Title

Progress towards the production of sustainable infrastructure materials from P. tricornutum and C. sorokiniana cultivated on food waste permeate

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Progress Towards the Production of Sustainable Infrastructure Materials from *P. tricornutum* and *C. sorokiniana* Cultivated on Food Waste Permeate

By

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THESIS

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Abstract

This thesis describes my research performed in the Franz Research Group while working towards my M.S. in Agricultural and Environmental Chemistry at the University of California, Davis. My research focused on remediating anaerobic digestate (food waste permeate, FWP) using the diatom *Phaeodactylum tricornutum* and marine microalgae *Chlorella sorokiniana* to produce sustainable biomass for composite materials.

Chapter 1 contains an overview of existing literature that provides the foundation for my research. This chapter discusses the implications of current practices in the construction industry, recent advances in moving towards sustainable practices within this industry, the status of microalgae use in wastewater remediation, and the potential for microalgae biomass in construction materials. Chapter 2 presents my research and efforts to overcome challenges with cultivating *P. tricornutum* in high ammonia-containing anaerobic digestate (food waste permeate, FWP) to produce biomass for sustainable concrete. In addition to dilution of the FWP, two methods to increase microalgal growth rate using FWP with high ammonia are discussed: 1) co-treatment of the FWP with nitrifying bacteria, and 2) silicon supplementation. Results of microplate screening and batch scale experiments for microalgae cultivation on variations dilutions of FWP and with different supplementation conditions are discussed. Chapter 3 presents results to produce an artificial lumber composite using biomass from *C. sorokiniana* cultivated on FWP.

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List of Abbreviations and Acronyms

AD ..... Anaerobic Digester

FWP ..... Food Waste Permeate

GHG ..... Greenhouse gas

mg ..... Milligram

NB ..... Nitrifing bacteria

NH_3-N ..... Ammonia-nitrogen

NO_2^- ..... Nitrite

NO_3^- ..... Nitrate

READ ..... Renewable Energy Anaerobic Digester

SAC ..... Surface Activation Catalyst

SCM ..... Supplementary Cementitious Materials

UCSD ..... University of California, San Diego

Chapter One: Anaerobic Digestate as a Nutrient Source for Microalgae Biomass to Produce Sustainable Materials

1.1. Bioremediation of Wastewater using Microalgae

Microalgae are a marine organism ranging from 0.2-200 μm in size that have been studied as a bioremediation mechanism and feedstock for high value bioproducts.[1] Microalgae have the potential to remove environmental pollutants such as nitrates and phosphates prominent in agricultural runoff and other water sources.[2] Microalgae use these chemicals as nutrients from the water, thus reducing contaminants in the water and allowing it to be recycled.[3–7] As such, the water quality is improved, and microalgae biomass is produced.

The biomass can be harvested for bioproducts that have the potential to be used in fuel, nutrition, and cosmetic industries. However, costs associated with cultivation and harvesting make microalgae bioproducts less competitive on the market. For example, a 2020 study in the U.S. estimated microalgae biofuels to be $25.68/gal for fuels produced from microalgae grown in wastewater.[8] Microalgae biomass, such as that of *Chlorella sp.*, is popular as a nutrition supplement.[9] Costs of whole microalgae biomass are lower ($44/kg), but a 2019 study found that targeted nutrition supplementation with microalgae beta-carotene or astaxanthin increases costs to $300-1,500/kg and $2,500-7,000/kg (in U.S. dollars).[9] These high costs are because biomass produced on wastewater cannot be used for human consumption; biomass must be produced on synthetic media for these applications. The costs of microalgae derived bioproducts are not competitive to traditional products such as gasoline or nutritional vitamins. As such, bioremediation of wastewater using microalgae creates an opportunity to reduce these costs by

producing multiple bioproducts such as remediated water and microalgae biomass that has potential in biofuels, fertilizer, animal feeds, and even infrastructure materials.

The production of microalgae biofuels from biomass cultivated on wastewater allows for spent microalgae biomass to be used as aquaculture feeds.[10,11] For example, lipid-extracted microalgae biomass contains nutrients and minerals that can replace fishmeal and soy protein concentrates in fish diets.[10,12] It can replace up to 10% of dietary protein without reduction in fish growth.[10,12] Further investigations of defatted, or lipid extracted, biomass in shrimp diets concluded that 6.51-13.3% replacement of fish meal and soy protein in shrimp diets did not affect survival, weight gain and growth.[10] A 2022 cost-analysis of aquaculture feed production from wastewater-produced microalgae biomass indicates that cost of feed is about $143.89/kg of biomass.[13] In addition to being used as animal feed, sustainable buildings have been designed with a microalgae biorefinery-based infrastructure to include photobioreactors that can produce microalgae on wastewater to provide remediated wastewater, reduction in energy costs and GHG emissions, and biofuel production.[14] Photobioreactors integrated into building designs create architectural issues, but these can be overcome with raw construction materials created from microalgae biomass, while simultaneously reducing GHG emissions and remediating wastewater. This thesis will discuss the production of sustainable concrete and lumber from microalgae biomass produced on wastewater.

1.1.1. Anaerobic Digestion

To target market competitiveness of microalgae bioproducts, the Franz lab in collaboration with the Zhang Lab at UC Davis explored cultivation of microalgae on food waste permeate (FWP) from the UC Davis Renewable Energy Anaerobic Digester (READ).[8] Anaerobic

digestion (AD) is the process of using anaerobic microbes to convert biomass and other organic wastes to biogas, mainly methane and carbon dioxide (**Figure 1**).[15] During this process, microbes break down organic waste such as food waste, manure, and crop waste in the absence of oxygen. Anaerobic digestion is a four step process: 1) hydrolysis, during which organic wastes are broken down into simple sugars and amino acids, 2) acidogenesis, during which the sugars are broken down into CO_2, NH_3, and volatile fatty acids, 3) acetogenesis, where these microbes further break down the volatile fatty acids into CO_2 and acetic acids, and 4) methanogenesis, where products of steps 1-3 are converted to biogas (primarily CO_2 and CH_4).[16] This process not only produces biogas, but also a digestate that consists of both solid and liquid waste. This digestate goes through an ultrafiltration process in which the liquid and solid components are separated.[15] The liquid component is rich in ammonia-nitrogen and is referred to as food waste permeate (FWP), and the solid waste is rich in phosphorus and is used as fertilizer.[15]

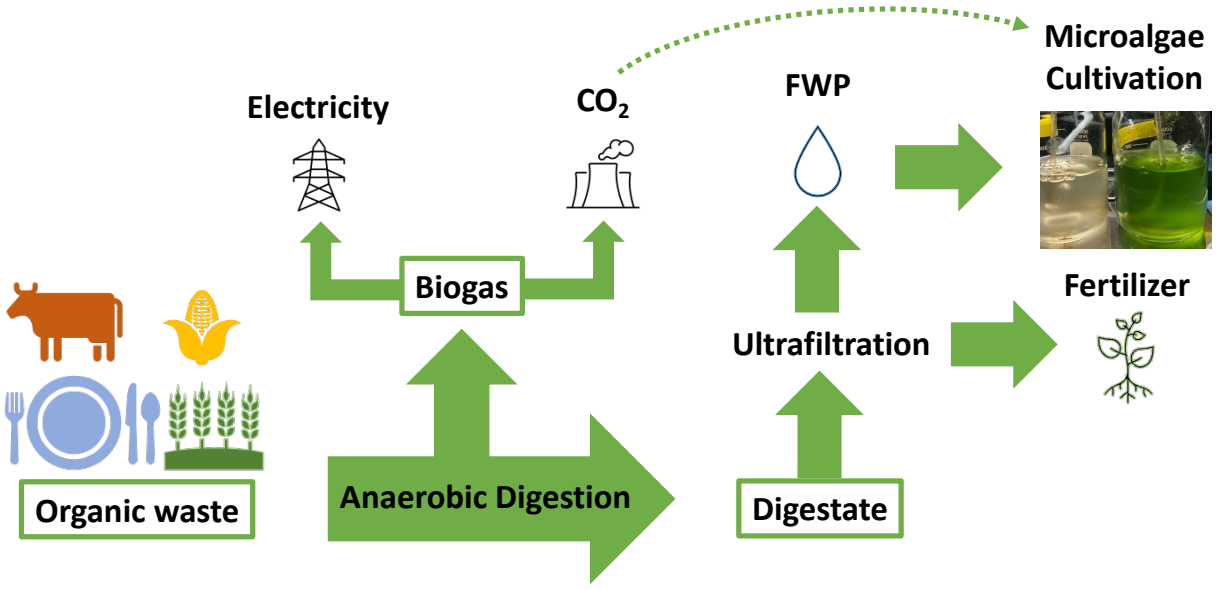

Figure 1. The process of anaerobic digestion and the production of nitrogen-rich food waste permeate (FWP) and fertilizer through ultrafiltration of the digestate for microalgae cultivation.

This anaerobic digestion process produces a nutrient-rich liquid that is ultrafiltered to produce a permeate that is ideal for microalgae cultivation (**Table 1**).[15] At READ, food waste and fertilizers from the campus serve as feedstocks for the digesters. The READ facility can treat ~50 tons of waste per day and produces ~15,000 tons of effluent per year.[8] This amounts to ~10,000 gallons of digestate per day with TKN of about 2,302 mg/L, about 21 tons of N per year.[8] Microalgae cultivation can be used to treat this digestate for reuse in agricultural practices. Research in the Franz lab has demonstrated nutrient remediation of this digestate, called food waste permeate (FWP), through the cultivation of freshwater microalgae species such as *Chlorella sorokiniana*, *Chlorella vulgaris* and *Scenedesmus obliquus*.[17] Biomass productivity reached 50-260 mg/day/L over the course of a 2-week cultivation period.[8] A major goal of the work described herein is to achieve similar biomass productivity by cultivating *P. tricornutum* in FWP, with the intent of utilizing the biosilica-rich biomass for sustainable concrete production.

1.1.2. Characteristics of FWP

Microalgae cultivation on the food waste permeate provides an opportunity while also posing some challenges. This permeate is rich in nitrogen, specifically ammonia-nitrogen (NH_3-N). The levels of ammonia-nitrogen in FWP from the UC Davis READ facility, which will be used in this study, were measured to be 2340 mg/L, which is approximately four times higher than what most microalgae species can tolerate.[18] Microalgae utilize nitrates/nitrites and phosphate to produce biomass; however, ammonia is the dominating nitrogen source in the food waste permeate. As a result, the investigation of methods to transform or decrease ammonia-nitrogen concentrations was one of the research goals for this project. Additionally, phosphates are a limiting nutrient in the food waste permeate because phosphate species, specifically

orthophosphates, are removed during the production of the permeate where solids are removed to produce a phosphorus-rich fertilizer co-product.

Table 1. Nutrient analysis of FWP from the UC Davis READ[19]

Parameter	FWP (mg/L)
TKN	2710 ± 127
NH_3-N	2340 ± 141
P	18.4 ± 2.3
K	1710 ± 28
Ca	6.9
Mg	8.4 ± 3.8
B	1.3 ± 0.1
Na	819.50 ± 0.09
TOC	20750 ± 6000
pH	8.4

*Measurements were performed by Denele Analytical, Inc.

1.2. Environmental impacts of traditional concrete production

While microalgae can be cultivated in wastewater to produce valuable bioproducts, the diatom *P. tricornutum* has unique properties that can target CO_2 reduction in concrete production. The global demand for concrete is approaching 30 billion tons per year, higher than any other material in the construction industry, including steel or wood.[20] Concrete is composed of cement, water, and granular rocks.[20] In the United States, over 80 million tons of cement are produced annually.[21] This production contributes 8-9% of global CO_2 and 2-3% of energy use.[20] It is projected that there will be a 50% increase in annual cement production by 2050, which will lead to an additional 85-105 Gt of CO_2eq and 420-505 TJ of energy over the next 33 years.[20] Most of these emissions are due to the calcination process of limestone and clay during the production of Portland cement.[22] During this process (**Figure 2**), fine raw materials are heated at temperatures

of 600-900 °C in a cement kiln to produce calcium oxide and carbon dioxide, and there is a subsequent kilning stage in which calcium silicates are formed with this calcium source (reaching temperatures of 1450°C).[22] There are direct emissions of CO_2 from limestone decarbonations, i.e., the calcination of $CaCO_3$ to CaO, and from thermal energy demand associated with the burning of fossil fuels to heat the kiln.[22] This stage in manufacturing makes concrete among the most difficult to decarbonize industrial sectors, with global burdens exceeding 7% of annual CO_2 emissions.[20] Meeting the high demand of cement while mitigating the environmental burdens associated with its demand poses a complex challenge.

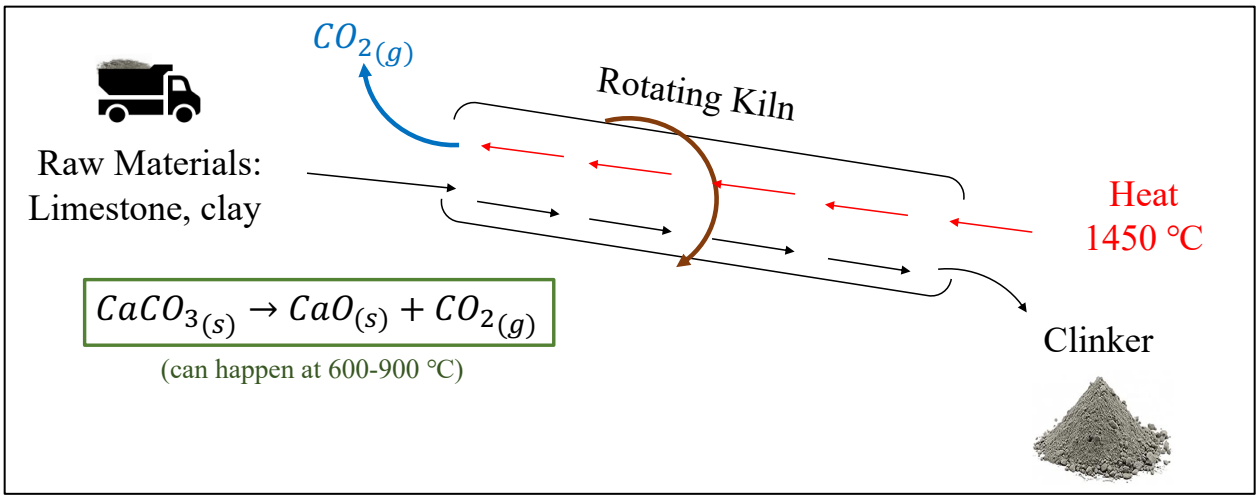

Figure 2. The process of limestone decarbonization, prior to Portland clinker production.

1.3. Solutions to reduce emissions from concrete production

Alternative processing routes are needed to reduce the carbon footprint of concrete production. Replacing the Portland cement requirement with materials like coal fly ash and ground granulated blast furnace slag is a common strategy.[23–25] Rice hull ash is an ideal candidate because it contains up to 20% amorphous silica that contributes to formation of

calcium silicate hydrate minerals that can improve strength in concrete.[23] Higher replacement of Portland cement with pozzolans, such as fly ash in concrete show slow strength development in early stages of the concrete life.[23,24] Given the need to use Portland clinker as efficiently as possible in concrete to mitigate GHG emissions, there is a need to investigate using alternative carbon-reducing materials.

Mineral admixtures can be added to concrete to improve performance and lower the amount of cement needed for any given mixture.[20] There is an opportunity to explore alternative admixtures that would reduce CO_2 emissions in the concrete production process rather than produce CO_2 emissions. One such solution is incorporating diatom biomass into concrete composites to replace some of these admixtures. Diatom biomass can be produced on wastewater to provide nutrient remediation and sequester CO_2 from the environment.

1.4. Diatom biomass as a solution to produce sustainable concrete

Marine diatoms are single cell eukaryotic microalgae that are photoautotrophic primary producers in the oceans.[26] Diatom biomass, specifically *Phaeodactylum tricornutum* biomass (**Figure 3**), is a new promising and potentially more rapid commercial application for algae biomass than algae-based biofuels. The biomass has potential to reduce costs associated with concrete production and reduce CO_2 emissions from cement production. Like trees, microalgae are carbon-storing. Microalgae biomass has been demonstrated to sequester 1.83 kg CO_2 per kg of biomass;[27] however, microalgae have a faster growth rate compared to trees (days/weeks vs. months/years).[5] Further, wastewater can be used as growth media as the source of nutrients and so that microalgae cultivation can simultaneously recycle nutrients and remediate wastewater.[5]

As such, microalgae provide a solution for long-term CO_2 storage while providing sustainable solutions to the demands of infrastructure materials.

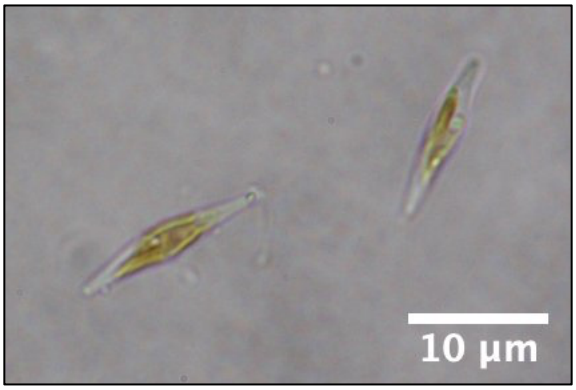

Figure 3. *P. tricornutum* cells in F/2 medium. Typical cell sizes range from 5-10µm.

A review of the literature demonstrates that biosilica from diatoms is a potential admixture where purified biosilica can contribute to desirable properties in concrete.[28] Preliminary diatom cultivation experiments suggest the use of biosilica from wastewater cultivation can be an economically feasible material for biomass use in concrete.[29] Microalgae, specifically the diatom *P. tricornutum*, has potential to be used because of its silica-based cell wall that can provide strength comparable to conventional additives.[5] The biomass of diatoms is chitin-containing biosilica and has an amorphous structure that can be a source of supplementary cementitious materials (SCMs), similar to fossilized diatom.[28] While fossilized diatom biomass, or diatomite, has been explored as a SCM, fossilized diatom substitution in Portland cement is limited to 20% due to its low amorphous silica content.[28] Preliminary studies show that diatomite with 83 wt.% biosilica can replace up to 40 wt.% Portland cement in concrete while maintaining compressive strengths.[28] These results present an opportunity to investigate the biosilica-based biomass of diatoms as a source of SCMs to replace Portland cement in higher wt. % in concrete.

Incorporation of diatom biomass has the potential to decrease the demand for the highest CO_2 emitting constituent in concrete (Portland cement clinker), decrease net CO_2 emissions by sequestering CO_2, and provide a regenerative source of SCMs.

1.5. Cost of traditional lumber production

As discussed above, the diatom *P. tricornutum* has potential to reduce carbon emissions during the concrete production process. Microalgae have potential to provide similar benefits in another construction material as an alternative to traditional lumber. The demand of natural woods is ~0.1 billion tons per year in the United States.[30] However, production costs have increased lumber prices to $600-800/ton from ~$250/ton over the past decade.[31] These costs are primarily due to the harvesting, treatment, and processing of timber, which is energy intensive.[32] The production of biomaterials for the construction industry represents an ideal market to harness the CO_2 sequestration ability of microalgae. A few billion tons of concrete are consumed every year with concrete production accounting for 5~8% human-related greenhouse gas (GHG) emissions: to manufacture one ton of ordinary Portland cement, about one ton of CO_2 is released.[20] If additional bioresources pathways for construction could be fabricated from microalgae biomass, it would not only improve the overall cost efficiency of microalgae production, but also pave the way for the next-generation green construction of carbon-negative buildings and infrastructures. This construction material use-case contributes to the commercial incentive for wastewater remediation using microalgae.

1.6. Microalgae biomass as a replacement for timber in artificial lumber

Microalgae-based infrastructure materials are a new promising and potentially more rapid commercial application for algae biomass than algae-based biofuels. The biomass has potential to both reduce costs and CO_2 emissions associated with lumber production. Like trees, microalgae are carbon-storing. Microalgae biomass has been demonstrated to sequester 1.83 kg CO_2 per kg of biomass.[27] For example, *Chlorella sp*. (**Figure 4**) can store 45-50 wt % atmospheric CO_2, like natural woods.[33] However, microalgae have a faster growth rate compared to trees (days/weeks vs. months/years). Further, wastewater can be used as growth media as the source of nutrients and so that microalgae cultivation can simultaneously recycle nutrients and remediate wastewater.[5] As such, microalgae provide a solution for long-term CO_2 storage while providing sustainable solutions to the demands of infrastructure materials.

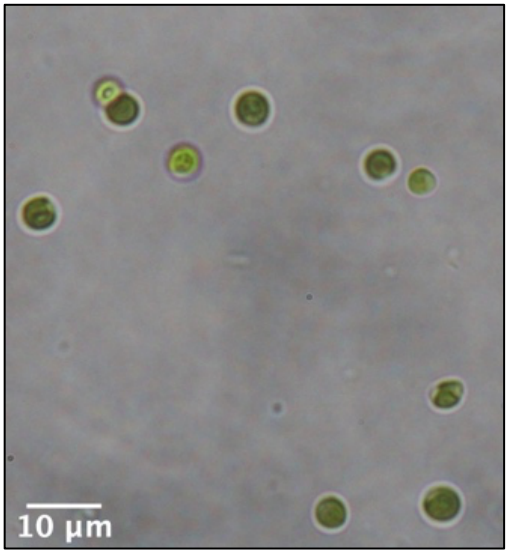

Figure 4. Brightfield microscopy of C. sorokiniana cells (size 2-4.5µm).

In addition to wastewater remediation, biofuel production from microalgae has been heavily explored.[34] However, microalgae derived biofuel is not competitive on the market with

estimates of $25.68/gal for biofuel produced from microalgae cultivated on anaerobic digestate (i.e. food waste permeate).[8] These costs are driven by the cost and energy that is required to harvest microalgae biomass and extract lipids for transformation into biofuels. In order to drive down costs and impact CO_2 reduction, microalgae production needs to be at a Gt/year scale. Production at this scale is being explored to produce biofuel, biogas, and fertilizers to bring down end-product costs. However, biomass incorporation into infrastructure materials is a new promising application to couple wastewater remediation using microalgae with sustainable material production.

Current research interests in mitigating CO_2 emissions from the construction industry create a unique opportunity to investigate microalgae biomass applications for sequestering CO_2 in sustainable materials production. Chapter Two describes the optimization of *P. tricornutum* cultivation in food waste permeate to produce silicate biomass that can serve as a supplementary cementitious materials. Chapter three describes the cultivation of *Chlorella sorokiniana* in FWP for artificial lumber applications. The production and characteristics of the artificial lumber are also discussed. This work utilizes food waste permeate to produce microalgae biomass that has the potential to overcome limitations associated with traditional concrete and lumber production in these regards. The wastewater remediation capability of *P. tricornutum* and *C. sorokiniana* are discussed, as well as challenges with optimizing growth in food waste permeate. The combined work of this thesis presents the successful development of a sustainable microalgae-based materials produced from biomass cultivated on food waste permeate produced from an anaerobic digester.

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Chapter Two: Evaluating the Growth of *P. tricornutum* on Food Waste Permeate

This chapter describes efforts at optimizing *P. tricornutum* growth in food waste permeate. An initial screening of *P. tricornutum* tolerance to FWP was conducted at the microplate (10 mL) scale to establish possible deficiencies in the FWP nutrient profile. Results of these studies advised further microplate experiments in which nitrifying bacteria were used as a co-remediation strategy to increase *P. tricornutum* tolerance to higher concentrations of FWP. Silicon supplementation was also explored at the microplate scale and was found to be suitable for batch scale studies of *P. tricornutum* cultivation in FWP. As a result, growth conditions for *P. tricornutum* cultivation in FWP were established.

2.1. Introduction

This chapter describes methods to increase the biomass productivity of *P. tricornutum* cultivated in food waste permeate (FWP). The main limitation with using FWP is that the high ammonia-nitrogen (NH_3-N) concentration is toxic to most microalgae.[1] Previous nutrient profiles of the FWP show that it contains toxic levels of NH_3-N (i.e. 2340 mg/L) and low levels of phosphate (i.e. 18.4 mg/L) (**Table 1**). As such, the effluent must either be pretreated, diluted, or co-treated to sustain microalgae growth. The goal of this research was to implement the minimum treatment required to meet the water quality and nutrient requirements to achieve optimum biomass production. I have already established baseline Kjeldahl nitrogen, ammonia, pH, nitrite, nitrate, and phosphorus levels in the effluent.[1]

2.1.1. Ammonia reduction using nitrifying bacteria

To target ammonia removal, *P. tricornutum* growth in FWP and subsequent co-cultures of nitrifying bacteria (NB) and *P. tricornutum* were investigated. Nitrifying bacteria consist of a consortium of bacterial species that can break down ammonia-nitrogen into nitrates and nitrites through nitrification.[2] First, ammonia-nitrogen (NH_3-N) is oxidized into nitrite (NO_2^-) using *Nitrosomonas sp.*, an ammonia oxidizing species of bacteria.[2,3] Then, the nitrite (NO_2^-) is oxidized to nitrate (NO_3^-) using *Nitrobacter sp.*, and *Nitrospina sp*., which are nitrite oxidizing species of bacteria.[2,3]

Methods of reducing NH_3-N concentration in wastewater streams using nitrifying bacteria is a prominent technique. Recent studies have demonstrated the use of nitrifying bacteria to reduce NH_3-N concentration in anaerobic digestate to allow for safe discharge into the environment.[1,4] Microalgae and nitrifying bacteria co-cultures have also been explored for NH_3-N reduction.[2,3,5,6] These studies demonstrated an increase in microalgae biomass concentration where co-cultures with nitrifying bacteria reduced NH_3-N concentrations.[3] In co-cultures with microalgae, nitrifying bacteria species use oxygen supplied by both aeration of the culture and microalgae photosynthesis to reduce NH_3-N concentration while maintaining total nitrogen concentration for microalgae growth.

2.1.2. Silicon's role in diatom growth

Diatoms are made up of silicified cell walls and are major contributors to the silicon cycle in oceans.[7] It is estimated that the silicon concentration of seawater is 90 μM.[8] During cell division, diatoms absorb silicon from the environment in low concentrations and transport it as silicic acid across the membrane.[9] Thus, silicon is vital in diatom cell growth. Since food waste

permeate does not contain sufficient silicon for diatom growth, two methods of silicon supplementation were explored to promote biomass productivity and biosilica accumulation in the cells.[1] The first was diluting FWP with seawater as opposed to tap water. The second attempt included dosing diluted FWP with silicon species ($Na_2SiO_3 \bullet 9H_2O$) added to F/2 growth media.[9,10]

2.2.Experimental Methods

2.2.1. *P. tricornutum* cultivation on tap water diluted food waste permeate (FWP)

A tiered culturing method was designed to evaluate the growth of *P. tricornutum* in dilutions of FWP with tap water, including 10%, 30%, 50%, 70% and 100% FWP. This two-step method consisted first of a 10 mL working volume microplate study performed in triplicate to monitor the growth of *P. tricornutum* in different dilutions of FWP, followed by a batch-scale study performed in triplicate to produce *P. tricornutum* in sufficient FWP dilutions. These dilutions of FWP were chosen based on previous work that studied *P. tricornutum* grown in dairy manure wastewater with similar nutrient profiles.[11] At batch scale, cultures were cultivated under 4-6% aeration with aquarium pumps, stirred with stir bars and maintained at a constant temperature of 23 ± 2°C with full spectrum lighting (High-Efficiency T-5 Grow Lights – Gardeners Supply Co, VT) at a 16:8 light/dark cycle (60-120 M photons/m/s). Stock cultures in exponential growth phase were used to inoculate experimental cultures at 10% v/v. Experimental cultures were cultivated to stationary phase as monitored through cell density measurements using a Thermo Scientific Genesys 10S Vis Spectrophotometer at 680 nm. After harvesting, biomass was stored for elemental silicon analysis and infrastructure material production. The culturing media (i.e. the food waste permeate) from batch scale experiments was also monitored

during the harvesting procedure and analyzed using Hach reagent kits to measure the change in ammonia-nitrogen (NH_3-N).

2.2.2. Nitrifying bacteria conversion of NH_3-N to NO_2^- and NO_3^-

Microalgae need both nitrates/nitrites and phosphate to produce biomass. To optimize nutrient levels in the FWP, I hypothesized that nitrifying bacteria (NB) could be utilized to break down NH_3-N into nitrates (NO_3^-) and nitrites (NO_2^-), which are absorbed by microalgae during growth. To test the hypothesis that co-cultivation of NB will reduce ammonia concentrations and support microalgae growth, NH_3-N concentrations and microalgae growth were measured over the course of the cultivation period when cultivated in diluted FWP. Tiered growth experiments were utilized to understand the role of nitrifying bacteria in *P. tricornutum* biomass productivity.

2.2.3. Evaluating Silicon Supplementation on *P. tricornutum* growth

Silicon supplementation in FWP was explored in two ways: 1) diluting FWP with sterile filtered seawater (collected from UC Davis College of Biological Sciences) as opposed to tap water and 2) diluting FWP with tap water and dosing it with silicon species, $Na_2SiO_3 \bullet 9H_2O$, added to F/2 growth media, at the same concentration of 1.0 mL/L media. Initial screening was performed using microplates with dilutions of 10%, 30%, 50%, and 70% FWP with F/2 medium cultures as a control. Results of microplate studies influenced batch scale cultivation under conditions outlined in 2.2.1.

2.2.4. Large-scale bucket cultivation of *P. tricornutum* in seawater diluted FWP

For purposes of increasing production of *P. tricornutum* biomass for infrastructure material studies, FWP cultivation was scaled to 15-L in buckets. Lab-scale studies that were upscaled from 300 mL to 1-L and 2-L studies influenced the design of the 15-L scale cultivation experiments by providing insight to *P. tricornutum* inoculation volume, FWP dilution, and cultivation period. Further, small-scale experiments highlighted the importance of light penetration to cultures on a large-scale. Reactor design consisted of clear buckets set on large stir plates set up in a fume hood to allow for light penetration (**Figure 5**). An air pump was used for aeration and T-5 Grow Lights were used to provide full spectrum lighting at a 16:8 light/dark cycle (60-120 M photons/m/s). *P. tricornutum* was harvested in 30% and 50% FWP diluted with seawater at this scale. The culture growth was monitored periodically through the growth period. As demonstrated by batch scale experiments (**Figure 6a**), bucket cultures were harvested no later than 15 days from the inoculation.

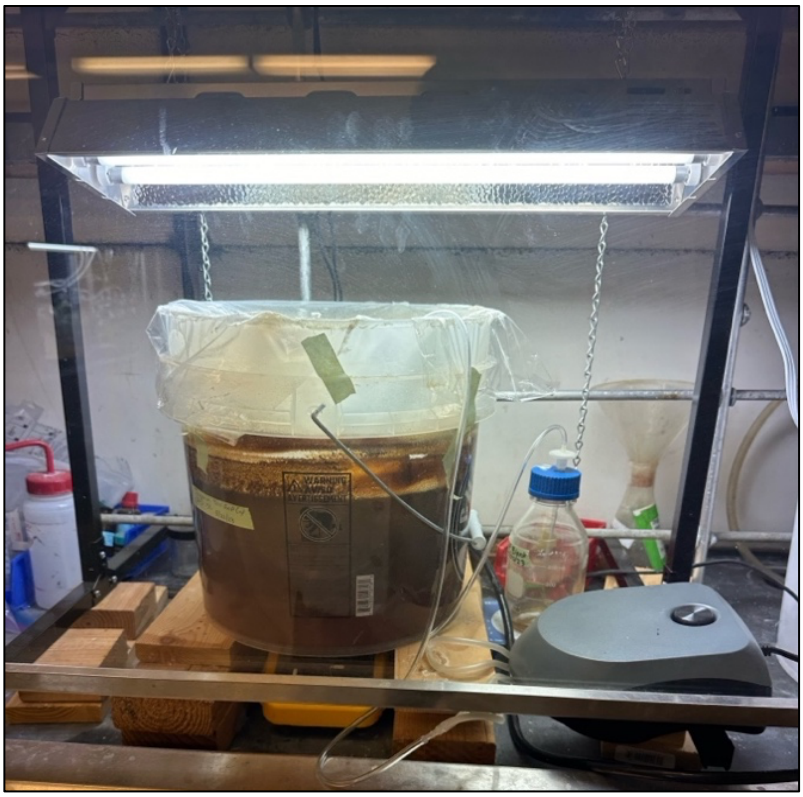

Figure 5. Large scale (15-L) cultivation setup of *P. tricornutum* in 50% FWP diluted in seawater.

2.3. Results and Discussion

2.3.1. *P. tricornutum* growth on food waste permeate (FWP)

At 30% FWP batch scale, *P. tricornutum* showed promising growth and biomass productivity (**Figure 6**). While using 30% FWP was identified to provide sufficient nutrients to sustain *P. tricornutum* growth, a method of co-culturing with nitrifying bacteria was also explored to reduce the dilution/pretreatment processing of the FWP and determine potential advantages for biomass production.

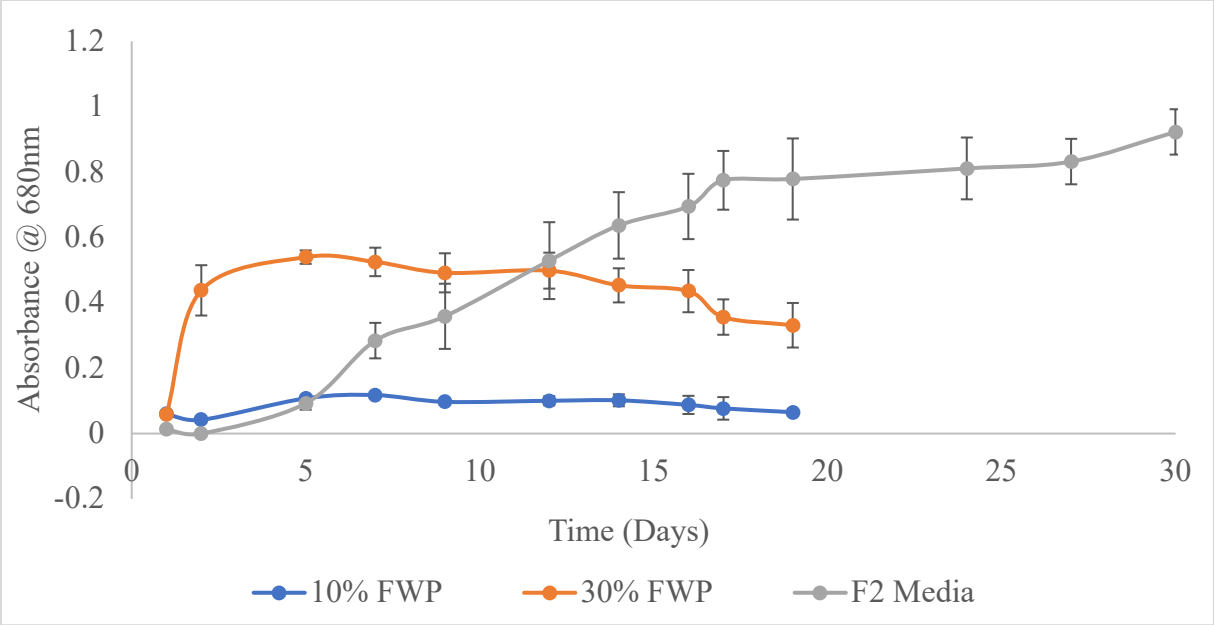

Figure 6. Growth curves for *P. tricornutum* grown on 10% vs 30% FWP (diluted in tap water) and compared with F/2 synthetic media.

2.3.2. Nitrifying bacteria and *P. tricornutum* co-cultures

Based on preliminary experiments indicating successful growth of *P. tricornutum* using 30% FWP, batch scale experiments of microalgae/NB co-cultures were conducted using 10%, 30% and 50% FWP to measure NH_3-N reduction and microalgae growth. *P. tricornutum* was inoculated at a 1:1 ratio with a commericially available nitrifying bacteria. The same culture growth conditions outlined in 2.2.1 were used for NB co-cultures.

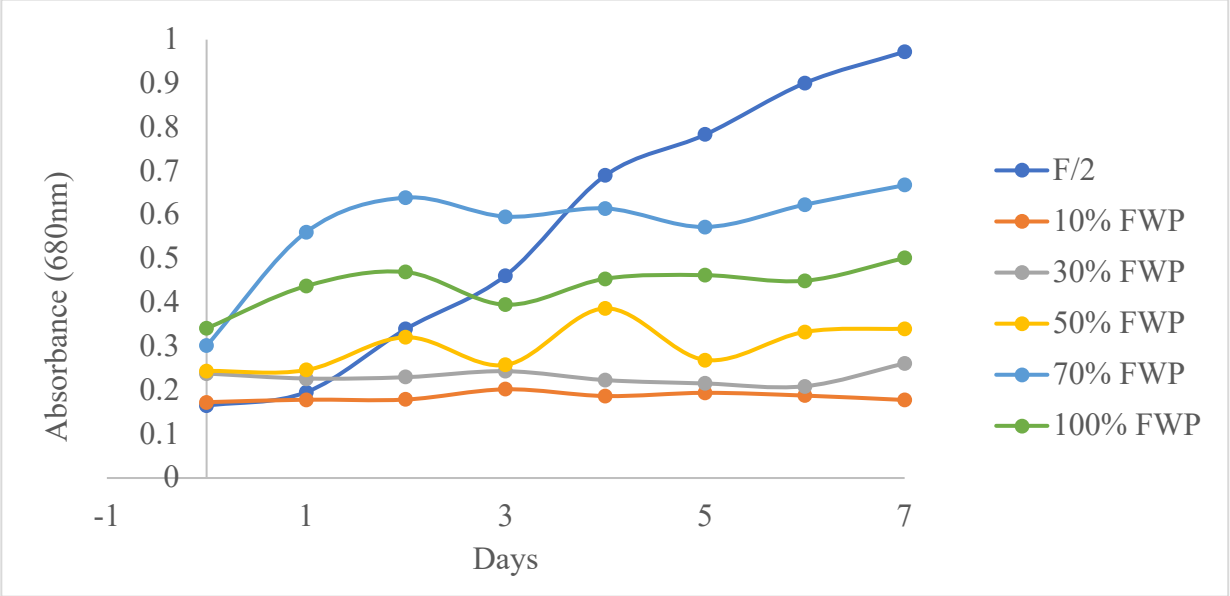

Figure 7. Initial microplate screening of *P. tricornutum* and nitrifying bacteria co-cultures in FWP diluted in tap water.

Initial microplate studies of diatom and nitrifying bacteria co-cultures indicated that 50%, 70% and 100% FWP provided nutrients for *P. tricornutum* growth, as demonstrated by growth curves with absorbance values higher than that of 10% and 30% FWP (**Figure 7**). For initial batch scale studies, 50% FWP was selected, and the study was repeated at batch scale with n = 3 and proper controls in place (**Figure 8**). For batch scale studies, NH_3-N data was collected to measure NH_3-N conversion to NO_2^--N and NO_3^--N (**Figure 9**). While growth data was inconsistent, it showed that nitrifying bacteria did not significantly increase biomass productivity when cultivated using 50% FWP diluted in tap water (**Table 2).**

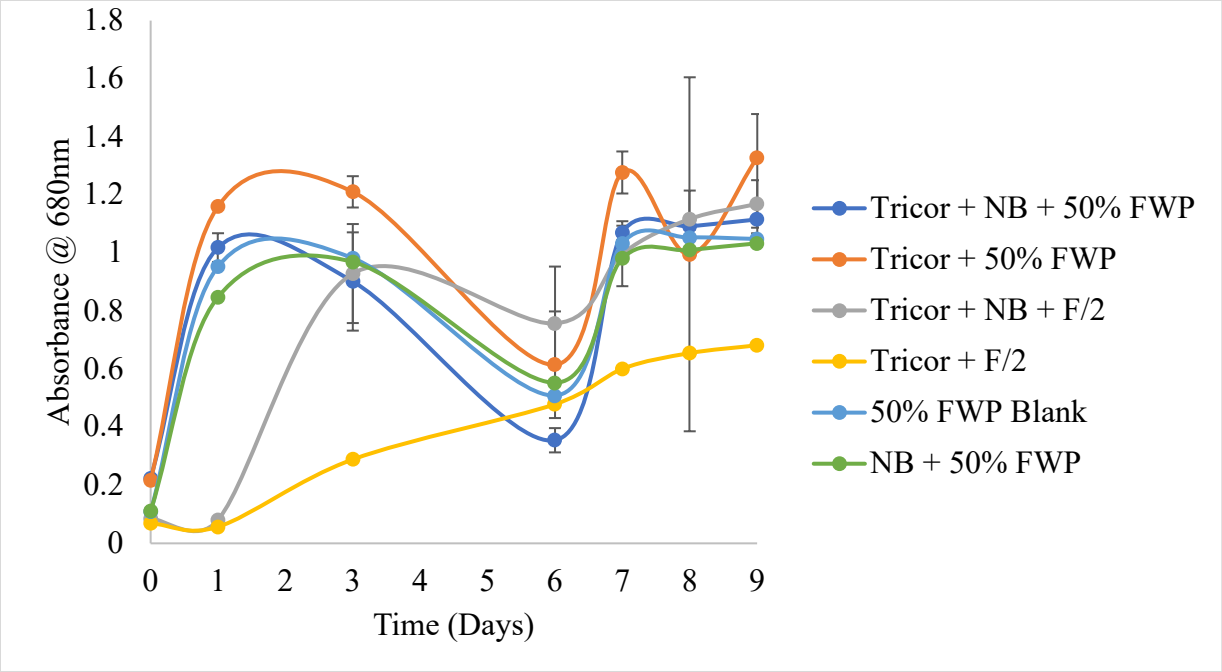

Figure 8. Batch scale screening of *P. tricornutum* and nitrifying bacteria co-cultures in 50% FWP diluted with seawater.

Co-cultures of nitrifying bacteria and *P. tricornutum* were harvested once cultures reached stationary phase, as determined based on absorbance readings at 680nm. Biomass productivity was calculated by multiplying the mass of the harvested biomass, the total volume of the culture at the time of harvest and the growth rate. Co-cultures of NB and *P. tricornutum* did not demonstrate increased biomass productivity in FWP. It is possible that the FWP was too concentrated for *P. tricornutum*, which resulted in cell death.

Table 2. Biomass data for *P. tricornutum* cultivated with nitrifying bacteria in 50% FWP[a]

Strain	Nitrifying Bacteria?	Growth Medium	Biomass Harvested (mg)[b]	Biomass Productivity (mg/L/day)[b]
P. tricornutum	-	F/2	93.5	34.6
	+		147.6 ± 2.7	54.7 ± 1.0
	-	50% FWP	234.3 ± 38.1	86.8 ± 14.1
	+		225.7 ± 5.0	83.6 ± 1.8

[a] Values are represented as mean ± standard deviation of triplicates

[b] Biomass based on harvesting at stationary phase (determined as 9 days in Fig. 7)

2.3.3. Nitrifying bacteria and *P. tricornutum* co-cultures: NH_3-N Reduction

Ammonia nitrogen concentration of the NB-diatom co-cultures was monitored using Ammonia Hach Kits. Total reduction of NH_3-N when cultivated using 50% FWP is presented in **Figure 9**. Cultures with nitrifying bacteria did not demonstrate increased reduction of NH_3-N concentration compared to cultures without nitrifying bacteria.

Figure 9. Percent reduction of NH_3-N concentrations after a 9-day cultivation period of P. *tricornutum* in 50% FWP with and without nitrifying bacteria (NB). Values are represented as percent reduction based on initial (day 1) and final (day 9) NH_3-N values calculated using standard NH_3-N concentration curves.

2.3.4. Microplate screening of *P. tricornutum* grown in FWP diluted with seawater

P. tricornutum growth on FWP diluted with both seawater and tap water supplemented with $Na_2SiO_3 \bullet 9H_2O$ was evaluated. Preliminary microplate studies showed that seawater supplementation promoted growth (**Figure 10**), specifically in higher concentrations of FWP compared to no supplementation. Both optical and spectral data of microplate experiments demonstrated that silica supplementation for FWP diluted in tap water did not promote growth, and results of these studies were not further investigated. As such, seawater dilution of FWP was determined to be optimal for *P. tricornutum* growth and was used in subsequent experiments.

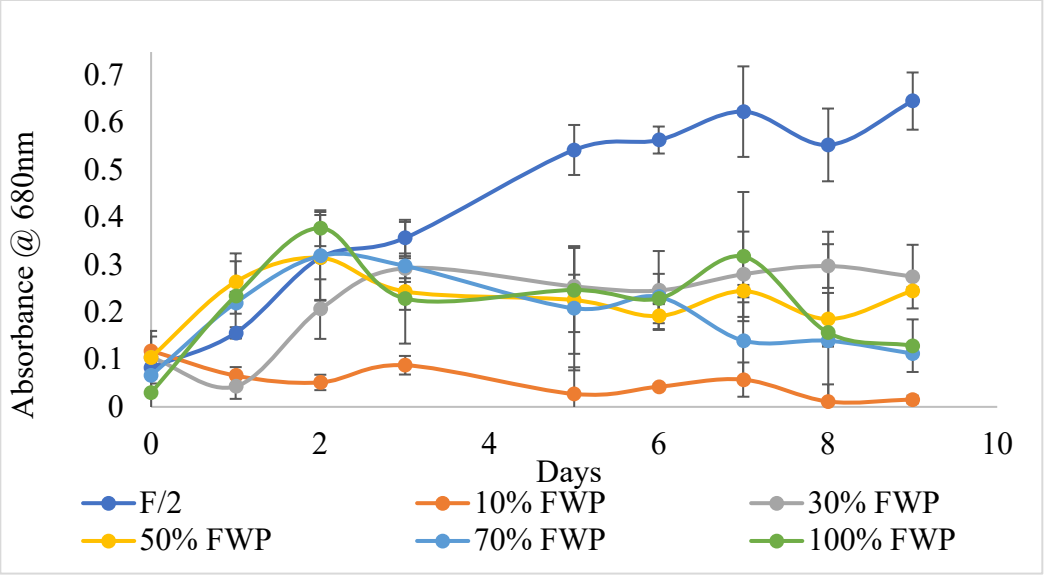

Figure 10. Initial microplate screening of *P. tricornutum* cultivated in FWP diluted with seawater.

2.3.5. Batch scale cultivation of *P. tricornutum* in FWP diluted with seawater

Based on microplate studies with seawater dilution, batch scale experiments with 30% and 50% FWP were set up to measure NH_3-N remediation. Culture conditions outlined in 2.1.1 were used for batch scale experiments, and P. tricornutum cultivated in F/2 growth media was used as a control. Cultures were cultivated in triplicate. Cultures were harvested once in stationary phase, which was reached at day 20 (**Figure 11**). An increase in biomass and biomass productivity was observed in cultures cultivated in FWP diluted with seawater (**Table 3**).

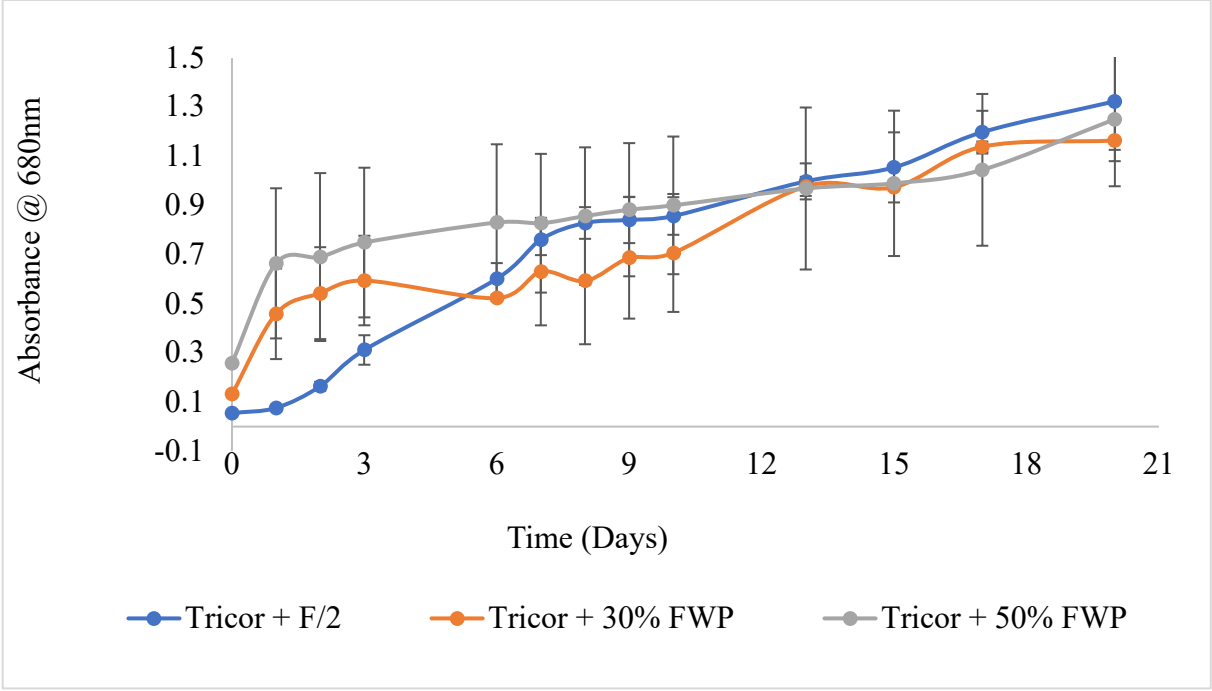

Figure 11. Batch scale growth curves of *P. tricornutum* (shortened to Tricor) cultivated in 30% and 50% FWP with seawater.

Table 3. Biomass data for *P. tricornutum* cultivated in 30% and 50% FWP diluted with seawater[a]

Microalgae	Growth Media	Biomass Harvested (mg)[b]	Biomass Productivity (mg/L/day)[b]
P. tricornutum	F/2	127.9 ± 11.6	21.3 ± 1.9
	30% FWP	200.5 ± 1.6	33.4 ± 0.3
	50% FWP	207.3 ± 11.4	34.6 ± 1.9

[a] Values are represented as mean ± standard deviation of triplicates
[b] Biomass based on harvesting at stationary phase (determined as 20 days in Fig. 10)

Ammonia nitrogen concentrations for FWP cultures were also monitored through the growth phase and percent reduction of NH_3-N is shown in **Figure 12**. An increased remediation of NH_3-N is demonstrated by these cultures as opposed to co-cultures of nitrifying bacteria and

P. tricornutum (**Figure 9**). Biomass from these studies was analyzed for elemental silicon analysis at the UC Davis Analytical Lab. Results indicate that *P. tricornutum* cultivated in F/2 media contained 1.20 wt. % silicon, biomass cultivated in 30% FWP contained 0.89 wt. % silicon, and biomass in 30% FWP diluted with seawater contained 1.42 wt. % silicon. The silicon content of food waste permeate cultured *P. tricornutum* is lower than that produced in optimized growth media produced biomass and literature values reporting 0.1-10% silicon.[9] However, diluting food waste permeate with seawater increases overall silicon in biomass.

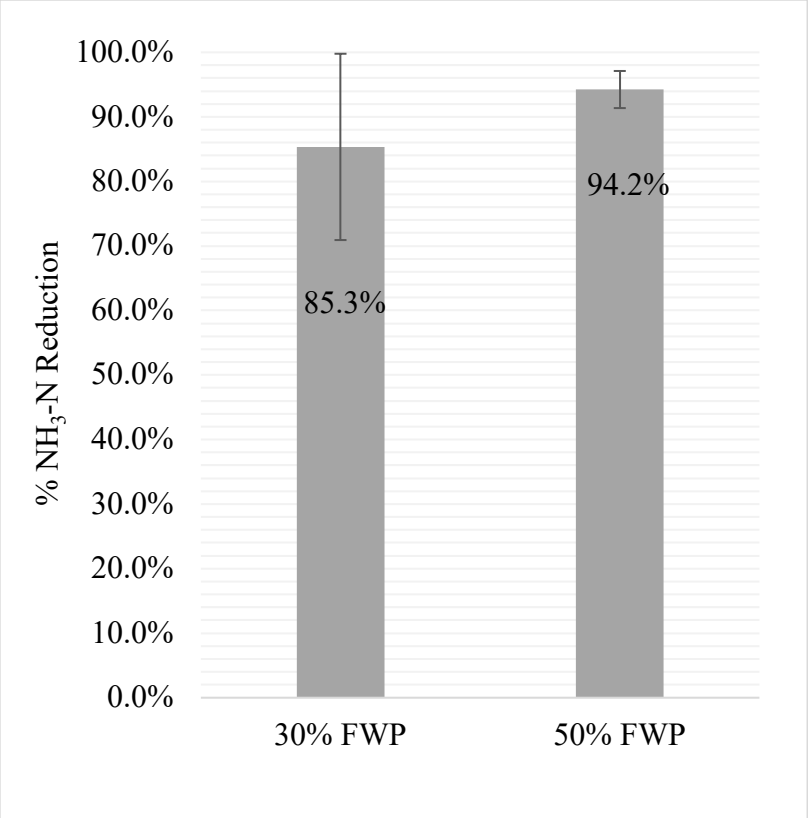

Figure 12. NH_3-N remediation of *P. tricornutum* cultivated in 30% and 50% FWP diluted with seawater.

2.4. Conclusions and Future Directions

Investigation of nitrifying bacteria and *P. tricornutum* co-cultures demonstrated that introducing nitrifying bacteria did not positively support the growth of *P. tricornutum* cultivated in FWP diluted with tap water and seawater. Future studies should investigate the effect of CO_2 supplementation on co-culturing with NB, and also consider monitoring nitrate/nitrite and total nitrogen content during the co-culturing process. Current studies were conducted under aeration using air pumps; increasing CO_2 aeration could promote growth in co-cultures. Additionally, dissolved oxygen concentration should be monitored through microalgae/NB cultivation to understand how O_2 levels are changing. Changes in dissolved oxygen concentration could explain the reduction of biomass and biomass productivity for NB co-cultures that are cultivated in FWP.

In conclusion, methods of cultivating *P. tricornutum* in food waste permeate were evaluated. While co-cultures with nitrifying bacteria did not enhance *P. tricornutum* growth in FWP, it was determined that dilution of FWP in seawater promoted growth of *P. tricornutum*. These results established baseline cultivation conditions for *P. tricornutum* in 30% and 50% FWP diluted with seawater. The biomass from these studies will be evaluated for use in diatom-based concrete.

Based on previous research using isolated biosilica in concrete production, future studies should explore 30% diatom incorporation to achieve competitive durability performance.[12] A large scale study should be conducted to establish upper and lower weight % limits that P. tricornutum biomass can be incorporated to achieve durability performance of concrete composites comparable to conventional concrete. Further, both dry biomass and semi-dry slurry of *P. tricornutum* biomass should be compared for incorporation into standard Portland cement

using each specific weight % to form a mortar-like consistency. Strength of the FWP-cultured diatom biomass should be compared to diatomaceous earth and Portland cement in concrete.

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Chapter Three: Production and Evaluation of *Chlorella sorokiniana* Biomass for Artificial Lumber Applications

Chapter Three describes experiments using *Chlorella sorokiniana* biomass as a wood replacement to create a composite material for artificial lumber production. This work was performed in collaboration with Professor Qiao's Lab in the Department of Structural Engineering at the University of California, San Diego (UCSD). Multiple microalgae biomass samples were provided for screening and testing in the Qiao lab. In order to produce artificial lumber composites from microalgae biomass, I compared FWP cultured microalgae biomass to previously explored artificial lumber replacement materials, specifically seaweed.[1] A key factor in strain selection was its ability to be cultivated in FWP and serve the dual purpose of wastewater remediation. Marine microalgae species *Chlorella vulgaris*, *Chlorella sorokiniana,* and *Scenedesmus obliquus* were screened in addition to the cyanobacteria *Spirulina platensis*, but *C. sorokiniana* was selected for further testing as an artificial lumber composite.

3.1. Introduction

3.1.1. Environmental impacts of lumber production

Current CO_2 emissions from the construction industry are more than 35 Bt/year in the United States.[2] As such, there have been advances to reduce these emissions by storing atmospheric CO_2 in construction materials. Chapter 1 described some of these emissions are due to concrete production practices, and supporting the expanded use of wood as an infrastructure material has been proposed as a means to curb total emissions from the construction industry.[3] While wood is a naturally carbon storing material used in lumber production, traditional

practices of the lumber industry create opportunities to further increase CO_2 reduction.[4] For example, timber forests are often far from final markets.[5] As such, there are opportunities to reduce emissions related to the transportation and processing of wood. Further, timber forests require large land use, which can be inefficient when compared to total CO_2 sequestration in wood. Lastly, wood is not easily moldable, so it cannot be used as a replacement in other infrastructure materials, such as concrete.[5]

3.1.2. Wastewater remediation using *Chlorella sorokiniana*

Microalgae monocultures have been used to remove chemical contaminants, specifically nitrates and phosphates, from water sources to allow for reuse in agricultural practices.[6–9] *Chlorella sorokiniana* is a freshwater microalgae species that has been studied for its ability to remediate wastewater across a variety of wastewater streams.[10–14] It demonstrated remediation of dairy wastewater treatment plant effluent, which contains high nitrogen and chemical oxygen demand content.[11] Biomass productivity reached 233 mg/L/day in dairy wastewater effluent, and over 80% remediation of nitrate, ammonia, and chemical oxygen demand was observed.[11] *C. sorokiniana* was also studied on swine wastewater, which is rich in nitrogen similar to the food waste permeate used in primary studies for this thesis. This study observed *C. sorokiniana* productivity of 360 mg/L/day in 50% swine wastewater diluted with BG-11 media and 97.0% remediation of total nitrogen through the growth phase.[12] A study of *C. sorokiniana* cultivated in anaerobic digestate of dairy wastewater, which has a high nitrogen profile, demonstrated 96.3% remediation of total nitrogen.[13] These studies conducted in different waste streams with high nitrogen content (greater than 500mg/L total nitrogen) demonstrate potential for *C. sorokiniana* viability and total nitrogen remediation in the ammonia-rich food waste permeate (FWP).

3.1.3. *Chlorella sorokiniana* in artificial lumber

Attempts at using *C. sorokiniana* biomass in artificial lumber are described herein (Figure 4). This strain was identified based on its rapid growth in diluted FWP and subsequent performance as a composite material.

Cultivation of *C. sorokiniana* can be scaled from lab scale (0.05-2 L) to open raceway ponds (100 L) (**Figure 13**). While lab scale harvesting utilizes centrifugation, large scale cultivation relies on filamentous or flocculating algae and omits the need for complicated drying.[15] *Chlorella sorokiniana* can be cultivated on minimally treated wastewater to remediate nitrogen and phosphorus species. One such source of wastewater is through the process of anaerobic digestion. Anaerobic digestion has been used increasingly to valorize organic wastes such as food, dairy and livestock waste through the generation of biogas, which addresses the problem of methane emissions, and produces a nutrient-rich liquid effluent.

Figure 13. Microalgae cultivation at different scales: (**A**) 300mL lab scale cultivation; (**B**) 100L open pond algae cultivation reactors at UC Davis, covered with clear 0.7-mm-thick plastic; (**C**) high density algae culture grown in 10% filtered food waste digestate.

3.2. Materials and Methods

3.2.1. Cultivation of *C. sorokiniana* on FWP

For this study, *C. sorokiniana* (UTEX 1230) was cultivated in aerated 30% FWP diluted in tap water (30/70 FWP/tap water) at laboratory batch scale (300 mL). Cultures were stirred with stir bars and maintained at a constant temperature of 23 ± 2°C with full spectrum lighting (60-120 M photons/m/s; High-Efficiency T-5 Grow Lights – Gardeners Supply Co, VT) at a 16:8 light/dark cycle. Stock cultures in exponential growth phase were used to inoculate experimental cultures at 10% v/v. Experimental cultures were cultivated to stationary phase as determined by cell density measurements using a Thermo Scientific Genesys 10S Vis Spectrophotometer at 680 nm. Microalgae cells were rinsed with deionized water, then harvested through centrifugation. Microalgae pellets were flash-frozen in liquid nitrogen and lyophilized to dryness. Dried biomass was weighed to determine biomass productivity and stored at –20°C until artificial lumber production by collaborators in the Qiao group at UCSD.

3.2.2. SAC compaction of *C. sorokiniana* biomass

Production of *C. sorokiniana* into lumber composites was performed by collaborators in the Qiao lab at UCSD. In short, powdered *C. sorokiniana* biomass was mixed with isopropyl alcohol, which acted as a surface activation catalyst (SAC). The SAC-activated biomass was transferred into a stainless-steel cylinder and compressed by a type-5582 Instron machine through a steel piston. Peak compressive stress was measured under a loading rate of 5 mm/min. This method produced a dark green solid, with the diameter around 19 mm and the height around 6~10 mm (Figure 14A). E-glass fibers were separated from a Wicks-1600-3 roving and attached to the upper and lower surfaces of the specimen by McMaster-7541A77 Devon epoxy (Figure

14B). The mass of the fiberglass was 1-2% of the *C. sorokiniana* mass. Flexural stress and flexural strain of this material were measured.

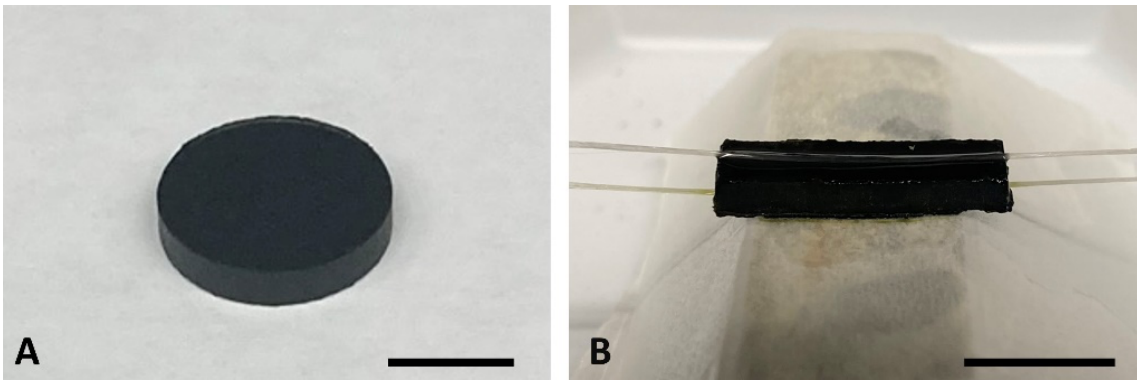

Figure 14. Photos of (**A**) a compacted solid microalgae cylinder and (**B**) an artificial lumber specimen strengthened by 2 wt.% fiberglass. Scale bar: 10 mm. Figure credits: Dr. Yu Qiao, UCSD.

3.3. Results and Discussion

3.3.1. *Chlorella sorokiniana* growth in FWP

Growth curves for *C. sorokiniana* cultivated in 30% FWP are shown in **Figure 15**. *C. sorokiniana* grown in BG-11 growth medium was used as a control and 30% FWP was used as a blank. Cultures were harvested at 25 days. A total of 59 ± 2 mg *C. sorokiniana* grown in 30% FWP was harvested, resulting in a biomass productivity of ~8mg/L/day. Cultures grown in BG-11 had higher productivity: a total of 250 ± 32 mg *C. sorokiniana* grown in BG-11 was harvested, resulting in a biomass productivity of ~33mg/L/day. This indicates that 30% FWP does not have optimal nutrients for *C. sorokiniana* growth. Future studies should include attempts at cultivating *C. sorokiniana* in different concentrations of FWP. However, for purposes of artificial lumber production for this study, 30% FWP allowed for enough biomass to be produced.

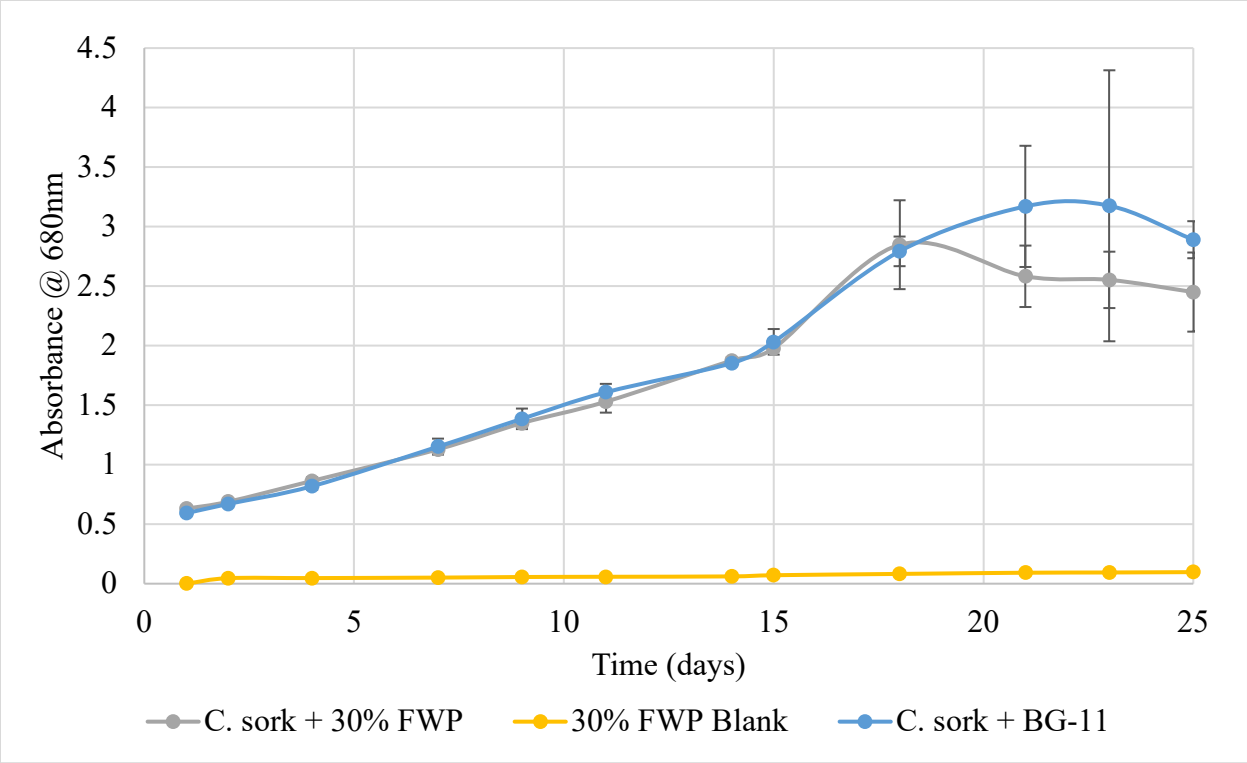

Figure 15. Growth curves for *C. sorokiniana* (referenced as C. sork) cultures in 30% FWP diluted in tap water.

3.3.2. Artificial lumber characteristics

In this study, a 100 wt. % microalgae composite material (without fiberglass reinforcement) withstood a flexural strength of 18 MPa before failure, which is weaker than softwoods (**Figure 16**).[5] This composite is brittle, as demonstrated by its maximum flexural strain of about 2%. This is attributed to the internal bonding of the material being limited to the *C. sorokiniana* cells and the lack of reinforcements to strengthen the material. As such, this material is not ideal for timber design due to its low flexural strength.

The addition of 1 wt. % fiberglass to the compacted microalgae increased the flexural strength of the artificial lumber to more than 40 MPa (**Figure 16**). This strength is approximately the same as the reference pinewood. The maximum flexural strain is 30-40% less than but on the

same order as the loading-carrying range of the reference pinewood. When the fiberglass content is increased to 2 wt.%, the flexural strength is 60-65 MPa, considerably above that of pinewood.

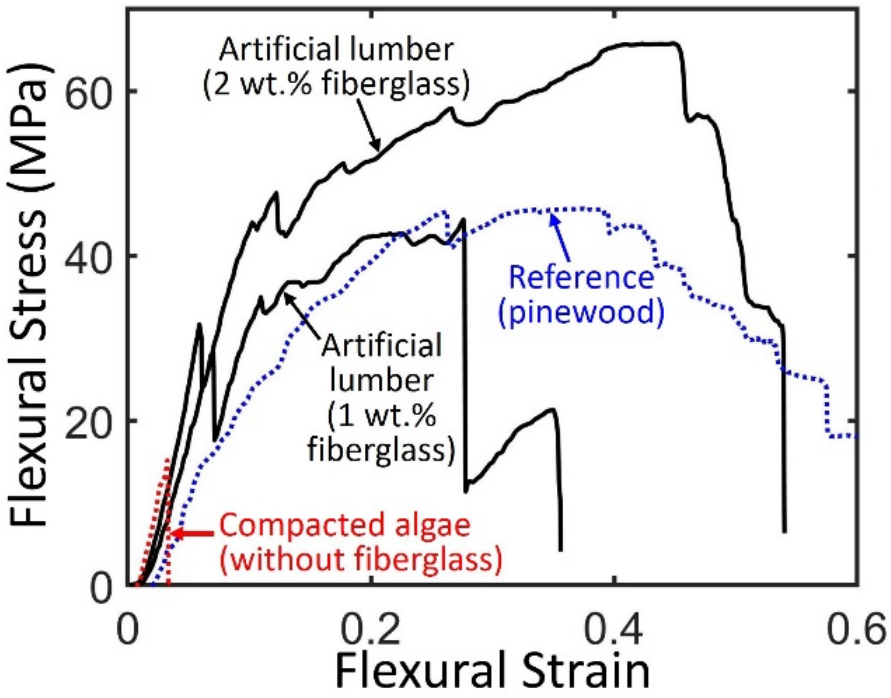

Figure 16. Flexural strain test curves for tested lumber composites: artificial lumber (without fiberglass), artificial lumber (with 1 wt. % fiberglass), artificial lumber (with 2 wt. % fiberglass), and reference pinewood. Figure credits: Dr. Yu Qiao, UCSD.

In traditional composite processing, the reinforcement content is usually 30-70 vol.% since a fiber content of less than 3-5% could weaken the material.[16] However, in the artificial lumber, the addition of minimal fiberglass reinforces the strength because it is strategically placed at the most critical load-carrying sites at the upper and lower surfaces.

3.4. Conclusion

A microalgae-based composite material as an artificial lumber was produced using *C. sorokiniana* biomass produced in 30% FWP. The 98-99% microalgae wt. % composites were

formed using a one-step self-assembly technique using isopropyl alcohol and were locally strengthened with 1-2 wt. % fiberglass. The flexural strength is 40-70 MPa, and the maximum flexural strain is more than 50%. The compaction is one-stepped, performed before the addition of fiberglass. The overall processing is fast, compatible with compression molding and additive manufacturing. Since the spent microalgae biomass can be recycled or upcycled, the artificial lumber offers a promising approach to store 30-50 wt.% atmospheric carbon. Further investigations should increase the scope of microalgae strain testing to include species of different biochemical properties, ash content, and wastewater remediation capacity.

3.5. References

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