ABSTRACT

Title of Dissertation: UTILIZING ALGAL TURF SCRUBBERS FOR BIOREMEDIATION AND BIOENERGY PRODUCTION

Danielle Marie Delp, Doctor of Philosophy, 2023

Dissertation directed by: Dr. Stephanie Lansing, ENST

This dissertation researched the conversion of algal biomass that was generated as a byproduct of bioremediation by algal turf scrubbers (ATS) into bioenergy via anaerobic digestion. Anaerobic digestion is a bacterial process that converts organic material into bioenergy in the form of biogas that contains methane (CH_4), the primary component of natural gas. Bioenergy yield was quantified as the volume of CH_4 generated from digestion of the algae in relation to seasonal changes in algal biomass yield, different digester operational parameters, co-digestion of the biomass with more conventional digestion feedstock, and flocculation pre-treatment for dewatering of algae prior to digestion.

The first study used a pilot-scale mesophilic digester at the Port of Baltimore (Baltimore, MD, USA) to continuously digest algae from a 122 m^2 ATS on the Patapsco River over two years. Biomass generation was significantly correlated to maximum daily air temperature, water temperature, and flow rate in Year 1 but only water flow rate in Year 2. Algae of the taxa Ochrophyta dominated the algal turf, especially the filamentous diatom *Melosira* sp., in both

years. In Year 1 of the study, two anaerobic digestion systems with variable hydraulic retention times (HRT), designated D1 (average HRT 45.0 ± 5.8 days) and D2-D3 (average HRT 61.0 ± 8.1 days) were used to digest the algae. The D1 generated 1090 L CH_4 from 2416 L of algae over a 39-day HRT (59.1 ± 8.9 L algae/kg VS), and D2-D3 generated 1170 L CH_4 from 2337 L of algae over a 53-day HRT (67.9 ± 11.0 L algae/kg VS). The difference in CH_4 yield with two different HRTs was not significant. In Year 2, only the D2-D3 was operated and was modified to test the use of active recirculation and heating to improve digestion efficiency and CH_4 yield. The D2-D3 system generated 4000 L of CH_4 (163 ± 42 L algae/kg VS) from 3310 L of algae in Year 2.

The second study consisted of laboratory-scale biomethane potential tests to test changes in CH_4 yield when algae harvested from an Anacostia River (Bladensburg, MD, USA) ATS was co-digested with three wastes (dairy manure, food waste, and poultry litter) at algae:waste loading ratios of at 1:1, 1:2, 1:5, and 1:10 by organic material, or volatile solids (VS), content. The algal biomass was the least efficient substrate at generating CH_4 when normalized by both mass VS digested (109 ± 4 mL CH_4/g VS) and total mass of substrate digested (0.687 ± 0.025 mL CH_4/g substrate). Co-digestion with all three of the wastes at all ratios tested significantly increased CH_4 generation efficiency per mass VS compared to only digesting algae. However, the high moisture content of the algae (95.2%) relative to the other co-digestion wastes (29.0-84.6%) significantly decreased CH_4 production on a mass basis for the dairy manure, food waste, and poultry litter when algae was added at any loading ratio. A lettuce growth experiment using the effluent of the digestion vessels showed no signs of acute toxicity when any of the diluted (8-fold) digester effluents were applied as fertilizer to the developing plants.

The third and final study consisted of flocculation experiments that tested 500-mL of algae using four experimental treatments ($FeCl_3$, electrocoagulation, chitosan, and *Bacillus* sp.

RP1137) to dewater algae harvested from the Anacostia River ATS and compared to gravity settling as a control. The experimental flocculants successfully increased the total solids (TS) of the ATS algae by 14-291% depending on the treatment, with electrocoagulation being the least effective and bacterial flocculation being the most effective flocculant. All treatments reduced total suspended solids (TSS) in the drained supernatant by >98%. The raw ATS algae and dewatered solids from the settling experiment were then digested for 35-days, with the algae yielding 49.6 ± 3.6 mL of CH_4/g VS. The dewatered solids had reduced digestion efficiency by 29.6-71.0% compared to untreated algae. Dewatering pre-treatment increased CH_4 yield from the algae when normalized by total g substrate fed to the reactor (1.65 ± 0.12 mL CH_4/g substrate) for all treatments except bacteria 1x, however the effect was only significant for solids dewatered with electrocoagulation.

The results from the three studies show that temperature drives algal growth patterns in temperate climates, which results in seasonally variable biomass yield from ATS, with a corresponding variability in CH_4 production due to inconsistent availability of the algal feedstock. Algae can be co-digested with agricultural and food wastes that are generated year-round to reduce variability in feedstock availability. Thickening and dewatering the algae improves CH_4 yield on a mass basis, however the digestion efficiency was reduced. In conclusion, the findings suggest that anaerobic digestion is a viable means of managing the algae harvested from ATS systems with and without co-digestion of the algal biomass.

Utilizing algal turf scrubbers for bioremediation and bioenergy production

by

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Dedication

To Mom, Dad, and Joe, and all the rest of my family and friends. Thank you so much for supporting me all these years.

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Chapter 1: Introduction

1.1: Algal turf scrubber (ATS)

The algal turf scrubber (ATS) is a bioremediation technology that uses a periphyton “turf” to remediate nutrients from flowing water. It was developed and patented in the 1980s by Dr. Walter Adey of the Smithsonian Institution and designed to promote periphyton growth like the highly productive algal communities that naturally form on coral reefs (Adey et al. 1982; Lewis 1977). A flow-way is lined with a textured material (e.g., netting, mesh, etc.) to mimic the rough surface of a coral. Water from a eutrophic body of water is then pumped across the lining in intermittent pulses, designed to mimic wave action in a shallow reef. The result is an ideal environment to stimulate periphyton development, with low to moderate wave energy, ample sunlight penetration, plentiful nutrients, and good benthic structure for holdfast attachment (Figure 1.1).

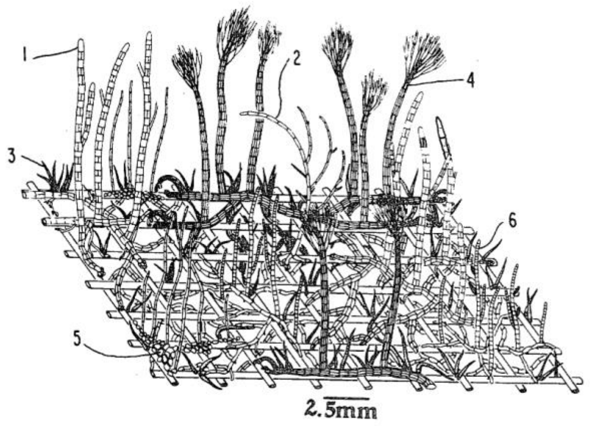


Figure 1.1: Patent illustration of an algal turf scrubber (ATS) lining and periphyton community (Adey, 1982).

The ATS is notable for being one of the only water treatment technologies capable of remediating non-point source nutrient pollution from the environment. The favorable growth conditions for the algae in the turf result in rapid algal biomass production, like that seen in the nuisance algal "blooms" that have been documented in eutrophic systems. An ATS turf consists of periphyton that is contained in a controlled space on land, however, and does not cause the same problems (e.g., hypoxia events, fish kills) associated with harmful algal blooms due to the algae being harvested and the biomass removed from the ecosystem. As dissolved nutrients are metabolized and sequestered in an ATS turf, those nutrients cease to be available to fuel algal blooms in the river.

In the forty years since the original patent of the ATS was approved and published, the field of algal bioremediation has developed and the technology available has diversified. The terms "algal flow-way" (AFW) and "algal flow-way technology" (AFT) have also been used to describe ATS and similar systems that utilize attached algal cultures to remediate nutrient nutrients from flowing water, including by United States Environmental Protection Agency (USEPA) in its expert panel recommendations for ATS (USEPA 2015b). Both terms are also used interchangeably in the literature, with additional confusion due to "ATS" having been trademarked since the original patent expired (Hydromentia 2022). This research studied ATS systems built according to the original design by Adey (1982) and operated according to subsequent best practice recommendations (Adey et al. 2013; USEPA 2015b), so the term "ATS" will henceforth be used exclusively even if a different term is used in the cited literature.

1.2: Managing algal byproduct from algal flow ways (ATS)

The algae on an ATS must be harvested periodically (weekly or biweekly) to remove accumulated biomass on the biofilm. Consistent harvests help maximize the rate of algal productivity and nutrient uptake by preventing the algae from reaching maturity and maintaining it in a state of constant growth. Gan et al. (2022) demonstrated that in a ATS biofilm reactor, the algae was able to assimilate up to 99% of N and 100% of P loaded with a 7-day period between harvests, exemplifying the high rate of algal growth and nutrient uptake with regular weekly harvests. Harvesting strategies may include any combination of manual scraping and vacuum suction at small- and pilot-scale, or heavy machinery removal at commercial scale (Adey et al. 2013; Hydromentia 2022). These methods all leave the algal holdfasts and fundamental structure of the periphyton intact post-harvest to allow the turf to regenerate rapidly.

The algae harvested from ATS is a mixture of water, biomass from a variety of in-situ micro- and macroalgal species, and debris (i.e., detritus and sediment) that become trapped in the periphyton. While ATS are designed primarily for removal and assimilation of dissolved carbon (C) and nutrients containing nitrogen (N) and phosphorus (P), heavy metals and other trace elements can also be assimilated by the algal turf. An elemental analysis by Kebede-Westhead et al. (2004) of ATS algae from an secondary treatment system receiving dairy manure digester effluent found that the primary components of the dry biomass were potassium (K, 14,700 mg/kg), calcium (Ca, 9700 mg/kg), magnesium (Mg, 2300 mg/kg), aluminum (Al, 1100 mg/ kg), iron (Fe, 580 mg/kg), zinc (Zn, 290 mg/kg), manganese (Mn, 240 mg/kg), and silicon (Si, 210 mg/kg), with copper (Cu), lead (Pb), and molybdenum (Mo) also detected at <10 mg/kg. Rothman et al. (2013) documented especially high concentration of Fe in algae from an ATS on the York River estuary (NY, USA) (0.39 ± 0.05 and 0.62 ± 0.03 mM, respectively, from the top and bottom of the ATS) and high uptake of Pb (87% removal) from the flowing water. Velu et al. (2020) demonstrated ATS-cultured *Tolypothrix* sp. removed >90% of dissolved Al, Sr, and Zn, 70-80% of dissolved Fe, and 65-75% of dissolved As when grown on synthetic ash dam water.

As the algal byproduct contains sequestered pollution, a waste management plan must be included for any ATS installation to prevent remediated contaminants from returning to the environment (USEPA 2015b). The literature indicates that an ATS can generate > 0.02 kg/m^2/day of dry algal biomass solids at peak productivity, which typically comprise 5-10% of the total wet mass of ATS algae (Bohutskyi et al. 2016; Mulbry et al. 2010; Witarsa et al. 2020). Assuming 5% dry solids, a single m^2 of ATS on a river could be expected to generate more than 0.4 kg of wet biomass per day, or 2.8 kg per 7-day growth cycle. A commercial ATS company in Florida (USA) designs ATS capable of processing up to 37,900 L/day contaminated water, which require large surface areas to accommodate (Hydromentia 2022). A review by Adey et al. (2011) estimated that widespread use of ATS for tertiary sewage or eutrophic surface water treatment in the midlatitudes of the USA could eventually produce up to 18 metric tons per hectare per year of wet biomass that would need to be processed. The findings by Kebede-Westhead et al. (2004) and Velu et al. (2020) both concluded that ATS algae could be safely and effectively used as a soil amendment and crop fertilizer despite metal uptake, and this is one of the current disposal strategies utilized by commercial ATS operators in the USA (Hydromentia, 2022).

An alternative approach would be to utilize ATS algae as a feedstock to generate renewable bioenergy. This would allow the carbon assimilated by the algae during bioremediation to effectively be recycled into a value-added product for the ATS operator’s use or profit. However, research into bioenergy derived from ATS algae is limited. Conversion of algal biomass into biodiesel, bioethanol, and biomethane fuels via transesterification, fermentation, and anaerobic digestion, respectively, have been demonstrated in the literature, but most of these studies utilized pure monocultures of algae rather than the heterogenous cultures an ATS generates. Adey et al. (2013) found that the lipid and carbohydrate content algae from a given ATS culture is variable according to which algal taxa are present, and that this changes over time with succession in the periphyton. This would make it inconsistent as a feedstock for transesterification or fermentation, which only convert the lipid or carbohydrate fractions of a waste, respectively, into biofuel. This research analyzed the conversion of algal biomass into biomethane using anaerobic digestion, due to its non-selective nature for organic substrates to convert into bioenergy.

1.3 Converting algal biomass to bioenergy via anaerobic digestion

An anaerobic digestion reactor uses an anaerobic slurry rich in microbes to degrade organic material into biogas rich in methane (CH_4), which may be burned directly for heat, converted into electricity by a generator or fuel cell, or upgraded into renewable natural gas. Digestion consists of four steps facilitated by different groups of bacteria: (1) hydrolysis to break down complex organic molecules into simpler ones, (2) acidogenesis to convert the simpler molecules into volatile fatty acids (VFAs) and alcohols, (3) acetogenesis to convert the VFAs and alcohols into acetic acid, and (4) methanogenesis to create CH_4 from the acetic acid, H_2, and CO_2 (Gerardi 2003). Carbon dioxide gas is also generated as a major secondary product, as are traces of other gases including ammonia (NH_3) and hydrogen sulfide (H_2S). Inorganic components of the degraded feedstock, including dissolved nutrients released from the organic phase, remain in the anaerobic slurry and are flushed out from the reactor periodically as effluent.

As with transesterification and fermentation, most research into anaerobic digestion of algae has only used monocultured species. Zamalloa et al. (2013) digested lab-grown dry lyophilized *Phaodactylum tricornium* and achieved 52% conversion of organic material, measured as chemical oxygen demand (COD), into biogas. Lu and Zhang (2016) showed that digestion of *Chlorella* sp. resulted in 43.3% reduction in organic material measured as volatile solids (VS) and generated 168.7 ± 12.5 L CH_4/kg VS fed. Tedesco and Stokes (2017) found that biogas production from industrial algal wastes is highly species-dependent, with residues from genera such as *Fucus* and *Laminaria* yielding the highest methane production (187-195 and ~100 L CH_4/kgVS, respectively) of those tested in the study. These results indicate that a given species of algae has a biomethane potential of approximately 100-200 L CH_4/ kg VS, but these findings are not directly comparable to the heterogenous algae generated by ATS.

Two studies have researched digestion of ATS algae specifically. Bohutskyi et al. (2016) produced ~200 L CH_4 kg/VS from lab-scale digestion of whole ATS cultures as well as algal residues left over from biodiesel production. Witarsa et al. (2020) examined digestion of ATS algae at both lab- and pilot-scale; laboratory-scale batch and continuous AD reactor studies showed methane potential of 158 ± 13 and 144 ± 7 L CH_4 kg/VS, respectively, for algae from a brackish ATS on the Patapsco River at the Port of Baltimore in Maryland. However, pilot-scale digestion of algae from the same ATS only yielded 107 ± 15 L CH_4/kg VS, while the concentration of CH_4 in the biogas was similar for all studies (60-66% CH_4). The CH_4 production from both studies were consistent with the those reported in the literature for AD of single-species algal cultures, showing that an ATS algae is not inferior to pure cultures as a feedstock for AD.

However, two questions remain unanswered that are vital to understanding the viability of anaerobic digestion for managing ATS algae if it were to be implemented at scale. First, it has been clearly demonstrated that the organic substrates present in ATS algae are dependent on the species present, which also have different biomethane potentials (Adey et al. 2013; Tedesco and Stokes 2017). There has been neither a mass balance directly assessing how temporal variability in ATS algae growth impacts the biomethane potential of the culture, nor of which specific climate variables are most strongly correlated to the total mass of VS generated. Second, the high moisture content of ATS algae indicates it may be relatively inefficient to use in mono-substrate digestion at scale, as the size and operational costs of a digester increase with the volume of waste that needs to be processed. These problems could be addressed via co-digestion of the algae with more conventional digestion feedstocks (e.g., dairy manure) or dewatering it prior to digestion, but there have been no studies on either of these topics using ATS algae. The studies in this dissertation were designed to address these gaps in the literature.

1.4 Research goal and background literature

The goal of this research was to quantify how the efficiency of anaerobic digestion of ATS algae changes according to seasonal variability in VS yield, during co-digestion with agricultural and food wastes, and with flocculation pre-treatment for dewatering the algae.

1.4.1 Seasonal growth trends of algal flow way (ATS) algae

Algal taxa are incredibly diverse in size, structure, and chemical composition. As their relative abundance in the periphyton culture on an ATS directly affects the chemistry of the algal byproduct, it is important to understand how variables that affect algal succession relate to seasonal changes in the mass of VS yielded from a harvest.

Adey et al. (2013) found that the overall rate of biomass production by a Wicomico River ATS (VA, USA) was most strongly influenced by temperature, followed by light availability and the degree of texture in the lining provided for algal attachment. Warmer temperatures and higher light availability were associated with increased growth due to more favorable conditions for metabolic processes and photosynthesis, respectively (Adey et al. 2013). Mulbry et al. (2010) found peak algal productivity between May-June during an eight-month study of two 1 m^2 ATS systems on the Patapsco and Patuxent Rivers in Maryland, with 7.2 ± 3.2 and 21.4 ± 8.5 g dry weight (DW) solids/m^2/day respectively (Mulbry et al. 2010). Peak biomass production from a study using four 1.6 m^2 ATS on a backwater of the Danube in Austria was also seen in the summer (July 12-Aug 9) with 9.2-11 g DW solids/m^2/day (Mayr et al. 2015), with no data from winter months due to local temperatures (<0°C) inhibiting ATS operation. This variability appears to be limited to temperate climates, however, as Chen et al. (2015) reported no significant seasonal change in biomass production during a ten-month study (Mar.-Dec.) from a sub-tropical ATS on the Jiulong River (Fujian Province, China).

As studies of anaerobic digestion of ATS algae are limited, it is unclear if the expected temporal variability in algal productivity in temperate climates would impact the consistency of bioenergy generated over time. Conventional on-farm and municipal wastewater anaerobic digesters can be operated year-round in the same climates, due to their respective feedstocks (animal waste and sewage) being generated consistently independent of climatic changes. Seasonal availability was specifically identified by Singh et al. (2022) as a major ongoing impediment to the commercial viability of lignocellulosic biomass-to-bioenergy operations. A better understanding of the mass balance of carbon and nutrients through a continuous digester processing ATS algae over time is thus needed to determine if anaerobic digestion would be effective and practical as an approach to manage the waste from ATS bioremediation at scale.

1.4.2 Co-digestion of algae with other feedstocks

Co-digestion is an established method for increasing the productivity of an AD reactor by introducing more diverse substrates for microbes to use (Lisboa and Lansing 2013). This could allow an ATS-AD operator to supplement the algal biomass with more traditional AD feedstocks (e.g., dairy manure, poultry litter, food waste) during periods of low algal productivity. Lu and Zhang (2016) reported that co-digestion of microalgae with septic sludge increased biogas production by up to 40% the production from sludge alone, with a 1:5 ratio of algae:sludge optimal for CH_4 production. Astals et al. (2015) had 33% higher yield of CH_4 during co-digestion of *Scenedesmus* sp. and pig manure due synergistic effects on biogas production. This result was verified by another algal co-digestion study with pig manure by Miao et al. (2014), which yielded 217 L CH_4/kg VS at an inoculum to substrate ratio of 2.0. As these papers all used monocultured algae, however, they are not directly comparable to the heterogenous ATS cultures.

Co-digestion in traditional on-farm AD reactors rather than algae-only reactors could also expand the scope of ATS implementation beyond the urban environments where ATS bioremediation has been focused to date. In Maryland, farmers that install ATSs could participate in the Water Quality Trading Program and benefit from energy generated by AD of the algal biomass. Pizarro et al. (2006) estimated that the yearly operational costs for an ATS installed on a hypothetical 1000-cow dairy could be decreased by 36% by incorporating an AD reactor to generate biogas from the algae for heating. There are no studies that empirically quantify co-digestion of ATS algae in an agricultural AD reactor, nor any that assess how adding algae to a conventional digester affects the safety of the resulting effluent for crop production, however.

1.4.3 Flocculation dewatering for suspended algal cultures

The moisture content of ATS algae increases its fluidity and makes it easier to pump, but the excessive moisture also dilutes the feedstock and reduces the efficiency of CH_4 production per unit of mass fed to the reactor. The moisture content of an ATS algae culture typically exceeds 90% (Bohutskyi et al. 2016; Witarsa et al. 2020). Given logistical challenges and the time needed to dry the large volume of algae continuously generated from a pilot-scale ATS, dewatering via flocculation may be more practical at scale.

A wide variety of flocculants have been tested with suspended algal cultures, due to its long history of use in municipal water treatment and the variety of flocculants available for experimentation (Alam et al. 2016; Ummalyma et al. 2017). Flocculation treatments include dosing with chemical (e.g., pH adjustment, polymers, metal flocculants) and biological agents (e.g., flocculating bacteria).

A bacterial flocculant was investigated by Powell and Hill (2013) and successfully achieved 70-98% flocculation of pure cultures of the microalgae *N. oceanica* using Bacillus sp. strain RP1137 at lab scale. Natural and synthetic polymers have also been explored as potential algal flocculants, including cationic guar gum, chitosan, and cationic polyacrylamide (Bannerjee et al. 2013; Lama et al. 2016; Musa et al. 2020). Van der Berg et al. (2020) also found that the species composition of the algae harvested with chitosan from a gravity-only control, further suggesting species-specific response to the treatment. Lama et al. (2016) concurred with these findings, saying that treatment response of algal cultures to flocculation treatment appears to be highly species dependent. Metal flocculants are the most used in traditional water treatment and are often used as positive control agents in studies of experimental flocculants (Lama et al. 2016; Ummalyma et al. 2017).

1.5 Research Objectives

This research aimed to assess if anaerobic digestion is an effective method for managing waste from ATS bioremediation systems over time, and how the digestion process could be improved by assessing the effects of co-digestion and thickening pre-treatment on CH_4 yield. Specific objectives were as follows:

Objective 1: Quantify changes in the total mass of VS generated by ATS in the Chesapeake Bay Watershed in relation to climatic variables over two growing seasons.

Objective 2: Test the hypotheses that increased HRT and use of active heating/recirculation systems will increase CH_4 yield from ATS-AD to greater than 150 L CH_4/kg VS.

Objective 3: Quantify CH_4 generation during co-digestion of ATS algae with dairy manure, poultry litter, and food wastes at four loading ratios.

Objective 4: Quantify the concentration of sulfur, metals, and other trace elements taken up by lettuce biomass grown after fertilization with effluent from co-digestion reactors processing ATS algae with food and agricultural wastes.

Objective 5: Apply flocculation pre-treatment to an ATS culture for thickening and quantify CH_4 generation during AD of pre-treated algae.

1.6 Research Approach

Objectives 1 and 2 (Chapter 2) were analyzed using algae from an ATS system at the Port of Baltimore, Dundalk Terminal. Algae was harvested over two growing seasons in 2018 and

2019 and the total mass of algae and organic material generated were quantified, with comparison to climate data over time. The algae were then digested at pilot-scale in a continuous anaerobic digestion system described in Witarsa et al. (2020) and modified to compare two different HRTs.

Objectives 3 and 4 (Chapter 3) consisted of a laboratory-scale batch anaerobic reactor experiment to co-digest algae from a small-scale ATS (Bladensburg, MD) with dairy manure, food waste, and poultry litter. A follow up greenhouse experiment used the reserved effluent from the batch reactors as fertilizer for lettuce plants, which were monitored for signs of acute toxicity. Leaf tissue analysis was also conducted to determine if metals present in the reactor effluent were assimilated by the plants.

In Objective 5 (Chapter 4), 500 mL subsamples of a composite algal culture from two ATS systems was subjected to a series of pre-treatments (i.e., gravity, electrocoagulation, FeCl3, chitosan, bacteria) to induce flocculation of the algae. Clarified supernatant was pipetted out, and the settled solids were digested in a batch-scale anaerobic reactor experiment to quantify changes in CH_4 generation between treated and untreated algae.

Chapters 2-4 will be presented in the form of manuscripts, designed for submission to a journal such as *Ecological Engineering* or *Bioresource Technology*. Chapter 5 will summarize the findings from each study and overall conclusions at the end of the dissertation.

Chapter 2: Determining the effect of climate on yield of algal flow way (ATS) algae and optimizing anaerobic digestion parameters for bioenergy generation

2.1 Introduction

Algal turf scrubbers (ATS) are among the few technologies capable of remediating non-point source nutrient pollution from eutrophic waters. An ATS consists of a shallow flow-way with a textured lining and a timed pump to deliver water to the ATS in pulses. The use of pulsed flow rather than continuous is designed to mimic natural wave activity in shallow ecosystems. These conditions encourage colonization of the ATS with in-situ algae species to form a periphyton turf that metabolizes nutrients and carbon from the water (Adey 1982). These systems have been adopted around the world since they were invented in the 1980s and have been the subject of multiple studies on tributaries of the Chesapeake Bay Watershed to support nutrient load reduction efforts in the bay (Adey et al. 2013; Mulbry et al. 2010) A logistical challenge that has limited widespread implementation of the ATS beyond pilot scale is how to manage the algal biomass that these systems generate. The accumulated algae must be harvested periodically (i.e., weekly) to maximize algae growth and prevent biofilm sloughing, and the resulting slurry consists of a heterogenous mixture of water, microalgae, macroalgae, detritus, and settled sediments.

Using the algae as a feedstock for anaerobic digestion would allow algae harvested from an ATS to be utilized for renewable energy production, with the sequestered nutrients recycled into the digester effluent (i.e., digestate) to use as a fertilizer resource. Anaerobic digestion involves a consortium of anaerobic microorganisms that degrade the organic components of biodegradable substrates (e.g., carbohydrates, lipids, proteins) into biogas, which has a high concentration of methane (CH_4) for use as bioenergy (Gerardi 2003). As organic material is degraded in the digester, organic N and P components of the feedstock are converted to inorganic forms, such as ammonium (NH_4) and orthophosphate (PO_4), which may be recovered as fertilizer (Mazzini et al. 2020). Anaerobic digestion is non-selective for organic substrates, unlike bioenergy conversion methods that use only a single component (i.e., fermentation of carbohydrates into bioethanol or lipids into biodiesel) and has proven capable of processing heterogenous wastes such as ATS algae (Bohutskyi et al. 2016; Witarsa et al. 2020)

Anaerobic digestion of algae harvested from an ATS has only been researched up to pilot-scale, and the literature has indicated that temporal variables may limit its viability as a mono-substrate for anaerobic digestion. It is known that the rate of algal growth and total biomass yielded from an ATS are significantly dependent on climatic variables, such as ambient temperature and sunlight availability (Adey et al. 2013). Mulbry et al. (2010) reported similar biomass generation rate of 21.4 ± 8.5 g DW/m^2/day from a Patuxent River ATS (Maryland, U.S.) but only during peak productivity (May -June) compared to only 1.2 ± 0.7 g DW/m^2/day during the winter periods (Dec -Feb). Chen et al. (2015) observed a smaller average range of productivity (17.6-25.4 g DW/m^2/day) over ten months on a sub-tropical ATS with no significant variability in growth attributed to seasonal temperature change.

Seasonal patterns in ATS biomass growth in temperate climates may also affect the species present due to successional changes over time. The composition of periphyton communities and bacteria are known to vary over time, especially during the early stages of biofilm development (Wang et al. 2022). Different alga have different CH_4 generation potential, as Tedesco and Stokes (2017) measured different yields from *Laminaria* sp. (187-195 L CH_4/kg VS), *Fucus* sp. (101-103 L CH_4/kg VS), and *Ulva* (72 L CH_4/kg VS). However, Mulbry et al. (2010) reported similar ash content (73-79%) between algae samples from a Patuxent River ATS over a 10-month study (June 2007-February 2008), which means that only 21-27% of the solids were volatile and capable of bioenergy production. There has not been a study specifically examining changes in the VS mass yielded from ATS-derived algae or its effect on CH_4 yield during digestion over time.

Separately, as there have only been two published studies on anaerobic digestion of ATS algae, it remains unclear what optimal operational parameters should be recommended for digesting ATS algae (Bohutskyi et al. 2016; Witarsa et al. 2020). The hydraulic retention time (HRT) needed to digest the algae is of particular importance, as it determines the size of the digester that needs to be built to process it – and in turn the cost required to implement digestion to dispose of the algae.

Bohutskyi et al. (2016) produced 200-210 L CH_4/kg VS from continuous digestion of algal biomass from an ATS and algal residues from biodiesel production, also with an HRT of 20 days. Laboratory-scale batch reactor experiments by Witarsa et al. (2020) measured a methane potential for algae from the same Patapsco River ATS at laboratory-scale batch reactor experiments to be 158 ± 13 L CH_4/kg VS with an HRT of 20 days, but only measured 107 ± 15 L CH_4/kg VS in continuous pilot-scale reactors using the same algae with an average weekly HRT of 45.4 ± 7.3 days attributed to a lower organic loading rate (OLR) at the higher HRT. Similarly, the algae used by Bohutskyi et al. (2016) also contained had a higher VS content (62-63% VS) compared to the batch reactor experiments of Witarsa et al. 2020 (23.4% VS). Because the OLR of the algae is dependent on how much biomass grows each week, a better understanding of optimal HRT together with the variables that influence biomass yield can help site operators better plan combined ATS and anaerobic digester systems.

This study digested algae from a Patapsco River ATS at the pilot-scale over two growing seasons (July - November in Year 1 and June – October in Year 2). The first research objective was to measure changes in algal biomass yield over multiple years and determine the water quality and climate variables that significantly affected the algal yield and VS content. The second objective was to quantify CH_4 yield from two digestion systems with a different HRT to determine if ATS algae would benefit from increased time for digestion. After the variable HRT was tested in Year 1, the system with the more efficient digestion was modified to test active heating and recirculation systems during Year 2 of digestion. This experiment was designed to better understand growth and digestion of ATS algae for potential widespread disposal of ATS algae in anaerobic digestion infrastructure in the Chesapeake Bay region.

2.2 Materials and methods

2.2.1 Algal turf scrubber (ATS) operation and biofilm characterizations

The ATS was a 61 x 2 m unit (122 m^2) constructed by the Port of Baltimore in 2013 at the Dundalk Terminal in Baltimore, Maryland, USA, at the same site described in Witarsa et al. (2020). The algae used in this experiment was harvested weekly during two growing seasons from June 14-November 1 (Year 1) and May 16-October 10 (Year 2) (Figure 2.1).

Figure 2.1: Pilot-scale algal turf scrubber (ATS) installed at the Port of Baltimore Dundalk Terminal (Baltimore, MD, USA).

The algal biomass was harvested weekly at 9:00 am to prevent afternoon temperatures from damaging the regenerating biofilm after harvest, according to best practices recommended by Adey et al. (2011). Prior to the harvesting, the flow of water to the ATS was stopped, and the system was allowed to rest for approximately 30 minutes to drain excess water. Each 20.3 m segment of the ATS between the water source and outflow were examined during the draining period to identify discrete colonies of visible algae. As new colonies occurred on the turf, a grab sample for species identification was taken using clean metal tweezers to transfer it to a sterile 50 mL centrifuge tube. These samples were analyzed using light microscopy for examination and species identification according to the key in Prescott et al. (1978).

Algal biomass harvesting was conducted by using rubber scrapers to detach the algae accumulated on the surface of the ATS mesh and push the algal biomass into a collection area, where it was transferred to a 2000 L storage tank using vacuum suction to be stored for anaerobic digestion.

Prior to starting the digester each year (Year 1 and Year 2), biomass from the first 4-5 harvests were composited in the storage tank storage to ensure sufficient feedstock would be available during the first week for feeding. As this composite did not reflect the chemical characteristics of a single harvest, algae feeding data to the digester from harvests 1-4 in Year 1 and 1-5 in Year 2 were omitted from the statistical analyses for this study.

2.2.2 Pilot-scale anaerobic digestion with variable hydraulic retention time (HRT) – Year 1

The pilot-scale digesters were continuous digestion reactors (Shenzhen Puxin Technology Co. Ltd., Shenzhen, China), labeled D1 (3.4 m^3), D2 (3.4 m^3), and D3 (1.2 m^3) as described in Witarsa et al. (2020). Each digester consisted of a flexible PVC bag contained in a metal frame and filled with 1.7 m^3 (D1 and D2) or 0.5 m^3 (D3) of liquid digestate. The remainder of the volume was left empty to allow biogas to accumulate in the headspace. Double-walled greenhouse plastic covered the metal frame for protection and insulation (Figure 2.2).

Figure 2.2: An anaerobic digester used at the Port of Baltimore Dundalk Terminal (Baltimore, MD, USA).

These systems were operated in series (D1-D2-D3) by Witarsa et al. (2020) with an overall 45.3 ± 7.3-day HRT for the three-digester system. In this experiment, D1 was disconnected and operated in parallel to D2-D3. As a result, two digestion systems were created with a 23% higher volume of digestate fluid in D2-D3 than D1. As D1 and D2 had the same volume of digestate, they were designed to act as replicates (Figure 2.3).

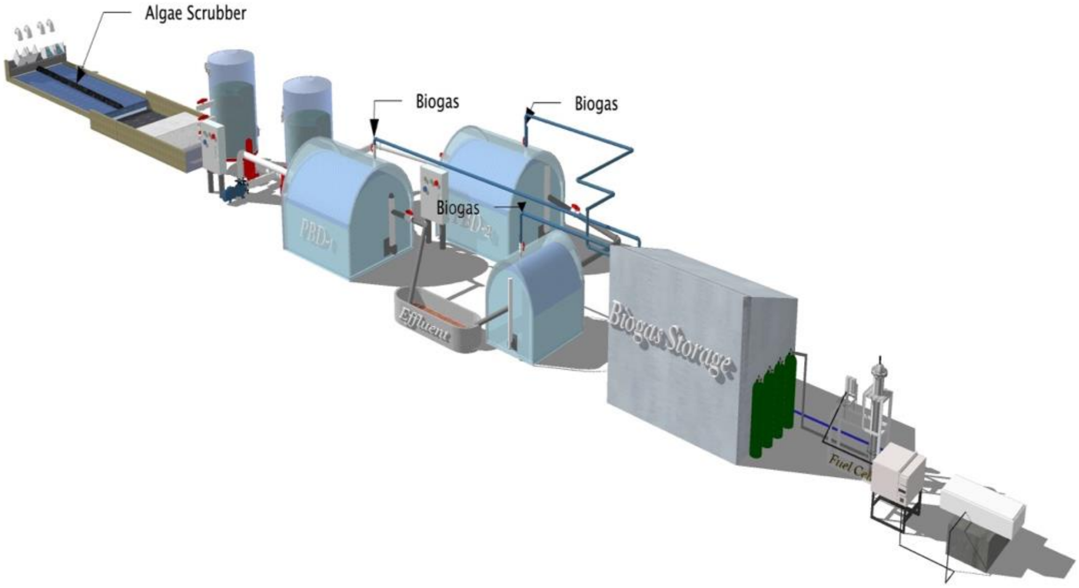


Figure 2.3: Diagram of the combined algal turf scrubber (ATS) and anaerobic digestion systems at the Port of Baltimore Dundalk Terminal (Baltimore, MD, USA).

All three digesters were inoculated on July 20th (Year 1) with 1.7 m^3 (D1 system) or 2.3 m^3 (D2-D3 system) of effluent from a dairy farm digester (MD, USA) that co-digested dairy manure with cranberry, chicken waste, and ice cream waste, as detailed in Lisboa and Lansing (2013) and Lansing et al. (2019). Each of the two systems (D1 and the D2-D2 series) was fed 568 L of algae incrementally in 3 feedings (M-W-F) to begin the semi-continuous digestion.

For all feedings, the algal biomass in the holding tank was recirculated using a gas-powered pump for five minutes to re-suspend settled solids and homogenize the feedstock before it was pumped into D1 and D2, with D3 fed by effluent displaced from D2. The rate of algal loading in each week following startup varied according to the rate of biomass production on the ATS, but an approximately equal volume of the available biomass was always loaded to D1 and the D2-D3 series to maintain the same proportional HRT differences between the two systems. Feeding was usually done on a M-W-F schedule, however during days 43-49 the schedule was adjusted to Tu-Th due to site access restrictions from a Monday holiday. To calculate how much algae would be fed to each system, equation 1 was used:

$$y = ((V/2)/7)*x \qquad \text{(Equation 1)}$$

V = Total volume of algae harvested in the given week

x = Number of days until next scheduled feeding

y = Volume of algae to feed to each digester system

As the algae was degraded, biogas was allowed to accumulate in the digester headspace which was tested for CH_4 (%), CO_2 (%), O_2 (%), H_2 (ppm), H_2S (ppm), and the balance gases (%, assumed N_2) in the field using a Landtec Biogas 5000+ Portable Gas Meter (Dexter, MI, USA) before each feeding. The total volume of biogas in each digester was measured weekly by collapsing the flexible biogas headspace and pumping the biogas through a biogas meter (China Coal, JBD2.5-SA, Shandong, China) into a holding bag, with the biogas flared.

Liquid samples of the algal influent (I) and digester effluents (D1E, D2E, and D3E) were taken during feedings. Initially, samples were taken three times per week after digester startup (Weeks 1-5). Sampling frequency was reduced to twice per week for Weeks 6-12, except during Week 9 when an additional sample was taken, and the digesters were re-inoculated.

Influent samples were taken while algal biomass was pumped into D1 or D2 from the holding tank. Effluent composite samples were taken over the first 10 minutes of drainage from each of the three digesters' outflow pipes as digestate was displaced by feeding. The digestate temperature was measured using a thermometer probe on the Landtec Biogas 5000+ meter. Samples were transported back to the laboratory and tested for pH (Accumet AB15 pH probe) and solids. Total solids (TS) and volatile solids (VS) were measured according to APHA methods 2540B and 2540E, respectively (APHA et al. 2005). The TS was determined by drying samples at 105 °C overnight and comparing sample mass before and after drying. The VS was determined by incinerating the dried TS samples at 550 °C and subtracting the ash mass from the TS mass to measure the volatilized mass.

The total carbon (TC) in the samples was estimated from the organic content based on VS using the Van Bemmelmen conversion factor of 1.742 shown in Equation 2 (de Castro Padrilha et al. 2020; Minasney et al. 2020).

$$C = VS/1.742 \qquad \text{(Equation 2)}$$

C = Total carbon (%)

VS = Volatile solids (%), wet weight basis

Sub-samples were acidified to pH <2.0 using Hach 5.25 N sulfuric acid standard solution (Loveland, CO, USA) to test for ammonium (NH_4), total Kjeldahl nitrogen (TKN), total phosphorus (TP), chemical oxygen demand (COD), and volatile fatty acid (VFA) analyses.

The COD was measured using acid digestion with high-range COD vial kits (Hach kit #2125915, Loveland, OH, USA). Samples for VFA analysis were centrifuged at 15,000 RPM for 20 minutes, and the supernatant filtered through a 0.22 µm nitrocellulose membrane. The concentration of acetic, propionic, butyric, and valeric acids in the filtrate were analyzed using an Agilent 7890A gas chromatograph (Santa Clara, CA, USA) equipped with a flame ionization detector (FID) and a DB-FFAP capillary column, set to an injection temperature of 250°C, detector temperature of 300°C, oven temperature of 106.5°C, and a 1.80 mL/min flow of carrier gases consisting of 10.6% H, 85.1% air, and 4.3% He.

Nutrients were measured using a Lachat Quickchem 8500 Series 2 ion flow analyzer (Lachat Instruments, Milwaukee, WI, USA). Ammonium samples were centrifuged at 15,000 rpm for 20 minutes, and the supernatant filtered through a 0.45 μm nitrocellulose membrane. Filtered samples were analyzed on the Lachat according to Hach Quickchem method 13-107-06-2-D. Total Kjeldahl nitrogen (TKN) and total phosphorus (TP) samples were acid digested prior to analysis using 2 mL of well-mixed sample digested with 1 Kjeltab Cu-1.5 reagent tablet (Foss) and 2.5 mL of concentrated sulfuric acid. Digested samples were analyzed for TKN and TP using Hach Quickchem methods 10-107-06-2-O and 13-115-01-1-B, respectively, on the Lachat instrument.

2.2.3 Pilot-scale anaerobic digestion testing recirculation and heating – Year 2

Only the in-series D2-D3 digesters were operated in Year 2, with the addition of recirculation heating units (CVL Tech, Maryland, USA) added to D2, while D3 remained unheated but receiving the heated effluent from D2. The D2 heater was programmed to recirculate the digestate at 30 L/min and maintain a mesophilic digestion temperature of 32 °C. To accommodate the new infrastructure required for the recirculation heating, the liquid volume in D2 was increased from 1.7 m^2 to 2.0 m^2 in Year 2, while the volume D3 remained the same (0.5 m^2). The inoculum used in Year 2 consisted of 2 m^3 of diluted biosolids (TS 1%) from a local municipal wastewater digester (Washington DC, USA).

The analyses of the biogas in the reactors in Year 2 was done according to the same methods described in Year 1, except that a new gas meter (Norgas Metering Technologies, Inc., NMT NG4, Fairfield, OH, USA) was used. Sample collection and preservation methods for the liquid influent and digester effluents was also the same, except that a once weekly influent and effluent sampling schedule was used throughout the entire experiment for the algal influent and digester effluents.

2.2.4 Weekly biomass productivity

The TS was used to approximate the dry biomass generated by the ATS during each week of the study (APHA et al. 2005). Prior ATS studies also utilize the method described in Kangas et al. (2017) to estimate algal productivity, which differs from the standard methods by including a sieving step to separately measure solids contained in both the macroalgal biomass and the fluid fraction of the harvest, which contains suspended microalgae. The drying method used in Kangas et al. (2017) also differed from the standard methods by utilizing air and oven drying at 25°C and 70°C, respectively, rather than the oven-only drying at a higher temperature (100°C) utilized in the standard methods. Both are similar in that they remove only water without volatilizing other components of the algae, however, so the results of the TS according to APHA et al. (2005) was acceptable to calculate generation of dry biomass/m^2/day according to Equation 3:

$$y = TS/A/x \qquad \text{(Equation 3)}$$

TS = Total solids (g)

A = Surface area of the algal turf scrubber (m^2)

x = Number of days of algal growth prior to harvest (days)

y = Rate of algal biomass production (g/m^2/day)

2.2.5 Climate and Patapsco River water quality data

Air temperature and precipitation data for the Baltimore region was obtained from the public database of the National Oceanic and Atmospheric Association’s nearest weather station at the Maryland Science Center in Baltimore, Maryland (39.2814°, -76.6111°). Water temperature and salinity data at the ATS was provided by staff at the Port of Baltimore Dundalk Terminal, measured using a YSI (Yellow Springs, OH, U.S.) handheld probe (Appendix Figures A.1-A.4).

2.2.6 Additional data

Unpublished annual reports detailing algal productivity from the Port of Baltimore for the five years prior to the start of this experiment (2013-2017) were provided by the previous operators of the ATS, Dr. Patrick Kangas and Dr. Peter May of the University of Maryland (Appendix Table A.1) (May et al. 2014; Selby et al. 2016; Selby et al. 2017; Smith et al. 2013; Smith et al. 2016).

Samples of frozen algae pre- and post-digestion samples were photographed using a scanning electron microscope (SEM) by the University of Maryland AIM Lab to visualize changes in the algal biomass during the digestion process.

2.2.7 Statistical analysis

All statistical analysis was performed using the R analytical software. Linear modeling was used to compare algal productivity parameters (biomass production, TS, VS, COD, and nutrients) to climate (average weekly maximum and minimum air temperature, weekly precipitation), water quality (salinity, water temperature), and operational (water flow rate) variables. An ANOVA test with Tukey-Kramer post-hoc analyses was used to compare CH_4 generation from the digestion reactors, normalized per kg algae fed and per kg VS fed.

Statistical analysis of algal biomass used data from harvests weeks 6-20 of ATS operations in Year 1 and weeks 5-20 in Year 2. The first 4-5 harvests in each year were consolidated after collection in the algae holding tank to stockpile sufficient volume for the digester startup procedures in the anaerobic digestion experiment.

2.3 Results and Discussion

2.3.1 Overview of algal flow way (ATS) periphyton community

The dominant algae on the ATS during both years of growth was *Melosira* sp., a filamentous diatom. Discrete colonies of the chlorophyte *Ulva intestinalis* also occurred within the turf of *Melosira* sp. along the entire length of the ATS. The more fragile ochrophyte *Vaucheria* sp. grew interwoven with the *Melosira* sp. filaments in the lower 20 m of the ATS only, where wave action from the pump dissipated. Colonies of cyanobacteria (*Oscillatoria sp.*) were observed inconsistently, only appearing on the surface of the ATS in spots without growth by *Melosira* sp. or the other species in the turf.

The taxa that formed visible colonies on the ATS are consistent with major species reported in studies of other ATS on tributaries of the Chesapeake Bay Watershed (Adey et al. 2013; Mulbry et al. 2010; Witarsa et al. 2020). Witarsa et al. (2020) also documented the dominance of *Melosira* sp. from the same Patapsco River ATS during a prior year of operations. The ATS studied by Mulbry et al. (2010) was located further downstream from this study on the Patapsco River also had periphyton dominated by filamentous diatoms, with *Ulva intestinalis* (previously identified in Mulbry et al. 2010 as *Enteromorpha*) also identified as a dominant species present year-round. Mulbry et al. (2010) also examined a Patuxent River (MD, USA) ATS and reported *Melosira* dominance at that site as well, however only for part of the year (winter/spring) while the chlorophyte *Spirogyra* and cyanobacteria dominated the turf for the remainder of the year. Adey et al. (2013) reported that ochrophytes, including *Melosira* sp., represented 54% of the 86 species present in on an ATS on the Great Wicomico River (VA, USA), however only 9% of the total biomass was *Melosira* sp.

The results suggest that ATS built on Chesapeake Bay tributaries were colonized by similar species, with dominance by ochrophyta for part or all the year. However, the literature indicates that the relative abundance of species and seasonal succession patterns in the periphyton differs between tributaries in the region.

2.3.2 Algae productivity and composition analysis

The ATS generated a total of 8,313 L of algae from 20 harvests in Year 1 (416 L/week) and 12,340 L of algae from 20 harvests in Year 2 (617 L/week). The wet algae contained 326 and 395 kg TS in Year 1 and Year 2, respectively, with 77.5 and 111 kg VS in Years 1 and 2, respectively. The overall rate of biomass production averaged 21.6 ± 1.1 g TS/m^2/day^1 during harvest weeks 6-20 of Year 1 and 29.8 ± 3.9 g TS/m^2/day^1 during harvest weeks 5-20 of Year 2 (Tables 2.1-2.3).

Table 2.1: Summary of algal biomass production from the 122m^2 algal flow way (ATS) at the Port of Baltimore. TS =Total Solids, VS = Volatile solids.

Year 1						Year 2					
Sample Date	**Harvest Week #**	**Wet algae (L)**	**TS (kg)**	**VS (kg)**	**g TS/m^2 /day**	**Sample Date**	**Harvest Week #**	**Wet algae (L)**	**TS (kg)**	**VS (kg)**	**g TS/m^2 /day**
Jul-19	1-5*	1136	50.3	11.6	-	20-Jun	1-4*	1,474	13.3	3.37	-
Jul-26	6	757	27.6	6.2	32.3	27-Jun	5	1,085	25.0	7.05	29.3
Aug-2	7	620	21.3	5.0	24.9	3-Jul	6	884	39.6	8.19	46.3
Aug-9	8	585	18.6	4.4	21.8	11-Jul	7	504	8.10	2.24	9.49
Aug-16	9	355	14.0	3.3	16.4	18-Jul	8	429	27.4	5.42	32.1
Aug-24	10	437	18.7	4.4	21.9	25-Jul	9	657	8.23	9.26	9.64
Aug-30	11	513	18.9	4.5	22.1	1-Aug	10	482	NA[#]	NA[#]	NA[#]
Sep-6	12	349	14.6	3.6	17.1	8-Aug	11	139	5.48	1.16	6.41
Sep-13	13	392	20.7	4.8	24.3	15-Aug	12	965	50.8	12.2	59.5
Sep-20	14	568	20.6	4.5	24.2	22-Aug	13	992	31.2	8.36	36.6
Sep-27	15	517	20.4	5.1	23.9	29-Aug	14	750	21.8	6.10	25.5
Oct-4	16	411	15.9	3.7	18.6	5-Sep	15	965	34.5	9.36	40.4
Oct-11	17	326	15.3	3.7	18.0	12-Sep	16	670	15.0	7.99	17.6
Oct-18	18	294	15.2	3.9	17.8	19-Sep	17	402	16.8	4.72	19.7
Oct-25	19	366	14.6	3.5	17.0	26-Sep	18	992	37.5	10.7	43.9
Nov-1	20	687	19.6	5.34	23.0	3-Oct	19	402	32.9	9.45	38.5
-	-	-	-	-	-	10-Oct	20	549	27.1	5.49	31.7
Sum		8313	326	77.5	-	Sum		12,340	395	111	-
Average		520 ± 53	20.4 ± 2.2	4.84 ± 0.49	21.6 ± 1.1	Average		725.9 ± 80.5	24.7 ± 3.2	6.9 ± 0.8	29.8 ± 3.9

*Algae stockpiled from multiple harvests prior to the start of the digestion experiment. Data excluded from statistical analyses.

[#] No solids data available.

Table 2.2: Summary of algal total solids (TS), volatile solids (VS), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), and total phosphorus (TP) concentrations in Year 1 and Year 2. Samples were collected once weekly from the holding tank following harvest (Weeks 5-20 in Year 1 and Weeks 4-20 in Year 2) and tested with analytical triplicates in the laboratory. Values given are the average from "n" number of weeks ± standard error. Year 2 calculations exclude data from Harvest Week 10 due to damaged sample.

Parameter	**Year 1 (n = 16)**	**Year 2 (n = 16)**
pH	7.2 ± 0.1	7.03 ± 0.6
COD (g/L)	11.8 ± 0.8	12.4 ± 1.4
TS (%*)	3.93 ± 0.15	3.97 ± 0.40
VS (%$^{\#}$)	24.1 ± 0.3	25.6 ± 0.8
TKN (mg N/L)	785 ± 66	806 ± 96
TP (mg P/L)	164 ± 32	142 ± 23

*Percentage on wet weight basis.

$^{\#}$Percentage on dry weight basis.

Table 2.3: Table of *p*-values from linear models of climate, water quality and operational variables to predict yield of L wet algae, kg total solids (TS), and kg volatile solids (VS) yield in weeks 4-20 of Year 1 and weeks 6-20 of Year 2.

	Year 1			**Year 2**		
Predictor Variable	**L wet algae**	**kg TS**	**kg VS**	**L Wet algae**	**kg TS**	**kg VS**
Weekly Average Maximum Air Temperature	0.047*	0.021*	0.037*	0.154	0.732	0.602
Weekly Average Minimum Air Temperature	0.073	0.029*	0.053	0.184	0.771	0.792
Weekly Precipitation	0.616	0.726	0.570	0.880	0.446	0.996
Water Temperature	0.025*	0.067	0.089	0.084	0.365	0.342
Salinity	0.083	0.278	0.319	0.283	0.817	0.905
Water Flow Rate	0.012*	0.084	0.991	0.034*	0.538	0.576

*Significant *p*-value.

In Year 1, the total yield of wet algae on a weekly basis was significantly predicted through a positively correlation by the weekly average maximum air temperature (p = 0.047, r^2 = 0.254) and water temperature (p = 0.025, r^2 = 0.352) . Wet algae yield was negatively correlated to the flow rate of water across the ATS (p = 0.012, r^2 = 0.423). Weekly average maximum air temperature was also positively correlated with TS (p = 0.021, r^2 = 0.326) and VS yield (p = 0.037, r^2 = 0.326) with a positive relationship. The TS was also significantly predicted by weekly average minimum air temperature (p = 0.029, r^2 = 0.297). In Year 2, only the flow rate of water across the ATS significantly predicted wet algae yield (p = 0.034, r^2 = 0.303), which was a negative correlation as in Year 1. None of the variables tested in the linear modeling were significantly correlated to the yield of TS or VS in Year 2.

The results suggest that temperature variables influenced the yield of wet and dry algal biomass and its organic content from the ATS in Year 1. These findings support the results of Adey et al. (2013) that temperature is among the strongest influences on the rate of biomass generation on an ATS. Ambient temperature is known to limit the rate of photosynthesis in autotrophs. Photosynthesis is facilitated in part by the activation state of the Rubisco enzyme, which is known to increase with temperature up to approximately 30 °C (Salvucci and Crafts-Brandner, 2004). The maximum and maximum daily temperatures each day thus effectively determine the maximum and minimum activation state of Rubisco, and in turn carbon assimilation and biomass growth.

The Year 2 results did not show a significant correlation between algal productivity and temperature. It is possible that the seasonal variability in temperature variables for Year 2 was too small to observe a significant effect during the study period. This is consistent with the findings of Chen et al. (2015), where consistent seasonal temperatures resulted in insignificant changes in algal yield between seasons. While the study period in Year 1 began one month later than in Year 2, air temperature data for the Baltimore region indicates that similar trends in both Year 1 and Year 2, with air temperatures above 25°C for most of the summer, peaking in July and August before declining in late September and early October. The peak temperature was higher and earlier during Year 2, however, with an average high of 35.9°C for the week of July 11 compared to the Year 1 peak of 34.0°C in the week of August 9.

The lower air temperatures in July of Year 1 were likely due in part to large storm events that occurred in the region that year. Year 1 of the study coincided with the wettest year on record for the Baltimore region, with July and November specifically having the highest precipitation recorded since 1889 and 1952, respectively, at the time (National Weather Service, 2019). During the last week of July of Year 1 the ATS site received 157.6 mm of rainfall, 134% more than the highest Year 2 rainfall of 67.1 mm during the week of August 22. The rainfall events in July of Year 1 corresponded to a 3.3°C decline in water temperatures over the course of the month, while water temperatures in July of Year 2 rose steadily over the same time frame.

Given the demonstrated positive correlation between algal yield and temperature variables in Year 1, reduced temperatures were expected to correspond with reduced biomass yield. Accordingly, Year 1 had 32.6% lower harvests of wet algae, 17.2% lower yield of TS, and 30.1% lower yield of VS than in Year 2. The overall ATS productivity of g algae/m^2/day was also 27.5% lower in Year 1. Summers are known to be peak seasons for algal growth on an ATS, so climatic instability that is sufficient to significantly impact algal growth during this time would be expected to greatly reduce overall algal yields for the entire year (Adey et al. 2013; Chen et al. 2015; Mulbry et al. 2010).

The only variable that was significantly influenced wet algae yield in both Year 1 and Year 2 was the volumetric flow rate of water from the pump across the ATS. The flow rate from the pump was lower in Year 2 on average (186 ± 7 L/min) compared to Year 1 (274 ± 18 L/min). Carpenter et al. (1991) showed that both the flow rate and method of flow (pulsed vs. continuous) significantly affected the growth rates of algae grown as periphyton on coral reefs. The flow rate of water over periphyton determines the rate at which nutrients and carbon are delivered to the algae, but continuous flow can cause "flattening" of the algal fronds that limits nutrient access to cells at the foundation of the turf (Carpenter et al. 1991). It was also documented by Zhang et al. (2014) that flow rates above 0.06 m/s increased turbidity of sediments and reduced algal growth due to reduced light penetration. Additionally, it has been demonstrated that high flow rates increase sheer on the turf and can cause holdfast detachment (Lau and Liu, 1993). In Year 1, the higher flow rate from the pump together with added flow from the high precipitation could have led to algae flattening and detachment to contribute to the lower algal yield compared to Year 2.

The overall productivity of the ATS during Year 1 and Year 2 (21.6 ± 1.1 and 29.8 ± 3.9 $g/m^2/day$, respectively) was within the range (19.6 ± 1.8 - 38.8 ± 3.8 $g/m^2/day$) reported by the operators of the ATS site in their records from five years of operations (2013-2017) prior to the start of this experiment (Ref. Appendix Table A.1). The Year 1 productivity was closest to the 2015 productivity (19.6 ± 1.8 $g/m^2/day$). The Year 2 productivity was more like that in 2016 (27.2 ± 2.4 $g/m^2/day$), which was also the median of the five prior years' data. It may be concluded that the productivity during Year 2 was more typical for this ATS site, while Year 1 productivity was lower than typical.

2.3.3 Anaerobic digestion of algae – Year 1

The CH_4 generation in all three digesters gradually increased over the first three weeks after activation and achieved stability by Day 21 (Week 3) of operation in Year 1. Data from the first 56 days of the digestion are presented and used in the statistical analysis.

The volume of algae fed to the digesters varied according to the volume of algal feedstock generated by the ATS in the corresponding week, with an accordingly variable HRT (Table 2.4). The D1 and D2-D3 systems were fed 2416 L (302 ± 45 L/week) and 2337 L (292 ± 47 L/week) of algae, respectively. The overall HRT was 39 days in D1 (weekly average 45.0 ± 5.8 days) and 53 days in D2-D3 (weekly average 61.0 ± 8.1 days). The D2-D3 system had a volume 29% greater than D1, which resulted in a 26% higher average HRT on a weekly basis and 36% higher HRT over the entire study. With a 56-day operational period in Year 1, D1 and D2-D3 processed algae for 1.44 and 1.06 total HRT cycles, respectively.

Table 2.4: Algae loading and resulting hydraulic retention time (HRT) for the D1 and D2-D3 systems in Year 1. ATS = algal flow way, HRT = hydraulic retention time.

		D1 System **$V = 1.7\ m^3$**		**D2-D3 System** **$V = 2.2\ m^3$**	
Digester Operational Period (days)	**ATS Harvest Week #**	**Algae fed (L)**	**HRT (days)**	**Algae fed (L)**	**HRT (days)**
1-7	1-4	568	21.0	568	27.1
8-14	5	386	30.8	371	41.5
15-21	6	308	38.6	312	49.4
22-28	7	293	40.6	292	52.8
29-35	8	166	71.7	189	81.3
36-42	9	241	49.3	196	78.7
43-49	10	265	44.8	248	62.1
50-56	11	187	63.5	161	95.5
Sum		2416	-	2337	-
Weekly Average		302 ± 45	45.0 ± 5.8	292 ± 47	61.0 ± 8.1

The D1 and the D2-D3 systems generated a total of 1557 and 1801 L of biogas, respectively, including 1087 L CH_4 from D1 (68.5 ± 2.3 L CH_4/week) and 1170 L CH_4 from D2-D3 (146.8 L ± 24 L CH_4/week) (Tables 2.5 and 2.6). The D1 and D2 digesters operated successfully as replicates, with no significant difference between the two for volume of L biogas or L CH_4 generated (*p*-values = 0.600 and 0.105, respectively). Both D1 and D2 generated significantly more L biogas then D3 (*p* values = 0.001 and 0.001, respectively) and L CH_4 (*p*-value > 0.001 and *p*-value = 0.004, respectively). However, the difference in production of L biogas (*p*-value = 0.726) and CH_4 (*p*-value = 0.899) was not significantly different between D1 and D2-D3. As the difference in L biogas generated was also not significant between D2 and D2-D3 (*p*-value = 0.127), the addition of D3 in series with D2 did not boost overall biogas generation significantly. However, the D2-D3 systems did generate significantly more L CH_4 compared to D2 alone (*p*-value = 0.024).

Table 2.5: Results from anaerobic digestion of algae with variable hydraulic retention time, Year 1.

	D1 System (HRT = 39 days)					**D2-D3 System (HRT = 51 days)**				
Digester Operational Period (days)	**Total Biogas (L)**	**Methane (%)**	**Methane (L CH_4)**	**L CH4 kg/VS**	**L CH4/ L algae**	**Total Biogas (L)**	**Methane (%)**	**Methane (L CH_4)**	**L CH4 kg/VS**	**L CH4/ L algae**
0-7	0.00	48.3	0.00	0.0	0.000	0.00	60.7	0.00	0.00	0.000
8-14	374	60.0	224	70.5	0.581	352	67.5	150	49.1	0.404
15-21	221	70.4	156	62.6	0.505	340	71.3	216	86.2	0.694
22-28	188	71.9	135	61.1	0.461	226	72.2	164	74.3	0.561
29-35	147	74.6	110	70.4	0.661	212	72.6	158	88.7	0.832
36-42	198	72.2	143	58.4	0.592	235	72.4	168	84.4	0.856
43-49	267	73.0	195	84.6	0.734	287	69.6	205	95.0	0.825
50-56	162	77.3	125	65.3	0.669	150	65.7	108	65.6	0.671
Sum	1560		1090			1802		1170		
Weekly Average	195 ± 38	68.5 ± 3.4	136 ± 24	59.1 ± 8.9	0.525 ± 0.081	225 ± 40	69.0 ± 1.5	146 ± 24	67.9 ± 11.0	0.606 ± 0.102

Table 2.6: Table of *p*-values from Tukey-Kramer HSD post-hoc analysis of ANOVA comparison of digester systems in Year 1.

Systems Compared	**Total biogas (L)**	**Methane (L CH_4)**	**L CH_4/kg VS**	**L CH_4/L algae fed**
D1 and D2	0.600	0.105	0.319	0.325
D1 and D3	0.001*	>0.001*	0.169	<0.001*
D1 and D2-D3	0.726	0.899	0.563	0.515
D2 and D3	0.024*	0.004*	0.979	0.001*
D2 and D2-D3	0.127	0.024*	0.025*	0.021*
D3 and D2-D3	>0.001*	<0.001*	0.010*	<0.001*

*Significant *p*-value.

The results indicate that D3 produced less biogas and CH_4 than the larger D1 and D2 digesters, which was expected both due to its smaller size and that it was fed with digested effluent from D2 rather than whole algae. The average 26% longer digestion time from operating D3 in series with D2 did consistently increase biogas and CH_4 yield compared to D1 alone. The significant difference between D2 and D2-D3 despite the insignificant comparison between D1 and D2-D3 is attributable to the slightly lower biogas yield in CH_4 in D2 compared to D1; of the 1170 L of CH_4 generated by D2-D3, 69.2% (810 L) was generated by D2, compared to the 1090 L from D1. It confirms that longer HRT does improve CH_4 generation, however the difference in HRT between D1 and D2-D3 was too small to observe a significant effect in this study.

There is no set standard for HRT or solids retention time (SRT) in anaerobic digesters, however 20 days is often used as a baseline for experiments, as research has indicated that most digestion products are generated during this time unless the substrate contains a large fraction of complex or non-digestible components such as lignin (Asam et al. 2011; Dai et al. 2014; Feng et al. 2019; Witarsa et al. 2020). Longer HRTs are known to correspond to higher bioenergy yield by providing more time and a more stable environment for the bacteria to break down the waste more completely, however it also increases the time required to process the waste (Dai et al. 2014; El-Hadj et al. 2007). As the results of this study indicate that an 51-day HRT did not significantly increase CH_4 yield compared to a 39-day HRT, it may be inferred that 39 days was sufficient to maximize total CH_4 yield from the ATS algal culture specific to the Port of Baltimore ATS in Year 1.

The efficiency of the digestion in Year 1 was lower in both D1 (59.1 ± 8.9 L CH_4/kg VS) and D2-D3 (67.9 ± 11.0 L CH_4/kg VS) than that during the previous year at the site with D1-D2-D3 in series (107 ± 15 L CH_4/kg VS) as reported by Witarsa et al. (2020) (Figure 2.4). The efficiency was also low when compared to more conventional digestion feedstocks (Moody et al. 2011). For example, Asam et al. (2011) generated 330 L CH_4/kg VS from digestion of raw pig slurry, 161-230 L CH_4/kg VS from processed animal wastes, and 236-361 L CH_4/kg VS from maize and grass sileage. The efficiency was also low compared to prior studies of anaerobic digestion of algae, which have measured >200 L CH_4/kg VS for some species (Chen et al. 2023; Tedesco and Stokes 2017).

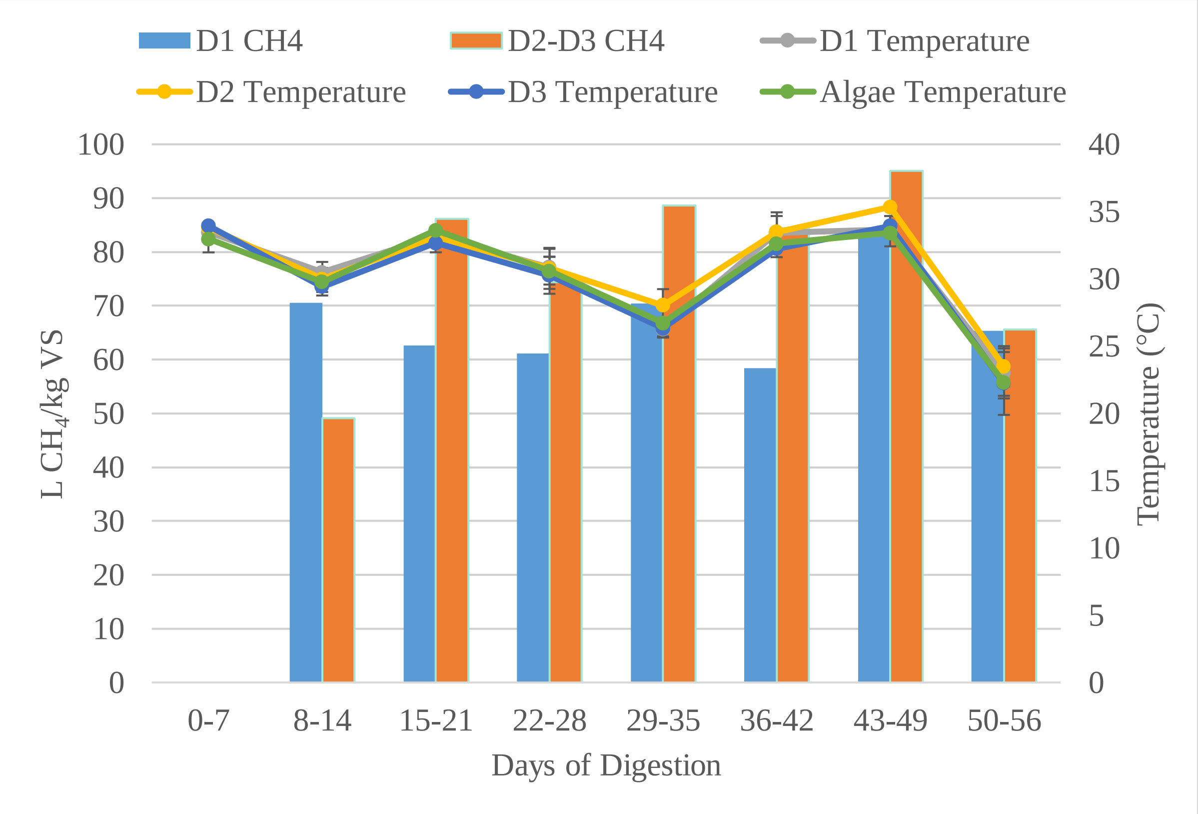


Figure 2.4: Methane (CH_4) generated per kg volatile solids (VS) in Year 1 from the anaerobic digesters processing algal turf scrubber (ATS) algae at the Port of Baltimore. D1 = Digester 1, D2-D3 = Digester 2 and 3 system.

As the individual digesters were operated according to the same parameters as in Witarsa et al. (2020) except for the modified HRT, the reduced efficiency was attributed to less complete digestion of the feedstock due to the lower HRT. While the difference in digestion efficiency between D1 and D2-D3 was not significant (*p*-value = 0.563) it was 14.9% higher on average in

D2-D2 compared to D1. As with total algal yield, it is possible that the difference in HRT tested in this study was not sufficiently large to observe a significant result.

The volume of CH_4 gained per L of algae processed was also low in both D1 (0.525 ± 0.081 L CH_4/L algae) and D2-D3 (0.606 ± 0.102 L CH_4/L algae). There was no significant difference between the D1 and D2-D3 systems (*p*-value = 0.515) but on average 15.4% more CH_4 was generated per L of algae fed in D2-D3. The results were comparable to the digestion efficiency per kg VS, due to the longer HRT in D2-D3 increasing time for bacteria to break down the organic components of the algae. The high water content of the algae (>96%) also contributed to the low CH_4 yield per L algae. Conventional digestion feedstocks such as manure and food wastes typically have relatively lower moisture content (>80%), and thus more digestible material is contained in each mass or volume of waste processed (Lisboa and Lansing 2013; Yarberry et al. 2020). While water is necessary to facilitate bacterial growth and biological functions, the water itself is a non-digestible component that does not contribute to bioenergy yield. It also dilutes the digestate and slows the digestion reaction by reducing interactions between the bacteria and the organic substrates they consume.

There was high weekly variability overall between the D1 and D2-D3 systems in terms of total biogas, total CH_4, digestion efficiency, and L CH_4/L algae. This variability is fundamentally attributable to the variable algal harvests during each week of the study. As previously discussed, the ATS harvest in Year 1 was low relative to Year 2 and prior years at the site. Stockpiling algae from multiple harvests prior to digester startup and high harvests during days 0-14 of digestion (>600 L algae/week) supported steady ramping up of CH_4 production with consistent feedings of >300 L/week of algae per system. Digestion efficiency stabilized at >55 L CH_4/kg VS around day 21 for both systems, however after day 21 the harvest volumes became smaller and more variable for the remainder of the experiment (448 ± 46 L/week). The highest yield of CH_4 from D1 was during Days 8-14 of digestion (224 L). The CH_4 yield of D2-D3 similarly peaked during Days 15-21, with 216 L generated in a single week. Despite more stable digestion after day 21, total L CH_4 yield declined subsequently in the following weeks the volume of algae available to feed also declined. The only exception to this was during digestion Days 43-49, which corresponded with a larger harvest of algae from the ATS during harvest weeks 9 and 10.

The low harvest volume also contributed to the long and variable average HRT for each digester system on a weekly basis; for example, although D1 operated as a standalone unit in Year 1 of this study, the average weekly HRT of the system (45.0 ± 5.8 days) was comparable to that of the D1-D2-D3 system in Witarsa et al. (2020) (45.3 ± 7.3 days) despite that system having a 2200 L higher capacity, simply due to the lower volume of algae processed per week.

The experimental design of the Year 2 study was designed to identify and modify operational parameters of the digesters to improve the efficiency of the digestion and total bioenergy yield per L of algae processed. The temperature of the algal feedstock in the storage tank and effluent inside the digesters was not controlled and fluctuated freely according to ambient temperatures at the Port (Appendix Figure A.1). This limited the operational time of the system, as the digester temperatures fell below mesophilic levels (30-35 °C) after 56 days of digestion. The temperature instability guided the experimental design in Year 2 to better control the environment for the microbes inside the reactors. A heating system that also recirculated the digestate was selected to also improve the homogeneity of the digestate, as problems with solids settling and clogging were documented throughout Year 1 of operations.

2.3.4 Anaerobic digestion of algae – Year 2

In Year 2, data from the D2-D3 system generated a higher volume of CH_4 than D1 in Year 1 (Tables 2.7 and 2.8). The HRT of D2-D3 in Year 2 was 59 days, with a weekly average of 77.3 ± 22.4 days. While the overall HRT was like Year 1, the lower and more variable average HRT due to low ATS productivity during harvest weeks 10 and 12 in Year 2. The increased harvest volumes overall compared to Year 1 allowed for 26.1% greater volume of wet algae to be converted into bioenergy, with 3310 L fed to D2-D3 in Year 2 over 70 days (331 ± 14 L/week). The D2-D3 system produced a total of 5510 L of biogas containing 4002 L of CH_4 (500 ± 159 L CH_4/week; 163 ± 42 L CH_4/kg VS; 1.17 ± 0.29 L CH_4/L algae fed) during Year 2. The addition of the heating system extended the operational period to 1.32 HRT cycles by controlling for ambient temperature instability (Figure 2.5).

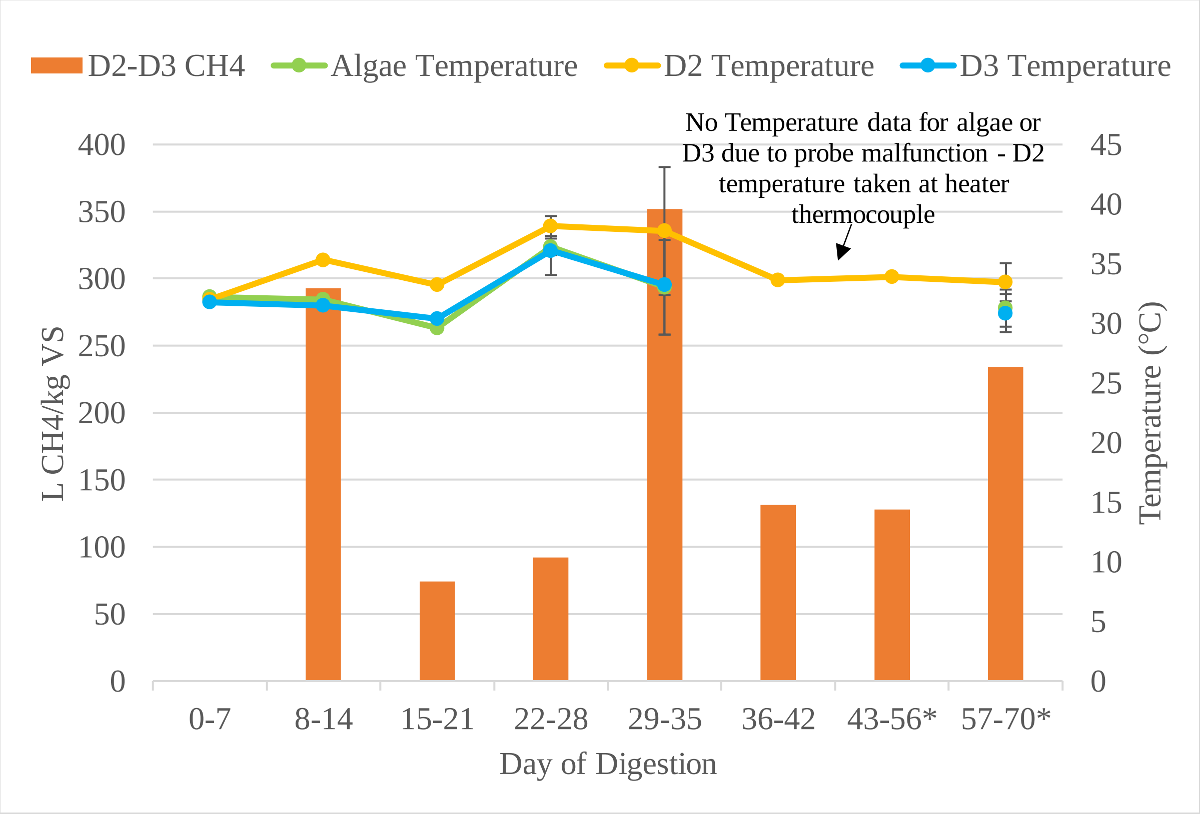
Table 2.7: Algae loading and resulting hydraulic retention time (HRT) for the D2-D3 system in Year 2. ATS = algal flow way, HRT = hydraulic retention time.

		D2-D3 System V = 2.5 m^3	
Digester Operational Period (days)	**ATS Harvest Week #**	**Algae fed (L)**	**HRT (days)**
0-7	1-5	335	52.2
8-14	6	402	43.5
15-21	7	543	32.3
22-28	8	442	39.6
29-35	9	252	69.4
36-42	10	214	81.6
43-49	11	328	53.3
50-56	12	241	72.6
57-63	13	69.7	251
63-70	14	482	36.3
Sum		3310	
Weekly Average		331 ± 14	73.2 ± 20.5

Table 2.8: Results from anaerobic digestion of algae with added heating and recirculation, Year 2.

	D2-D3 (HRT = 52.9 days)				
Days	**Total Biogas (L)**	**Methane %)**	**Methane (L)**	**L CH_4/kg VS**	**L CH4/L Algae**
0-7	0.00	15.5	0.00	0.00	0.00
8-14	518	32.7	236	293	0.59
15-21	391	59.7	263	74.4	0.48
22-28	487	75.0	378	92.2	0.85
29-35	479	77.6	394	352	1.56
36-42	462	75.8	356	131	1.66
43-56*	1348	75.2	1025	128	1.80
57-70*	1826	73.6	1352	234	2.45
Sum	5511		4004		
Average	689 ± 209	60.6 ± 8.4	500 ± 160	163 ± 42	1.17 ± 0.29

*Biogas measurement taken every 2 weeks instead of every week as in prior weeks.



*Biogas measurement taken every 2 weeks instead of every week as in prior weeks.

Figure 2.5: Methane (CH_4) generated per kg volatile solids (VS) in Year 2 from the Port of Baltimore, and algae and digester temperature data. Temperature data taken once weekly except in weeks indicated with error bars, which are the average of n = 2-4 measurements ± standard error.

The D2-D3 digestion was 140% more efficient in Year 2 than in Year 1. It was comparable to the biomethane potential of ATS algae from the Port of Baltimore in a 2017 harvest (158 ± 13 L CH4/kg VS) that was measured in laboratory-scale batch digestion by Witarsa et al. (2020). As the volume of digestate in D2 increased, the overall HRT in Year 2 of the D2-D3 system increased to 59 days compared to 53 days in Year 1. In Year 1, the 23.5% longer HRT of the D2-D3 system provided a small (6.83%) increase in digestion efficiency compared to D1. In Year 2, however, the 15.6% longer HRT of the D2-D3 system compared to itself in Year 1 corresponded to a 140% increase in efficiency. As the species composition and overall chemical parameters of the algal feedstock were similar between Year 1 and Year 2, the large change in digestion efficiency is attributable to the heating and recirculation added to D2 in Year 2.

The heating system would have increased the stability of the digestion by eliminating variability due to digester cooling at night, as ambient temperatures often fell below mesophilic levels even during the hottest parts of the summer (Appendix Figure A.2). This may have even resulted in excess heating in some weeks, however, as digester temperatures approached 40°C during days 22-35 of the digestion. This allowed the digestion to be extended an additional 2 weeks of sustained mesophilic digestion in Year 2 compared to Year 1.

The consistently high ambient temperature in Year 2 also increased the temperature of the algal influent in its storage tank to ~30°C during most weeks of the digestion, compared to Year 1 when the algae temperature routinely fluctuated below 25°. This sustained temperature would have been within the range required for mesophilic anaerobic digestion, so the enclosed algae holding tank may have acted as a hydrolysis pre-treatment despite not being actively heated.

Hydrolysis is the first step of anaerobic digestion and involves breaking down raw organic substrates into simpler molecules for other microbial consortia to convert into CH_4 and other digestion products. As a result, the rate of hydrolysis in a digester is generally considered the primary limiting factor in the rate of anaerobic digestion (Menzel et al. 2020). Park et al. (2005) demonstrated that hydrolysis pre-treatment of waste activated sludge increased COD and VS breakdown during digestion by 88.9 and 77.5%, respectively, with a corresponding increase in CH_4 yield of 530 L CH_4/kg VS compared to without pre-treatment. Hydrolysis pre-treatment was not an objective of this study, however, so it is recommended that future analyses examine the effect of ambient temperature control on algal conditions in storage prior to anaerobic digestion.

The use of recirculation heating can improve the digestion process by better homogenizing the digestate. Mixing and recirculation of digestate are common practices in digesters, as agitation prevents solids and bacterial cells from settling out of suspension and improves the frequency of interactions between synergistic bacterial consortia (Lindmark et al. 2014). Low-intensity, intermittent, or partial mixing is known to be beneficial to substrate degradation, especially during the initial stages of digestion (Kariyama et al. 2018; Lindmark et al. 2014). The energy required to operate pumps and engines for digestate homogenization decreases the net energy obtained from the digestion to the point that Kariyama et al. (2018) concluded that an increase in digestion efficiency is not sufficient to offset enough to be worth the sustainability lost from continuous mixing. Continuous mixing has also demonstrated negative effects on microbial communities in digestate by breaking up functional groups of synergistic bacteria with sheer stress (Dapelo and Bridgeman, 2018). Wu (2010) also concluded that recirculation mixing specifically is the least efficient compared to two other common methods of digester homogenization (mechanical mixing and bubbling biogas). Dapelo and Bridgeman (2018) demonstrated that bubbling biogas especially could achieve digestate homogenization with 300 second intervals of active homogenization, without the need for continuous operation.

2.3.5 Mass balance and algal turf scrubber (ATS) scale-up

The C, N, and P content of the algae harvested in Year 1 and Year 2 is summarized in Table 2.9 and 2.10. In Year 1, the ATS generated 326 kg of algae containing 44.7 kg of C, 5.94 kg of N, and 1.13 kg of P sequestered from the Patapsco River, with an average C:N Ratio of 7.99 ± 1.05 : 1. Each L of raw, wet algae thus contained 5.53 ± 0.24 g C, 0.780 ± 0.070 g N, and 0.143 ± 0.026 g P. Effluent from the D1 and D2-D3 systems had an average reduction in total C of 63.9 ± 2.8% and 54.7 ± 4.0%, respectively, compared to the algal influent, which was utilized by bacteria in the reactors to form the CO_2 and CH_4 in the biogas. While the D2-D3 system had a higher HRT, the similar C utilization rate indicates that 26% additional digestion time did not result in higher degradation of organic material in Year 1. In Year 2, the effluent from the D2-D3 system had 99.7 ± 0.1% reduced C in the effluent compared to the influent, indicating near-total breakdown of the C available for anaerobic digestion.

Table 2.9: Results of mass analysis of the algae harvested from the Port of Baltimore ATS in Year 1.

Harvest Week#	Total kg TS	Total kg VS	Kg C	kg Total N	kg Total P	C:N Ratio
1-5*	50.3	11.6	6.65	0.582	0.086	11.4 : 1
6	27.6	6.24	3.57	0.433	0.060	8.26 : 1
7	21.3	5.00	2.87	0.622	0.310	4.62 : 1
8	18.6	4.41	2.55	0.431	0.067	5.91 : 1
9	14.0	3.34	1.94	0.342	0.057	5.69 : 1
10	18.7	4.44	2.60	0.432	0.063	6.02 : 1
11	18.9	4.46	2.62	0.337	0.047	7.76 : 1
12	14.6	3.57	1.96	0.244	0.042	8.02 : 1
13	20.7	4.80	2.39	0.363	0.064	6.57 : 1
14	20.6	4.51	2.64	0.126	0.021	20.9 : 1
15	20.4	5.08	2.81	0.261	0.040	10.8 : 1
16	15.9	3.75	2.22	0.408	0.059	5.43 : 1
17	15.3	3.67	2.02	0.337	0.054	6.00 : 1
18	15.2	3.94	2.47	0.367	0.058	6.73 : 1
19	14.6	3.52	2.14	0.372	0.054	5.75 : 1
20	19.6	5.34	3.25	0.285	0.048	11.4 : 1
Sum	326	77.7	44.7	5.94	1.13	-
Average	18.4 ± 0.9	4.4 ± 0.21	2.54 ± 0.12	0.357 ± 0.029	0.07 ± 0.017	7.99 ± 1.05 : 1

*Algae stockpiled from multiple harvests prior to the start of the digestion experiment.

Table 2.10: Results of mass analysis of the algae harvested from the Port of Baltimore ATS in Year 2.

Harvest Week#	**Total kg TS**	**Total kg VS**	**Kg C**	**kg Total N**	**kg Total P**	**C:N Ratio**
1-4*	6.89	1.61	0.922	0.124	0.004	7.45 : 1
5	25.0	7.05	4.08	1.590	0.439	2.56 : 1
6	39.6	8.19	4.85	0.505	0.077	9.61 : 1
7	8.1	2.24	1.32	0.432	0.049	3.05 : 1
8	27.4	5.42	3.18	0.382	0.060	8.31 : 1
9	8.23	9.26	5.26	0.549	0.088	9.58 : 1
10	NA#	NA#	NA#	NA#	NA#	NA#
11	5.48	1.16	0.66	NA#	NA#	NA#
12	50.8	12.2	6.87	NA#	NA#	NA#
13	31.2	8.36	4.80	0.562	0.086	8.54 : 1
14	26.9	6.10	3.59	0.341	0.078	10.5 : 1
15	34.5	9.36	5.54	0.604	0.130	9.17 : 1
16	15.0	7.99	4.74	0.706	0.120	6.72 : 1
17	16.8	4.72	2.71	0.337	0.056	8.02 : 1
18	37.4	10.7	5.97	0.775	0.131	7.69 : 1
19	32.9	9.45	5.15	0.617	0.107	8.34 : 1
20	27.1	5.49	3.11	0.588	0.114	5.28 : 1
Sum	393	109	62.7	8.11	1.54	-
Average	24.6 ± 3.3	6.83 ± 0.81	3.92 ± 0.46	0.580 ± 0.09	0.110 ± 0.027	7.49 ± 0.68 : 1

*Algae stockpiled from multiple harvests prior to the start of the digestion experiment.

#No data available.

The results of the mass balances for Year 2 were used to predict carbon sequestration and bioenergy yield from a 20-week (140-day) growing season of operations at a hypothetical 1-hectare scale-up of the ATS and AD systems at the Port of Baltimore site (Table 2.11). It is estimated that the biogas produced from the hypothetical site would generate 774 kWh of electricity over 20 weeks (5.53 kWh/day), assuming a 35% conversion efficiency, valued at approximately $115 USD. This is a substantial 389% increase over the 1-ha system modeled by Witarsa et al. (2020) using data from the Port of Baltimore ATS in the prior study at the site due to the higher digestion efficiency observed in Year 2 of this study. It would be 73.8% less kWh/day, however, than Lansing et al. (2008) reported from a generator fueled by biomethane from a digester processing a more conventional manure feedstock. While the predicted dollar value of the electricity from digestion of ATS algae is low, it still has value as a renewable bioenergy that can be used on-site to offset the need for electricity from the power grid which may be derived from fossil fuels. This benefits the sustainability of the bioremediation and bioenergy operations and helps incentivize the installation of ATS for bioremediation of impaired waterways by providing a side benefit to the operator that would not be obtained if the algae were landfilled or disposed of as wastewater in the municipal sewer, as was previously done to dispose of algae from the Port of Baltimore ATS prior to the installation of the digesters in 2017.

Table 2.11: Estimated carbon sequestration and bioenergy generated from a 1-hectare scale-up of the combined algal turf scrubber (ATS) and anaerobic digestion at the Port of Baltimore.

Parameter	**Rate of generation (1 ha ATS)**	**Total per growing season (n = 140 days)**
Wet algae harvested	7230 L/day	1.01 *10^6 L
Dry algae harvested (TS)	32.9 kg/day	4,610 kg
Algae organic material (VS)	9.14 kg/day	1,280 kg
C sequestered	5.25 kg/day	735 kg
CH_4 generated*	1,490 L CH_4/day	2.09 * 10^5 L CH_4
BTU of CH_4 generated[#]	54.7*10^4 BTU/day	7.65 * 10^6 BTU
Electricity generated[#]	5.53 kWh/day	774 kWh
Value of electricity[#]	$0.83/day	$115.00

*Value calculated using 163 L CH4/kg VS measured from D2-D3 in Year 2 of the pilot-scale digestion at the Port of Baltimore, with VS generation of 9.14 kg/ha/day.

[#] Assumes conversion of CH_4 into electricity in a generator with 35% efficiency. Conversion factors from United States Energy Information Administration's energy calculation tools (USEIA 2023a; USEIA 2023b; USEIA 2023c)

One challenge to scale-up operations for the Port of Baltimore site is the high ash content (~75%$_{\mathrm{DW}}$) of the algae observed in both Year 1 and Year 2, which indicated that over half of the solids in the biomass slurry were non-digestible and may have contributed to the regular pipe clogging during Year 1. This is attributable in part to the biogenic silica in the frustules of the diatoms that made up most of the algal culture, which persisted through digestion without breaking down (Figures 2.6 and 2.7). The high ash content is consistent with that measured by Witarsa et al. (2020) during the previous year, (80.5%$_{\mathrm{DW}}$) when the turf was also dominated with diatoms. Biogenic silica was not quantified directly, however the ~30% ash content reported by Bohutskyi et al. (2016) from an ATS algae dominated by Chlorophytes (e.g. *Cladophora* sp., *Rhizonium* sp.) that lack a silica frustule suggests that over half of the ash content could be attributable to silica. The ash content was also high compared to conventional anaerobic digestion feedstocks, such as dairy manure (20-60%$_{\mathrm{DW}}$), poultry litter (25-45%$_{\mathrm{DW}}$), and food waste (<10%$_{\mathrm{DW}}$) (Hidalgo-Sanchez et al. 2023; Lisboa and Lansing 2013; Lisboa and Lansing 2014; Pin Viso et al. 2022; Yarberry et al. 2019).

Figure 2.6: Scanning electron microscope (SEM) imagery of a sample of algal turf scrubber (ATS) algae pre-digestion collected during harvest week 6 of Year 1. Diatom frustules are visible throughout the image.

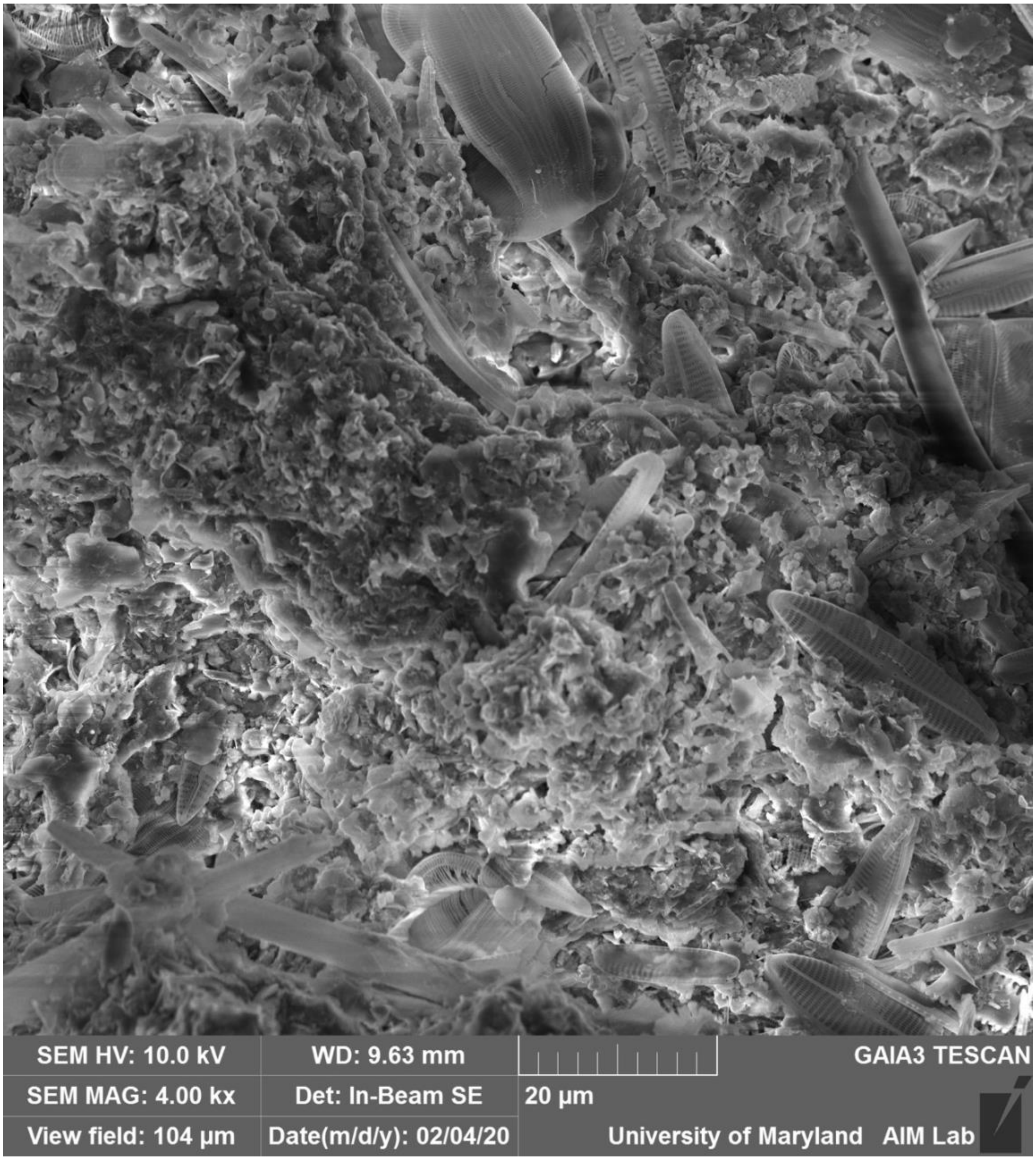


Figure 2.7: Scanning electron microscope (SEM) imagery of a sample of effluent from digester 1 (D1) collected on day 21 of digester operations in Year 1. Undigested diatom frustules are visible at right.

Strategies to harvest intercellular products of diatoms include ultrasonication and pre-treatments using thermal and chemical disruption (Ruiz-Dominguez et al. 2022; Van Eynde et al. 2014; Yatipanthalawa et al.; 2022). For example, Ruiz-Dominguez et al. (2022) reported effective recovery of fucoxanthin via supercritical fluid extraction pre-treatment of diatoms prior to anaerobic digestion, however post-extraction the methane potential of the cells was reduced by 26.5%. Thermohydrolysis may also be an effective approach for future studies to degrade the silica and more easily digest the organic fraction of the cells into bioenergy and has shown promising results in enhancing alcoholic fermentation of cyanobacteria to increase yields of bioethanol by up to 98% compared to a raw control (Nowicka et al. 2019).

2.4 Conclusions

Algae from the pilot-scale ATS on the Patapsco River at the Port of Baltimore was successfully converted into bioenergy via anaerobic digestion over two growing seasons. The results indicate that the productivity of biomass from an ATS each year fundamentally limits the amount of bioenergy that can be derived from digestion. This rate of productivity can vary greatly between years, with the second year of this study yielding 133% of the harvest volume from than the previous year.

The species that colonize the “turf” of an ATS appear to be consistent within systems in the same watershed, however the relative dominance of species present may vary depending on the water source and site. Some of those species, such as the *Melosira* sp. diatoms that made up much of the algal culture used in this experiment, have high ash content due to non-digestible components and may benefit from longer anaerobic digestion times.

Longer digestion time in the D2-D3 system was associated with increased with 6.84% higher CH_4 yield compared to D1 in Year 1, although the average 26% difference in HRT between the systems was insufficient to observe a statistically significant difference between the two. In Year 2, the addition of a recirculation and heating system in D2 was associated with a large increase in CH_4 yield, High ambient temperatures were also associated with 224% more L CH_4 generated and 140% greater digestion efficiency. This was attributed to the addition of heating or mixing improving digestion stability and organic substrate utilization.

Longer HRT, heating, and mixing benefited the efficiency of ATS algae digestion and energy generated. The CH_4 yield from digestion of ATS algae is low compared to agricultural feedstocks, so it is important to optimize the process to maximize the generation of the algae as a value-added product. It is recommended that planning for new ATS systems using digestion as the management strategy begin with tests of small-scale (~1 m^2) ATS systems on the proposed site to characterize the expected “turf” and adjust digestion parameters accordingly. Co-digestion in existing digestion infrastructure with conventional digestion feedstocks could also be considered as an alternative to on-site mono-substrate algae digestion, which could reduce variability of bioenergy yield from irregular harvests.

Chapter 3: Co-digestion of algae from algal flow ways in farm-based digesters

3.1 Introduction

In an algal flow way (ATS), water from a eutrophic body of water is pumped across a shallow flow-way cultivation system with a textured lining. In-situ algal species capable of forming periphyton colonize the lining and develop into a biofilm fed by nutrients in the eutrophic water (Adey 1982). Thus, a contained algal “bloom” is created on land that continuously metabolizes the dissolved pollution upstream to reduce impairments to downstream water quality. The algal biomass is periodically harvested from the ATS to maintain continuous algal growth and uptake of pollutants (Adey et al. 2013). The algal byproduct contains sequestered pollutants as well as settled sediments. The ATS can contribute to nutrient load reduction in watersheds with high levels of nitrogen and phosphorus pollution, such as the Chesapeake Bay Watershed.

The Chesapeake Bay is the largest estuary in the United States of America, with a watershed draining 175,000 km^2 of land within six states and the District of Columbia, including 34,000 km^2 containing 87,000 farms used to grow crops such as corn, soy, wheat, hay, pasture, vegetables, and fruit (USEPA 2010b). A 2015 assessment of animal agriculture statistics for the same area estimated that up to 58,000 of the farms in the region may include poultry or livestock operations (USEPA 2015a). This is important for nutrient management in the region because fertilizer and animal waste can be washed away by runoff during precipitation events and containment is needed to keep compliance with discharge regulations.

Point source nutrient pollution was addressed in 2010 by the United States Environmental Protection Agency (USEPA) in the Total Maximum Daily Load (TMDL) plan for the Chesapeake Bay Watershed (USEPA 2010a). The TMDL regulations for the watershed set annual discharge limits for N and P discharges across the entire watershed were set at 84.3 million and 5.67 million kg, respectively, while sediment was to be limited to 2.95 billion kg. If adhered to, these restrictions represented respective declines of 25, 24, and 20% of N, P, and sediment, respectively, from annual discharges reported in when the TMDL was implemented in 2010 (USEPA 2010a). In its midpoint assessment of Maryland's progress on implementing TMDL programs, the USEPA assessed that the state had failed to meet its midpoint goals for N reduction due to missed milestones in the agricultural sector and unexpected changes in agricultural production (USEPA 2018).

The ATS is included in the Chesapeake Assessment Scenario Tool (CAST) modeling program that states use to verify their individual TMDL (Chesapeake Bay Program, 2020). In Maryland, ATS are additionally eligible for incentives through the state's Water Quality Trading Program (MD Code Regs. 26.08.11; MDE, 2018; MDE et al. 2019; Witarsa et al. 2020). However, ATS have so far been considered primarily an urban nutrient remediation system with limited use in the agricultural sector. Urban ATS installations allow otherwise unproductive land in developed areas such as decommissioned docks, unused parking lots, and capped waste sites to be used for bioremediation. They also usually have established infrastructure (e.g. electricity) already in place nearby to support the ATS. The USEPA expert panel on ATS did consider both urban and agricultural ATS in its recommendations to the Chesapeake Bay Program on how to implement ATS and verify their effectiveness, however the panel could not agree on guidance for agricultural ATS due to the variability in nutrient output and management plans between farms and a lack of data available on agricultural ATS (USEPA 2015b). The largest urban areas in Maryland are sited at the mouths of Chesapeake Bay tributaries, and a 2010 assessment of land use in Maryland indicated that "developed land" covered a smaller area (673,000 ha) than "agriculture" land (772,000 ha), and only 26.7% of the total land area in the state (MDP 2010).

Limiting ATS to urban and developed areas would prevent 30.6% of land covered by agriculture to also benefit from its non-point source remediation efforts and state incentive programs, despite the literature indicating that ATS can also be used in an agricultural context (MDP 2010). Kangas and Mulbry (2014) demonstrated that ATS were capable of remediating 125 mg N/m^2/day and 25 mg P/m^2/day from an agricultural drainage ditch at peak flow. Kebede-Westhead et al. (2004) utilized ATS as secondary treatment for nutrient remediation of dairy manure anaerobic digester effluent with further elemental analysis, and documented uptake of Al (1100 mg/kg), Ca (9700 mg/kg, Cd (0.43 mg/kg), Cu (56 mg/kg), Fe (580 mg/kg), Pb (5.0 mg/kg), Mg (2300 mg/kg), Mn (240 mg/kg), Mo (3.0 mg/kg), K (14,700 mg/kg), Si (210 mg/kg), and Zn (290 mg/kg). Both cited papers indicated that the rate of ATS sequestration of N and P was associated increased loading rates, and Kebede-Westhead et al. (2004) showed the same association between loading rate and algal uptake of Al, Ca, Cu, Fe, Mg, Mn, and Zn (Kangas and Mulbry, 2014). Mulbry et al. (2008) indicated that ATS bioremediation of dairy manure digester effluent could generate up to 25 g/m^2/day of algae at an N loading rate of 2500 mg N/m^2/day.

The algal byproduct from the ATS could be disposed of using on-farm anaerobic digestion to convert the carbon in the biomass into bioenergy. The process of anaerobic digestion (AD) utilizes a series of bacterial processes (hydrolysis, acidogenesis, acetogenesis, and methanogenesis) that occur in the absence of oxygen to break down organic wastes into biogas containing methane (CH_4), which may be used to generate energy (Gerardi 2003). Nutrients in the waste remain in the liquid digestate of the reactor in aqueous form and is typically recycled on to crop fields as fertilizer. The United States Department of Agriculture has documented 65 functional digesters in the Chesapeake Bay watershed as of May 2022 for processing food and agricultural wastes (USEPA 2022).

Anaerobic digestion is non-selective in converting carbon components of waste into bioenergy and is well-suited to a complex feedstock like ATS algae, but research on the topic has been limited (Bohutskyi et al. 2016; Witarsa et al. 2020; Yue et al. 2014). Bohutskyi et al. (2016) produced ~200 L CH_4/kg VS volatile solids (VS) from laboratory-scale digestion of ATS biomass as well as algal residues left over from biodiesel production. Witarsa et al. (2020) reported laboratory-scale batch, laboratory-scale semi-continuous, and pilot-scale AD studies with 158, 144, and 107 L CH_4/kg VS, respectively, for algae generated by a brackish ATS on the Patapsco River at the Port of Baltimore in Maryland. While the efficiency of CH_4 generated per mass of organic material are comparable to CH_4 yield from more conventional digestion feedstocks, the high moisture content of the algae in these studies (>90%) suggests that the algae is not a productive substrate when digested alone on a per total wet biomass basis. Furthermore, the rate of biomass generation from ATS can vary seasonally with changes in temperature and sunlight availability so it is unlikely that ATS algae alone would be sufficient to sustain year-round operation of an anaerobic digester at commercial scale in temperate climates (Adey et al. 2013).

This study explored feasibility of integrating ATS algae waste into existing digestion infrastructure as a co-digestion feedstock. Lisboa and Lansing (2013) increased CH_4 yield by 67-2940% from dairy manure by incorporating food waste into the digester as a co-digestion substrate. Previous studies have indicated that co-digestion of algae with other wastes could increase CH_4 yield from algae by up to 40% (Astals et al. 2015; Lu and Zhang 2016). Most previous research has only focused on co-digestion of waste with mono-cultured algae, however, which are not directly comparable to the heterogenous multi-species cultures of in-situ taxa that are generated by ATS.

Three wastes already used as feedstocks for AD in Chesapeake Bay Watershed (dairy manure, food waste, and poultry litter) were selected as co-digestion substrates for a laboratory-scale batch reactor experiment (USEPA 2022). A follow up plant growth study used reserved effluent from the batch reactors to fertilize lettuce plants to determine if metals known to accumulate in ATS will be passed on to plants receiving land-applied reactor effluent. The goal was to determine if ATS algae could be added to established agricultural digesters without significantly negatively impacting the CH_4 yield to the farmer or the health of their crops receiving effluent from co-digestion of ATS algae.

3.2 Materials and methods

3.2.1 Collection and characterization of algae and waste co-digestion feedstocks

A 71 x 168 cm (1.2 m^2) ATS sited on the Anacostia River at Bladensburg Waterfront Park in Bladensburg, Maryland was used to supply algae (Figure 3.1). The ATS was activated in September and run for one month with harvests every ten days, with the algal biomass used in this study collected at the end of the one-month operation and frozen at -20°C before use in the co-digestion studies. Microscopy was used to identify the filamentous diatom Melosira sp. (~70%) and the chlorophyte Spyrogyra sp. (~30%) as the dominant species in this culture (Figure 3.2).

Figure 3.1: Small-scale algal flow way (ATS) system on the Anacostia River used to supply algae for this experiment, before (left) and after (right) 10 days of biofilm growth.

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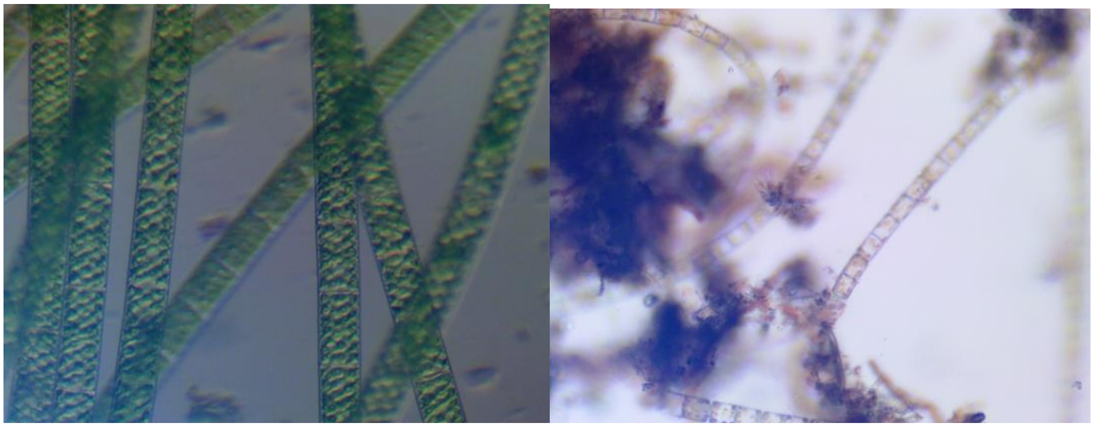


Figure 3.2: Microscope images of *Spyrogyra* sp. (left) and *Melosira* sp. (right) taken from samples of the algal biofilm from the Bladensburg Waterfront Park algal flow way (ATS) system.

Dairy manure (DM) was collected from a dairy farm in Beltsville, Maryland that utilized a scrape manure collection system and was refrigerated for 24-48 h before experimental use. Food waste (FW) was prepared using the methods described in Yarberry et al. (2019), which consisted of 506 g of white bread, 907 g of pork and beans, and 176 g of potato flakes that were homogenized via blending and refrigerated for 24-48 h before experimental use. Poultry litter (PL) was collected from a poultry farm on the Eastern Shore of Maryland and frozen at -20°C before use in the co-digestion studies. All biomass substrates were tested for TS and VS according to APHA methods 2540B and 2540E, respectively, prior to refrigeration or freezing (APHA et al. 2005) (Table 3.1).

Table 3.1: Total solids (TS) and volatile solids (VS) of algae and waste biomass substrates used in the batch digestion experiments. Values given are mean % wet weight ± standard error (n = 3).

Material	**TS (%)**	**VS (%)**
Algae	4.82 ± 0.02	0.61 ± 0.00
Dairy Manure (DM)	15.4 ± 0.0	13.3 ± 0.1
Food Waste (FW)	45.3 ± 0.3	43.6 ± 0.2
Poultry Litter (PL)	71.0 ± 0.1	50.0 ± 0.3

3.2.2 Collection and preparation of anaerobic digestion (AD) inoculum

The AD inoculum culture was a mixture from two source: 1) liquid inoculum (TS = 1.11} 0.00%, VS = 0.71 } 0.00%) supplied from a continuous laboratory digester at the University of Maryland (College Park, Maryland US) inoculated with dairy manure and fed with glucose powder, and 2) dewatered Class A biosolid known as "BLOOM" (TS = 33.8 } 0.4%, VS = 17.7 } 0.2%) generated by thermohydrolysis and anaerobic digestion at Blue Plains Advanced Wastewater Treatment Plant in Washington, DC (US) (Alvarez-Campos and Evanylo 2019; DC Water 2020). The inoculum mixture used in the experiment was prepared in batches consisting of 1110 g of liquid inoculum and 390 g of solid inoculum, stirred until homogenous (TS = 8.48 ± 0.09%, VS = 4.53 ± 0.04%).

3.2.3 Batch reactor anaerobic digestion (AD) experiment setup

The batch reactors were 250 mL glass AD reactors for all treatments except algae only (bottles #4-6), which were digested in 500 mL glass AD reactors due to the high mass of algae (264.228 g) needed to obtain a 2:1 inoculum:substrate loading ratio (Figure 3.3). Each AD reactor was loaded with 5 g total VS at a 2:1 inoculum: substrate ratio, with two parts (3.333 g VS) supplied by the inoculum and one part (1.667 g VS) by the experimental substrate. There were 17 treatments tested in triplicate, with four mono-substrate treatments for the algae-only, DM-only, FW-only, and PL-only, and twelve co-digestion treatments consisting of algae + waste mixed at a 1:10, 1:5, 1:2, or 1:1 ratio by VS mass. A triplicate control treatment was also included to obtain a baseline of biogas production from the organic material in the inoculum alone, as it was used to seed all other treatments. During data analysis, the baseline CH_4 generation from the control was subtracted from that of the experimental reactors to measure only the bioenergy generated from digestion of the substrate being tested (Table 3.2.)

Figure 3.3: Example of a batch-scale reactor experiment setup using glass bottles, with rubber septa attached.

Table 3.2: Experimental Setup of the batch scale reactor test. Reactors 1-3 were loaded with inoculum only to provide a baseline of biogas and methane production, 4-15 were mono-substrate reactors, and 16-48 co-digestion reactors with different ratios of algae:waste by VS. Dairy Manure, FW = Food Waste, and PL = Poultry Litter.

Reactor #	Treatment	Inoculum (g)	Algae (g)	DM (g)	FW (g)	PL (g)	Total Substrate Mass (g)	Total Mass (g)
1-3	Inoculum only	73.6	-	-	-	-	0	73.6
4 – 6	Algae only	73.6	264	-	-	-	264	338
7-9	DM only	73.6	-	12.5	-	-	12.5	86.2
10-12	FW only	73.6	-	-	3.85	-	3.85	77.5
13-15	PL only	73.6	-	-	-	3.33	3.33	73.6
16-18	Algae+DM 1:1	73.6	132	6.27	-	-	138	212
19-21	Algae+DM 1:2	73.6	88.1	8.36	-	-	96.4	170
22-24	Algae+DM 1:5	73.6	44.0	10.5	-	-	54.5	128
25-27	Algae+DM 1:10	73.6	24.0	11.4	-	-	35.4	109
28-30	Algae+FW 1:1	73.6	132	-	1.92	-	134	208
31-33	Algae+FW 1:2	73.6	88.1	-	2.57	-	90.6	164
34-36	Algae+FW 1:5	73.6	44.0	-	3.21	-	47.2	121
37-39	Algae+FW 1:10	73.6	24.0	-	3.50	-	27.5	101
40-42	Algae+PL 1:1	73.6	132	-	-	1.67	133	206
43-45	Algae+PL 1:2	73.6	88.1	-	-	2.22	90.3	162
46-48	Algae+PL 1:5	73.6	44.0	-	-	2.78	46.8	118
49-51	Algae+PL 1:10	73.6	24.0	-	-	3.03	27.1	97.7

After each bottle was loaded with inoculum and substrate, the headspace was flushed with nitrogen gas (N_2) for 30 seconds to displace oxygen in the headspace and create an anaerobic environment. Rubber self-healing septa were used to create an airtight seal and reinforced with sealant (Silicone Max, DAP Products, Baltimore, Maryland) to prevent leaks. Reactors were incubated in the dark at mesophilic temperatures (35°C) with continuous agitation (120 rpm) for 62 days.

3.2.4 Biogas analysis

Biogas volume was measured using a 50 mL wet-tipped frictionless glass syringe to extract accumulated biogas in the headspace of each reactor. The gas pressure in the reactor and syringe barrel equalizes when the needle is inserted into the rubber septa, which displaces the plunger to a volume equal to the volume of gas present in the reactor. Measurements were taken every 4-6 hours during the first 48 hours of incubation, every 12 hours during the next 48 hours, daily, and then reduced to every other day and bi-weekly, as biogas production declined.

Following each biogas volume measurement, gas composition was also measured using an Agilent 7890A gas chromatograph (GC) to determine the concentration of CH_4 and carbon dioxide (CO_2) according to published protocols (Achi et al. 2020; Lisboa and Lansing, 2013; Witarsa et al. 2020). The GC was equipped with a thermal conductivity detector (TCD) and porous layer open tubular (PLOT) Q column. The injection and detector temperatures were 250°C, oven temperature was 60°C, and flow of He carrier gas was 8.6 mL/min. After gas analysis, each bottle’s self-healing septa was reinforced with silicone gel to minimize leaks and maintain the anaerobic environment inside the bottle. The percentage of CH_4 in the biogas was multiplied by its volume to determine the volume of CH_4 generated.

3.2.5 Liquid sample analysis

The pH of the reactor fluid was tested pre- and post-digestion using an Accumet AB15 meter. Samples used in volatile fatty acid (VFA) and ammonium (NH_4) analyses were acidified to pH <2.0 immediately after collection using 1.5 N sulfuric acid. The TS and VS were measured according to APHA Methods 2540B and 2540E, respectively (APHA et al. 2005) of the individual inoculum, algae, and waste fed to each reactor. Post-digestion TS and VS values were measured directly from combined substrates at the end of the experiment. The VFA analyses were conducted using a Agilent 7890A GC equipped with a flame ionization detector (FID) and DB-FFAP capillary column to determine the specific organic acids. The injection temperature was 250°C, detector temperature 300°C, oven temperature 106.5°C, and carrier gas flow (10.6% H, 85.1% air, 4.3% He) 1.8 mL/min.

Pre- and post-digestion ammonium (NH_4) in the liquid was analyzed using a Lachet QuickChem 8500 Series 2 flow meter (Hach, Loveland, CO, USA) and QuickChem Method 10-107-06-2-O. Frozen samples were sent to an external laboratory (Agrolab, Inc., Harrington, DE) for total nitrogen (N), total phosphorus (P), nitrate (NO_3), C:N ratio, and mineral analyses to obtain a complete chemical profile of the influent and effluent. The remaining effluent after analysis was composited for each set of triplicate reactors and frozen at -20°C for use in a lettuce growth experiment.

3.2.6 Lettuce growth experiment

Diluted digested effluent from the batch experiments was used in an indoor plant growth experiment. Buttercrunch lettuce was used as a model organism in this experiment due to its rapid growth and ease of growth in a greenhouse environment. All plant cultivation was conducted at the University of Maryland Research Greenhouse complex at the College Park campus.

The lettuce was grown in 4.5” square plastic pots with 4.5” rock wool cubes (Cultilene, Rijen, Netherlands) as growth media. All blocks were rinsed and saturated with water prior to use to wash away particles and residues from manufacturing. The pots connected to an automatic watering system that delivered 100 mL of water to each pot 1-2 times daily, according to the needs of the plant. Each block was planted with two seeds, watered thoroughly, and allowed to germinate for 48-36 hours. The plants were then allowed to develop for approximately 10 days, until secondary leaves began to show, at which point seedlings were thinned to 1 plant per pot.

The AD effluent was diluted 8-fold to create fertilizer, which was applied to each pot immediately after thinning by pipetting 50 mL of liquid to the roots of the seedlings. All fertilization tests were tested using four replicate pots. Light was supplied on a 16:8 hour light:dark cycle by the greenhouse’s high-intensity discharge (HID) lamps on a timer. The greenhouse’s automatic climate control was set to maintain a constant temperature of 20°C.

The plants were grown for two months until they reached maturity. The automatic watering was stopped 12 hours prior to harvest allow excess water to drain. Each whole plant was cut at the point the roots entered the rock wool, placed in a paper bag, and dried in an oven at 50°C for four days. Replicate plants from each treatment were composited, stored at room temperature inside the drying bags and, sub-samples from each composite were sent to an external laboratory (Agrolab Inc., Harrington, DE) for plant tissue analysis of elemental composition.

3.2.7 Statistical analysis

All data analysis was done in RStudio. The volume of CH_4 generated from mesophilic digestion at 35°C (after subtracting inoculum biogas production) was normalized according to two parameters: the mass of VS (i.e., organic material) in each reactor (1.667 g) and the total mass of substrate loaded, which varied according to the treatment (Table 2.2). The VS normalization shows the efficiency of bioenergy production from an AD substrate, while the mass normalization show the bioenergy production in terms of the total mass of substrate, including water content which does not convert to bioenergy. ANOVA was used to find if there is a significant difference in CH_4 volume produced between each of the different mono- and co-digestion treatments. Tukey-Kramer HSD post-hoc analysis was used to provide pairwise comparisons for all treatments.

3.3 Results and discussion

3.3.1 Reactor solids, organics, and pH analyses

The algae had the highest water content of the four substrates digested (4.82 ± 0.02% TS and 0.61 ± 0.00% VS), followed by DM (TS = 15.4 ± 0.0%, VS = 13.3 ± 0.1%), FW (TS = 45.3 ± 0.3% TS, VS = 43.6 ± 0.2%), and PL (TS = 71.0 ± 0.1%, VS = 50.0 ± 0.3%). Due to the low VS concentration in the algae, a larger mass was needed to provide the necessary mass of VS to reactors. This effect was most pronounced in 1:1 co-digestion reactors, for example algae+DM 1:1 was loaded with 132.11 g of algae and 6.27 g DM for a 1:1 ratio by VS (Tables 3.1 and 3.2).

The high water content had a clear dilution effect in the co-digestion reactors, such as algae+FW 1:10 (TS = 8.88%, VS = 4.95%) and the lowest in algae+FW 1:1 (TS = 6.49% TS, VS = 2.40%) (Table 3.3). Post-digestion, the algae mono-substrate treatment (VS = 0.103 ± 0.012%)

had 93% VS reduction compared to pre-digestion, followed by FW (VS = 3.17 ± 0.92%; 51% change), DM (VS = 4.25 ± 0.09%; 27% change), and PL (VS = 4.85 ± 0.13%; 25% change). In the co-digestion treatments, the change in VS varied from 40-74% for algae+DM reactors, 36-58% for algae+FW reactors, and 24-31% for algae+PL reactors. The dilution effect was also observable during VFA analysis of the pre-digestion reactors. While each reactor had the same mass of VS added initially, VFA concentrations ranged from 497 mg/L in the algae-only reactor to 1310 mg/L in the DM only reactor. The addition of algae appeared to have a strong dilution effect that increased with the addition of more algae, mirroring the results of the TS and VS analyses. The magnitude of the dilution effect was approximately 2-fold between the mono-substrate DM, FW, and PL reactors and their respective algae+waste 1:1 co-digestion reactor (Figure 3.4).

Table 3.3: The total solids (TS) and volatile solids (VS) of the laboratory-scale reactors before and after 62 days of digestion. The initial TS and VS loaded in each reactor was calculated from the data in Table 3.1, while final the TS and VS values are shown as the mean ± standard error of the triplicate reactors from Table 3.2. DM = Dairy Manure, FW = Food Waste, and PL = Poultry Litter.

	TS (% wet weight)		**VS (% wet weight)**	
Treatment	**Initial**	**Final**	**Initial**	**Final**
Mixed Inoculum	8.48	8.15 ± 0.53	4.53	3.99 ± 0.24
Algae	5.62	0.239 ± 0.025	1.47	0.103 ± 0.012
DM	9.49	8.08 ± 0.19	5.80	4.25 ± 0.09
FW	10.3	7.41 ± 0.97	6.47	3.17 ± 0.92
PL	11.7	9.45 ± 0.23	6.79	4.85 ± 0.13
Algae+DM 1:1	6.40	3.04 ± 2.67	2.35	0.94 ± 0.81
Algae+DM 1:2	6.93	2.08 ± 1.55	2.93	0.75 ± 0.54
Algae+DM 1:5	7.79	5.71 ± 1.05	3.90	2.34 ± 0.43
Algae+DM 1:10	8.40	4.06 ± 1.71	4.58	1.90 ± 0.81
Algae+FW 1:1	6.49	3.35 ± 1.55	2.40	1.01 ± 0.45
Algae+FW 1:2	7.09	5.83 ± 0.27	3.04	1.94 ± 0.12
Algae+FW 1:5	8.12	6.84 ± 0.13	4.14	2.68 ± 0.05
Algae+FW 1:10	8.88	7.16 ± 0.16	4.95	3.08 ± 0.09
Algae+PL 1:1	6.70	5.99 ± 0.30	2.42	1.83 ± 0.09
Algae+PL 1:2	7.46	6.23 ± 0.11	3.08	2.10 ± 0.07
Algae+PL 1:5	8.79	7.61 ± 0.23	4.24	3.11 ± 0.11
Algae+PL 1:10	9.78	8.70 ± 0.31	5.12	3.76 ± 0.08

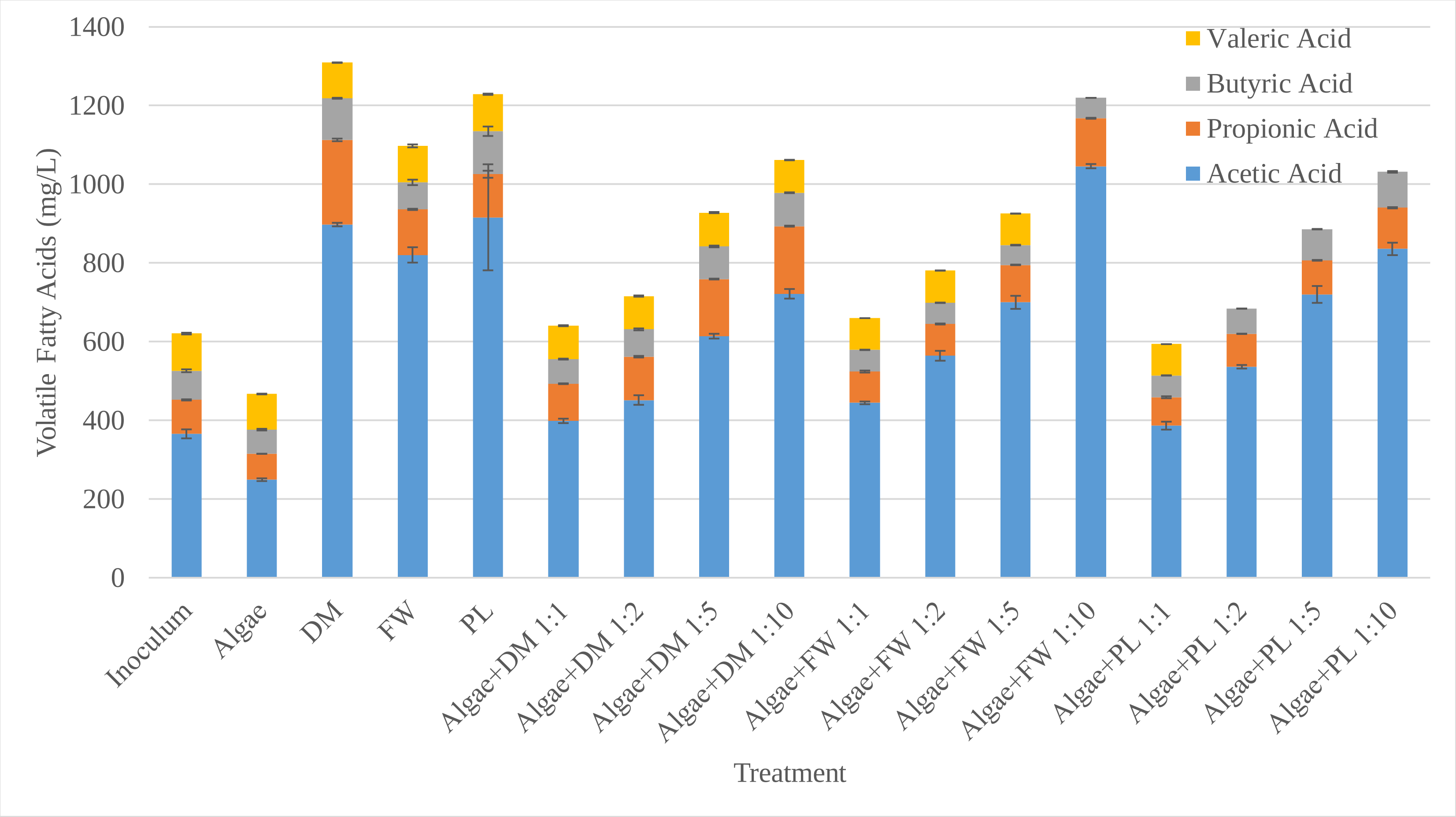


Figure 3:4 Results of the volatile fatty acid (VFA) analysis on pre-digestion samples from the batch reactor experiment. DM = Dairy Manure, FW = Food Waste, and PL = Poultry Litter.

The water content of algae, especially microalgal cells in suspended culture, is a known impediment to its use in biomass-to-bioenergy, especially at scale. In the context of anaerobic digestion, excess water in an anaerobic digester is non-productive mass that takes up space and reduces the energy gained per volume of waste processed. Further, dilution slows chemical reactions by reducing interactions between the digestate bacteria and the organic material in the substrates. The volume taken up by substrates with high water content also increases the cost and logistical challenges of storage pre-digestion (Li et al. 2014). Asam et al. (2011) yielded only 10 L CH_4/kg substrate from digestion of raw pig slurry compared to 55 L CH_4/kg substrate from slurry that had undergone solids separation. Wall et al. (2012) also demonstrated that dewatered food waste required less energy to transport to an anaerobic digestion site, however co-digestion of dewatered food waste with manure resulted in low digestion efficiency (30% of VS utilized) compared to digesting manure alone due to the high solids reducing the rate of hydrolysis in the reactor.

A balance is needed between the low and high solids substrates in the reactor, to reach the ideal for wet digestion (~10%) to optimize CH_4 yield (Tumusiime et al. 2022). The mono-substrate algae reactor had a TS of just 5.62% compared to the manure, FW, however the 1:10 co-digestion reactors had TS close to the ideal (8.40-9.47%).

The pH of all reactors after the inoculum and substrates were combined was neutral (7.27-7.47), except the FW reactors, which were slightly acidic pre-digestion (6.32 - 6.85), with all reactors neutral by the end of the experiment (7.02-7.59). The co-digestion reactors with 1:1 algae:waste were almost neutral post-digestion (7.07-7.16) (Table 3.4).

Table 3.4: pH of lab-scale reactors before and after 62 days of incubation. Initial pH was n = 1, while the final pH is shown as the mean ± standard error of the triplicate reactors (n = 3). DM = Dairy Manure, FW = Food Waste, and PL = Poultry Litter.

Treatment	Initial pH	Final pH
Mixed Inoculum	7.47	7.59 ± 0.03
Algae	7.31	7.02 ± 0.04
DM	7.27	7.40 ± 0.03
FW	6.58	7.57 ± 0.02
PL	7.38	7.58 ± 0.04
Algae+DM 1:1	7.38	7.07 ± 0.02
Algae+DM 1:2	7.30	7.20 ± 0.01
Algae+DM 1:5	7.38	7.34 ± 0.02
Algae+DM 1:10	7.38	7.34 ± 0.01
Algae+FW 1:1	6.85	7.11 ± 0.01
Algae+FW 1:2	6.62	7.26 ± 0.03
Algae+FW 1:5	6.58	7.42 ± 0.03
Algae+FW 1:10	6.32	7.47 ± 0.01
Algae+PL 1:1	7.45	7.16 ± 0.02
Algae+PL 1:2	7.40	7.26 ± 0.01
Algae+PL 1:5	7.33	7.48 ± 0.02
Algae+PL 1:10	7.34	7.54 ± 0.01

3.3.2 Ammonium analysis

Of the mono-substrate reactors, PL had the highest initial concentration of NH_4 (1650 ± 25 mg N/L), followed by DM (1390 ± 13 mg N/L), FW (1320 ± 30 mg N/L) and algae (325 ± 3 mg N/L). The NH_4 in the algae reactors was low despite the high concentration present in the inoculum added to all treatments (1500 ± 29 mg N/L), which highlights the strong dilution effect observed during the solids and organics analyses. The co-digestion reactors containing PL had the highest NH_4 concentration overall (682 – 1450 mg/L), followed by the DM reactors (623 – 1030 mg/L), and FW reactors (493 – 1080 mg/L). Post-digestion, the ammonium increased in all the reactors, which was expected due to the conversion of organic N to ammonium ions during the AD process. (Gerardi 2003) (Table 3.5).

Table 3.5: Ammonium (NH_4) data from the batch reactor experiment (n = 3). Values given are as mean ± standard error. DM = Dairy Manure, FW = Food Waste, and PL = Poultry Litter.

Treatment	**Pre-Digestion NH_4 (mg N/L)**	**Post-Digestion NH_4 (mg N/L)**
Mixed Inoculum	1500 ± 29	1800 ± 46
Algae	325 ± 3	531 ± 9
DM	1390 ± 13	1850 ± 52
FW	1320 ± 30	2200 ± 97
PL	1650 ± 25	2500 ± 31
Algae+DM 1:1	623 ± 11	841 ± 33
Algae+DM 1:2	684 ± 4	957 ± 30
Algae+DM 1:5	850 ± 21	1270 ± 24
Algae+DM 1:10	1030 ± 37	1430 ± 73
Algae+FW 1:1	493 ± 8	824 ± 29
Algae+FW 1:2	668 ± 8	1020 ± 40
Algae+FW 1:5	983 ± 22	1480 ± 18
Algae+FW 1:10	1080 ± 23	1760 ± 120
Algae+PL 1:1	682 ± 10	905 ± 25
Algae+PL 1:2	940 ± 48	1060 ± 11
Algae+PL 1:5	1500 ± 67	1580 ± 35
Algae+PL 1:10	1450 ± 48	1990 ± 90

The accumulation of ammonium molecules in reactor digestate can have a toxic effect on the microbes in an anaerobic digester and has been associated with inhibited methanogenesis at concentrations ~1900-2000 mg/L without preemptive sludge adaptation or corrective action (Koster 1983; Yenigun and Demirel 2013). The mono-substrate FW and PL treatments exceeded this threshold (2200 ± 97 and 2500 ± 31 mg N/L, respectively) while DW approached it (1850 ± 52 mg N/L). The dilution effect of the algae appears to have been beneficial in this experiment to prevent the ammonium concentration from reaching this threshold during co-digestion, as none of those treatments exceeded the 1900 mg N/L inhibition level except the algae+PL 1:10 co-digestion reactor (1990 ± 90 mg N/L), indicating that PL specifically may benefit from higher algae loading for ammonium control.

3.3.4 Methane production

A summary of biogas and CH_4 production data from the batch reactor experiment is provided in Table 3.6 normalized by both the mass of substrate VS fed to each reactor (1.667 g for all treatments) and the total mass of substrate fed to each reactor, which varied by treatment (Table 3.2). The concentration of CH_4 in the biogas increased at a similar rate for all reactors, stabilizing around Day 10 and remaining above 50% for all mono- and co-digestion substrate treatments until the end of the experiment. The average CH_4 concentration was also similar between all treatments (48.9-58.6%). Of the mono-substrate treatments, the algae-only reactors had the highest peak CH_4 concentration (73.5%), while the highest peak of the co-digestion treatments was algae+DM 1:1 (77.3%), nearly 20% higher than peak generation from algae:DM 1:10 treatment (60.1 %). For the DM reactors, the addition of algae appears to have increased the percent CH_4 in the biogas, creating higher quality biogas for use in energy generation. The FW and PL reactors did not exhibit this trend, however, and peak CH_4 concentrations were similar between their respective mono-digestion reactors (FW 72.1%, PL 70.4%) and co-digestion reactors (FW 70.2-73.8%, PL 66.4-70.2%) (Figures 3.5 and 3.6).

Table 3.6: Summary of biogas and CH_4 generation during batch reactor digestion. All values are the average of data from n = 3 reactors over 62 days of incubation, ± standard error. DM = dairy manure, FW = food waste, PL = poultry litter.

Treatment ID	**Total Biogas (mL)**	**mL Biogas/g VS**	**mL Biogas/ g substrate**	**Average CH_4 Concentration (%)**	**Total CH_4 (mL)**	**mL CH_4/ g VS**	**mL CH_4/g substrate**
Algae	230 ± 10	138 ± 6	0.87 ± 0.04	54 ± 3.6	182 ± 7	109 ± 4	0.687 ± 0.025
DM	897 ± 22	538 ± 13	71.5 ± 1.8	49.8 ± 3	498 ± 14	299 ± 8	39.7 ± 1.1
FW	1280 ± 43	768 ± 26	333 ± 11	48.9 ± 4.2	640 ± 39	384 ± 23	166 ± 10
PL	927 ± 20	556 ± 12	278 ± 6	53.1 ± 3.3	504 ± 12	302 ± 7	151 ± 4
Algae+DM 1:1	522 ± 29	313 ± 17	3.77 ± 0.21	58.6 ± 3.3	422 ± 23	253 ± 14	3.05 ± 0.17
Algae+DM 1:2	622 ± 4	373 ± 2	6.45 ± 0.04	54.1 ± 3.1	424 ± 6	255 ± 3	4.40 ± 0.06
Algae+DM 1:5	616 ± 90	370 ± 54	11.3 ± 1.6	52.1 ± 3.2	391 ± 47	235 ± 28	7.18 ± 0.86
Algae+DM 1:10	812 ± 10	487 ± 6	22.9 ± 0.3	51.9 ± 3.0	488 ± 5	293 ± 3	13.8 ± 0.1
Algae+FW 1:1	772 ± 15	463 ± 9	5.76 ± 0.11	57.9 ± 3.1	524 ± 10	314 ± 10	3.91 ± 0.12
Algae+FW 1:2	933 ± 41	560 ± 25	10.3 ± 0.5	56.6 ± 3.7	559 ± 33	336 ± 20	6.17 ± 0.36
Algae+FW 1:5	1240 ± 20	746 ± 12	26.3 ± 0.4	52.8 ± 4.2	664 ± 15	398 ± 9	14.0 ± 0.3
Algae+FW 1:10	1300 ± 39	781 ± 23	47.3 ± 1.4	51.5 ± 4.0	664 ± 36	399 ± 22	24.1 ± 1.3
Algae+PL 1:1	488 ± 4	293 ± 2	3.65 ± 0.03	56.6 ± 2.9	385 ± 7	231 ± 4	2.88 ± 0.06
Algae+PL 1:2	525 ± 33	315 ± 20	5.82 ± 0.36	55.7 ± 2.9	370 ± 31	222 ± 19	4.10 ± 0.34
Algae+PL 1:5	715 ± 13	429 ± 8	15.3 ± 0.3	53.5 ± 3.3	424 ± 3	254 ± 2	9.06 ± 0.06
Algae+PL 1:10	786 ± 17	472 ± 10	29.1 ± 0.6	53.2 ± 3.2	445 ± 12	267 ± 7	16.0 ± 0.5

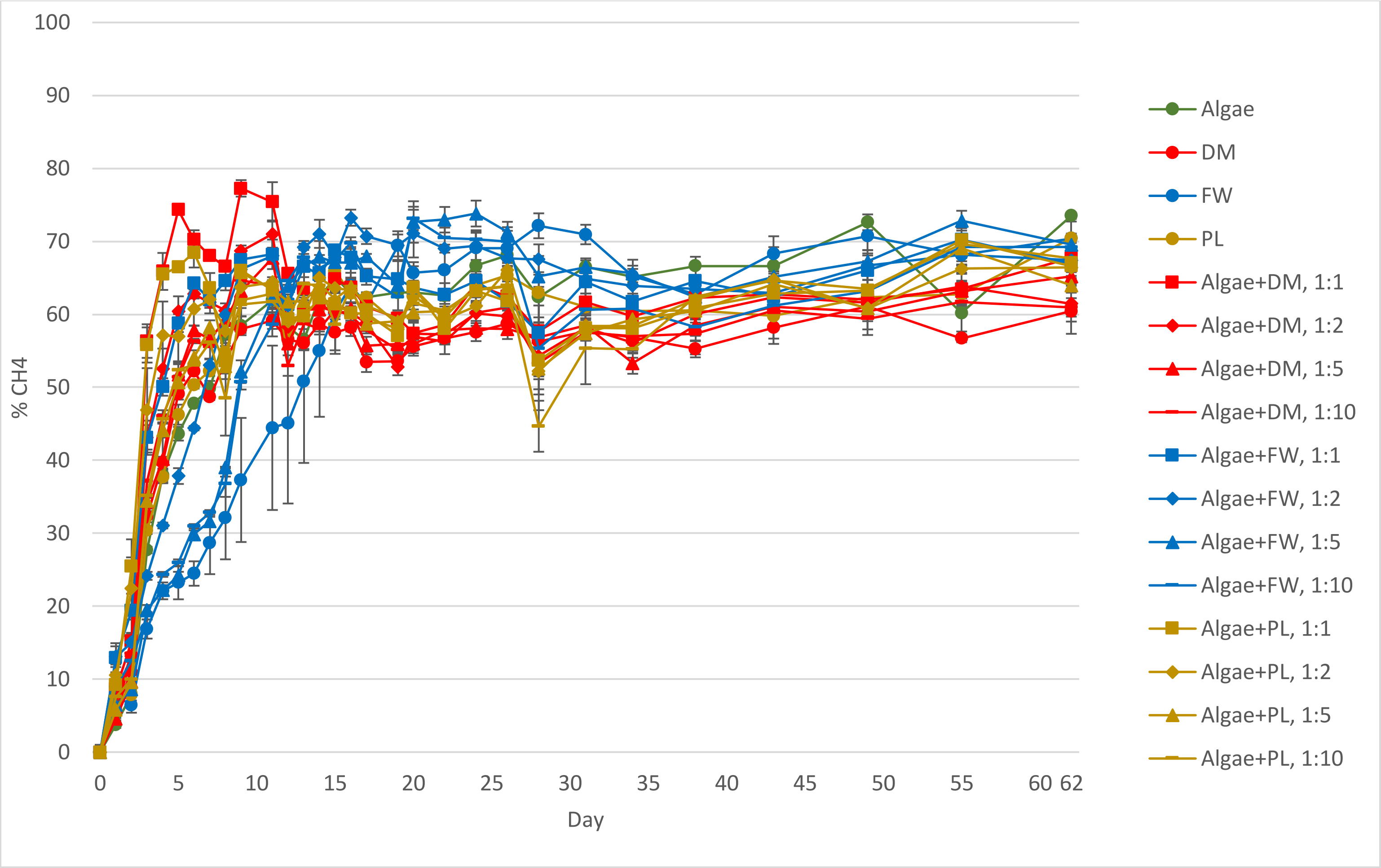


Figure 3.5: Methane (CH_4) concentration in biogas collected from the triplicate feedstock reactors and inoculum reactors in the batch reactor experiment. The standard error of the triplicate reactors is shown in the error bars. DM = Dairy Manure, FW = Food Waste, and PL = Poultry Litter.

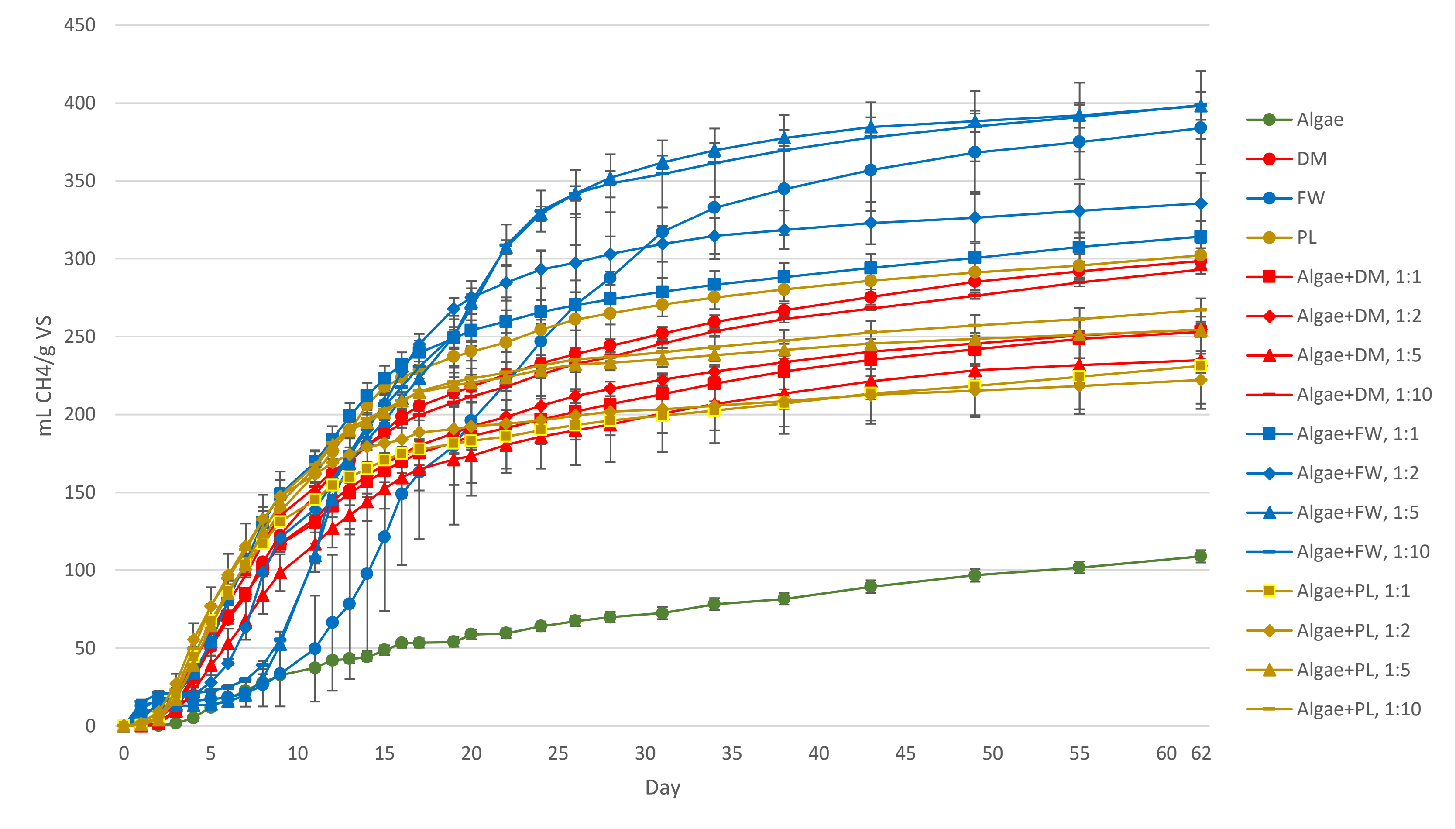


Figure 3.6: Cumulative methane (CH_4) production in the batch-scale reactor experiment, with CH_4 production normalized by g volatile solids (VS) of the substrates. The standard error of the triplicate reactors is shown in the error bars. DM = Dairy Manure, FW = Food Waste, and PL = Poultry Litter.

When normalized by g VS fed to the reactors, the algae-only treatment was significantly lower than all other treatments (109 ± 4 mL CH_4/g VS; *p*-value < 0.001). The FW was the most efficient mono-substrate treatment overall (384 ± 23 mL CH_4/g VS). The FW co-digestion reactors were also highly efficient, with algae+FW 1:10 yielding the highest biomethane potential in the study (399 ± 22 mL CH_4/g VS) and algae+FW 1:5 had the second highest (398 ± 9 mL CH4/g VS). There was no statistical difference between the biomethane potential of the FW mono-substrate and the four algae+FW co-digestion reactors (*p*-values ranging from 1.00 to 0.072). The addition of algae to FW digestion did not significantly decrease CH_4 generation at any of the VS ratios tested. This indicates that ATS algae can replace some of the VS in FW reactors without significantly reducing the efficiency of the reaction.

PL was the second most efficient mono-substrate treatment (302 ± 7 mL CH4/g VS). Each of the PL co-digestions had a progressively lower biomethane potential as the mass of algae in a treatment increased, but there was no statistically significant difference between the PL mono-substrate and three of the algae+PL co-digestion treatments (1:10, *p*-value = 0.902; 1:5, *p*-value = 0.538; 1:1, *p*-value = 0.062), except the algae+PL 1:2 treatment, which was significantly lower than PL-only (*p* = 0.020). The findings suggest that algae may be added to PL digesters for co-digestion without significantly impacting digester efficiency only at higher (e.g. 1:10) co-digestion ratios.

DM was the third most efficient mono-substrate treatment (299 ± 8 mL CH_4/g VS), with similar CH_4 production between the PL and DM mono-substrate and co-digestion reactors, with reduced CH_4 potential with increased algae addition, yet, no statistical difference between the DM mono-substrate and the four algae+DM co-digestion reactors (1:10, *p*-value = 1.000; 1:5, *p*-value = 0.139; 1:2, *p*-value = 0.662; 1:1, *p*-value = 0.612). As with FW, there was not a significant negative impact on CH_4 potential when ATS algae was co-digested with DM at these four loading ratios).

Co-digestion was expected to increase CH_4 yield and digestion efficiency due to synergistic effects observed when the bacteria in a digester are provided more complex and varied substrates (Lisboa and Lansing 2013). Wall et al. (2012) increased methane yield 2-fold during co-digestion of blended food processing waste with manure at a loading ratio of 2:2:1 (by weight) compared to the dairy manure only in semi-continuous digesters. However, the loading ratio in Wall et al. (2012) was based on total substrate weight and was also specifically chosen to optimize the C:N ratio for the digester in question. While the reactor loading in this study was done based on VS ratio rather than ideal C:N, it is an approach that should be considered for future studies of co-digestion of ATS algae to maximize CH_4 yield.

The biomethane potential of the algae was within the expected range (100-200 L CH_4/kg VS) for algae digestion reported in the literature (Tedesco and Stokes 2017; Witarsa et al. 2020, Bohutskyi et al. 2016). It was 32.3% less than the CH_4 production of Witarsa et al. (2020) batch digestion of ATS algae from a different river in the Chesapeake Bay Watershed, and 46.5 less then reported by Bohutskyi et al. (2016). As reported by Tedesco and Stokes (2017), the CH_4 potential from a given algae culture can vary widely depending on the species being digested. It is possible that variations in the composition of the algal culture between prior ATS digestion studies and this one accounts for this difference, indicating site-specific biomethane potential.

The digestion efficiency of the algae (109 ± 4 mL CH_4/g VS) was 70% less than the DM (299 ± 8 mL CH_4/g VS) and PL (302 ± 7 mL CH_4/g VS), and 75% less than of the FW (384 ± 23 mL CH_4/g VS). This is consistent with studies of animal and food wastes in the literature, with a range from 200-400 L CH_4/kg VS (Moody et al., 2011; 2019; Asam et al. (2011). The CH_4

potential of FW cultures tends to vary depending on their composition, however the results are comparable with those of Yarberry et al. (2019) which reported 450 - 500 mL CH_4/g VS from the same blend used in this study tested with different inoculum.

The degree of increased efficiency from co-digestion reactors compared to the algae alone (103-266%) was higher than expected from prior studies of algal co-digestion by Astals et al. (2015) and Lu and Zhang (2016) which only reported up to a 40% increase in digestion efficiency compared to algae alone. However, the algae used by Astals et al. (2015) had TS of 6.8% and VS of 3%, indicating a moisture content comparable to the ATS algae but 391% more organic content. A higher baseline CH_4 yield would thus be anticipated, which would explain the smaller degree of increase seen in that study compared to this one (Astals et al. 2015).

While the digestion efficiency was low for the algae relative to the other mono-substrate reactors, the high reduction in VS in the algae-only reactor (93.0%; Table 3.3) compared to the DM, FW, and PL mono-substrate reactors (2.95-36.7%) during digestion indicates high efficiency of substrate conversion into bioenergy compared to the other substrates. This is consistent with findings by Wall et al. (2012) that increased solid content does increase CH_4 yield, but it can also decrease digestion efficiency and carbon utilization by slowing hydrolysis. The high moisture content of the algae could be beneficial as a diluent for high-solids co-digestion substrates.

When the CH_4 data was normalized by total substrate mass loaded into each reactor, the FW mono-substrate treatment produced the most CH_4 (166 ± 10 mL CH_4/g substrate), followed by PL (151 ± 4 mL CH_4/g substrate) and DM (39.7 ± 1.1 mL CH_4/g substrate). Algae mono-substrate was an order of magnitude lower than these (0.687 ± 0.025 mL CH_4/g substrate), reflecting its high moisture and low percentage of digestible components (VS) in its overall mass relative to the other wastes. The difference between the algae and the FW, DM and PL were all significant (*p*-value < 0.001 for all). All co-digestion treatments with algae yielded ≤ 24.1 mL CH_4/g substrate. Co-digestion of algae with waste resulted in significantly less CH_4/g substrate then each given waste's respective mono-substrate reactor (p-value < 0.001 for all) (Figure 3.7).

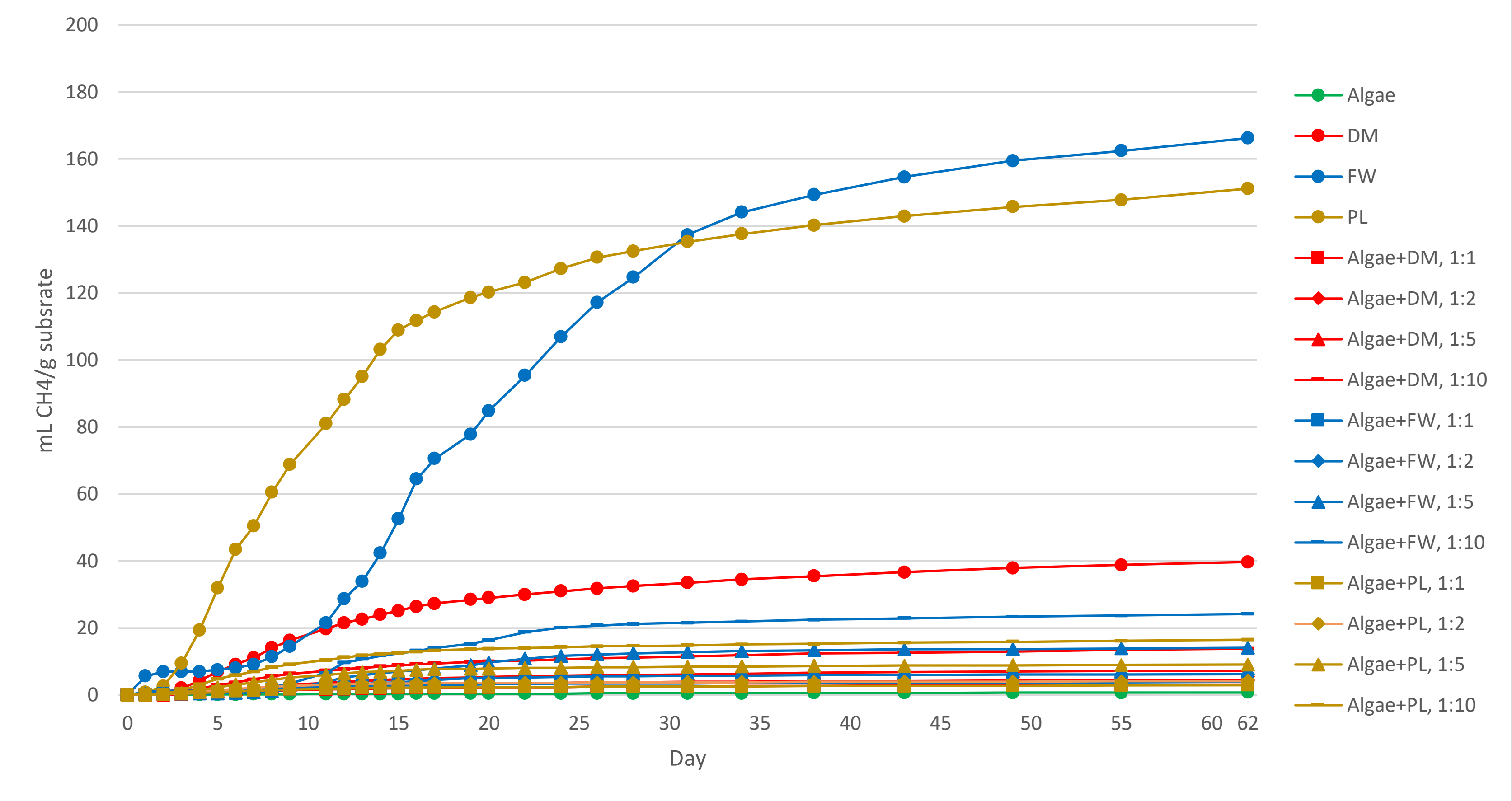


Figure 3.7: Cumulative methane (CH_4) production in the batch-scale reactor experiment, with CH_4 production normalized by g of substrate fed to each reactor. The standard error of the triplicate reactors is shown in the error bars. DM=Dairy Manure, FW=Food Waste, and PL=Poultry Litter.

The reactors with higher CH_4 per mass substrate corresponded to those with the highest initial TS and VS (Table 3.3). The results were anticipated from the TS and VS analyses as discussed previously and confirmed that the high water content of the ATS algae resulted in reduced CH_4 yield per substrate mass. This effect is exemplified in Asam et al. (2011) generated 55 mL CH_4/g substrate from dewatered pig slurry compared to 10 mL CH_4/g substrate from raw slurry. The significant loss of CH_4 per mass substrate even in treatments with relatively low loading of algae (1:5 and 1:10 VS ratios) indicates the strength of the dilution effect from using such a dilute substrate.

The water content of the algae is of practical, financial, and logistical concern for digester operators who would have to store, transport, and process the high moisture ATS algal waste in their established digesters (Wall et al. 2012). The organic content in the algae is digested relatively efficiently, as shown in the VS normalization (Figure 3.6, Table 3.3), however this benefit should be weighed against the high amount of water and non-digestible mass of the algae.

No significant synergistic effects on digestion efficiency or CH_4 yield were observed when algae was co-digested with wastes in this study. Chen et al. (2023) observed that co-digestion of dairy manure and Chlorella at an 8:2 loading ratio by mass increased CH_4 yield by 9.3-22.8% and 3.9-16.7% compared to digestion of the manure and algae alone, respectively. Assessment of Chen et al. (2023) indicated that the carbohydrate to protein loading ratio is crucial to generate the synergistic effect. Dhungana et al. (2022) also asserted the importance of the weight mixing ratio for optimizing anaerobic co-digestion, finding that the ideal mix of food waste, poultry litter, and goat manure for optimized bioenergy yield was 2:2:1 by weight.

3.3.5 Reactor effluent elemental analysis

The algae-only reactor contained the lowest concentration of all analytes except for Al (260 mg/L pre-digestion, 307 mg/L post-digestion) (Tables 3.7 and 3.8). This was expected due to the presence of diatoms, such as *Melosira* sp. in the algal culture, as their cell characteristic walls (i.e., frustules) contain aluminum and silica. Iron was the analyte with the highest concentration in all treatments, explained by the high Fe concentration in the inoculum only treatment (2420 mg Fe/L pre-digestion and 5150 mg Fe/L post-digestion). The iron can bind with the S in the reactors during digestion, suppressing H_2S generation in the biogas by forming the solid precipitate iron sulfide (Ge et al. 2013). The concentration of S did not appear to change pre-and post-digestion for any treatment, indicating it was retained in the effluent rather than converted to gaseous H_2S.

Table 3.7: Results of metal and sulfur analysis for the batch reactor experiment, pre-digestion samples. Data provided by Agrolab (Harrington, DE). DM=Dairy Manure, FW=Food Waste, and PL=Poultry Litter.

Treatment	**S (mg/L)**	**Ca (mg/L**	**Mg (mg/L)**	**Na (mg/L)**	**Zn (mg/L)**	**Fe (mg/L)**	**Mn (mg/L)**	**Cu (mg/L)**	**B (mg/L)**	**Al (mg/L)**
Mixed Inoculum	859	1140	266	270	29.2	2420	16.8	15	4.5	146
Algae	223	425	177	97.3	11.6	1060	116	4.1	1.8	260
DM	951	1520	431	383	40.8	2600	23.7	18.5	4.6	191
FW	919	1790	385	467	38.9	3320	23.1	19.2	6.2	193
PL	1070	1170	362	551	27.9	1550	20.6	18.7	4.1	113
Algae+DM 1:1	361	665	227	147	18.2	1540	114	7.5	2.7	284
Algae+DM 1:2	386	579	205	202	12.7	999	60.4	5	1.9	161
Algae+DM 1:5	485	574	221	256	11.9	817	33.1	4.8	1.6	113
Algae+DM 1:10	676	1170	320	321	30.6	2260	55.9	13.8	4.4	209
Algae+FW 1:1	309	618	199	147	12.2	1170	90.7	4.8	2	214
Algae+FW 1:2	330	639	192	199	7.7	870	55.4	3	1.5	112
Algae+FW 1:5	541	1120	273	299	18.5	1840	64.8	8.5	3.4	186
Algae+FW 1:10	607	1270	284	357	20.7	2110	52.6	9.5	3.9	169
Algae+PL 1:1	354	496	199	164	12.5	1010	78.2	5.8	1.8	209
Algae+PL 1:2	451	622	227	232	16	1210	76.4	8.1	2.4	213
Algae+PL 1:5	712	963	297	331	25.8	1870	71.8	14	4	225
Algae+PL 1:10	834	1200	324	391	33	2220	61.8	19.2	4.8	227

Table 3.8: Results of metal and sulfur analysis for the batch reactor experiment, post-digestion. Data provided by Agrolab (Harrington, DE). DM=Dairy Manure, FW=Food Waste, and PL=Poultry Litter.

Treatment	**S (mg/L)**	**Ca (mg/L**	**Mg (mg/L)**	**Na (mg/L)**	**Zn (mg/L)**	**Fe (mg/L)**	**Mn (mg/L)**	**Cu (mg/L)**	**B (mg/L)**	**Al (mg/L)**
Mixed Inoculum	981	2160	357	298	298	5150	35.5	33.7	9.9	307
Algae	108	424	150	82.5	82.5	988	97.1	3.7	1.7	246
DM	777	2040	381	389	389	3820	31.5	25.6	7.3	227
FW	761	1780	319	404	404	4150	28.4	27.3	7.7	246
PL	1010	2300	481	505	505	4590	45.8	42.6	9.6	279
Algae+DM 1:1	304	886	257	180	180	2220	159	10.1	3.9	427
Algae+DM 1:2	366	1030	281	223	223	2380	129	12.3	4.3	363
Algae+DM 1:5	558	1450	331	266	266	3270	111	19	6.1	360
Algae+DM 1:10	677	1740	367	303	303	3690	91.3	22.5	6.5	350
Algae+FW 1:1	344	941	268	186	186	2560	179	12.1	4.4	465
Algae+FW 1:2	380	1010	261	220	220	2620	136	13.5	4.6	386
Algae+FW 1:5	606	1460	320	294	294	3800	128	22.1	6.8	416
Algae+FW 1:10	604	1380	301	348	348	3440	82	21.2	6.2	319
Algae+PL 1:1	421	2520	330	248	248	2660	178	17.1	4.9	502
Algae+PL 1:2	410	1840	307	283	283	2330	143	16.7	4.4	398
Algae+PL 1:5	691	1590	372	341	341	3490	127	27.2	6.8	389
Algae+PL 1:10	768	1690	375	394	394	3600	92.8	29.9	7.3	329

Ca (425-1790 mg/L) and S (223-1070 mg/L) had the next highest concentration in all reactors both pre- and post-digestion after Al and Fe, especially in the DM, FW, and PL mono-substrate treatments (919-1070 mg S/L; 1170-1790 mg Ca/L) (Tables 3.7 and 3.8). Ca and S are crucial micronutrients for plants to construct, respectively, the cell wall and intracellular cellular components involved in cellular signaling and metabolism (e.g. chlorophyll) (Narayan et al. 2022). Kaya et al. (2020) demonstrated that soils amended with S-enriched amendments increased both fresh and dry shoot yield from maize plants by ~42% compared to untreated plants under P deficiency and water stress conditions, concluding that S played an important role in maintaining resiliency of plants to stress. This study demonstrates broadly how sulfur-rich effluent from anaerobic digesters could benefit soils as a sulfur amendment, but also how the dilution effect of the algae can also reduce the concentration of beneficial components of the fertilizer.

The Na concentration in the effluents varied from 82.5 mg/L in the algae only reactor to 505 mg/L in the PL mono-substrate treatment. Lettuce plants are sensitive to salt stress, so dilution was required prior to lettuce fertilization (Table 3.8). Fertilizer analysis (Table 3.9) showed that an 8-fold dilution was sufficient to dilute the Na and maintain sufficient NH_4 to support lettuce growth, with the same dilution factor used for all treatments to maintain the unique differences in other trace elements (Tables 3.7 and 3.8).

Table 3.9: Nitrogen-phosphorus-potassium (NPK) profile of composite effluents from the batch reactor experiments, prior to dilution. Data provided by Agrolab (Harrington, DE). DM=Dairy Manure, FW=Food Waste, and PL=Poultry Litter.

Treatment	**NH_4^+ (mg N/L)**	**Total N (mg N/L)**	**P (mg P_2O_5/L)**	**K (mg K_2O/L)**	**NPK Ratio***
Mixed Inoculum	790	3790	4550	1180	3:4:1
Algae	319	460	497	648	1:1:1
DM	764	3300	3510	1390	2:3:1
FW	866	5590	3660	1280	4:3:1
PL	870	4100	4580	2170	2:2:1
Algae+DM 1:1	534	1270	1370	844	2:2:1
Algae+DM 1:2	694	2170	1700	1020	2:2:1
Algae+DM 1:5	633	2360	2580	1080	2:2:1
Algae+DM 1:10	420	2930	3060	1180	2:3:1
Algae+FW 1:1	519	1710	1560	861	2:2:1
Algae+FW 1:2	580	2080	1810	865	2:2:1
Algae+FW 1:5	778	2420	2890	1024	2:3:1
Algae+FW 1:10	854	3200	2720	1160	3:2:1
Algae+PL 1:1	574	1350	1780	1050	1:2:1
Algae+PL 1:2	682	1870	1720	1230	2:1:1
Algae+PL 1:5	874	3370	3010	1520	2:2:1
Algae+PL 1:10	931	4050	3360	1690	2:2:1

*Calculated using Total N, P_2O_5, and K_2O data.

3.3.6 Lettuce growth experiment

No symptoms of acute toxicity were observed on any of the lettuce plants, indicating that dilution was successful in reducing Na concentration in the effluents to safe levels for fertilization without inducing salt stress. The overall nutrient uptake and assimilation analysis indicated similar tissue composition for all fertilizers. The results of the plant tissue analysis indicated that Mo, B, Mn, Cu, and Zn were similar between all fertilization treatments, including the two controls. Concentrations of Mn, Fe, and Al in the plant tissue varied the most highly between treatments, with plant tissue receiving algal digestion effluent containing the highest Mn (252 ppm), Al (46 ppm), and Fe (66 ppm) of the mono-substrate reactors (Figures 3.8 and 3.9). All the plants fertilized with effluent from co-digestion treatments had elevated Mn and Al compared to their respective mono-substrates, suggesting that the ATS algae was a good source of bioavailable Mn and Al (Figure 3.9). Of the plants fertilized with effluents, the algae-only effluent yielded the lowest amount of whole (3.3 ± 0.20 g) and dry (0.517 ± 0.035 g) biomass on average (Appendix Table B.1). These findings empirically confirm speculation by Kebede-Westhead et al. (2004) that used the results of elemental analysis of ATS algae to suggest that while ATS algae do assimilate heavy metals during growth, the concentration may not be sufficient to prevent lettuce growth in plants at the dilution level tested.

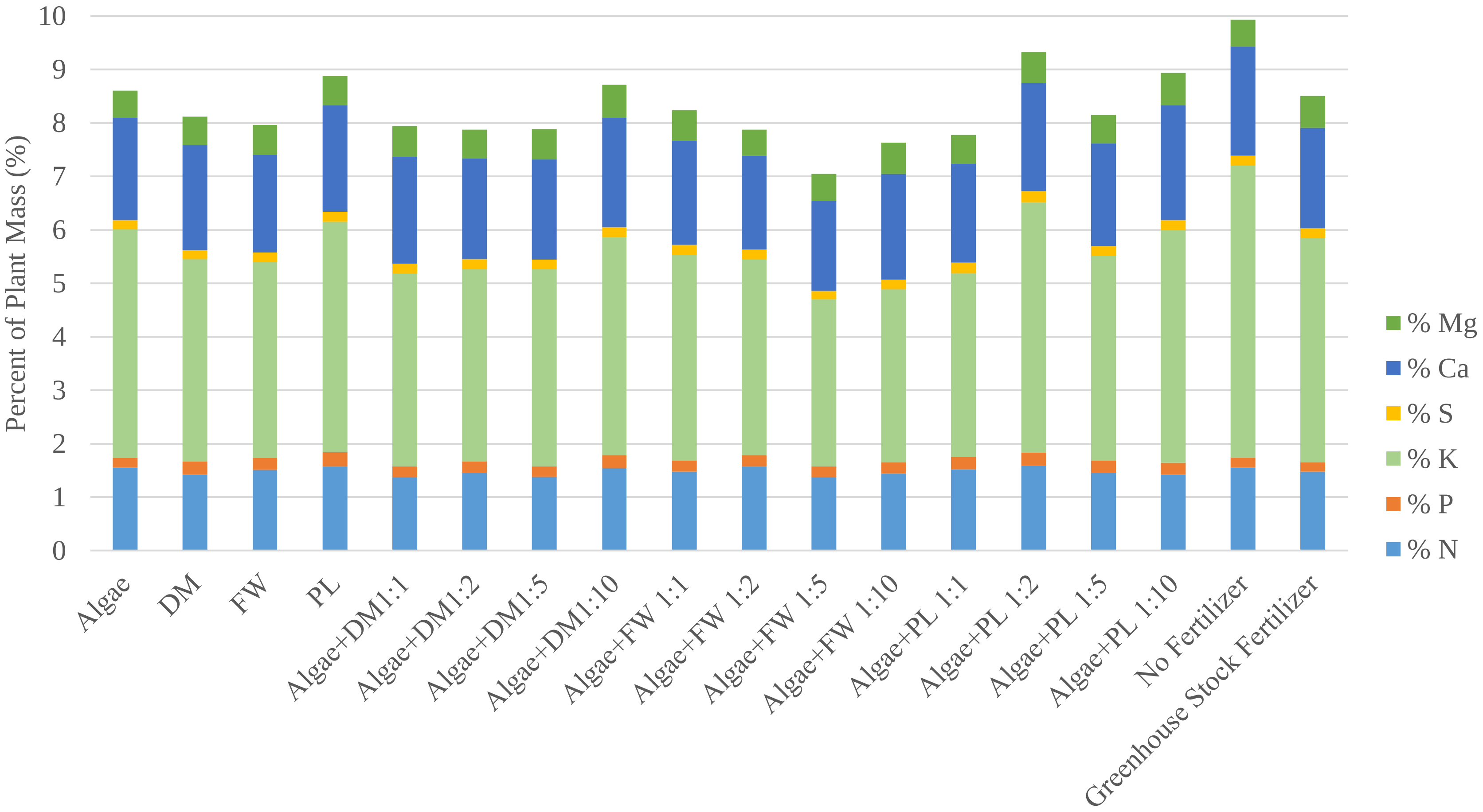


Figure 3.8: Results of principal component elemental analysis for lettuce from the effluent fertilization experiment. DM = Dairy Manure, FW = Food Waste, PL = Poultry Litter.

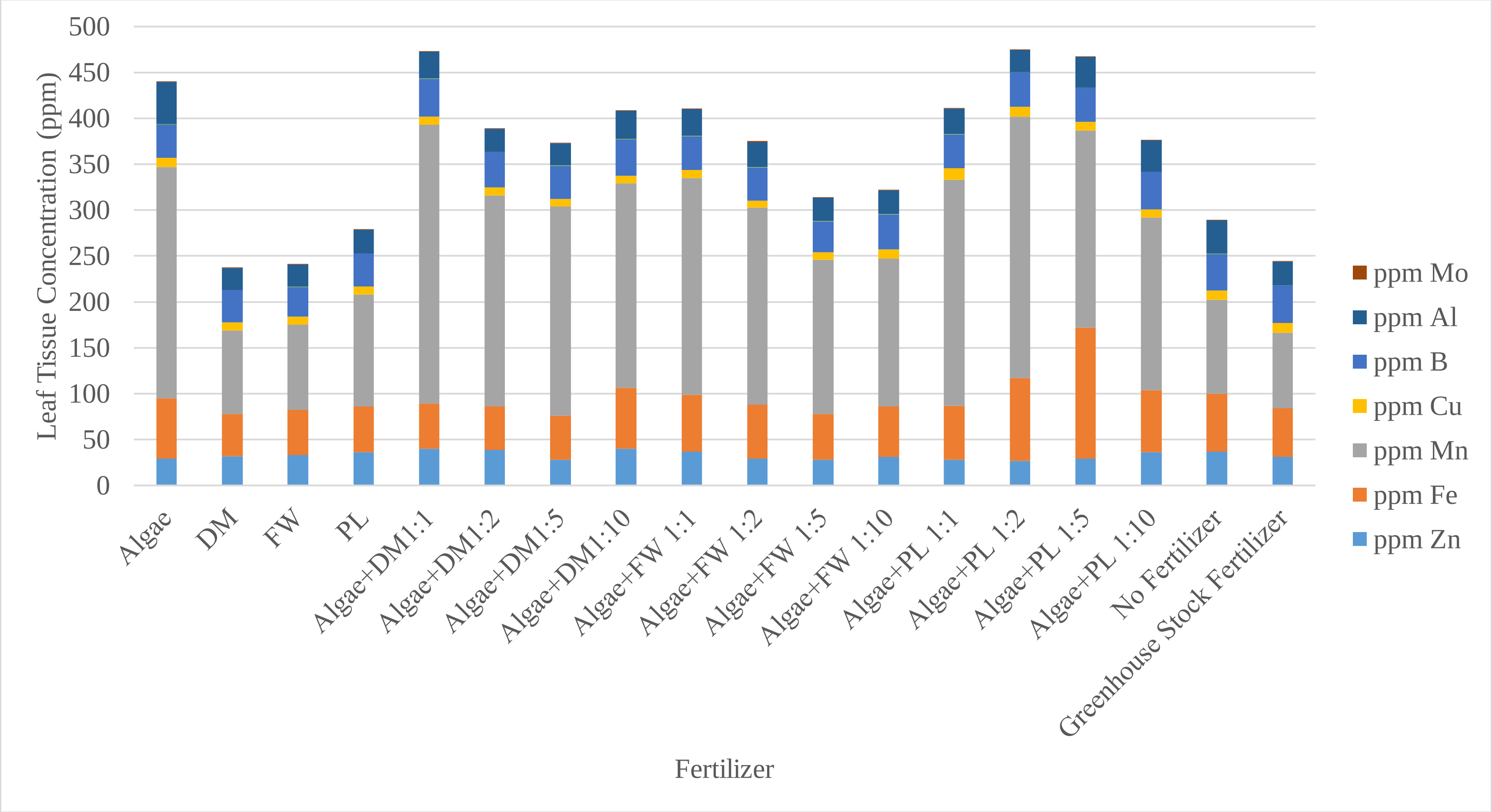


Figure 3.9: Results of trace mineral analysis for lettuce from the effluent fertilization experiment. DM = Dairy Manure, FW = Food Waste, PL = Poultry Litter.

3.4 Conclusions

Algae from an ATS was co-digested successfully with DM, PL, and FW at 1:1, 1:2, 1:5, and 1:10 VS loading ratios. Digestion efficiency (VS normalization) of was significantly higher when algae was co-digested with dairy manure or food waste at the four loading ratios tested for DM and PL. A significant reduction in efficiency was observed in PL digesters with higher volumes of algae was loaded. The high water content of the algae resulted in lower CH_4 production when normalized by total mass introduced as a co-digestion feedstock, however, CH_4/mass substrate remained significantly higher in the co-digestion reactors compared to when the algae culture was digested alone. It is recommended that co-digestion of algae with any of the three wastes be used instead of mono-digestion of algae alone, due to higher CH_4 yield by both normalization criteria tested.

The water content of the algae also resulted in a more dilute effluent post-digestion. While this was beneficial in reducing the concentration of undesired components such as Na in digestion effluents when they were used to make fertilizer, it also diluted the N and P concentration and reduced the amount of nutrients delivered to lettuce seedlings during fertilization.

Despite the challenges presented by the water content of the algae, it also provides some benefits to digester operators. First, the more dilute algae had more complete conversion of organic components (VS) to CH_4 compared to the DM, FW, and PL reactors, with increased VS utilization in co-digestion reactors with higher quantities of algae. The dilution effect also demonstrated increased resistance to ammonium accumulation inside the reactors, indicating that supplementing reactors with algae could balance ammonium accumulation and mitigate the risk of ammonia toxicity. Finally, the algae could be used in place of fresh water as a diluent for co-digestion with high-solids substrates (e.g., PL). Dewatering pre-treatment could reduce the moisture content of the algae; however, it is unclear how its digestibility would change after dewatering treatment. The next step in research of bioenergy derived from ATS algae should be pre-treatment to further optimize both mono- and co-digestion of the algae.

Chapter 4: Flocculation pre-treatment for dewatering algal flow way (ATS) algae for anaerobic digestion

4.1 Introduction

Algal flow ways (ATS) are bioremediation systems designed to encourage the growth of algal periphyton, which is used to metabolize nutrients from eutrophic water. An ATS consists of a long, shallow channel with a textured lining. Pumps deliver water to the channel, where the rough lining mimics the rock and corals that in-situ periphyton species colonize in their natural habitat (Adey et al., 2013). This periphyton "turf" develops quickly by taking up excess nutrients present in the water. Through harvesting the algae before it reaches maturity, continuous algal growth and nutrient uptake is facilitated resulting in bioremediation of eutrophic waters.

Disposing of the algal byproduct from an ATS via anaerobic digestion is an emerging topic in the literature (Bohutskyi et al., 2016; Witarsa et al., 2020). Anaerobic digestion reactors use a series of biochemical reactions facilitated by bacteria to convert the organic material, or volatile solids (VS), in a waste into methane (CH_4), a gaseous fuel. As the organic material breaks down, organic nutrients are also freed from the biomass into the digester slurry. In the case of ATS algae, this would allow the remediated nutrients to be recovered and recycled as a fertilizer.

However, the high water content typical of ATS algae (>90%) may limit its feasibility as a feedstock for anaerobic digestion (Bohutskyi et al., 2016; Witarsa et al., 2020). This results in a far larger quantity of algal biomass needed to supply a reactor with the same amount of organic material as a drier digestion feedstock (e.g., manure). This was confirmed by Witarsa et al.

(2020) in batch-scale anaerobic reactor studies, where air-dried (21.5% moisture) ATS algae generated 86% more CH_4 than algae with 61.5% moisture, and 652% more CH_4 than algae digester, as harvested (93.3% moisture) when normalized by the overall substrate mass added to the digester. Yet, the digestion efficiency, measured as the conversion of the VS in the biomass to CH_4 was 24 to 35% higher moisture content algae compared to the air-dried algae (Witarsa et al. 2020).

An alternative to air drying for dewatering is to encourage solids settling and thickening in the algal slurry. When suspended solids settle out from a mixture, clarified supernatant can be drained to produce a thickened sludge. Larger solids may settle out via gravity with time, but fine particles may remain suspended without additional treatment to induce flocculation into larger clumps. Flocculation is currently considered one of the most promising methods for harvesting cells of monocellular algal species from suspension in fluid growth media, with a wide range of physical, chemical, and biological flocculation treatments tested in the literature (Alam et al. 2016; Ummalyma et al. 2017). In this study, four coagulation treatments ($FeCl_3$, chitosan, electrocoagulation, and *Bacillus* RP1137) were tested to thicken ATS algae and reduce its water content prior to anaerobic digestion, with comparison to gravity settling as a control.

Metal coagulants, including iron salts, are traditionally used in wastewater treatment and often used as control treatments in studies of experimental flocculants (Lama et al. 2016; Ummalyma et al. 2017). Lama et al. (2016) reported a consistent 90% flocculation rate in all microalgae tested with Fe salts during the experiment, with the optimal dosage (0.03-0.30 g $FeCl_3$ per g algae) varying by algal species.

Natural and synthetic polymers, such as guar gum, cationic polyacrylamide, and chitosan, have demonstrated >90% flocculation efficiency of monocellular algae cells in solution

(Bannerjee et al., 2013; Lama et al., 2016; Musa et al., 2020). Chitosan, a polymer derived from crustacean shells, has been tested on a wide variety of algal species. Lama et al. (2016) found the effectiveness of chitosan flocculation was species-dependent, with some groups achieving >90% cell flocculation (chlorophytes, cyanobacteria), but others (chrysophytes, diatoms, pavlovophytes, and cocoliths) having little or no significant response. Van der Berg et al. (2020) researched chitosan flocculation of mixed algal cultures in effluent from wastewater stabilization ponds and found the best overall dosage was 0.368 g/L with a 2-hour resting period. Van der Berg et al. (2020) also found that the species composition of the algae harvested with chitosan differed from a gravity-only control, further suggesting species-specific response to the treatment. Lama et al. (2016) concurred with these findings, reporting that treatment response of algal cultures to flocculation treatment appears to be highly species dependent.

Electrocoagulation uses an electrical current flowing between two electrodes to interfere with the electrical interactions between the water and suspended solids, allowing the latter to settle out of solution despite their small size. It is currently an emerging technology for use in wastewater treatment, including for the removal of nuisance cyanobacteria and for microalgae harvesting (de la Fuente et al., 2019; Luckakova et al., 2022; Pearsall et al. 2011). At pilot-scale, Luckakova et al. (2022) achieved >85% flocculation of *Chlorella vulgaris* cells in a continuous harvesting system and demonstrated that the flocculation is also effective at scale.

Bacterial flocculation is a novel approach, with *Bacillus* sp. strain RP1137 investigated by Powell and Hill (2013) used as a bacterial flocculant and achieving 70-98% flocculation of pure cultures of the microalgae *N. oceanica* at lab scale. Powell and Hill (2014) determined that the flocculation was due to charge neutralization of calcium ions on cell surfaces during interactions between the bacteria and algal cells. As with metal flocculation, bacterial flocculation was also determined to be pH dependent (9.00 - 10.00) (Powell and Hill 2013; Powell and Hill 2014).

Most of the previous studies only tested flocculation response of mono-cultured algae and the species-specific response to treatments reported by multiple species studies suggest that the findings in the literature may not be directly comparable with a heterogenous culture such as ATS algae. This research examined the response of an ATS algal culture to a range of flocculation and coagulation treatments by quantifying changes in total solids (TS), total suspended solids (TSS), and VS.

The objective of thickening was to maximize removal of water in the settled solids resulting from these treatments, to increase TS and VS content for more optimal CH_4 yield per mass VS and mass substrate in anaerobic digestion. Observing which treatment minimized TSS remaining in the clarified supernatant post-thickening was a secondary objective of the thickening treatments, to ensure maximum harvest of microalgal cells and debris. The final objective was to measure changes in CH_4 yield during laboratory-scale batch anaerobic digestion of raw and dewatered ATS algae to quantify changes in substrate digestibility due to pre-treatment. This was the first study of its kind to apply established flocculation and coagulation treatments to dewater ATS algae, and the first to consider this approach as an alternative to the slower process of evaporative drying previously used in the literature (Witarsa et. al 2020).

4.2 Materials and methods

4.2.1 Algae composite collection and species composition analysis

The algae used in this experiment was a composite of cultures harvested from two freshwater ATS. The ATS unit 1 was a small-scale (1 m^2) system inside a laboratory at the

University of Maryland (College Park, MD) that was used as a demonstration model for ATS systems. It recirculated tap water and Anacostia River water from a holding tank, which had been inoculated with a sample of periphyton from a dock on the Anacostia River (Washington D.C., USA). Light was supplied 24 hours a day using a white hydroponics grow light (60 W, Feit Electric) suspended approximately 4” above the turf. This system was operated continuously for 5 months before harvesting the periphyton. The water recirculation system was stopped 1 hour prior to harvest and excess water allowed to drain off. A wet/dry vacuum used to detach the algae from the surface of the ATS for harvesting.

ATS unit 2 was a small-scale system (2 m^2) sited on the Anacostia River at Bladensburg Waterfront Park (Bladensburg, MD). This system was operated for two months and was harvested weekly using the same method used for unit 1. The algae used in the flocculation experiment was collected during the second and third weekly harvest from unit 2; the algae was stored via refrigeration during the time between the two harvests (Figure 4.1).

Figure 4.1: Algal turf scrubbers (ATS) unit 1 (left) and unit 2 (right) that were used to supply algae for the coagulation dewatering experiment.

The ATS algae from unit 1 was collected within 24 hours of the flocculation experiment and stored in a refrigerator when not in use. Algae from unit 2 was also stored in a refrigerator during the 7 days between the first and second harvest, with the latter also occurring within 24 hours of the start of the flocculation experiment. To composite, both cultures were brought up to room temperature and added to a mixing tub for homogenization.

4.2.2 Flocculation experimental design

Sets of 1-L graduated plastic Imhoff settling cones were used as vessels for the flocculation experiment (Figure 4.2). A set of triplicate cones were set in a metal frame, with the bottoms plugged. During each flocculation test, each triplicate cone was filled with 500 mL of homogenized algae treated with a given flocculant. After each flocculation treatment was applied, the algal biomass was mixed vigorously for 60 seconds with a stir rod, then allowed to rest and settle for 60 minutes. The clarified supernatant was decanted using a 50 mL pipette to carefully remove any fluid visible above the boundary with the settled solids. Both the supernatant and solids were reserved and refrigerated immediately. Supernatant was tested for TSS, TS, and VS according to APHA Methods 2540D, 2540B and 2540E, respectively (APHA et al. 2005). The TS and VS were measured sequentially by first drying the sample at 105 °C overnight and then incinerating at 550 °C for 2 hours to compare removal of water and organic material, respectively (APHA et al. 2005). The TS results were the sum of the total dissolved solids (TDS) and TSS in each sample. The TSS fraction of the TS was separately quantified by filtering a measured volume of sample (2-300 mL) through a fiberglass TSS filtration membrane with vacuum suction, which were dried at 105 °C and then weighing to measure retained solids (APHA et al. 2005). Settled solids were tested for TS and VS using the same methodology (APHA et al. 2005). The TS was reported as a percentage of the wet weight of the algal substrate for consistency with past studies of ATS algae digestion. The VS was reported as both wet and dry basis percentages of the raw algae and the TS, respectively.

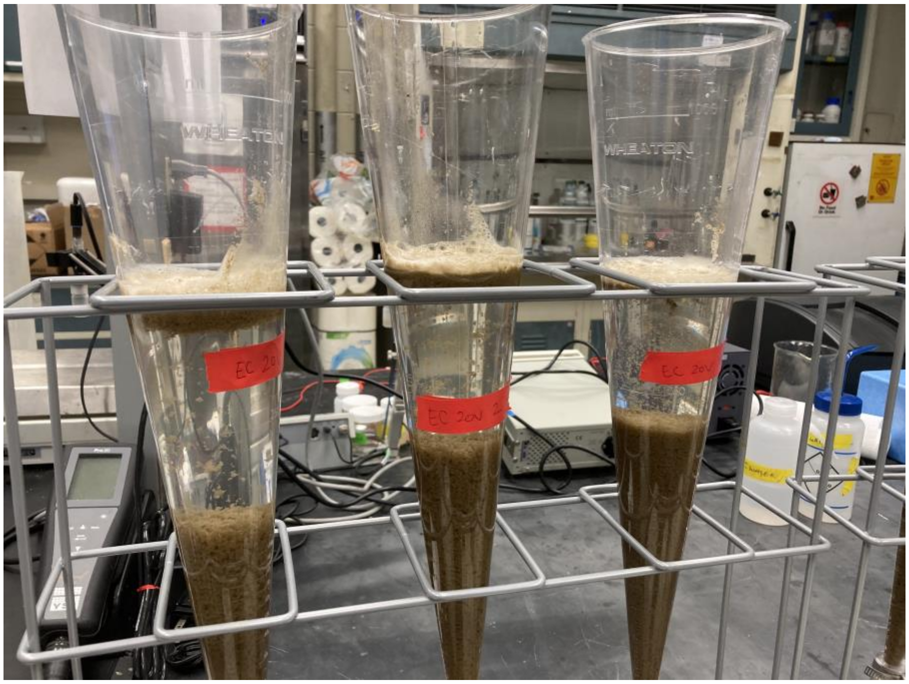


Figure 4.2: Imhoff settling cone setup during the coagulation dewatering experiment.

Between tests, the cones were washed with laboratory glassware detergent (Alconox), sonicated for 10 minutes, and rinsed with deionized water to remove any flocculation residues. Cones were subsequently dried in an oven at 50°C for one hour to remove residual moisture from cleaning.

4.2.3 Flocculation methods

The metal flocculant used was iron (III) chloride hexahydrate ($FeCl_3 * 6H_20$) delivered at dosages of 5, 20, and 50 mg $FeCl_3$/L. The iron for each cone was first dissolved in a weigh boat using ~3 mL of water temporarily transferred from inside that cone. The water containing the dissolved iron was then returned to the cone, and vigorously mixed with a stir rod for 60 seconds to incorporate.

The chitosan test used a 5g/L stock mixture of chitosan (Spectrum Chemical Manufacturing Corp., New Brunswick, NJ, USA) prepared by mixing 5g of powdered chitosan in 1 L of deionized water for 1 hour to homogenize. The stock fluid was then applied to each cone for final concentrations of 25, 50, and 100 mg chitosan/L.

Electrocoagulation was tested by temporarily transferring the algae from the Imhoff cones to a series of three 600-mL aluminum cylinders (3” diameter) designed to convey electric current to steel tube brushes (1¾” DS/DS.010 HC, Mill Rose Company, Mentor, OH, USA) used as electrodes inside. Alligator clamps wired to a 3645-A DC power supply (0 – 36 V, 3 Amp) were set at opposite sides on the outside of the cylinders and applied electricity throughout the column at one of three voltages (10, 20, or 30 V) for ten minutes each. Following treatment, the algae was returned to their respective Imhoff cones for settling.

The bacterial coagulant was prepared using an isolated culture of *Bacillus* RP1137, supplied by the Institute of Marine and Environmental Technology (Baltimore, MD, USA) on a Marine Broth 2216 agar plate. The bacteria was cultured on liquid Marine Broth 2216 according to the methods from Powell and Hill (2013) and frozen as 0.7 mL aliquots with 0.3 mL of sterile glycerol at -80°C. Prior to this experiment, six frozen aliquots were thawed and revived for 24 hours on 20 mL of sterile marine broth each using the same parameters as detailed in Powell and Hill (2013). Each mature 20 mL culture was then used to inoculate 200 mL of fresh growth media, which was allowed to incubate for 36 hours. At the end of the incubation, the six cultures were composited for a total of 1.2 L of liquid bacteria. Half (600 mL) was centrifuged down to harvest the bacterial cells, which were re-suspended using 60 mL of fresh Marine Broth 2216 for a 100x concentrated bacterial solution. Flocculation was induced by applying 2.5 mL of bacteria culture from the 1x or 100x solutions to the Imhoff cones, to test the efficacy of flocculation from both the raw and concentrated solutions.

Finally, a control treatment using only gravity was compared with the experimental flocculants. A 500 mL sample of algae composite was added in triplicate to the Imhoff cones, and the algae was mixed and allowed to settle using the same methodology as the other treatments, with no flocculant applied and the algae allowed to settle for 60 minutes prior to solids and supernatant separation.

4.2.4 Batch-scale anerobic digestion reactor experimental design

The most effective dosage of each experimental flocculation treatment was determined by comparing the increase in VS concentration relative to the raw algae, with the flocculation treatments used in anaerobic digestion reactors consisting of electrocoagulation (10 V), $FeCl_3$ (50 mg/L), Chitosan (100 mg/L), and Bacteria (1x concentration). The triplicate settled solids from these flocculation treatments were composited, as were those from the gravity-only, and all were refrigerated for use in a batch anaerobic digestion experiment to determine the effect of pre-treatment on the CH_4 potential of the algae.

A total of six treatments were tested in the digestion experiment, testing the raw algae (control) and settled solids from each of the four flocculation treatments and gravity settling as digestion substrates. The anaerobic digestion reactors consisted of 250-mL glass serum bottles loaded with 3 g VS at a 2:1 ratio of inoculum:substrate according to the VS of each material. An additional treatment containing only inoculum was also tested to account for baseline CH_4 generation by organic material in the inoculum. The inoculum was supplied from a laboratory-scale continuous food waste digester at the University of Maryland, College Park (USA). After the reactors were loaded, the headspace of each was purged using N_2 gas before sealing with rubber septa to create an anaerobic environment. The reactor bottles were incubated together in the dark at 35°C with agitation (120 RPM) for 34 days.

4.2.5 Analytical methods

The TSS, TS, and VS of liquid samples in both the flocculation and anaerobic digestion experiments were tested according to standard methods for wastewater (APHA 2005). Efficacy of a given thickening treatment was determined via comparison of the TS and VS in the settled solids fraction of the treated algae with the raw algae; higher % TS and VS in the settled solids, and correspondingly low TSS remaining in the decanted supernatant, indicated more successful treatment. The pH was measured with an Accumet AB15 meter (Fisher Scientific) on all liquid samples.

For the anaerobic digestion experiment, the volume of biogas generated was measured by observing the displacement of a wet tipped glass syringe through the rubber septa. The concentration of CH_4 in the biogas was tested using gas chromatography with an Agilent HP 7890A thermal conductivity detector (TCD) and porous layer open tubular (PLOT) Q column (injection temperature = 250°C, oven temperature = 60°C) with He as a carrier gas at 8.6 mL/min. Gas volume and concentration were initially tested daily, with sampling slowing as the rate of gas production in the reactors declined over time.

4.2.6 Statistical analysis

All statistical analyses were conducted in RStudio. ANOVA was used to compare TS, VS, and TSS for liquid samples in the flocculation experiment. In the anaerobic digestion experiment, ANOVA was used to compare CH_4 generation, normalized by the g algae VS fed to each reactor and by the total g of algae loaded to the reactors.

4.3 Results and discussion

4.3.1 Algae species identification and solids characterization

The algae from ATS unit 1 consisted primarily of Spirogyra (~60%) and *Vaucheria* sp. (~40%). The first harvest from unit 2 was primarily the filamentous diatom *Melosira* sp. (>90%) with small, discrete strands of the ochrophyte *Vaucheria* sp. identified within the *Melosira* sp. turf. The second harvest from Unit 2 was also dominated by *Melosira* sp. (~80%) and *Vaucheria* (~5%), but colonies of *Oscillatoria* sp. (~5%) and *Spirogyra* sp. (~10%) were also identified. The final mixture of algae consisted of approximately 8 L of algae from unit 1 and 9 L of algae from each of the harvests of unit 2, for a total of ~26 L of algae composite. The taxa present, specifically the prevalence of ochrophytes, is consistent with other studies of ATS in the Chesapeake Bay Watershed (Adey et al., 2013; Mulbry et al., 2010; Witarsa et al., 2020).

The algae composite had a TS of 4.02 ± 0.08%, a VS of 17.0 ± 1.0% TS, and a TSS of 36.7 ± 0.8 g/L. These values were comparable but slightly lower than the TS (6.7%) and VS (23.4% TS) values reported by Witarsa et al. (2020) for ATS algae on a Patapsco River (MD, USA). The solids content of the ATS algae culture used in this experiment indicate that each 500-mL subsample of algae dewatered contained ~20.1g of TS to coagulate, including 18.4g of TSS.

4.3.2 Gravity thickening

The flocculation treatments reduced the moisture content of the algae by 0.53-12.2% compared to the raw algae (Table 4.1). Gravity thickening yielded a settled substrate with a TS 11.8 ± 0.3% and VS 2.18 ± 0.02% concentration, a 293% and 261% increase from the raw algae, respectively. (Table 4.2). Supernatant remaining from gravity thickening had a TSS of 0.479 ± 0.016 g/L, a decline of 645% compared to the raw algae.

The algal composite used in this analysis had both a higher initial TS (42.4 ± 1.5 g/L) and final TS after gravity thickening (126 ± 4 g/L) than Ortiz et al. (2022)'s study of multi-stage microalgal thickening, where the initial TS (0.1-1 g/L) and final TS (6.4 g/L) of multi-species microalgae that was gravity-thickened, however, that study also had a higher increase in TS concentration (6,400%) relative to its original culture (Ortiz et al. 2022). The algal composite from ATS consisted of both micro-and macroalgal species, so the high solids in this study are attributable to the presence of larger particles of algae that more readily settled out of solution. Additionally, only a single step of gravity thickening was used in this analysis. A multi-stage approach as demonstrated by Ortiz et al. (2022) could be of consideration for future analyses.

Table 4.1: The total solids (TS) and volatile solids (VS) concentrations of the settled solids with flocculation or gravity settling, with the algae composite showing the values prior to treatment.

	TS (%)	**TS (g/L)**	**VS ($\%_{\text{Wet Weight}}$)**	**VS ($\%_{\text{TS}}$)#**
Algae Composite	4.02 ± 0.08	42.4 ± 1.5	0.686 ± 0.051	17.0 ± 1.0
Gravity	11.8 ± 0.3	126 ± 4	1.79 ± 0.09	14.6 ± 0.2
$FeCl_3$ 5 mg/L	10.4 ± 0.9	111 ± 10	1.44 ± 0.1	14.6 ± 0.2
$FeCl_3$ 20 mg/L	10.8 ± 0.3	116 ± 2	1.49 ± 0.05	15.1 ± 0.7
$FeCl_3$ 50 mg/L	11.9 ± 0.6	127 ± 7	1.8 ± 0.18	15.2 ± 0.2
Chitosan, 25 mg/L	13.7 ± 0.2	148 ± 3	1.94 ± 0.09	14.1 ± 0.4
Chitosan, 50 mg/L	14.4 ± 0.2	174 ± 21	2.19 ± 0.03	15.0 ± 0.4
Chitosan, 100 mg/L	14.7 ± 0.8	138 ± 27	2.22 ± 0.06	13.9 ± 0.3
Electrocoagulation 10 V	9.9 ± 0.5	105 ± 5	1.48 ± 0.08	10.4 ± 4.2
Electrocoagulation 20 V	4.6 ± 0.8	44.3 ± 8.4	0.63 ± 0.11	13.8 ± 0.6
Electrocoagulation 30 V	5.65 ± 0.3*	56.7 ± 2.8	0.82 ± 0.08*	15.0 ± 1.1
Bacteria, 1x	15.7 ± 0.6	165 ± 9	2.29 ± 0.09	13.9 ± 0.4
Bacteria, 100x	14.9 ± 0.3	158 ± 2	2.18 ± 0.02	15.2 ± 0.6

*Value given from analysis of duplicate samples rather than triplicates.

#Value reported on a dry weight basis, as a fraction of the TS.

Table 4.2: The total solids (TS), volatile solids (VS), and suspended solids (TSS) of the clarified supernatant in the flocculation experiment, with comparison to the untreated algae composite.

	TS (%)	**VS ($\%_{\text{Wet Weight}}$)**	**VS ($\%_{\text{TS}}$)#**	**TSS (g/L)**
Algae Composite	4.02 ± 0.08	0.686 ± 0.051	17.0 ± 1.00	35.7 ± 0.8
Gravity	0.081 ± 0.006	0.026 ± 0.008	30.7 ± 8.80	0.479 ± 0.016
$FeCl_3$ 5 mg/L	0.059 ± 0.006	0.013±0.007	22.9 ± 11.9	0.315 ± 0.031
$FeCl_3$ 20 mg/L	0.066 ± 0.014	0.023±0.003	37.3 ± 6.5	0.926 ± 0.672
$FeCl_3$ 50 mg/L	0.231 ± 0.101	0.202±0.098	70.7 ± 19	0.177 ± 0.034
Chitosan, 25 mg/L	0.066 ± 0.009	0.020 ± 0.000	31.2 ± 4.50	0.394 ± 0.094
Chitosan, 50 mg/L	0.100 ± 0.008	0.031 ± 0.013	30.1 ± 10.0	0.381 ± 0.043
Chitosan, 100 mg/L	0.080 ± 0.010	0.020 ± 0.000	23.6 ± 2.60	0.361 ± 0.08
EC 10 V	0.043 ± 0.020	0.033 ± 0.020	54.2 ± 27.3	0.056 ± 0.006
EC 20 V	0.692 ± 0.673	0.676 ± 0.676	33.2 ± 33.2	0.068 ± 0.01
EC 30 V	0.036 ± 0.012	0.010 ± 0.010	16.7 ± 16.7	0.063 ± 0.015
Bacteria, 1x	0.114 ± 0.015	0.046 ± 0.018	37.0 ± 13.0	0.718 ± 0.181
Bacteria, 100x	0.153 ± 0.017	0.059 ± 0.000	39.2 ± 4.20	0.398 ± 0.053

#Value reported on a dry weight basis, as a fraction of the TS.

4.3.3 Bacterial flocculation

The bacterial treatments were the most effective at increasing solids concentration, with bacteria 1x having the highest final TS and VS concentrations of 15.7 ± 0.6 and 2.29 ± 0.09, respectively. The 84.3% moisture content of the settled solids in the bacteria 1x treatment was lower than raw algae but still higher than the 21.5% final TS reported by Witarsa et al. (2020) from air dried ATS algae. The TSS of the bacteria 1x (0.315 ± 0.031 g/L) and bacteria 100x (0.479 ± 0.016) supernatants was comparable to the TSS from gravity settling (0.398 ± 0.053 g/L). Both bacteria 1x and 100x significantly increased TS (p-value < 0.001) and VS (p-value < 0.001) and reduced TSS (p-value < 0.001) compared to the raw algae.

Powell and Hill (2013) successfully flocculated 70-95% of a pure microalgal culture of *Nannochloropsis oceanica* using the *Bacillus* sp. strain RP1137. The TSS reduction in the clarified supernatant of this study was higher for both the bacteria 1x and 100x (98.9%) compared to Powell and Hill (2013). While this suggests higher cell recovery in the settled solids fraction, the similar TSS results for the gravity thickening treatment indicate that the effect may be due to gravity rather than from the bacterial treatment. While Powell and Hill (2013) did not assess moisture reduction by decanting and harvested cells via centrifuging, it is important to consider for commercial dewatering that decanting would require less energy input. The VS in the final solids was approximately half that reported for dairy manure from flush systems (Lisboa and Lansing 2013).

4.3.4 Chitosan flocculation

The second most effective treatment at thickening the solids was chitosan, which yielded similar TS (13.7 - 14.7%) and VS (1.94 - 2.22%) concentrations at all three dosages tested, with increased thickening at higher dosages. Chitosan was the most effective treatment at reducing supernatant TSS, however, with Chitosan 50 mg/L supernatant having the lowest TSS all treatments tested (0.056 ± 0.006) and Chitosan 100 mg/L having the third lowest (0.068 ± 0.010).

All the chitosan treatments significantly increased TS (*p*-value < 0.001) and VS (*p*-value < 0.001) and reduced TSS (*p*-value < 0.001). The maximum concentration tested in this study (100 mg/L) was lower than the optimal concentration determined by Van der berg et al. (2020) (368 mg/L) for flocculation harvest of microalgal cells in wastewater ponds. The 100 mg/L maximum dosage in this study was chosen based Lama et al. (2016), that determined the minimum effective dosage of chitosan for flocculation of twelve algal species were <100 mg/L. Chitosan was noted by Lama et al. (2016) to have a species-specific effect on flocculating algae, with freshwater species being the most effectively harvested (>90% cell recovery) with chitosan. Beach et al. (2012) also determined that 100 mg/L was optimal for >90% cell flocculation of *Neochloris oleoabundans*, and demonstrated that harvesting efficiency actually declined at concentrations >100 mg/L for that species. The low TSS remaining in the supernatant from flocculating the ATS algae suggests that 100 mg/L was optimal for the heterogenous mixture of algae in the freshwater ATS algae mixture.

4.3.5 Metal flocculation

The $FeCl_3$ treatment demonstrated a similar trend to chitosan, with similar TS (10.4 - 11.9%) and VS (1.44 - 1.80%) in the settled solids for each treatment with slight increases with an increased dosage. $FeCl_3$ was less effective at removing TSS from the supernatant, however, as the TS (0.361 - 0.718) was comparable to or greater than that of the gravity thickening control treatment. $FeCl_3$ at all concentrations tested significantly increased TS (*p*-value <0.001 for all) and VS (*p*-value < 0.001 for all) and decreased TSS (*p*-value < 0.001 for all).

The range of $FeCl_3$ concentrations tested here was lower than the 30 - 300 mg/L tested by Lama et al. (2016) but were still effective at flocculating the ATS algae. The color of the supernatant was visibly dyed red in all treatments from the addition of an iron salt, however, which may be undesired if decanted fluid from dewatering is returned to the source river. The findings from Lama et al., (2016) suggest that a higher dose of iron flocculant is needed than the 30 mg/L indicated by Poon and Chu (1999) for primary treatment of general sewage systems. The 50 mg/L maximum concentration tested in this experiment is above the range needed to flocculate sewage wastewater, but on the lower end of the range of that which the literature suggests is optimal for pure microalgal cultures. If the effective dosage for $FeCl_3$ is like existing standards for wastewater treatment and anaerobic digestion infrastructure, it implies that ATS algae could be readily integrated into existing those systems.

4.3.6 Electrocoagulation

The electrocoagulation yielded variable TS, VS, and TSS results by treatment level with no clear trends. The 20 V treatment had the lowest TS of the flocculation treatments overall (4.6 ± 0.8%) and a VS concentration that was lower than the raw algae (0.63 ± 0.11%), followed by the 30 V treatment TS (5.65 ± 0.3%) and VS concentration (0.82 ± 0.08%) was only half that of the 10 V treatment (1.48 ± 0.08%). The TSS results were similarly inconsistent, ranging from the second lowest in the study (Electrocoagulation 10 V, 0.063 ± 0.015 g/L) to the second highest after the raw algae (Electrocoagulation 20 V, 0.926 ± 0.672 g/L). Of the three voltages tested, only 10 V significantly increased TS (p-value < 0.001) and VS (p-value < 0.001), however all three treatment levels significantly reduced TSS (p-value < 0.001 for all).

The results of the electrocoagulation experiment suggest that low voltages are more effective at increasing TS and VS in settled solids, which is likely attributable to higher voltages lysing or otherwise damaging the algal cells (de la Fuente et al., 2019). This would explain why the TSS was successfully decreased for all three treatments, but the higher voltages ended up with less TS and VS in the settled portion if contents of the cells were released into the water column. It is unclear if the cellular contents were lost during decanting, but this would have implications for anaerobic digestion if crucial intracellular carbon components (e.g. lipids) were lost due to cellular disruption.

4.3.7 Nutrient and pH analysis of flocculated algae

The pH of the initial algae mix prior to flocculation was 7.55. After flocculation and separation of the supernatant from settled solids, the pH remained mostly unchanged by the gravity, EC 10 V, and the chitosan and bacteria treatments (Table 4.3). The EC at higher voltages (20 V and 30 V) were associated with ~0.30 decline in pH in the supernatant, and a smaller decrease in the settled solids. The both the supernatant and $FeCl_3$ had large decreases in pH that increased with treatment concentration, to the point that the $FeCl_3$ 50 mg/L supernatant was slightly acidic after separation (6.66 ± 0.03). This was expected due to the acidic nature of $FeCl_3$ in solution. This effect on solids pH is of concern if the solids are anaerobically digested, due to acid inhibition of the digester bacteria. Digestate naturally becomes more acidic over time due to the accumulation of volatile fatty acids (VFAs) in the digestate, and pH may need to be actively controlled for if it falls to acidic levels (Gerardi 2003).

Table 4.3: Results of pH analysis on supernatant and settled solids fractions of algae samples after flocculation and coagulation treatment. EC=Electrocoagulation. Values given are equal to average of triplicate measurements ± standard error.

	Supernatant pH	**Settled Solids pH**
Gravity	7.59 ± 0.07	7.41 ± 0.01
EC 10 V	7.60 ± 0.03	7.66 ± 0.02
EC 20 V	7.33 ± 0.06	7.43 ± 0.04
EC 30 V	7.32 ± 0.02	7.47 ± 0.02
$FeCl_3$ 5 mg/L	7.52 ± 0.01	7.28 ± 0.00
$FeCl_3$ 20 mg/L	7.43 ± 0.01	7.03 ± 0.02
FeCl3 50 mg/L	6.66 ± 0.03	7.31 ± 0.01
Chitosan 25 mg/L	7.44 ± 0.01	7.55 ± 0.01
Chitosan 50 mg/L	7.58 ± 0.01	7.57 ± 0.01
Chitosan 100 mg/L	7.58 ± 0.00	7.6 ± 0.01
Bacteria 1x	7.63 ± 0.08	7.56 ± 0.01
Bacteria 100x	7.51 ± 0.01	7.56 ± 0.00

Acidic pretreatments can benefit digestion by increasing the rate of hydrolysis and cell breakdown, with Park et al. (2005) demonstrating how chemical hydrolysis can exceed the rate of biological hydrolysis that typically occurs in digesters and increase overall CH_4 yield over time. Devlin et al. (2011) increased CH_4 yield during digestion by 14.3% by acidic pre-treatment of waste activated sludge by reducing pH to 2.0 prior to digestion. The pH reduction incurred by the $FeCl_3$ in this experiment was small and the final solids remained approximately neutral, however digesters fed with algae pretreated with $FeCl_3$ may require more active management of reactor pH.

4.3.8 Anaerobic digestion of flocculated algae

The CH_4 generation in the batch digestion experiment was low overall. The untreated algae reactors generated 66.2 ± 4.8 mL CH_4, with an average digestion efficiency of 49.6 ± 3.6 mL CH_4/g VS (Figure 4.3). This less than half the 158 ± 13 mL CH_4/g VS shown by Witarsa et al. (2020) from ATS algae on the Patapsco River (Baltimore, Maryland, USA), and a quarter of the 200 mL CH_4/g VS reported by Bohutskyi et al. (2016) from digestion of ATS algae. The EC 10 V and $FeCl_3$ 50 mg/L treatments yielded similar results, generating 46.6 ± 4.1 mL CH_4 (34.9 ± 3.0 mL CH_4/g VS) and 45.6 ± 8.1 mL CH_4 (34.1 ± 6.1 mL CH_4/g VS), respectively. The chitosan 100 mg/L generated less total CH_4 (34.5 ± 10.4 mL CH_4) then the electrocoagulation and $FeCl_3$ treatments, however the efficiency of the chitosan digestion (33.6 ± 0.7 mL CH_4/g VS) was comparable to those treatments. Treatments with the lowest CH_4 yield were those thickened with gravity only (19.4 ± 7.3 mL CH_4; 14.5 ± 5.4 mL CH_4/g VS) and bacteria 1x (19.9 ± 7.6 mL CH_4; 15.0 ± 5.7 mL CH_4/g VS).

Each of the thickening pre-treatments resulting in lower total CH_4 yield and efficiency. The reduction in efficiency compared to the untreated algae was significant for the gravity (*p*-value = 0.007) and Bacteria 1x (*p*-value = 0.008) treatments. No significant reduction in digestion efficiency occurred for the EC 10 V (*p*-value = 0.452), $FeCl_3$ 50 mg/L (*p*-value = 0.396), or chitosan 100 mg/L (*p*-value = 0.083) treatments (Figure 4.3). Wall et al. (2012) found that dewatered substrates generate higher volume of CH_4 per total mass digested, however added solids can slow the rate of hydrolysis compared to more dilute substrates. Fujishima et al. (2000) had up to 60.9% less carbohydrate removal efficiency and 25.9% less VS removal efficiency when sewage sludge was dewatered from 97% to 89% moisture. A decline in CH_4 generation when moisture fell below 91.1% disrupted the bacterial community of the sludge, including an order of magnitude fewer glucose consuming and acidogenic bacteria that are crucial to anaerobic digestion.

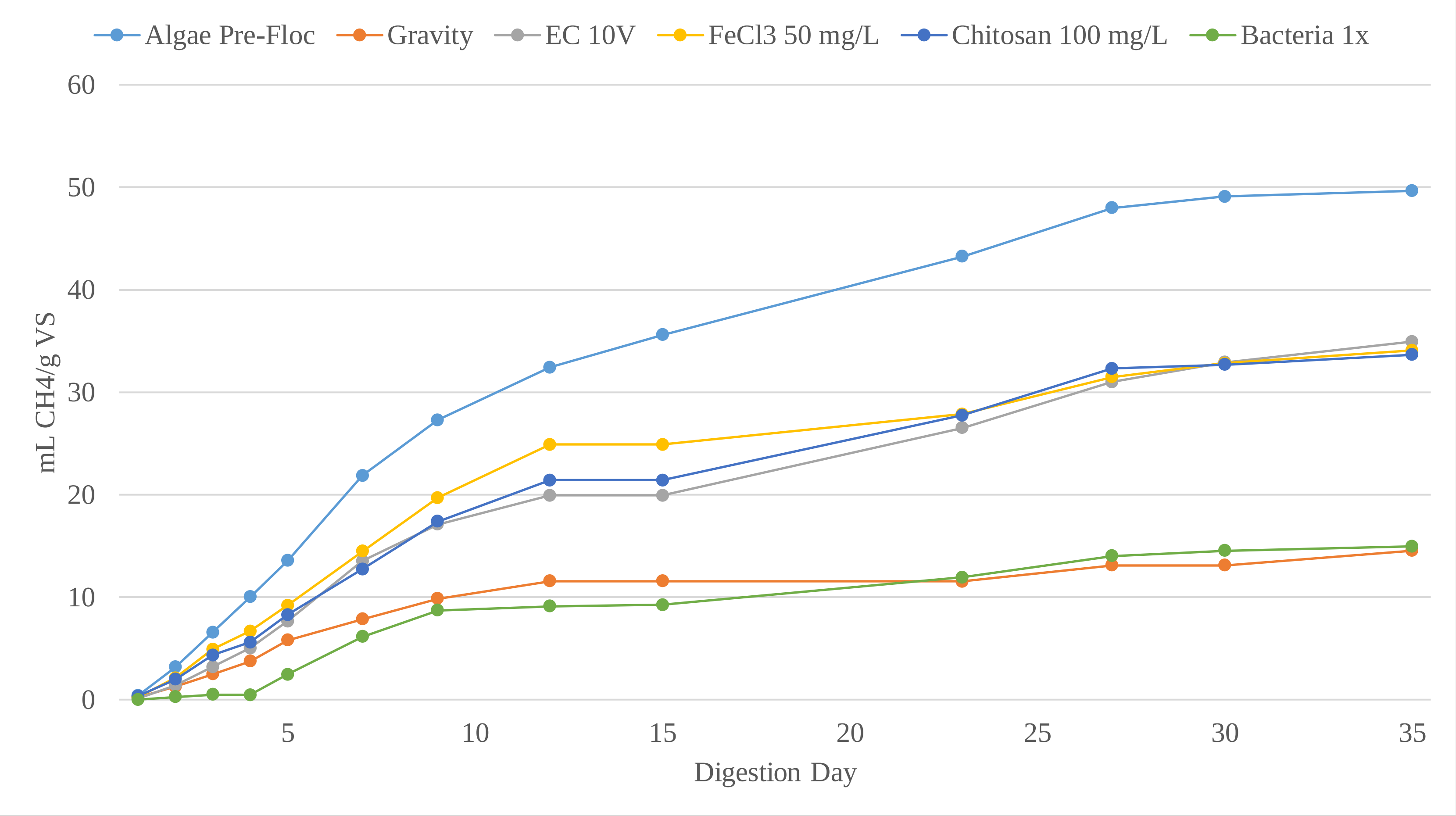


Figure 4.3: Normalized cumulative CH_4 generation per g volatile solids (VS) fed to the reactors in the anaerobic digestion experiment. Values given are the results of analysis of triplicate reactors ± standard error, except for the chitosan 100 mg/L treatment (light blue) which reports the results of duplicate reactors.

When CH_4 yield was normalized by total g of substrate fed to each reactor, the raw algae had 1.65 ± 0.12 mL CH_4/g substrate. The gravity treatment had reduced yield (1.46 ± 0.55 mL CH_4/g substrate) while the bacteria 1x treatment only slightly increased yield (2.00 ± 0.76 mL CH_4/g substrate) compared to the untreated algae. The $FeCl_3$ 50 mg/L and chitosan 100 mg/L generated 3.45 ± 0.62 and 4.21 ± 0.09 mL CH_4/g substrate, respectively, an increase of 109% and 155% from the raw algae. The EC 10 V treatment had the highest CH_4 yield per mass substrate (17.9 ± 1.06 mL CH_4/g substrate), an increase of 985%. Of the five flocculation methods tested, only electrocoagulation significantly increased bioenergy yield per L algae (*p*-value <0.0001) with no significant increase from gravity (*p*-value > 0.999), bacteria 1x (*p*-value > 0.999), $FeCl_3$ 50 mg/L (*p*-value = 0.556), or chitosan 100 mg/L (*p*-value = 0.672) (Figure 4.4).

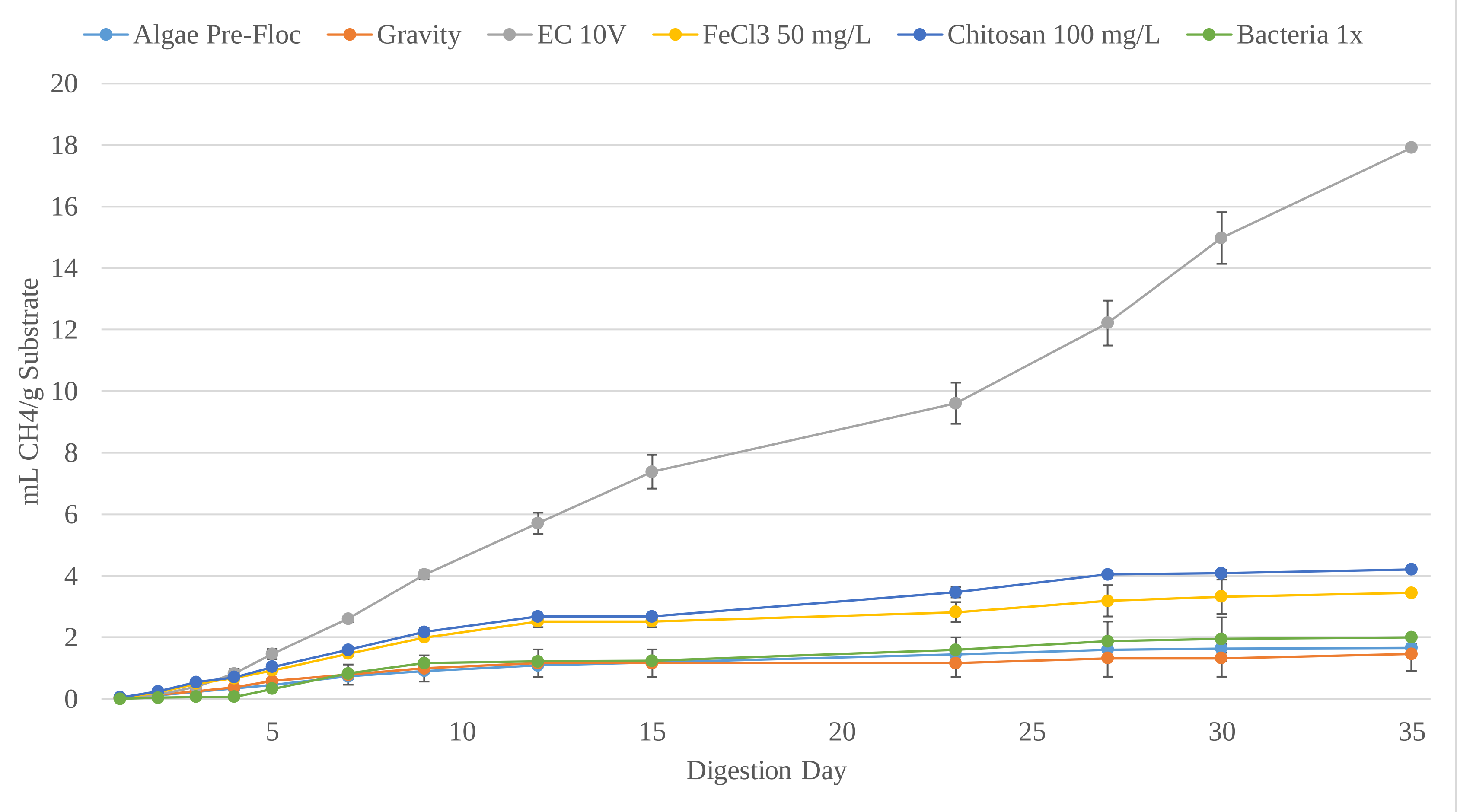


Figure 4.4: Normalized cumulative CH_4 generation per g substrate fed to the reactors in the anaerobic digestion experiment. Values given are the results of analysis of triplicate reactors ± standard error, except for the chitosan 100 mg/L treatment (light blue) which reports the results of duplicate reactors.

The effectiveness of the EC 10 V when normalized by both VS and total substrate mass was not anticipated due to not yielding solids with the highest TS and VS in the flocculation experiment. The previously discussed cell rupture effect of electrocoagulation could have effectively acted as an additional hydrolysis pre-treatment by preemptively breaking down the algal cells. Lee et al. (2014) increased CH_4 yield from digestion of the filamentous chlorophyte (*Hydrodictyon reticulatum*) from 167 to 384 mL CH_4/g VS by using ultrasound to similarly disintegrate the algal cells prior to digestion. Passos et al. (2014) demonstrated a similar effect with ultrasound on microalgae and increased organic matter solubilization, hydrolysis rate, and methane yield by 16-100%, 25-56%, and 6-33%, respectively, with digestion efficiency increasing from 147.7 up to 196.4 mL CH_4/g VS. Yatipanthalawa et al. (2022) utilized combined ultrasound and polymer treatment to treat the diatom *Navicula* and successfully increased the effectiveness of lipid extraction for bioenergy generation by removing the extracellular components of the diatom. If similar disruption to the frustule occurred in the EC reactors, it would have effectively acted as a combined hydrolysis and flocculation pre-treatment.

The algae thickened with chitosan had CH_4 yield comparable performance to the $FeCl_3$,. The effectiveness of the treatment confirms the findings of Musa et al. (2020), which increased microalgal cell recovery from 7 to 94% by using a cationic polymer flocculant, the same group to which chitosan belongs, to induce charge neutralization of the algae and promote “bridging” (i.e. clumping) between cells. Bannerjee et al. (2013) similarly recovered up to 94.5 and 92.2% of *Chlorella* sp. and *Chlamydomonas* sp. from suspended culture by utilizing modified cationic guar gum, compared to <20% from raw guar gum. The species-specific treatment effect reported by Lama et al. (2016) did not appear to greatly disrupt the flocculation effect on the ATS algae polyculture in this study. However, it should be noted that the cost of $FeCl_3$ and chitosan are different. At time of writing, 1 kg of $FeCl_3 * 6H_20$ retails at approximately $271 USD, compared to $2,156 USD for the same mass of chitosan (Fisher Scientific 2023a; Fisher Scientific 2023b). If the two perform comparably under the same conditions without further optimization of the digestion process, it is recommended that $FeCl_3$ be used instead.

Both bacteria and gravity thickening were the least productive treatments when normalized by VS and substrate mass. The former could be explained by the pH range of the raw algae (7.55) being outside the range deemed optimal for the flocculation treatment by Powell and Hill (2013) (>9.0), however this would not account for its high TS and VS after the flocculation test. The final TS of the bacteria 1x and bacteria 100x samples were both significantly greater than that of the gravity control (p-values = 0.002 and 0.016, respectively), so the high TS cannot be explained by the effect of gravity alone. It is recommended that further investigation into the use of *Bacillus* sp. RP1137 investigate other parameters also assessed by Powell and Hill (2013) on flocculation, such as salinity.

Gravity thickening is not recommended as the sole dewatering pre-treatment for ATS algae due to its low digestion efficiency, despite its low cost and ease of use compared to the other treatments used in this study. While it resulted in 194% increase in TS, during digestion it yielded significantly reduced digestion efficiency compared to the raw algae and no significant benefit on bioenergy yield per substrate mass digested. Incorporating multi-step gravity thickening for algae as in Ortiz et al. (2022) or utilizing it as a secondary process with the other treatments utilized in this experiment may also be a viable approach for future studies.

4.4 Conclusions

Coagulation and flocculation treatments successfully thickened ATS algae by reducing the water content of the raw algae culture by up to 12.1%. The degree of dewatering was less then reported by previous studies that used air drying to treat ATS algae, however all the thickening treatments tested except electrocoagulation resulted in large and consistently significant increases in both TS and VS. Also, electrocoagulation increased the efficiency of CH_4 yield during anaerobic digestion compared to digesting algae alone despite lower solids recovery then from the other treatments. At a 10 V treatment level, CH_4 yield from electrocoagulation outperformed conventional wastewater coagulants such as $FeCl_3$, which was attributed to cell disruption that occurred when electrical current was applied to the coagulation chamber. Thus, electrocoagulation could be effective as both a flocculation and hydrolysis pre-treatment and it is recommended in particular that future studies with ATS algae specifically consider this approach.

Additionally, it is important to consider the time scale of this study relative to those in the literature, which often conduct flocculation tests over 24 hours (Ortiz et al., 2022, Witarsa et al. (2020). This study effectively removed up to 11% of the moisture in the algae within a single hour, which could potentially be improved with longer flocculation time or multi-step combined treatments. It is recommended that further studies to dewater ATS algae also explore the potential of other novel treatments currently being explored in the literature, such as osmotic filtration and rotary drum dewatering (Munshi et al., 2018; Shao et al. 2015).

All the tested treatments also successfully removed 97-99% of the TSS in the raw algae from suspension with small variations in efficacy between treatments. While the supernatant was not used in the algal digestion study that followed the flocculation, the reduced solids are important to consider for discharge permits that may limit sediment content when returning the decanted fluid back to the river treated by the ATS. Further studies should also more closely examine the supernatant for cellular debris, nutrients, and organic material that may be released if the cell wall is disrupted by treatments such as electrocoagulation.

Flocculation and thickening are a fast and relatively easy approach to implement even at scale to dewater ATS algae. Additionally, except for electrocoagulation, the methods require no additional energy input for peripheral equipment (e.g., drying fans), which improves the sustainability of the process for using the algae in anaerobic digestion systems. It is recommended that methods that use the most effective treatments from this study (electrocoagulation, $FeCl_3$, and chitosan) be considered for pre-treating ATS algae for anaerobic digestion in the future.

Chapter 5: Research Conclusions

5.1 Overall conclusions

This research clearly demonstrates that anaerobic digestion of algae from algal turf scrubbers (ATS) is effective at processing the algal biomass into bioenergy as a value-added product for site operators. Each of these studies contributed to the understanding of combined anaerobic digestion and ATS. Chapter 2 built on prior anaerobic digestion and ATS research by focusing on the temporal variability in the algal feedstock over multiple growing seasons as well as how that affects bioenergy yield over time. Chapter 3 and Chapter 4 were the first of their kinds to assess known approaches to co-digesting and dewatering algae, respectively, using the unique algal cultures specific to ATS. The algae these systems can and should be treated as a valuable bioenergy feedstock rather than a waste, and while anaerobic digestion requires time, energy, and capital investment to incorporate into ATS bioremediation, it is worthwhile to dispose of the waste while generating valuable bioenergy.

The results from Chapter 2 indicate that the species composition of ATS algal cultures sited on the same river are consistent, though the relative abundance of them may vary over time and between systems. Temperature appears to be the most important variable determining the rate of algal yield each year, with unstable summer temperatures due to extreme climate events associated with reduced yields. This can correspond to lost bioenergy generation, as the availability of the feedstock (i.e. how much C was sequestered from the environment) directly limits how much CH_4 can be created by digestion. The ambient temperature also directly limits the operational time of unheated digesters, further affecting the consistency of CH_4 generation.

Also, algal cultures high in species with non-digestible components such as diatoms may benefit from higher digestion HRTs and hydrolysis pre-treatment to improve the rate of digestion.

The results from Chapter 3 indicate that ATS algae can be successfully co-digested with dairy manure, poultry litter, and food waste substrates. This alleviates the impact of seasonal instability in biomass yield on bioenergy generation and allows operators to benefit from renewable biomass-to-bioenergy operations year-round. However, the high moisture content of the ATS algae culture tested was observed diluting the drier agricultural and food waste substrates, but not enough to significantly reduce digestion efficiency for dairy manure at any of the loading ratios tested. Poultry litter and food waste were similarly unaffected when small amounts of algae were added to the waste, which realistically would be the most likely scenario for actual on-farm digesters where such wastes are generated daily. Still, the water content of the algae did significantly reduce the productivity of the digestion per actual mass of waste processed, which would necessitate a larger digester (and associated increased cost) to manage without dewatering.

The results of Chapter 4 directly approached algal dewatering using flocculation and coagulation to thicken the algal culture and found that any form of gravity or flocculation treatment except electrocoagulation consistently and significantly increased the TS and VS present in the resulting algal solids, while also removing >97% of the TSS present in the raw algae. Electrocoagulation was especially promising, having the highest rate of CH_4 generation per mass algae processed and comparable digestion efficiency to $FeCl_3$ and chitosan, despite not thickening the algae to the same degree or with the same consistency as those treatments. Electrocoagulation additionally appears to act as a hydrolysis pre-treatment by lysing the cellular membranes of the algae, which can also contribute to its solids' performance during anaerobic digestion.

5.2 Recommendations

These findings indicate that anaerobic digestion can be broadly recommended as a management option for ATS operators to dispose of their algal byproduct. While the approach is still new and the optimal parameters for digester operation and feedstock management are still being studied, assessments of the pros and cons of the process have led to the development of several recommendations for effective future research and operation of these combined technologies.

It is recommended that planning for new ATS begin with testing a small-scale (1 m^2) at the potential installation site, to characterize the in-situ algal species that will colonize the ATS. Characterizing the volume of algae and composition of the "turf" at this site, preferably over a full year, will allow the operator to plan more effectively how much algae will need to be processed and physical and chemical properties. While this is a time-intensive approach and may not be feasible for all installations, it is strongly recommended to account for the inconsistent composition of ATS turfs between sites and over time. This is particularly important if the turf will contain large amounts of diatoms, as additional pretreatment may be needed to better hydrolyze intercellular components within the silica frustule.

It is also recommended that future studies test additional pre-treatment approaches, with an emphasis on dewatering and hydrolysis methods. Dewatering can reduce the carbon utilization of the algal substrate, however excessive water in the algae can reduce the efficiency of a digestion as well, as discussed in Chapters 3 and 4. Hydrolysis is suspected to have occurred unintentionally twice over the course of this study, first with the elevated heat in the algae storage tank in Year 2 of the Chapter 2 digestion study, and second by the electricity applied to the algal cultures during electrocoagulation treatments in Chapter 4. Both instances were associated with unexpected increases in digestion performance, but as hydrolysis was not specifically quantified within the scope of the study, the results were inconclusive.

Future research should also include formal life cycle assessment (LCA) as part of the methodology of the study. While Chapter 2 included a cost assessment, it did not include an assessment of the sustainability of combined ATS and anaerobic digestion compared to past methods of algal disposal (e.g. landfill). Numerous ATS studies in the literature have suggested using the algal byproduct for bioenergy to recycle the remediated carbon, however, there has yet to be an empirical study proving that this is true. Including LCA would also allow for more direct comparisons of the sustainability of bioenergy derived from ATS algae compared to traditional fossil fuels. Additional cost assessments should also be paired with LCA, as LCA does not account for the financial aspect of a project, but this is a relevant component that ATS operators must consider when choosing the best approach to managing their algal byproduct.

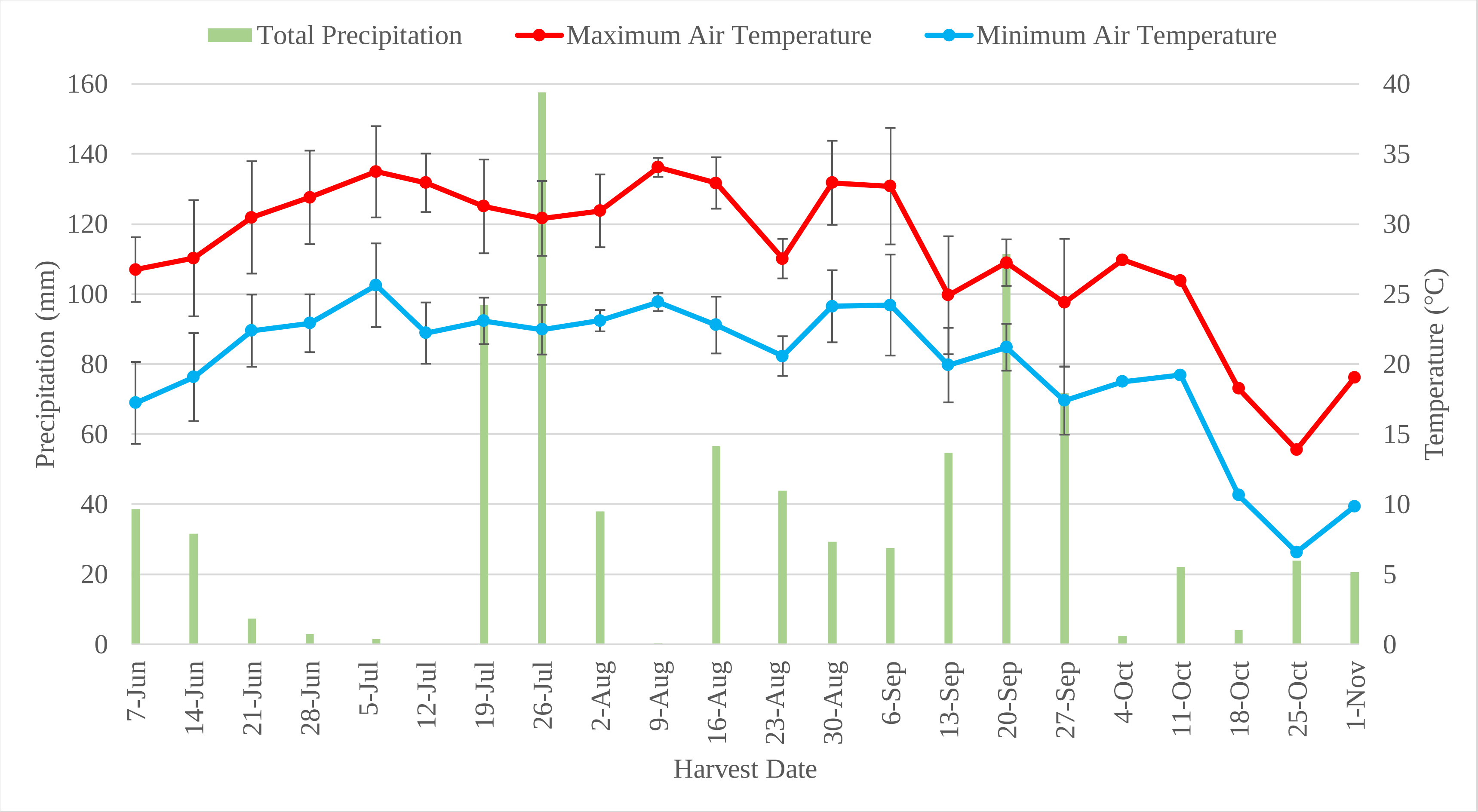
It is also recommended that more comprehensive studies be developed to assess the safety and efficacy of fertilizer derived from effluent from reactors processing ATS algae. This is a particular concern in the case of algae from ATS such as that at Port of Baltimore site, which was located on a tidal river with salinity up to 10.8 ppm measured during the study period. As any salt in the algae or its liquid growth media would persist in the reactor effluent post-digestion, this could make the effluent unsuitable as a fertilizer. Alternative disposal strategies for the algae should also be studied, to account for scenarios where fertilizer use is not feasible. These studies should include full mass balances of nutrient transformations through the digestion process as well, to ensure that the fate of the nutrients remediated by the ATS are accounted for and returned to the environment with sustainable application of effluent fertilizer.

Related to the previous recommendation, testing for contaminants of concern such as polychlorinated biphenyls (PCBs) and per- and poly-fluorinated substances (PFAS) should be conducted on both the ATS algae both pre- and post-digestion. Both ATS sites used in this research were sited on rivers with known histories of contamination with pesticides and industrial chemicals. It is unclear if these substances could accumulate in the algal turf, either via metabolism of the algae or adherence to the algal fronds. If these substances were digested and then land applied, there is a chance that fields could become contaminated. This has been an emerging concern in recent news as some sites that received land applied municipal biosolids have been found to have elevated levels of chemicals such as PFAS. Assessments of fertilizer derived from ATS algae digestate should include contaminant of concern (COC) assessment to ensure that a process designed to sustainably combine bioremediation and renewable bioenergy generation does not come at the cost of new environmental degradation.

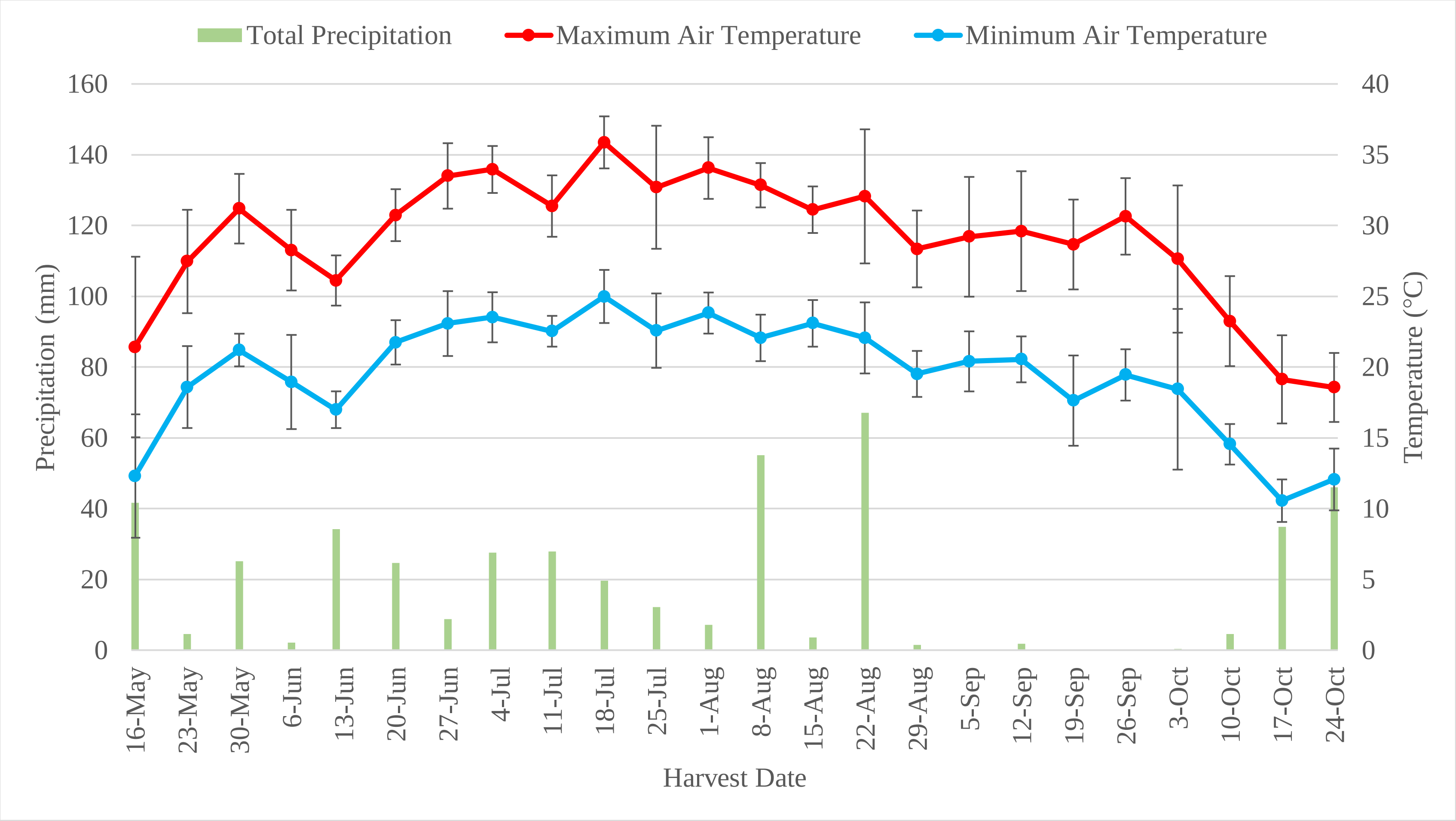
5.3 Wider implications of anaerobic digestion of algal turf scrubber (ATS) algae

The use of anaerobic digestion and related waste to energy approaches (e.g., thermohydraulic) is growing in Maryland to manage the vast quantity of food and animal waste the state generates every year. Two large-scale anaerobic digesters are operational at Kilby Farm in Cecil County and the Maryland Bioenergy Center in Jessup, MD. Municipal wastewater facilities such as the Blue Plains Advanced Wastewater Treatment Plant in nearby Washington, D.C. also utilize anaerobic digestion as part of their routine water treatment processes (DC Water 2023). Smaller experimental and pilot-scale digestion systems are also operational throughout the state, especially on the Eastern Shore where poultry farming is widespread. The state has incentive programs such as the Maryland Animal Waste Technology grants, that are encouraging additional installations of novel waste management technology in the future (MDA 2023). The infrastructure for anaerobic digestion in Maryland is increasing and could provide an option for support for future widespread ATS use in the state.

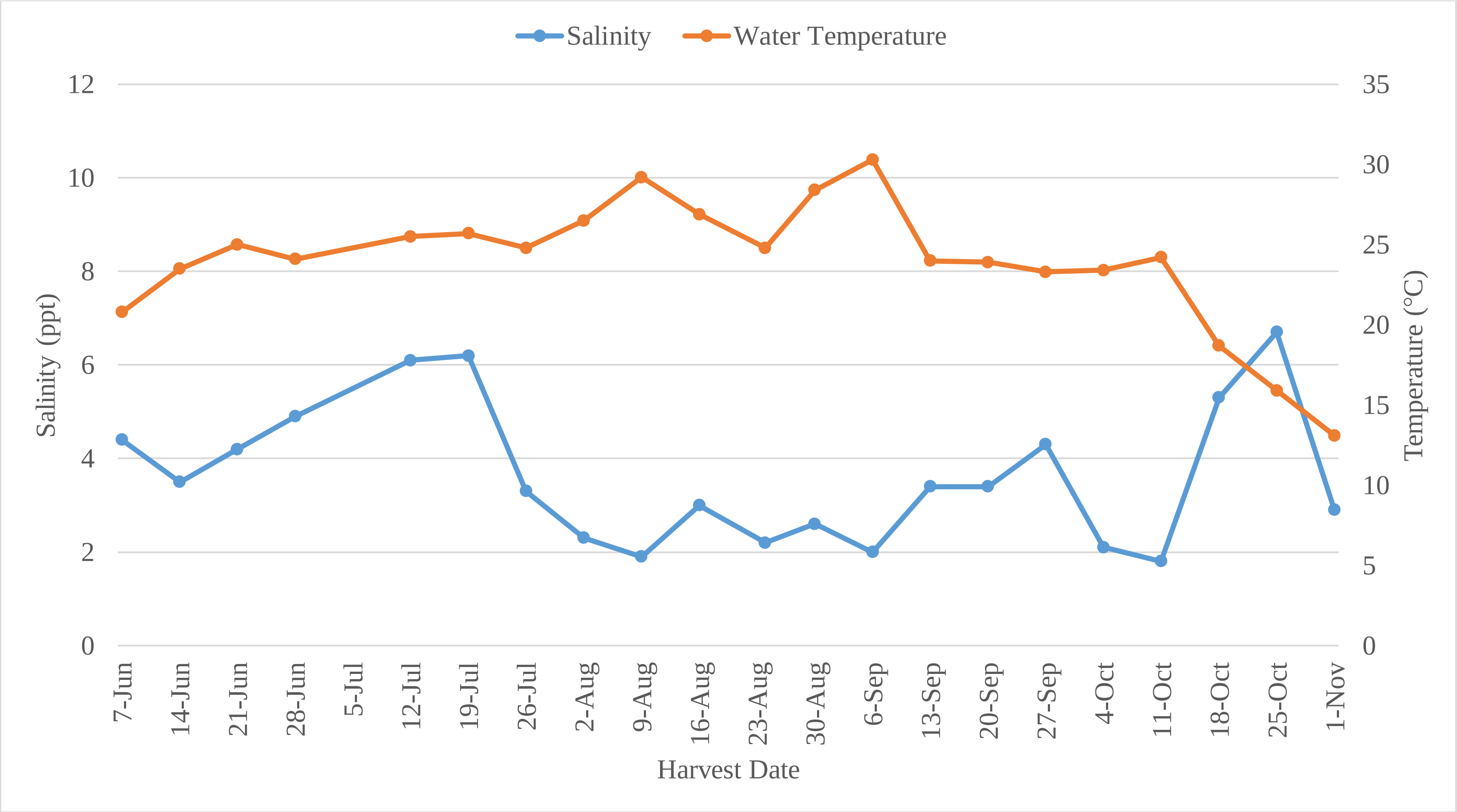
Appendix A: Supplemental Data for Chapter 2



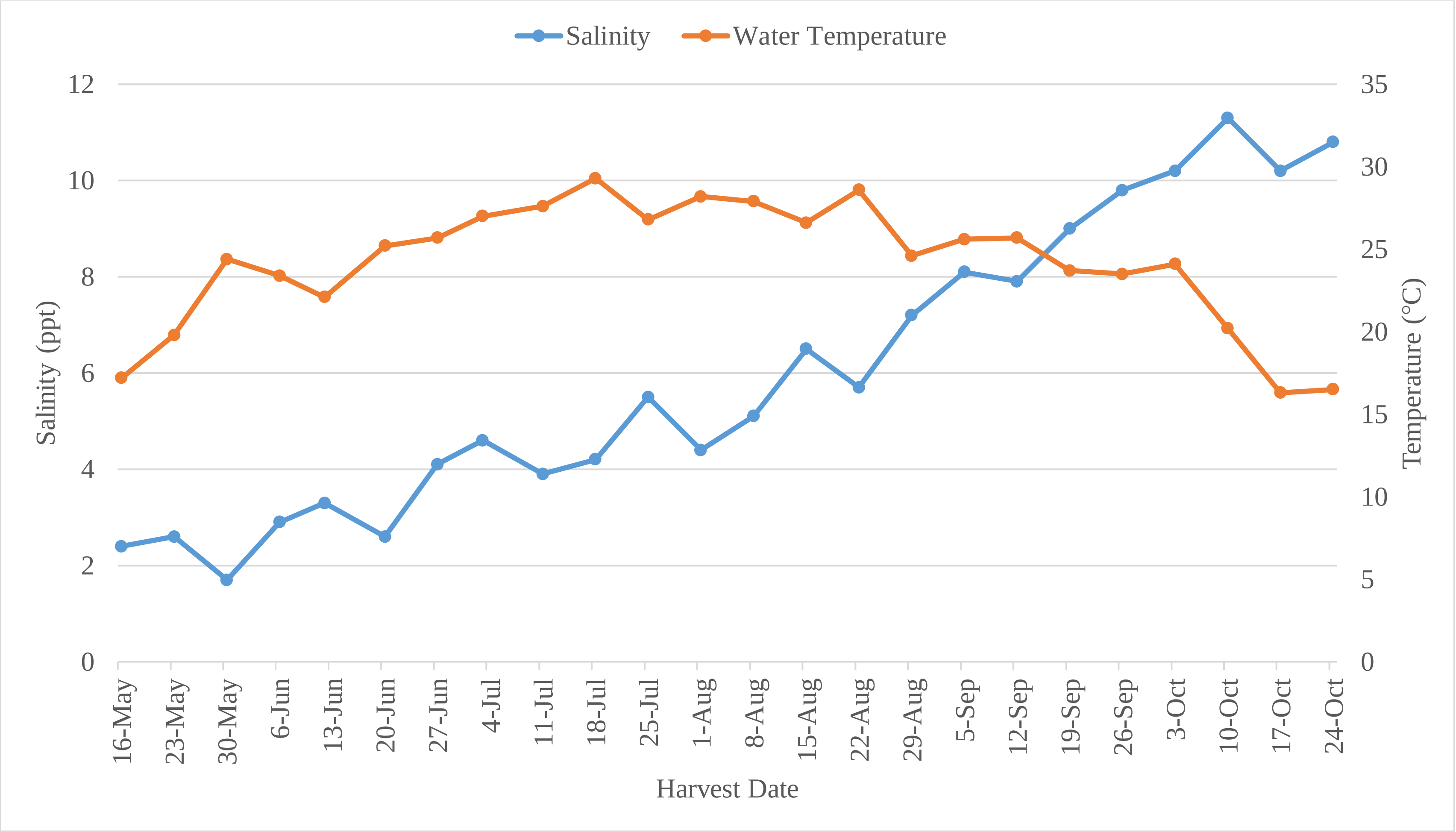
Appendix Figure A.1: Air temperature and precipitation data for the Baltimore area during algal flow way (ATS) operations, Year 1. Precipitation data calculated as the sum for each week of ATS operations. Temperature data is weekly averages (n = 7 days), ± standard error for the week of the harvest.



Appendix Figure A.2: Air temperature and precipitation data for the Baltimore area during algal flow way (ATS) operations, Year 2. Precipitation presented as the sum for the week of the harvest. Temperature data is averages for the week of harvest (n = 7 days), ± standard error for the week of the harvest.



Appendix Figure A.3: Salinity and water temperature data for water pumped from the Patapsco River to the algal turf scrubber (ATS) at the Port of Baltimore Dundalk Terminal (Baltimore, MD, USA) in Year 1.



Appendix Figure A.4: Salinity and water temperature data for water pumped from the Patapsco River to the algal turf scrubber (ATS) at the Port of Baltimore Dundalk Terminal (Baltimore, MD, USA) in Year 2.

Appendix Table A.1: Summary of algal turf scrubber (ATS) growth and productivity during five years prior to the Chapter 2 experiments, 2013-2017. Data retrieved from unpublished site operation reports by the Port of Baltimore and University of Maryland. (May et al. 2014; Selby et al. 2016; Selby et al. 2017; Smith et al. 2013; Smith et al. 2016).

	2013		**2014**		**2015**		**2016**		**2017**	
Harvest Week #	**Date**	**g/m^2/day**	**Date**	**g/m^2/day**	**Date**	**g/m^2/day**	**Date**	**g/m^2/day**	**Date**	**g/m^2/day**
1	Jul-9	27.6	Jun-18	28.3	Apr-30	21.3	Sep-9	43.9	Aug-31	46.6
2	Jul-17	44.3	Jun-25	33.9	May-7	17.7	Sep-15	33.5	Sep-21	42.8
3	Jul-30	30.4	Jul-2	31.6	May-14	22	Sep-22	26.8	Sep-28	53.7
4	Aug-6	35	Jul-9	47.5	May-21	14.4	Oct-6	28	Oct-5	46.4
5	Aug-15	18.3	Jul-16	30.1	May-28	22.7	Oct-13	21.8	Oct-12	43
6	Aug-21	20.7	Jul-23	35.8	Jun-4	-	Oct-20	20.6	Oct-19	27.9
7	Aug-28	20.4	Jul-30	52.8	Jun-11	7.5	Oct-28	27.1	Oct-26	34.9
8	Sep-3	25.2	Aug-8	45.9	Jun-18	18.6	Nov-3	18.3	Nov-2	38.3
9	Sep-11	19.3			Jun-25	28	Nov-10	21.2	Nov-16	16
10	Sep-17	24.5			Jul-2	22.8	Nov-22	30.5		
11	Sep-24	28.2			Jul-9	21.4				
Average		26.7 ± 2.3		38.2 ± 3.2		19.6 ± 1.8		27.2 ± 2.4		38.8 ± 3.8

Appendix B: Supplemental Data for Chapter 3

Appendix Table B.1: Results of plant biomass analysis for the lettuce growth experiment in Chapter 3. Plant and dry biomass values are averages from four replicate plants, + standard error.

	Fertilizer pH	**Plant Biomass (g)**	**Dry Biomass (g)**
Control Stock Fertilizer	4.01	5.28 + 0.16	0.966 + 0.082
No Fertilizer	NA	2.28 + 0.26	0.318 + 0.018
Algae	7.55	3.28 + 0.2	0.517 + 0.035
DM	8.32	4.53 + 0.34	0.683 + 0.089
FW	NA*	3.98 + 0.28	0.655 + 0.056
PL	8.40	4.08 + 0.15	0.659 + 0.014
Algae+DM 1:1	7.62	3.58 + 0.26	0.611 + 0.025
Algae+DM 1:2	7.86	3.33 + 0.35	0.565 + 0.082
Algae+DM 1:5	8.05	4.03 + 0.33	0.696 + 0.074
Algae+DM 1:10	8.04	3.98 + 0.18	0.653 + 0.04
Algae+PL 1:1	7.64	3.05 + 0.12	0.578 + 0.03
Algae+PL 1:2	7.97	3.35 + 0.36	0.542 + 0.103
Algae+PL 1:5	7.94	4.25 + 0.15	0.714 + 0.043
Algae+PL 1:10	8.11	3.93 + 0.22	0.675 + 0.039
Algae+PL 1:1	7.83	3.38 + 0.18	0.597 + 0.031
Algae+PL 1:2	7.97	3.33 + 0.23	0.564 + 0.046
Algae+PL 1:5	8.04	4.23 + 0.05	0.704 + 0.038
Algae+PL 1:10	8.12	3.95 + 0.06	0.674 + 0.023

Bibliography

33 USC § 1313 (2000). Water quality standards and implementation plans.

Achi, C., Hassanein, A., Lansing, S. (2020). Enhanced Biogas Production of Cassava Wastewater Using Zeolite and Biochar Additives and Manure Co-Digestion. Energies, 13(2), p 491.

Adey, W. (1982). Algal Turf Scrubber, US4333263A. US Patent and Trademark Office.

Adey, W., Kangas, P., Mulbry, W. (2011). Algal Turf Scrubbing: Cleaning Surface Waters with Solar Energy while Producing a Biofuel. Bioscience, 61(6), 434-441.

Adey, W.H., Miller, J.B., Hayek, L.C., Thompson, J.G., Bertman, S., Hampel, K., Puvanendran, S. (2013). Algal Turf Scrubber (ATS) Floways on the Great Wicomico River, Chesapeake Bay: Productivity, Algal Community Structure, Substrate and Chemistry. Journal of Phycology, 49, 489-501

Adey, W., Luckett, C., Smith, M. (1996). Purification of industrially contaminatedgroundwaters using controlled ecosystems. Ecological Engineering, 7, 191-212.

Alam, M.A., Vandamme, D., Chun, W., Zhao, X., Foubert, I., Wang, Z., Mulart, K., Yuan, Z. (2016). Bioflocculation as an innovative harvesting strategy for microalgae. Reviews in Environmental Science and Biotechnology, 15, 573-583.

American Public Health Association (APHA). (2005). Standard Methods for the Examination of Water and Wastewater, 21st ed.; APHA:Washington, DC, USA, 2005. American Water Works Association/American Public Works Association/Water Environment Federation. doi:10.2105/AJPH.51.6.940-a.

Asam, Z-u-Z., Poulsen, T.G., Nizami, A-S., Rafique, R., Kiely, G., Murphy, J.D. (2011). How can we improve biomethane production per unit of feedstock in biogas plants? Applied Energy, 88(6), 2013-2018.

Astals, S., Musenze, R.S., Bai, X., Tannock, S., Tait, S., Pratt, S., Jensen, P.D. (2015). Anaerobic co-digestion of pig manure and algae: Impact of intracellular algal products recovery on co-digestion performance. Bioresource Technology, 181, 97-104.

Banerjee, C., Ghosh, S., Sen, G., Mishra, S., Shukla, P., Bandopadhyay, R. (2013). Study of algal biomass harvesting using cationic guar gum from the natural plant source as flocculant. Carbohydrate Polymers, 92(1), 675–681.

Beach, E.S., Eckelman, M.J., Cui, Z., Brentner, L., Zimmerman, J.B. (2012). Preferential technological and life cycle environmental performance of chitosan flocculation for harvesting of the green algae *Neochloris oleoabundans*. Bioresource Technology, 121, 445-449.

Bohutskyi, P., Chow, S., Ketter, B., Shek, C.F., Yacar, D., Tang, Y., Zivojnovich, M., Betenbaugh, M.J., Bouwer, E.J. (2016). Phytoremediation of agriculture runoff by filamentous algae polyculture for biomethane production, and nutrient recovery for secondary cultivation of lipid generating microalgae. Bioresource Technology, 222, 294-308.

Carpenter, R.C., Hackney, J.M., Adey, W.H. (1991). Measurements of primary productivity and nitrogenase activity of coral reef algae in a chamber incorporating oscillating flow. Limnology and Oceanography, 36(1), 40-49.

Chen, N., Li, J., Wu, Y., Kangas, P.C., Huang, B., Yu, C. (2015). Nutrient removal at a drinking water reservoir in China with an algal floway. Ecological Engineering, 84, 506-514.

Chen, R., Zhou, J., Zheng, X., Jiang, L., Duan, N. (2023). Unveiling the synergy of Chlorella sp. and cattle manure co-digestion under high feeding load. Energy, 270, 126877.

Chesapeake Bay Program. (2020). Chesapeake Assessment and Scenario Tool (CAST) Version 2019. Chesapeake Bay Program Office.

Dai, X., Gai, X., Dong, B. (2014). Rheology evolution of sludge through high-solid anaerobic digestion. Bioresource Technology, 174, 6-10.

Dapelo, D. and Bridgeman, J. (2018). Assessment of mixing quality in full-scale, biogas-mixed anaerobic digestion using CFD. Bioresource Technology, 265, 480-489.

de Castro Padrilha, M.C., Vicente, L.E, Dematté, J.A.M., dos Santos Wendriner Loebmann, D.G., Vicente, A.K., Salazaar, D.F.U., Guimãrez, C.C.B. (2020). Using Landsat and soil clay content to map soil organic carbon of oxisols and Ultisols near São Paulo, Brazil. Geoderma Regional, e00253.

de la Fuente, A., Muro-Pastor, A.M., Merchán, F., Madrid, F., Pérez-Martinéz, J.I., Undabeytia, T. (2019). Electrocoagulation/flocculation of cyanobacteria from surface waters. Journal of Cleaner Production, 238, 117964.

Devlin, D.C., Esteves, S.R.R., Dinsdale, R.M., Guwy, A.J. (2011). The effect of acid pretreatment on the anaerobic digestion and dewatering of waste activated sludge. Bioresource Technology, 102(5), 4076-4082.

DC Water (2020). Fresh Bloom for Agriculture Fact Sheet.

DC Water (2023). Blue Plains Advanced Wastewater Treatment Plant Brochure.

Dhungana, B., Lohani, S.P., Marsolek, M. (2022). Anaerobic Co-Digestion of Food Waste with Livestock Manure at Ambient Temperature: A Biogas Based Circular Economy and Sustainable Development Goals. Sustainability, 14(6).

El-Hadj, T.B., Dosta, J., Mata-Alvarez, J. (2007). Start-up and HRT influence in thermophilic and mesophilic anaerobic digesters seeded with waste activated sludge. Chemical and Biochemical Engineering Quarterly, 21(2), 145-150.

Feng, Q., Song, Y.C., Kim, D.H., Kim, M.S., Kim, D.H. (2019). Influence of the temperature and hydraulic retention time in bioelectrochemical anaerobic digestion of sewage sludge. International Journal of Hydrogen Energy, 44(4), 2170-2179.

Fisher Scientific. (2023a). Ferric Chloride Hexahydrate, (Crystalline), MP Biomedicals.

Fisher Scientific (2023b). Chitosan, MP Biomedicals™.

Fujishima, S., Miyahara, T., Noike, T. (2000). Effect of moisture content on anaerobic digestion of dewatered sludge: ammonia inhibition to carbohydrate removal and methane production. Water Science and Technology, 41(3), 119-127.

Gan et al. (2022). Optimizing nutrient removal and biomass production of the Algal Turf Scrubber (ATS) under variable cultivation conditions by using Response Surface Methodology. Frontiers in Bioengineering and Biotechnology, 10: 962719.

García-Casal, M., Pereira, A.C., Leets, I., Ramírez, J. Quiroga, M.F. (2007). High Iron Content and Bioavailability in Humans from Four Species of Marine Algae. The Journal of Nutrition, 137, 2691-2695.

Ge, H., Zhang, L., Batstone, D.J., Keller, J., Yuan, Z. (2013). Impact of Iron Salt Dosage to Sewers on Downstream Anaerobic Sludge Digesters: Sulfide Control and Methane Production. American Society of Civil Engineering, 594-601.

Gerardi, M. (2003). The Microbiology of Anaerobic Digesters. John Wiley and Sons, Inc.

Hidalgo-Sánchez, V., Behmel, U., Hofmann, J., Borges, M.E. (2023). Enhancing Biogas Production of Co-Digested Cattle Manure with Grass Silage from a Local Farm in

Landshut, Bavaria, through Chemical and Mechanical Pre-Treatment and Its Impact on Biogas Reactor Hydraulic Retention Time. Sustainability, 15(3), 2582.

Hydromentia. (2022). Algal Turf Scrubber®.

Kangas, P. and Mulbry, W. (2014). Nutrient removal from agricultural drainage water using algal turf scrubbers and solar power. Bioresource Technology, 152, 484-489.

Kangas, P., Mulbry, W., Klavon, P., Laughinghouse, H.D., Adey, W. (2017). High diversity within the periphyton community of an algal turf scrubber on the Susquehanna River. Ecological Engineering, 108, 564-572.

Kariyama, I.D., Zhai, X., Wu, B. (2018). Influence of mixing on anaerobic digestion efficiency in stirred tank digesters: A review. Water Research, 143, 503-517.

Kaya, C., Şenbayram M., Akram, N.A., Ashraf, M., Alyemeni, M.N., Ahmad, P. (2020). Sulfur-enriched leonardite andhumic acid soil amendments enhance tolerance to drought and phosphorus deficiency stress in maize (Zea mays L.). Scientific Reports, 10:6432.

Kebede-Westhead, E., Pizarro, C., Mulbry, W.W. (2004). Treatment of dairy manure effluent using freshwater algae: Elemental composition of algal biomass at different manure loading rates. Journal of Agricultural and Food Chemistry, 52(24), 7293-7296.

Koster, I.W. (1986). Characteristics of the pH-influenced adaptation of methanogenic sludge to ammonium toxicity. Journel of Chemical Technology and Biotechnology, 36(10), 445-455.

Lama, S., Muylaert, K., Karki, T.B. et al. (2016). Flocculation properties of several microalgae and a cyanobacterium species during ferric chloride, chitosan and alkaline flocculation. Bioresource Technology, 220, 464-470.

Lansing, S., Hüselmann, B., Choudhury, A., Schueler, J., Lisboa, M.S., Oechsner, H. (2019). Food waste co-digestion in Germany and the United States: From lab to full-scale systems. Resources, Conservation, and Recycling, 146, 104-113.

Lansing, S., Víquez, J., Martínez, H., Botero, R., Martin, J. (2008). Quantifying electricity generation and waste transformations in a low-cost, plug-flow anaerobic digestion system. Ecological Engineering, 34(4), 332-348.

Lau, Y.L. and Liu, D. (1993). Effect of flow-rate on biofilm accumulation in open channels. Water Research, 27(3), 355-360.

Lee, K., Chantrasakdakul, P., Kim, D., Kong, M. (2014) Ultrasound pretreatment of filamentous algal biomass for enhanced biogas production. Waste Management, 34(6), 1035-1040.

Lewis, J.B. (1977). Processes of organic production on coral reefs. Biological Reviews of the Cambridge Philosophical Society, 52(3), 305-347.

Li, J.J., Zicari, S.M., Cui, Z.J., Zhang, R.H. (2014). Processing anaerobic sludge for extended storage as anaerobic digester inoculum. Bioresource Technology, 166, 201-210.

Lisboa, M.S. and Lansing, S. (2013). Characterizing Food Waste Substrates for Co-Digestion through Anaerobic Toxicity Assays (ATA) and Biochemical Methane Potential (BMP). American Society of Agricultural and Biological Engineers Conference. Paper number 131619080, Kansas City, Missouri, July 21 - July 24, 2013.

Lisboa, M.S. and Lansing, S. (2014). Evaluating the toxicity of food processing wastes as co-digestion substrates with dairy manure. Waste Management, 34(7), 1299-1305.

Lu, D. and Zhang, X.J. (2016). Biogas Production from Anaerobic Codigestion of Microalgae and Septic Sludge. Journal of Environmental Engineering, 142(10): 04016049.

Luckakova., S., Branyakova, I., Kovacikova, S., Masojidek, J., Ranglova, K., Branyik, T., Rizicka, M.C. (2022). Continuous electrocoagulation of Chlorella vulgaris in a novel channel-flow reactor: A pilot-scale harvesting study. Bioresource Technology, 351.

Maryland Department of Agriculture (MDA). (2023). Animal Waste Technology Grants.

Maryland Department of the Environment (MDE). (2018). MDE Approved BMPs for Trading.

Maryland Department of the Environment (MDE), Maryland Department of Natural Resources (MDNR), Maryland Department of Agriculture (MDA), and Maryland Department of Planning (MDP). (2019). Maryland's Phase III Watershed Implementation Plan to Restore Chesapeake Bay by 2025.

Maryland Department of Planning (MDP). (2020). A Summary of Land Use Trends in Maryland: The Maryland Department of Planning 2010 Land Use/Land Cover product.

MD Code Regs. 26.08.11 (2018). Maryland Water Quality Trading Program.

May, P., Kangas, P., Smith, J., Selby, B. (2014). A comparison of methods for estimating algal biomass productivity at the Port of Baltimore Algal Turf Scrubber. Unpublished report to the Port of Baltimore.

Mayr, M., Jerney, J., Schagerl. (2015). Combating planktonic algae with benthic algae. Ecological Engineering, 74, 310-318.

Mazinni, S., Borgonovo, G., Scaglioni, L., Bedussi, F., D'Imporzano, G., Tambone, F., Adani, F. (2020). Phosphorus speciation during anaerobic digestion and subsequent solid/liquid separation. Science of the Total Environment, 734, 139284.

Menzel, T., Neubauer, P., Junne, S. (2020). Role of Microbial Hydrolysis in Anaerobic Digestion. Energies, 13(21), 5555.

Miao, H., Wang, S., Zhao, M., Huang, Z., Ren, H., Yan, Q., Ruan, W. (2014). Codigestion of Taihu blue algae with swine manure for biogas production. Energy Conversion and Management, 77, 643-649.

Minasney, B., McBratney, A.B., Wadoux, A.M.J.-C., Akoeb, E.N., Sabrina, T. (2020) Precocious 19th century soil carbon science. Geoderma Regional, e00306.

Mulbry, W., Kangas, P., Kondrad, S. (2010). Toward scrubbing the bay: Nutrient removal using small algal turf scrubbers on Chesapeake Bay tributaries. Ecological Engineering, 36, 536-541.

Mulbry, W., Kondrad, S., Buyer, J. (2008a). Treatment of dairy and swine manure effluents using freshwater algae: fatty acid content and composition of algal biomass at different manure loading rates. Journal of Applied Phycology, 20, 1079-1085.

Mulbry, W., Kondrad, S., Pizarro, C., Kebede-Westhead, E. (2008b). Treatment of dairy manure effluent using freshwater algae: Algal productivity and recovery of manure nutrients using pilot-scale algal turf scrubbers. Bioresource Technology, 99, 8137-8142.

Munshi, F.M., Church, J., McClean, R., Maier, N., Sadmani, A.H.M.A., Duranceau, S.J., Lee, W.H. (2018). Dewatering algae using an aquaporin-based polyethersulfone forward osmosis membrane. Separation and Purification Technology, 204, 154-161.

Musa, M., Wolf, J., Stephens, E., Hankamer, B., Brown, R., Rainey, T.J. (2020). Cationic polyacrylamide induced flocculation and turbulent dewatering of microalgae on a Britt Dynamic Drainage Jar. Separation and Purification Technology, 233, 116004.

Narayan, O.P., Kumar, P., Yadav, B., Dia, M., Johri, A.K. (2022). Sulfur nutrition and its role in plant growth and development. Plant Signaling and Behavior, 2030082.

Nowicka, A., Zieliński, M., Dębowski, M. (2019). Microwave support of the alcoholic fermentation process of cyanobacteria *Arthrospira platensis*. Environmental Science and Pollution Research, 27, 118-124.

Ortiz, A., Garcia, J., Ugetti, E., Diez-Montero, R. (2022). Optimization of multi-stage thickening of biomass in a demonstrative full–scale microalgae-based wastewater treatment system. Separation and Purification Technology, 281, 119830.

Park, C., Lee, C., Kim, S., Chen, Y., Chase, H.A. (2005). Upgrading of anaerobic digestion by incorporating two different hydrolysis processes. Journal of Bioscience and Bioengineering, 100(2), 164-167.

Passos, F., Astals, S., Ferrer, I. (2014). Anaerobic digestion of microalgal biomass after ultrasound pretreatment, Waste Management, 34(11), 2098-2103.

Pearsall, R.V., Connelly, R.L., Fountain, M.E., Hearn, C.S., Werst, M.D., Hebner, R.E., Kelley, E.F. (2011). Electrically dewatering microalgae. IEEE Transactions on Dielectrics and Electrical Insulation, 18(5), 1578-1583.

Pin Viso, N.D., Rizzo, P.F., Young, B.J., Gabioud, E., Bres, P., Riera, N.I., Merino, L., Farber, M.D., Crespo, D.C. (2022). The Use of Raw Poultry Waste as Soil Amendment Under Field Conditions Caused a Loss of Bacterial Genetic Diversity Together with an Increment of Eutrophic Risk and Phytotoxic Effects. Soil Microbiology.

Pizarro, C., Mulbry, W., Blersch, D., Kangas, P. (2006). An economic assessment of algal turf scrubber technology for treatment of dairy manure effluent. Ecological Engineering, 26, 321-327.

Poon, C.S., Chu, C.W. (1999). The use of ferric chloride and anionic polymer in the chemically assisted primary sedimentation process. Chemosphere, 39:10, 1573-1582.

Powell, R.J. and Hill, R.T. (2013). Rapid Aggregation of Biofuel-Producing Algae by the Bacterium *Bacillus* sp. Strain RP1137. Applied and Environmental Microbiology, 79(19), 6093-6101.

Powell, R.J. and Hill, R.T. (2014). Mechanism of Algal Aggregation by *Bacillus* sp. Strain RP1137. Applied and Environmental Microbiology, 80(13), 4042-4050.

Prescott, G., Bamrick, J., Cawley, E., Jaques, W. (1978). How to Know the Freshwater Algae, 3rd Edition. Mc-Graw Hill Science/Engineering/Math.

Rothman, N.J., Canuel, E.A., Beck, A.J. (2013). Trace metal cycling in an algal floway system. Ecological Engineering, 52, 290-297.

Ruiz-Dominguez, M.C., Salinas, F., Medina, E., Rincón, B., Martin, M.A., Gutiérezz, M.C., Cerezal-Mezquita, P. (2022). Supercritical Fluid Extraction of Fucoxanthin from the Diatom *Phaeodactylum tricornutum* and Biogas Production through Anaerobic Digestion. Marine Drugs, 20(2), 127.

Salvucci, M.E. and Crafts-Brandner, S.J. (2004). Mechanism for deactivation of Rubisco under moderate heat stress. Physiologia Plantarum, 122(4), 514-519.

Selby, B., Kangas, P., May, P., Mulbry, W. Calahan, S. (2016). Algal biomass productivity at the Port of Baltimore Algal Turf Scrubber: Fall 2016. Unpublished report to the Port of Baltimore.

Selby, B., May, P., Calahan, S., Blair, A., Kangas, P. (2017). Algal biomass productivity at the Port of Baltimore Algal Turf Scubber: 2017. Unpublished report to the Port of Baltimore.

Shao, P.H., Darcovich, K., McCracken, T., Ordorica-Garcia, G., Reith, M., O'Leary, S. (2015). Algae-dewatering using rotary drum vacuum filters: Process modeling, simulation and techno-economics. Chemical Engineering Journal, 268, 67-75.

Singh, N., Singhania, R.R., Nigam, P.S., Dong, C-D., Patel, A.K., Puri, M. (2022) Global status of lignocellulosic biorefinery: Challenges and perspectives. Bioresource Technology, 344, 126415.

Smith, J., Selby, B., Kangas, P., May, P. (2013). Progress report on the Port of Baltimore algal turf scrubber project, December 2013. Unpublished report to the Port of Baltimore.

Smith, J., Selby, B., Kangas, P., May, P., Mulbry., W. (2016). Final progress report on the Port of Baltimore algal turf scrubber project. Unpublished report to the Port of Baltimore.

Tedesco, S., Stokes, J. (2017). Valorisation to biogas of macroalgal waste streams: a circular approach to bioproducts and bioenergy in Ireland. Chemical Papers, 71:721-728.

Tumusiime, E., Kirabira, J.B., Musinguzi, W.B. (2022). Optimization of substrate mixing ratios for wet anaerobic digestion of selected organic waste streams for productive biogas systems. Energy Reports, 8, 10409-10417.

Ummalyma, S.B., Gnansounou, E., Sukumaran, R.K., Sindhu, R., Pandey, A., Sahoo, D. (2017). Bioflocculation: An alternative strategy for harvesting of microalgae – An overview. Bioresource Technology, 242, 227-235.

United States Energy Information Administration (USEIA). (2023a). British Termal Units. Retrieved from: https://www.eia.gov/energyexplained/units-and-calculators/british-thermal-units.php

United States Energy Information Administration (USEIA). (2023b). Energy Conversion Calculators. Retrieved from: https://www.eia.gov/energyexplained/units-and-calculators/energy-conversion-calculators.php

United States Energy Information Administration (USEIA). (2023c). Maryland State Profile and Energy Estimates. Retrieved from: https://www.eia.gov/state/data.php?sid = MD

United States Environmental Protection Agency (USEPA) (2010a). Chesapeake Bay Total Maximum Daily Load.

United States Environmental Protection Agency (USEPA). (2010b). Guidance for federal land management in the Chesapeake Bay Watershed.

United States Environmental Protection Agency (USEPA). (2015a). EPA's Assessments of Animal Agriculture Programs in the Chesapeake Bay Watershed. Retrieved from https://www.epa.gov/chesapeake-bay-tmdl/epas-assessments-animal-agriculture-programs-chesapeake-bay-watershed.

United States Environmental Protection Agency (USEPA). (2015b). Nutrient and Sediment Reductions from Algal Flow-way Technologies: Recommendations to the Chesapeake Bay Program’s Water Quality Goal Implementation Team from the Algal Flow-way Technologies BMP Expert Panel.

United States Environmental Protection Agency (USEPA). (2018). EPA Evaluation of Maryland’s 2016-2017 and 2018-2019 Milestones.

United States Environmental Protection Agency (USEPA). (2022). AgSTAR Livestock Anaerobic Digester Database.

Van der Berg, M.F., Botha, A-M., Bierman, A., Oberholster, P.J. (2020). Harvesting Microalgal Biomass from a Cultured Algae-Based Wastewater Pond System. Journal of Environmental Engineering, 146(12), 04020133.

Van Eynde, E., Lenaerts, B., Tytgat, T., Verbruggen, S.W., Hauchcorne, B., Blust, R., Lenaerts, S. (2014). Effect of pretreatment and temperature on the properties of *Pinnularia biosilica* frustules. Royal Society of Chemistry, 4, 56200-56206.

Velu, C., Cirés, S., Brinkman, D.L., Helmann, K. (2020). Bioproduct Potential of Outdoor Cultures of Tolypothrix sp.: Effect of Carbon Dioxide and Metal-Rich Wastewater. Frontiers in Bioengineering and Biotechnology, 8:51.

Wall, D.M., Wu-Haan, E., Safferman, S.I. (2012). Reconstitution of dewatered food processing residuals with manure to increase energy production from anaerobic digestion. Biomass and Bioenergy, 46, 429-434.

Wang, C., Cai, Q., Feng, B., Feng, S., Tian., C., Jiang, X., Wu, X., Xiao, B. (2019). Improving the performance of shipboard rotary drum filters in the removal of cyanobacterial blooms by cationic polyacrylamide flocculation. Separation and Purification Technology, 215, 660-669.

Wang, J., Peipoch, M., Guo, X.X., Kan, J.J. (2022). Convergence of biofilm successional trajectories initiated during contrasting seasons. Frontiers in Microbiology, 13, 991816.

Witarsa, F., Yarberry, A., May, P., Kangas, P., Lansing, S. (2020). Complementing energy production with nutrient management: Anaerobic digestion system for algal turf scrubber biomass. Ecological Engineering, 143.

Wu, B. (2010). CFD simulation of gas and non-Newtonian fluid two-phase flow in anaerobic digesters. Water Research, 44, 3861-3874.

Yarberry, A., Lansing, S., Luckarift, H., Diltz, R., Mulbry, W., Yarwood, S. (2019). Effect of anaerobic digester inoculum preservation via lyophilization on methane recovery. Waste Management, 87, 62-70.

Yatipanthalawa, B.S., Ashikkumar, M., Scales, P.J., Martin, G.J.O. (2022). Ultrasound-Assisted Extracellular Polymeric Substance Removal from the Diatom Navicula sp.: A Route to

Functional Polysaccharides and More Efficient Algal Biorefineries. ACS Sustainable Chemical Engineering, 10(5), 1795-1804.

Yenigun, O., Demirel, B. (2013). Ammonia inhibition in anaerobic digestion: A review. Process Biochemistry, 48(5-6), 901-911.

Yue, D., You, F., Snyder, S.W. (2014). Biomass-to-bioenergy and biofuel supply chain optimization: Overview, key issues and challenges. Computers and Chemical Engineering, 66, 36-56.

Zamalloa, C., De Vrieze, J., Boon, N., Verstraete, W. (2012). Anaerobic digestibility of marine microalgae Phaeodactylum tricornutum in a lab-scale anaerobic membrane bioreactor. Applied Microbiology and Biotechnology, 93, 859-869.

Zhang, H., Chen, R., Li, F., Chen, L. (2014). Effect of flow rate on environmental variables and phytoplankton dynamics: results from field enclosures. Chinese Journal of Oceanology and Limnology, 33, 430-438.