and have a similar density as water and are difficult to remove

from the water column (Grima, 2003). Chemical coagulants and flocculants can be added to allow for more rapid settling, and dissolved air flotation, or centrifugation may be used; however, adding chemicals and power are detrimental to cost and environmental sustainability of algae biofuels (Lundquist et al., 2010). Inorganic coagulants may also inhibit the digestion process (Gouleke 1956, Ummalyma, 2017).

Bioflocculation and autoflocculation are alternative processes to aid harvesting and thickening with lower chemical and energy inputs (Ummalyma, 2017). Bioflocculation is caused by bacterial-algal interactions, and microalgal-fungus associations and has achieved harvest efficiencies over 97% (Ummalyma, 2017). Autoflocculation involves adjusting pH to 9 or greater to promote calcium precipitation and sweep flocculation (Golueke and Oswald, 1965; Ummalyma, 2017). These methods for flocculation can improve the overall cost and process efficiency but require further R&D to be incorporated into a full-scale algae biofuel production system.

2.6 Algae digestion

Algal cells are resistant to bacterial breakdown during digestion (Gouleke et al, 1956). This is attributed to living algal cells surviving the conditions within the digester. Living algal cells are known to resist bacterial attack and hydrolysis (Gouleke et al, 1956). Additionally, recalcitrant material in the algal cell such as polyphenols, cellulosic fibers, and lignin resist digestion (Ward, 2014). During digestion, algae are broken down in three fractions: storage products that decompose within a few hours, material representing 30-70% of the mass that decomposes within a year, and the recalcitrant fraction that resists decomposition (Jewell et al., 1971).

Temperature, pH and alkalinity are three important digestion parameters. Organic acids accumulate at lower temperatures due to lower utilization of acetic and propionic acid by methanogens (Gouleke et al, 1956; Foree et al., 1970). In general, rates of solids stabilization and chemical oxygen demand (COD) stabilization increases at higher temperatures (>25˚C), but no significant difference was observed when digesters were operated at temperatures less than 25˚C (Foree et al., 1970). Alkalinity greater than 2500 mg $CaCO_3$/L with a pH of 6.3 or greater had no significant effect on methane production and/or COD stabilization; however, lower pH and alkalinity risked inhibition of the methanogens due to low pH (Foree et al., 1970).

Unmixed and poorly mixed digesters may experience longer solids residence times (SRT) that may result in solids accumulation. The organic loading rate (OLR), SRT and temperature can also affect the accumulation and conversion of solids to produce higher methane yields (McCarty, 1964; Sialve, 2009). Longer detention times increase gas yields and volatile solids destruction (VSD) (Eisenberg et al., 1980). A longer SRT leads to more solids stabilization, even in colder temperatures, because more time is given for the hydrolysis step. Higher temperatures do not require as long of an SRT but may have an additional cost due to heating. This cost is negligible if residual heat from combustion process is used to heat digestate. Research found that covered lagoon type earthen digesters with long hydraulic residence times, up to 40 days, were more cost-effective than other digester types even with low organic loading rates (Eisenberg et al., 1980).

2.7 Nutrient and CO_2 recycling

The return of the nutrient-rich digested algae and CO_2 from the combustion of biogas to the raceway ponds creates a nearly closed-loop system for growing algae (Lundquist, 2010; Ward, 2014). Bioavailable nutrients can be recycled into algal ponds instead of adding costly and unsustainable fertilizer (Sialve et al., 2009; Ward, 2014). One ton of algal biomass requires a minimum of 45 kg of nitrogen and 4 kg of phosphorus, from stoichiometry, with an 11:1 nitrogen to phosphorus (N:P) ratio (Ward, 2014). N:P ratios from digester effluent can range from 10:1 to 17:1 indicating digester effluent can provide enough of both nitrogen and phosphorus for recycling (Ward, 2014; Schamphleaire, 2009). CO_2 addition from combustion, flue gas, or wastewater is needed in algae ponds to provide carbon needed to re-assimilate these nutrients (Park, 2011).

2.8 Recent Cal Poly algae digestion research

Previous theses (Fresco, 2016; Hill, 2014) focused on maximizing the biogas production and nutrient solubilization from the anaerobic digestion of algae and laid the foundation for the experiments conducted herein. In batch digesters, compared to controls, sonicated algae feed had a 35% higher methane yield, from 0.276 to 0.315 L CH_4/g VSin; 60-70% higher volatile solids destruction, and an 86-94% higher nitrogen solubilization (Hill, 2014). Digesters fed semi-continuously generally have different methane yields and nutrient solubilization than batch digesters (Krikorian, 2017; Gunaseelan, 1997), so semi-continuous digestion of sonicated wastewater-grown algae was tested in this research.

Lab-scale digesters in Fresco (2016) tested the effects on methane yields and nutrient solubilization from digesters operated with a variable organic loading rate versus a

constant 0.25 g VS/L-day, varying temperatures versus a constant 30˚C and mixing with a constant OLR. The digesters fed at a variable organic loading rate gave a 25% higher methane yield (0.20 L CH_4/g VSin) than mixed digesters fed at a constant rate (0.16 L CH_4/g VSin). Unmixed, unheated digesters with variable feed achieved the highest yield of the study at 0.30 L CH_4/g VSin.

Fresco (2016) also operated unmixed, unheated, variable feed pilot digesters, work that was extended in the present research. Pilot-scale digesters were operated for 81 days (12/17/2014 - 3/8/2015) at steady state by Fresco. The average percent organic nitrogen and particulate phosphorus remaining in the digestate was 22% and 41%, respectively, with the remainder being solubilized. The yield and volumetric productivity was 0.19 L CH_4/g VSin and 0.022 L CH_4/L-day, respectively. Past research established that 1.2-L lab-scale unmixed digesters operating at a constant 20˚C produced similar results to the 1,134-L pilot-scale digesters, but the lab-scale digesters achieved a 55% higher methane yield (Fresco, 2016). This lab versus pilot comparison was improved and continued in the present research.

2.9 Experimental objectives

The body of algae digestion research includes the study of residence time, cell disruption, temperature, organic load, and co-digestion and their effects on methane yields (Sialve, 2009; Marsolek, 2014). However, to-date, no long-term studies have been found on the most basic and straightforward algae biofuel scenario: the growth and digestion of bioflocculated wastewater algae in unmixed, unheated pilot digesters. Furthermore, resolubilization of N and P during algae digestion has been studied, so far, only in the

short term (Fresco, 2016). The present research measured methane yield and productivity, volatile solids destruction, and N and P solubilization in unmixed, unheated pilot digesters over 15 consecutive months. In addition to studying seasonal effects, this research explored optimizing methane production and nutrient solubilization through mixing, heating, varied organic load, and feed pretreatment by sonication.

The goal of this research was to maximize mass yields (L CH_4/g VS in), volumetric methane production (L CH_4/L digester) and nutrient solubilization from the anaerobic digestion of algae biomass with low-cost, practical, and low energy demand methods. The research was accomplished in three experiments.

In the first experiment two 1,893-L (1,136-L working volume) anaerobic digesters were operated outdoors. The goal of this experiment was to determine the effect of seasonal environmental conditions on biogas yield and nutrient solubilization for unheated unmixed pilot-scale digesters.

In the second experiment six 2-L (1.2-L working volume) lab-scale anaerobic digesters were operated to determine the effect of mixing and pretreatment of the feed by sonication.

In the third experiment, the pilot-scale digesters were compared to the lab-scale digesters operated under similar conditions. The goal of experiment three was to determine if lab digester results can be scaled to pilot digesters.

3 Materials and methods

3.1 Experimental overview

This research involved operation of 1.2-L laboratory and 1135-L pilot digesters, and the collection and preparation of the digester feedstock. Algal biomass was grown and harvested from 33-m^2 raceway ponds located at the Algae Field Station (AFS) at the City of San Luis Obispo Water Resource Recovery Facility (SLOWRRF) from August 13, 2014 to March 17, 2016 (Figure 2). The influent and source of nutrients for all raceway ponds was clarified municipal wastewater from the full-scale treatment plant. The number of raceway ponds used to grow the algal biomass changed from three ponds to two ponds on May 3, 2015. The ponds were operated in parallel with the hydraulic residence time (HRT) ranging from 2-4 days depending on the season (more information on pond operation can be found in the theses of Robert, 2015; Reiff, 2015 and Bowen, 2018). HRT, for both ponds and digesters, was defined as the average time a particle of water remained in the reactor assuming perfect mixing (Equation 1).

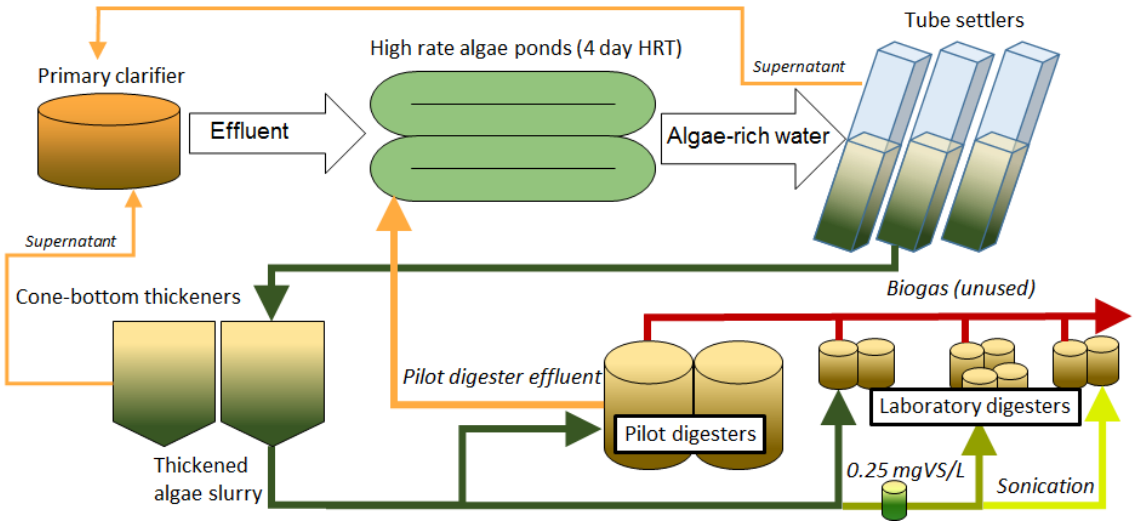

Figure 2 - Process flow from the primary clarifier effluent into the algae raceway ponds to the collection and digestion of the algal biomass.

Equation 1- Hydraulic residence time (HRT)

$$Hydraulic\ Residence\ Time\ (days) = \frac{V_{reactor}}{Q}$$

$Vreactor$ = Volume of liquid digestate (L)

Q = Volumetric flowrate of algal biomass (L/day)

Algal biomass grown in the raceway ponds was harvested at mid-depth from the pond using an impeller pump (Flotec FP0F360AC, Delavan, Wisconsin and Little Giant 360, Oklahoma City, Oklahoma) at a rate less than the pond HRT. Pond 1 was pumped into a tube settler at an average rate of 3.4 L/min and pond two was pumped into a tube settler at an average rate of 5.8 L/min. Pumps were activated for two to three minutes every 13-20 minutes, depending on the pump efficiency. Settled biomass from the bottom of the

tube settlers was removed using a peristaltic pump into two 1893-L (500 gallon) cone bottom thickening tanks operating in series for additional settling. Thickened biomass was collected from the bottom of the thickening tanks daily. A minimum volume of 56-L (15 gallons) was collected and used as the feed for all experiments. Thickened algae, ranging from 0.5% - 2.8% VS was fed directly to the pilot digesters, and a sample was collected in a screw-top HDPE bottle and taken to the lab for further analysis. Lab digester feed was collected from the thickener every two to three days, stored in a screw-top HDPE bottle and refrigerated at 4˚C.

Variables in this research included mixing, cell-disruption by sonication, temperature, organic loading, hydraulic residence time, and digester geometry. The outputs from digesters resulting from the controlled variables were referred to as "digester performance" and were used to examine the effects on energy output in the form of methane yield (L CH_4/g VS_{in}), volumetric production (L CH_4/Ldig-day), and nutrient solubilization. The analysis of the results focused on the digester performance of three main experiments (Table 1).

Table 1- Experimental Design with dependent and independent variables. All digesters operated in duplicate at a 40-day HRT

Experiment	Digester Name	Label	Average Organic Loading Rate (g VS/L-day)	Average Operating Temperature (°C)	Mixing	Volume
1	Varied feed, Unheated, Unmixed	Pilot	0.15 (range: 0.01-0.65)	16^0 C **	Unmixed	300 Gallons (1136 L)
2	Constant feed, Heated, Unmixed	CHU	0.25	30^0 C	Unmixed	1.2 L
	Sonicated feed, Heated, Unmixed	SHU	0.25*	30^0 C	Unmixed	1.2 L
	Constant feed, Heated, Mixed	CHM	0.25	30^0 C	Mixed	1.2 L
(Fresco, 2016)	Varied feed,Unheated, Unmixed	VUU	0.15 (range: 0.01-0.65)	20^0 C	Unmixed	1.2 L
	Varied feed, Heated, Mixed	VHM	0.15 (range: 0.01-0.65)	30^0 C	Mixed	1.2 L
	Constant feed, Heated, Mixed	CHM***	0.25	30^0 C	Unmixed	1.2L
3	Lab: Varied feed, Average Daily Field Temperature, Unmixed	VAU	0.15 (range: 0.01-0.65)	14^0 C	Unmixed	1.2 L
	Field: Varied feed, Unheated, Unmixed	VAU,P	0.15 (range: 0.01-0.65)	14^0 C	Unmixed	300 Gallons (1136 L)

**100 mL of 1% VS thickened algal biomass sonicated daily for 10 minutes using Analog Branson sonifier 250 with 1.27 cm tapped tip.*

***Average of continuous temperature results over 24-hours over entire duration of experiment.*

**** Duplicate lab-scale digesters were operated between the thesis of Fresco (2016) and this research for constant feed, heated and mixed digesters (CHM)*

Experimental results from a previous experiment were used in the final analysis of this research (Figure 3). Experiment 1 data includes data collected by Elai Fresco from August 13, 2014 to March 30, 2015 (Fresco, 2016). The reminder of the data was collected for this research from April 1, 2015 to March 17, 2016. Overall, Experiment 1 encompasses all lab-scale experiments. The lab-scale VUU and VHM digesters also conducted by Fresco (2016) from August 13, 2014 to April 2, 2015 were also included in the final analysis. Finally, Experiment 2 was conducted from April 24, 2015 to February 11, 2016 and Experiment 3 was conducted from October 22, 2015 to March 17, 2015.

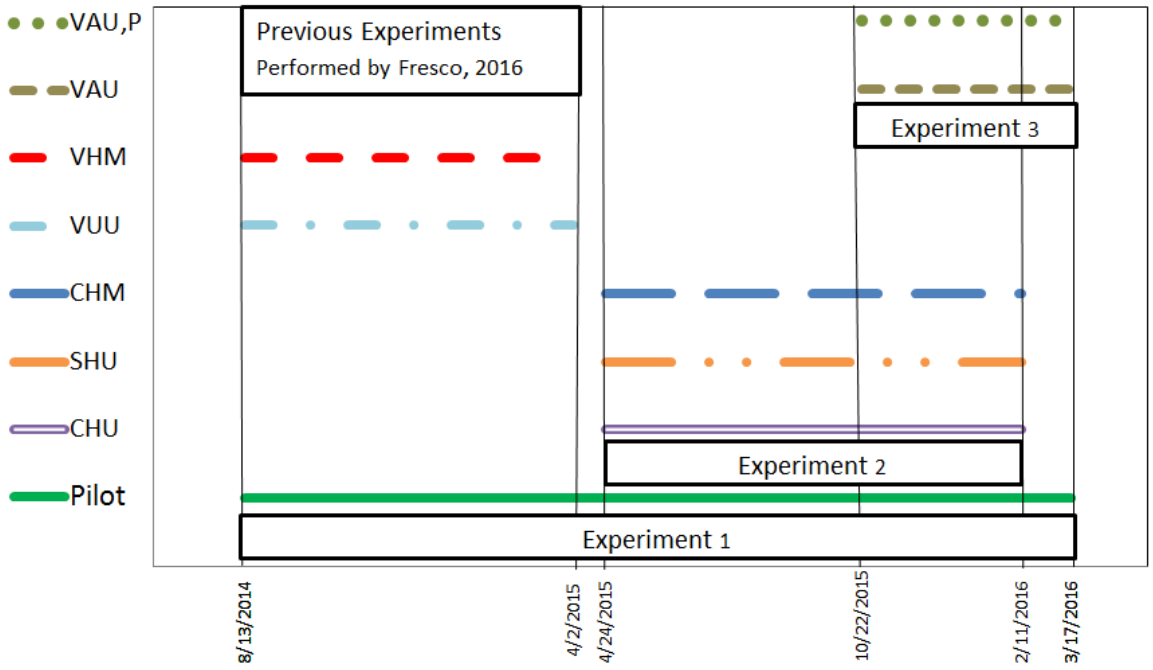

Figure 3- Experimental timeline.

3.1.1 Experiment 1: Overview

In Experiment 1 the effects of seasonal temperature and organic load on biogas production, nutrient solubilization, organic solids destruction and accumulation, and overall digester health were evaluated in unmixed replicate outdoor anaerobic digesters with a 40-day HRT located at the SLOWRRF (Table 2) for 15 months. Each digester contained 1135-L of unmixed liquid digestate with a head-space volume of 758-L. The pilot-scale digesters were unmixed and exposed to seasonal ambient temperatures to mimic the low-cost full-scale option of unmixed covered lagoon anaerobic digesters. Digester feed was harvested and thickened from raceway ponds that also experienced seasonal changes in biomass productivity and settleability and influenced the organic load to the pilot digesters. Uncontrolled variables included the variable digestibility of the algal- bacteria polyculture, and the effects of shading (Section 4.3.3).

Table 2- Variables for pilot-scale digesters in Experiment 1.

Independent	Dependent (measured)	Controlled	Uncontrolled
Seasonal Variation in Temperature	Biogas Production (Methane Yield)	HRT	Algae composition of feed
	Nutrient Solubilization (N, P)	Unmixed	OLR
	VS Destruction/Accum (% VSD, COD accum)	Reactor Volume	Digester Shading
	Digester Health (pH, Alk)		

3.1.2 Experiment 2: Overview

Experiment 2 setup contained two simultaneous experiments that tested the effects of (1) digester mixing and (2) cell disruption by sonication on biogas production, nutrient solubilization, organic solids destruction and accumulation, and overall digester health (Table ##). The six digesters were operated in duplicate semi-continuously with a 40-day HRT for 31 weeks and contained 1.2-L of digestate with a head-space volume of 0.8-L. The first set was named SHU [sonicated feedstock, heated to 30℃, unmixed, 0.25 g VS/L-day feed at 1% VS]. The second set was named CHM [constant 0.25 g VS/L-day feed at 1% VS, heated to 30℃, mixed, no sonication]. The third set was named CHU [constant 0.25 g VS/L-day feed at 1% VS, heated to 30℃, unmixed]. Uncontrolled variables affecting Experiment 2 included inadvertent mixing from operating small, lightweight digesters, and varying resistance of the algal cells to disruption by sonication.

Table 3- Variables for the lab-scale Experiment 2.1 on sonication and Experiment 2.2 on mixing.

Independent	Dependent (measured)	Controlled	Uncontrolled
Cell Disruption by Sonication	Biogas Production (Methane Yield)	HRT	Resistance to cell disruption
Shorter SRT by mixing	Nutrient Solubilization (N,P)	Feed Pretreatment	Inadvertent mixing
	VS Destruction/Accum (% VSD, COD accum)	Unmixed	Sloughing from walls
	Digester Health (pH, Alk)	Operating Temperature	
		OLR	

3.1.3 Experiment 3: Overview

In Experiment 3 the biogas production, nutrient solubilization, organic solids destruction and accumulation, and overall digester health of the lab-scale (1.2-L) and pilot-scale (1135-L) digesters were compared. The lab-scale digesters were operated under the same conditions as the pilot-scale digesters (EXP 1) with a 40-day HRT, unmixed and received the same feed and organic load. The lab digesters were operated at the average pilot digester temperature from the previous day and did not experience the diel fluctuations in ambient temperature. Lab-scale digester results were compared to pilot-scale digester results to determine if trends in the dependent variables were consistent between the two digester sizes (Table 3).

Table 4- Variables for pilot-scale digesters in experiment 3.

Independent	Dependent (measured)	Controlled	Uncontrolled
Reactor Geometry	Biogas Production (Methane Yield)	Daily Average Temperature	Diel field temperature variation
	Nutrient Solubilization (N, P)	HRT	
	VS Destruction/Accum (% VSD, COD accum)	Unmixed	
	Digester Health (pH, Alk)	Organic Loading Rate	
		Algae Composition of Feed	

* Continuously varying ambient temperatures controlled the pilot digester temperatures, but the lab digester temperature was adjusted to the 24-hour average of the pilot diel temperature variation.

3.2 Experimental apparatus

3.2.1 Cultivation system

The algae biomass used in Experiments 1, 2 and 3 was generated from several 33-m^2 (30-cm deep) raceway ponds located at the AFS at the SLOWRRF that were diluted with primary clarifier effluent (Bowen, 2018). Primary clarifier effluent was selected due to the abundance in carbon, bacteria, and nutrients (nitrogen and phosphorus) needed for growing algae. The raceway ponds grew bioflocculated cultures and no chemical coagulants were used in this study.

Concentrated algae slurry was collected daily to be used as the feedstock for digesters in all experiments. Collection of the algae slurry was achieved by using a system of pumps,

tube settlers and cone bottom thickening tanks. Impeller pumps were used (Flotec FP0F360AC, Delavan, Wisconsin) from October 11, 2014 until July 17, 2016 when they were replaced with submersible centrifugal pumps (115-V/ 1.6 Amp Pony Pump 360, Little Giant, Oklahoma City, Oklahoma). Pumping schedules were adjusted weekly as pumps were repaired or the efficiency changed due to wear. The pumps were programed to collect 1,135-L per day to fill the two 570-L cone bottom thickening tanks. The resulting thickened algal slurry was collected daily from the bottom of the cone bottom tanks and used as the digester feed.

To collect thickened biomass from the thickener tanks, supernatant was first decanted leaving 30-L of thickened biomass at the bottom of the tank. The subnatant slurries were drained into a 57-L pail which was mixed by stirring before samples were collected. This collection of slurry for feeding and analysis was performed 7 days per week. The remainder of the pail was mixed and pumped with an impeller pump into two pails until each contained 28.4-L. For pilot-scale digesters in Experiment 1 and 3, the 28.4-L of slurry was pumped into each pilot digester via a quick-connect fitting on the lowest port of the digester. The pumping process was supervised to make sure no air was pumped into the digester. Feed was collected for use in the lab-scale digesters.

Depending on the concentration of volatile solids (VS), the lab digester feed was either thickened or diluted to reach 10 g VS/L. When the algae slurry was less than 10 g VS/L, additional thickening was performed by gravity settling for at least 24-hrs in a 4°C refrigerator in a 250-mL HDPE bottle. After the setting period, the results of the solids test from the previous day were used to calculate the volume of the supernatant to remove

to achieve 10 g VS/L (Equation 2). If the sample was greater than 10 g VS/L, then tap water was added to reach the desired solids concentration. Lab digesters were run semi-continuously by feeding and draining 30-mL daily to maintain the hydraulic residence time (HRT) of 40 days. Feeding occurred 7 days per week. The algae feed was from the same sample used to feed the pilot digesters.

Equation 2- Feed sample thickening

$$C_{sample} * V_{sample} = C_{desired} * V_{desired}$$

Where:

Csample = Volatile solids concentration of sample from previous day (g VS/L)

Vsample = Volume of sample (L)

Cdesired = Volatile solids concentration desired (g VS/L)

Vdesired = Desired volume of feed (L)

3.2.2 Biogas collection system

The biogas collection system for each digester consisted of custom-built wet gas tipping meter, tubing, a 4-channel pulse data logger (Onset HOBO UX120-017, Bourne, Massachusetts), an in-line septum for sampling, and a Tedlar® gas sampling bag (Figure 4). The pressure in the gas systems was approximately 10 cm of water (the depth in the tipping meters). Gas meters were initially calibrated with a compressed air gas flow meter (Gasmet, A.E.D., Chicago Illinois) and periodic calibration checks were performed by injecting known volumes of air into the meter using medical grade syringes.

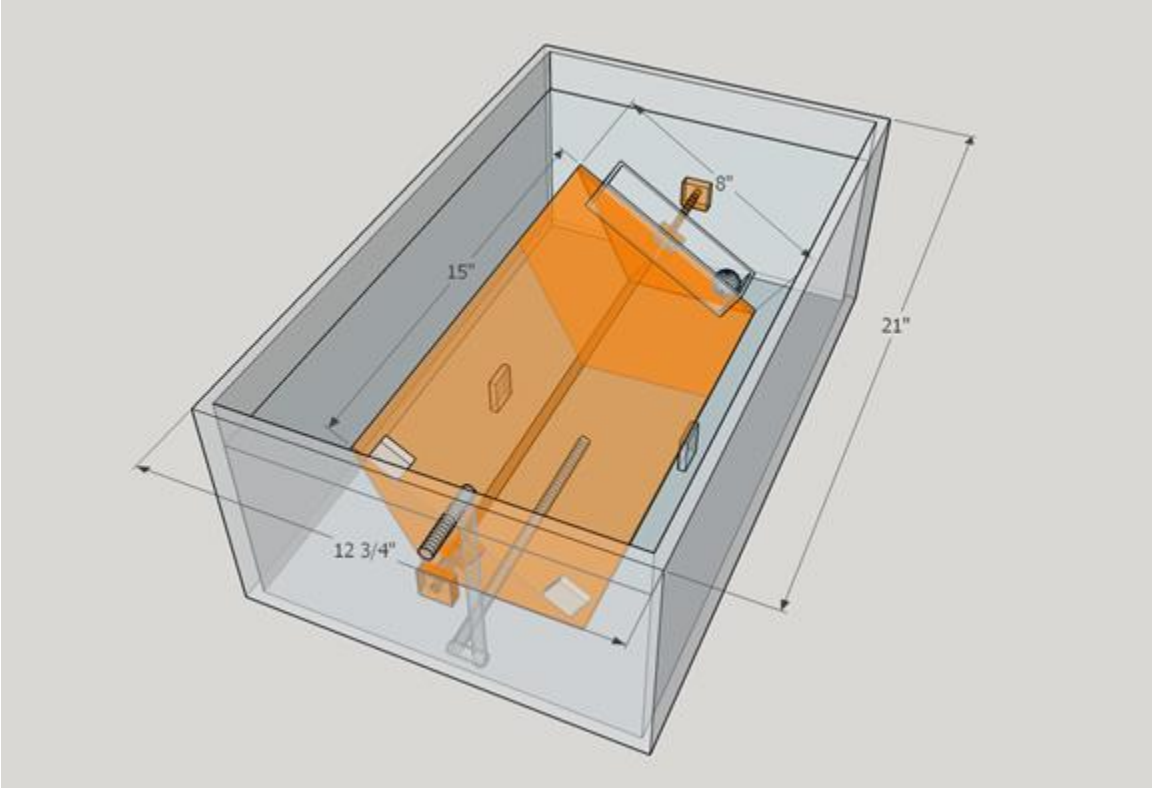

Figure 4- Schematic of wet gas tipping meters used to measure the volume of gas produced from pilot digesters, lab digester meters were similar but smaller in length and width.

The biogas flowed from the digesters into the tipping meter as follows (*Figure 5*):

1. Gas from the digester flowed to the dual-chambered tipping platform sitting submerged in a water-filled tank. A one-way valve was placed just prior to the submerged rod to prevent any water backflow into the digester.

2. A submerged rod with a small hole was placed directly below the tipping platform to allow the gas in. As gas filled the chamber on the tipping platform the buoyant force caused the platform to overcome its weight and tip to release the gas. Tipping was assisted by a steel marble counter weight.

3. As the platform tipped the magnet would close the reed switch and send a signal to the logger indicating a tip had occurred. The volume of gas required to cause a tip was measured and the total volume of gas produced was calculated by multiplying this by the number of times the reed switch closed.

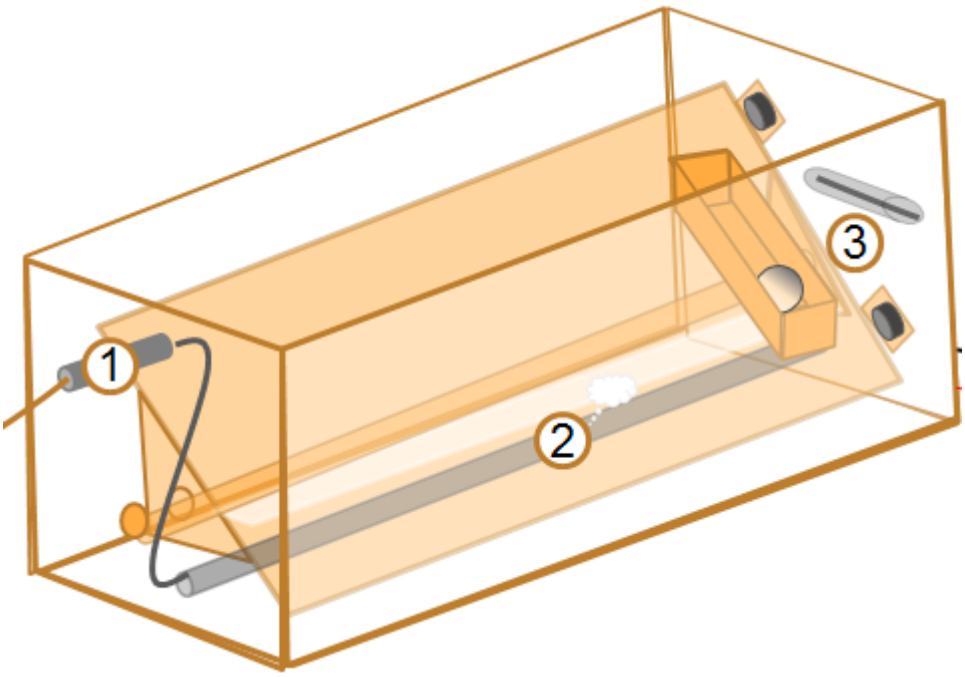

Figure 5- Schematic of the process flow for biogas produced from digesters. Biogas produced by the digesters would flow through a one-way valve (1) into a tube located below the tipping platform (2) where a known volume of gas would cause the tipping platform to tip and trigger a reed switch to open or close (3). The reed switch would send a signal to the data logger. The volume of gas produced was calculated by multiplying the number of tips by the tipping volume.

It was assumed that the temperature of the gas passing through the laboratory tipping meters was room temperature and remained at a constant 21˚C throughout the experiment. Temperature of the biogas produced from the pilot gas meters was measured by a temperature probe placed inside of the tipping gas meter from December 17, 2014 to the end of the experiment (Onset HOBO UX120-017, Bourne, Massachusetts). The

volume of gas collected was corrected to standard temperature (273 Kelvin) and pressure (1 atm) using the Ideal Gas Law.

3.2.3 Pilot digesters

The two outdoor pilot-scale digesters were built at the AFS at the SLOWRRF for the preceding research (Fresco, 2014). The digester vessels consisted of custom 2.9-m^3 vertical cylindrical tanks (Chem-Tainer translucent HDPE, product TC4676IC, CO_2 permeability = 16 cc*mm/m^2*day*bar). Ports along the length of the digester were added to allow flexibility in sample collection, feeding and draining, and biogas collection. An additional internal dead-end port was added for temperature monitoring (Figure 6). Both digesters had an 1893-L capacity but were operated with 1135-L of liquid and 758-L of headspace. To reduce diel fluctuations in pilot digester temperature and prevent algae photosynthesis in the digesters, each digester was enclosed in reflective bubble insulation (TekFoil Reflective/Bubble/Reflective Insulation, Dyersville Iowa). The bottom of the digesters rested on the earth without insulation.

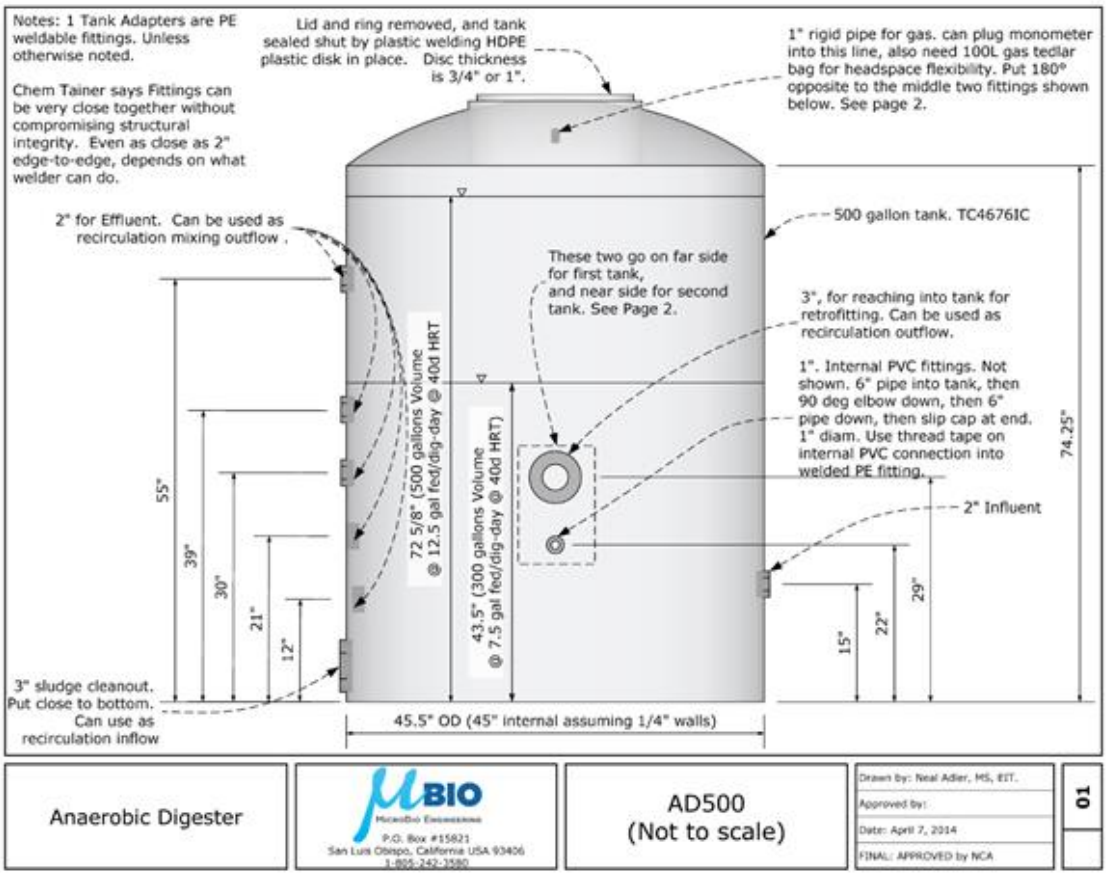

Figure 6- Schematic of the pilot digesters used in this research (Neal Adler, MicroBio Engineering Inc.)

Algae slurry was fed through the port located at 12” (30 cm) from the base and effluent was removed through the port located at 39” (99 cm) from the base (Figure 7; Item 1 and 2). Digestate temperature was measured continuously by a temperature probe inserted into a 2.5-cm diameter capped dead-end PVC fitting filled with water located at 22” (56 cm) from the base. The biogas filled the 758-L headspace of each digester and then flowed through tubing (2.5-cm diameter, Tygon formulation E-3603, CO_2 permeability = 2330 cc*mm/m^2*day*bar) into an innertube (butyl rubber, CO_2 permeability = 9720 cc*mm/m^2*day*bar) (Items 3 and 4). The inner tube provided a volume buffer for diel volume changes in the biogas due to temperature fluctuation. When the inner tube was

full, the biogas flowed to a tipping gas meter (Item 5). The inner tube was usually full by mid-day after digester temperature increased. The gas meter would tip when 250 mL of biogas filled one side of the dual-chambered platform. The tipping caused a magnet to trigger a reed switch. The reed switch signal was recorded by a data logger (Item 6). The number of tips was used to calculate biogas volume. Meters were calibrated prior to start of Experiment 1 by Elai Fresco and calibrated again on April 20th, 2014.

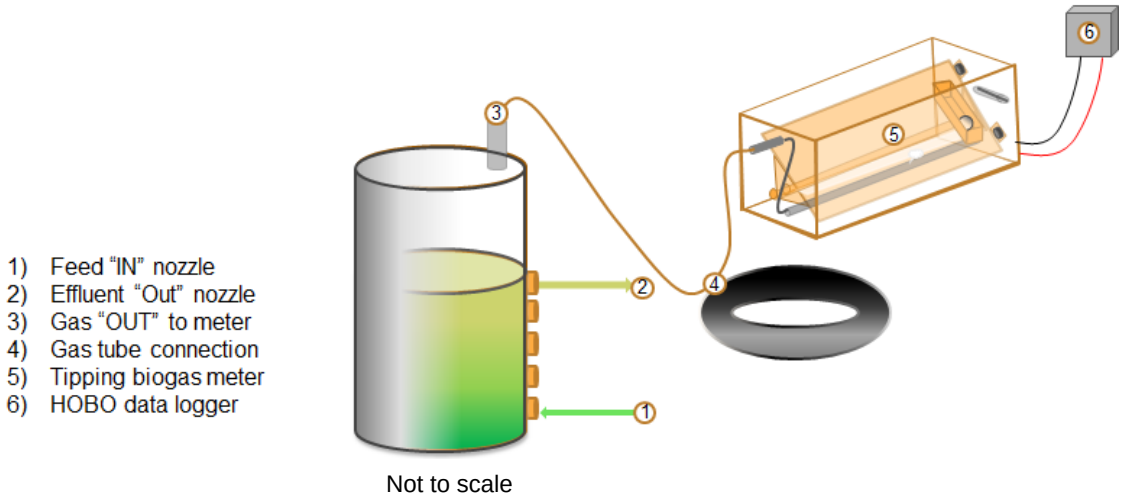

Figure 7- Schematic of gas collection system for the two pilot digesters 1. Feed port 2. Digester effluent port 3. Biogas outlet port 4. Innertube 5. Tipping gas meter 6. Datalogger

3.2.4 Laboratory digester apparatus

The lab digesters used in the present research were originally used by Olivas (2015) and Fresco (2016), and the current methods of digester preparation were the same as Fresco. Materials for all laboratory digesters were chosen to reduce the permeability of biogas through the reactor and gas collection lines. The digester vessels were 2-L FLPE fluorinated polyethylene bottles (Nalgene, CO_2 permeability = 8900 cc*mm/m^2*24 hr*bar). Biogas collection tubing was minimized to reduce leaks (MasterFlex 06409-17 TYGON, with an inner diameter of 0.6 cm).

The lab digesters consisted of eight main components (Figure 8) (1) Feed was added into each digester through the feeding tube with a syringe. (2) Before feeding, a syringe was attached to the effluent tube and used to remove effluent. To prevent removal of settled biomass in the unmixed digesters the effluent tube was located just below the digestate liquid level inside the digester. (3) Gas flowed through leak tested tubing in the top of the digester. (4) The temperature port contained a temperature probe installed in dead-end tubing in the digester (Hydrofarm MTPRTC, Petaluma, California). (5) Each digester had a Tedlar bag connected to the gas tube that served to equalize volume during draining and feeding, and, if deflated, indicate leaks. Gas samples were collected with a syringe from an in-line septa on the biogas tubing. (6) A tipping gas meter was used to determine the volume of biogas produced. (7) An Onset HOBO Data Logger recorded each platform tip which corresponded to a known gas volume. (8) The digester temperature was monitored and controlled using seedling heat mats wrapped around the digesters with thermostats connected to the temperature probe (Hydrofarm MT10006, Petaluma, California).

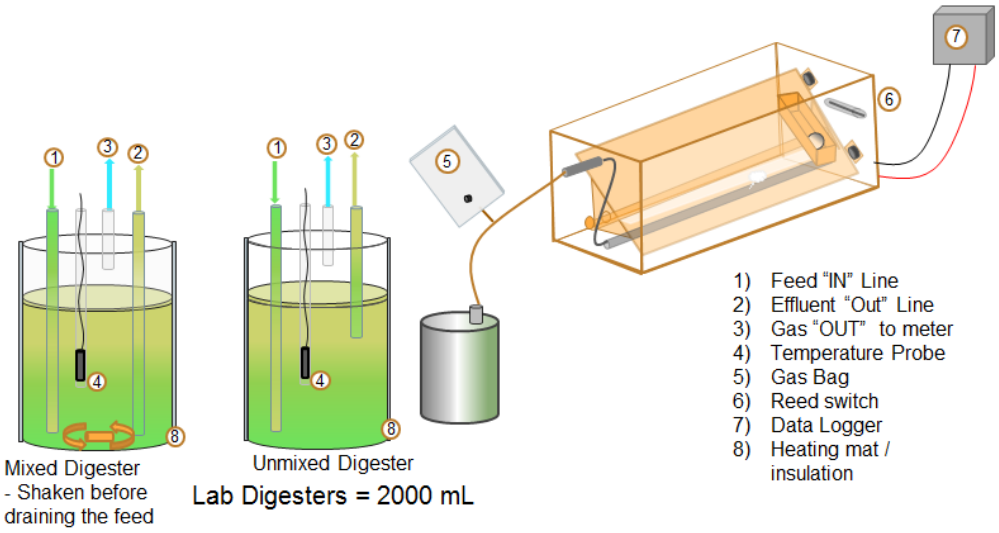

Figure 8– (Right) Schematic of gas collection system for laboratory digesters including the 2-L digester, Tedlar gas bag with sampling septum, the gas tipping meter and the data loggers. (Left) Mixed and unmixed digesters with ports.

3.2.5 Experiment 2: Mixing and sonication equipment

The CHM digesters were mixed in Experiment 2. Magnetic stir bars (3.8-cm Teflon-coated) were placed inside of digesters before leak testing. Stir plates (200 Mini Stirrer Cat No. 58940-158, VWR, Radnor, Pennsylvania) were used from April 27-August 31, 2015 and operated at 805 rpm. The stir plate was replaced on August 31, 2015 by a VWR (120 Mini Stirrers 986965 Cat No. 12620-998, VWR, USA) stir plate and calibrated to 800 rpm using a stroboscope.

Sonication was used as a pretreatment of the algae feed for the SHU digesters. The digesters were fed 7 days per week, and the feed was sonicated each day. A 75-mL sample was placed in a 100-mL HDPE sample bottle and the tip of the sonifier was placed at about mid depth below the liquid level. An analog Branson Sonifier 250 (Danbury, Connecticut) with a 1.3-cm tapped horn (Fisher Scientific, #4137) was used to

sonicate the 10 g VS/L sample (Figure 9) for 10 minutes. Temperature of the algal slurry consistently reached 70℃ with 10 minutes of sonication. The sample was then cooled to 35℃ prior to feeding the digesters.

Figure 9- The SHU digester feed was a 10 gVS/L sonicated algal slurry. An analog Branson Sonifier 250 was used to lyse the algal cell walls prior to feeding.

3.2.6 Experiment 3: Incubator temperature control

A temperature programmable incubator, rather than heating mats, was used to control the temperature of the lab-scale VAU digesters used in Experiment 3. A mini-refrigerator was modified to be an incubator using a dual temperature controller (STC-1000 A400-P version, Youkong, Guangdong, China) (Figure 10 and Figure 11). The temperature was set daily to match the average pilot digester temperature from the prior day. A mercury thermometer in water was placed inside of the incubator as an additional temperature check. Lab digester digestate temperature readings were checked against the mercury thermometer and settings on the controller during the experiment with adjustments made as needed.

Figure 10 and Figure 11- The temperature-controlled incubator housed the lab-scale digesters for Experiment 3.

3.2.7 Digester setup and operation

Each digester was soaked in a bleach-water solution (1:4 v/v) for at least 24 hours. The bottles were cleaned thoroughly with a bottle brush to remove any residue from prior experiments. After the digesters were cleaned, they were sealed, and leak tested. Silicone caulking (Alex Plus acrylic latex caulk plus silicone 300-mL) was used generously on all connections, ports and seams and let dry overnight. Then a leak test was performed using graduated cylinders. A 0.6-cm diameter tube was used to connect the gas port of the digester to the bottom of an elevated 1.5-L graduated cylinder filled with water and capped with foil. The water surface in the cylinder was approximately 30 cm higher than the digester. If the water level in the cylinder dropped overnight, then the digester was re-sealed with silicon and re-tested. This was repeated until no leaks were detected.

3.2.8 Experiment 1: Inoculation and setup

The pilot-scale digesters were inoculated with SLOWRRF digester effluent in 2014 (Fresco, 2016). Inoculum was digested municipal wastewater sludge collected from the third-in-series, full-scale mesophilic digester operated by the SLOWRRF staff. The full-scale digesters were fed a mixture of sludge from the primary and secondary clarifiers, which had been thickened by dissolved air flotation and ferric chloride. The three digesters were operated at a combined 60-day HRT, with the first two digesters heated to 32°C and mixed, and the final digester unheated and unmixed. A total of 2270 liters of digestate (“seed”) was collected from the effluent of the third digester and pumped into the pilot-scale digesters. The digesters were leak tested prior to the inoculation.

Additional methods for leak testing and inoculation of the pilot digesters can be found in the thesis of Fresco, 2016.

3.2.9 Experiment 2: Inoculation and setup

For Experiment 2, the lab-scale digesters were inoculated on April 20, 2015. The seed was collected from the same source with the same methods used to inoculate the pilot digesters. Once digestate was collected, approximately 8-L of seed was stirred in a 20-L pail on a stir plate with a 15-cm stir bar. Then each leak-tested digester was filled with 1200-mL with the mixed seed and the headspace was purged with nitrogen gas for one minute at 15 psi. When purging the digesters, the gas bag and tubing from the gas collection system were attached to ensure the oxygen was completely removed from the system. Then the cap was placed on the digester and was sealed with silicon. The seed pH, alkalinity, solids, total ammonia nitrogen (TAN), and phosphorus was measured on the same day as inoculation (see analytical section below).

3.2.10 Experiment 3: Inoculation and setup

In Experiment 3 two lab-scale digesters were operated inside a temperature-controlled incubator. Digester materials and digester preparation for Experiment 3 were identical to the setup for Experiment 2 lab digesters, except they were inoculated with mixed effluent from the pilot-scale digesters collected on September 14, 2015. To collect the seed for the lab-scale digesters both pilot digesters were mixed by connecting the inlet of an impeller pump to the feeding port of the pilot digester and the outlet of the pump to the effluent port of the digester, created a circular mixing pattern within each digester for 15 minutes.

After mixing, 3-L were collected from each digester, inter-mixed in a 20-L pail, and stored covered at room temperature until the Experiment 3 lab digesters were filled to 1,200-mL on October 1, 2015 using the methods described in Section 2.2.2.

Further digester startup efforts were needed. On October 16, 2015, it was discovered that one of the lab digesters was exposed to ambient air via a missing gas sampling port septum. A septum was installed immediately, but subsequent measurements of biogas methane content for this digester were consistently 30% CH_4 or less. On October 22, 2015, both Experiment 3 lab digesters were disassembled, the digestate inter-mixed, and the digesters restarted, per the procedure of Section 2.2.2.

3.2.11 Corrections to biogas collection system

During Experiment 2 and 3, biogas yield and production were negatively affected by occasional operational problems. The apparatus and operating methods were adjusted to avoid the problems. Early in the lab experiments, the in-line gas bags were inflated and the Tedlar bags were pressurized which contributed to leaks. Starting November 13, 2015, the gas bags were emptied daily by gently squeezing out all of the gas. Over the course of the experiments when leaks were discovered they were repaired, but it generally took three to five days for gas composition and production to recover to normal levels.

3.3 Analytical procedures

3.3.1 Gas chromatography

The gas collection system and gas chromatography (GC) were used in the analysis of the biogas. The biogas composition was determined using a gas chromatograph (GC) (SRI 8610, Torrance CA). The GC used a thermal conductivity detector with a 1.8-m packed column including concentric inner and outer columns for separation of oxygen, nitrogen, methane and carbon dioxide (Alltech CTR I, Deerfield Illinois). Ultra-high purity argon gas with a flow rate of 0.91 mL/min at 55°C was used as the carrier gas. The GC calibration was checked daily using atmospheric air, and when results were outside of the acceptable range of 78% nitrogen and 21% oxygen (+/- 5%) the machine was calibrated. The machine was calibrated a total of 3 times, at the beginning of Experiment 2, at the beginning of Experiment 3 and during January 2016.

Pilot digester gas samples were collected by connecting a 500-mL Tedlar gas bag to the sampling port located near the inner tube on the gas line. Air contamination was removed from the bag by filling the bag by pressing on the inner tube. Then the bag was disconnected and the gas was removed. This occurred a minimum of three times before the gas sample was collected and transferred to the lab. The lab digester gas samples were collected in a 1-mL manual syringe (Agilent Technologies, Santa Clara California) from the septum located on each digester gas tube. To remove air contamination in the syringe, the gas was pulled into the syringe and removed at least three times before collecting the sample taken to the GC, which was in the same room as the lab digesters.

Oxygen and nitrogen levels were monitored in the digesters to monitor for leaks. Nitrogen gas in the GC runs was considered acceptable up to 5% N_2 by volume. Leaks in the gas collection system were detected during GC analysis when the sample contained greater than 5% nitrogen by volume, or any oxygen was observed with methane results below 30%. If the gas collection bags did not fill with gas within 24-48 hours, it was also a sign of a potential leak in the system and a GC test was run to confirm. If a gas leak was suspected the gas collection system was checked for possible holes, breaks, and leaks.

The volume and composition of the biogas were used to calculate the volumetric productivity and mass yield of methane. Volumetric productivity is the volume of methane produced per volume of digester and is a useful factor when capital costs associated with digester volume are considered (Equation 3). The mass yield is the volume of methane produced per gram of volatile solids (VS) fed and is a helpful tool to measure the efficiency of conversion of digester feed to biogas (Equation 4). Finally, the mass yield of methane per gram of VS destroyed was determined. The VS destruction yield was a measure of waste stabilization (Equation 5).

Equation 3– Volumetric methane productivity

$$Volumetric\ CH_4\ yield = \left(\frac{L_{CH_4}}{L_{digester} * day}\right) = \frac{V_{avg} * C_{avg}}{Vol}$$

Equation 2

Equation 4– Mass methane yield, VS introduced

$$Mass\ CH_4\ yield = \left(\frac{L_{CH_4}}{g\ VS_{in} * day}\right) = \frac{V_{avg} * C_{avg}}{VS_{avg}}$$

Where:

$Vavg$ = Average daily biogas production during the representative period (liters/day)

$Cavg, weekly$ = Average fraction of CH_4 in biogas during the representative period (unitless)

Vol = Digester volume (liters)

$VSavg$ = Average mass of influent volatile solids introduced daily during representative period (grams)

Equation 5– VS destroyed yield

$$Mass\ CH_4\ yield = \left(\frac{L_{CH_4}}{g\ VS_{destroyed} * day}\right)$$

$LCH4$ = total methane production over entire experiment duration (liters)

$VSdestroyed$ = total volatile solids destroyed over entire experiment duration (grams)

3.3.2 Solids

Total solids (TS) and volatile solids (VS) content on a mass per volume basis were determined daily for the digester feeds and effluent samples. All samples were run in triplicate and the average was used as the reported value. If one sample was greater than 10% different from the average it was discarded. The volume used in the determination depended on the solids from the previous day. A 5-mL syringe was used if the solids content from the day before was below 0.5% VS, otherwise a 3-mL syringe was used. During winter the feed collected from the field was low in VS and 10-mL samples were used in analysis. Samples were well-mixed prior to sampling with an electric drink mixer.

The sample was then placed on a pre-weighed aluminum weighing dish (Thermo Fisher Scientific, No. 09-732-100, Waltham, Massachusetts). The weight of the sample and the weighing dish was recorded and then placed into a 105°C drying oven. Once the sample

was dried, the total solids was recorded by weighing the aluminum tray with the dried sample. The samples were then placed into a 550°C oven for 15 minutes to determine the VS (Standard Methods 2540B and 2540E). The weight of the tray plus the ash that remained was recorded. Assuming the density of the samples were equivalent to water the solids concentrations were given in percent total solids or percent volatile solids (Equation 6).

Equation 6- % TS or % VS

$$\%\, Total\ or\ volatile\ solids = \left(\frac{mg\ TSorVS}{L_{sample}} * \frac{L_{sample}}{1{,}000{,}000\ mg_{sample}}\right) * 100$$

3.3.3 Volatile solids destruction

VSD is a measure of solids stabilization and the conversion of feed mass to methane (Sialve, 2009). In this study, VSD was determined by calculating the reduction in VS added, as a ratio (Equation 7). The VSD for each digester was averaged between the duplicates.

Equation 7- VS Destruction (VSD)

$$\%\, Volatile\ solids\ destruction = 1 - \left(\frac{\sum mg\ VS_{out}}{\sum mg\ VS_{in}}\right)$$

Where:

VS_{out}= Sum of the volatile solids in the effluent collected during steady state operation (mg/L)

VS_{in}= Sum of the volatile solids in the influent collected daily (mg/L)

3.3.4 Corrections made to the draining methods

Straining of solids during draining was discovered in Experiment 2 when the CHM digester showed inconsistent effluent results during the representative steady state period (Figure 12). Corrections were made to daily feeding procedures for all Experiment 2 and Experiment 3 lab-scale digesters to prevent solids straining. Corrections included making sure no bends existed in the plastic tubing during digester effluent removal. This correction was made for all lab digesters on December 3, 2015.

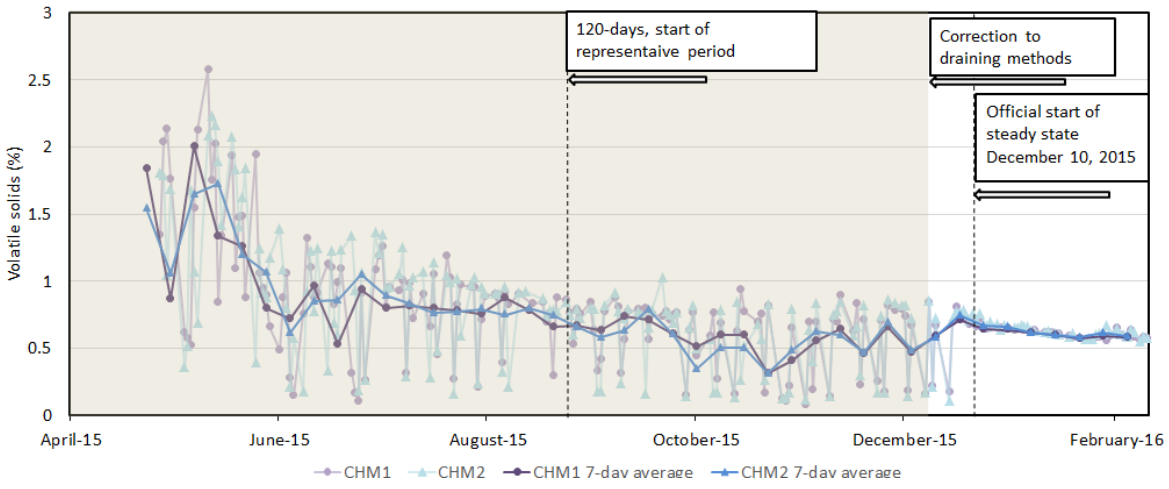

Figure 12- Draining methods for all lab-scale digesters in Experiment 2 and 3 were corrected to prevent straining on December 3, 2015. Steady state started for Experiment 2 lab-scale digesters after December 10, 2015 when digester effluent VS achieved steady effluent results.

3.3.5 pH and alkalinity

At the AFS, the pilot digester pH was measured before draining to ensure it was at least 6.70. A pre-calibrated dual pH and temperature probe (Oakton waterproof pH meter, EW-35631-00, Cole-Parmer, Vernon Hills, Illinois) was used to immediately measure the pH and temperature. The sample was taken as quickly as possible to prevent any loss of CO_2. If the pH was below 6.70, the pilot digesters were not drained or fed, and any loss

of volume was added during the next feeding. If the pH was at or above 6.70 then the draining and feeding process would continue and a well-mixed sample was collected.

pH was measured for the samples from the pilot-scale digesters and the lab-scale digesters with a Mettler Toledo Inlab 4B pH probe with a gel type electrode calibrated before each use. pH of the lab samples was taken immediately. Field samples were analyzed within 5 hours of collection. No significant difference in pH was measured between the pH taken at the AFS and the pH taken in the lab.

During Experiment 3, when the pH of the pilot-scale digester was below 6.70 the pilot- and lab-scale digesters were not drained or fed. A 250-mL sample was collected from the pilot-scale digesters 3 times per week to measure alkalinity. Over Experiment 3 the field digesters were not fed for 56 days due to low pH.

Alkalinity was measured three times per week for all digesters. Alkalinity was measured as $CaCO_3$ using acid titration to a pH of 4.5 using 0.2 N H_2SO_4 (APHA 4230D). The titration setup included a 15 mL sample beaker, a Mettler Toledo Inlab 4B pH probe, 100-mL graduated titration glass apparatus, and a magnetic stir bar and stir plate. Samples were titrated slowly, until the pH reached 4.5. The volume of acid consumed during the titration was recorded and used for the calculation of alkalinity.

3.3.6 COD methods

Field and lab samples were collected once per week for total and soluble chemical oxygen demand (tCOD and sCOD) analysis. COD samples were preserved by adding concentrated H_2SO_4 until the pH of the sample reached 2.0 and then refrigerated at 4°C.

sCOD samples were prepared using a centrifuge (US Centrifuge Model M212, Indianapolis Indiana) at 10,000 RPMs for 10 minutes. After centrifugation was complete the sample was filtered through a 1.2-µm pore-size glass fiber filter (Fisher G4). CHEMetrics 0-1500 ppm vials were used in the determination of total and soluble COD (closed reflux APHA 5220-D). Data were excluded when the tests failed QA/QC.

COD was used to perform a mass balance. A COD to VS ratio was calculated using the tCOD results and the corresponding VS results for that sampling day. Ratios were discarded if value was greater than one standard deviation from the mean ratio. The average COD to VS ratio used for all samples was 1.41 (±0.12). This ratio was applied to all days with VS data to complete the mass balance. The COD converted to gas was calculated by using the ideal gas law to determine the mols of CH_4 produced and the mass of COD in the gas. From August 2014 to March 2015 the COD of the biogas was calculated for the pilot digester using overall the average percent of methane produced. After March 2015 the COD in the biogas was calculated using the weekly average percent methane. The COD mass balance can be found in section 4.2.

3.3.7 Nitrogen

Nitrogen analysis included total nitrogen (TN) and total ammonical nitrogen (TAN). TN was the total concentration of nitrogen put added and removed from the digesters and TAN was the total soluble nitrogen available for nutrient recycling. Nutrient solubilization, or the conversion of organic nitrogen in TN to TAN was one of the main goals of this research. The percent organic nitrogen (Equation 8) remaining represented the amount of organic nitrogen not solubilized in the digester.

Equation 8- Percent particulate nitrogen remaining

$$\%\ Particulate\ nitrogen\ remaining = \left[1 - \left(\frac{TAN_{eff}}{TN_{eff}} - \frac{TAN_{inf}}{TN_{inf}}\right)\right] * 100$$

Where:

TAN_{eff} = total ammonia nitrogen in effluent (mg/L)

TAN_{inf} = total ammonia nitrogen in influent (mg/L)

TN_{eff} = total nitrogen in effluent (mg/L)

TN_{inf} = total nitrogen in influent (mg/L)

3.3.8 Total nitrogen

If TN samples were not analyzed immediately, a 15-mL sample was preserved by acidification to pH 2.0 using concentrated H_2SO_4 and stored in the refrigerator at or below 4°C. TN data for Experiment 1 from August 1, 2014 to May 27, 2015 and Experiment 2 and 3 on May 19 and May 26, 2015 was determined by using a CNS benchtop analyzer (VarioMAX, Elementar Analysensysteme, Hanau Germany) with the Dumas method by combusting the samples at 900°C. Additional information on nitrogen determination using the Elementar can be found in the thesis of Fresco (2016).

TN analysis was also conducted using the modified Total Kjeldahl Nitrogen (TKN) analysis outlined in APHA 4500-N_{org} B from April 28, 2015 to August 17, 2015. Additional details on the TKN analysis of digester samples can be found in the thesis of

Hill (2014). QC samples used in the TKN analysis consisted of spirulina and ammonia in conjunction with two splits and one spike.

TN samples collected after August 17, 2015 used the Hach Total Nitrogen Persulfate Digestion Method (10072), a modified analysis based on the APHA standard methods 1995 sections 4500 N-C. Samples were analyzed at room temperature and neutralized, if needed, to a pH between 6 and 8 by adding drops of 5N NaOH to the diluted sample. Samples were then prepared by mixing with a hand-held mixer for at least 1 minute. A sample volume of 0.5 mL was pipetted into the HR Total Nitrogen Digestion vials with the Total Nitrogen Persulfate Powder Pillow, shaken, and left to react for 30 minutes in a heating block set to 105°C. Once samples were removed and cooled, reagents containing Sodium Metabisulfate, white quartz sane, urea, and Chromotropic Acid Disodium Salt were added before pipetting a 2-mL sample into the NitraVer X Test ‘N Tube. Once a yellow color developed in the vial, a spectrophotometer set at an absorption of 410 nm was used to analyze the TN content.

3.3.9 Total ammonium nitrogen

A Timberline Instruments ammonia analyzer (4500-N D - Conductometric Determination of Inorganic Nitrogen, TL-2800, Boulder Colorado) was used to determine the TAN concentrations. The analyzer used a caustic solution (pH 11-13) to convert the ammonium in the samples into ammonia gas before running the sample through an ammonia-selective membrane coupled with an electrical conductivity detector to give the concentration of TAN in the sample.

Samples were prepared weekly from the TN sample by first separating the liquid and solids by centrifugation (US Centrifuge Model M212, Indianapolis Indiana) at 10,000 RPM for 10-minutes. The separated liquid was collected by vacuum filtration using a 1.2-µm pore-size glass fiber filter (Fischer G4) and acidified to a pH of 2.0 with concentrated H_2SO_4. Acidification was performed after centrifugation to prevent any loss of NH_3 during the vacuum filtration process.

3.3.10 Phosphorus

Bioavailable phosphorus, which includes adsorbed and soluble orthophosphate, can be used in the recycling of nutrients in algal raceway ponds. Total phosphorus (TP) is the measure of all phosphorus in the sample including particulate and organic phosphorus, while total reactive phosphorus (TRP) is the portion of soluble and adsorbed phosphorus in the digestate available by uptake in nutrient recycling. The percent particulate phosphorus remaining (Equation 9) represented the amount of organic and particulate phosphorus not solubilized in the digester.

Equation 9- Percent particulate phosphorus remaining

$$\%\ Particulate\ \ phosphorus\ remaining = \left[1 - \left(\frac{TRP_{eff}}{TP_{eff}} - \frac{TRP_{inf}}{TP_{inf}}\right)\right] * 100$$

Where:

TRP_{eff} = total reactive phosphorus in effluent (mg/L)

TRP_{inf} = total reactive phosphorus in influent (mg/L)

TP_{eff} = total phosphorus in effluent (mg/L)

TP_{inf} = total phosphorus in influent (mg/L)

Acid-washed glassware was used for all phosphorus tests. All glassware was soaked in an acid-bath solution for 30-minutes, rinsed for 15-minutes in a tub of deionized water with a final rinse with deionized water prior to use.

3.3.11 Total phosphorus (TP)

Two methods were used for the analysis of total phosphorus (TP). All samples were prepared by acidifying to pH 2.0 using concentrated H_2SO_4 and stored in a 4°C refrigerator in 50-mL Falcon tubes. TP for all digesters was determined weekly using Kjehdahl digestion (sulfuric-nitric acid digestion, APHA 4500-P B) and colorimetric measurement (vanadomolydbophosphoric acid colorimetry, APHA 4500-P C) from April 17, 2015 to July 29, 2015. For all samples analyzed after July 29, 2015 the Hach Total Phosphorus Analysis (TNT 845) kit adapted from the APHA 1500-P E, Ascorbic Acid Method was used.

3.3.12 Total reactive phosphorus (TRP)

TRP was used to measure the dissolved and adsorbed phosphorus using the total reactive phosphorus Absorbic Acid Method (TRP, APHA 4500-P. A, P.B & P.E) with the Vanadate-molybdate reagent (APHA 4500-P.C). Reagent was added prior to filtration through a 0.45 µm filter to desorb the phosphorus adsorbed to the solids within the sample. This is a more accurate representation of the bioavailable phosphorus available for nutrient recycling than soluble phosphorus alone.

3.3.13 Quality assurance and control

Quality assurance and control (QA/QC) for all lab analysis consisted of duplicate samples ("splits") and a spike analysis. A split sample tolerance of ±10% from the original sample and spikes within ±15% were accepted as passing and the results were acceptable for use in the analysis. The matrix spike included a known sample concentration with an additional known standard concentration. The percent recovery of the total concentration of analyte was measured (Equation 10).

Equation 10- Matrix spike Calculation

$$C_{spike} = \frac{C_{sample}V_{sample} + C_{standard}V_{standard}}{V_{spike}}$$

Where:

C_{sample} = Concentration of sample (mg/L)

V_{sample} = Volume of sample (L)

$C_{standard}$ = Concentration of standard (mg/L)

$V_{standard}$ = Volume of standard in spike (L)

V_{spike} = Total volume

3.3.14 Biogas collection corrections

The most common cause of incorrect gas production was leaks in the system at the fittings, the sampling septa's, or Tedlar biogas collection bags. The digesters were siliconed at the fittings and Tedlar bags were replaced when leaks were discovered. No leaks were found in the pilot-scale digester over Experiment 1. Five leaks were discovered in Experiment 2 in the Tedlar bags.

Digester biogas volume was not recorded for some of the digesters prior to steady state operation. One CHM digester had no gas data until July 27, 2015 due to a data logger. During this period the duplicate CHM digester produced 4.1-L of biogas. SHU and CHU digesters had no gas data until July 17, 2015 due to malfunctioning data loggers. A 3.3% difference between the average daily biogas production of the duplicate CHM digesters was achieved when unaccounted for biogas was corrected. The following steps were taken to correct for the missing gas data:

1. Total number of tips during steady state operation (171 days) was determined for each digester.

2. Average number of tips per day was determined for each digester

3. The average number of tips was multiplied by the volume of each tip for each gas meter to determine the rate of biogas production when loggers were not operational.

4 Results

Results for digester performance for all digesters are described in the following section. Throughout this text, ± values in parentheses indication one standard deviation of mean for duplicate digesters.

4.1 Determination of a representative period

For the present study, comparisons of digester performance were made after steady state had been approximately reached by each digester. These relatively steady performance periods are termed representative periods. Digesters were seeded with 100% digestate from the last-in-series SLOWRRF digester. According to continuous-flow stirred tank reactor hydraulics theory, the inoculum was 95% washed-out and replaced with algae

feed after three residence times (120 days) (Equation 11). The representative period was defined as three residence times after inoculation and when effluent pH, alkalinity, TAN, TRP and solids concentrations stabilized. Determination of a representative period was based on Fresco (2016).

Equation 11– Concentration of nonreactive compounds in an ideal mixed digester.

$$C = C_0 e^{-t/T}$$

Where:

C = Concentration of nonreactive compounds at time, t

C_0 = initial concentration of a nonreactive compound (g VS/L)

T = Hydraulic residence time (days)

The pilot digesters used in this study were inoculated 9 months prior to the present study, on August 1, 2014 and the representative period started on December 17, 2014 (Fresco, 2016). For Experiment 2 in the lab, the mixed digesters (CHM) were used to determine the representative period. These digesters were inoculated on April 24, 2015 and achieved steady state on December 10, 2015 (Figure 13). The representative period includes 10 weeks of data collected for all tests with passing QA/QC results. For Experiment 3, the VAU digesters were seeded with a 1:1 blend of digestate from the two pilot digesters.

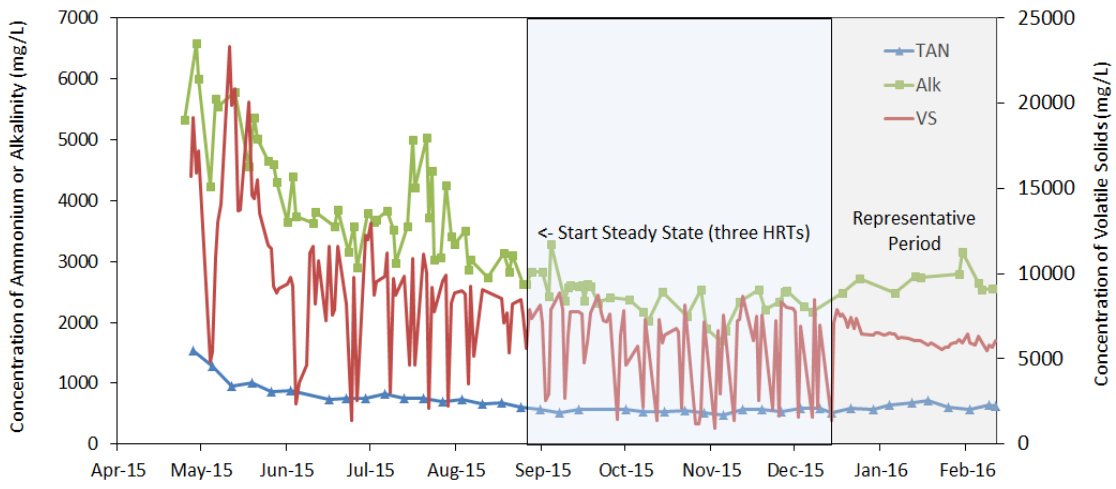

Figure 13- CHM: TAN, alkalinity and volatile solids concentrations. The shaded area indicates three residence times were reached on August 24, 2015 (120 days of digester operation) but the representative period started on December 10, 2015 when the effluent VS stabilized.

4.2 Determining condition of digestate

Experiment 1 and 2 digesters were mixed on September 14, 2015 and pH, alkalinity, soluble and total COD, soluble and total nitrogen, soluble and total phosphorus and total and volatile solids concentrations were measured. The information collected was used to create mass balances for the COD, nitrogen and phosphorus. Mass balances were used to analyze the mass of VS, COD, nitrogen and phosphorus added and removed from the digester. A single average mass was determined by the product of the average mass from each digester. Digester inoculum and digester feed were considered the "Inputs" and the digester effluent and the final volume of digestate were considered the "outputs" for the mass balances. Because VS was measured daily, versus the weekly measurements of the other constituents, correlation ratios were developed during the representative period to determine the proportion of COD, TN and TP added and removed from the digesters

based on the volatile solids concentration (Table 5). Individual ratios were discarded if the difference from the mean ratio was greater than one standard deviation. The average constituent to VS ratio was used as a multiplier to the daily influent or effluent solids concentration (Equation 12). The average theoretical COD/VS ratio (COD:VS) given by Foree et al. (1970) was 1.4, like the ratio given by McCarty et al. (1964) for wastewater solids of 1.42 g COD/g VSS. In this study, a COD: VS ratio of 1.405 g COD/g VS was calculated and was applied to all inputs and outputs to the digesters.

Table 5- Nutrient/volatile solids (VS) ratios for the nitrogen and phosphorus mass balances. Final indicates the end point average ratio of the constituent to the VS concentration for digestate within each digester at the end of the experiment.

	Experiment 1		Experiment 2						Experiment 3			
	Pilot		CHM		CHU		SHU		VAU		VAU,P	
Ratio	$\frac{TN}{VS}$	$\frac{TP}{VS}$	$\frac{TN}{VS}$	$\frac{TP}{VS}$	$\frac{TN}{VS}$	$\frac{TP}{VS}$	$\frac{TN}{VS}$	$\frac{TP}{VS}$	$\frac{TN}{VS}$	$\frac{TP}{VS}$	$\frac{TN}{VS}$	$\frac{TP}{VS}$
Seed	0.125	0.028	0.136	0.014	0.136	0.014	0.136	0.017	0.171	0.012	0.171	0.012
Feed	0.100	0.033	0.090	0.014	0.090	0.014	0.085	0.017	0.100	0.033	0.100	0.033
Effluent	0.338	0.086	0.203	0.033	0.200	0.040	0.392	0.073	0.365	0.155	0.365	0.130
Final	0.119	0.015	0.158	0.033	0.092	0.031	0.079	0.036	0.110	0.013	0.119	0.015

Equation 12- Determination of the average effluent nutrient concentration

$$C = VS_{day} * \left(\frac{C_{N,P}}{VS_{N,P}}\right)_{average}$$

Where:

C = Average effluent nutrient concentration

$C_{N,P}$ = Concentration of the nutrients from the TP or TN analysis

$VS_{N,P}$= Concentration of the VS from the day of the TP or TN analysis

VS_{day} = Daily variable concentration of the volatile solids removed in the effluent

Chemical oxygen demand (COD) was evaluated using a mass balance for the entire duration of the experiment (Figure 14). CODin added to the digester included the initial inoculum and daily feed, and the CODout included the COD removed in the effluent, the end point digestate volume and the methane accumulated in the headspace, and the biogas that left the digesters (Equation 13 and Equation 14). The recovered COD was less than the added COD for all digesters with an average recovery of 62%, 89% and 79% for Experiments 1, 2 and 3, respectively. In Experiment 2, the unmixed digesters (CHU, SHU) had on average 8% more COD removed as methane than the mixed digester (CHM). The unaccounted COD totaled 46 kg for the longest operating pilot digesters in Experiment 1, an average of 18 g of unaccounted for COD from the lab digesters in Experiment 2, and an average of 10 kg and 9 g of unaccounted for COD in the pilot and lab digesters in Experiment 3, respectively.

The total biogas volume collected during Experiment 2 was corrected because data loggers were not recording biogas volume consistently. To account for the volume of COD lost, the average number of tips for each digester for the steady state period was multiplied by the volume of gas in each tip. This steady state volume was used to determine the average volume of gas lost per day. This method accounted for the following portions of the missing COD from each digester set: 16.5 g COD (± 0.020) for the CHM digesters, 22 g COD (± 0.96) for the CHU digesters, and 21.1 g of COD (± 3.15) for the SHU digesters. CHM digesters had the least amount of COD remaining in the final volume of digestate at the end of the experiment because digestate was mixed.

Equation 13 – COD mass balance

$$COD_{in} = COD_{out}$$

Where:

$$COD_{in} = COD_{seed} + COD_{feed}$$

$$COD_{out} = COD_{eff} + COD_{gas} + COD_{final}$$

Equation 14 – Conversion of gas to COD

$$COD_{gas} = \left[\frac{P_{std} * V_{gas} * (\%\ CH4)}{R\ *\ T_{std}}\right] * \frac{2_{mol\ O_2}}{1_{mol\ CH_4}} * Atomic\ Mass\ of\ O_2$$

Where:

$P, T_{std} = 1.0039$ atm, 293.15 K

$V_{gas} = Daily\ volume\ of\ biogas\ (L)$

$$R = 0.08206\ \frac{L * atm}{K * mol}$$

Nitrogen and phosphorus were also evaluated using a mass balance (Figure 15 and

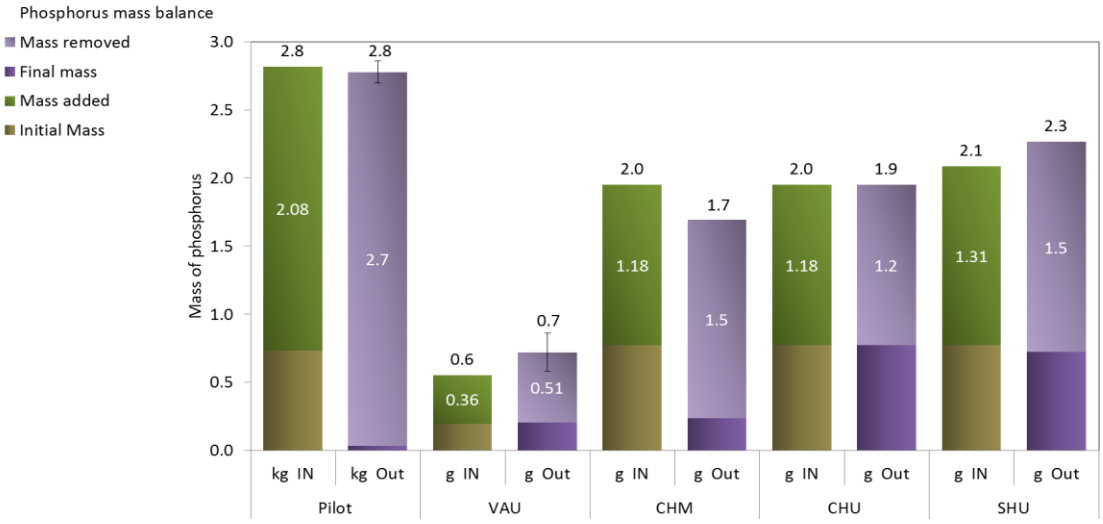

Figure 16). The nitrogen and phosphorus added to the digester included the initial inoculum and daily feed, and the nitrogen and phosphorus out included the material removed in effluent and the final digestate (Equation 15). Recovery of the added nitrogen was 111%, 103% and 86% for Experiments 1, 2, and 3, respectively. Recovery of the added phosphorus was 99%, 98% and 137% for Experiments 1, 2 and 3, respectively.

Equation 15- Nitrogen and phosphorus mass balance

$$N, P_{in} = N, P_{out}$$

Where:

$$N, P_{in} = N, P_{seed} + N, P_{feed}$$

$$N, P_{out} = N, P_{eff} + N, P_{final}$$

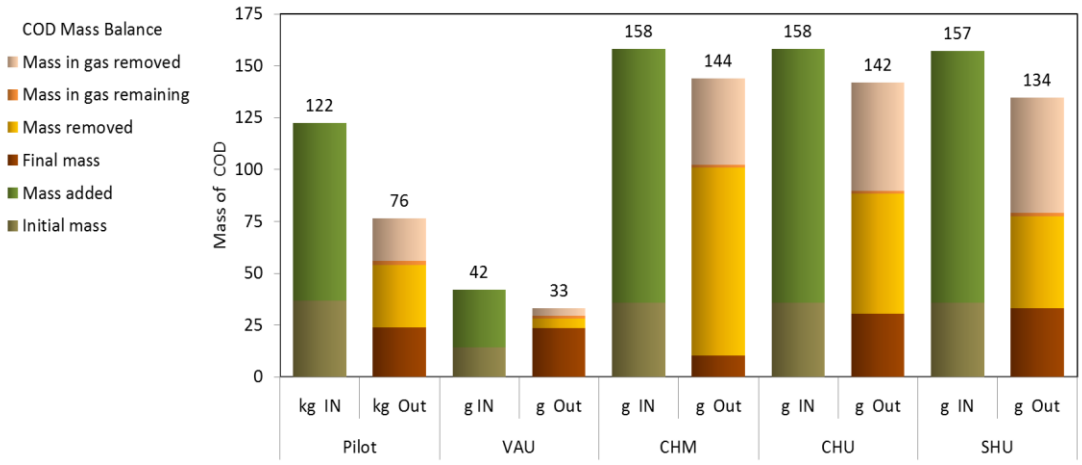

Figure 14 Mass balance of chemical oxygen demand (COD) initially and added (mass added) and the mass of COD removed in the digestate (Mass removed) and the total COD removed in the form of solids and methane gas. Mass was averaged between the average masses of each digester.

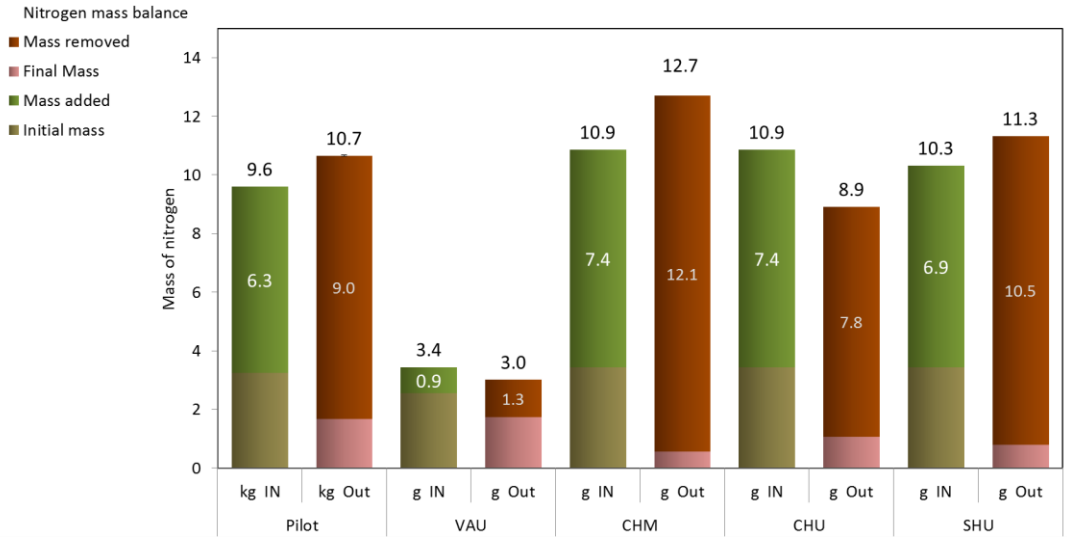

Figure 15 Nitrogen mass balance, including the initial and added nitrogen (TNin), the mass of nitrogen removed (TNout) and the TN remaining inside the digester at the end of the experiment (Final mass). The TNout error bars represent one standard deviation from the average of the duplicate digesters.

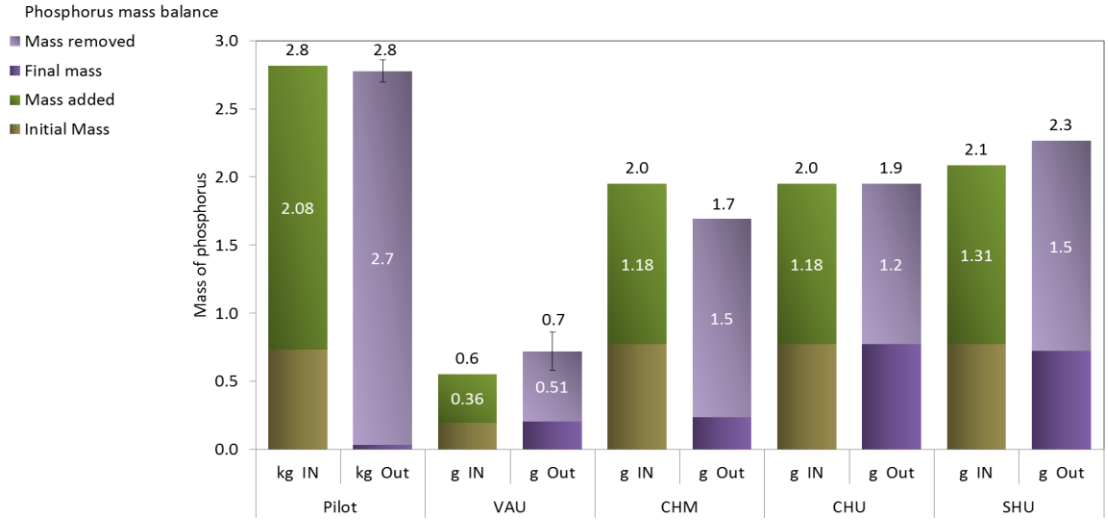

Figure 16 Phosphorus mass balance, including initial and added mass (TPin), mass removed (TPout) and the TP within the digestate at the end of the experiment (Final mass). The TPout error bars represent one standard deviation from the average of the duplicate digesters.

4.3 Experiment 1: One-year operation of the pilot-scale digesters

This section describes the results of the year-long operation of the pilot digesters.

4.3.1 Field Conditions - Seasonal and diel temperatures

Digesters in Experiment 1 experienced diel and seasonal variability in temperatures due to exposure to ambient temperatures. On an average day during the summer season (June 27, 2015), the range in field temperature for the ambient air, soil, gas meter liquid, and digester were 12.4˚C, 1.6 ˚C, 4.4 ˚C and 0.25 ˚C, respectively (Figure 17). Temperature affected the biogas production rates. Daily maximum and minimum temperatures were usually 3 hours earlier for the ambient air than the soil and gas meter temperatures. It was previously discovered that the liquid temperature in the digester closely followed soil temperature trends, so missing temperature data (due to probe malfunction) was replaced with soil temperature from the California Irrigation Management Information System (CIMIS) (Fresco, 2014). A total of 64-days of temperature data, of the 594-day experiment, were replaced with average soil temperature. Each season was determined by the spring and fall equinox or the summer and winter solstice dates (Table 6). Digestate temperatures were recorded over five seasons where the summer achieved the highest temperatures averaging 21˚C and the winter achieved the lowest temperatures of 14˚C (Figure 18). Temperatures were averaged over the day and then averaged between the two digesters.

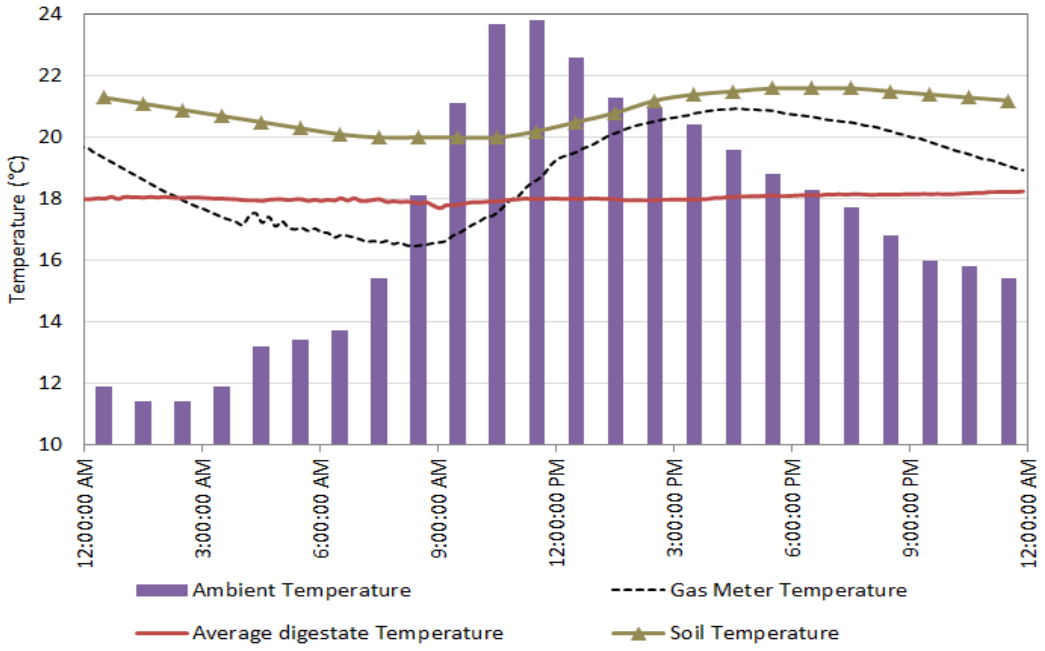

Figure 17- A single day of temperature data (June 27, 2015): ambient air, soil, digestate and gas meter.

Table 6- Seasonal variation in average digester temperature was broken into five seasons.

Season	Date Range	Average Temperature (°C)
Winter 2014	12/21/14-03/20/15	14
Spring 2015	03/21/15-06/20/15	17
Summer 2015	06/21/15-09/20/15	21
Fall 2015	09/21/15-12/20/15	17
Winter 2015	12/21/15-03/17/16	14

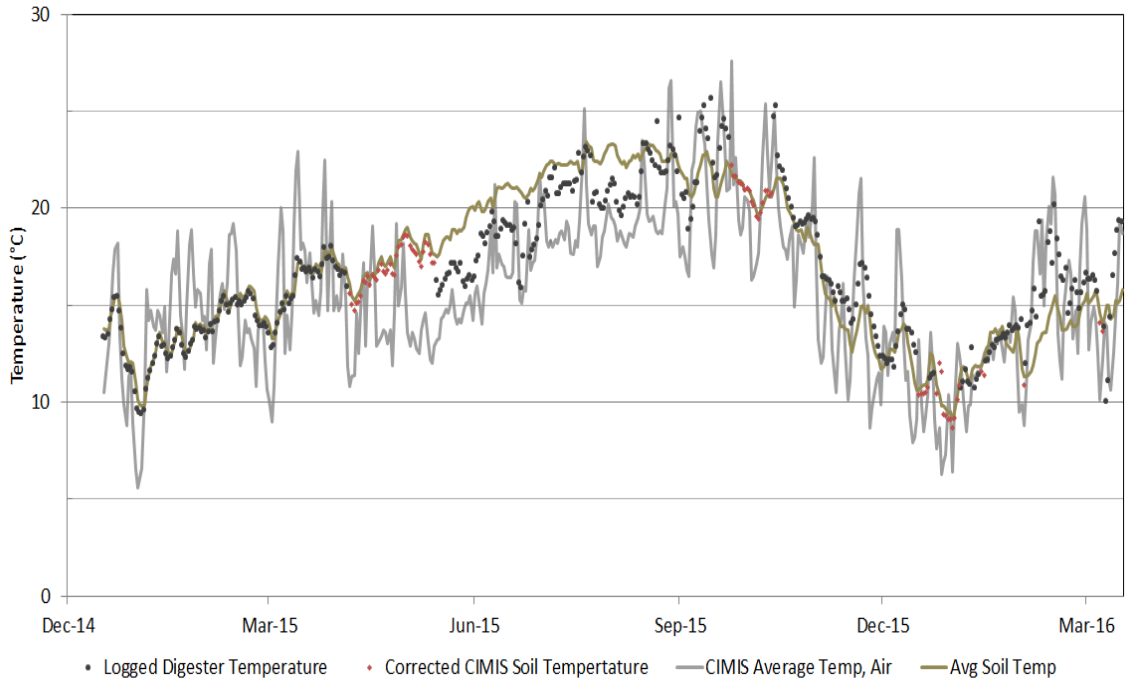

Figure 18- Average daily digestate temperature compared to ambient air temperature and soil temperature data collected by CIMIS.

4.3.2 Field Conditions - Variable Organic Loading Rate

Organic loading rate depended on the availability of biomass which was controlled by algae pond productivity and biomass settleability for harvesting. Factors affecting settleability and harvestability of algal biomass included the character and size of the algal species, addition of flocculants, pond operation (Bowen, 2018) and environmental variables such as weather or exposure to sunlight and growth-medium characteristics (Shelef et.al., 1984). Additional factors affecting settleability of algae biomass were raceway pond HRT, the F/M ratio, native algae cultures, and the structure of the flocs (Ripley, 2013). In past research, algal species grown in wastewater ponds were variable (Chen, 2015) and methane potentials ranged widely (e.g., from 0.59-0.79 L CH_4/gVS (Sialve, 2009). In this research, algal and bacterial species present varied naturally in the

outdoor ponds, which might have had an important effect on the digestibility of the biomass harvested. The relationship between the algal species and their digestibility and harvestability was beyond the scope of this research.

Raceway volatile suspended solids (VSS) data (Reiff, 2015; Roberts, 2015; Bowen, 2018) were compared to the corresponding digester feed VS. In general, as more biomass was produced in the ponds, more biomass was collected and harvested for the digester feed (Figure 19). In spring 2015, the number of ponds used to grow the algal biomass decreased from three ponds to two ponds (Bowen, 2018). Although less pond volume was harvested during the spring of 2015, an increase was seen in the pond VSS and in the thickened algae VS collected in the algae thickening tanks. An opposite trend was seen in pond algal production in the winter seasons. The average digester OLR for the entire duration of the experiment was 0.29 g VS/L-day, with an average steady state OLR of 0.24 g VS/L-day. Spring had the highest average organic loading rate of 0.24 g VS/L-day and winter 2015 had the lowest of 0.10 g VS/L-day (Figure 20). Yearly variably was also large, with 20% higher average OLR in winter 2014 (0.12 g VS/L-day) than in winter 2015 (0.10 g/L-day).

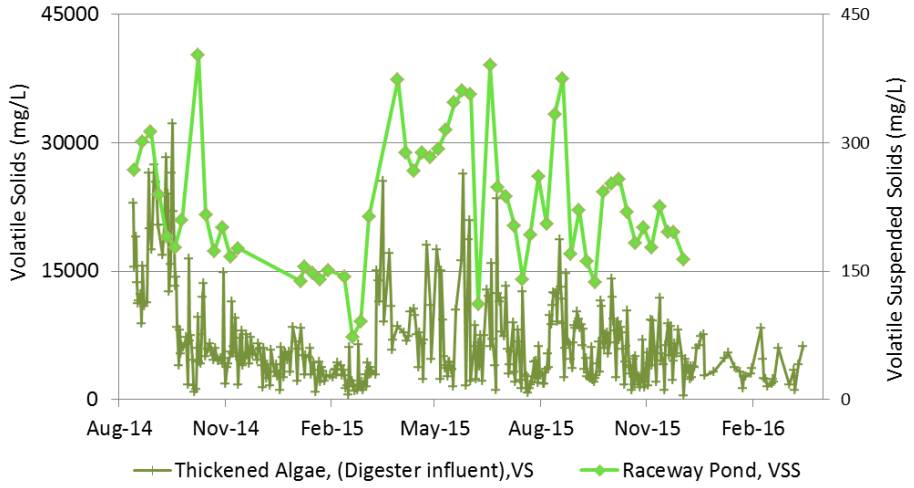

Figure 19- Concentration of volatile suspended solids (VSS) in the algal raceway ponds concurrently with the volatile solids (VS) harvested from the algae gravity thickening tanks.

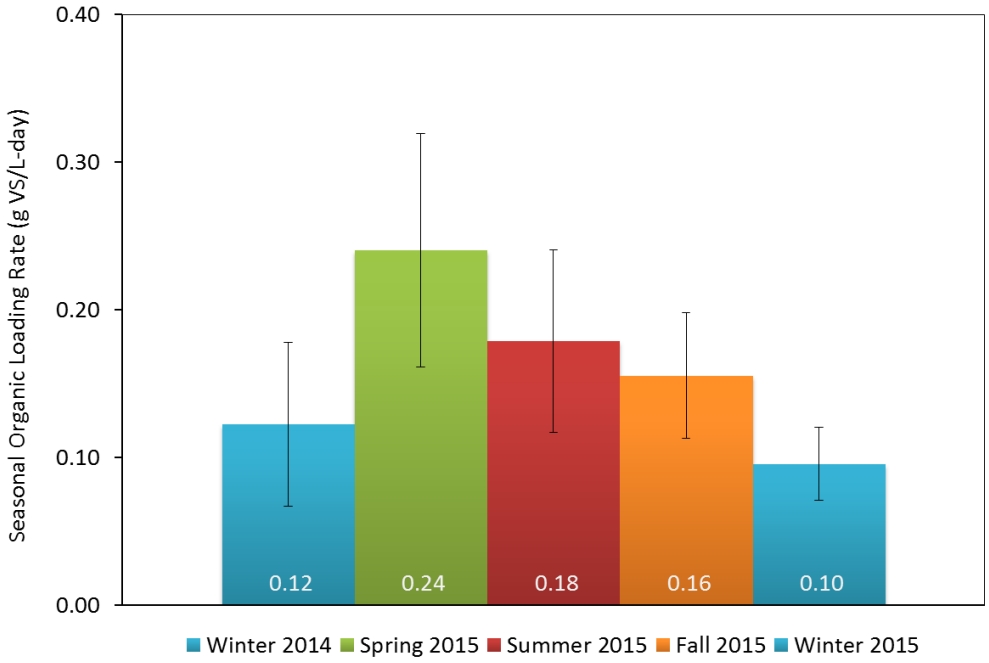

Figure 20 - Seasonal variation of the organic load fed into the digesters. Mean volatile solids collected from the gravity harvesting system from winter 2014 was 4.3 g VS/L, spring 2015 was 8.5 g VS/L, summer 2015 was 6.3 g VS/L, fall 2015 was 5.5 g VS/L, and winter of 2015 was 3.4 g VS/L.

4.3.3 Field Conditions - Digester shading

Due to space restrictions at the field site the east digester shaded the west digester (Figure 21). The east digester had an average liquid temperature of 16.8℃ (standard deviation of 3.6) and the west digester had an average liquid temperature of 16.6℃ (standard deviation of 3.8) indicating that shading did not have a major effect on temperature. To confirm this mass yield was also compared (Figure 22). The average mass yield for the east digesters was within one (+/-) standard deviation of the west digester indicating the west digester liquid temperature and mass yield was not affected by the shading caused by the east digester.

Figure 21- East digester shaded the west digester daily due to proximity of the digesters, located at the AFS.

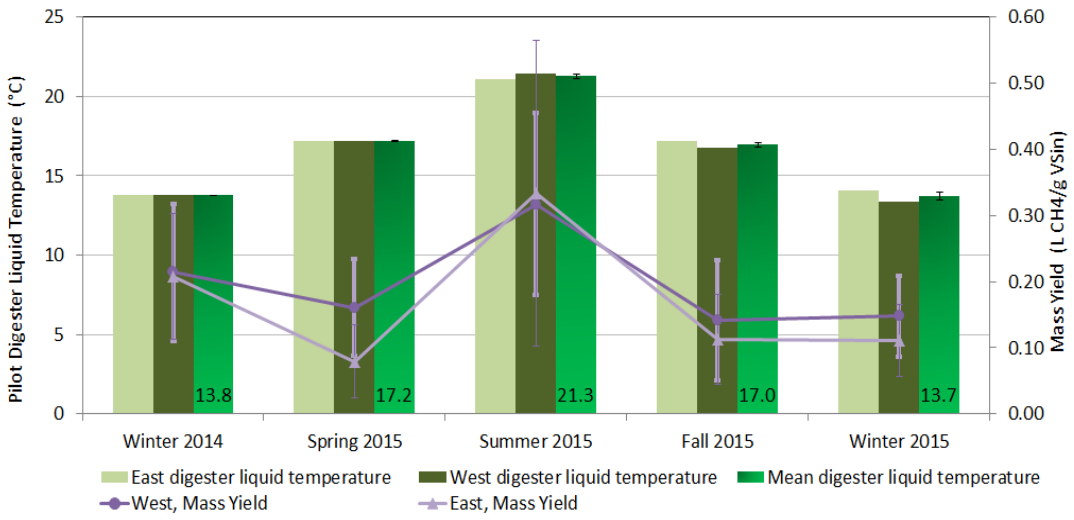

Figure 22- Mass yields for the east digester and west digester was compared during five seasonal changes in digestate temperature.

4.3.4 pH and alkalinity

pH and alkalinity were used to determine the health and stability of the digester. Pilot digester pH and alkalinity appear to have been affected by the seasonal temperatures and the organic loading rate. One year of pH data collection showed that pH decreased in colder temperatures and increased during warmer temperatures (Figure 23). The average pH values for winter 2014 and 2015 were 6.73 and 6.66, respectively, when average temperature was 14˚C. Spring 2015, summer 2015, and fall 2015 saw higher pH results of 6.73, 6.83, and 6.83 at average temperatures of 17˚C, 21˚C, and 17˚C, respectively. The average pH for Experiment 1 was 6.80 (+/- 0.003) and the average alkalinity was 1846 mg $CaCO_3$/L (+/- 8.36 mg $CaCO_3$/L).

This research was conducted in a relatively temperate Mediterranean climate with ambient air temperatures ranging from -1.4˚C to 38˚C. In more extreme climates the effect of temperature might have a greater effect on pH. It was determined in Foree

(1970) that pH levels below 6.3 lead to decreased COD stabilization, and that at a pH of 6.7, the lowest acceptable limit for bicarbonate alkalinity in a healthy digester is 1000 mg $CaCO_3$/L (McCarty, 1964). Digester pH and alkalinity was monitored daily to ensure pH stayed above 6.7 and alkalinity remained above 1000 mg $CaCO_3$/L. The pH of the effluent from each digester was monitored daily, and when the pH was below 6.7 the digesters were not drained, or fed. Digesters were not fed due to low pH in spring 2015, summer 2015 and fall 2015 a total of 21, 6, and 17 days, respectively. No draining or feeding occurred 28 times in winter 2014 and 55 times in winter 2015 (Figure 24). The higher number of no-feed days in winter in 2015 could be due to the decrease in the average organic loading rate from 4307 mg VS/L-day to 3370 mg VS/L-day from 2014 to 2015, respectively. Normally, lower OLR would be thought to lower the risk of pH drop, but in this case, lower VS concentration meant high water content, and the water had low alkalinity.

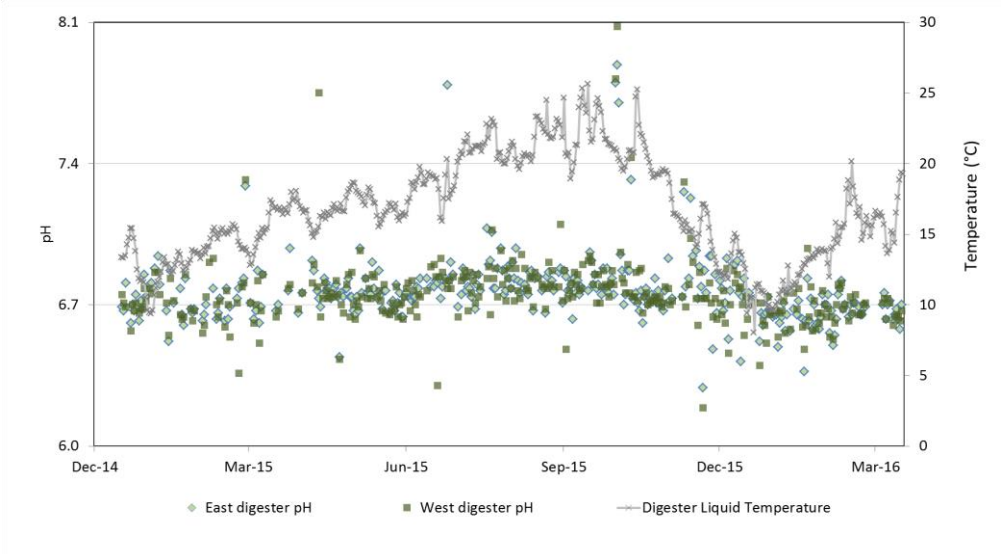

Figure 23- pH ranged from 6.63 to 7.94 and digester liquid temperatures ranged from 9˚C to 25˚C.

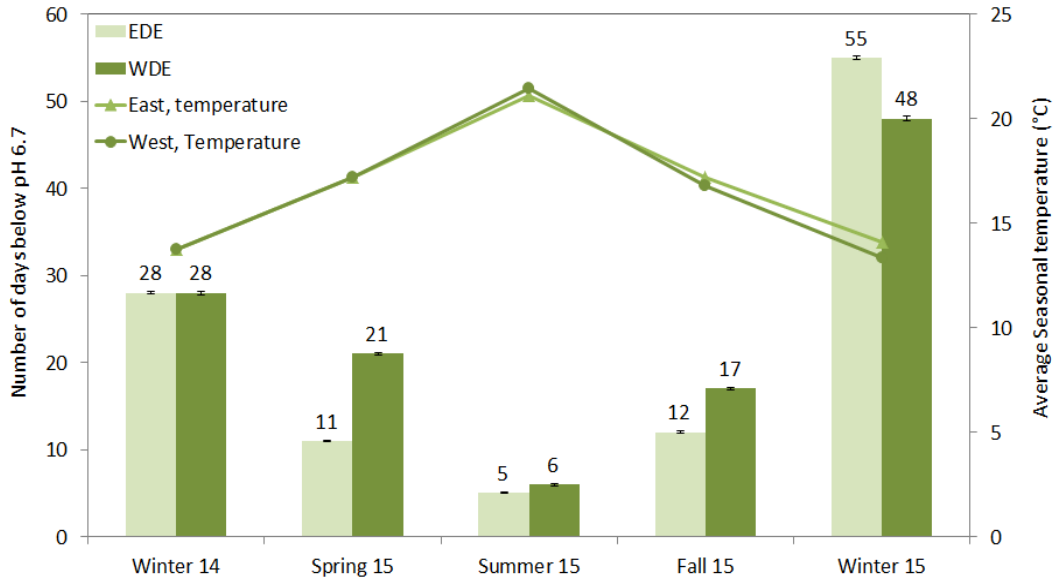

Figure 24- Temperature and the number of days below the pH feeding threshold of 6.7. EDE is East Digester Effluent and WDE is West Digester Effluent.

Like pH, alkalinity was a measure of digester health and indicated the buffering capacity of the digestate to resist changes in pH. Digestate alkalinity may have been affected by the digester temperature, concentration of hydrolysable biomass in the feed, HRT, and its partial pressure of carbon dioxide (McCarty, 1964; Metcalf et al., 1991). Low temperatures (below 20°C) coupled with a low alkalinity may have caused volatile acid concentrations to build to unhealthy levels reducing pH and biological activity (McCarty, 1964). The alkalinity of the pilot digesters ranged from 1166 mg $CaCO_3$/L to 3987 mg $CaCO_3$/L throughout the duration of the experiment with the lowest average alkalinity of 1166 mg $CaCO_3$/L occurring in November 2015 when temperatures dropped below 10˚C. Evidence from this study shows that alkalinity increased when temperatures and OLR increased and decreased when temperatures and OLR decreased (Figure 25). The effect on consumption of VFAs and production of alkalinity when digesters are fed low OLR in

colder temperatures are speculated to have an impact on metabolic rates of the digester bacteria but is outside the scope of this research.

The concentration of CO_2 in the biogas seems to have correlated with alkalinity, which, in turn, seems to have correlated with temperature and/or OLR (Figure 26).

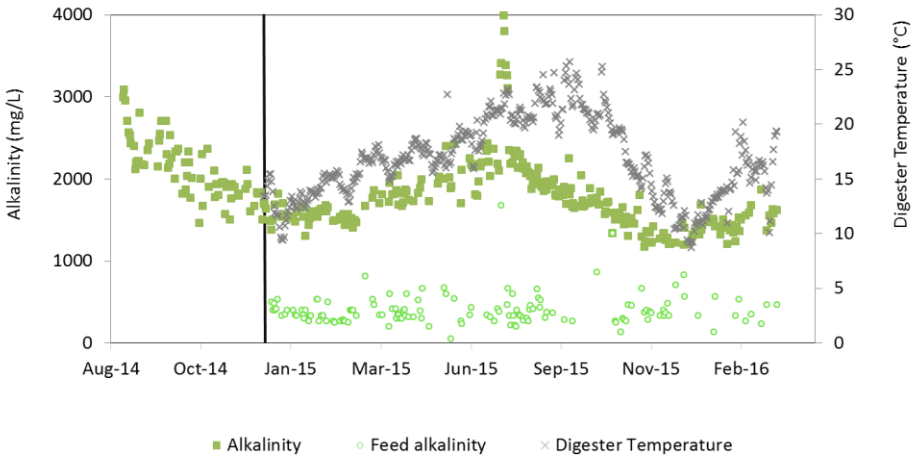

Figure 25- Alkalinity increased during warmer temperatures seen during spring and summer and decreased during late fall, winter and early spring seasons when temperatures dropped.

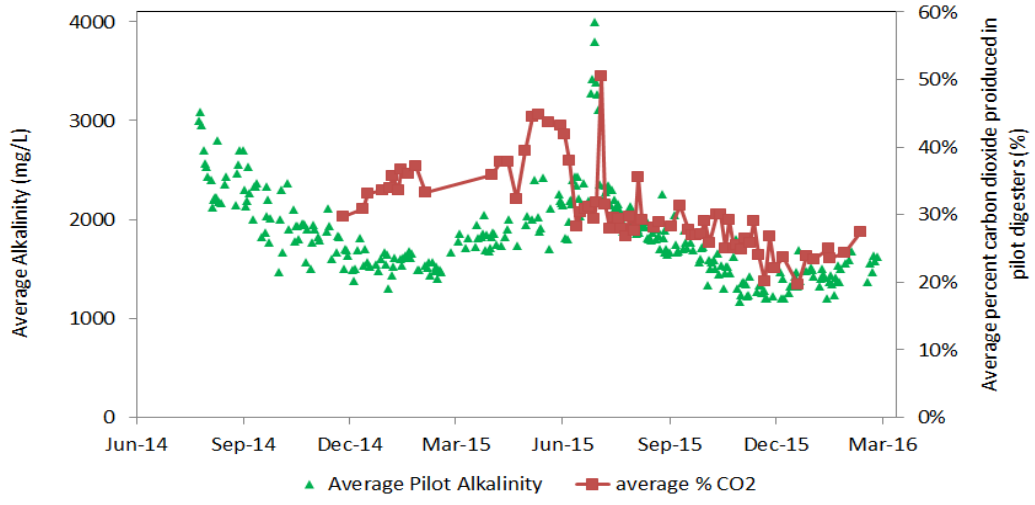

Figure 26- Alkalinity and the carbon dioxide content of the digestate increased and decreased at similar rates over the one-year experiment.

4.3.5 Accumulation and nutrient solubilization

Accumulation of solids at the bottom of the unmixed digesters increases the SRT leading to a higher VSD with more available nutrients for recycling, but it may also lead to increased capital or O&M costs of future algae biofuel systems. Pilot digesters experienced an average 2.7% mass accumulation over 15 months of operation (Figure 27).

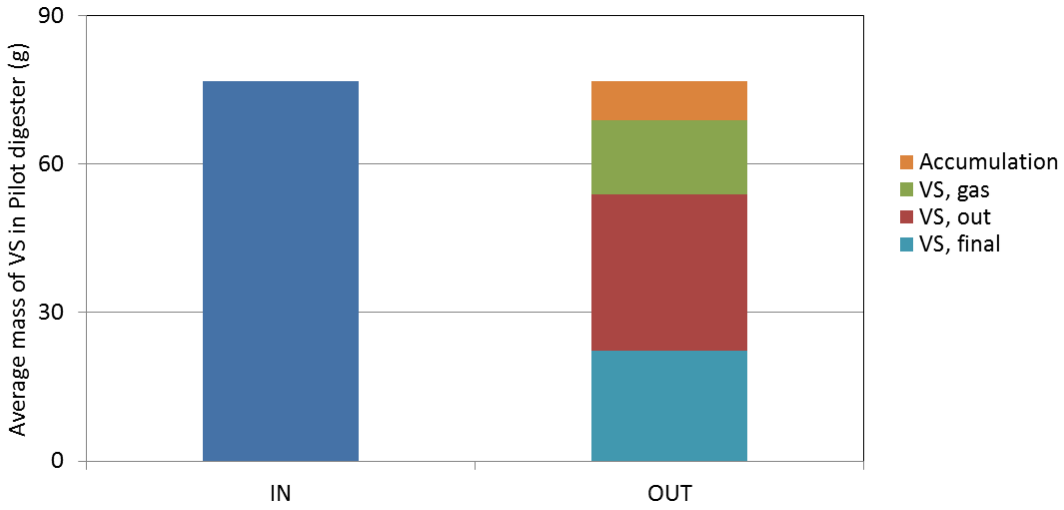

Figure 27 - Average mass of VS added to pilot digesters, the mass of solids removed as particles, mass lost as gas and accumulation.

Nutrient solubilization and solids destruction was measured as percent solubilization of particulate remaining of total nitrogen and total phosphorus and % VSD. Overall, the average percent particulate phosphorus remaining was 3% less (33%) than the percent particulate nitrogen remaining (36%), and the average VSD was 57% during the steady state operation (Figure *28*). VSD increased during summer with an average 61% VSD and decreased during winter with an average 29% VSD (Figure 29). In addition, effluent TAN concentrations increased when temperatures increased, but a similar trend was not seen by the effluent TRP results (Figure 30).

Some digestate solids did not settle and were removed in the daily draining of the digester effluent. The average solids concentration in the effluent was 80 mg VS/L. The increased VSD and decrease in effluent solids during warmer seasons which are seasons when biomass productivities increase is ideal for recycling digester effluent back into ponds because fewer light blocking particles are introduced.

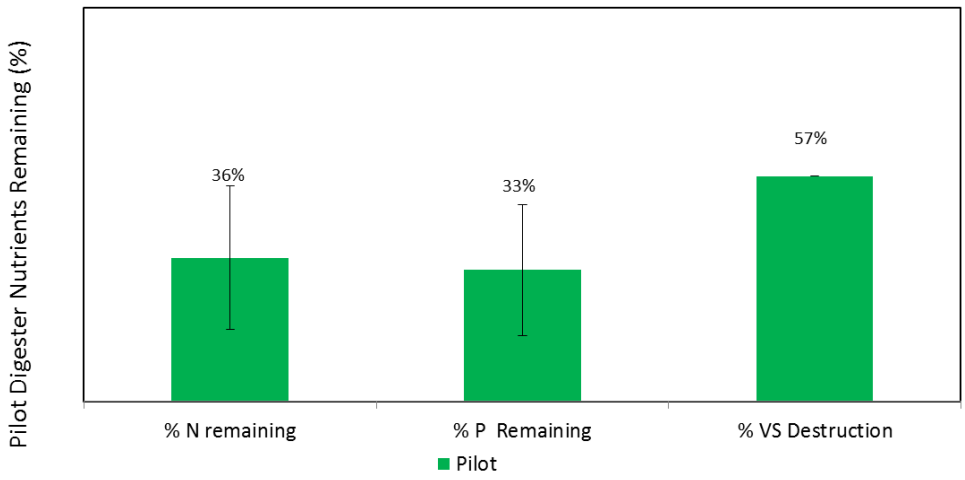

Figure 28- Mean total particulate nitrogen and phosphorus remaining in the digestate and the mean VSD for the pilot-scale digesters.

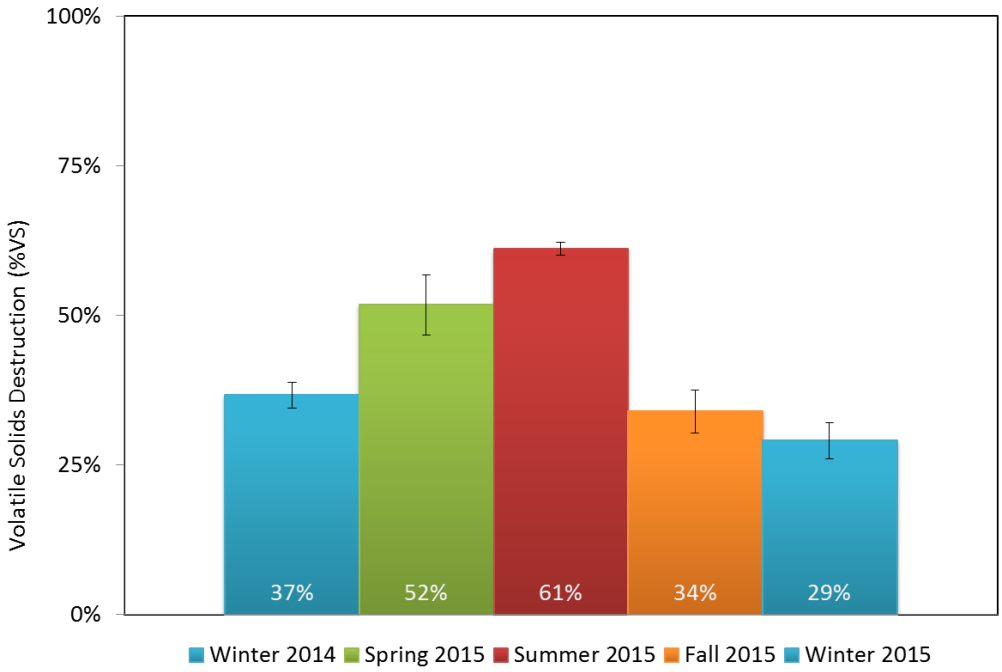

Figure 29- Average seasonal VSD for pilot digesters. Error bars represent one standard deviation from the mean.

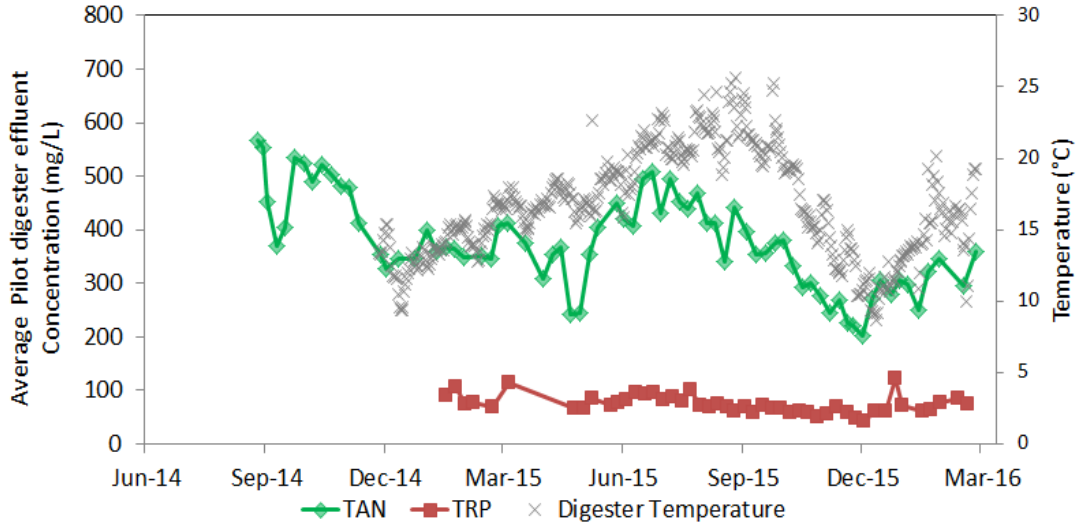

Figure 30- Soluble forms of nitrogen and phosphorus in the digestate effluent and the digestate temperature.

4.3.6 Biogas yield and production

Biogas yield was measured in terms of mass yield (L CH_4/ g VSin), volumetric productivity (L CH_4/L digester), and mass yield destruction (L CH_4/ g VS destruction). The pilot digesters were exposed to temperatures predominantly in the psychrotrophic temperature range (< 20˚C). Steady state encompassed five seasons starting in the winter of 2014 and ending in winter of 2016, with seasonal mass yields ranging from 0.11-0.46 L CH_4/g VSin at 14-21˚C (Figure 31). Mass yield and concentration of solids fed to digesters increased when temperatures increased.

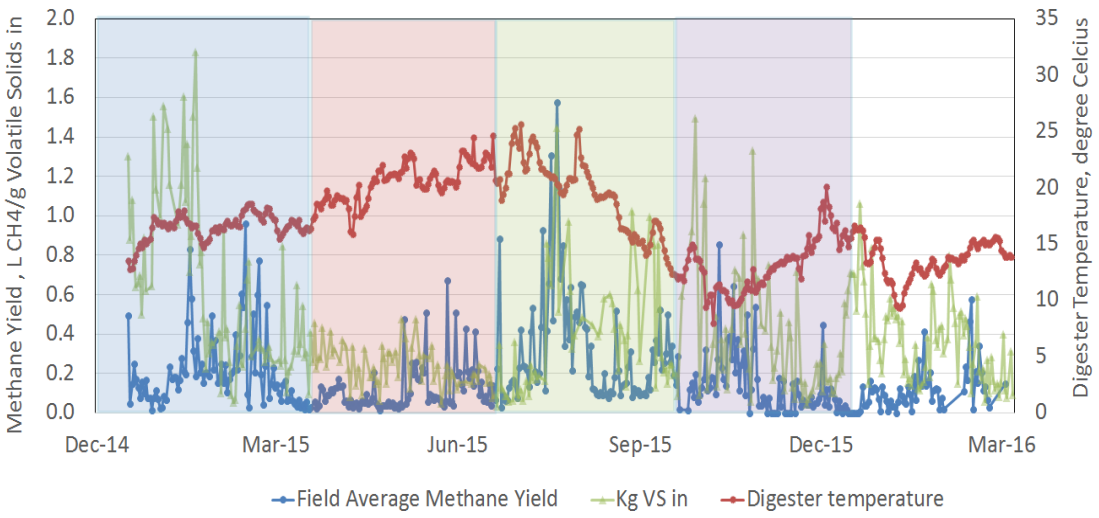

Figure 31- Pilot digesters average mass yield, organic loading rate and digester liquid temperature.

Digester operating conditions play a key role in digester health and digester biogas yields. Two periods of abnormal operation occurred in spring 2015 for the east digester (exposure to air and a clogged effluent gas line) causing the spring 2015 mass yield (0.08 L CH_4/g VS) to be 50% less than the west digester yield (0.16 L CH_4/g VS). Except for

spring 2015, the average mass yields of the duplicate digester were within one standard deviation of each other (Figure 32). Including spring 2015, the east and west digester biogas yields were 0.20 L CH_4/g VSin and 0.17 L CH_4/ g VSin, respectively. The difference between the two digesters might be due to shading that caused a slight difference in operating temperature.

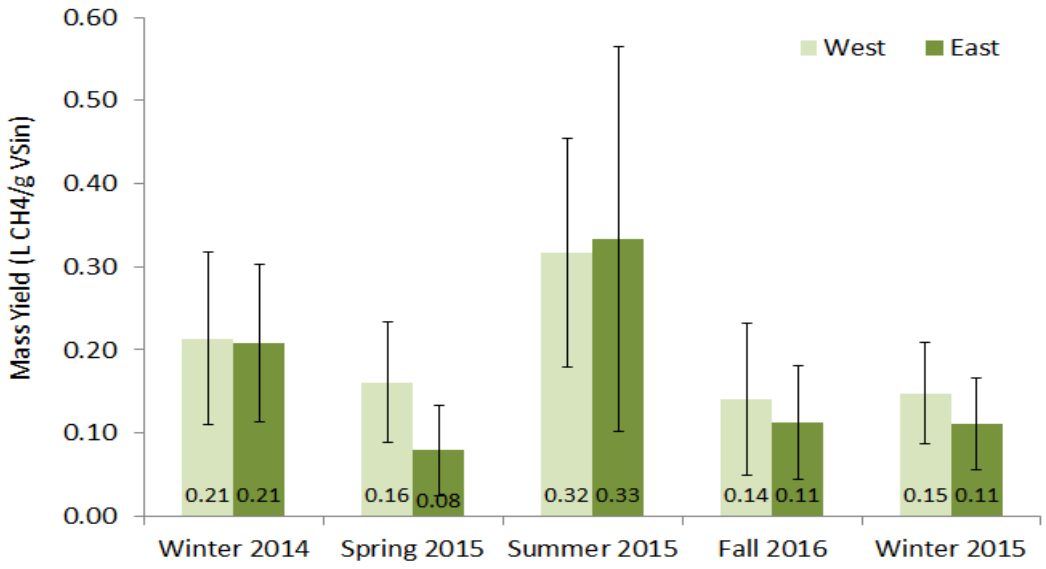

Figure 32- East digester and west digester average mass yield for the 5 seasons. Error bars indicate one standard deviation from the mean.

Higher digester temperatures resulted in higher methane yield with a mass yield of 0.46 L CH_4/g VSin at an average of 21˚C (Figure 33). This is better than the range determined by Eisenberg et al. (1980), with unmixed earthen microalgae digesters with biogas yields from 0.210-0.320 L CH_4/g VS at 25-40˚C. Conversely, colder temperatures resulted in a decrease in mass yield during the winter 2014 with a mass yield of 0.19 L CH_4/g VS at 14 ˚C and winter 2015 with a mass yield of 0.11 L CH_4/g VS at 14˚C. Possible explanations for the winter 2015 mass yield to be less than the winter 2014 mass yield

include the decrease in temperature of 0.1˚C between the two winter seasons or the decrease in the average %VS in the OLR from 43% (winter 2014) to 34% (winter 2015).

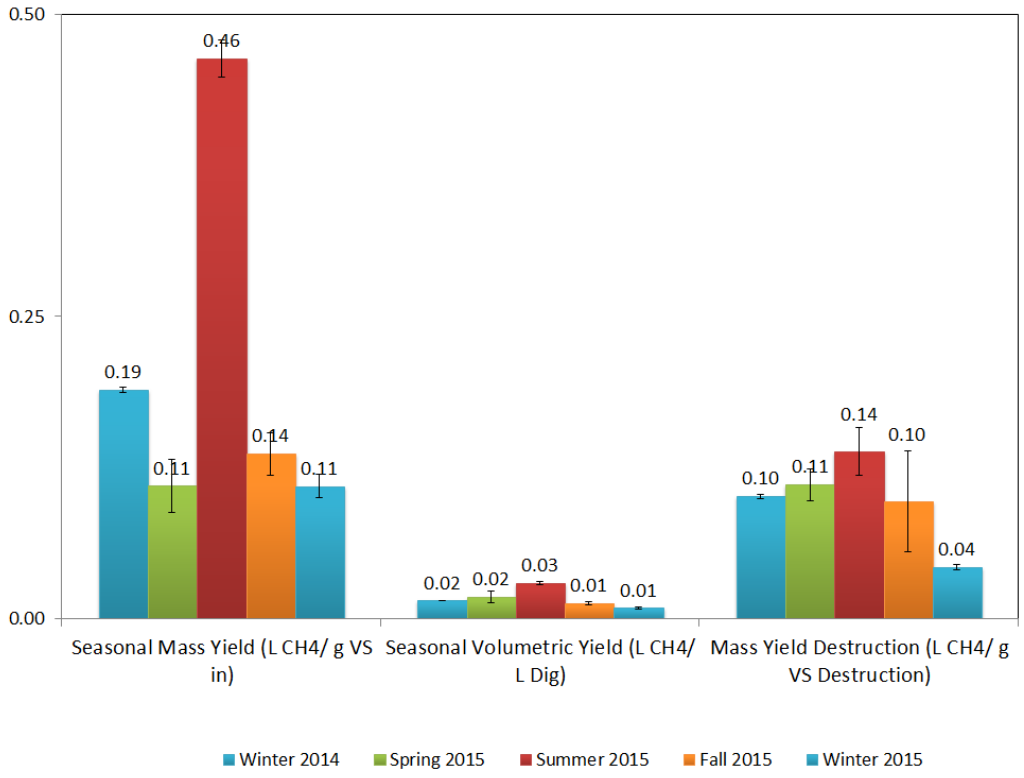

Figure 33- *Mass, volumetric, and VS destruction yield for the pilot digesters over five seasons. The gas was corrected to 20℃. Error bars represent one standard deviation from the mean.*

4.4 Experiment 2: Effects of mixing and sonication on digester performance

This section describes laboratory digester experiments.

4.4.1 Nutrient solubilization, VSD and solids accumulation

Digester effluent characteristics from mixed digesters (CHM) and unmixed digesters fed sonicated feed (SHU) were compared to an unmixed digester (CHU) to determine the effects of mixing and sonication. All digesters were operated at a 40-day HRT, with a constant temperature

of 30˚C, and an organic load of 0.25g VS/L-day. Organic nitrogen solubilization to ammonia (TAN) and particulate phosphorus solubilization to bioavailable phosphorus (TRP) was measured. TAN and TRP data were collected from April 24, 2015 until February 11, 2016 for the feeds and effluents from the digesters. Initial digester TAN concentrations were an average TAN of 1530 mg N/L (Figure 34). Initial concentration of TRP was almost the same in the unmixed digesters with an average of 55 mg P/L (Figure 35). Soluble nutrient concentrations in the effluent were greater than the influent for all digesters. Effluent soluble nitrogen of the CHM, CHU, and SHU was greater than the influent by 152%, 160% and 157%, respectively. Effluent soluble phosphorus of the CHM, CHU and SHU digesters was 159%, 166% and 162% greater than the influent, respectively.

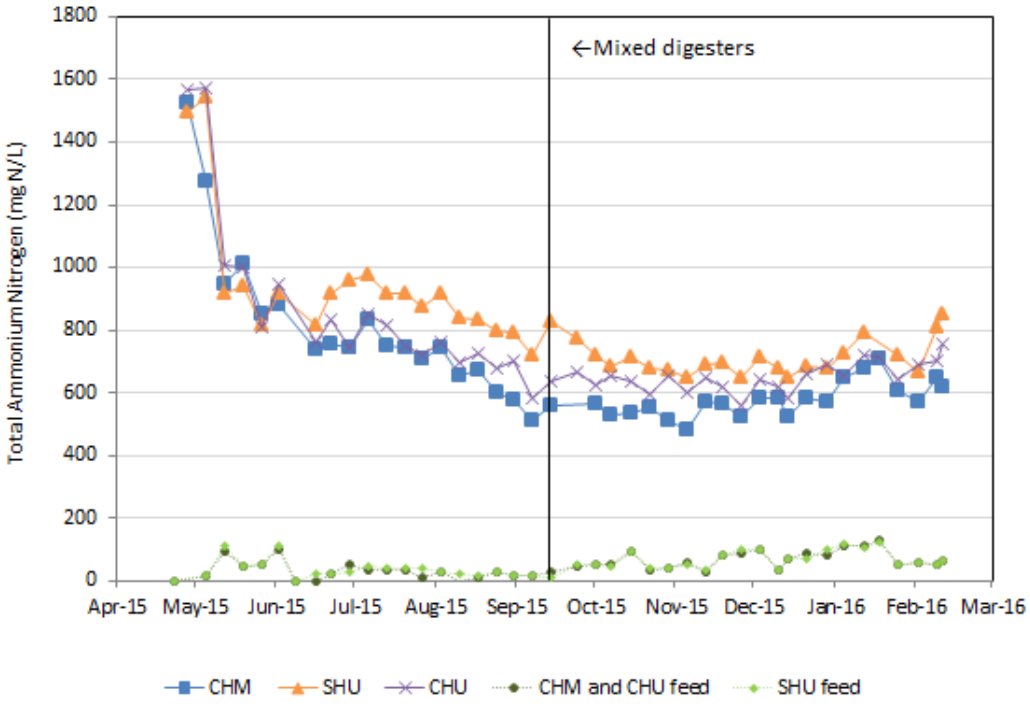

Figure 34- *The total ammonium nitrogen (TAN) in the effluent from the 1.2-L lab-scale digesters for the full duration of Experiment 2 starting on April 24, 2015 and ending on February 11, 2016. During steady state (right of vertical line) TAN concentrations were below the inhibitory ammonia nitrogen concentration of 1500 mg N/L. All digesters were shaken and mixed to determine condition of digestate on September 14, 2015.*

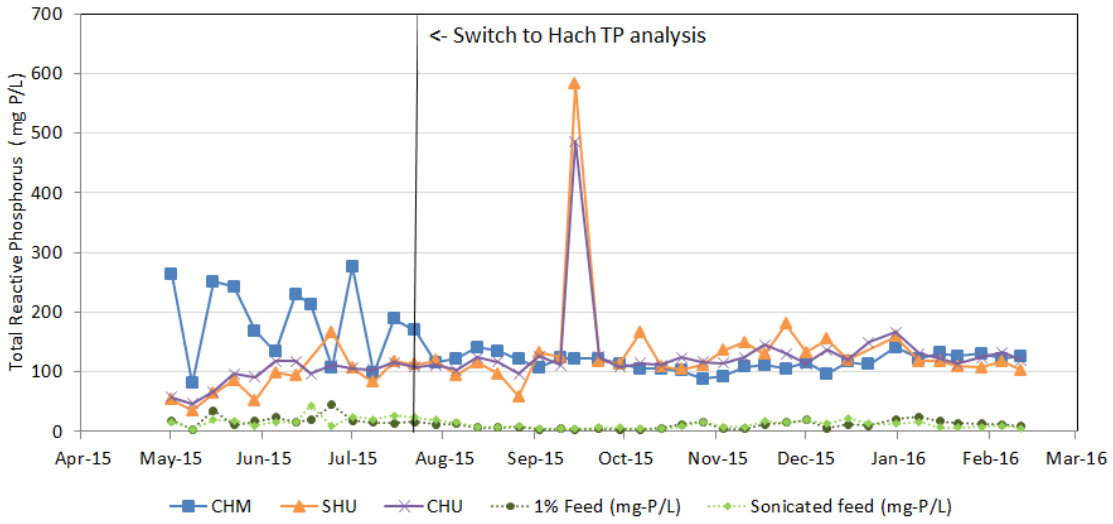

Figure 35 - TRP in the influent and effluent from the 1.2-L lab-scale digesters for Experiment 2 starting on April 24, 2015 and ending on February 11, 2016. Changing from the Kjeldahl TP test to the Hach TP analysis on July 22, 2015 resulted in steadier effluent results from mixed digesters. A spike in TP was observed in unmixed digesters when they were mixed on September 14, 2015.

The feed TAN and TRP was the same for all reactors, but typically the effluent of the SHU digesters had the highest TAN concentrations while CHM had the lowest. Overall, the average effluent concentration of TAN for the CHM, CHU and SHU digesters was 576 mg N/L (±7.5), 650 mg N/L (±11.4), and 722 mg N/L (±14.1), respectively. The average effluent concentration of TRP for the CHM, CHU and SHU digesters was 123 mg P/L (±0.72), 131 mg P/L (±2.65), 123 mg P/L (±3.58), respectively. Absence of mixing led to greater solubilization of nitrogen and phosphorus by 12% and 7% (CHM, CHU), respectively, while sonicating feed further increased the solubilization of nitrogen by 10% but decreased the solubilization of phosphorus by 7% (CHU, SHU) (

Figure 36).

Effluent solids and TRP had the greatest increase in concentration when unmixed digesters were mixed on September 14, 2015. Digesters were mixed by shaking to determine condition of

digestate only once throughout the experiment. CHU and SHU digesters were not mixed again after this onetime event. When the unmixed CHU and SHU digesters were mixed, the TRP increased by 115% and 130%, respectively, and the effluent solids concentration increased by 128% and 146%, respectively (Figure 37). Mixing the digesters increased the average TAN of the CHU and CHM digesters by 3% but the greatest increase was seen by the SHU digesters of 13%. The increase in TRP after mixing may be due to the increase in effluent solids and associated adsorbed phosphorus. Adsorbed phosphorus is a source of bioavailable phosphorus retained on the cell wall of the algal biomass which can be used in nutrient recycling.

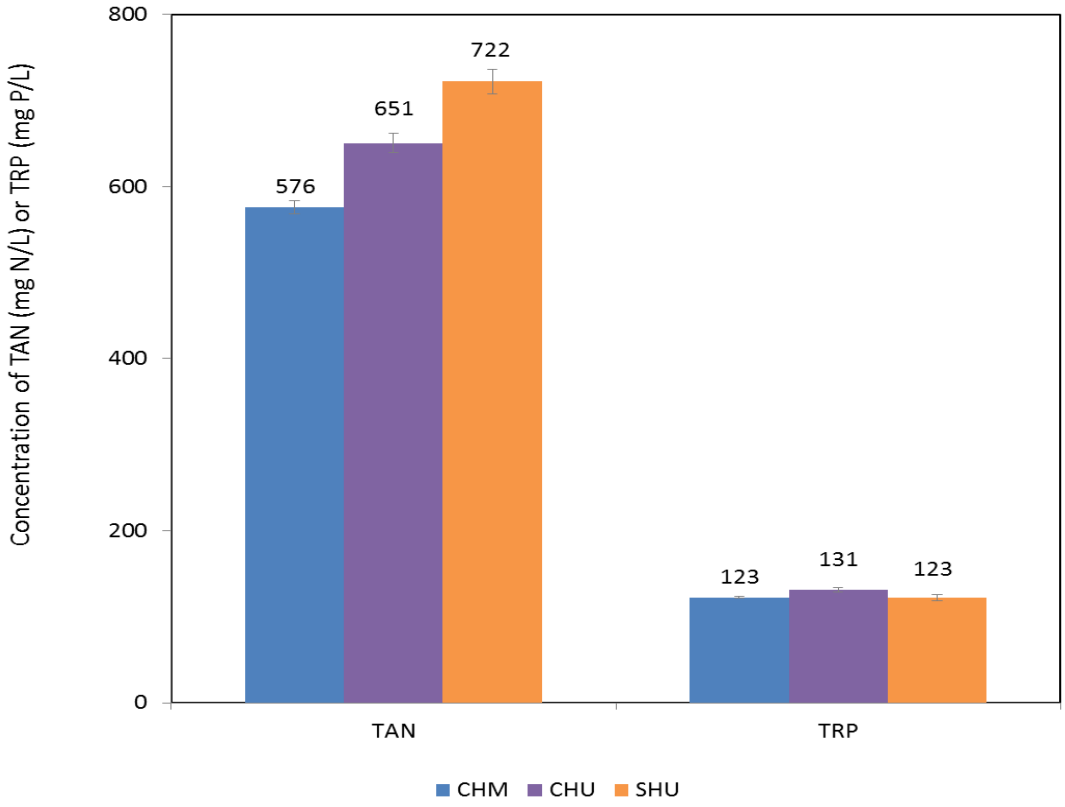

Figure 36 – Average effluent TAN and TRP results from CHM, CHU and SHU digesters.

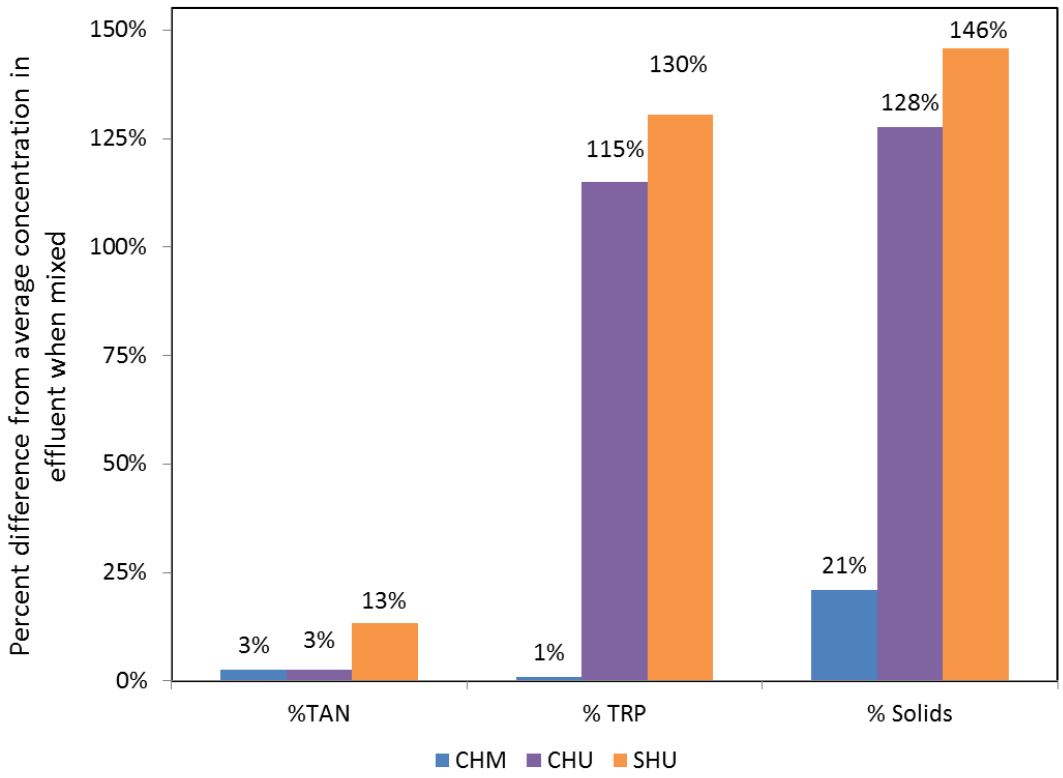

Figure 37- The percent difference from the steady state average in TAN, TRP and effluent solids when digesters were mixed on September 14, 2015.

Nutrient solubilization and VSD were improved by sonication and in the absence of mixing. Both the control (CHU) and the sonicated digester (SHU) were consistently unmixed throughout the experiment. Eliminating mixing and sonicating the feed led to a 6% higher accumulation than the unsonicated and unmixed digester (SHU, CHU) resulting in a longer SRT (Figure 38). Sonication may have increased the dewatering ability of the digester leading to a thicker, denser accumulation of solids (Zhang, 2011). Sonicating the algae feed increased the VSD by 14% (SHU average VSD 75%) and eliminating mixing increased the VSD by 8% (CHU average VSD 61%) (Figure 39). The sonicated unmixed digesters had the least amount of particulate remaining nitrogen (36%), and phosphorus (28%) and the highest VSD (75%) of all treatments. Sonicating improved the nitrogen and phosphorus solubilization by 10% and 4%, respectively, while

eliminating mixing improved nitrogen and phosphorus solubilization over the mixed and unsonicated digesters (CHM) by 4% and 10%, respectively. This likely indicates that sonication had the greatest effect on nitrogen solubilization (6% increase over CHU), compared to eliminating mixing (4% increase over CHM); however, considering the energy required for sonication, the elimination of mixing (a net energy gain) was an energy efficient way to increase nitrogen solubilization. Similarly, sonication improved phosphorus solubilization (4% increase over CHU) and eliminating mixing had the greatest increase on phosphorus solubilization (10% increase over CHM) indicating eliminating mixing would also be the most energy efficient method for increasing phosphorus solubilization.

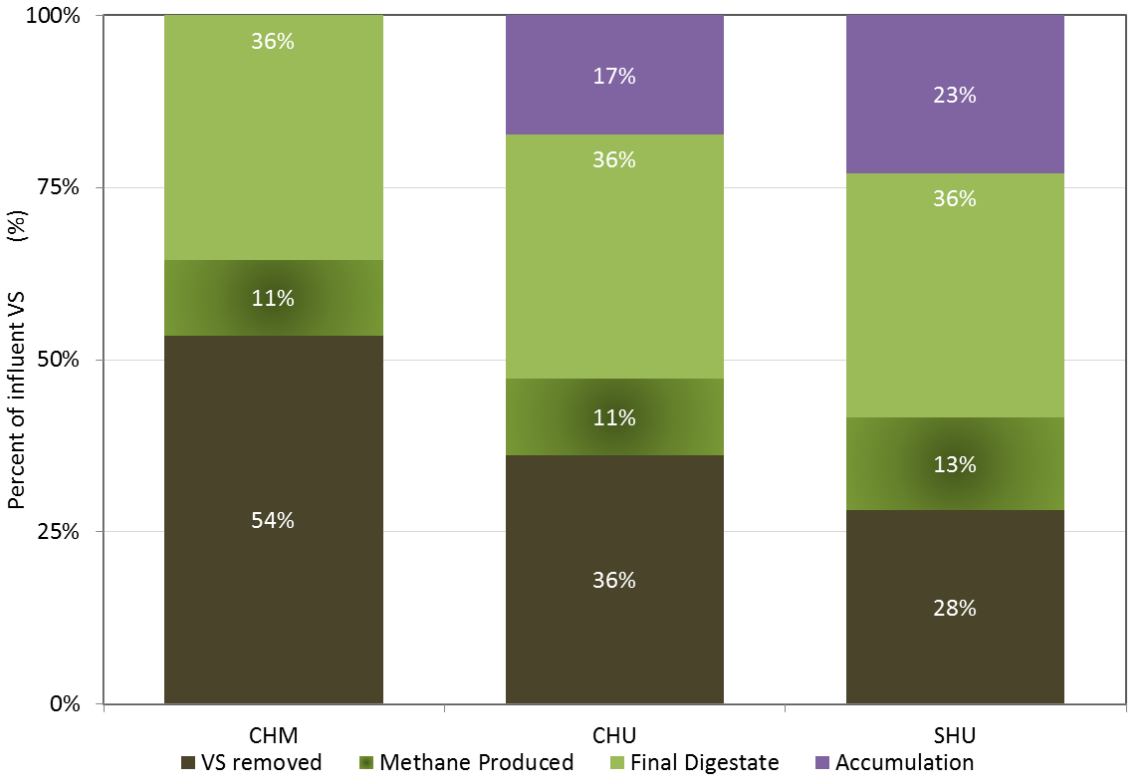

Figure 38- Mass balance on the volatile solids (VS) in and out of the 1.2-L lab digesters. The VS removed term represents the mass of the biomass removed in the liquid effluent during the steady state operating period. The loss of biomass due to the conversion of biomass to biogas is presented in terms of grams of VS loss due conversion to CH_4. Unmixed digesters had an average 44% of biomass removed as methane which is 10% greater than the mixed digester (34%). Conversion of biomass to methane used conversion factors of 1.42 g VS/L CH_4 and 0.35 L CH_4/g COD.

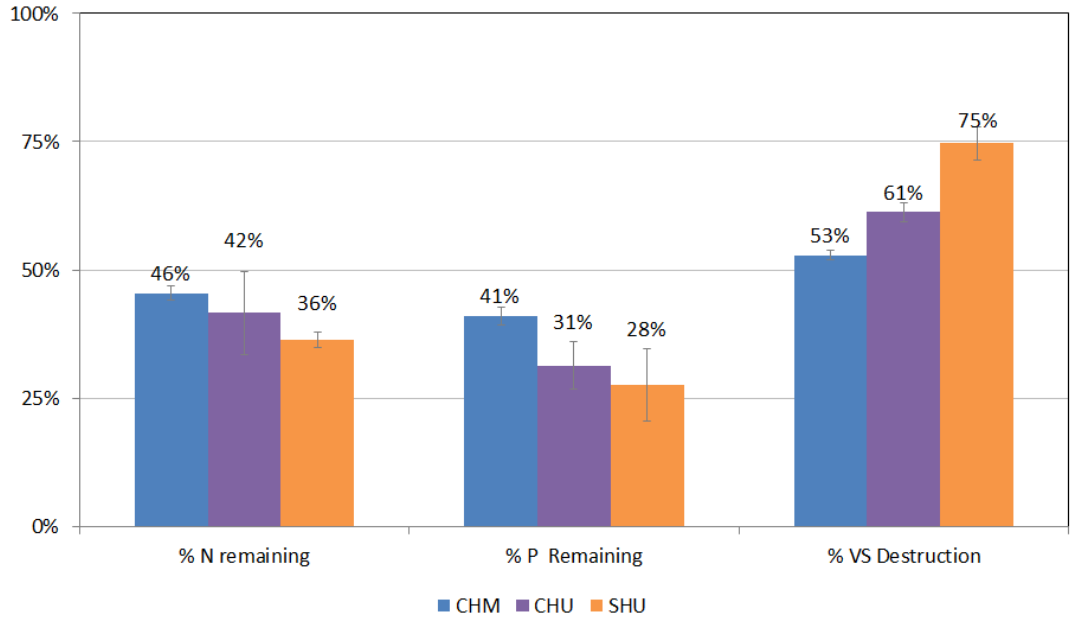

Figure 39- Nutrient solubilization between the 1.2-L lab digesters for the CHM, CHU and SHU digesters. Error bars represent one standard deviation from the mean.

4.4.2 Biogas condition and production

Biogas volume was measured continuously and the percent of methane in the biogas were measured twice a week by GC. Biogas yields were calculated by first multiplying the daily volume (mL) of biogas produced by average weekly methane percentages. Average weekly methane was applied to the days where GC data were not collected. The proportion of methane in the biogas for all digesters ranged from 55% to 76% CH_4 by volume during the steady state operation (Figure 40). This was similar to results found in literature, where algae digesters operated semi-continuously at 35˚C, with a 33-day HRT and fed an average OLR of 0.97 g VS/L-J had a methane percentage of 68% -72% CH_4 (Samson et al., 1982). Factors influencing the proportion of methane in the biogas include the digestate pH and the oxidation state of the biomass fed into the digester (Sialve et al., 2009). Average weekly methane volume produced was highest for SHU and lowest for CHM at the start of steady state. Averages were taken using the weekly GC data and the average weekly volume of biogas produced per day. Average steady

state daily production of biogas for the SHU, CHU and CHM digesters was 79, 68, and 56 mL/day, respectively. After January 3, 2016 all digesters experienced an average decrease in methane produced of 313 mL/day (± 150 mL/ day) with the greatest decrease seen by the CHM digesters of 462 mL/day. This decrease may be explained by the breakdown of lipids, proteins and carbohydrates in the biomass changing the methane potential of the algae during the colder temperatures experienced by the raceway ponds growing the algae (Buswell, 1952).

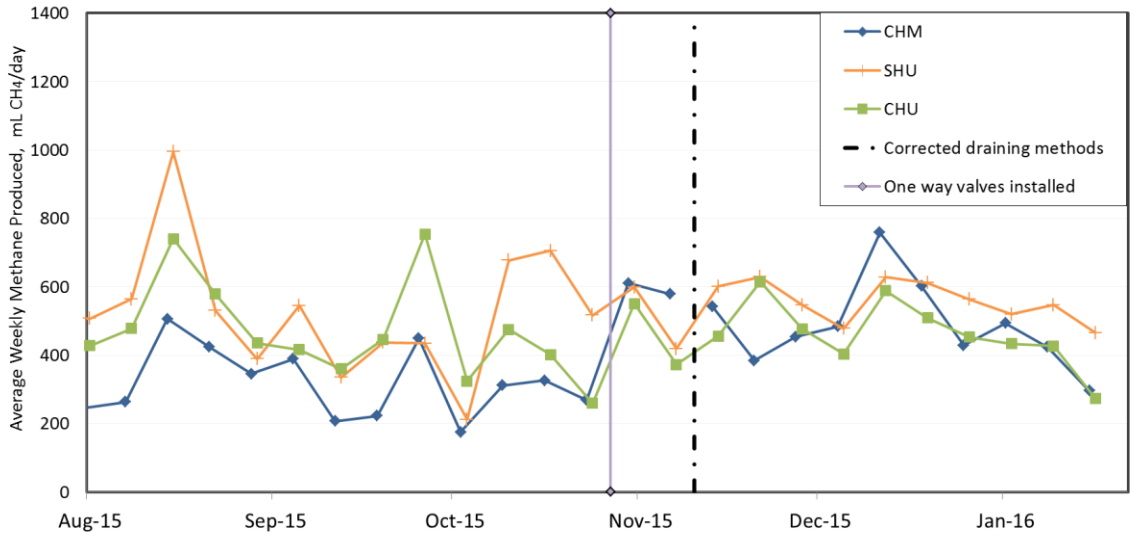

Figure 40- Steady state cumulative volume for the SHU, CHU and CHM digesters operating in the steady state conditions for 171 days. Average biogas production was steadier after one-way valve were installed and the draining methods were corrected to prevent solids straining.

4.4.3 Effect on biogas yield

The effects of mixing and sonication on methane production was evaluated by determining the average mass yield (L CH_4/ g VS in), volumetric productivity (L CH_4/ L digester) and mass yield from VS destruction (L CH_4/g VS destroyed). Biogas yields were collected during the representative period including 10-weeks of steady state data. The highest volumetric productivity of 0.070 L CH_4/L digester and the highest mass yield of 0.28 L CH_4/g VSin were produced by the unmixed and sonicated feed (SHU) digester (Figure 41). The unmixed digester

(CHU) resulted in the lowest volumetric and mass yields of 0.058 L CH_4/L and 0.24 L CH_4/g VSin, respectively. The mixed digester (CHM) had the second highest volumetric productivity of 0.061 L CH_4/L digester, mass yield of 0.25 L CH_4/g VSin and the highest VSD yield of 0.63 L CH_4/ g VSD. The CHU and SHU digesters achieved the lowest VSD yields of 0.56 L CH_4/g VSD and 0.52 L CH_4/g VSD, respectively. SHU had the highest %VSD of 75%, but the lowest VSD yield possibly due to the conversion of low methane potential algal components resulting from the increased breakdown of biomass from the sonication process (Buswell, 1952; Zhang, 2011). Not mixing the digester increased the mass yield by 10% and sonication increased the mass yield by 17%. The net energy production from the unmixed and sonicated digesters versus the net energy from the unmixed digesters with only a 7% improvement in mass yield might not justify the extra costs for sonication as a feed pretreatment. The mixed and heated digester in Experiment 2 achieved a 22% higher mass yield than the mixed and heated digester operated by Fresco (2016) though fed an identical OLR, but with different algae genera. Unmixed digesters with no feed pretreatment or low-energy pretreatment, such as heating, might maximize the net energy from anaerobic digestion of whole-cell or residuals from the biorefinery process of algae.

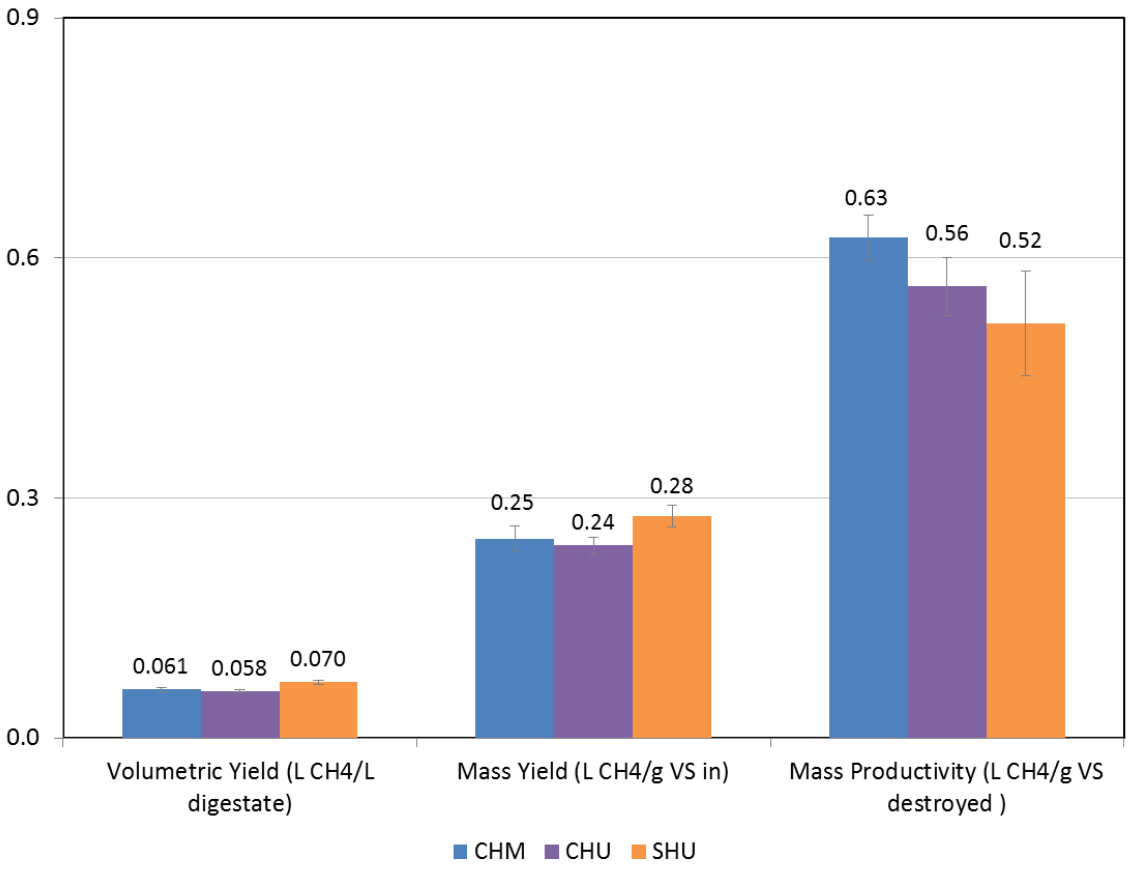

Figure 41- CHM, SHU, CHU were evaluated by the volumetric productivity (L CH4/L digester), mass yield (L CH4/g VS in) and VSD yield (L CH4/g VS destroyed) for the representative data collection period from December 4, 2015 to February 11, 2016. Error bars represent one standard deviation from the mean.

4.4.4 Energy balance for sonication

An energy balance was performed for the unmixed and sonicated feed digesters (SHU) and the unmixed digesters (CHU) to evaluate if the energy output from sonication justified the energy input. Input energy (Equation 16) quantifies the energy required for the sonication treatment and the output energy (Equation 17) quantifies the energy produced from methane production. Equations were adapted from Cho et al. (2013) and Hill (2014). Net energy was determined using the input energy of 7.50 kJ/ g VSin for sonication determined in the thesis of Hill (2014).

Equation 16- Input energy

$$E_{in}\left(\frac{kJ}{g\ VS_{initial}}\right) = \frac{[Power\ (Watts) * Time\ (seconds)]}{1000\ \left(\frac{W}{kW}\right) * volatile\ solids\ (g\ VS_{initial}) * effective\ volume\ (L)}$$

Equation 17- Output energy

$$E_{out}\left(\frac{kJ}{g\ VS_{initial}}\right) = \frac{35.8\frac{kJ}{L\ CH_4} * Y_{CH_4}\left(\frac{mL}{g\ VS_{initial}}\right) * R}{1000(\frac{mL}{L})}$$

The methods and assumptions from Hill (2014) were used, where critical assumptions for this energy balance include:

- 100% recovery for all methane produced by SHU (i.e. R=1)
- Heating value of methane is 35.8 kJ/L CH_4 (Metcalf & Eddy, 2003)
- Average cumulative mass yield of 0.28 L CH_4/g VSin is representative of steady state conditions
- Input energy for sonication remains at 7.50 kJ/g VSin (Hill, 2014)
- Output energy of SHU methane productions was 10.02 kJ/g Vsin

Although both digesters resulted in a positive net energy the unmixed digesters (CHU) achieved a greater positive net energy than the unmixed and sonicated feed digesters (SHU). The net energy of the CHU digester was 6 kJ/g VSin greater than the SHU digester which achieved a positive energy balance of 2.52 kJ/g VSin. Increasing the OLR of the feed may increase the mass yield and ultimately the net energy because this research focused on biogas yields from an OLR of 0.25 g VS/L-day (Rodrigues, 2015). From a previous study, digesters sonicated for 10 minutes, fed an OLR of 61.8 g VS/L-day, and operated at a 42-day HRT achieved a mass yield of 0.315 L CH_4/g VSin with a resulting net energy of 3.78 kJ/g VSin (Hill, 2014). Although sonication can achieve a positive energy balance, the increased energy input of sonication or the additional costs of

improving the collection of harvesting the algae may not be justified if eliminating mixing can achieve a greater net energy production.

4.5 Experiment 3: Scalability of pilot-scale digesters to lab-scale digesters

The main objective of Experiment 3 was to determine the scalability of the lab digester results to the field digesters. Previous research determined lab-scale digesters (VUU) operated at a constant temperature of 20˚C without mixing achieved comparable results for pH (3.8-4.3% higher for VUU), and soluble nutrients (5.8-9.4% higher N and 2.1-5.1% higher P) when fed identical feed to the pilot-scale digester. A major difference between the two geometries was that the mass yield results were 56% higher for lab-scale VUU digesters (Fresco, 2016). The average daily temperature in lab-scale digesters from Fresco (2016) did not account for the diel temperature variation in the pilot digesters, discussed in 4.3.1. Experiment 3 was conducted to determine if operating lab-scale digesters at variable field temperatures would result in more comparable results for mass yield. Digester performance and scalability in this experiment was evaluated using the following criteria: pH, alkalinity, concentration of soluble nutrients, and biogas yield.

VAU,P indicated the pilot-scale digesters and VAU indicated the lab-scale digesters that were operated from October 22, 2015 to March 17, 2016. These results were also compared to the full-length results of the pilot-scale experiment, referred to as “pilot,” operated from August 8, 2014 to March 17, 2016. The VAU digesters were assumed to be immediately at steady state because they were inoculated with mixed digestate collected from the pilot digesters on September 14, 2015. For this experiment, some data gaps exist when the VAU, and VAU,P digesters were not fed or drained because pH was at or below 6.7. Feeding and draining was skipped a total of 78 days during this experiment. The average solids concentration fed to VAU and VAU,P digesters was 0.09 g VS/L-day which was lower than the average feed solids of the overall pilot experiment of 0.15 g VS/L-day.

4.5.1 Temperature variation

This experiment was performed from October 22, 2015 to March 17, 2016. The digesters experienced a decline in temperatures for the first half of the experiment in the winter followed by a temperature increase in spring. Field temperature was recorded continuously from a probe within the pilot digesters, VAU temperature was recorded once per day as an average of the duplicate digesters. In the lab, the thermometer, placed in a beaker of water within the incubator, was read once per day. The experimental average temperatures for the VAU, P, VAU and the thermometer within the incubator were 14.4 ˚C (± 0.5 ˚C), 14.36 ˚C (± 0.02 ˚C) and 13.3˚C (± 2.5˚C), respectively (Figure 42). Although average digester temperatures of the VAU and VAU,P were almost identical, the thermometer more closely matched the daily trend in changing average pilot digester temperatures because it measured the temperature inside of the incubator (Figure 43). This indicates the incubator environment matched the temperature of the pilot digester liquid. Average difference between the VAU, P and the VAU digester liquid temperature was a maximum of 2˚C but the lab digester liquid temperature was consistently higher.

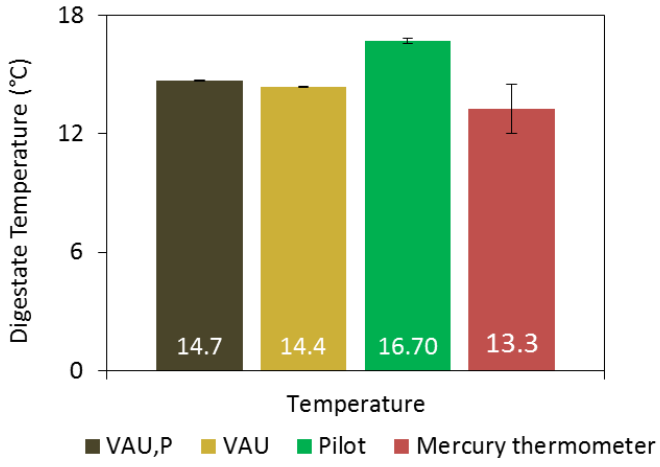

Figure 42- Average digestate temperatures of the VAU, VAU,P, and Pilot digesters with the average organic loading rate for Experiment 3. Error bars represent one standard deviation from the mean.

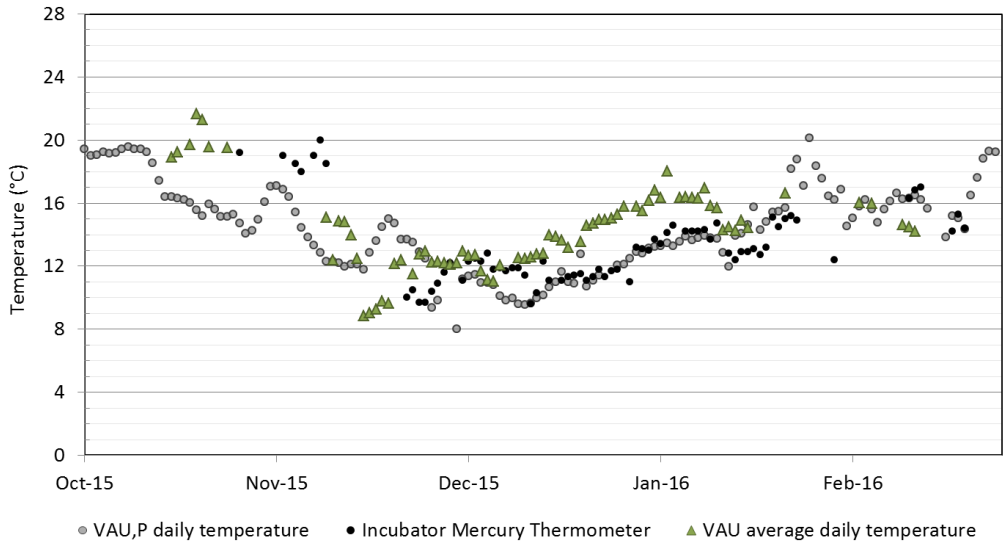

Figure 43- VAU and VAU,P temperatures were collected using HOBO data loggers and the average of the VAU,P were mimicked by the VAU digesters, placed in a temperature controlled incubator.

4.5.2 pH and alkalinity

The low OLR (< 0.1 g VS/L-day) and the low operating digester temperature possibly caused a low alkalinity and pH (McCarty, 1964). The average alkalinity and pH of the VAU and VAU,P digesters were 6.74 (± 0.004) and 1630 (± 5.2) mg $CaCO_3$/L, and 6.71 (±0.003 and 1423 (± 67.1) mg $CaCO_3$/L, respectively (Figure 44). The difference in pH and alkalinity was likely because pH and alkalinity was checked daily in the pilot scale digesters and the VAU digesters were only measured when the pilot pH was greater than 6.7 and there was digester influent and effluent. Lab digesters were not sampled because the volume required for sample was significant to overall digester volume if there was no feed to replace removed volume. Overall the pH and alkalinity of the lab-scale digesters was like the field digesters, but these results could be improved if lab-scale pH and alkalinity had been measured daily.

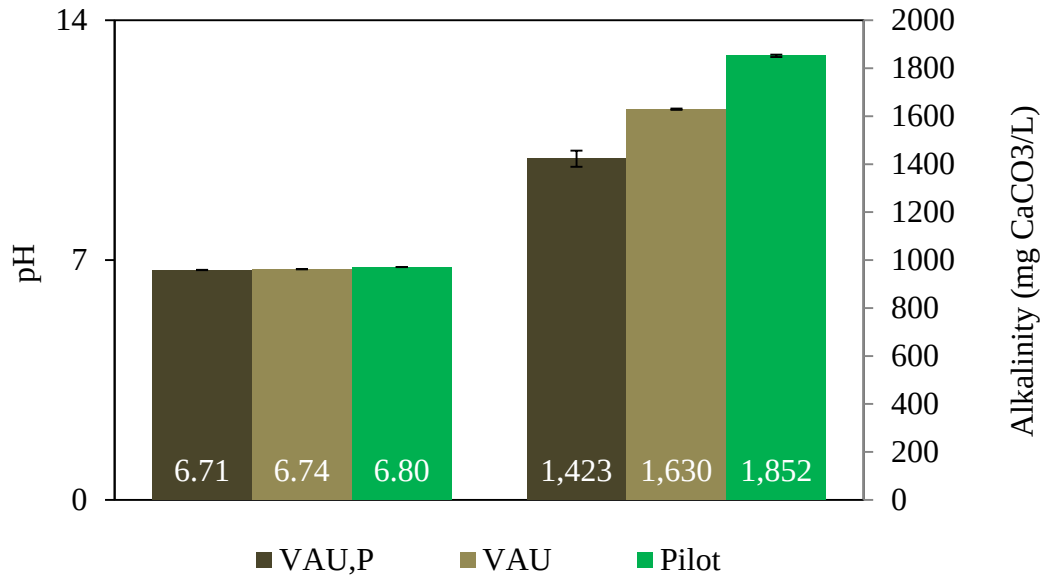

Figure 44- *Mean pH and alkalinity of the VAU,P, VAU digesters with the mean pilot pH and alkalinity for comparison.*

4.5.3 Scalability of effluent characteristics

A nitrogen, phosphorus, and COD mass balances were performed on VAU,P and VAU to compare to the full length pilot-scale experiment in Experiment 1. Constituent to biomass ratios were developed for the seed, feed, effluent, and final digestate to determine the average proportion of nitrogen, phosphorus and COD in the VS (

Table 7). VS data had more data points than the weekly nutrient and COD tests, especially because the number of sampling days was limited by the low pH. The mass of nitrogen, phosphorus, and COD added included the initial inoculum, and the mass added from the daily feed. The mass removed was determined from the effluent VS concentration and the final concentration at the end of the experiment, COD also was removed with gas production. The nitrogen, phosphorus, and COD average mass recoveries for the VAU digester were 85 %, 130 %, and 78 %, respectively. Nitrogen, phosphorus, and COD average mass recoveries for the VAU,P digester were 87 %, 144 %, and 80 %, respectively (Figure 45, Figure 46, and Figure 47).

Having less than 100% recovery of mass may be due loss of ammonia or methane in the nitrogen and COD mass balances or could be due to loss of volume during daily draining and feeding. Ratios used for calculations can be improved with a longer experiment (greater than 8 weeks) to help narrow down the best nutrient to VS ratios to be used. Although the mass balances had imperfect recoveries, the total mass added and removed the nitrogen, phosphorus, and COD percent differences were similar and indicate the two geometries can be used as proxies for each other.

Table 7 – Phosphorus and nitrogen to VS ratios used for determining the mass of phosphorus added and removed from the Pilot and VAU digesters for Experiment 3.

	Pilot		VAU		VAU,P	
Ratio	$\frac{TN}{VS}$	$\frac{TP}{VS}$	$\frac{TN}{VS}$	$\frac{TP}{VS}$	$\frac{TN}{VS}$	$\frac{TP}{VS}$
Seed	0.125	0.028	0.171	0.012	0.171	0.012
Feed	0.100	0.033	0.100	0.033	0.100	0.033
Effluent	0.338	0.086	0.365	0.155	0.365	0.130
Final	0.119	0.015	0.110	0.013	0.119	0.015

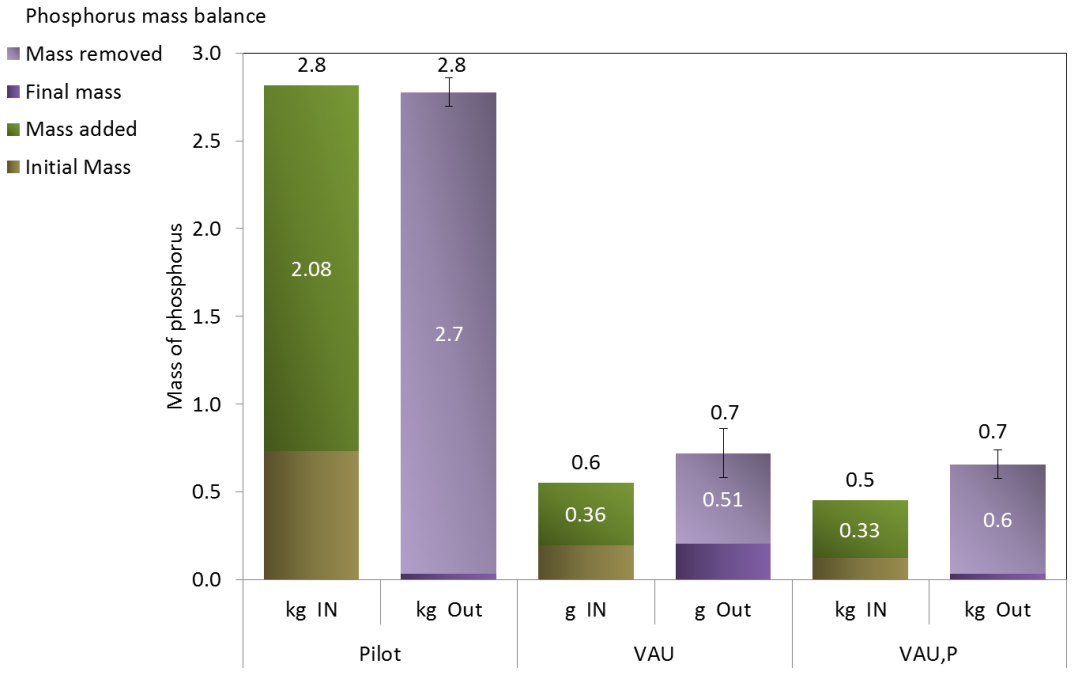

Figure 45- Phosphorus mass balance for Experiment 3 digesters, VAU and VAU,P, and the pilot digester for reference.

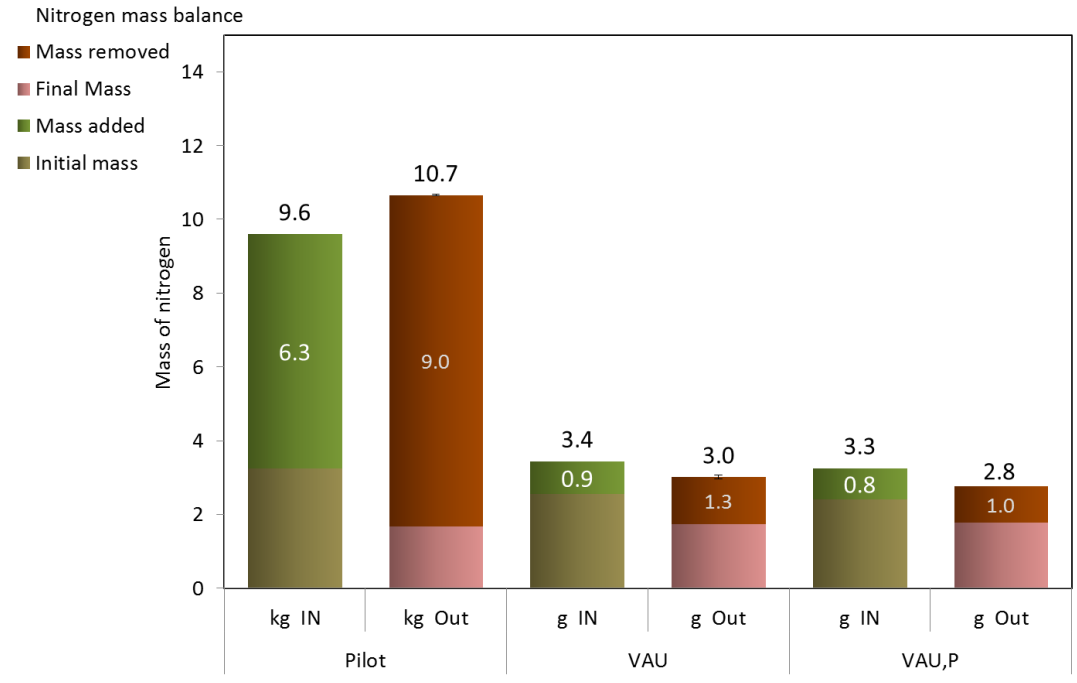

Figure 46- Nitrogen mass balance for Experiment 3 digesters, VAU and VAU,P, and the pilot digester for reference.

A COD mass balance was performed (Figure 47). A COD/VS ratio of 1.406 was used to as multiplier for all VS data collected for the VAU,P and VAU digesters, discussed in Section 4.2. Methane lost from methane produced as COD was 5.6 g COD/L and 2.9 g COD/L for the VAU,P and VAU digesters, respectively. The difference between the COD added and removed for the VAU,P and VAU digesters was 80% and 78% indicating there might have been unaccounted for COD due to less than 100% recovery of biogas and loss of volume when draining or feeding. As mentioned previously, the mass added and removed were not identical but the difference between the inputs and outputs was similar enough to assume the two different volumes are reasonable proxies for each other.

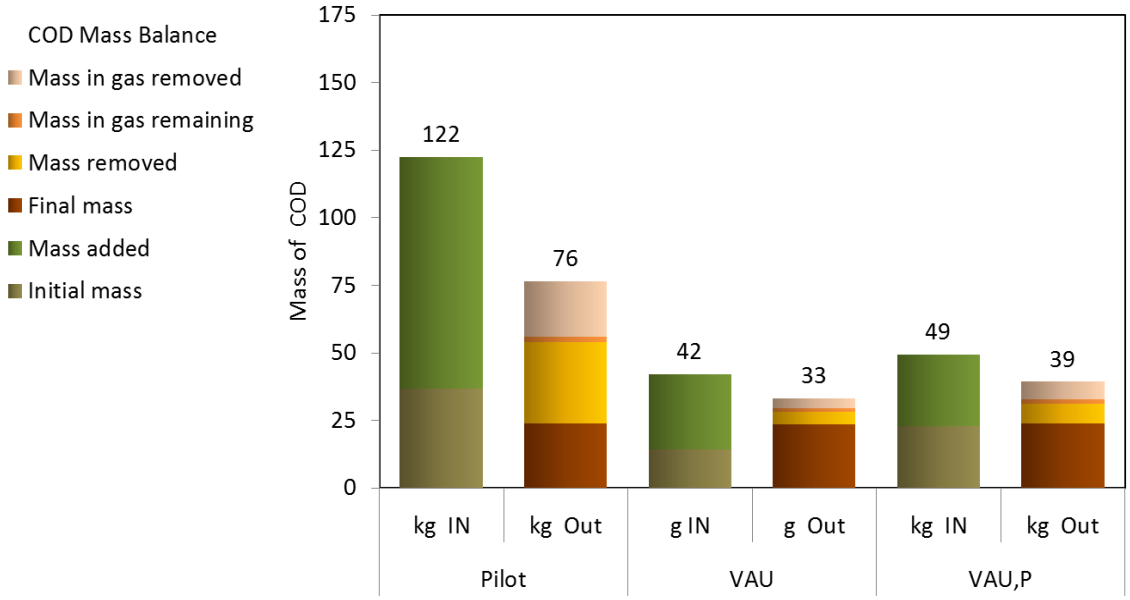

Figure 47- COD mass balance was 78% and 80% different for the VAU and VAU,P digesters including the total COD added and total COD removed. Differences may be due to biogas leaks or loss of volume during daily draining and feeding.

Nitrogen and phosphorus solubilization and the VSD were compared between the VAU and VAU, P digesters. The average TAN from the VAU and VAU,P effluent was 365±10 mg N/L and 304±15mg N/L, respectively (Figure 48). TRP from the VAU and VAU,P was 73±0.01 mg-P/L and 63±0.33 mg-P/L, respectively. Interestingly, the average TAN and TRP of the lab-scale

VAU was only 4% greater than the overall pilot-scale average indicating the lab-scale VAU digesters were more similar to the overall pilot averages than the VAU,P. Visual inspection of the effluent from the VAU digester had noticeably less in solids, indicating the settling was better in the VAU digesters. A depth to diameter ratio was determined for the VAU,P and VAU digesters of 0.97 and 1.2, respectively, possibly explaining the 20% increase and 16% increase in TAN and TRP, respectively. VAU achieved an average VSD of 56% compared to 54% for the overall pilot experiment and 51% for the VAU, P digester (Figure 49). Nitrogen was solubilized better in the VAU,P digesters (22% N remaining) than in the VAU digesters (23% N remaining). Higher solubilization of phosphorus (20% P remaining) was observed by the VAU digesters than by the VAU,P digesters (15% P remaining). Exposure to diel temperature shifts in the VAU,P digesters or improved settling in the VAU digester due to the higher diameter to depth ratio can also explain the 5% increase in VSD and 5% greater solubilization of phosphorus. Results from a previous scalability analysis exhibited a 5.8-9.4% increase in soluble nitrogen and 2.1-5.1% increase in soluble phosphorus from the pilot-scale digesters (Fresco, 2016).

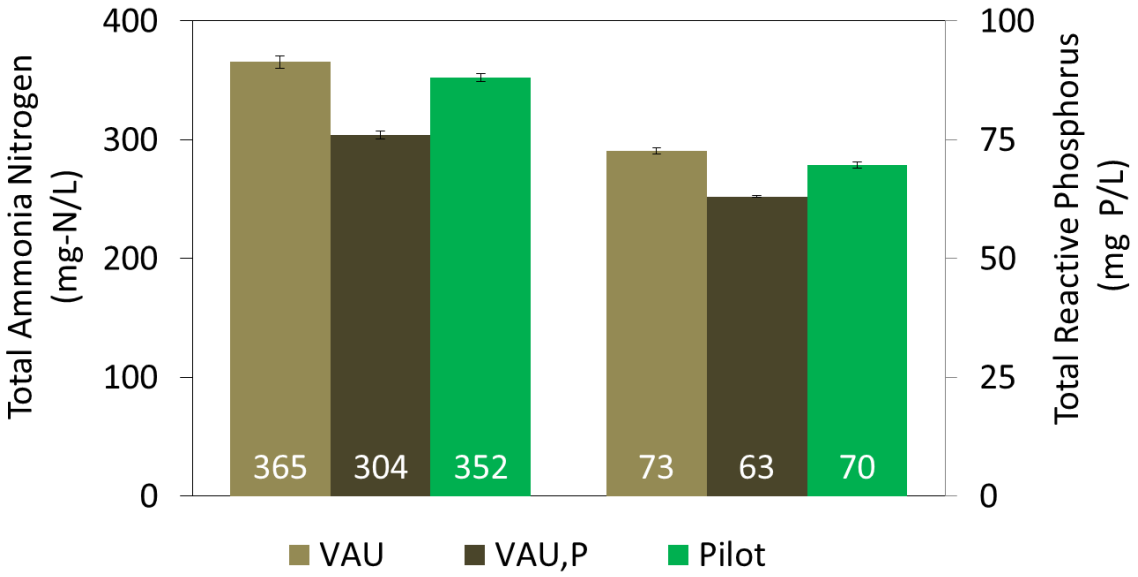

Figure 48- TAN and TRP concentrations were highest for VAU and lowest for VAU,P digesters. Pilot TRP and TAN concentrations are included for comparison. Error bars represent one standard deviation from the mean.

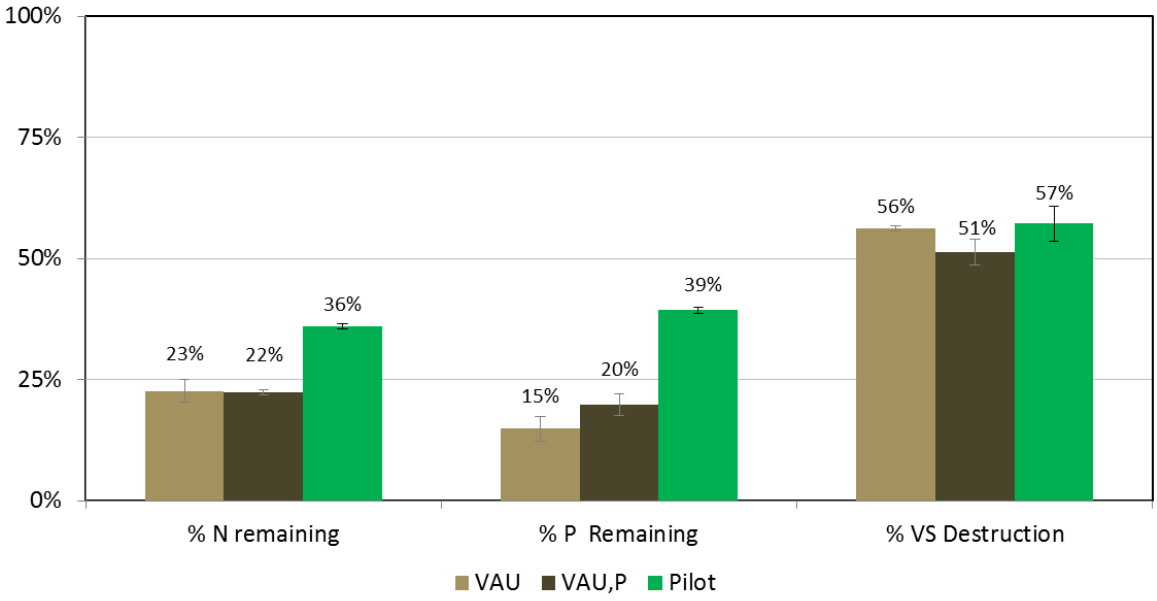

Figure 49- Solubilization of N or P remaining and the VS destruction of VAU, VAU,P and Pilot digesters in Experiment 3.

4.5.4 Scalability of biogas yield

Biogas was analyzed based on the mass yield (L CH_4/g VSin), volumetric productivity (L CH_4/ L digester), and VSD yield (L CH_4/g VSD). Results from a similar previous scalability study experienced an increase in biogas yield for the lab-scale digester (VUU) operated at a constant temperature of 43% in VSD yield (VUU, 0.03 L CH_4/g VSD; pilot, 0.022 L CH_4/g VSD), 51% in volumetric productivity (VUU, 0.37 L CH_4/L; pilot, 0.22 L CH_4/L), 56% in mass yield (VUU, 0.30 L CH_4/g VS; pilot, 0.19 L CH_4/g VS) (Fresco, 2016). In this research, the lab-scale digester (VAU) mass yield was 47% higher than the pilot digester (VAU,P) with average mass yield from VAU and VAU,P of 0.19 L CH_4/g VS and 0.12 L CH_4/g VS, respectively (Figure 50). Volumetric productivity of VAU and VAU,P digesters were 28% different with yields of 0.011 L CH_4/L and 0.008 L CH_4/L, respectively. VSD yield of VAU (0.014 L CH_4/g VSD) was 102% less than the VAU,P digesters (0.043 L CH_4/g VSD). The higher mass and volumetric productivity of the VAU digesters, and the decrease in VSD yield may be due to the OLR fed being harvested during colder temperatures that contained algal mass with low methane potential (Buswell, 1952; Zhang, 2011). The lab-scale digesters operated at the average daily temperature of the pilot-scale digester produced identical mass yield results but 50% and 154% less volumetric productivity and VSD yields, respectively. In comparison, digesters operated at the average daily (24-hour) temperature of the pilot-scale digester improved the mass yield by 9% but decreased the similarity in results for volumetric productivity and VSD yield when compared to the results in Fresco (2016). Overall, lab-scale digesters operated at the average daily temperature had substantial differences from the pilot digesters even though improvements in similarity were seen for mass yield. In the future, copying reactor geometry exactly and mimicking diel temperature changes might result in even more comparable results.

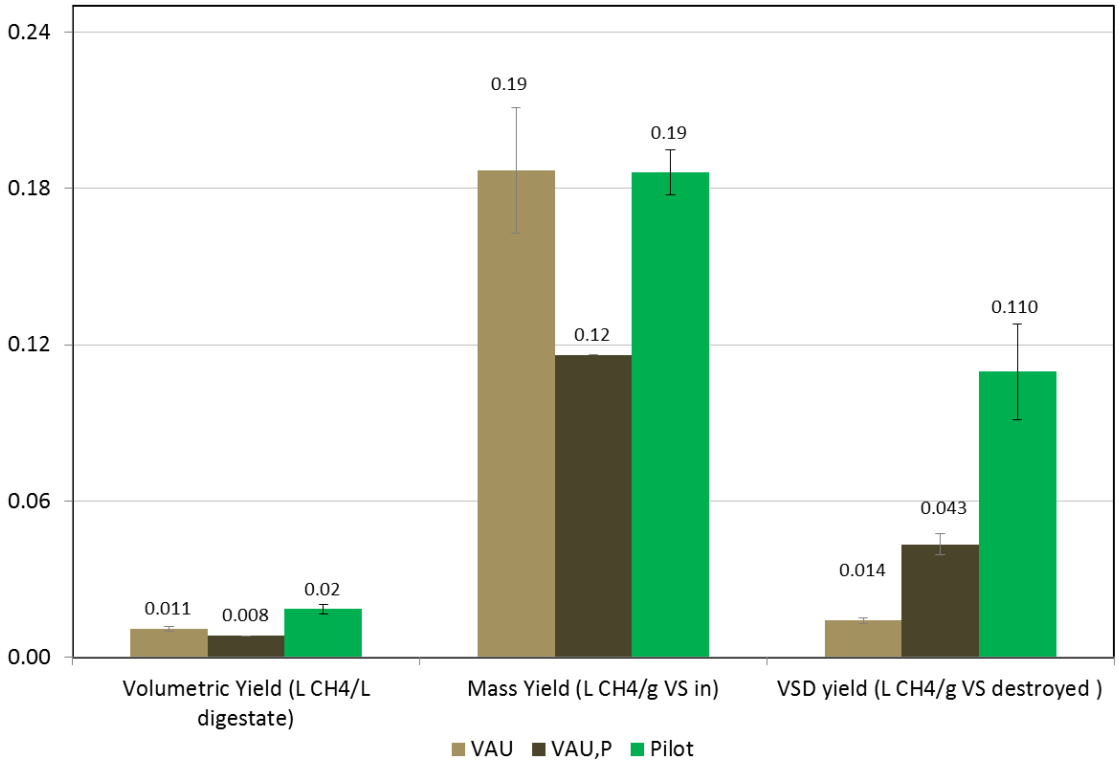

Figure 50 - Comparisons for biogas yield for the VAU, VAU,P and Pilot digesters.

5 Discussion

Overall the results from pilot-scale digesters (Pilot) and five sets of 1.2-L digesters operated at 40-day HRT were compared. The digesters used in Fresco (2016) were setup and operated the same as the digesters used in this study and included VUU [40-day HRT, 1.2-L, variable organic load, unheated at a constant 20˚C, unmixed], VAU [40-day HRT, 1.2-L, average organic load 1% VS, unmixed], and CHM; an identical replicate between the two studies. Independent variables analyzed included mixing, heating, variable temperature, pretreatment of feed with sonication, and variable organic load. Dependent variables included pH, alkalinity, biogas yields, VSD and nutrient

solubilization (Table 8). The lab-scale digester mass yields ranged from 0.20 to 0.30 L CH_4/g VSin and the volumetric productivity ranged from 0.001 to 0.065 L CH_4/L. The one year long pilot-scale experiment resulted in the highest average CH_4 content of 67% but the lowest mass and volumetric productivities of 0.19 L CH_4/ g VSin and 0.019 L CH_4/L digester, respectively. The variable feed, unmixed, and unheated (VUU) digester resulted in the highest mass yield of 0.30 L CH_4/ g VSin but the sonicated feed and unmixed digester (SHU) resulted in the highest volumetric productivity of 0.070 L CH_4/L digester. Sonicated and unmixed digesters achieved percent particulate nitrogen remaining of 28% and the highest %VSD of 75%, but the variable feed unmixed and unheated digester from Fresco (2016) achieved percent particulate remaining phosphorus of 22%. Mixed digesters fed a constant OLR of 0.25 g VS/L-day from this study achieved a mass yield 44% greater (0.25 L CH_4/ g VSin) than the mixed digester fed an identical organic load from Fresco (2016) (0.16 L CH_4/ g VSin).

Table 8 - Summary of experimental setup and digester results for biogas yields and nutrient solubilization. Percent remaining nitrogen and phosphorus describes the average amount of particulate nitrogen or phosphorus not removed in the effluent from the digester. Soluble nutrients added in the feed could lead to false increase in the digester's ability to solubilize nutrients.

Experiment	Digester Name	Label	Average Organic Loading Rate (g VS/L-day)	Average Operating Temperature (°C)	Mixing	Volume	HRT (d)	pH	Alkalinity (mg $CaCO_3$/L)	CH4 (% Vol)	Mass Yield (L CH_4/ g VS in)	Volumetric productivity (L CH_4/L)	% P remaining	% N remaining	% VSD
1	Varied feed, Unheated, Unmixed	Pilot	0.15 (0.01-0.65)	16º C [1]	Unmixed	1136 L[2]	40	6.80	1,852	**67%**	0.19	0.019	39%	36%	57%
2	Constant feed, Heated, Unmixed	CHU	0.25	30º C	Unmixed	1.2-L	40	7.11	2,494	63%	0.24	0.058	31%	40%	61%
	Sonicated feed, Heated, Unmixed	SHU	0.25[3]	30º C	Unmixed	1.2-L	40	7.21	3,062	66%	0.28	**0.070**	**28%**	36%	**75%**
	Constant feed, Heated, Mixed	CHM	0.25	30º C	Mixed	1.2-L	40	7.18	2,776	68%	0.25	0.061	41%	45%	53%
(Fresco, 2016)	Varied feed,Unheated, Unmixed	VUU	0.15 (0.01-0.65)	20º C	Unmixed	1.2-L	40	7.0	1,748	65%	**0.30**	0.034	41%	**22%**	72%
	Constant feed, Heated, Mixed	CHM[4]	0.25	30˚ C	Unmixed	1.2-L	40	7.25	2,438	62%	0.16	0.038	55%	46%	45%
	Varied feed, Heated, Mixed	VHM	0.15 (0.01-0.65)	30º C	Mixed	1.2-L	40	7.16	1,562	63%	0.20	0.023	55%	40%	42%
3	Lab: Varied feed, Average Daily Field Temperature, Unmixed	VAU	0.15 (0.01-0.65)	14º C	Unmixed	1.2-L	40	6.74	1,638	41%	0.19	0.011	15%	23%	56%
	Field: Varied feed, Unheated, Unmixed	VAU,P	0.15 (0.01-0.65)	14º C	Unmixed	1136 L	40	6.71	1,421	62%	0.12	0.008	20%	22%	51%

[1] Variable temperatures ranged from 8.5˚C to 26˚C
[2] Volume of digestate was 300 gallons
[3] Sonicated for 10 minutes prior to feeding
[4] Digester was fed an OLR of 0.25 g VS/L-day, mixed, and heated to 30˚

5.1 Optimization of nutrient solubilization

Solubilization of nitrogen and phosphorus were evaluated on the percent of particulate nutrients remaining in the digester to account for the soluble forms of nutrients added with the feed. Eliminating mixing (CHU, CHM) improved the nitrogen and phosphorus solubilization by 4% and 10%, respectively (Figure 51). Sonicating the feed further improved the nitrogen and phosphorus solubilization by 7% and 3%, respectively. VSD was improved by eliminating mixing from digesters with the unmixed digesters (65% ± 9%) resulting in an average increase of 18% more VSD than the mixed digesters (47% ± 8%). Overall, unmixed, heated and fed pretreated feed had more solubilization of nutrients and a greater % VSD even when fed a low and variable OLR. Nitrogen solubilization in the lab-scale unmixed digester was improved by 23% when warmed to a constant 20˚C and fed low and variable (VUU) compared to the pilot scale unmixed and unheated digesters also fed a low and variable OLR; however, solubilization of particulate phosphorus was improved by 11% in the pilot-scale digester. Nutrient solubilization and VSD from an unmixed digester fed a variable organic load could be optimized if the average operating temperature was increased. Additional solubilization and VSD may be achieved with pretreatment but the net energy would need to be justified by the biogas yields.

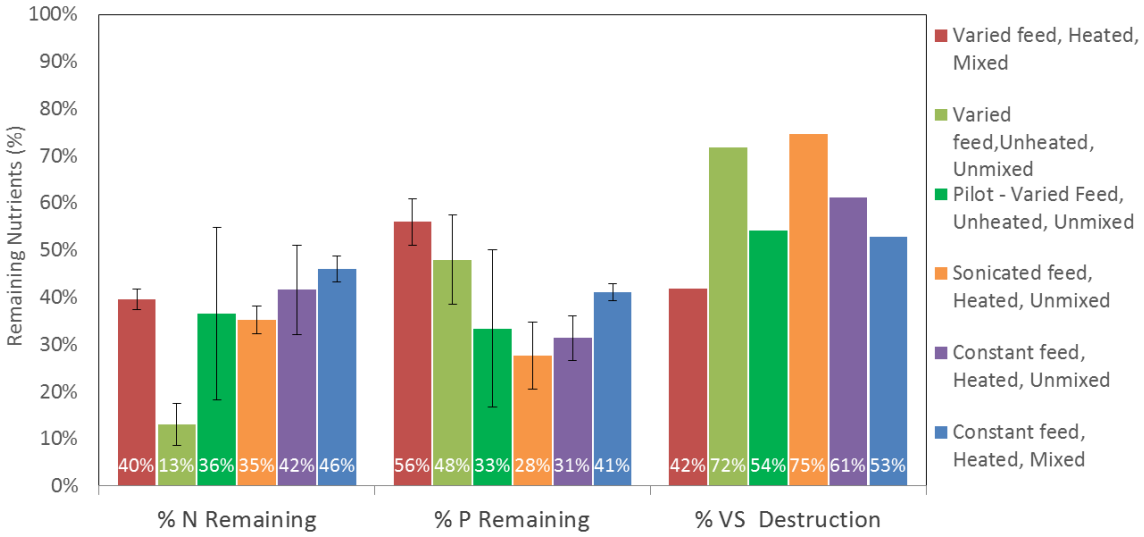

Figure 51 - Nutrient solubilization and %VSD of the 1.2-L lab digesters (VHM, VUU, SHU, CHU, and CHM) and pilot scale digesters. Error bars represent one standard deviation from the mean of each digester over the duration of the experiment. Percent remaining nutrients accounts for the particulate matter remaining in the digester (Equation 8 and Equation 9).

5.2 Optimization of biogas yield

The highest mass yield of 0.30 L CH_4/ g VSin was achieved with the VUU digesters [lab-scale 1.2-L digesters, 0.12 g VS/L-day OLR variable, heated to 20˚C and unmixed] followed closely by the SHU digesters [lab-scale 1.2-L digesters, 0.25 g VS/L-day day OLR variable, heated to 30˚C and unmixed] with a mass yield of 0.28 L CH_4/ g VSin. Both digester sets were unmixed, but the OLR for the VUU digesters was 0.12 g VS/L-day and variable whereas the organic load for the SHU digesters was 0.25 g VS/L-day and constant. The higher mass yield in the VUU digesters was attributable to the low and variable OLR. The temperature of the SHU digesters was 10˚C higher and the feed was sonicated which in most cases would result higher yields; however, in this case the lower organic load offset the benefits of heating and sonication in unmixed digesters. This was further confirmed in the literature where the benefits of unmixed digesters on gas yields decreased with higher organic loads for algae digesters (De la Rubia et al., 2006). Therefore, optimization of the mass yield is highly dependent on the feed solids and subsequent solids

accumulation. More research should be done to determine the organic load where the mass yield is optimized by not mixing. In general, higher temperatures, feed pretreatment by sonication, and lower organic yields increased the mass yield.

Optimization of volumetric productivity is highly dependent on the OLR, accumulation and resulting SRT. Digesters fed variable feed of an average 0.12 g VS/L-day achieved an average volumetric productivity of 0.034 L CH_4/L (± 0.008) and the digesters fed a constant 0.25 g VS/L-day resulted in an average volumetric productivity of 0.063 L CH_4/L (± 0.006). The sonicated constant feed and unmixed digester (SHU) achieved the highest volumetric productivity of 0.070 L CH_4/L followed closely by the unsonicated constant feed and mixed digester (CHM) with a volumetric productivity of 0.061 L CH_4/L (Figure 52 and Figure 53). Both digesters were fed the same OLR of 0.25 g VS/L-day but sonicating the digester feed and eliminating mixing resulted in a higher volumetric productivity. Similar to mass yield results the accumulation (23%) in the SHU resulted in a longer SRT. Sonication was expected to result in higher biogas yields but the small improvement in volumetric productivity may not justify the increased energy costs. Additionally, since sonication also increases the feed temperature sonication may be masking a less energy intensive pretreatment process of exposure to elevated temperatures prior to feeding. Overall, algae digestion, whole cell or residuals from the biorefinery process, does make algae biomass a more viable biofuel especially if biogas yields are maximized with low energy operation such as heating from combustion processes waste heat or fed a greater average OLR from efficient harvesting systems.

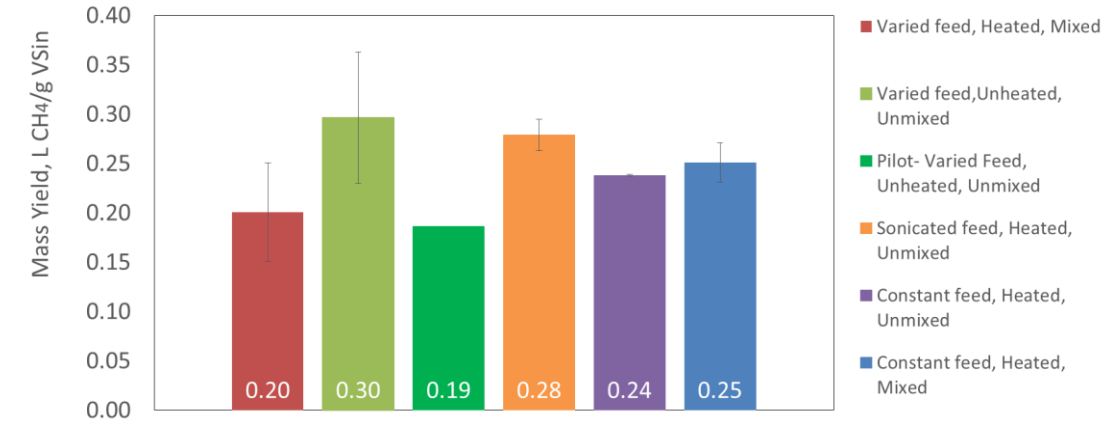

Figure 52- Mass yield (L CH4/g VSin) for five lab-scale digesters (VHM, VUU, SHU, CHU, and CHM) and one pilot-scale digester

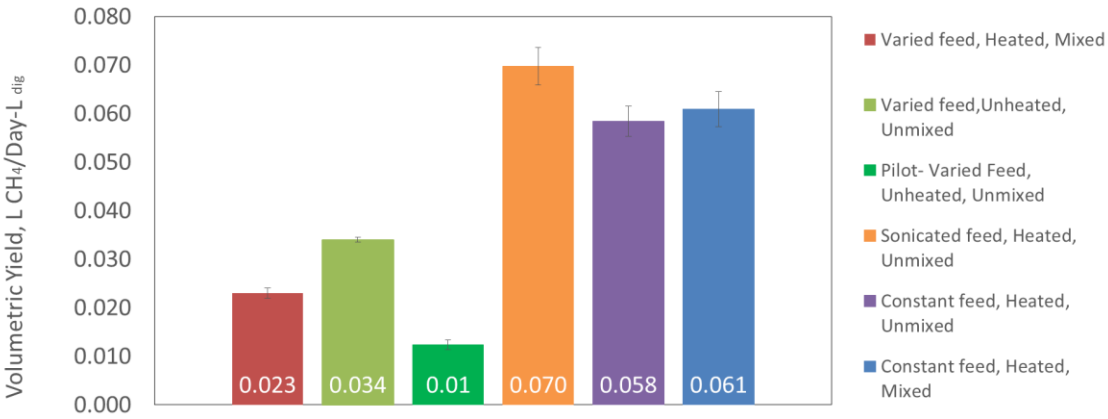

Figure 53- Volumetric productivity (L CH4/ L) for five lab-scale digesters (VHM, VUU, SHU, CHU, and CHM) and one pilot-scale digester

6 Conclusions

This research involved a microalgae biofuel production system in which algal biomass grown on clarified municipal wastewater was harvested and digested in pilot-scale anaerobic digesters, and the recycled back to algae ponds for 15 months. In parallel work, lab-scale experiments were conducted on digester performance as affected by mixing, heating, load variability, and feed sonication. One set of lab digesters was

operated under similar conditions as the pilot digesters, allowing the assessment of the scalability of the lab results.

6.1 Effects of seasonal temperature and variable organic load in a pilot-scale digester

The pilot digesters, with a 40-day HRT, were operated unheated, unmixed, and with a variable feeding rate to mimic a full-scale covered lagoon-type digester. Unheated digesters with low organic loading and a long HRT are expected to have yields like heated digesters with high organic loading and a short HRT (Eisenberg et al., 1980). Pilot digester temperatures ranged from 9˚C to 26˚C, averaging 16.7˚C. The pilot digester organic load ranged from 0.01 - 0.81 g VS/L-day (average 0.17 g VS/L-day). The variable organic load of the pilot digesters was largely due to seasonal variation in algae biomass production and harvestability. Over the five seasons of operation, beginning with winter in 2014, the average seasonal digester temperatures were 14˚C, 17˚C, 21˚C, 17˚C, and 14˚C with resulting average seasonal mass yields of 0.19, 0.11, 0.46, 0.14, and 0.11 L CH_4/g VSin, respectively. The variable-feed pilot digesters produced an average mass yield of 0.19 L CH_4/g VSin. Digester pH and alkalinity decreased during colder winter temperatures when influent was low in alkalinity. This suggests that an ideal covered-lagoon algae digester would have a longer winter and shorter summer residence time. During colder temperatures, the low alkalinity can be mitigated with a longer residence time and possibly with alkaline treatment. During warmer temperatures, when nutrient and CO_2 demands are higher in algae ponds, a shorter residence time can provide more nutrients to grow additional biomass.

6.2 Effects of sonication as a feed treatment with a low organic load

Sonication of algae feed increases biogas yield and nutrient solubilization by disrupting algae cell walls (Rodrigues, 2015). High nutrient solubilization is desirable in systems where new algae are grown on water fertilized with digestate. In the present lab research, nitrogen and phosphorus solubilization was 10% and 11% higher, respectively, in digesters fed sonicated feed compared to the unsonicated control digesters. The percent of total feed nutrients remaining in the digestate as particulate nutrients was 36% N and 28% P for the sonicated-feed digesters versus 40% N and 31% P for the control digesters.

Sonication improved the methane yields and productivity (0.28 L CH_4/g VSin, 0.065 L $CH_4/L_{digester}$) by 14% and 18%, respectively, over unsonicated-feed control digesters (0.24 L CH_4/g VSin, 0.050 L $CH_4/L_{digester}$). Despite the improvement in biogas yield, the net energy produced from the sonicated digesters was projected to be 2.52 kJ/ g VSin which was 6 kJ/g VSin less than the net energy production of the unsonicated digesters.

6.3 Effects of digester mixing

For lab digester with the same organic loading (0.25 g VS/L-day) mixing was found to improve biogas yields and productivity slightly by 4% and volatile solids destruction by 8% but decrease the solubilization of nutrients. Solids accumulation in unmixed digesters was 17% higher than in mixed digesters, and the resulting longer SRT resulted in higher particulate nutrient solubilization (13% less total influent N remaining and 27% less total influent P remaining). The results of Fresco (2016) that showed that mixed lab digesters with a constant organic load of 0.25 g VS/L-day had a decrease in mass yield compared to unmixed digesters with an average variable organic load of 0.12 g VS/L-day. These

results indicate the importance of organic loading and mixing on mass yield. At higher VS concentrations it was likely that mixing helped liberate gas trapped in the liquid but at lower VS concentrations in unmixed digesters the longer SRT led to greater improvements in mass yield (De la Rubia, 2006)

The present results on mixed digesters agree with De la Rubia (2006) that, in general, greater stabilization and methane yields can occur with lower OLRs and longer SRTs. Furthermore, when unmixed and fed a low OLR, the yield can be even higher due to in-digester settling leading to the SRT being longer than the HRT. The VUU digesters of Fresco (2016), operated at 20˚C and fed a low average variable organic load of 0.12g VS/L-day, achieved the highest mass yield (0.30 L CH_4/g VSin) of the Fresco and present experiments. The periodic removal of settled solids is, of course, a maintenance issue to be considered. In summary, mixing digesters when OLR rates are high, during warmer seasonal temperatures, may increase the biogas yield; however, it may also decrease nutrient solubilization. The preferred operational scheme is likely dependent on site nutrient requirements and available organic load of the feed.

6.4 Scalability of lab-scale digester results to pilot-scale digesters

Lab digester results were found to represent pilot-scale results in some respects. The lab and pilot digesters were both unmixed, fed the same variable organic loading rate, and experienced the same average daily temperatures. Average pH of the pilot digesters was 6.71, which was close to the 6.74 average pH of the lab digesters. The average alkalinity of the lab digesters was 14% greater than the pilot digesters with their average alkalinity of 1,422 mg $CaCO_3$/L (±67.1) for VAU and 1,330 mg $CaCO_3$/L (± 5.2) for VAU,P but

this may be explained due to sampling bias where the pilot digesters were sampled much more frequently than the lab digesters. The lab and pilot digesters solubilized nitrogen essentially equally, but the lab digesters solubilized more phosphorus than the pilot digesters by 29%. These results contradict the results in Fresco (2016) in which lab digesters, operated at 20˚C and fed a variable OLR, solubilized 11% more nitrogen than the pilot digesters, but the pilot digesters solubilized 7% more phosphorus. Overall the pilot and lab digester pH, alkalinity, and soluble nutrients all responded similarly to changes in temperature and organic load.

Results from a previous scalability study indicated lab digesters operated at a constant temperature instead of the variable seasonal temperature experience by the unheated pilot digesters lead to a difference in mass yield of 56% (Fresco, 2016). In the present study, the average lab digester mass yield of 0.19 L CH_4/g VSin was greater than the average mass yield of the shortened Experiment 3 average of the pilot digester of 0.12 L CH_4/g VSin by 47%. Interestingly, when average yield of the lab-scale digester was compared to the15-month average of the pilot digester in Experiment 1, the average mass yields were identical. These results indicate mimicking the variability in temperature *did* decrease the difference in mass yield between the pilot and lab digesters.

6.5 Limitations of this study and suggestions for future research

This research was a continuation and culmination of two prior thesis projects testing the effectiveness of feed pretreatment, mixing, volume, variable organic load, variable seasonal temperatures, and heating (Hill, 2014; Fresco, 2016). Nonetheless, the present

research had its limitations that might be overcome in future work. The recommendations based on the limitations and results of this research include:

- Assure the availability of the algae feed supply to achieve the optimal digester loading year-round. Feed harvest efficiency could be improved at full-scale by using dissolved air floatation (DAF) and or chemical flocculants but the increase in cost may not be justified.

- Increase the number of replicate digesters (n = 2 in the present work) to allow greater statistical power.

- Further study the relationship between organic loading and mixing on mass yield nutrient solubilization to determine precisely the organic load where mixed digesters become preferable to unmixed digesters.

- Compare mixed to unmixed pilot digesters and better simulate the diel and seasonal temperature variations of full-scale digesters, which have less extreme temperature swings than the pilot digester of the present study.

- There was no direct comparison to test the variables of the variable feed, unmixed, unheated digester, which experienced the highest mass yield of 0.30 L CH_4/g VSin. A future study might include an unmixed and unheated digester fed a constant OLR.

- Algae genera's effect on the digestion process were not studied or controlled and the effect is still unknown under unmixed, unheated in a pilot-scale setup.

- Furthermore, if lab-scale digesters were able to mimic the diel variability in addition to the average daily temperature variation the difference in mass yield might be further reduced.

- Ultimately, research should be conducted on long-term piloting of a wastewater-based algae integrated biorefinery, such as started by Bowen (2018), Zardouzian (in preparation), and the author, in which algae digestate nutrients were recycled to algae raceways to grow more digester feed.

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