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Integrated Strategies For Sustainable Wastewater-Based Algal Biofuel Production And Environmental Mitigation In The Us

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INTEGRATED STRATEGIES FOR SUSTAINABLE WASTEWATER-BASED ALGAL BIOFUEL PRODUCTION AND ENVIRONMENTAL MITIGATION IN THE US

by

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DISSERTATION

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DEDICATION

I dedicate my dissertation work to my family and many friends. A special feeling of gratitude to my wife whose words of encouragement and push for tenacity ring in my ears. I also dedicate this dissertation to my many friends who have supported me throughout the process. I will always appreciate all they have done.

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BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
DOE	Department of Energy
EIA	Energy Information Administration
FU	Functional Unit
GWP	Global Warming Potential
HRAPs	High-Rate Algal Ponds
HRT	Hydraulic Residence Time
HTL	Hydrothermal liquefaction
LCA	Life Cycle Assessment
LCO	Life Cycle Optimization
LE	Lipid extraction
LP	Linear Programming
MOO	Multiobjective Optimization
MP	Microwave pyrolysis
O.D.	Optical Density
OP	Open Ponds
PAR	Photosynthetically Active Radiation
PBRs	Photobioreactors
SEHR-LCA	Spatially-Explicit-High-Resolution Life Cycle Assessment
SS	Suspended Solids

TIC	Total Inorganic Carbon
TKN	Total Kjeldahl nitrogen
TOC	Total Organic Compound
TP	Total Phosphorus
TSS	Total Suspended Solids
SWEET lab	Sustainable Water-Environment-Energy Technologies (SWEET) Laboratory
WWTPs	Wastewater Treatment Plants
μ	Specific Growth Rate

CHAPTER 1 INTRODUCTION

1.1. Overview

Algae can be found in different size and shape in the environment. Microalgae are unicellular species and their size can be from few micrometers to few hundred micrometers. Microalgae have drawn increasing attention as a promising source for biofuel production due to their unique and desirable characteristics, including rapid growth and capability of growing in poor quality water. However, there remain a number of challenges such as harvesting cost, nutrient cost, lower biomass yields in some water resources before the technology can be deployed on a large-scale (NationalResearchCouncil(NRC) 2012, Vasudevan, Stratton et al. 2012, DOE 2015). Key barriers that hinder the utilization of algae-based biofuels are high cost and limited capacity for scaled-up production of algal biofuel feedstock. Wastewater can be used as free nutrients and water resources for growing microalgae. Studies have indicated that wastewater, which is currently underused, could be one of the most favorable resources for algae feedstock production because it (1) provides ample supply of nutrients and water, (2) can support a large capacity for biofuel production (up to 5 billion gallons of algal biofuel per year could be generated with municipal wastewater in the U.S. (Lundquist 2015), and (3) can be integrated into existing public infrastructure, rather than creating new industrial systems (Lundquist, Woertz et al. 2010, Clarens, Nassau et al. 2011, Pittman, Dean et al. 2011, Orfield, Fang et al. 2014).

Many researchers have found that the integration of municipal wastewater treatment and algae cultivation, whereby partially treated wastewater with rich nutrients is recycled into algae cultivation ponds, could deliver significant sustainability benefits compared to the two standalone entities (Colosi, Resurreccion et al. 2015). Growing algae in wastewater is considered as a sustainable potential for algal biofuel production. A number of studies investigated the potential of the synergies of algae biofuels and wastewater; from empirical selection of algal strains to pilot-scale algae cultivation systems and energy conversion pathways (Li, Chen et al. 2011, Park, Craggs et al. 2011, Zhou, Li et al. 2011, Zhou, Schideman et al. 2013). Despite such progress and promise, no large-scale algae-wastewater facilities emerged.

Currently, the most economical method for the commercial scale of using wastewater algae is open pond system, which is an autotrophic mode of algae cultivation (Li, Chen et al. 2011, Pittman, Dean et al. 2011). These open ponds are shallow ponds with depths of up to 0.5 meters, and algae are growing in a continues suspended medium culture (Pittman, Dean et al. 2011). The open type cultivation systems like a raceway pond have several disadvantages due to contamination and evaporation problems. Also, it needs a large surface area for photosynthesis (Hoh, Watson et al. 2015).

This research contains both modeling and lab experiments to address the aforementioned challenges. I have evaluated the potential of using different modes of cultivation, such as autotrophic and mixotrophic modes. In the heterotrophic method, organic carbon sources are the main source for providing energy. Mixotrophic mood uses both light and organic carbon sources for energy (Agwa, Ibi et al. 2013, Perez-Garcia and Bashan 2015). Besides that, I have evaluated algae biofilm attached growth as a new way to grow microalgae. Algae biofilm growth showed higher algal biomass productivity and easy harvest of algal biomass by easily scrapping biomass from the substrate (Christenson and Sims 2011).

For the modeling section, I have applied life cycle assessment (LCA) to assess the integration of algae-wastewater systems. LCA is a widely accepted quantitative accounting tool for evaluating the environmental effects of products, processes, or services by computing the energy/material inputs and wastes released to the environment. LCA can also be used to assess potential environmental impacts of those energy, materials, and wastes (Christiansen, Hoffman et al. 1995). LCA has become an actively researched area and has been increasingly applied in academic and industrial fields for environmental impact assessments. Due to the tremendous interest in algae as an alternative energy source, many researchers have generated a significant body of life cycle studies in algae-to-energy systems over the past decade. However, state of the art algal LCA studies have primarily focused on "snapshots in time" analyses; e.g., the use of simple linear models and generalized parameters without systematic consideration of geographic diversity and development timing.

In this study, for the first time, I evaluated the potential of biofuel production from wastewater with a Spatially-Explicit-High-Resolution Life Cycle Assessment (SEHR-LCA) model and as the lab work also evaluated the potential of using attached growth and mixotrophic growth for producing biofuels. For this purpose, each wastewater treatment plant (WWTP) around the US has been analyzed by Spatial Analysis Models using GIS software, MS Excel, Crystal ball and MATLAB. This new dynamic SEHR-LCA method is uniquely suited for the holistic and accurate assessment of algae-to-energy systems. I developed a spatially explicit lifecycle methodology for algae cultivation in the U.S. and model spatially specific impacts and source availabilities for rational selection of appropriate locations for large-scale algal bioenergy systems. The necessary modeling data will be gathered from the literature review and laboratory experiments in Sustainable Water-Environment-Energy Technologies (SWEET) Laboratory here at Wayne State University.

1.2. Problem Statement

Due to the lack of integrity for spatial and optimized LCAs in the evaluation of wastewater algal cultivation, this study for the first time has addressed the overall environmental and land resource usage impacts of algae cultivation from wastewater resources in the US in a point by point analysis. A spatial and data analysis LCA model will be developed for the research and the potential of wastewater based algal biofuel production with the integration of CO_2 sources will be evaluated. The environmental impacts of cultivation will be minimized based on the Life Cycle Optimization (LCO) model which will be developed using MATLAB software. Additionally, the cultivation method such as mixotrophic biofilm growth has be evaluated since it has the potential of higher yield and easier cultivation. This research will provide more precise and understandable results for the potential and availability of wastewater resources in the US for algal biofuel production and CO_2 sequestration.

1.3. Research Objectives

The research objectives in this work are:

(1) Preparing the first point by point wastewater treatment plant analysis for the continental US by doing spatial analysis

(2) Analyzing the mixotrophic attached growth algae cultivation in wastewater and the potential of using these methods for mass algae cultivation. Currently the autotrophic mode is the primary way to produce wastewater algae. Evaluating the Mixotrophic cultivation methods can give us a new outlook for other potential methods of using wastewater resources for algae cultivation.

Evaluating the biofilm attached growth of algae as a method for algae cultivation. Collecting algae from the suspended medium is one of the main energy consuming steps in the algae farms. Attached growth can be a potential way of collecting algae more easily.

(3) Evaluating the environmental impacts of the wastewater algal biofuel in the US by doing LCA

(4) Preparing a Life Cycle Optimization (LCO) model for evaluating the potential methods to minimize the environmental impacts

1.4. Significance

This innovative research has addressed a point by point wastewater algae production around the US with the combination of SEHR-LCA and LCO. For each WWTPs a full-scale analysis of the availability of solar, land, CO_2 resources, and other optimized factors has been carried out.

Results of this study contributed to our understanding of the real potential of wastewater algae cultivation and its environmental impacts in the US and proposed a framework for optimization studies in a life cycle assessment studies.

Also, different modes of growth, such as mixotrophic and biofilm attached growth are evaluated in the lab for better understanding the potential of using those methods of growing algae for wastewater algal cultivation.

1.5. Dissertation Structure

In this research, a combination of targeted laboratory studies and LCA modeling are used to achieve the research objectives. Figure 1 describes the entire methodology of this research and figure 2 shows different stages of this research.

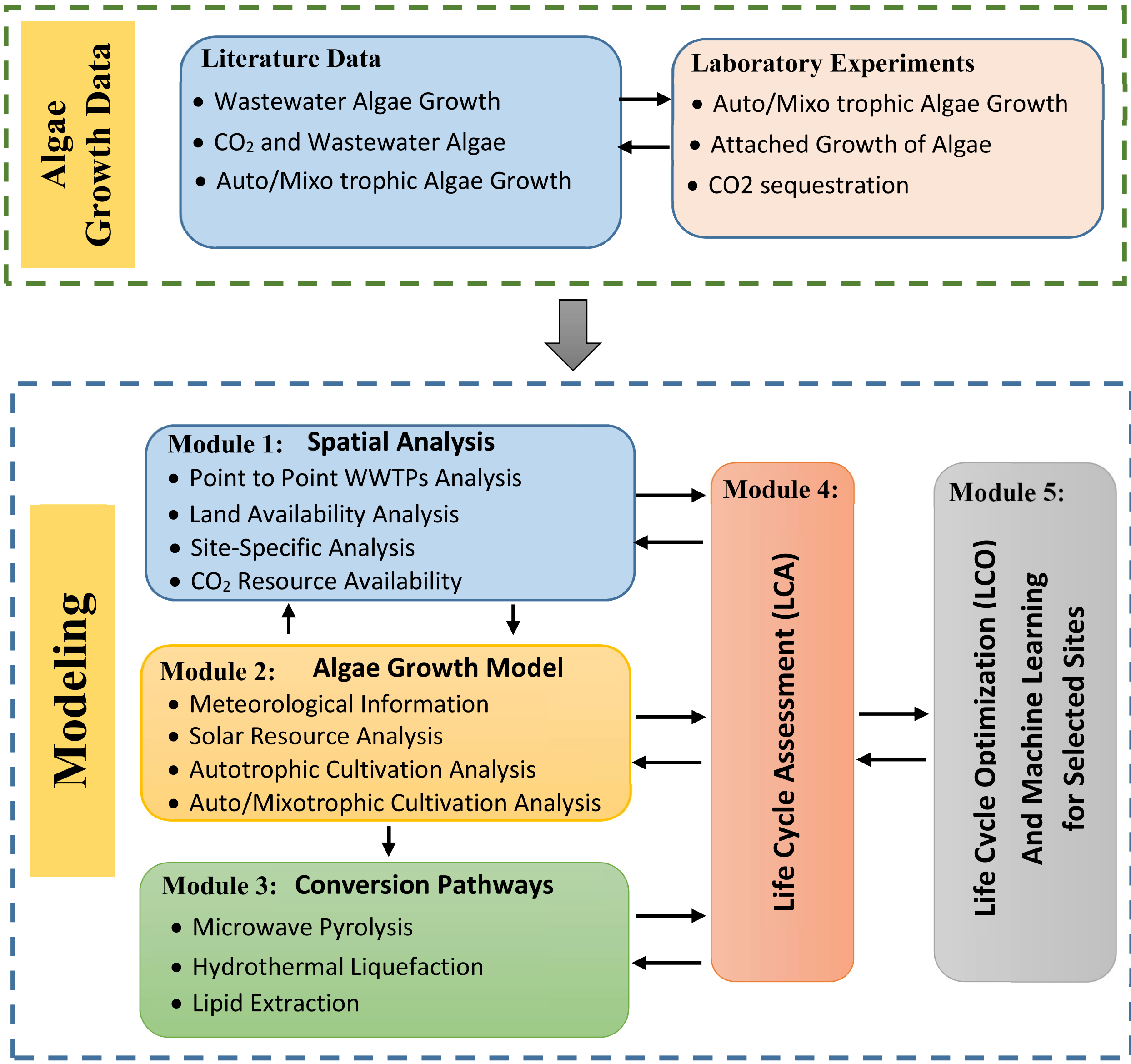

Figure 1. Methodology for different modules of the research

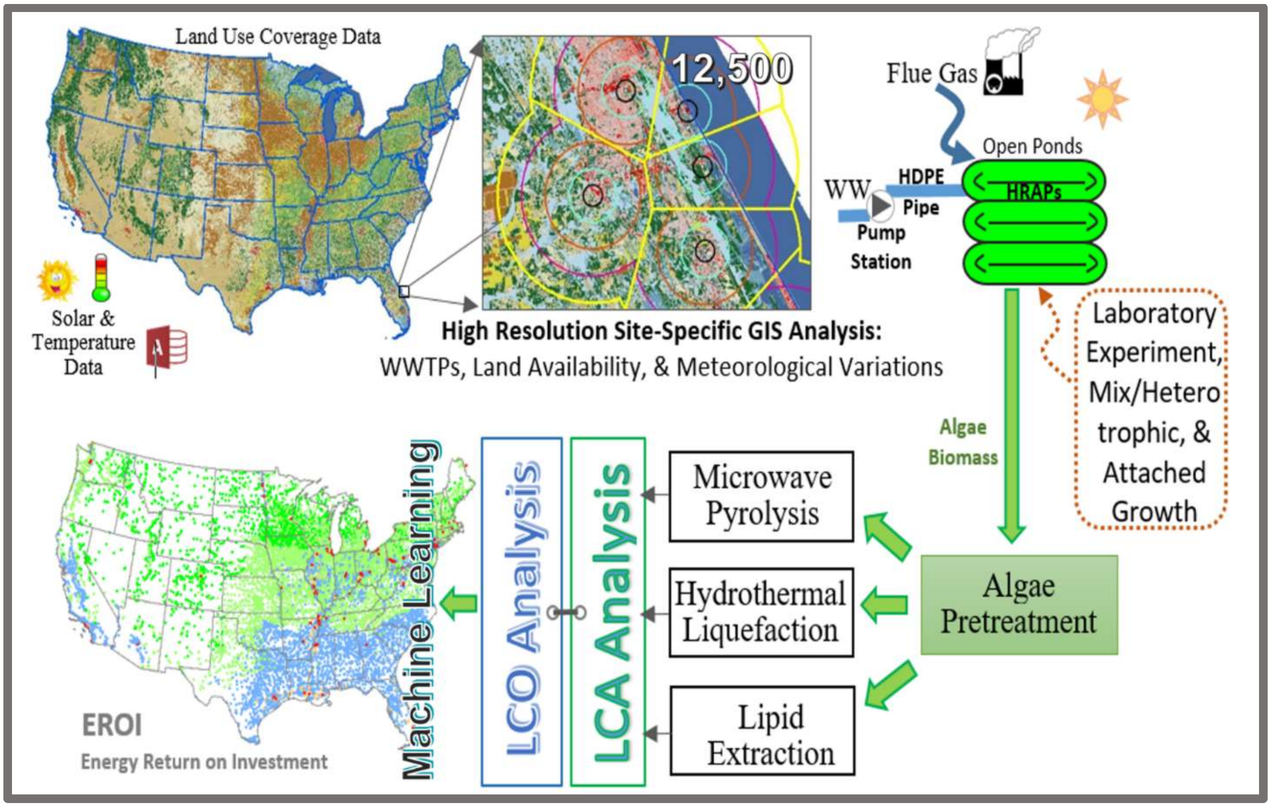

Figure 2. Different Section of the Dissertation

CHAPTER 2 SPATIALLY EXPLICIT LIFE CYCLE ASSESSMENT

Most of the work in this chapter has already published in Algal Research journal (Roostaei and Zhang 2017).

2.1. Background

A careful and thorough literature review is essential for understanding the gap between parts of knowledge. In the following part of this report, some of the most important articles related to this area of research are reviewed. First, current state of the art facilities using municipal wastewater for algal biofuel production are evaluated. Next, application of CO_2 emission in algae cultivation is reviewed. After that, the LCA studies for algae biofuel and wastewater algae biofuel are analyzed. Finally, the optimization methods for doing the LCA is reviewed and the results of some of the previous studies for LCO is presented.

2.2. Algal Based Biofuel

Biofuels produced from renewable biomass have the potential to replace a significant fraction of the fossil fuel need. However, concern has grown that the use of food crops to produce ethanol, biodiesel, or other renewable fuels will increase food prices while having little impact on greenhouse gas emissions. According to the Energy Independence and Security Act of (EISA) 2007, the US should produce more than 35 billion gallons per year by the year 2022, to move the United States toward greater energy independence and security. This main purpose of this act is to increase the production of clean, renewable fuels, promote research on and deploy greenhouse gas capture and storage options, and to improve the energy performance of the Federal Government.

Based on EISA 2007, the U.S. targets at 5 billion gallons of advanced oil in 2022 (Figure 3). Algal biofuel is one of the most promising biofuel resources. However, there is a significant gap between the targeted goals and current production of advanced biofuels. Therefore, a spatial analysis, which can predict the real potential of algal biofuel production, is warranted (GPO 2007, MSU 2015).

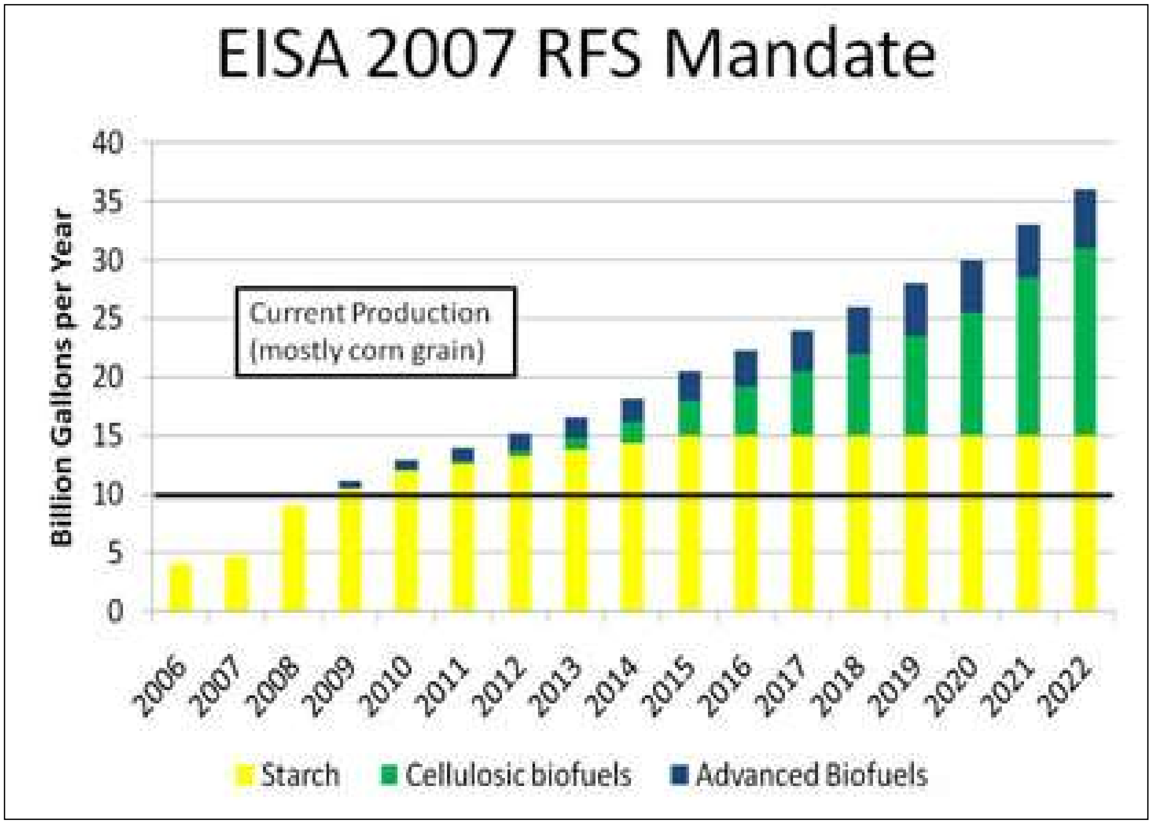

Figure 3. Biofuel production goals for year 2022 taken from (MSU 2015)

Prior work, in particular the Aquatic Species Program sponsored by the U.S. Department of Energy, suggested that algae would be capable of producing oil suitable for conversion to biodiesel with an aerial productivity 20–40 times higher than that of oilseed crops such as soy and canola (Paul Abishek, Patel et al. 2014). However, economic studies suggested that large-scale algae cultivation solely for biofuel production would not be economically feasible, reemphasizing the integration of biofuels production and wastewater treatment with CO_2 supplementation, as first proposed by Oswald and Golueke in 1960. In particular, the assimilation of wastewater nutrients by algae followed by algae harvesting via sedimentation was considered potentially practical and economical approaches to biofuel production. Use of algae for municipal wastewater treatment in ponds is well established (Oswald, Lee et al. 1978) and algae-based treatment of dairy and swine waste have also been investigated (Mulbry et al. 2008; An et al. 2003).

In future applications, CO_2 could be supplied by flue gas collected from power plants and other sources. A schematic of one envisioned process is shown in Figure 4 the CO_2 supplementation of algae cultures to increase productivity in the laboratory scale, have been studied for many years, as well as the use of flue gas as a CO_2 source. In outdoor ponds, supplementation of CO_2 to promote nutrient removal has also been studied (Sheehan, Dunahay et al. 1998). However, the production of lipids has not been measured in these studies. Lipid content for pure cultures of algae has been reported to range from 1–85% and the lipids exhibit varying carbon chain lengths, degrees of unsaturation, and polarity (Chisti 2007, Woertz, Feffer et al. 2009).

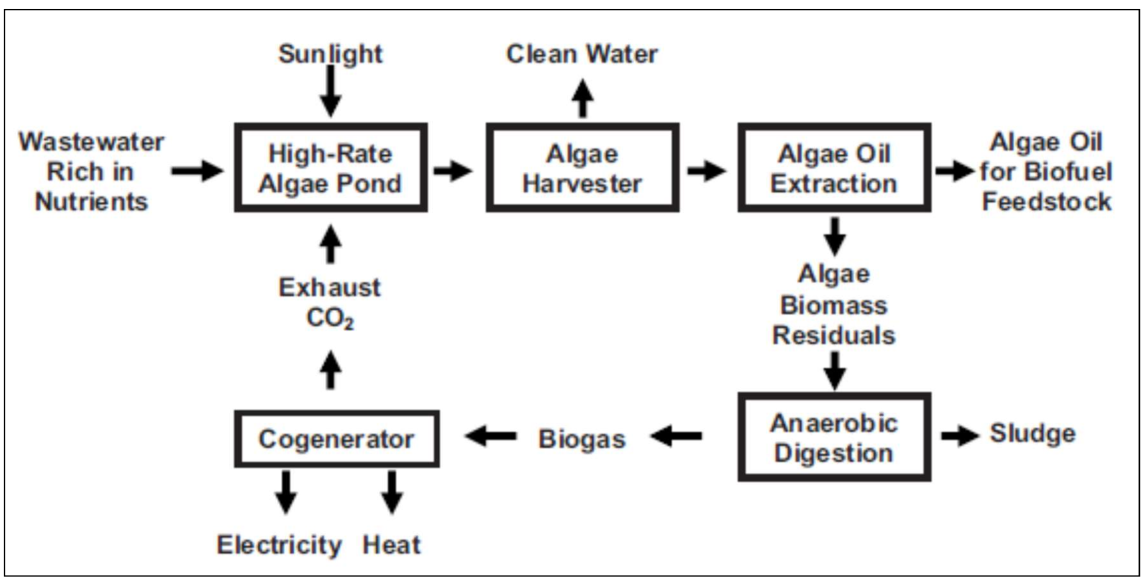

Figure 4. Simplified process flow diagram envisioned for algae wastewater treatment and liquid biofuel production taken from (Woertz, Feffer et al. 2009)

However, the lipid content and the lipid productivity of wastewater pond algae, have rarely been reported. Furthermore, lipid content, fatty acid profile and biomass productivity depend on environmental conditions, culturing methods, and growth phase. In particular, nitrogen limitation decreases growth rate, which can lead to decreased overall lipid productivity (Woertz, Feffer et al. 2009).

This problem was investigated by Shifrin and Chisholm (1981), but maximizing lipid productivity remains an outstanding problem (Shifrin and Chisholm 1981). While a few studies have reported the lipid content of waste has grown algae cultures (e.g., 25%, (Enssani 1987), lipid productivities for waste-grown polycultures have not been reported previously. Their research presented was conducted to determine the lipid content and lipid productivity of microalgae grown for nutrient removal from two types of wastewater dairy and municipal (Woertz, Feffer et al. 2009).

2.3. State of the Art on Municipal Wastewater Algal Biofuel Production

Conventional municipal sewage treatment consists of a primary treatment phase for the sedimentation of solid materials, a secondary treatment phase in which suspended and dissolved organic materials are removed, and a tertiary treatment phase in which final treatment of the water is performed before being discharged into the environment. It is during the tertiary phase that the removal of many dissolved inorganic compounds, including Nitrogen (N) and Phosphorous (P) takes place and here it is the potential to use microalgae in N and P removal. Certain unicellular green microalgae species are particularly tolerant to sewage effluent conditions, most notably those of the *Chlorella* and *Scenedesmus* genus, and so a majority of studies have examined the growth of these species (Mallick 2002).

Microalgae have been observed to be very efficient at removing N and P from sewage-based wastewater in either a free-swimming suspension or in an immobilized form. For example, various species of *Chlorella* and *Scenedesmus* can provide high removal efficiencies (>80%), and in many cases almost complete removal of ammonia, nitrate and total P from secondary treated wastewater indicating the potential of microalgae for tertiary sewage treatment. Many of these experiments were performed under laboratory-based batch culture conditions with the microalgae showing high growth rates over the batch growth period. Krishna *et al.* also compared the growth of *S. obliquus* under semi-continuous culture conditions and found that initial growth over four cultivation cycles (every 35 h with fresh wastewater added at the start of each cycle) was much higher than in batch culture, possibly due to eventual nutrient depletion in the batch, but after four cycles of culture growth and chlorophyll content of the cells decreased significantly, indicating a collapse of the culture (Krishna, Dev et al. 2012).

Studies have also shown microalgae to grow and efficiently remove nutrients from primary settled sewage wastewater. For example, *C. vulgaris* was demonstrated to remove over 90% of N and 80% of P content from the primary treated sewage (Hammouda, Gaber et al. 1995). This study compared the effect of various algal starting inoculum densities with inoculum ranging from a density of 1×10^7 cells mL^{-1} to a low-density inoculum of 5×10^5 cells mL^{-1}. It was found that growth rates were not significantly different between various densities and apart from the lowest starting inoculum density, the total amounts of nutrients removed from all treatments were equivalent. This suggests that effective wastewater growth and nutrient removal is not significantly dependent on starting cell density (Hammouda, Gaber et al. 1995).

A second recent study characterized *Chlorella minutissima*, which was identified in wastewater treatment oxidation ponds in India. *C. minutissima* was able to grow well in high concentrations of raw sewage and dominate the subsequent pond stages in the oxidation pond system. Analysis has found that this species can grow heterotrophically in the dark, mixotrophically in the light utilizing a variety of organic carbon substrates over a wide pH range, and in the presence of salt. Furthermore, it can utilize either ammonia or nitrate as an N source.

The growth studies of this algae have shown to be highest under mixotrophic (photoheterotrophic) conditions with biomass productivity of 379 mgL^{-1} after ten days of growth compared to the biomass of 73.03 mgL^{-1} under photoautotrophic conditions. This species could, therefore, become a good candidate for high biomass productivity in a wastewater high-rate pond system. All of these experiments have also demonstrated that chlorophyte microalgae such as Chlorella can grow well even in very raw wastewater conditions (Chinnasamy, Ramakrishnan et al. 2009).

2.3.1. Application of Algae on Wastewater Recourses Treatment

Application of microalgae in the wastewater industry is still relatively limited and only seen on a small scale for wastewater treatment. For example, algae may be seen in conventional oxidation (stabilization) ponds or the more developed suspended algal pond systems such as high-rate algal ponds (HRAPs) which are shallow raceway-type oxidation ponds with mechanical mixing and have been shown to be highly effective for wastewater treatment (Hoffmann 1998). Figure 5 shows some of the places that wastewater can be used for algae treatment.

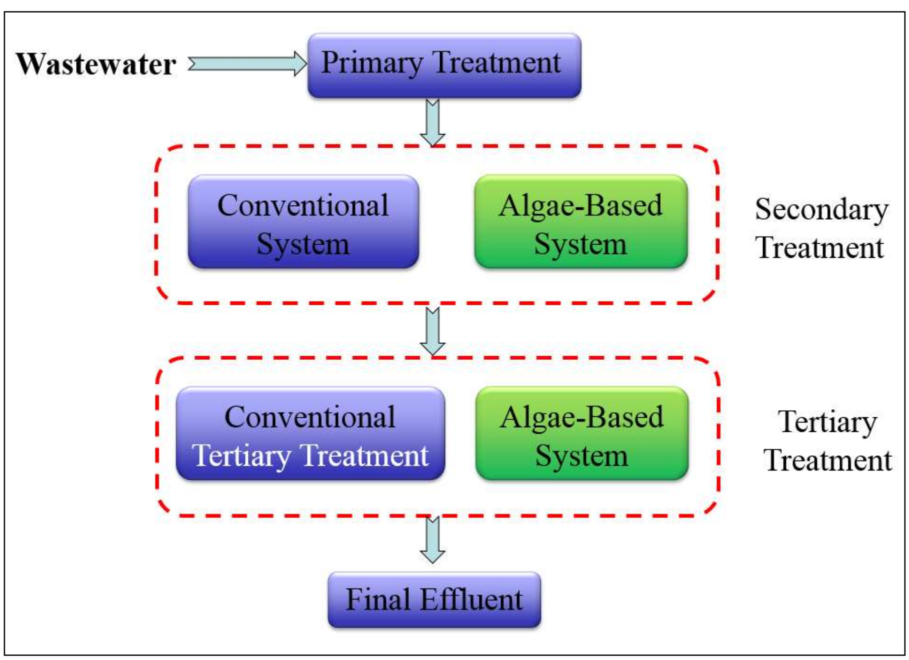

Figure 5. Potential stages for using algae-based treatment system in WWTPs

Most of the research on algal wastewater treatment has come from the analysis of laboratory small-scale and pilot pond scale cultures, as well as from experimental high-rate algal ponds. There remains a gap here because using algae in wastewater treatment has not been fully researched. As with any growth medium, critical variables must be studied such as pH and temperature of the growth medium, the concentration of essential nutrients (including N, P and organic carbon), and the availability of light, O_2 and CO_2. For example, the growth of microalgae in primary settled sewage water was shown to increase significantly under long photoperiod conditions and following addition of CO_2, while increased temperature decreased algal biomass (Chinnasamy, Ramakrishnan et al. 2009).

A major difference between wastewater media and other growth media is the high concentration of nutrients in wastewater such as N and P. Much of the N is often in the form of ammonia which at high concentration can inhibit algal growth. The presence of toxins such as cadmium or mercury, or organic chemicals provides other critical factors of algal growth in wastewater. This will particularly be an issue with industrial-derived wastewaters. Biotic factors that may negatively impact algal growth include pathogenic bacteria or predatory zooplankton. In addition, other microorganisms in the wastewater might out-compete the microalgae for essential nutrients. The starting density of microalgae in the wastewater is also most likely to be a critical factor for the growth of the whole population (Pittman, Dean et al. 2011).

These variables will differ depending on the wastewater type and from one wastewater treatment site to another. Furthermore, there will be variation in the ability of different algal species to tolerate particular wastewater conditions. Unicellular chlorophyte microalgae have been shown to be particularly tolerant to many wastewater conditions and very efficient at accumulating nutrients from wastewater (González, Cañizares et al. 1997).

Chlorella and *Scenedesmus* are usually predominant of the phytoplanktonic communities in oxidation ponds and high-rate algal ponds. Nevertheless, there is variation in effectiveness between chlorophyte species. For example, *Chlorella Vulgaris* was more effective than *Chlorella kessleri* at accumulating N and P from wastewater in one study, while Pittman et al. research found that *Scenedesmus obliquus* grew better in municipal wastewater than *C. vulgaris* (Pittman, Dean et al. 2011).

Nitrogen is removed from wastewater in algal ponds due to the assimilation of nitrogen by the algae, desorption of Ammonia into the atmosphere, and natural nitrification-denitrification in the pond. According to Rothermel, et al. (2011) in addition to nutrient removal, chemical oxygen demand (COD), total inorganic Carbon (TIC), and heavy metals can also be removed from wastewater through microalgal treatment (Rothermel 2011).

The research of Woertz et al. (2009) has indicated that dissolved oxygen production and nutrient assimilation are primary contributors to algae growth in wastewater treatment ponds. However, the Carbon: Nitrogen and Carbon: Phosphorus ratios in domestic sewage C:N 3.5:1; C:P 20:1 and dairy lagoon water C:N 3:1; C:P 10:1 are low compared to typical ratios in rapidly growing algae biomass C:N 6:1; C:P 48:1. This lack of carbon leads to limitations in algae production and incomplete assimilation of wastewater nutrients by algae (Woertz, Feffer et al. 2009).

Results of Woertz et al. (2009) showed that lipid contents of the algae from the municipal wastewater experiments ranged from 4.9–11.3% of (Volatile Suspended Solid) VSS by weight (Table 1). Despite the relatively low lipid contents observed, short residence times and high biomass production rates resulted in lipid productivities ranging from 9.7 mg/L/day (air-sparged) to 24 mg/L/day (CO_2-sparged 3-day HRT).

Table 1: Lipid productivity of Municipal Wastewater Culture (Taken from Woertz, Feffer et al. 2009)

Sample	VSS (mg/l)	Lipid (%)	Lipid Content of Culture medium (mg/L)	Lipid productivity (mg/L/day)
CO_2 4-day HRT	843	4.9	41.5	10.4
CO_2 3-day HRT	813	9.0	73.3	24.4
Air 3-day HRT	317	9.3	29.2	9.7
CO_2 3-day HRT	412	11.3	46.2	23.1

Potential biomass production estimates for algae grown on wastewater nutrients in the Tampa Bay area, FL was calculated by Dalrymple. Table 2 represents the results of this work (Dalrymple, Halfhide et al. 2013).

Table 2. Potential biomass production estimates for algae grown on wastewater nutrients in the Tampa Bay area, FL (Taken from Dalrymple, Halfhide et al. 2013)

Description	Source	Flow rate MGD	Nitrogen (mg L^{-1})	Algae biomass (tons yr^{-1})	CO_2 consumed (tons yr^{-1})	Indoor area (ha)	Outdoor area (ha)
Wastewater	HFC AWTP	3.0	30	1,956	3,026	179	179
Centrate	HFC AWTP	0.5	427	4,660	7,179	182	80
Wastewater	WTS	5.0	10	1,091	1,681	598	598
Total				**7,716**	**11,889**	**959**	**857**

Figure 6 shows the basic operating principles for the algal production integration with wastewater treatment (Dalrymple, Halfhide et al. 2013). This picture shows one of the most promising ways of producing algae to a higher degree of concentration. Laboratory results showed that by the end of a 14-day batch culture, algae could remove ammonia, total nitrogen, total phosphorus, and chemical oxygen demand (COD) by 93.9%, 89.1%, 80.9%, and 90.8%, respectively, from raw centrate, and the fatty acid methyl ester (FAME) content was 11.04% of dry biomass providing a biodiesel yield of 0.12 g-biodiesel/L-algae culture solution. The system could be successfully scaled up, and continuously operated at 50% daily harvesting rate, providing a net biomass productivity of 0.92 g-algae/(L day) (Li, Chen et al. 2011, Pittman, Dean et al. 2011).

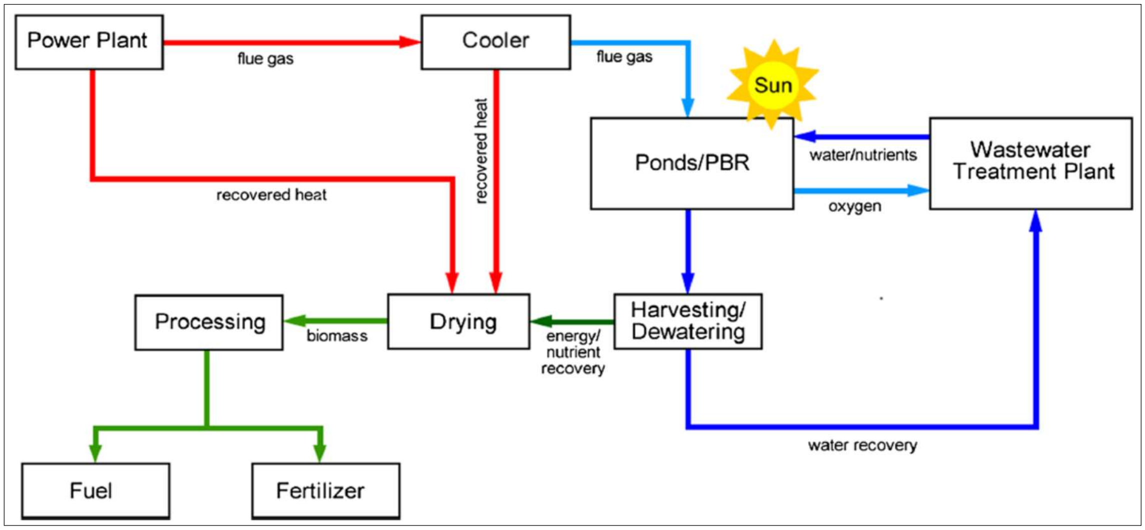

Figure 6. Basic operation principles for the algal production integration with wastewater treatment Taken from (Dalrymple, Halfhide et al. 2013)

2.3.2. Municipal Wastewater Recourses in the US

Wastewater is essentially the water supply of the community after it has been used in a variety application. Wastewater is defined as a combination of one or more of domestic effluents industrial effluents, stormwater and other urban run-off, agricultural, horticultural and aquaculture effluents. Municipal wastewater usually consists of blackwater (from toilets, etc.), greywater (kitchen and bathing wastewater), and water from institutions, including hospitals and commercial establishments (Corcoran 2010). Wastewater is about 99 percent water by weight, and 1 percent is made up primarily of organic solids that are suspended or dissolved in the water. Most of the organics found in wastewater can be decomposed by natural biological processes (Hammouda, Gaber et al. 1995).

Wastewater characteristics in table 3 were estimated based on literature (EPA 2002, Gross 2005). Main nutrients required for algae growth include N, P, and C. There are some challenges for lipid accumulation and cell growth rate in wastewater. These challenges include growth environment (Solar radiation, Temperature, CO_2 availability), algal species, and cultivation conditions (Lundquist 2008, Woertz 2008, Su, Mennerich et al. 2011). One of the main challenges for municipal wastewater algal cultivation is that the ratio of carbon to nitrogen (C/N) and carbon to phosphorus (C/P) is low compared to the typical ratios in rapidly growing algal biomass (C/N 6:1; C/P 48:1) (Metcalf and Eddy 1991, Dalrymple, Halfhide et al. 2013). Therefore, CO_2 supply from flue gas was considered in the model. The quality of wastewater changes based on the removal efficiency of each treatment stage. In terms of algae growth, the most common nutrients are N, P and CO_2. So, I considered the removal efficiency of these nutrients at each stage.

Another study has shown that major Wastewater Treatment Technologies in the U.S. are Activated Sludge (6,800 Facilities, $25{,}000\times10^6$ gallon per day), Biofilm system (2,500 Facilities, $6{,}000\times10^6$ gallon per day) and Ponds (5,100 Facilities, $2{,}000\times10^6$ gallon per day) (Lundquist 2008). This result shows that the capacity of WWTPs is consistent with the report by EPA 2008 report.

Table 3. Raw municipal wastewater characteristics (Metcalf and Eddy 1991)

Component	Concentration Range	Typical Concentration
Total Suspended Solids, TSS	155 – 330 mg/L	250 mg/L
5-Day Biochemical Oxygen Demand, BOD5	155 – 286 mg/L	250 mg/L
pH	6 -9 s.u.	6.5 s.u.
Total Coliform Bacteria	10^8 – 10^{10} CFU/100mL	10^9 CFU/100mL
Fecal Coliform Bacteria	10^6 – 10^8 CFU/100mL	10^7 CFU/100mL
Ammonium-Nitrogen, NH4-N	4 - 13 mg/L	10 mg/L
Nitrate-Nitrogen, NO3-N	Less than 1 mg/L	Less than 1 mg/L
Total Nitrogen	26 – 75 mg/L	60 mg/L
Total Phosphorus	6 - 12 mg/L	10 mg/L

2.3.3. Other Wastewater Resources for Algal Growth

In this section, I briefly discuss other wastewater resources such as agricultural, industrial and artificial wastewater resources for growing microalgae. Compared to municipal, domestic sewage-based wastewater, agricultural wastewater, which is often derived from manure, can be very high in N and P content (Wilkie and Mulbry 2002). Despite these high nutrient concentrations, studies have demonstrated the efficient growth of microalgae on agricultural waste, and as with municipal wastewater, microalgae are efficient at removing N and P from manure-based wastewater (González, Cañizares et al. 1997, Wilkie and Mulbry 2002). For example, the green algae *Botryococcus braunii* grew well in piggery wastewater containing 788 mg L^{-1} NO_3 and removed 80% of the initial NO_3 content (An, Sim et al. 2003). Studies of algal-mediated nutrient recovery from dairy manure have assessed the potential of benthic freshwater algae rather than planktonic (suspended) algae due to the potentially higher nutrient uptake rates in some species of benthic algae. These species include *Microspora willeana, Ulothrix sp*. and *Rhizoclonium hierglyphicum*. Using a semi-continuous cultivation method where the benthic algae was grown in recycling wastewater with fresh manure added daily, algal growth rates and nutrient uptake were found to be high and equivalent to values from algae grown in municipal wastewater (Wilkie and Mulbry, 2002).

Another potential resource is industrial wastewater. There is significant interest in the use of algae for remediation of industrial-derived wastewaters, predominantly for the removal of heavy metal pollutants (cadmium, chromium, zinc, etc.) and organic chemical toxins (hydrocarbons, biocides, and surfactants), rather than N and P (Mallick, 2002). Due to generally low N and P concentration and high toxin concentrations, algal growth rates are lower in many industrial wastewaters. Consequently, there is less potential for utilizing industrial wastewaters for large-scale generation of algal biomass. Furthermore, municipal and agricultural waste is likely to be more widely available and more uniform in characteristic than the variable constituents of different industrial wastewaters. However, one recent study, which may suggest the potential for some industrial wastewaters in providing resources for the generation of significant algal biomass came from the analysis of wastewater from the carpet mill effluent (Chinnasamy et al., 2009).

Carpet mill wastewater (and a small proportion of municipal wastewater) from the city of Dalton, GA, USA, makes up 100–115 million L of wastewater per day. The wastewater includes process chemicals and pigments used in the mills, plus a range of inorganic elements including low concentrations of metals, and relatively low concentrations of total P and N. This wastewater was shown to be low enough in toxins and had enough P and N to support algal growth, with two freshwater microalgae *B. braunii* and *Chlorella saccharophila*, and a marine alga *Pleurochrysis carterae*, able to grow particularly well on the untreated wastewater (Chinnasamy et al., 2009). With the considerable amount of wastewater available from this industry, a significant amount of biomass and potentially also biodiesel could be generated from this resource.

The last resource that is discussed here is artificial wastewater. Some studies have examined algal growth and nutrient removal characteristics using artificial wastewater (Aslan and Kapdan 2006), (Lee and Lee 2001); (Voltolina, Cordero et al. 1999). Utilization of an artificial medium has benefits such as ease of use for initial laboratory-based experiments. It also allows for simplified analysis of the major components in a wastewater medium without one needing to consider unknown variables such as biotic components. Most artificial wastewater media are composed of inorganic constituents, including high concentrations of specific nutrients and will lack solid organic material and other potential toxins. Therefore, there may be some drawbacks in using artificial wastewater to assess conditions in real wastewater. Direct comparisons of artificial wastewater with municipal wastewater have found that although nutrient removal rates are equivalent, microalgal growth rates are higher in artificial wastewater (Ruiz-Marin et al., 2010). Because of the better understanding of the quality of artificial wastewater, I used it for some of our experiments to have a better understanding of the characterization of the experiments. Although other wastewater resources can be used for algae growth, municipal wastewater is the most significant source for such purpose, and it would be the primary focus of this research.

In summary, municipal wastewater resources around the US have an excellent potential for use as a medium for algae production. However, many challenges like contamination control, land resource availability, CO_2 supplementation and low productivity need to be overcome to understand the real potential of using wastewater for algae cultivation. This research has addressed the resource availability for each WWTP, point-by-point, in the US.

2.4. CO_2 Sequestration by Wastewater Algae

CO_2 is the major source of greenhouse gases which contributes to global warming. Previous studies show that power plants which are using fossil fuel contribute to around one-third of the total CO_2 released from fuel combustion (Razzak, Hossain et al. 2013). The ability of microalgae to use CO_2 for growth makes microalgae cultivation an attractive alternative to CO_2 sequestration. Furthermore, adding CO_2 to wastewater helps algae to grow more effectively. The idea is to use CO_2 emission that is released from coal and natural gas power plants, which contribute to the reduction of greenhouse gas emissions, and as a result, decreases the GWP (Yun, Lee et al. 1997, Razzak, Hossain et al. 2013). Figure 7 shows the algae production integration with CO_2 sequestration and wastewater treatment (Lundquist 2008). In this research, I have used some laboratory experiments for evaluating the effect of adding CO_2 to the algal wastewater medium and see the effects on algae growth.

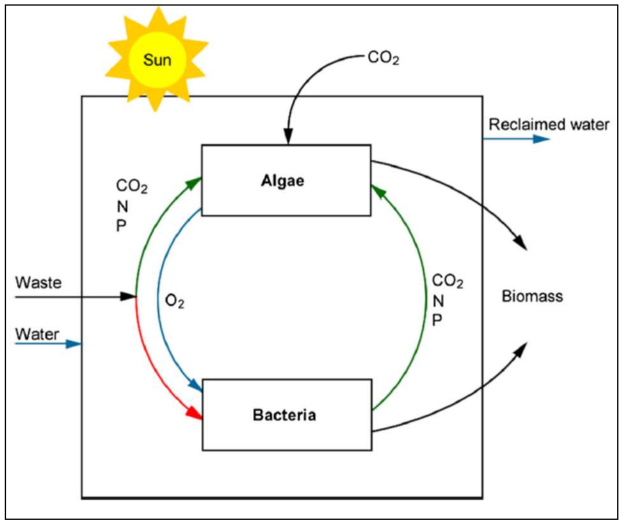

Figure 7. Algae production integration with power generation and wastewater treatment (taken from (DOE 2010)

2.5. Life Cycle Assessment (LCA)

In the late 1980s, the environmental implications of resource and energy use emerged as a serious consideration, especially with the problem of acid rain in Europe and North America and the growing awareness of the potential global greenhouse effect. These two problems joined the growing list of environmental problems arising from the disposal of wastes. Doubts arose about the ultimate ability of the earth's natural systems to deal with these wastes, and the pressure was placed on manufacturers to reduce the environmental impacts of their products (Ross and Evans 2002). This technology has been used in many fields for quality assessment, remanufacturing (Gavidel and Rickli 2017, Gavidel and Rickli 2018), engineering redesign and many researchers has developed tools and platform for performing better sustainability analysis (Aliabadi and Huang 2015).

To understand the whole environmental impacts of any steps in the process we need to consider the comprehensive life cycle of a product from raw material extraction, through manufacturing, to final disposal. This led to the realization that the environmental impacts resulting from a product or service could only be properly understood with a comprehensive assessment, whereby all processes from raw material extraction to the final disposal had been evaluated. This shift away from the project- or process-specific impacts to a system-wide cumulative impact assessment approach, was the catalyst for increasing interest in the use of LCA, an evaluation technique that assesses the environmental impacts of a product or service from "cradle to grave" (Ross and Evans 2002).

The basic concept of LCA is that all environmental burdens connected with a product or service have to be assessed, back to the raw materials and down to waste removal. This basic idea is undoubtedly true, and LCA is the only environmental assessment tool which avoids neglecting some environmental impacts that have to be assessed (Klopffer 1997).

The philosophy adopted by LCA is that the true extent of the environmental burden can only be understood if all steps in the whole life cycle of the product or service are accounted for in the final analysis. ISO has sponsored the development of a series of international standards to describe a consistent methodology, which helps to understand the whole procedure better. The emerging ISO 14040 series of standards, which is part of the ISO 14000 series on environmental management, is the result (Ross and Evans 2002). Figure 8 shows the basic steps of an LCA study.

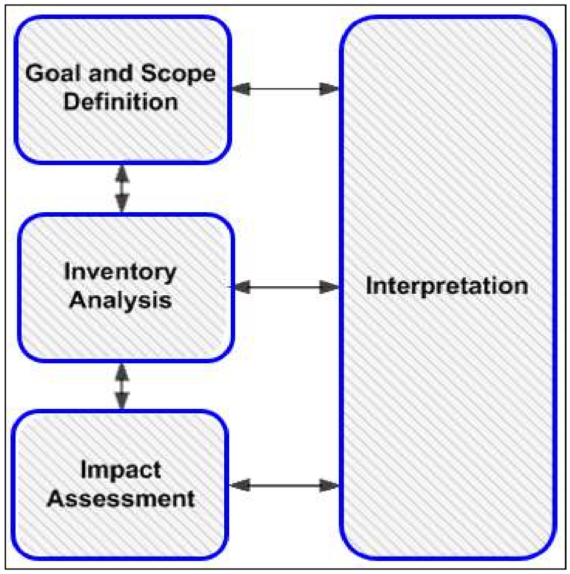

Figure 8. Illustration of LCA phases (taken from (ISO 1998)

Recent LCA work on hypothetical large-scale algae-to-energy systems suggests that cultivation impacts are perhaps the most environmentally burdensome components of the overall algae-to-fuel life cycle (Resurreccion, Colosi et al. 2012). Open Ponds (OP) systems are less expensive and require less energy to construct and operate than PBRs (Benemann and Oswald, 1996; Fischer et al., 2011). They are also easily deployed and scaled up (Davis et al., 2011), but because they are not enclosed, they are susceptible to contamination and evaporation.

PBR systems are more complex and thus more expensive to build and operate than OP systems (Molina-Grima et al., 2003; Chisti, 2007), but they provide better control of species composition and growth conditions (Travieso et al., 2001), improving overall biomass yield and/or lipid yield, and also increase the energy density (MJ/kg) of the harvested algae. In this research, I have evaluated different options for algae cultivation like OP with autotrophic conditions, and OP with mixotrophic and heterotrophic conditions.

Previous studies have mostly focused on one wastewater treatment plant (Mu, Min et al. 2014) or some sections of the states (Fortier and Sturm 2012). Some studies evaluated the continental US wastewater was focused on the population in each county, and not the WWTPs which is the location where all wastewater is gathered (Orfield, Keoleian et al. 2014, Sharma, Brandes et al. 2015). In this study, for the first time, I have evaluated the US potential of algal wastewater treatment based on the WWTPs location which is the point wastewater is collected. All the resource such as land and CO_2 availability will be evaluated based on this location, and the environmental impacts of each process will be calculated on SEHR-LCA, and these impacts will be minimized on LCO model.

2.6. Methodology for Modeling and analysis

2.6.1. Spatial Analysis (Module 1)

Spatial Analysis (SA) can be defined as "a study in depth of the patterns of points, lines, areas, and surfaces depicted on maps of some sort or defined by coordinates in two- or three-dimensional space" (Hägerstrand, 1973). In this section, different resources for spatial modeling are reviewed.

Municipal wastewater. Spatial wastewater resource data for each WWTP, including capacity and population served, was extracted from the Clean Watersheds Needs Survey (EPA 2008) by using "Exist Total Flow" (wastewater generated by population plus infiltration). Data shows that there are around 17,000 WWTPs for the continental U.S., and the yearly flow rate is roughly about 34,200 Million Gallon / Day (1.3×10^8 m^3/day). By filtering out WWTPs with very small capacity (less than 0.05 MG/D), 12,452 WWTPs with a total capacity of 33,576 MG/D, accounting for 99.7% of the total wastewater flow, were included in this analysis. Primary or secondary wastewater effluent was chosen for algae cultivation, as previous studies suggest that solid material contained in wastewater prior to the primary clarifier could damage pumps and reduce their operational life (Lundquist, Woertz et al. 2010, Dalrymple, Halfhide et al. 2013, Craggs, Park et al. 2014). The nutrient profile (nitrogen, phosphorous, and COD) of wastewater was determined by literature (EPA 2002, Gross 2005)

Land availability. National Land Cover Database (NLCD 2011) map, published by USGS, was used for land availability analysis, and site selection around each WWTP for a total of 12,452 WWTPs across the U.S. (USGS 2011). Suitable land for algae cultivation is non-agricultural, undeveloped, or low-density developed, and non-environmentally sensitive, including grassland/herbaceous, shrub/scrub, and barren land (Lundquist, Woertz et al. 2010, Sharma, Brandes et al. 2015). The analysis was performed by considering the land availability in 1, 2.5, 5, 7.5, and 10 km radius distance from the wastewater treatment plant. This method has been applied in the study of land availability in Kansas for up to 2.5 km ((Fortier and Sturm 2012). In this analysis, I extended the radius up to 10 km to analyze land availability for the 99.7% WWTPs identified earlier. To avoid land overlapping around different WWTPs, the Thiessen Polygon method from ArcGIS toolbox was used.

Solar Radiation: Daily solar radiation, averaged value over surface cells of 0.1 degrees in both latitude and longitude, (or about 10 km in size), was used in this research. This data was extracted by using the State University of New York/Albany satellite radiation model, developed by Dr. Richard Perez and collaborators at the National Renewable Energy Laboratory, and other universities for the U.S. Department of Energy (Perez 2012). Figure 9 is a sample of data that is used in the spatial analysis model.

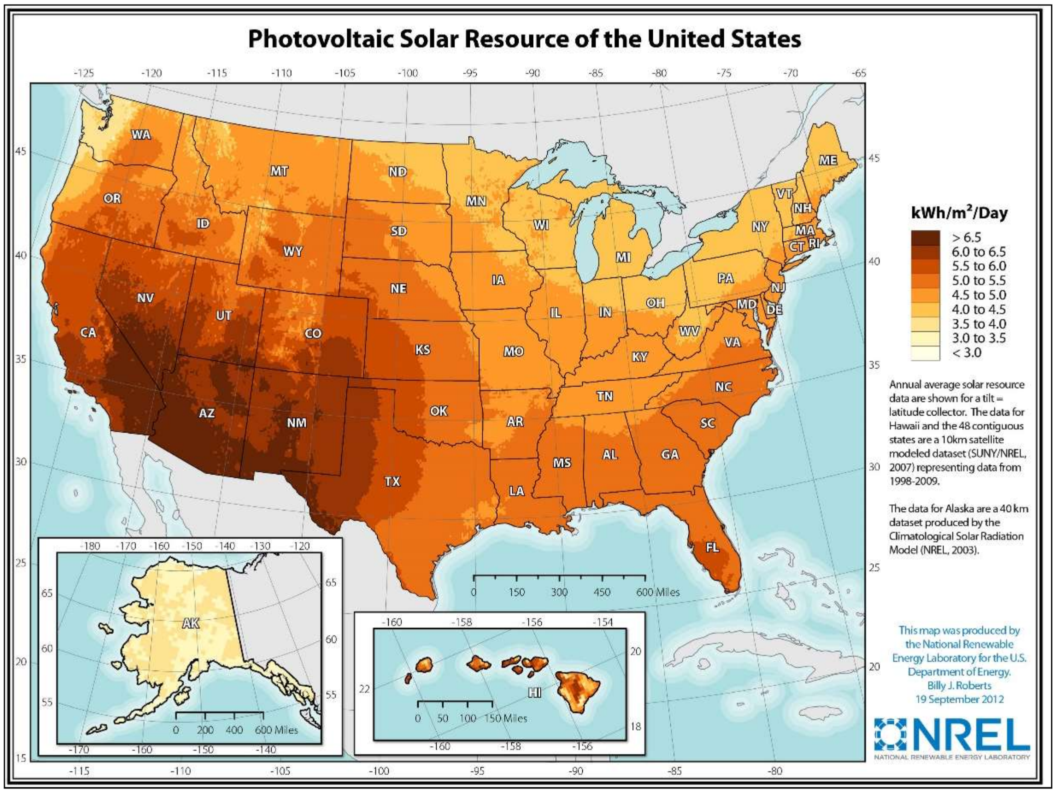

Figure 9. Solar resources data sample (taken from (NREL 2012)

Average Monthly Temperature Base on Daily Data: Different types of algae species can grow in a wide range of temperatures, from almost 0 to 35 degrees centigrade. The effect of temperature and flue gas adding to microalgae system has been discussed by Cassidy, 2011. The algae species modeled in this analysis, *Chlorella Vulgaris*, can grow in temperatures as high as 30-35 °C, but the optimum temperature ranges from 25 to 30°C (Cassidy 2011).

In this study, I considered 10°C as the minimum possible temperature for growth of algae cultivation. The Normal Mean Temperature data that spans 30 years (from 1991-2010) are based on the PRISM Climate data (PRISM 2015). A sample map of this data for April is shown in Figure 10.

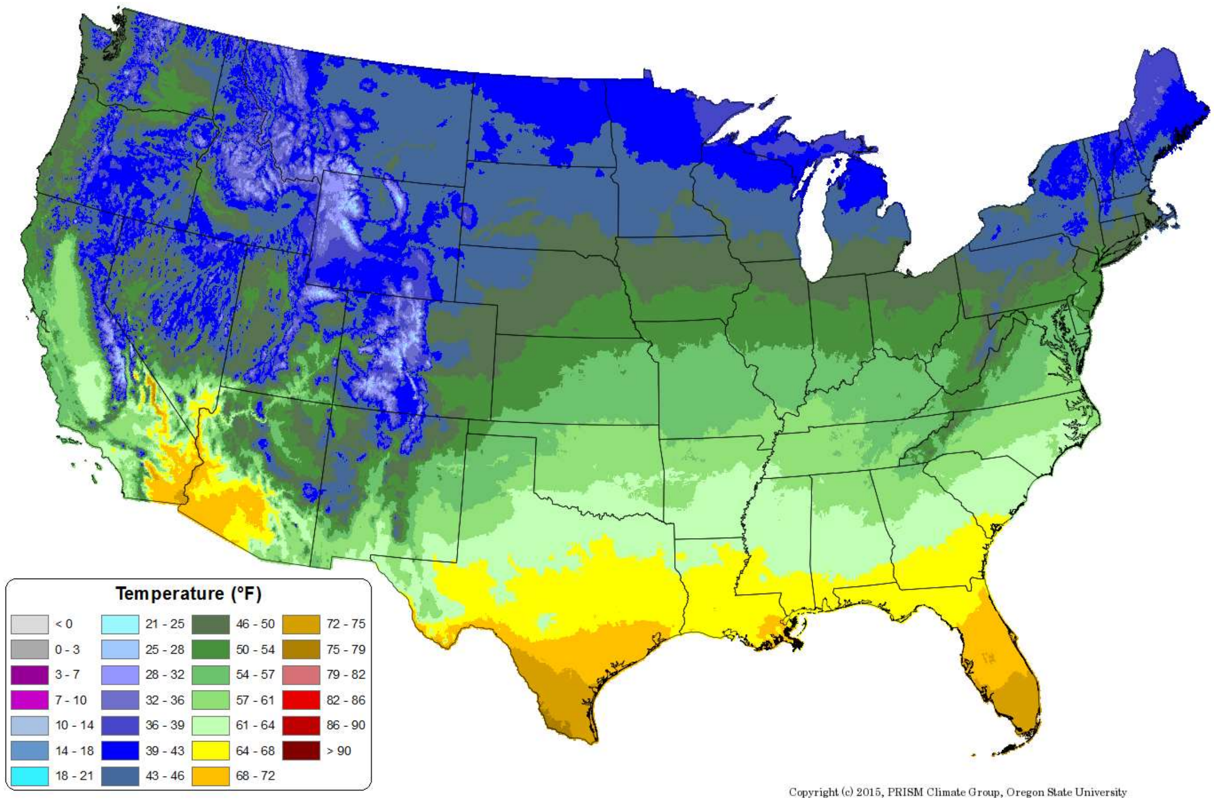

Figure 10. Normal Mean Temperature for 30-yrs: April, (PRISM 2015)

Evaporation: Evaporation varies based on the temperature and solar resources. One of the main disadvantages of open pond microalgae growth systems is the vast volume of water required to make-up the evaporation loss. Some references suggested 1.5 cm/day for evaporation loss (Rogers, Rosenberg et al. 2014). In this study, I considered 1.5% of the wastewater volume lost daily due to evaporation. This amount was considered in the calculation for land resource demand.

Land Coverage Data: Information regarding land coverage was gathered from Multi-Resolution Land Characteristics Consortium (MRLC). I used National Land Cover Database 2011 (NLCD 2011) which is the most recent national land cover product. NLCD 2011 has 16-class land coverage classification schemes, which have been applied consistently across the United States, with a spatial resolution of 30 meters (USGS 2011).

HDPE Pipe and Wastewater Pumping: Wastewater is considered to be pumped to certain areas where enough land is available. High-Density Polyethylene Pipe (HDPE) has been used worldwide for water distribution and transmission systems. Some of the main advantages of using HDPE pipes are: chemical and abrasion resistance, construction advantages, flexibility and fatigue resistant, cost-effectiveness, long-term and permanent placement, easy handling, and better hydraulic properties. Table 4 provides some of the main environmental burdens associated with HDPE. These values will be used for LCA calculation.

Table 4. HDPE pipe key parameters for modeling

Energy needed for Production	74.9 MJ/kg HDPE	(Europe 2008)
Life Time Span	30 yr	Project life time
GWP	1.96 kg CO_2 eq	(Europe 2008)
Eutrophication Potential	0.43E-3 kg PO_4 eq	(Europe 2008)

The following equation (on figure 11) was extracted based on the information of HDPE pipe production (JMEagle 2015). This equation gives the pipe weight per meter for different diameters.

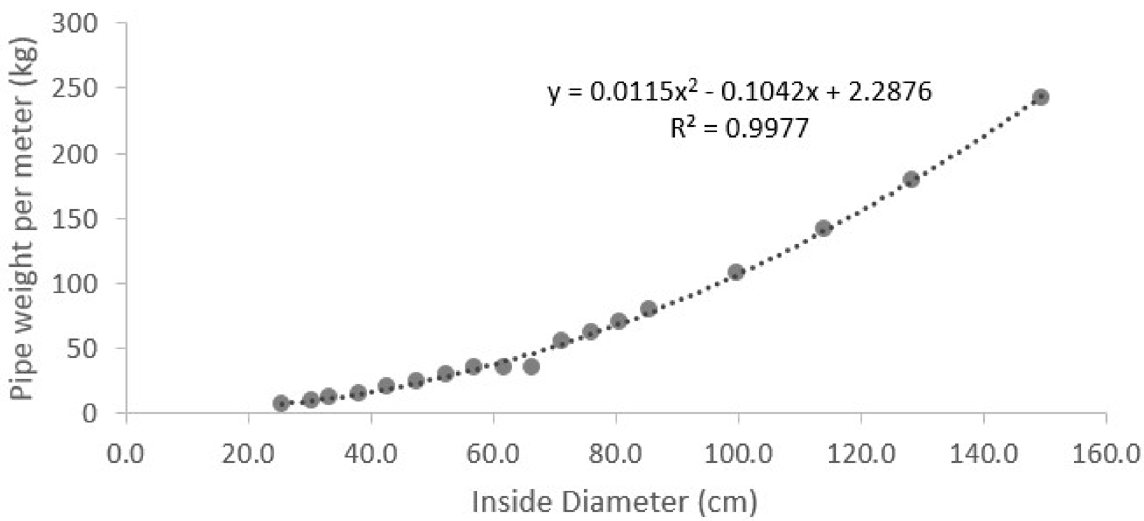

Figure 11. The relation between HDPE pipe inside diameter and the weight developed

The pumping energy demand is calculated based on the equation (1):

$$P = \frac{Q\gamma h_t}{3.6 \times 10^6 \eta_T} \qquad \text{Eq. (1)}$$

P: Hydraulic Power (kW)

Q: Flow capacity (m^3/h)

γ : specific weight = 9810 N/m^3

ht= Sum of Static and dynamic head required

ηT= Total efficiency of pump %

In this research, I considered a 20m static head as an average. This is based on the average of 50 WWTPs around the US from different regions. However, for the future research it may be needed to consider the elevation difference between WWTP and the place which land is available. In terms of dynamic head loss, the optimum diameter (head loss as a result of friction being minimized) is used. As a result, the average velocity in the pipe is set to be 1 m/s which is the optimum velocity for reducing head loss in the pipe. The dynamic head was calculated based on the Darcy-Weisbach equation. The total efficiency of the pump and shaft (ηT) is considered to be 65% as a conservative assumption for calculating the energy demand.

2.6.2. Algae Growth Model (Module 2)

Because of the ease of operation and low cost, open pond systems (OPs) are currently the most promising systems for algal biomass production at large scale (Kumar, Mishra et al. 2015). Previous studies have reported that the productivity of algae dry biomass ranges from 0.12 to 0.48 $g.L^{-1}.d^{-1}$, or 8 to 20 $g.m^{-2}.d^{-1}$ (Brennan and Owende 2010, Craggs, Park et al. 2014). Likewise, algal oil yield varies from 2.3 to 25 $m^3.ha^{-1}.yr^{-1}$ (Quinn and Davis 2014). Among different OPs cultivation strategies, High-Rate Algal Ponds (HRAP) is the most studied system with relatively low environmental impact (Grobbelaar 2009, Stephenson, Kazamia et al. 2010, Vasudevan, Stratton et al. 2012). *Chlorella* sp. is the predominant phytoplankton in HRAPs and WWTP clarifiers (Canovas, Picot et al. 1996), and also one of the most studied algae species for biofuel production (Chinnasamy, Ramakrishnan et al. 2009, Wang, Min et al. 2010). Therefore, *Chlorella* *sp.* in HRAP was chosen as the algae cultivation system in this study. The modeling parameters are presented in Table 5.

For constructing the HRAPs, concrete was chosen as a construction material because of its long lifespan and less seepage in comparison to the earthen ponds. Data regarding the concrete design and environmental burdens of concrete was extracted from SimaPro and other references (Eamon, Wu et al. 2014). Table 5 shows the data for concrete used in the model. The pond size was determined from the study of Ben-Amotz, 2008 (Ben-Amotz 2008).

Table 5. HRAPs key parameters for modeling

Parameters	Value	References
CO_2 Embedded on 1 cubic meter of concrete	100 kg of CO_2	Nonstructural Concrete (NRMCA 2008)
Water usage per 1 cubic meter of concrete	4.8 m^3	Waterwise.org.uk
Length of the pond	150 m	Assumed base on (Ben-Amotz 2008)
Width of the Pond	10 m	Assumed base on (Ben-Amotz 2008)
Concrete thickness on the Bottom	0.07 m	Assumed base on (Ben-Amotz 2008)
Concrete thickness on the Wall	0.10 m	Assumed base on (Ben-Amotz 2008)
Depth of pond + free wall	0.40 + 0.20 m	Assumed base on (Ben-Amotz 2008)
Lifetime Span	30 years	Project lifetime
Embodied Energy of non-structural mass concrete	0.77 MJ/kg	(Hammond and Jones 2008)

* The lifetime span of HDPE pipes is between 50-100 years. However, in this study, I considered it to be equal to the HRAPs construction ponds.

Different formulas are suggested for modeling the algae biomass yield. Here I have presented some of the studies for predicting biomass production. Algae grow by converting solar energy during photosynthesis to chemical energy stored in the form of oils and other biomasses. Wigmosta et al. have used the following equation to predict the rate of biomass production (P_{mass} in mass per unit area per unit time) (Weyer, Bush et al. 2010, Zemke, Wood et al. 2010, Wigmosta, Coleman et al. 2011):

$$P_{mass} = \frac{\tau_p C_{PAR} \varepsilon_a E_s}{E_a} \qquad \text{Eq. (2)}$$

$$\varepsilon_a = \frac{E_c \varepsilon_p \varepsilon_b}{Q_r E_p} \qquad \text{Eq. (3)}$$

Where E_s is the full-spectrum solar energy at the land surface (MJ/m^{-2}), C_{PAR} is the fraction of photosynthetically active radiation (PAR), τ_p is the transmission efficiency of incident solar radiation to the pond microalgae, ε_a is the efficiency by which algae converts photons to biomass and E_a is the energy content per unit biomass ($MJ\ kg^{-1}$). Where the photon energy E_p ($MJ\ mol^{-1}$) converts PAR as energy to the number of photons and p accounts for reductions in photon absorption due to suboptimal light and water temperature. The quantum requirement Q_r is the number of photons required to liberate one mole of O_2 and together with the carbohydrate energy content Ec represents the conversion of light energy to chemical energy through photosynthesis. Table 6 shows the values of those parameters in equations 2 and 3.

Table 6. OPs microalgae biomass growth model parameters taken from (Wigmosta, Coleman et al. 2011)

Term	Theoretical Maximum Case	Units
τ_p	0.95	
C_{PAR}	0.46	
E_a	38	$MJ\ kg^{-1}$
E_p	0.2253	$MJ\ mol^{-1}$
Qr	8	$mol\ mol^{-1}$
E_c	0.4825	$MJ\ mol^{-1}$
ε_b	1.0	
ε_p	1.0	
ρ_{oil}	0.92	
f_{oil}	1.0	

Shen et al. used the following equation for predicting algae biomass productivity in wastewater (Shen, Yuan et al. 2009):

$$BY = \frac{QT\eta}{Ec(1-L)+E_l L} \qquad \text{Eq. (4)}$$

Where:

Q is the month-average PAR energy per day (kWh/m2-day);

T is cultivation time (operation days in the month when the temperature is above 10 °C);

η is the theoretical final PAR conversion efficiency (3.2%) (Larkum 2010);

Ec is the energy necessary for building one gram of carbohydrate (17KJ/g);

E_l is the energy necessary for synthesizing one gram of lipid (38KJ/g);

L is the lipid content ($\frac{\text{g lipids}}{\text{g algae}}$) of the algae by dry weight.

Previous studies used equations to predict the algae mass in wastewater, but they did not consider the effect of temperature and seasonal variations (Orfield, Keoleian et al. 2014). Equation number 4 has also taken temperature into consideration, as T is the number of days in the month for operation when the temperature is above 10 °C.

In this research based on previous work (Clarens, Resurreccion et al. 2010, Zhang, White et al. 2013) a GIS-based spatial explicit algae growth model was developed. Specifically, algal biomass production, water / nutrient demand, material input/output, and energy consumption were computed by site-specific meteorological information (solar radiation, temperature, precipitation, and evaporation) incorporated into a mass and energy balanced algal open pond model (Clarens, Resurreccion et al. 2010, Zhang, White et al. 2013, NREL 2015), including available wastewater and land resources from the RA module. Algae cultivation was assumed to occur in months where the average monthly temperature was greater than 10°C (Lavens and Sorgeloos 1996). Site-specific biomass yield had a substantial effect on land analysis and was calculated based on the formula as a function of solar radiation (Photosynthetically Active Radiation or PAR), temperature, and conversion efficiency (Cunningham, Heim et al. 2010, Larkum 2010). Specifically, solar radiation was the average value over surface cells of 10 km in size, and data were extracted from the model developed by Dr. Richard Perez and collaborators at the National Renewable Energy Laboratory and other universities for the U.S. Department of Energy. Temperature variations were obtained based on PRISM Climate data that is a 30-year Normal Mean Temperature database. Model outputs were calculated on a monthly basis in operational periods when the temperature is above 10 ˚C.

2.6.3. Conversion to Biofuel Pathways (Module 3)

Mass and energy balance methods were used to develop the biomass harvest and bio-oil conversion model. Processing and modeling parameters were determined based on previous studies (Clarens, Resurreccion et al. 2010, Du, Li et al. 2011, Resurreccion, Colosi et al. 2012, Chaiwong, Kiatsiriroat et al. 2013, Mu, Min et al. 2014). Three conversion pathways were examined for bio-oil production: lipid extraction, microwave pyrolysis, and hydrothermal liquefaction. Lipid extraction (LE) is the most studied conversion pathway, consisting of algal lipid extraction and anaerobic digestion of residual non-lipid biomass for nutrient recycling and by-product generation (bio-electricity and fertilizer) (Clarens, Resurreccion et al. 2010, Zhang, Liu et al. 2015). The LE technology is mature, but its energy yield is relatively low because lipid is the only energy carrier. Microwave pyrolysis (MP) uses uniform internal heating of large biomass particles to generate bio-oil, combustible biogas, and biochar. This process does not require agitation or fluidization, and, as such, the bio-oil contains fewer particles (ashes) (Du, Li et al. 2011). The main disadvantage of MP is the necessity for removing nitrogen and oxygen from crude oil, which needs more energy (Du, Li et al. 2011). Hydrothermal liquefaction (HTL) has gained increasing interest as it is more energy efficient method. The main advantages of HTL are that it can convert non-lipid compounds to bio-oil and does not require energy-intensive processing such as drying (Mu, Min et al. 2014). However, the complexity of the conversion mechanisms, as well as the difficulty of maintaining the constant property of biomass feedstock, makes it hard to improve conversion efficiency for higher bio-oil yield (Barreiro, Prins et al. 2013, Mu, Min et al. 2014). The detailed information regarding energy requirements for each conversion pathway are presented in section 4.4 of SI.

Three pathways are selected based on the previous studies (Stephenson, Kazamia et al. 2010, Du, Li et al. 2011, Roberts, Fortier et al. 2013, Mu, Min et al. 2014), including microwave pyrolysis (MP), hydrothermal liquefaction (HTL), and lipid extraction (LE). Tables 7 to 9 describe key parameters and assumptions for these three pathways.

Table 7. Design Parameters for Microwave Pyrolysis taken from (Du, Li et al. 2011)

Process operation	Value	Unit
Microwave power	750	W
Temperature	569 ± 42	°C
Bio-oil Yields	28.6	wt.% algae
Bio-oil yields HHV	39.0	MJ/kg
Char Yields	24	wt.% algae
Char HHV	10	MJ/kg
Char Recycle	0.2	To algae
Gas Yields	26	wt.% algae
Gas LHV	15.52	MJ/kg
Gas Content H2	28	wt.% gas
Gas Content CO	15	wt.% gas
Gas Content CO2	25	wt.% gas
Gas Content CH4	25	wt.% gas

Table 8. Design parameters for HTL taken from (Roberts, Fortier et al. 2013)

Pretreatment	Value	Unit
Heat required	0.53/0.75/1.0	MJ/kg Water
Temperature	569 ± 42	°C
HTL		
Heat required	0.72/1.03/1.40	kWh/kg-TS
Ash free content	90	% of dry weight algae
Biocrude Yields	44.5 ± 4.7	% afdw (Roberts, Fortier et al. 2013)
HHV	39	MJ/kg biocrude oil (Roberts, Fortier et al. 2013)
C	78.7	Wt.% biocrude (Roberts, Fortier et al. 2013)
Char Yields	21.0 ± 8.6	% afdw (Roberts, Fortier et al. 2013)
HHV	9 (8-10)	MJ/kg bio char oil
C	20	% biochar (Roberts, Fortier et al. 2013)
Gas Yields	16 ± 8	% afdw (Roberts, Fortier et al. 2013)
C	25	% biochar (Roberts, Fortier et al. 2013)

Table 9. Design parameters for Lipid Extraction (LE)

Pretreatment	Value	Unit
Thickening electricity use	26,372	MJ/ha/year (Resurreccion, Colosi et al. 2012)
Homogenization electricity use Raceways Pond	5.9	MJ/kg of biodiesel (Stephenson, Kazamia et al. 2010)
Oil Extraction electricity use Raceways Pond	2.4	MJ/kg of biodiesel (Stephenson, Kazamia et al. 2010)
Anaerobic Digestion electricity demand	38,532	(MJ/ha/Year) (Resurreccion, Colosi et al. 2012)
Anaerobic Digestion Heat demand	1,515	(MJ/ha/Year) (Resurreccion, Colosi et al. 2012)

2.6.4. Life Cycle Assessment (Module 4)

Results from Modules 1-3 were used for LCA to account for two types of seasonal and site-specific environmental impacts: energy use and greenhouse gas emission. The functional unit (FU) was defined as 50,700 MJ/year, the average energy embodied in gasoline required for driving a compact car by an American (US.DOT 2014, US.DOT 2015). System boundaries were “cradle-to-gate”, encompassing all processes associated with algal bio-oil production with wastewater, including: pond instruction, algae cultivation, bio-oil conversion, by-product generation, and extraction of raw resources for the production of required energy/material inputs. The

Environmental burdens associated with infrastructure and equipment were calculated by multiplying required material inputs and their corresponding impact factor obtained from the Ecoinvent database (Weidema 2007). These burdens were divided by the assumed project lifetime (30 years) for direct comparison with annual impacts arising from operations. All facilities associated with WWTPs were excluded from analysis because they would already be in place at all WWTPs. However, environmental impacts associated with nitrogen and phosphorous removal by algae were considered as credits, as algae cultivation replaced the corresponding N and P treatment from WWTPs. LCA boundaries for our research is presented in figure 12.

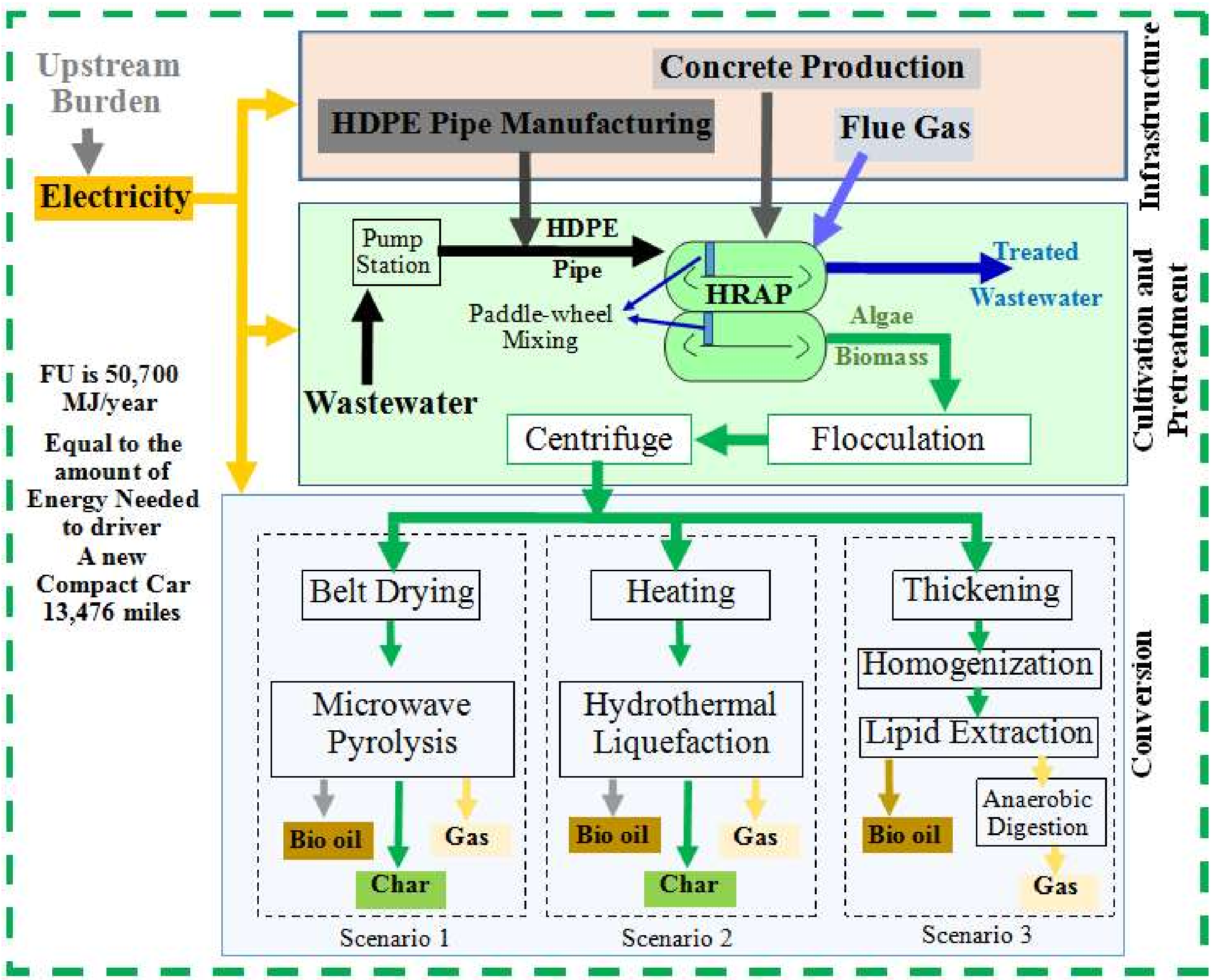

Figure 12. System boundaries and processes for life cycle assessment

In conclusion, reviewing the combined literature in this research subject, one is able to ascertain that a large gap in understanding the realistic potential of using wastewater for algal cultivation exist. SEHR-LCA and LCO model will help us to have a better understanding of resource availability (such as land, CO_2) and laboratory experiments will evaluate the use of new methods of growing wastewater algae. In the next section, I have evaluated the methodology that I used to reach our proposed research objectives.

Based on the research objective the following sections are presenting the primary results for spatial analysis and life cycle assessment model. The information for doing the model have gathered from different kinds of literature and the Spatially-Explicit-High-Resolution Life Cycle Assessment (SEHR-LCA) model for wastewater-based algal biofuel production has produced. The primary results of this model for resource availability and environmental impacts (Green House Gas emissions) have been calculated. This part of the research is completed, and the results are published in "Algal Research" journal, one of the high-ranked journals in the field of algae and biofuel. In this part of the proposal, I discussed the results that I have achieved up to this point of the research. First of all, some laboratory experiments with Detroit wastewater that I have done on SWEET Lab has been discussed. In the next step, the results of special analysis and evaluation of potential wastewater algae cultivation are discussed. Finally, the primary results of LCA studies on environmental impacts of algal wastewater are reviewed.

2.7. Results

2.7.1. Spatial Analysis and Potential algae cultivation in wastewater in the US

Wastewater resource. There are total 12,452 municipal WWTPs with a capacity of 0.05 MG/D (190 m^3/day) and above across the U.S., accounting for 99.7% of the total wastewater flow (Figure 13). Most WWTPs, 73% of the total WWTPs, have the capacity of 0.1-10 MG/D, accounting for 33.4% of the total wastewater flow, followed by WWTPs of 10 – 50 MG/D (27% of the total wastewater flow), > 100 MG/D (26% of the total wastewater flow), 50 – 100 MG/D (13% of the total wastewater flow), and 0.05 – 0.1 MG/D (0.6% of the total wastewater flow). The majority of WWTPs are located in the middle-to-east and fewer on the west coast of the US, in accordance with population distribution. Large metro areas, such as Detroit, Chicago, Los Angeles, usually have WWTPs with large capacity (>100 MG/D), which indicates the popularity of centralized wastewater infrastructures. This part of the research has been published recently in Algal Research journal (Roostaei and Zhang 2016).

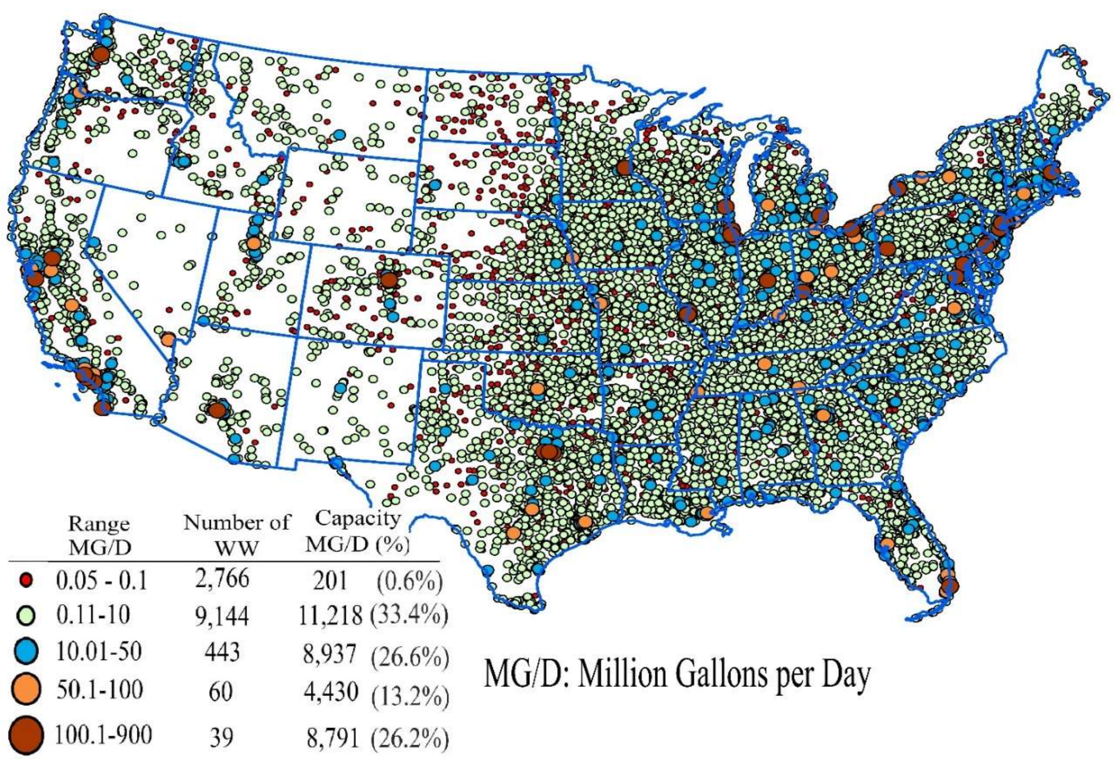

Figure 13. Wastewater treatment plants and their corresponding treatment capacity (wastewater flow) across the continental U.S.

2.7.2. Land Availability

High-Resolution analysis of land resource around each WWTP was conducted to assess the land availability in 1, 2.5, 5, 7.5, and 10 km radius, respectively. The required land of algae open-pond for each WWTP was determined by pond depth, evaporation, infrastructure land usage (pump station, etc.), and pond hydraulic retention time. The land analysis was first performed for 1 km radius around the WWTP. If not, enough land was available, then 2.5 km radius was analyzed, followed by 5, 7.5, and 10 km radius, respectively. Our analysis results show that algae facility located in further than 10 km of the WWTP is not likely to be energy favorable due to the increasing amount of energy required for wastewater pumping. Therefore, land resource in 10 km radius would be first considered for algae cultivation. For those WWTPs where the land requirement could not be met in the range of 10 km, energy efficiency was used as the criteria for site selection. Specifically, the wastewater would be pumped further for algae cultivation until energy return on investment (EROI), determined by LCA module, reached to 1.0. Results of land analyses show that only 8,507 WWTPs, accounting for 16% of the total wastewater flow, have the capacity to locate algae facility in 1 km radius (Table 10). These WWTPs usually serve small communities/populations with low wastewater capacity (StateRule 2008). The number of WWTPs with available land in 2.5, 5, 7.5, and 10 km radius is 2,401, 808, 197, and 58, respectively. In sum, 11,971 of the 12,452 WWTPs could co-site algae facilities in 10 km radius, accounting for 69% of total wastewater flow. Figure 14 shows the results of land analysis. Those areas with black color are the available land in different radius by removing the effect of overlapping. For infrastructure facilities such as pumping station, flue gas house, and other utilities, 12.5% extra area was considered for land area demand (Ben-Amotz 2008).

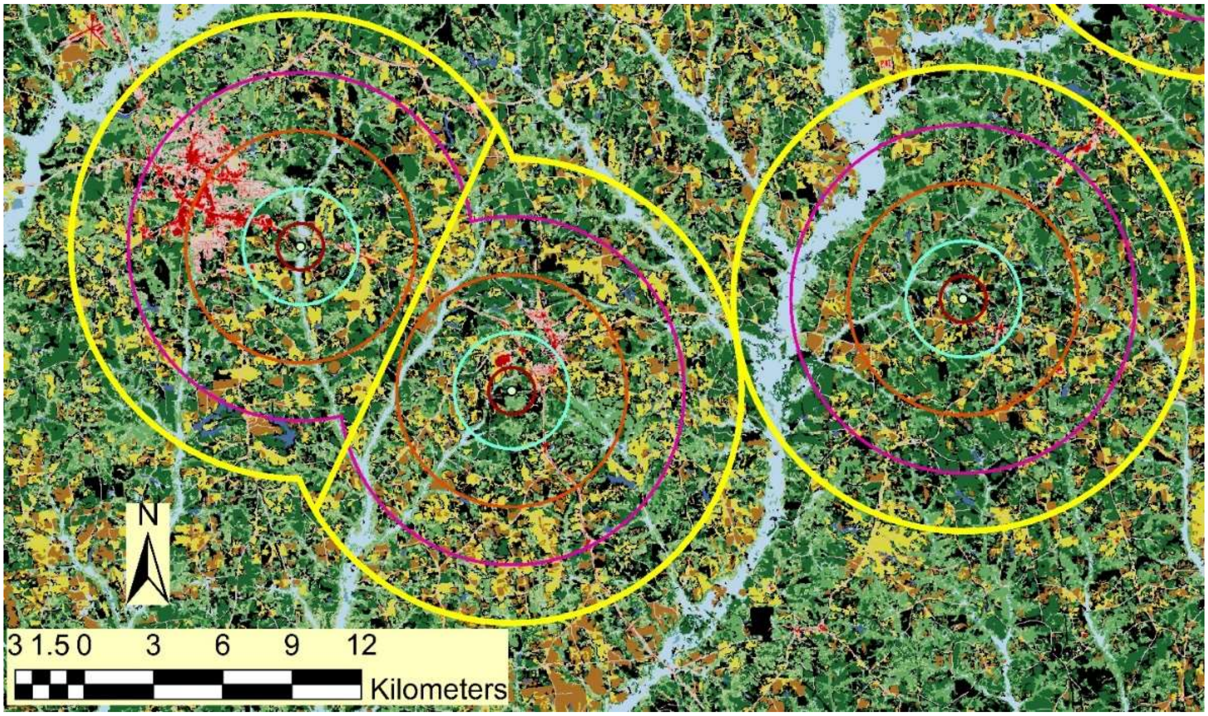

Figure 14. Land Availability Analysis on the different radius and removing the effect of overlapping

The results of this analysis are presented in table 10.

Table 10. Land availability for WWTPs in different radius

Radius, km	Number of WWTPs with enough land	Capacity (10^{+6} G/d)	Percentage of total Wastewater flow
0 – 1 km	8,507	5,250	16%
1 – 2.5 km	2,401	6,150	18%
2.5 – 5 km	808	5,810	17%
5 – 7.5 km	197	2,830	8%
7.5 – 10 km	58	850	3%
> 10 km	481	12,692	38%

These results imply the importance of land resources for co-siting algae facilities when using municipal wastewater for algal biofuels. This constraint has not been fully considered in previous LCA or GIS studies (Quinn and Davis 2014). For example, Orfield et al. (2014) performed a GIS analysis to estimate algal bio-oil production potential through flue gas and wastewater co-utilization without land analysis. Chiu et al. (2013) analyzed water availability, wastewater resources, and suitable lands in the development of algal bio-oil (Chiu and Wu 2013, Orfield, Keoleian et al. 2014). However, they assumed all the wastewater effluent could be used for algae cultivation without considering the co-siting of algae and wastewater facilities. Figure 15 shows the variations in algal biomass productivity across the continental US. Part A depicts annual average yield, and part B shows monthly average yield in four representative WWTP sites. Cultivation seasons are those months when the average temperature is above 10 °C. Four stars represent four representative WWTPs.

Interestingly, for most WWTPs with small wastewater capacity, the land demanding for algae cultivation could be met within 1 km radius. The larger capacity the WWTP has, the less land demanding could be met. This raises the question of how to scale the facilities: centralization or decentralization? There have been many debates regarding this issue, both for bioenergy facilities and wastewater infrastructures. Some studies found that large-scale centralized facilities are more cost-efficient, especially from an economic perspective; others argued that decentralized facilities could have more environmental benefits (Stephenson, Kazamia et al. 2010, Davis, Aden et al. 2011, Davis, Fishman et al. 2014). The results of this study suggest that decentralization could have greater potential for wastewater-based algae bioenergy systems, which aligns with the increasing interest of decentralized water infrastructures for wastewater reclamation (Massoud, Tarhini et al. 2009, Libralato, Ghirardini et al. 2012). However, further research is warranted to investigate to what extent the scale could be optimized for both environmental and economic benefits.

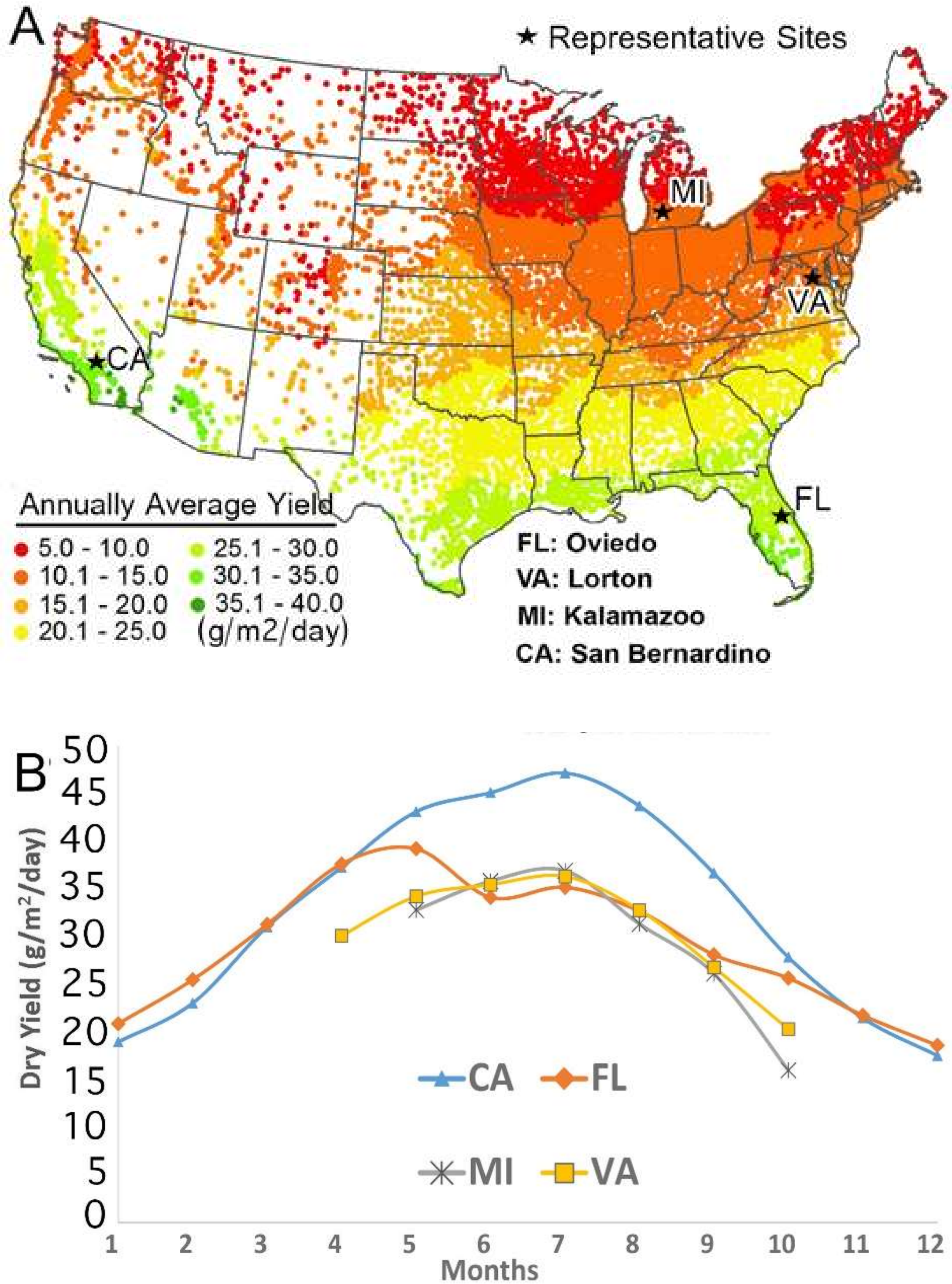

Figure 15. A) Annually average yield for each WWTPs, and B) monthly average yield in four representative WWTP sites

2.7.3. Life Cycle Assessment (LCA) Analysis

In this work, the developed High-Resolution-Spatially-Explicit Life Cycle Assessment model (HRSE-LCA) allowed a variation of environmental impacts to be studied in more detail because environmental impacts can be calculated for each WWTP and every month, avoiding a large area and long-time averaging. Energy use and greenhouse gas (GHG) emissions were chosen as two environmental impact factors. Energy use is discussed in detail for seasonal and site-specific variation. For GHG emission, only total emissions are presented here, since GHG emission ties with energy efficiency and show the same variation pattern.

2.7.3.1 Energy Efficiency

The LCA results (Figure 16) show significant variations in energy efficiency among different conversion pathways, cultivation seasons, and wastewater treatment plants. Energy efficiency (energy return on investment, EROI)): the ratio of energy output to energy input with greater value being more energy favorable. **A, B, C**: energy efficiency (yearly average) of individual WWTP across the continental U.S for Microwave Pyrolysis (A), Hydrothermal Liquefaction (B), and Lipid Extraction (C), respectively. **D**: Monthly variations of energy efficiency in four representative WWTPs for the best performance scenario (Hydrothermal Liquefaction).

Bio-oil Conversion Pathway. All conversion pathways are independent from location, and their modeling is based upon the total amount of algal biomass production. Among the three conversion pathways (Figure 16 A, B, C), hydrothermal liquefaction (HTL) is the best performance scenario, where most WWTPs can generate positive energy output (EROI >1). This is because HTL has the best energy output (0.98 billion gallon /yr bio-oil + 1.9 million tons biochar + 1.4 million tons biogas) and relatively low energy input compared to Microwave Pyrolysis and

Lipid Extraction, since HTL does not require intensive energy procedure such as drying and can convert 50-60% of the total biomass to bio-crude oil (Barreiro, Prins et al. 2013, Mu, Min et al. 2014).

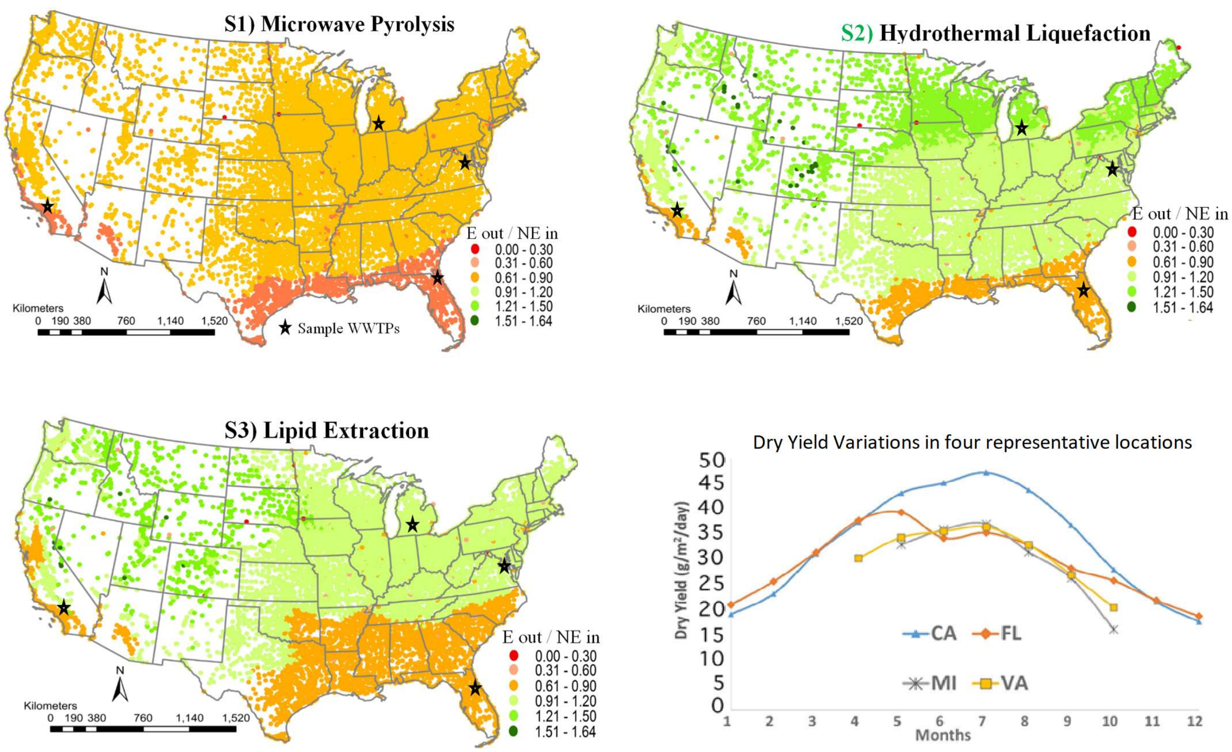

Figure 16. Variations of energy efficiency among different conversion pathway scenarios (Roostaei and Zhang 2017)

Microwave Pyrolysis (MP) produces second large energy output (0.77 billion gallons/yr bio-oil + 1.8 million tons biochar + 2.4 million tons biogas) but has the worst energy performance (no WWTP producing positive energy output). This is mainly due to the high heat and electricity requirement for pretreatment and microwave generation. Lipid extraction (LE) produces the least energy (0.57 billion gallons/yr + 0.74 million tons biogas) among the three conversion technologies because lipid composition in algae is lower than carbon content that can be converted into bio-oil via thermochemical conversion. Nevertheless, compared to MP, lipid extraction has better energy performance (some WWTPs have net energy output), because it requires less heat and electricity. When compared to conventional fossil fuel (EROI: 13) (GREET 2015), wastewater-based algal bio-oils are not energy competitive (EROI $\leq$2) (GREET 2015), but they do perform much better than pathways with synthetic fertilizer and fresh water (Clarens, Nassau et al. 2011, Libralato, Ghirardini et al. 2012).

Site-Specific and Seasonal Variations. When examining the site-specific variations (take HTL scenario as the example), it is surprising that energy performance is opposite to the productivity. For example, warm climates have higher yearly productivity but exhibit poorer energy performance compared to cold climates. Further analysis reveals that this is mainly due to seasonal variation. Figure 16 D shows that there is a large variety in energy efficiency among different seasons. Because of lower productivities, the EROI in the winter season (December, January, February) decreases by more than 50% compared to the summer season. Therefore, warm climates with all-season operation have lower yearly average energy efficiency than cold climates where oil production only occurs in optimal months (April to October). If winter operation is shut down, energy efficiency in warm climates will outperform that in cold climates (data not shown). The regression between algal biomass yield and energy performance (SI) suggests that it will not be energy favorable if the productivity is below 20 g/m^2-d (based on operational days). Our results suggest that winter shutdown may be necessary even in warm climates if winter productivity remains low. These results indicate that it is warranted to develop cultivation technology in cold weather for productivity improvement.

Energy Allocation. To understand the driving force for energy efficiency, four WWTPs in different climate (from very cold to very warm) were selected to analyze the energy allocation for different processes including wastewater pumping, algae cultivation, biomass harvesting and pretreatment, bio-oil conversion, and energy credits from by-products (biochar and biogas) and wastewater treatment. Figure 17 shows the allocation of energy use for four representative WWTPs in California (CA), Florida (FL), Michigan (MI), and Virginia (VA). Y-axis is the energy use per functional unit (50,700 MJ/yr). MP, microwave pyrolysis; LE, lipid extraction; HTL, hydrothermal liquefaction.

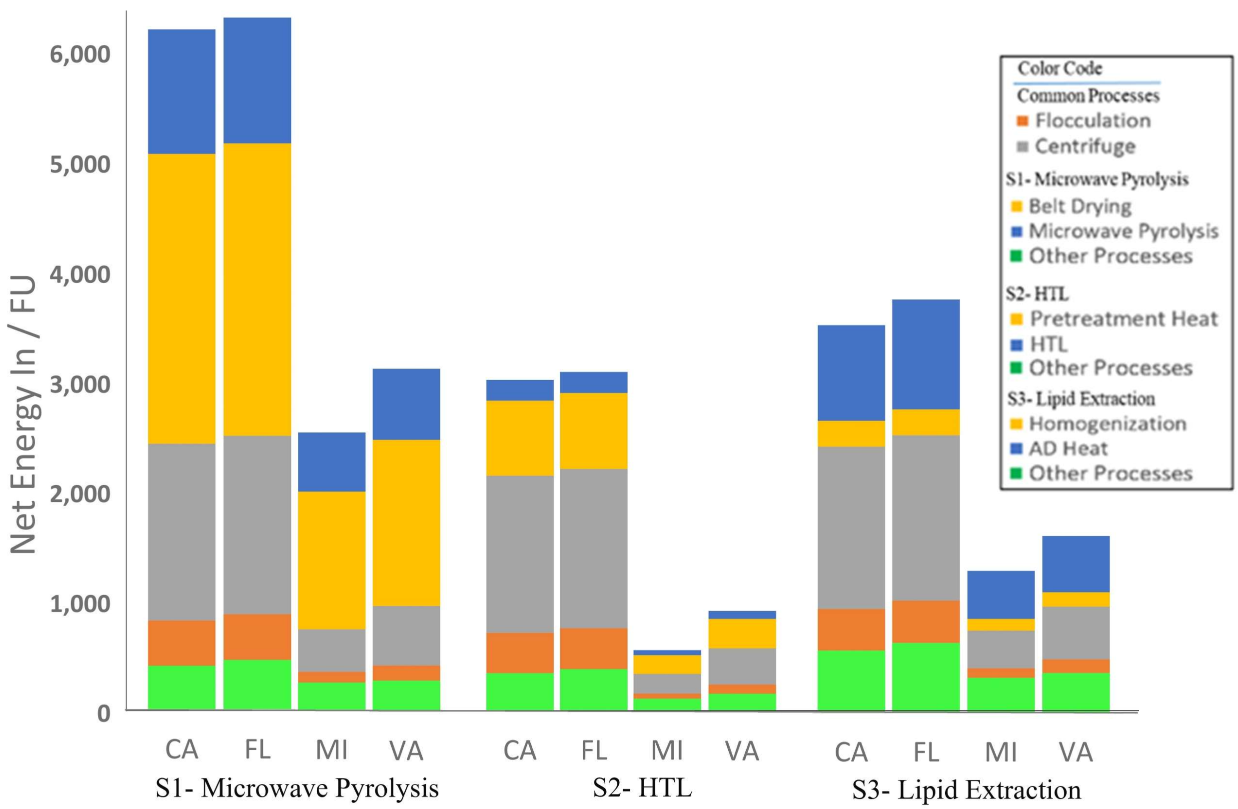

Figure 17. Net energy input per FU for each scenario and four representatives

These four WWTPs have the same distance for wastewater pumping (5 km) and the same wastewater flow (around 100,000 m^3/day). For all cases in different locations, WWTPs, and bio-oil conversion scenarios, the top two driving forces for energy burden are biomass harvesting/pretreatment and bio-oil conversion (contributing to 60-80% of total energy use), mainly from the electricity and heat used for process operations. The MP conversion pathway is the most burdensome process, accounting for about 50% of total energy use. In HTL and LE, biomass harvesting/pretreatment is the top contributor (30-50% of total energy use). In contrast to the freshwater-based system, energy use for cultivation has much less impact on total energy use. This is mainly due to the replacement of synthetic fertilizer that is very energy intensive. Previous studies indicate that energy burden associated with fertilizer could contribute up to 30% of total energy use (Clarens, Resurreccion et al. 2010, Colosi, Zhang et al. 2012).

Wastewater pumping is a considerable contributor to energy use in wastewater-based algae systems (20-30% of total energy use), from both electricity used for pumping and upstream burden associated with pipe construction. This indicates that land availability around WWTPs has significant impact on the performance of wastewater-algae systems. Further analysis suggests that energy efficiency will drop below one if the land is not available in 10 km (SI). Ironically, WWTPs with abundant wastewater resources usually located in well-developed metro areas, where land is limited. As discussed in Section 3.2, about 40% of wastewater resources could not be utilized due to the short of land resource. Land availability plays a significant role for wastewater-algae systems because it not only determines the feasibility of co-siting algae facilities but also affects the overall cost. This is evidenced in Figure 16 A-C, as most of the large WWTPs (red dots) in metro areas are not energy favorable. According to the EPA survey (EPA 2016), U.S. needs $271 Billion investment to maintain and/or improve the nation's wastewater infrastructures. Algal cultivation is an opportunity for wastewater treatment and bioenergy generation. This study suggests that, for those WWTPs need redesign or reconstruction, decentralization could be one solution for wastewater utilization/reclamation such as algal biofuel production.

2.7.3.2 Greenhouse Gas Emission

The main processes contributing to greenhouse gas emission include pipe production, concrete production, and CO_2 emission from electricity used for operations. Upstream impact of GHG emissions from electricity and construction materials are calculated based on US mix electricity (0.8 kg of CO_2 equivalent/ kWh^{-1}) and Ecoinvent Database (Mu, Min et al. 2014). Similar to energy efficiency, the total GHG emissions vary significantly among different scenarios (MP, HTL, and LE) and locations (Figure 18), from -2,677 to 29,486 kg/FU, with the best performance scenario as HTL, followed by LE and MP. High electricity demand for MP is the main reason for large GHG emission. The site-specific differences are in accordance with the variations of energy efficiency, better performance in a colder climate (MI, VA) than in warmer climate (CA, FL). This is attributed to the same reason causing the variations of energy efficiency, all-season operation in a warm climate with lower average productivity while optimal-season operation in a cold climate with higher productivity.

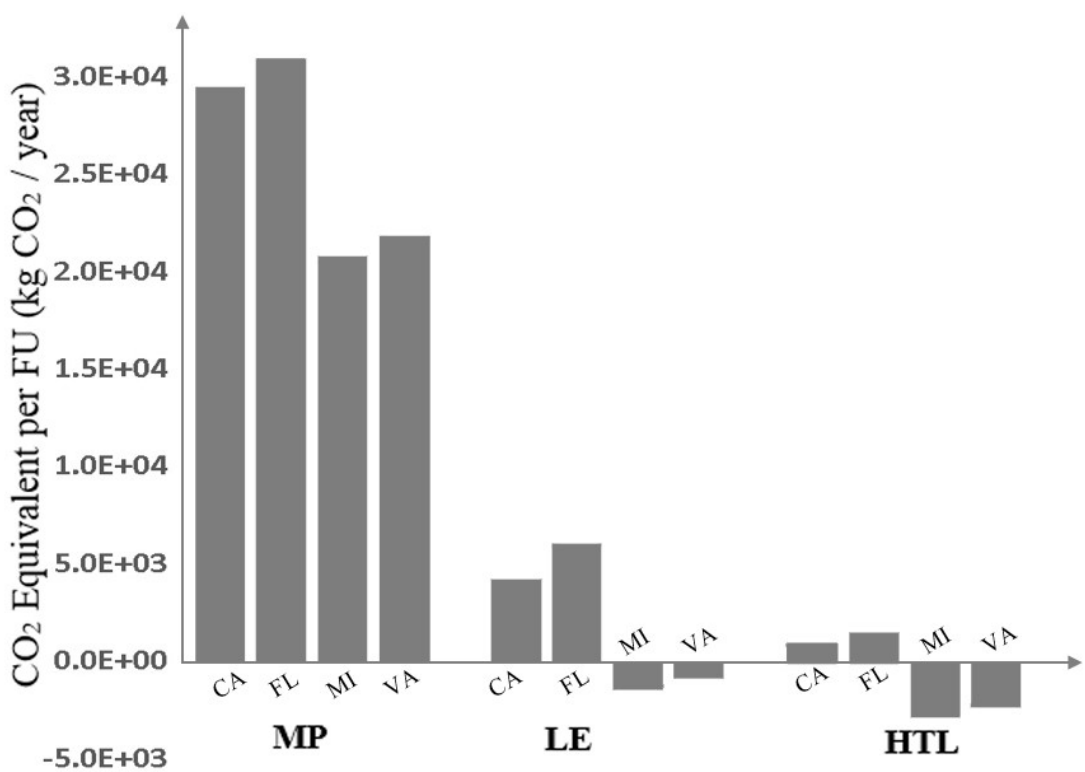

Figure 18. Total greenhouse gas emissions per functional unit in four representative WWTPs (MP: Microwave Pyrolysis, LE, Lipid Extraction, and HTL, Hydrothermal Liquefaction)

While not competitive to conventional fossil fuel in energy efficiency, wastewater-based algal could offer significant benefits in GHG control. GHG emissions for the best performance scenario (HTL) are 4 -7 times lower than that of conventional fossil fuels (GREET 2015), with negative GHG emissions in some cases (LE and HTL in MI and VA). Flue gas uptake by algae biomass and wastewater treatment credit play a major role in reducing GHG emission.

2.7.3.3. Spatial Analysis and Potential Algae Cultivation from Wastewater in the US

Spatial Analysis (SA) can be defined as an in-depth study of the patterns of points, lines, areas, and surfaces depicted on maps of some sort or defined by coordinates in two- or three-dimensional space' (Hägerstrand, 1973). One of the purposes of this research is to evaluate the potential of algae cultivation in the U.S. GIS software is used for building the models of spatial analysis.

Table 11 shows the Primary results of algae cultivation in the U.S. based on the counties and solar radiation.

Table 11. Results of special Analysis for Algae cultivation for each state in the US

State	Scenario 1 Biocrude Oil (Liter/year)	Scenario 2 Biocrude Oil (Liter/year)	Scenario 3 Biocrude Oil (Liter/year)
AL	*45,456,791*	*57,981,249*	*33,774,713*
AR	26,170,570	33,381,202	19,444,916
AZ	66,305,941	84,574,850	49,265,777
CA	440,542,370	661,922,576	327,326,062
CO	33,112,992	42,236,432	24,603,185
CT	24,924,066	31,791,256	18,518,755
DC	28,277,461	36,068,593	21,010,351
DE	7,694,843	9,814,961	5,717,322
FL	195,286,518	249,092,734	145,099,249
GA	70,292,948	89,660,375	52,228,152

State	Scenario 1 Biocrude Oil (Liter/year)	Scenario 2 Biocrude Oil (Liter/year)	Scenario 3 Biocrude Oil (Liter/year)
AL	*45,456,791*	*57,981,249*	*33,774,713*
AR	26,170,570	33,381,202	19,444,916
AZ	66,305,941	84,574,850	49,265,777
CA	440,542,370	661,922,576	327,326,062
CO	33,112,992	42,236,432	24,603,185
HI	18,967,079	24,192,973	14,092,672
IA	25,838,516	32,957,660	19,198,198
ID	10,401,506	13,267,375	7,728,392
IL	159,040,761	202,860,384	118,168,398
IN	60,199,321	76,785,707	44,728,516
KS	24,967,943	31,847,222	18,551,356
KY	31,145,958	39,727,432	23,141,665
LA	60,988,313	77,792,086	45,314,743
MA	47,105,500	60,084,218	34,999,716
MD	33,628,195	42,893,586	24,985,984
ME	7,746,785	9,881,214	5,755,915
MI	85,487,647	109,041,587	63,517,920
MN	26,948,609	34,373,611	20,023,005
MO	62,400,495	79,593,359	46,364,004
MS	26,465,089	33,756,868	19,663,746
MT	4,770,610	6,085,030	3,544,596
NC	60,218,752	76,810,492	44,742,954
ND	3,074,451	3,921,538	2,284,339
NE	14,796,195	18,872,908	10,993,676
NH	6,561,240	8,369,023	4,875,047
NJ	89,430,204	114,070,415	66,447,267
NM	12,769,281	16,287,530	9,487,665
NV	30,385,395	38,757,314	22,576,561
NY	195,081,282	248,830,950	144,946,757
OH	128,915,273	164,434,586	95,784,949
OK	34,434,541	43,922,100	25,585,105
OR	31,537,519	40,226,877	23,432,597
PA	113,354,459	144,586,386	84,223,155
RI	8,160,949	10,409,490	6,063,642
SC	48,734,321	62,161,819	36,209,941
SD	3,542,876	4,519,025	2,632,381
TN	61,185,505	78,043,610	45,461,258
TX	249,470,102	318,205,221	185,358,031
UT	28,615,100	36,499,260	21,261,219

State	Scenario 1 Biocrude Oil (Liter/year)	Scenario 2 Biocrude Oil (Liter/year)	Scenario 3 Biocrude Oil (Liter/year)
AL	*45,456,791*	*57,981,249*	*33,774,713*
AR	26,170,570	33,381,202	19,444,916
AZ	66,305,941	84,574,850	49,265,777
CA	440,542,370	661,922,576	327,326,062
CO	33,112,992	42,236,432	24,603,185
VA	55,428,216	70,700,047	41,183,552
VT	2,493,806	3,180,910	1,852,915
WA	59,024,143	75,286,739	43,855,351
WI	39,454,726	50,325,469	29,315,138
WV	14,375,826	18,336,718	10,681,339
WY	2,885,636	3,680,699	2,144,048
Total (L/yr)	2,918,096,625	3,722,103,667	2,168,166,199
Total Billon Gallon/year	0.77	0.98	0.57

For the purpose of showing in more detail, four representative WWTPs (San Bernardino (CA), Oviedo (FL), Lorton (VA), and Kalamazoo (MI)) were selected for detailed discussion. These WWTPs had nearly the same capacity and located in the same pumping distance, but the solar radiation and temperature are different. Table 12 describes the information for these selected WWTPs and also presents the average yearly algae yield. Tables 13 to 15 show the results of LCA for the four selected WWTPs.

Table 12. Four representative WWTPs at different regions with pumping distance 5km

State	City	Longitude (W)	Latitude (N)	Capacity m^3/day	Average Solar Radiation (kwh/m^2/day)	Average 30-year temperature °C	Days of Operation per year	Average Algae Yield in the year (g/m^2/day)
CA	San Bernardino	117.292	34.0775	100,000	5.48	18.7	365	32.8
FL	Seminole	81.225	28.6237	113,000	4.88	22.3	365	29.2
MI	Kalamazoo	85.5731	42.3064	105,000	3.80	9.2	184	11.5
VA	Fairfax	77.2033	38.6982	105,000	4.18	13.8	214	14.7

Table 13. Results for S1. Microwave Pyrolysis in four representative WWTPs

State	Algae biomass yield Annual (kg/year)	Total Bio-oil production (m^3/year)	Total Energy Production Bio-oil and coproducts (MJ/year)	Total Energy Required (MJ/year)	Total Energy Credit (MJ/year)	Total Energy Out / (Total Energy in – Total Credit)	Number of FU production
CA	11,848,372	3,400	2.03E+08	3.65E+08	4.22E+07	0.63	2,607
FL	11,946,537	3,410	2.04E+08	3.76E+08	4.25E+07	0.61	2,628
MI	5,761,818	1,600	9.86E+07	1.56E+08	2.05E+07	0.73	1,268
VA	6,926,525	2,000	1.19E+08	1.91E+08	2.47E+07	0.71	1,524

Table 14. Results for S2 HTL in four representative WWTPs

State	Algae biomass yield Annual (kg/year)	Total Bio-oil production (m^3/year)	Total Energy Production Bio-oil and coproducts (MJ/year)	Total Energy Required (MJ/year)	Total Energy Credit (MJ/year)	Total Energy Out / (Total Energy in – Total Credit)	Number of FU production
CA	11,848,372	4,700	2.21E+08	2.47E+08	4.22E+07	1.08	3,325
FL	11,946,537	4,750	2.23E+08	2.57E+08	4.25E+07	1.04	3,352
MI	5,761,818	2,300	1.07E+08	9.92E+07	2.05E+07	1.36	1,617
VA	6,926,525	2,750	1.29E+08	1.22E+08	2.47E+07	1.33	1,944

Table 15. Results for S3 LE in four representative WWTPs

State	Algae biomass yield Annual (kg/year)	Total Bio-oil productio n (m^3/year)	Total Energy Production Bio-oil and coproducts (MJ/year)	Total Energy Required (MJ/year)	Total Energy Credit (MJ/year)	Total Energy Out / (Total Energy in – Total Credit)	Number of FU production
CA	11,848,372	2,860	1.45E+08	2.67E+08	4.22E+07	0.64	1,937
FL	11,946,537	2,880	1.46E+08	2.85E+08	4.25E+07	0.60	1,953
MI	5,761,818	1,400	7.05E+07	1.12E+08	2.05E+07	0.77	942
VA	6,926,525	1,670	8.47E+07	1.36E+08	2.47E+07	0.76	1,132

Relations among energy efficiency, pumping distance, and biomass productivity are presented in figure 19 and 20.

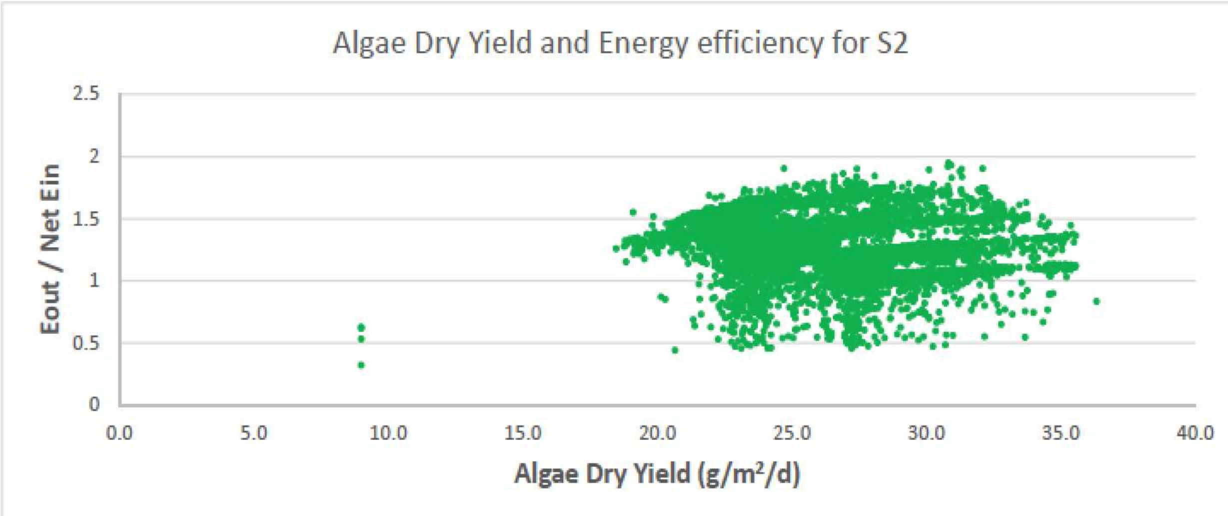

Figure 19. The regression of biomass productivity and energy efficiency

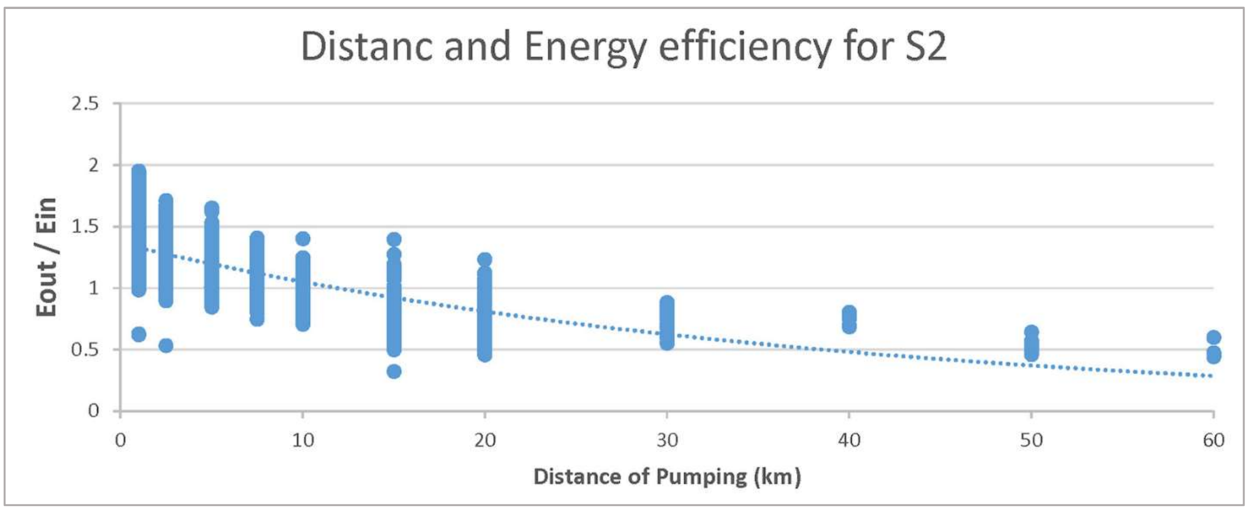

Figure 20. The impact of land availability on energy efficiency

2.8. Conclusions

In this chapter I have reviewed a spatial explicit life cycle assessment model for evaluating each WWTP around the US in term of algae cultivation. The structure and assumptions of LCA has been introduced in this chapter. The results indicated that we could grow algae in almost 60% of total wastewater available in the US with the potential of 0.98 billion gallons per year bio-oil.

CHAPTER 3 AUTOTROPHIC AND MIXOTROPHIC ATTACHED GROWTH

3.1. Background

Researchers around the world have been working on sustainable methods for cultivation of algae in wastewater (Hammouda, Gaber et al. 1995, Pittman, Dean et al. 2011, Mehrabadi, Craggs et al. 2016, Mehrabadi, Craggs et al. 2016, Mehrabadi, Craggs et al. 2017). The current system of algae cultivation mostly focused on phototrophic growth in which light is the main source of energy, and it has used in different methodologies such as open pond systems and closed photobioreactors are used for commercial cultivation (Mehrabadi, Craggs et al. 2017, Mehrabadi, Farid et al. 2017). Mixotrophic cultivation could be the next movement for algae cultivation which uses an additional source of energy besides light, which is usually a carbon source (Wang, Yang et al. 2014). These two systems of cultivation are usually in suspended growth mode in which algae cells are suspended in the medium, which minimizes the settlement and attachment of cells onto bioreactor surfaces (Kesaano 2015). Such a system causes a massive demand for harvesting energy for algae cells after treatment has happened which could reach up to 20-30% of total costs (Grima, Belarbi et al. 2003, Dassey and Theegala 2013). Small diameters of algae cells and also surface charge positive make the harvesting algae very energy intensive (Brentner, Eckelman et al. 2011). Another possibility for reducing the energy is to grow algae on a biofilm surface. In this method algae will attach to a surface and there will be lower energy cost for harvesting the algae (Johnson and Wen 2010, Ozkan, Kinney et al. 2012, Lee, Oh et al. 2014, Zhang, Liu et al. 2017). Combination of attached growth algae in mixotrophic cultivation is the new idea that is proposed in this research.

Depending on the type of algae, different ways of cultivation like autotrophic, mixotrophic or heterotrophic can happen in open ponds. Autotrophic species are photosynthetic like plants.

Heterotrophic species get their energy from organic carbon compounds in much the same way as yeast, bacteria, and animals. Mixotrophic species can use sunlight or organic or inorganic carbon, whatever they can get (Li, Tsai et al. 2014). Some microalgal species are not truly mixotrophic but have the ability to switch between phototrophic and heterotrophic metabolisms, depending on environmental conditions. For example, *Chlorella* sp. can grow in three different modes of cultivation (Perez-Garcia, De-Bashan et al. 2010, Perez-Garcia, Escalante et al. 2011).

Microalgae mixotrophic cultivation in comparison to autotrophic cultivation has some advantages like higher growth rate, prolonged exponential growth phase, and reduction of photo-inhibitory effect (Wang, Yang et al. 2014, Lowrey, Brooks et al. 2015, Perez-Garcia and Bashan 2015). Mixotrophic cultivation has the potential to remove organic carbon and several types of nitrogen and phosphorus compounds from wastewater (Li, Tsai et al. 2014). This process requires changing the culture medium's organic substrate, which stimulates specific metabolic and biosynthetic pathways. On the other hand, there are some limitations for mixotrophic cultivation, namely: demand for organic carbon substrates increases the energy and costs; bacteria and other microorganisms, which compete with microalgae may grow faster and produce contamination; and microalgal species that can grow heterotrophically and mixotrophically are limited (Abreu, Fernandes et al. 2012).

Wastewater is considered one of the promising mediums for algae cultivation because of it negligible cost and availability. The potential of wastewater algae growth in the US had been evaluated in previous research (Orfield, Keoleian et al. 2014, Roostaei and Zhang 2017). Autotrophic cultivation of wastewater algae, however, is limited by light limitation, fluctuation in temperature, CO_2 concentration, ammonium and phosphates concentrations (Ozkan, Kinney et al. 2012). Wastewater microalgae cultivation yield can be increased by heterotrophic or mixotrophic growth because in case of light limitation, microalgae can get the energy demand from other sources (Perez-Garcia, Bashan et al. 2011).

Wang et al. (2010) discussed a detailed comparison of autotrophic and mixotrophic attached growth algae cultivation by using MB3N, wastewater after secondary clarifier (SWW), and wastewater after primary clarifier (PWW) as a medium and *Chlorella* and *Scendesmus* species as the algae. Previous research has been shown that these two algae is the dominant algae in the algal farms and also has a great potential for producing biofuels (Wang, Min et al. 2010).

Another study evaluated how *Chlorella vulgaris*, *Nitzschia amphibia* and *Chroococcus minutus* attached to hydrophobic (such as Perspex, Titanium and Stainless Steel 316-L), hydrophilic (glass) and toxic (copper, aluminum brass and admiralty brass) materials. In addition, the study also examined several factors such as surface roughness, pH of the medium, culture density, age, cell viability and also the presence of organic and bacterial films that influenced the attachment of N. amphibia (Sekar, Venugopalan et al. 2004).

Coupons of the size 2 × 2 × 0.5 cm were used in the experiment. The metal coupons were polished up to 400 grits and wiped with acetone to remove any oil or dirt and were left out to air dry.

The results for the influence of wettability and material composition on adhesion are as follows: After 48 hours, *C. vulgaris* was maximum on stainless steel, followed by titanium, perspex, and glass. The colonization was poor on aluminum brass, admiralty brass, and copper. The rate of an attachment for *N. amphibia* was higher than *C. vulgaris*; maximum colonization of N. amphibia occurred on titanium, followed by stainless steel, perspex, and glass. The colonization was poor on copper and its alloys. For *C. minutus*, it colonized titanium panels better than stainless steel, perspex and glass. Its colonization was however poor on copper and its alloys. The density of attached algal cells varied with time among the three organisms. The attachment was highest in *N. amphibia*, followed by *C. minutus* and least in *C. vulgaris*. Moreover, the results also showed that the attachment was higher on the rougher coupons (for both titanium and stainless steel coupons) , and was significantly lower on smoother surface (Sekar, Venugopalan et al. 2004).

pH is an important factor in natural biofilms and may vary in different layers of the biofilm: The adhesion of *N. amphibia* was studied on both titanium and glass coupons that were in media of different pH's (6, 7, 8 and 9). The results showed that the attachment on both materials varied from the different pH's. The attachment was much higher at pH of 7, 8 and 9, compared to pH 6 on titanium coupons. On glass, the attachment was higher at pH of 9 when compared to pH 7 and 6 . Furthermore, the adhesion of *N. amphibia* on titanium and glass coupons covered with organic films displayed that the organic films increased the algal attachment on both titanium and glass.

Additionally, different culture densities of N. amphibia were used to study adhesion of the diatom on titanium and glass coupons. The results found that as culture density increased, adhesion increased as well. For instance, there was maximum attachment on the 2.3 × 105 cells ml versus minimum at 2 × 102 cells ml−1, for both titanium and glass coupons. In conclusion, the surface property and composition of the material play an important role in microalgal attachment to its surface. The attachment is also influenced by pH, organic film, culture age, culture density, cell viability and bacterial films (Sekar, Venugopalan et al. 2004).

Michel Vert (2010) describes biofilm as a way that "Aggregate of microorganisms in which cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS) adhere to each other and/or to a surface" (Vert, Doi et al. 2012). Microalgae are used for recycling carbon dioxide and converting them into renewable bioproducts, but they create challenges; the low biomass concentration of current suspension culture systems leads to high water requirements, inefficient harvesting and high liquid transportation costs. In this article, the authors discuss how biofilms can be used to improve microalgal production, the development of biofilm and review biomass productivities of current biofilm cultivation systems (Berner, Heimann et al. 2015).

Formation of microfilms begins when microalgae adhere to a surface, either a direct interaction between cells and surface or by secondary colonization of an existing. As they grow, they also produce a sticky matrix substance of EPS. The EPS matrix stores water and other chemicals and can sometimes, protect the cells against harmful chemicals or environmental conditions. Additionally, as biofilms grow, they form a 3D s, multi-layer structure and these structures. Biofilm cultivation systems are categorized into three groups; the first two categories, the microalgae are submerged either submerged continuously or intermittently submerged under a layer of medium. The third category uses a leaky substrate that supplies nutrients and moisture to the microalgae which grow on the outside, exposed to the surrounding gas.

The constantly submerged systems are generally constructed as flow cells or channels. For the constantly submerged system, the microalgae are grown on a solid surface and then covered by a thin layer of medium. Flow is provided by pumping and inclines with smaller angles. The highest productivity was on the constantly submerged system. The intermittently submerged systems come in two varieties: those based on the algal turf scrubber (ATS) and systems with moving surfaces. ATS is quite similar to the constantly submerged system. But the flow rate of the medium is varied to create periodic submersion (Berner, Heimann et al. 2015). The Advantage of these periodic surges is that the biofilm is periodically replenished by fresh medium, but is directly exposed to light and the gas phase in between waves. ATS system has excellent productivity; The ATS is the most mature and popular individual design for algal biomass cultivation and has been successfully used in wastewater remediation studies, including a very large scale for phosphorus remediation of a creek in Florida (USA) (Adey, Kangas et al. 2011). The other intermittently submerged systems include a number of innovative designs in which the surface moves through the (stationary) liquid medium to provide the necessary medium flow for biofilm cultivation. The movement intermittently submerges the biofilm to provide hydration and fresh nutrients and then exposes the cells directly to light and the gas phase the rest of the time (Berner, Heimann et al. 2015).

In recent years, biofilms microalgae are receiving much attention. There were innovation designs put in place that can change microalgal cultivation. To sum it all up, microalgal biofilms play a vital role in the future of industrial photosynthetic biomass production. The authors of this article identified the knowledge gaps and standardization requirements in order to as a certain driver governing microalgal biofilm establishment and development.

In the following section the material and methods that I have used to do our experiment have been reviewed.

3.2. Material and Method

3.2.1. Laboratory Experiments for Autotrophic / Mixotrophic Attached Growth

3.2.1.1. Materials as the Attached Surface

First, laboratory studies were carried out to investigate mixotrophic conditions for suspended and attached growth (algal biofilm). The next paragraphs has described some of the methodologies I used for this part of the experiment.

For autotrophic mode, I used to experiment with different materials of varying roughness to find out which combinations are better for the attached growth. The selected material with the best roughness were used for mixotrophic. Different conditions of the experiment are listed below:

- Materials (four materials: SS, stainless steel. PP, polypropylene. PMMA, acrylic. PC, polycarbonate)
- Roughness (four Roughness 60, 220, and 400 grit sandpaper)
- Mediums (three Mediums: MB3N, PWW)
- Algae Types (*Chlorella, Scenedesmus*)
- Growth Modes (Autotrophic and Mixotrophic by adding Glucose)
- Replicate (At least two)

I have taken samples at the exponential growth phase and stationary phase of the algae growth. The coupons have submerged inside the algae medium when algae are in those two growth phases. According to our previous experiments and literature review, the time to reach log and stationary phases are shown in figure 21.

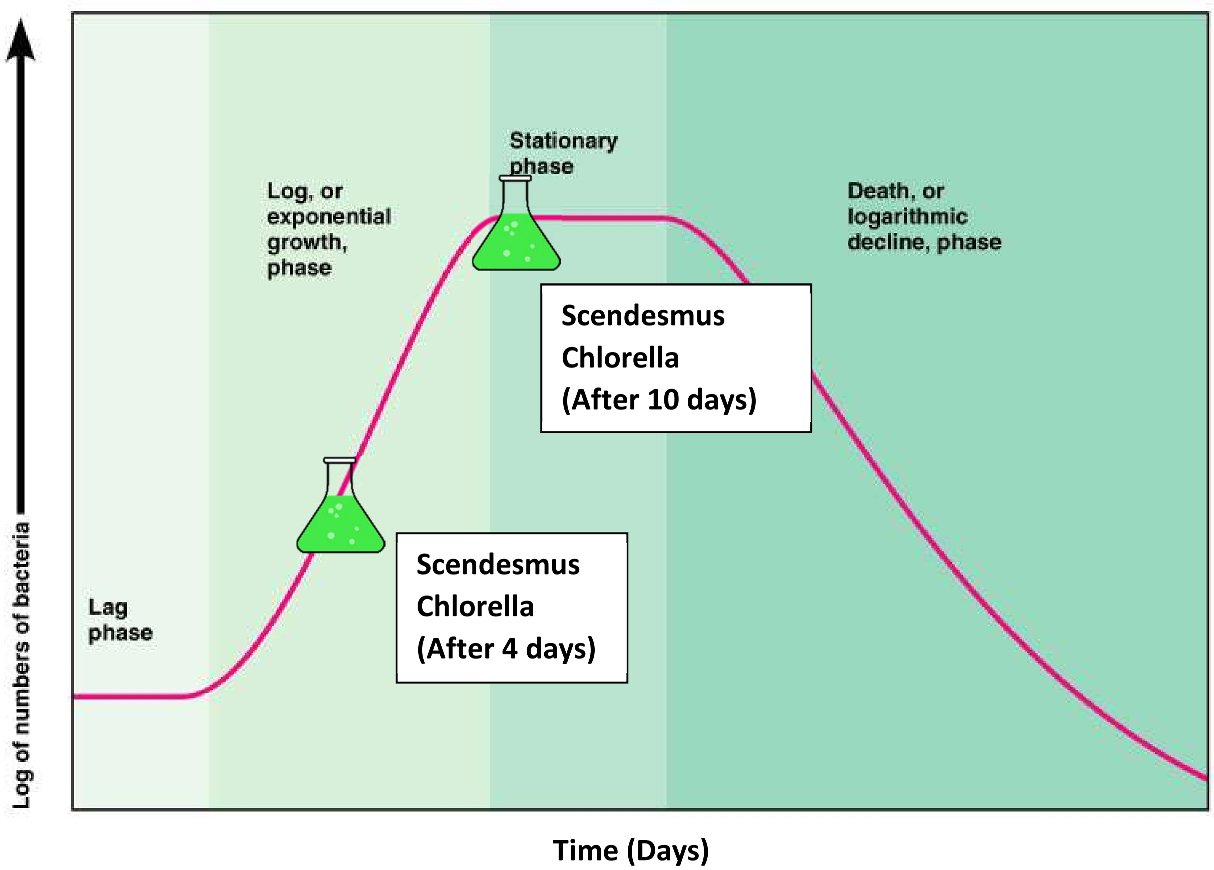

Figure 21. Sampling algae at a different stage for attached growth experiment

Initially, the algae have grown for more than 2 weeks during the stationary phases. During the exponential phases, I took 50 ml algae and centrifuge it, moving the algae pallet to a new medium. Fifty ml algae were added to 250 ml of medium.

We need six liters of algae for each run, so 1200 ml of currently well-growing algae from phase one was centrifuged and added to 6 liters of algae. After three days I moved the medium to a container, submerge the coupons inside the water, and start the reading.

During the stationary phases, I used the algae we had to that point, or we can again centrifuge 1200 ml and put it in a new medium, and after ten days, I moved the algae to the plate and submerge the coupons on the plate and read the data based on the following times.

Time of Sampling: (8 samplings): 12 hrs, 1, 2, 3, 4, 6, 8, and 10 days

Method: I prepared the material first. For each roughness, I used eight small pieces. Like below. The growth area for the sampling reading is 1 square inch. The rest of the area is for handling the coupons when took a sample (figure 22).

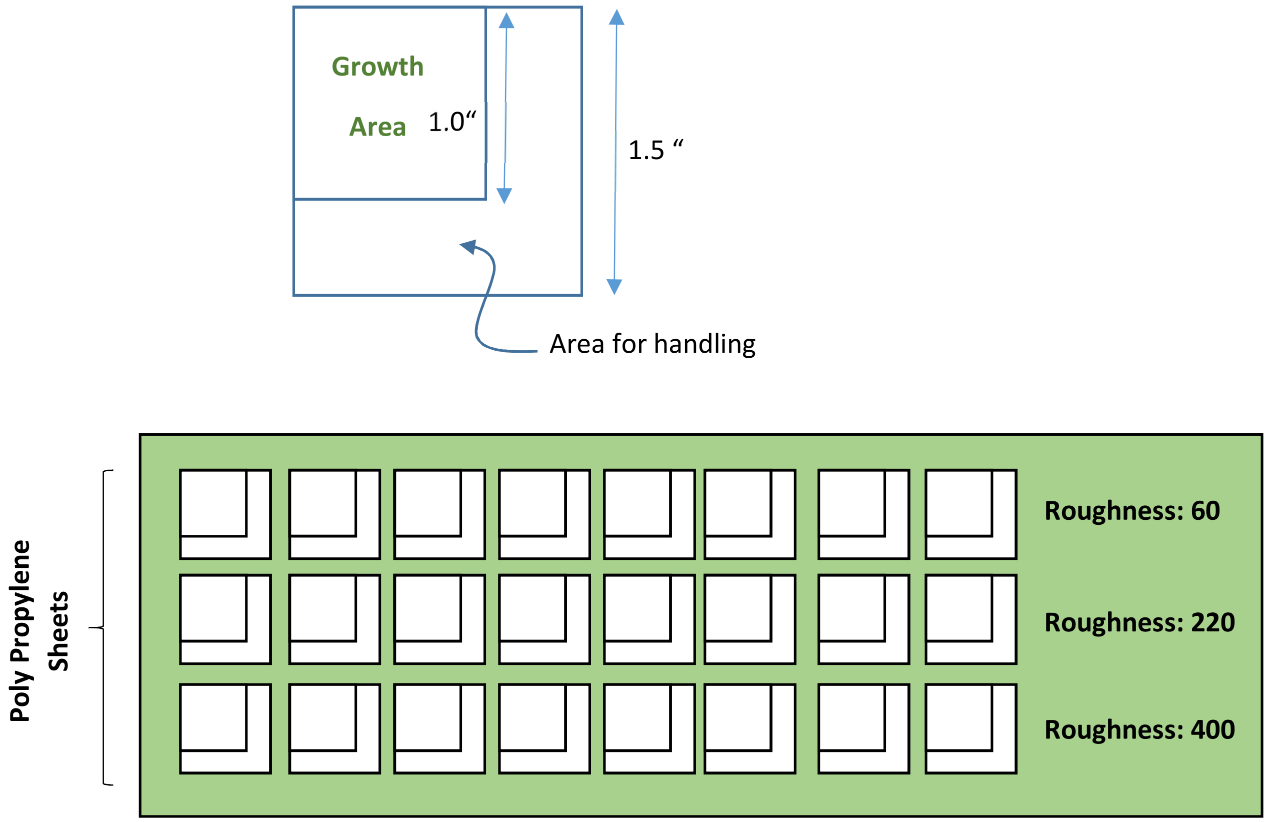

Figure 22. Plan view of the experiment

Four materials that have been used in this experiment are polypropylene (PP), acrylic (PMMA), polycarbonate (PC) and stainless steel (SS). These materials have been selected based on low cost and higher surface energy and hydrophobicity properties (Lee, Oh et al. 2014, Gross, Zhao et al. 2016). All these materials have been cut to 1.5 * 1.5-inch coupons, and then roughness has been added to the surface. For PC, PP, and PMMA coupons, I used roughness at levels of 60, 220 and 400 grit. For SS material I used just 220 grit, but because it was difficult to add the roughness to the steel material, I considered with 220 grit roughness and without it. In each coupon, the algae growth area for sample reading is 1 square inch. The rest of the area is for handling the coupons when sampling. The plan view of the experiment is presented in figure 23. Each experiment has been conducted in a replicated manner.

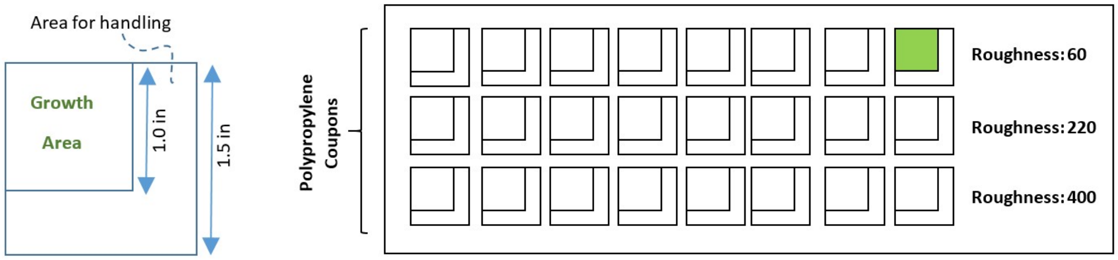

Figure 23. The coupons design for the experiment and plan view for experiment PP coupons

Four aquariums with the dimension of 1 foot * 2 foot have been used simultaneously for these experiments (Figure 24). One as an autotrophic and the other one as mixotrophic. The other two is considered as the replicate for the first set. In each of them, a submersible pump was used to make the circulation. This circulation helped the medium reach the coupons continuously. The flow rate on average was considered 29.4 ml/s. Considering the plug flow in the system, the velocity of the system was 0.289 cm/s. The details for calculation are provided in supporting information. The light density was 100 µmol.m^{-2}s^{-1} and for 12 hours from 8 am to 8 pm and then off for the dark cycle.

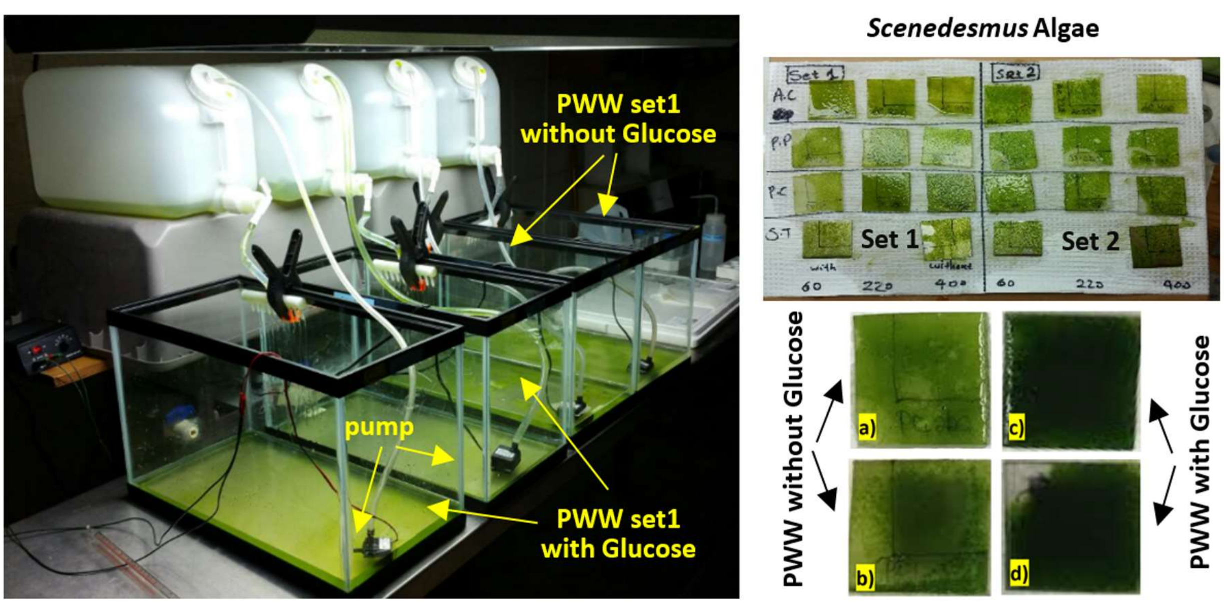

Figure 24. System design for plug flow condition

3.2.1.2. Medium and Algae Species and Growth

Autotrophic and mixotrophic cultivation have been performed in MB3N, SWW, and PWW mediums. For mixotrophic cultivation, I have added 1 g/l glucose. Algae species that have been used are *Chlorella minutissima* and *Scendesmus dimorphus*.

3.2.2. Sampling Methods (OD, Dry Mass, and Cell Count)

All experiments have been conducted in the lab with a room temperature of 20 °C. Optical density at a wavelength of 680 nm (OD_{680}) using UV–visible spectrophotometer (Thermo Scientific) has been used for daily microalgae growth. The setup for all experiments was considered with the initial OD_{680} of 0.1±0.01. The dry mass of initial conditions shows 65.5 mg/l and 75.3 mg/l concentration and the (2.44 g/m^2 and 3.01 g/m^2) for *Chlorella vulgaris* and *Scenedesmus dimorphus* respectively.

Samples have been taken on a daily basis. I took four additional samples in different time frames (1^{st}, 3^{rd}, 5^{th}, 7^{th} , 9^{th} and 10^{th} day) for further analysis such as algae, lipid and bacteria cell counting, using flow cytometer and also for dry mass and ash content analysis.

Sampling started after the first day of experiments by taking one coupon from each set (considering 2 replicates). The area of one square inch was washed with 50 ml distilled water. Then the OD of the 50 ml washed algae was measured. Then the remaining sample has been filtered using 1.22 μm filter. The weight of the filter before filtering was been recorded and then the filter containing the algae was put inside the oven at 110 °C for 3-4 days. Then the dry algae mass was recorded. The result is the algae mass per 1 square inch per time passed.

3.3. Results

3.3.1. Laboratory Experiment for Algae Cultivation and Wastewater Growth

Primary results for autotrophic cultivation of algae with wastewater samples are presented here. These samples are gathered from the Detroit wastewater treatment plant at different (primary and secondary clarifier) stages. Optical Density (OD) and Mass growth are measured for Growing algae in Detroit wastewater. Tables 16 and 17 show the results for these experiments. Each experiment was replicated. To see the effect of algae and bacteria together, first, wastewater filtered by 1.0 µm filter and then the algae collected from the wall of that clarifier is inoculated inside the samples. 1.0 µm filter can remove dirt, but the bacteria can pass through it. For the next experiment the filtered wastewater was autoclaved to kill all the bacteria and then a species of wastewater which is purified in auger was inoculated into the sample.

Figure 25 and 26 show that both experiments “algae and bacteria” and “algae alone” can utilize wastewater as a medium and grow happily. The main focus of our experiments was to grow certain species like *Chlorella* and *Scenedesmus* inside the wastewater algae. Attached growth and heterotrophic growth experiments have been started and the research results is presented in the main thesis manuscripts. The results indicate that both OD and algae mass is increasing in the wastewater. Specifically, algae grow better in the first five days, but the bacteria community overcomes the growth of algae usually after 6 days. To continue growing algae, we need to add new medium (wastewater) to the samples.

Table 16. Results of algae growing in wastewater collected after primary clarifier of Detroit WWTPs

Time of sampling	Tests	Algae and Bacteria		Algae	
		S1	S2	S3	S4
0	OD reading	**0.088**	**0.092**	**0.067**	**0.065**
	Filter Mass (gr)	0.0405	0.0417	0.0763	0.0766
	Filter and Algae (gr)	0.044	0.0433	0.0785	0.0781
	Mass (gr)	**0.0035**	**0.0016**	**0.0022**	**0.0015**
After 1 day	OD reading	**0.150**	**0.153**	**0.119**	**0.113**
	Filter Mass (gr)	0.0776	0.0778	0.0776	0.0779
	Filter and Algae (gr)	0.0819	0.0814	0.0806	0.0802
	Mass (gr)	**0.0043**	**0.0036**	**0.003**	**0.0023**
After 2 days	OD reading	**0.252**	**0.250**	**0.192**	**0.183**
	Filter Mass (gr)	0.0769	0.0773	0.0774	0.0772
	Filter and Algae (gr)	0.0835	0.0831	0.0823	0.0815
	Mass (gr)	**0.0066**	**0.0058**	**0.0049**	**0.0043**
After 3 days	OD reading	**0.423**	**0.399**	**0.354**	**0.313**
	Filter Mass (gr)	0.0759	0.0761	0.0751	0.0758
	Filter and Algae (gr)	0.0876	0.0865	0.0865	0.0836
	Mass (gr)	**0.0117**	**0.0104**	**0.0114**	**0.0078**
After 4 days	OD reading	**0.600**	**0.565**	**0.568**	**0.502**
	Filter Mass (gr)	0.0757	0.0764	0.0758	0.0755
	Filter and Algae (gr)	0.0928	0.0909	0.0924	0.0893
	Mass (gr)	**0.0171**	**0.0145**	**0.0166**	**0.0138**
After 5 days	OD reading	**0.660**	**0.670**	**0.648**	**0.593**
	Filter Mass (gr)	0.0743	0.0749	0.0752	0.0751
	Filter and Algae (gr)	0.0957	0.0961	0.0979	0.0945
	Mass (gr)	**0.0214**	**0.0212**	**0.0227**	**0.0194**
After 6 days	OD reading	**0.801**	**0.752**	**0.731**	**0.751**
	Filter Mass (gr)	0.0747	0.0751	0.0757	0.0761
	Filter and Algae (gr)	0.0979	0.0985	0.1014	0.1022
	Mass (gr)	**0.0232**	**0.0234**	**0.0257**	**0.0261**
After 7 days	OD reading	**2.110**	**2.071**	**2.110**	**2.050**
	Filter Mass (gr)	0.076	0.0759	0.0761	0.0764
	Filter and Algae (gr)	0.1369	0.1383	0.1306	0.146
	Mass (gr)	**0.0609**	**0.0624**	**0.0545**	**0.0696**

Table 17. Results of algae growing in wastewater collected after secondary clarifier of Detroit WWTPs

Time of sampling	Tests	Algae and Bacteria		Algae	
		S5	S6	S7	S8
0	OD reading	**0.080**	**0.092**	**0.083**	**0.095**
	Filter Mass (gr)	0.0772	0.0797	0.079	0.078
	Filter and Algae (gr)	0.0812	0.0816	0.0802	0.0811
	Mass (gr)	**0.004**	**0.0019**	**0.0012**	**0.0031**
After 1 day	OD reading	**0.140**	**0.135**	**0.140**	**0.156**
	Filter Mass (gr)	0.0776	0.0769	0.0765	0.0767
	Filter and Algae (gr)	0.0844	0.0836	0.0838	0.0848
	Mass (gr)	**0.0068**	**0.0067**	**0.0073**	**0.0081**
After 2 days	OD reading	**0.198**	**0.155**	**0.166**	**0.191**
	Filter Mass (gr)	0.0756	0.0753	0.0752	0.0757
	Filter and Algae (gr)	0.0849	0.0829	0.0849	0.087
	Mass (gr)	**0.0093**	**0.0076**	**0.0097**	**0.0113**
After 3 days	OD reading	**0.224**	**0.223**	**0.238**	**0.253**
	Filter Mass (gr)	0.0757	0.0751	0.0752	0.075
	Filter and Algae (gr)	0.0859	0.0844	0.0851	0.0877
	Mass (gr)	**0.0102**	**0.0093**	**0.0099**	**0.0127**
After 4 days	OD reading	**0.298**	**0.292**	**0.280**	**0.345**
	Filter Mass (gr)	0.0747	0.0748	0.0743	0.0743
	Filter and Algae (gr)	0.0887	0.089	0.0864	0.092
	Mass (gr)	**0.014**	**0.0142**	**0.0121**	**0.0177**
After 5 days	OD reading	**0.300**	**0.308**	**0.323**	**0.370**
	Filter Mass (gr)	0.0753	0.0756	0.0752	0.075
	Filter and Algae (gr)	0.0892	0.0896	0.088	0.0898
	Mass (gr)	**0.0139**	**0.014**	**0.0128**	**0.0148**
After 6 days	OD reading	**0.370**	**0.395**	**0.450**	**0.470**
	Filter Mass (gr)	0.0758	0.076	0.0765	0.0767
	Filter and Algae (gr)	0.0928	0.0927	0.0933	0.0966
	Mass (gr)	**0.017**	**0.0167**	**0.0168**	**0.0199**
After 7 days	OD reading	**1.720**	**1.680**	**1.725**	**1.786**
	Filter Mass (gr)	0.0772	0.0766	0.0768	0.0772
	Filter and Algae (gr)	0.1204	0.1201	0.1232	0.1394
	Mass (gr)	**0.0432**	**0.0435**	**0.0464**	**0.0622**

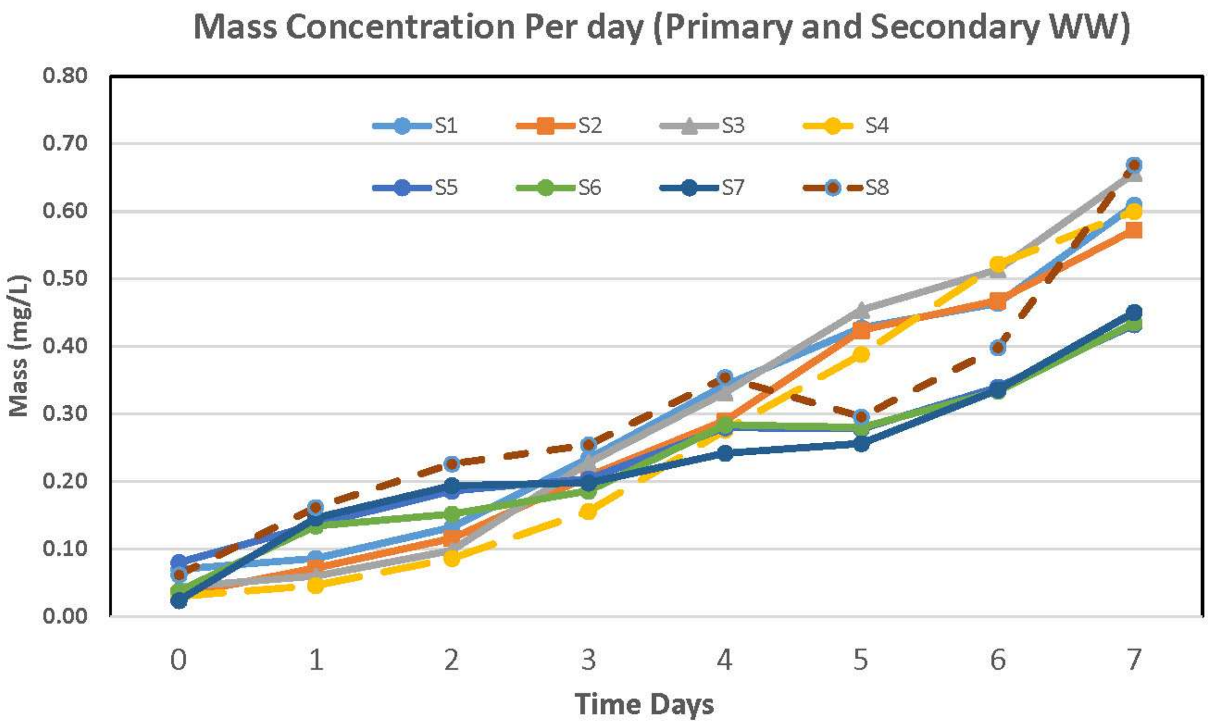

Figure 25. Results of algae and Bacteria mass growth in wastewater samples

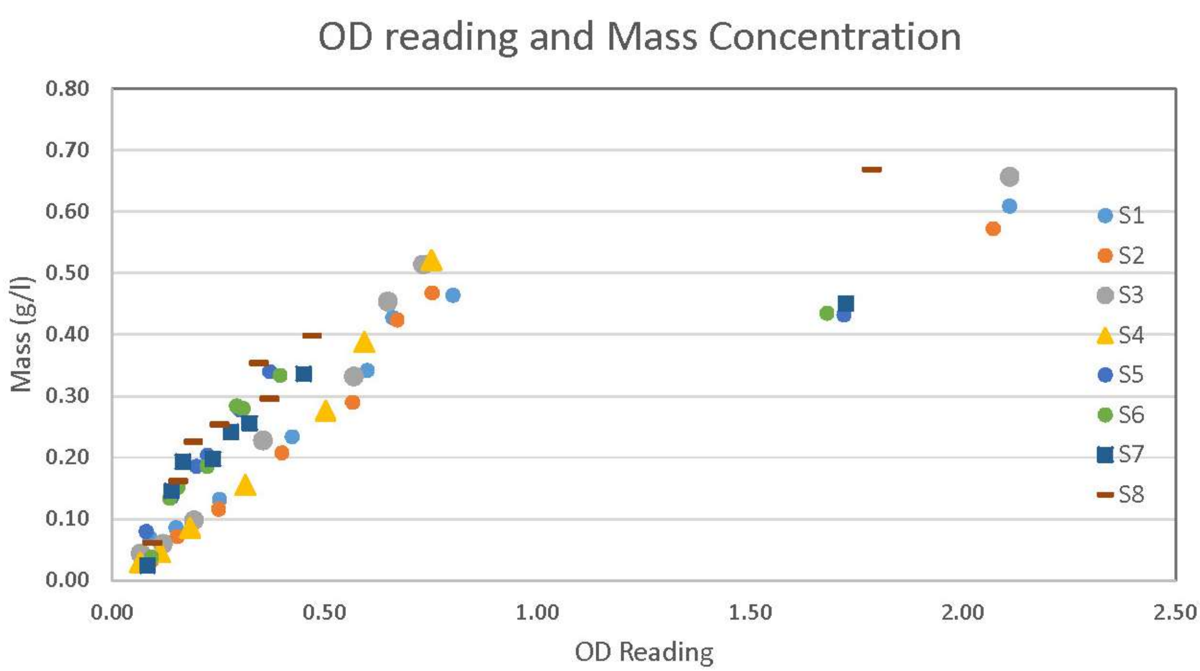

Figure 26. Results of Mass and OD for algae and Bacteria in wastewater samples

S1 and S2 are algae and bacteria samples after primary clarifier, S3 and S4 is just algae samples. The same for secondary clarifier, S5 and S6 are algae, and bacteria samples and S7 and S8 are algae samples (Table 16 and 17).

3.3.2. Mass Results

Mass Analysis was done based on filtering the solutions with a 1.22 µm filter (GLASS FB PPR GFC 4.25CM). For each set of experiments, three filters were put inside the oven and furnace as the controls. Oven temperature was set at 105-110 °C, and after 4 days the dry algae mass was recorded. Then the samples were put in the furnace at 288 °C for 2 hours. Mass of controls and samples have been measured.

3.3.3. ANOVA statistical Analysis

Two-way analysis of variance (ANOVA) was used to compare the effects of parameter that were testing such as material (4 types PP, PMMA, PC, and SS), roughness (grit 60, 220, 400), medium (MB3N, PWW, and SWW), algae type (Chlorella and Scendesmus), and finally growth condition (Autotrophic and Mixotrophic). Parameters that I measured are OD, Dry Mass, Algae Cell Count, Ash Content, Lipid Count, Bacteria Cell Count, and Hydrophobicity. SPSS software Version 23 has been used in this analysis. I focused on algae mass as dependent variables and the roughness, growth condition, and algae type as independent variables. For 650 data reading the statistical analysis shows growth condition ($F=304.37$ and $P<0.001$) and algae type ($F=240.80$ and $P<0.001$) have a statistically significant effect on the results of algae growth per day. On the Other hand, roughness has no statistically significant effect on the growth rate ($F=1.689$ and $P=0.186$ which is >0.05). More detail is provided in the supporting information. Figure 27 shows the boxplot of results for growing different algae types, materials, and growth modes in each day (D).

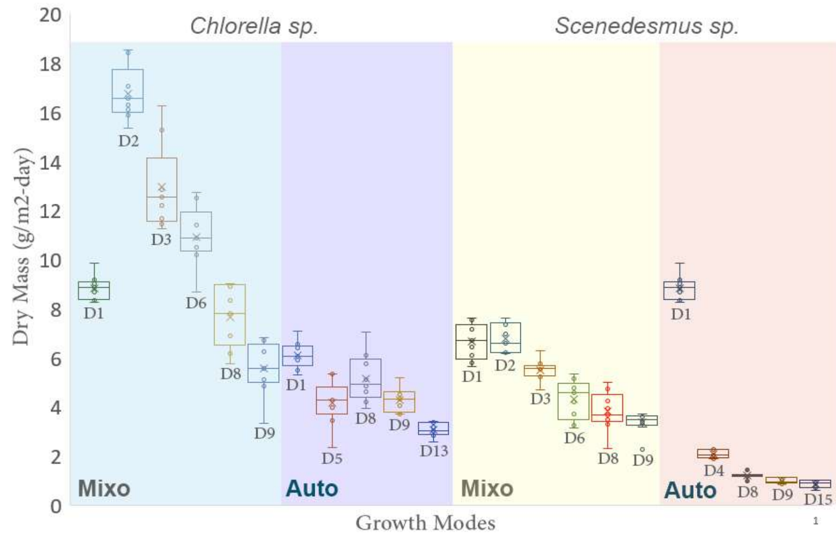

Figure 27. Boxplot for algae growth in mixo/autotrophic growth with three roughness, error bars are standard deviations

The same analysis for comparing just mixotrophic and autotrophic growth shows that there is a statistically significant difference between the autotrophic and mixotrophic growth and also types of algae species. $F_{0.05;3,176} = 2.6049$ but the analysis shows F=111.69 which again indicated that the there is a statistically significant difference among the samples means. More detail is provided in the supporting document.

3.3.4. Optical Density, Mass and Cell Count

The results are presented in terms of OD, Mass (accumulated and per day mass growth), and cell count per time passed from the beginning of the experiment. The results indicated mixotrophic attached growth experiments have a higher algae yield in almost all setups when I used MB3N. This clearly indicates that using mixotrophic attached growth experiments have a great potential for algae cultivation. Figure 28 shows the accumulative growth results of MB3N results for all coupons in autotrophic and mixotrophic growth condition. This figure also shows the average daily growth for each coupon in mixotrophic and autotrophic growth condition.

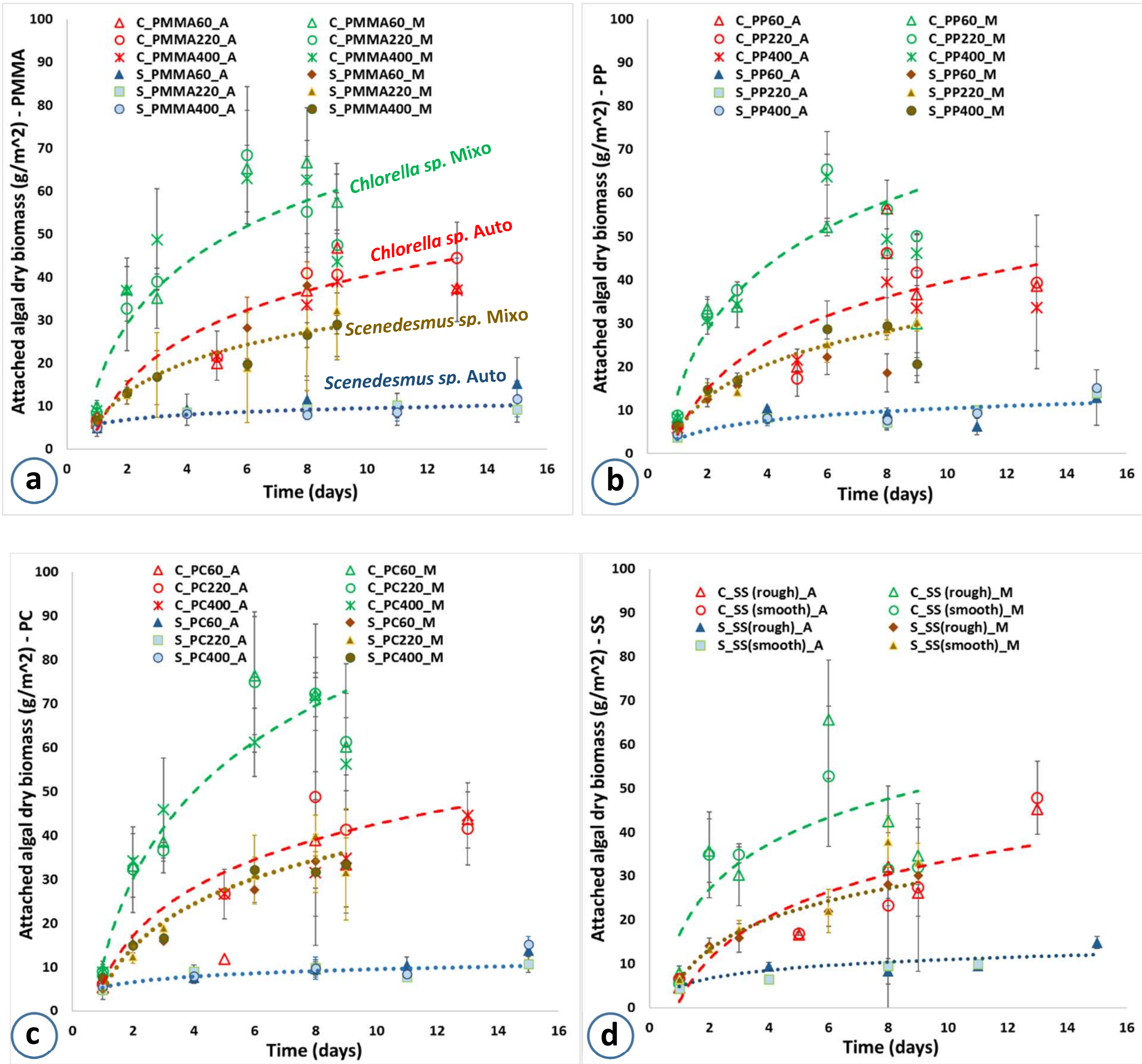

Figure 28. The results of attached growth in different coupons in MB3N medium,

Parts a to d are PMMA, PP, SS, and PC coupons receptively trend for each set of coupons is for roughness 220, I considered SS with roughness (rough) and without roughness (smooth). The line colors in all graphs are the same of graph a. Error bars are standard deviations

Figure 29 shows the result of maximum growth after some days for each cultivation mode. Autotrophic cultivations reach to the maximum growth around 8-9 days and mixotrophic growth reach to the maximum growth around 4-5 days after starting the experiment.

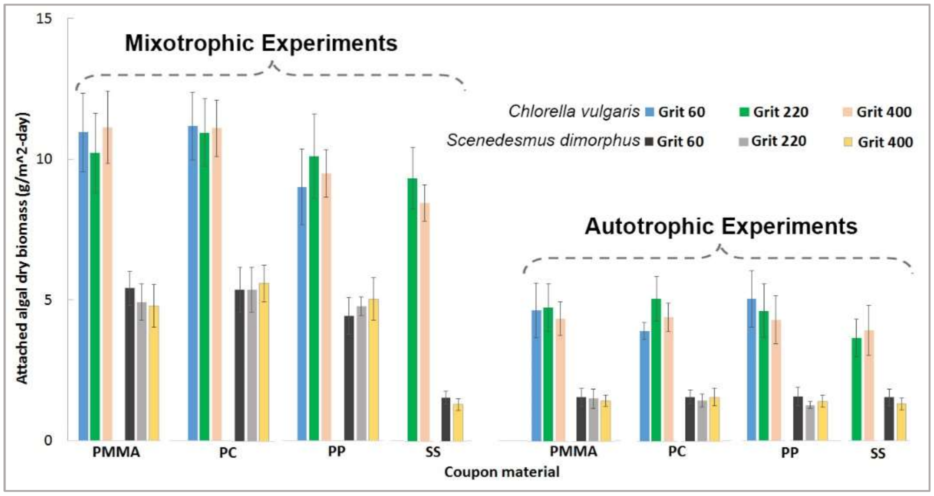

Figure 29. Attached daily Growth Rate of Algae cultivation for MB3N experiment, error bars are standard deviations

Based on the results, PC materials have the best growth rate among other four materials followed by PMMA coupons. In most of the experiment, coupons with grit 220 show slightly better growth results. The results show that in MB3N experiment setup the *Chlorella* algae has a higher potential growth (reach 10 $g/m^2/day$) than *Scenedesmus* algae (reach to 10 $g/m^2/day$). These provide a great opportunity for considering mixotrophic growth as an option to have a higher yield for algae cultivation, although the challenges of using the low-cost resource of carbons are still prevalent. I have used glucose in this experiment which is an expensive resource. Future research needs to focus on the area of using cheap resources of organic carbon such as wastewater.

For SWW and PWW experiment was conducted in the same conditions, but we selected the representative coupons with the higher growth rate. Coupons for PMMA material with the roughness of 220 grit is one of the coupons with a higher growth rate of algae. Therefore, I have done some more experiment with this material with wastewater medium which was taken after secondary and primary clarifier in the Detroit wastewater treatment plant. *Scenedesmus* and *chlorella* algae with the initial OD_{680} of 0.1 ± 0.01 concentration were grown in these mediums. OD, dry mass and the number of algae cell, lipid incident, and bacteria cell were recorded. I used wastewater with and without glucose. The experiment with glucose as 1 g/l concentration of glucose. The results at this experiment clearly showed that wastewater without glucose has a higher growth and attachment rate that wastewater with glucose, in contrast with the MB3N experiment. Wastewater contains organic carbon and it has already served as a medium for mixotrophic growth as well. Adding glucose to it causes the bacteria to grow much faster than the algae. This can be clearly observed since our samples became cloudier because of bacterial growth. Also, the attachment is firmer on the surface of the wastewater sample without glucose. *Chlorella* and *Scenedesmus* experiment I have seen that a higher growth rate in the first four days in the wastewater with glucose. This can be seen in figure 30 for both algae.

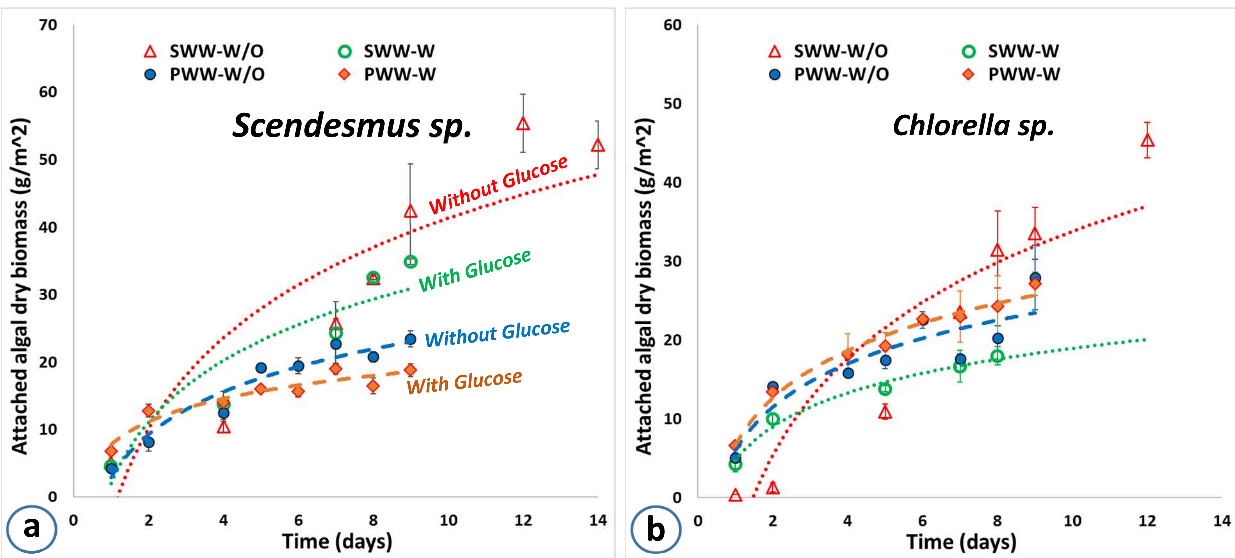

Figure 30. The results for Attached growth dry biomass a. Chlorella algae b. Scenedesmus, in PWW and SWW (without glucose are W/O and with glucose are W) error bars are standard deviations

The same analysis for the days that I can see maximum growth have been conducted in this experiment. The results are presented in Figure 31 which show in both PWW and SWW the maximum growth for autotrophic (indicated in the figure with A) is happening around 7-9 days after starting the experiment. On the other hand, for the mixotrophic experiment, the maximum growth is happening after 3-4 days of the experiment. To compare the results with MB3N medium, I have conducted a similar experiment with coupons 220 in both MB3N. The results are added to Figure 31 to compare the growth in all mediums. As it can be seen in MB3N medium, the mixotrophic attached growth is showing more than double growth than autotrophic. However, the PWW and SWW the mixotrophic growth are less or equal to autotrophic growth. This still showing some promising results because the maximum growth is happening after 3-4 days which is half of the autotrophic growth which is happening after 7-9 days.

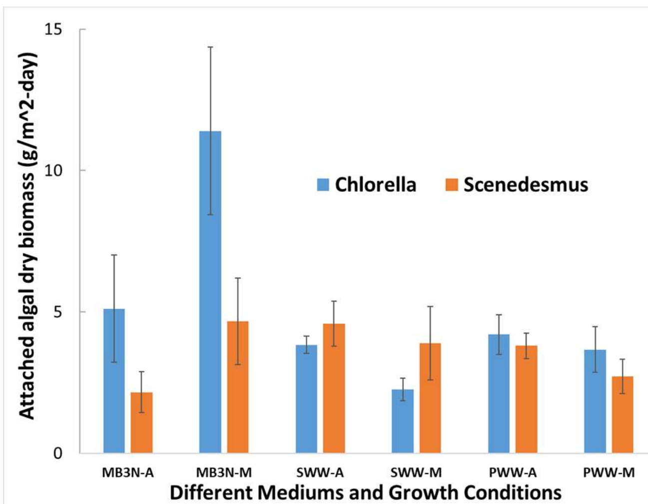

Figure 31. Average daily growth in the PMMA 220 grit coupons in three mediums with two growth conditions

3.3.5. Lipid Productivity

Because lipid is the main source of biofuel in algae cultivation, I conducted counting it in the experiment. The Lipid productivity results for MB3N and PW experiments has been measured by taking extra samples (two replicates) for further analysis in the Flow Cytometer. Algal lipid productivity was measured using BD Accuri C6 Plus Flow Cytometer. The results of this analysis in figure 32, indicate that lipid productivity in mixotrophic growth has higher lipid incidents (from 2 to 13 times higher than mixotrophic).

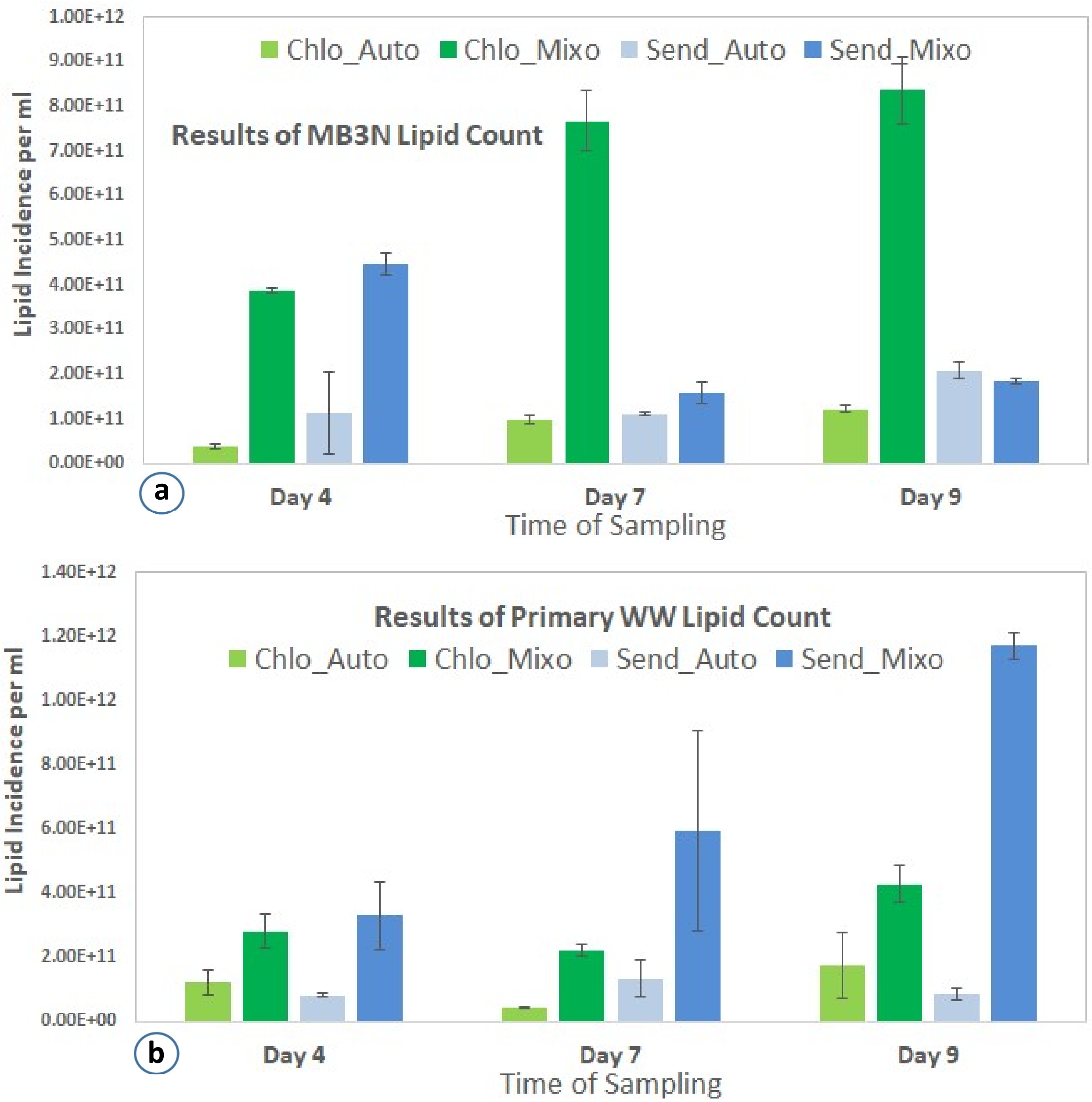

Figure 32. Lipid Productivity of Chlorella and Scenedesmus in PW

3.3.6. Hydrophobicity Analysis

Material surface physicochemical properties play an important role for the initial colonization of algae cells (Finlay, Callow et al. 2002). Hydrophobic interaction is one of the mechanisms that affects algae-material attachment (Gross, Zhao et al. 2016). In this research, I have calculated the hydrophobicity effect of different materials that I used. KSV contact angle measurement system has used for calculating the surface angle. The original surface angels of the four materials (PP, PMMA, PC, and SS) have been calculated and presented in the following figure 33 and table 18.

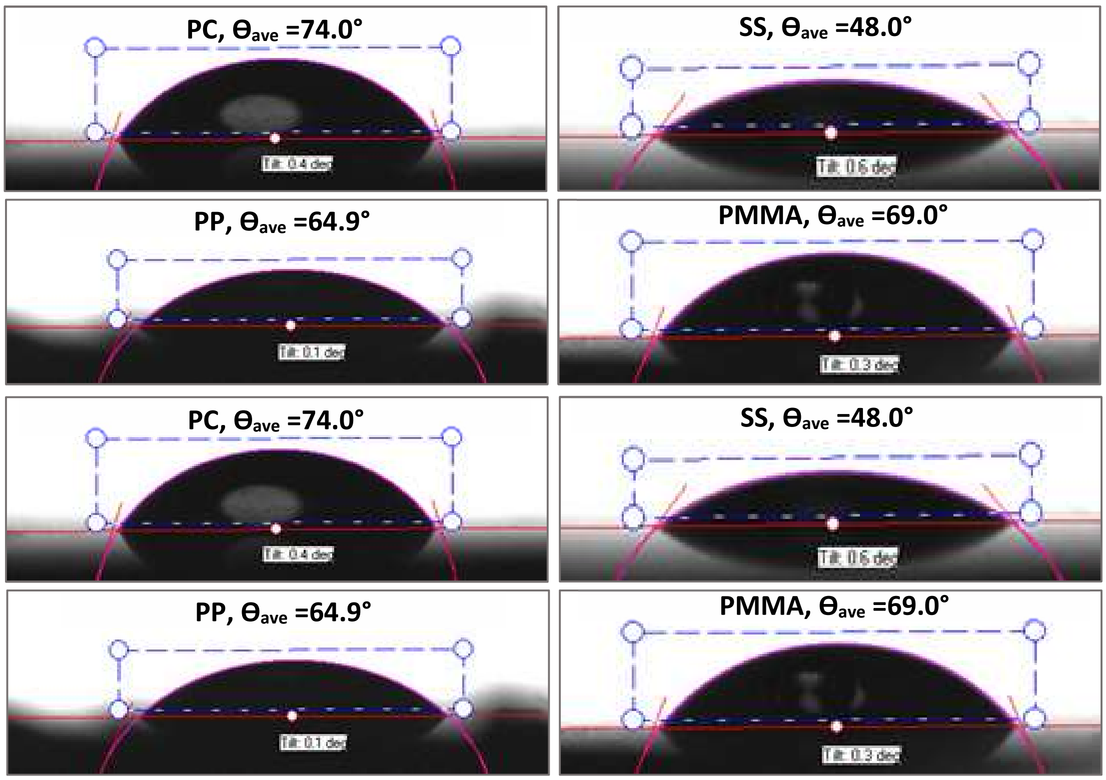

Figure 33. The hydrophobicity test results for the experiment material

The results for other materials is presented in the following table.

Table 18. Results of angle measurement for different materials

Material	Water, OD=0.00		
	Θ left	Θ left	**Θ ave**
SS	49.2	46.7	**48.0**
PMMA	69.4	68.6	**69.0**
PP	65.0	64.7	**64.9**
PC	73.2	74.9	**74.0**

The results of this analysis clearly indicated that there is a relationship between hydrophobicity and attached growth. PC and PMMA which are materials with a higher level of hydrophobicity have the higher growth level as well.

3.3.7. Ash Content Analysis

Results for ash content at different stages of growth is presented in the following table. For mixotrophic the samples from 4th day of growth and for autotrophic the results from 8th day of growth is presented in the table 19. Two Replicates were taken in each set.

Table 19. Ash Growth results for

Medium	Algae	Ash content % (STD)	
		Growth Mode Mixotrophic - 4th Day	Growth Mode Autotrophic - 8th Day
MB3N	Chlorella	9.1% (0.5%)	16.8% (1.2%)
	Scendesmus	8.5% (0.5%)	15.3% (1.1%)
PWW	Chlorella	2.9% (0.6%)	13.5% (1.9%)
	Scendesmus	6.7% (3.2%)	16.7% (2.4%)

Based on these results, the ash content of mixotrophic samples are lower than autotrophic samples. However, both of the methods for cultivations have an acceptable range ash contents.

3.4. Conclusion

This article compared the mixotrophic attached growth cultivation of algae with autotrophic attached growth cultivation. MB3N and Detroit Primary and Secondary clarifier wastewater (PWW and SWW) mediums have been used, and the experiment was performed 12 hours' light and 12 hours' dark cycle condition. Glucose with the concentration of 1 g/l have been used as organic carbon source in the mixotrophic experiment. The results indicate that mixotrophic attached cultivation has higher algae yield in MB3N medium in comparison to autotrophic cultivation (algae dry mass of 11 g/m^2/d). However, adding glucose to PWW or SWW has prohibited the attachment of algae to the surface, and the wastewater without glucose shows slightly higher growth (algae dry mass of 5 $g/m^2/d$). Although the growth results in wastewater mediums are not higher than autotrophic growth, the shorter period (3-4 days) for reaching to the maximum in comparison with autotrophic (7-9 days) is a favorable factor because shorter hydraulic retention time can reduce the are needed. The results show that PC and PMMA coupons with the roughness of 220 grit, which is in the middle of our roughness setup, have a better range of cultivation. This article shows that attached growth in mixotrophic can be used as a promising way of cultivation in algal farms. However, further research is needed for designing the system that effectively harvests the biofilm algae which is grown on the surface of materials.

CHAPTER 4 LIFE CYCLE OPTIMIZATION AND MACHINE LEARNING FOR SUSTAINABILITY

4.1. Introduction

In this section, I have evaluated the possibility of using CO_2 emission from power plants in algal pond cultivation. In addition, a multi-objective life cycle optimization (LCO) and machine learning were developed for life cycle assessment of algae-to-energy systems.

4.2. Life Cycle Optimization (LCO)

Optimization has been used for years in many fields such as chemical process, operations, health and many other fields (Olya, Shirazi et al. 2013, Moradi-Aliabadi and Huang 2016). Studies regarding optimization on algal open ponds are rare and almost new. Many parameters affect the optimization decisions. Some of the earliest studies by Ritchie and Larkum adjusted pond depth to optimize water temperatures (Ritchie and Larkum 2012). Michels et al. studied the effect of changing the algal concentration on the optimization of light availability and the minimization of biomass respiration losses (Michels, Slegers et al. 2014). The most recent studies about operation parameters have been optimized in response to seasonal meteorological fluctuations to maximize algal productivity and minimize water demand (Béchet, Shilton et al. 2016). Yue et al. (2016) introduced, for the first time, a framework for a hybrid model that integrated LCA and Multiobjective Optimization (MOO). MOO deals with multiple criteria decision making, that is a concern with mathematical optimization problems, involving more than one objective function to be optimized simultaneously. LCA is almost based on decision making, and the optimization problem will inevitably be a multiobjective problem (Azapagic and Clift 1999). For this reason, a life cycle optimization (LCO) model needs not only optimized environmental impacts (like global warming) but also the ability to compare different alternatives, and identify both ecologically and economically better decisions (Yue, Pandya et al. 2016). Process-based, input-output (IO), and hybrid LCA are the three conventional methods in LCA that can be used for an LCO (Lenzen and Crawford 2009, Yue, Pandya et al. 2016).

In this research, based on our research objectives, I used MATLAB programing language or other optimization tools to develop an optimization model. By doing LCO, I minimized the overall environmental impacts of the proposed algal wastewater system. The comprehensive SEHR-LCA provided the necessary data for LCO models such as distance for providing CO_2 resources or pumping wastewater.

The primary purpose of this section is to evaluate the optimization methods to minimize or maximize objectives while satisfying constraints. The framework for doing life cycle optimization (LCO) is based on minimization of environmental impacts (like greenhouse gas) for the whole system. I used optimization toolbox option available on software like Matlab or write code for an optimization algorithm. As mentioned in the literature review, because of the nature of the decision-making process in the LCA context, the optimization problem will inevitably be a multi-objective one. There are many different programming techniques for solving multi-objective optimization. Selecting a particular method will depend on the problem that we have. Based on the results of Azapagic and Clift, multi-objective linear programming (LP) can be used effectively for this purpose. A general form for optimization problem in LP model has the following form (Azapagic and Clift 1999):

$$Z = \sum_{i=1}^{I} z_i x_i \qquad \text{Eq. (5)}$$

subjected to:

$$\sum_{i=1}^{I} \mathrm{a}_{\mathrm{j,i}} \mathrm{x}_{\mathrm{i}} \le \mathrm{e}_{\mathrm{j}} \quad j = 1, 2, ..., J \qquad \text{Eq. (6)}$$

and

$$x_i \geq 0 \quad i = 1, 2, \ldots, I \qquad \text{Eq. (7)}$$

Where Eq. (5) represents an objective function and Eq. (6) and (7) are the linear constraints in the system. In the context of LCA, the general LP model has the same format. However, in LCA studies constraints are present from the cradle to the grave. In addition, the outputs are also treated as activities, and objective functions include the environmental burdens. So, I can present that by: to minimize

$$B_m = \sum_{i=1}^{I} bc_{m,i} x_i \qquad \text{Eq. (8)}$$

Where bc_{mi} is burden m from process or activity x_i. Now, the objective function can also be defined as the environmental impacts: to minimize

$$E_k = \sum_{m=1}^{m} ec_{k,m} B_m \qquad \text{Eq. (9)}$$

Where the $ec_{k,m}$ represents the relative contribution of burden B_m to impact E_k.

The procedure for this section of research will develop more in the future, and we may find and use better tools and methods that model and minimize more environmental impacts. There are some suggestions for LCO framework that we may use, or we may develop our framework for optimization.

Figure 34 shows the coal and natural gas power plants in the US. Our goal is to use this source of emission for algae cultivation.

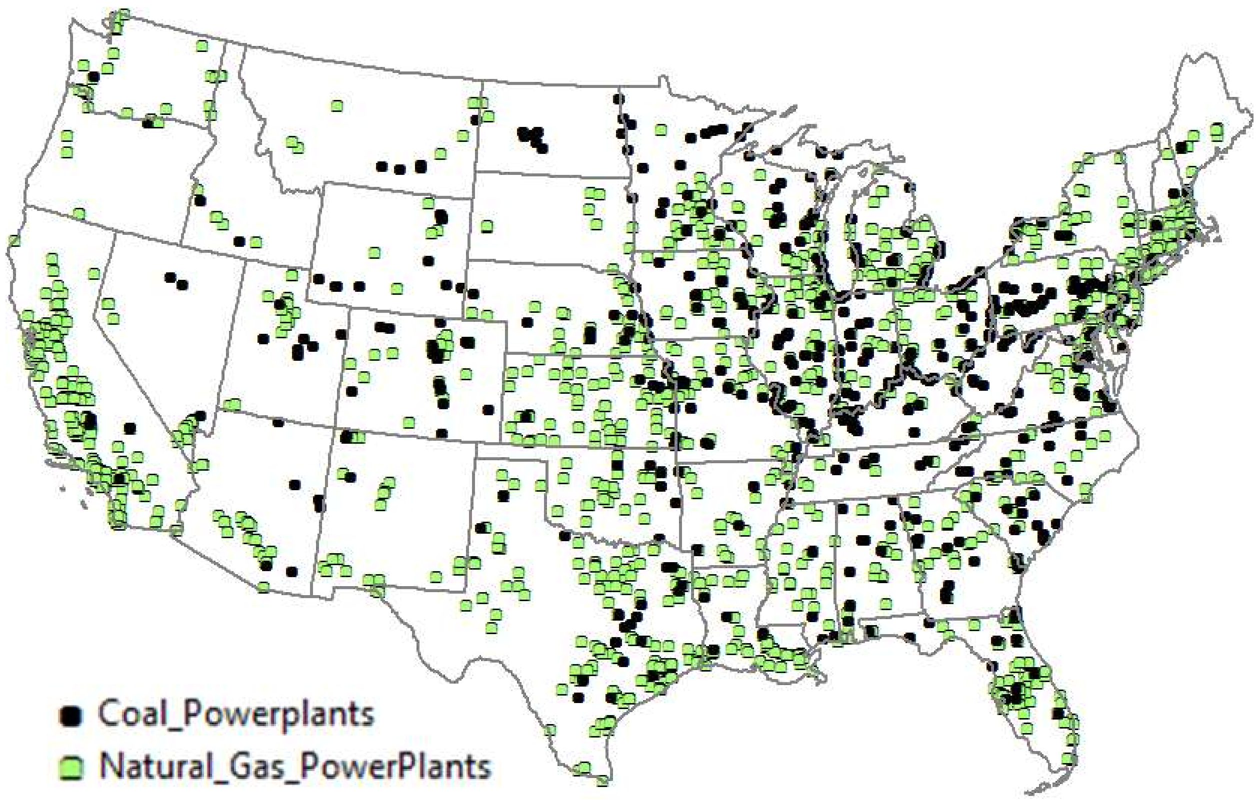

Figure 34. Powerplants as a source of CO2 in the US for algae cultivation

If I add the WWTPs location to this map, we can see a better picture of the accessibility of these emission resources to the WWTPs. For example, in the Michigan state, we will have the following map.

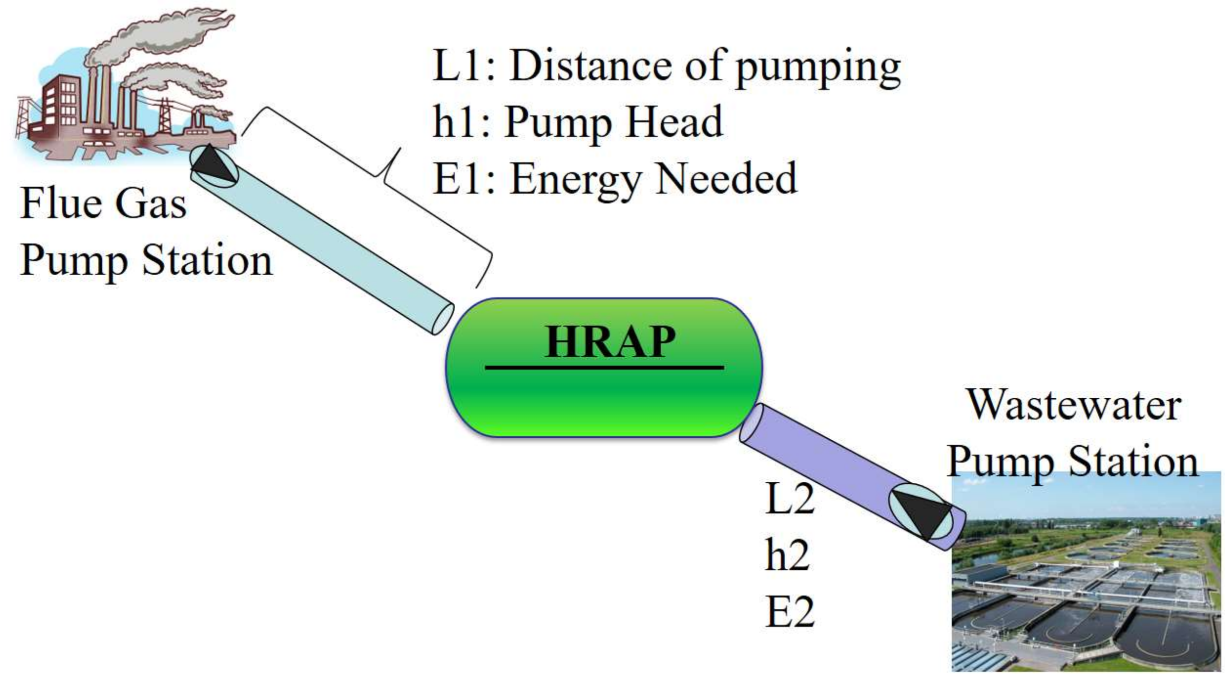

Figure 35. The possibility of using CO2 gas and wastewater

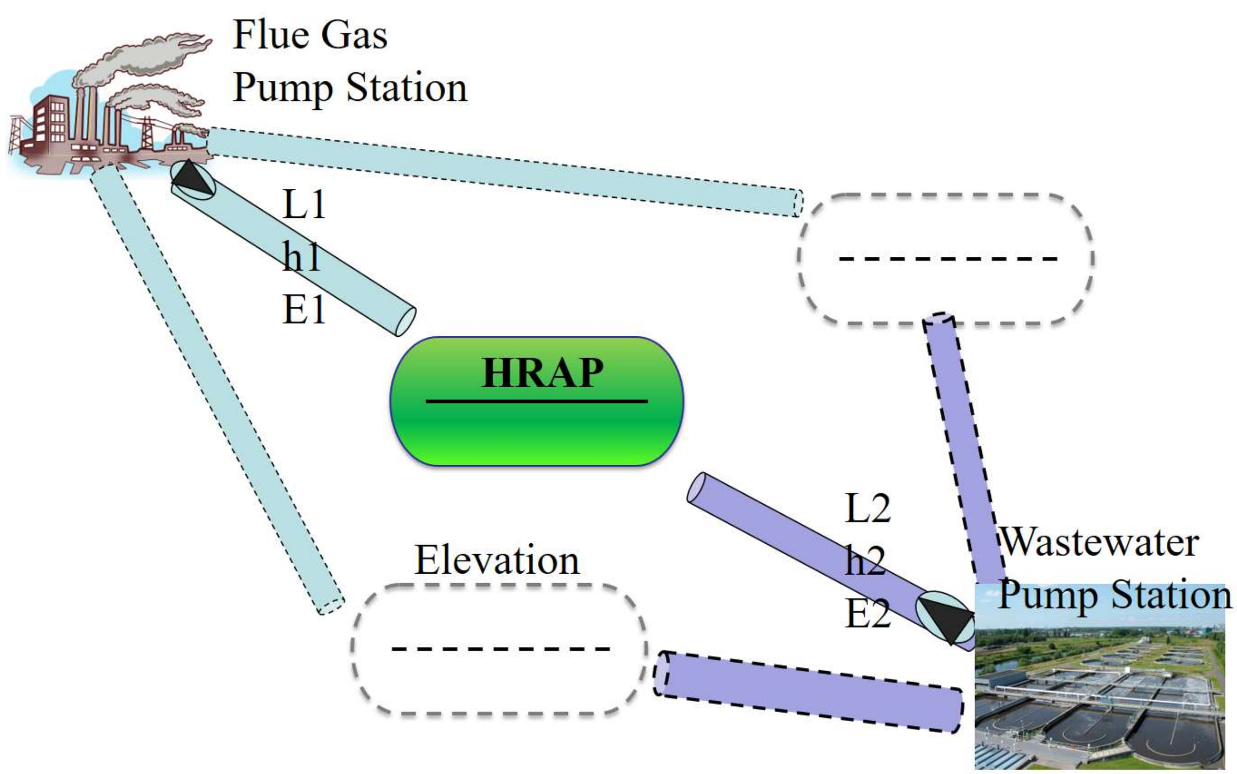

Figure 36. Evaluation of different position for HRAP to reduce the environmental burdens

Selecting the land in this problem are based on the spatial analysis. We have worked on four different locations around the US as the case studies.

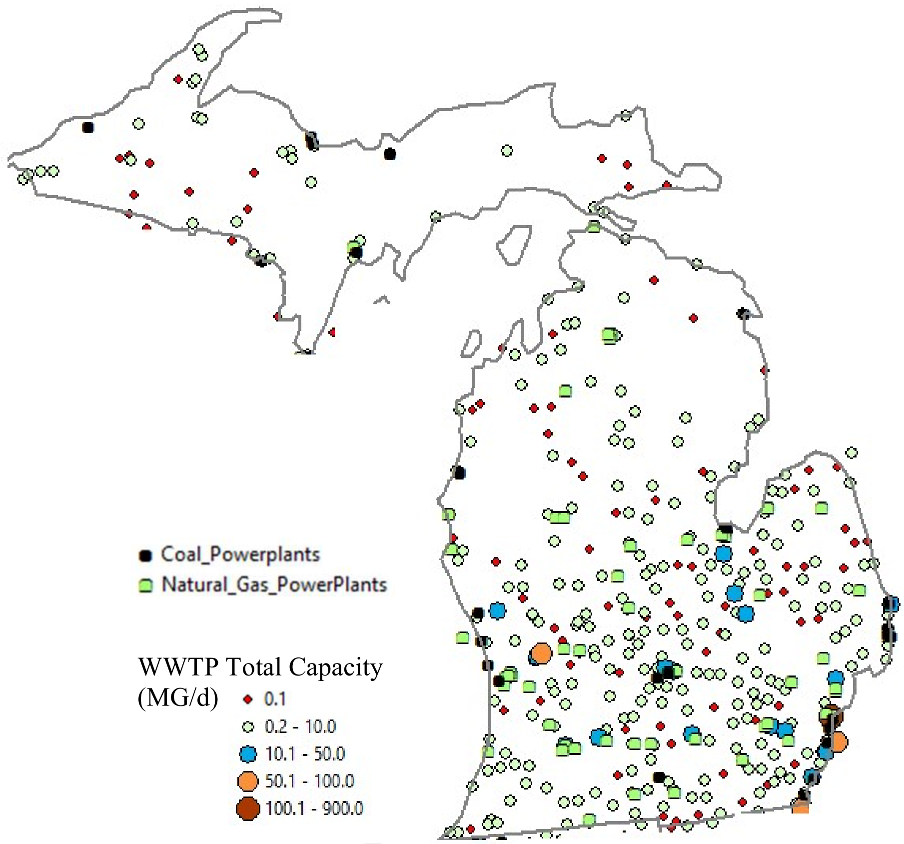

Figure 37. WWTPs and Powerplants spatial location in the state of Michigan

Our studies in the first chapter have evaluated the point by point location of using wastewater as a source for algae cultivation. In this section, I have integrated CO_2 emission resources for optimizing the cultivation and reducing environmental emissions.

In a hypothetical distributed network of pipes that transfer CO_2 from coal power plant to the nearby WWTPs and algal pond, there is a possibility to optimize the environmental effect of the project. This could have been done by an optimization model. I have considered the following hypothetical situation.

Optimization function 1: Sum of the amount of energy that is needed to pump wastewater and CO2 gas to a location. This function depends on static and dynamic head that is needed to be provided by pumps.

Optimization function 2: Sum of the amount of water that is needed for HDPE pipe and Steel pipe production

Optimization function 3: Sum of the amount of eutrophication effect that is due to HDPE and Steel pipe production and the pumping.

The hypothetical model of this optimization is presented in Figure 38. The pipe for pumping CO_2 is going to bring the gas closer to the wastewater treatment plants.

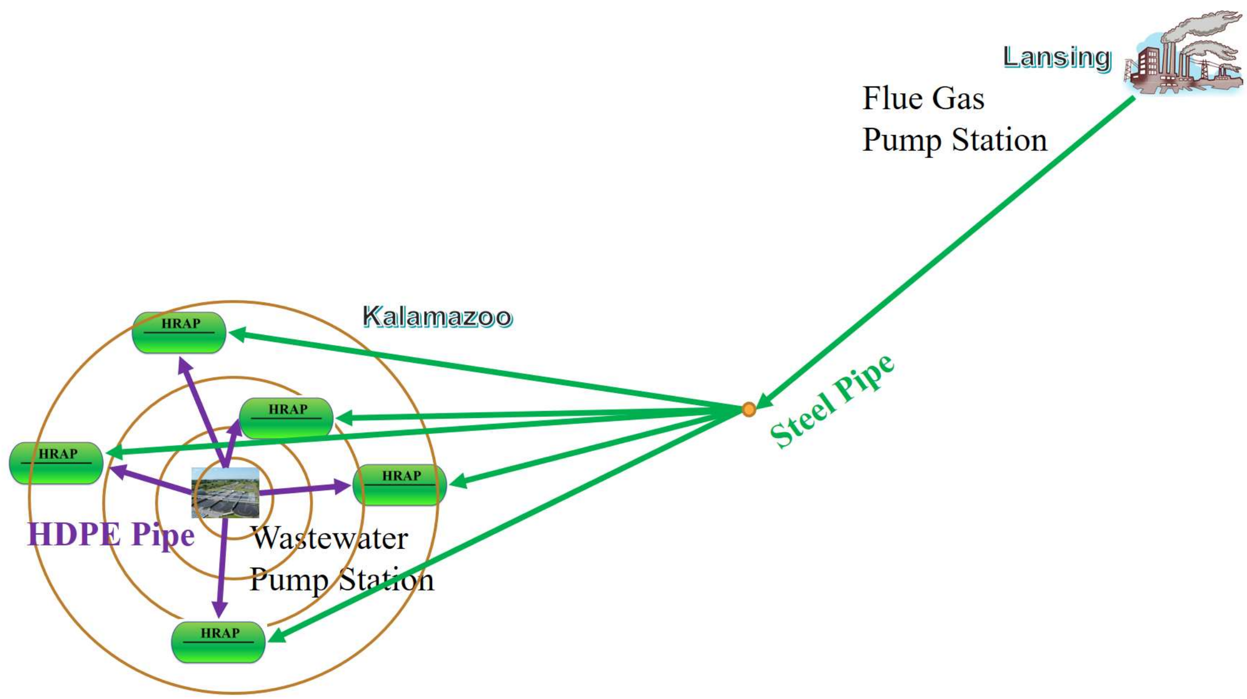

Figure 38. Hypothetical model of using CO2 gas in HRAP treatment system

The independent variables in this model are:

L1: Length of the steel pipe in pumping the gas

L2: Length of the HDPE pipe in pumping the wastewater

H1: Total dynamic and static head of pumping CO_2 gas to the location of HRAP

H2: Total dynamic and static head of pumping wastewater to the location of HRAP

I have used MATLAB and Multiobjective Optimization Using the Genetic Algorithm to solve this optimization problem. The results indicate that the optimized condition is happening when the WWTP is close to HRAP. For example, in the radius of 5 km from WWTP, we can have an optimize situation for optimization function.

However, in terms of realistic applications of these suggestions, there is many parameters that need to be considered. For example, the flue gas is not purified, and it may cause the algae to die. Also, we have to consider the effect of pH after adding CO_2 to the algal pond. Acidic pH can cause the algae to die very fast. In general, there are many parameters that need to be considered for suggesting these techniques for the future of algal biofuel production.

4.3. Machine Learning for LCA results prediction

Machine learning has been used in many fields such as medicine, autonomous cars, manufacturing, etc (Olya, Shirazi et al. 2013, Gavidel and Rickli 2015, Nezhad, Zhu et al. 2016, Aguwa, Olya et al. 2017, Nezhad, Sadati et al. 2018, Sadati, Chinnam et al. 2018). There is a vast potential for using these techniques in environmental engineering applications (Roostaei 2018). Since many of parameters can affect the results of a life cycle assessment (LCA), it is important to evaluate some new methods for predicting the results of an LCA study. Machin learning applications have been used in many fields such as medicine (Olya, Shirazi et al. 2013, Nezhad, Zhu et al. 2016, Nezhad, Sadati et al. 2018). Our research indicates that applications of machine learning for LCA studies is limited. Slapnik et al. have presented one of the primary applications of machine learning in the LCA (Slapnik, Istenič et al. 2015). This research indicates that normalization is an essential part of the interpretation.

Based on some previous research I have developed a machine learning model that can predict the global warming potential based on some input parameters such as:

X1: Exist total flow m3/d

X2: Area needed (m2)

X3: Solar annual (kWh/m2/day)

X4: Day more than 10 C in Annual

X5: Biomass yield annual (kg/year)

X6: Total energy output from biomass (MJ/year)

X7: Total pumping Distance (m)

X8: Total head static and dynamic (m)

X9: Total energy needed for operation (MJ/year)

X10: Pretreatment heat required (MJ/year)

X11: HTL heat required (MJ/year)

X12: Extraction heat required (MJ/year)

X13: Extraction electricity required (MJ/year)

The parameters that I used to predict is the Total CO_2 Emission divided by the Functional Unit (FU)which is depicted in chapter two section 2.6.4. I ran the machine learning model for Hydrothermal Liquefaction (HTL) which is the best scenario of our LCA analysis.

Y: Total CO_2 Emission / FU in HTL Scenario (kg CO_2 / year-FU)

The result of this model is a table with 12,454 rows of information and 13 independent variables as X and one dependent variable as Y which are presented in table 20.

I considered that the ML model uses 80% (9,964 rows of information) of the data for training the model and 20% (2,490 rows of information) of the data for testing. These rows are selected randomly from the dataset. Choosing 80% for training and 20% for testing is a common procedure in the ML studies. The ML method used was the Multiple Linear Regression models which is one of the most used models in the problem that has multiple independent variables that affecting one dependent variable. I have used the Python language for this test. Most of the code is dependent on the library, and for that, I have imported different libraries.

After running the model, one can see that the prediction of Y based on the test data set. These results are presented in figure 40.

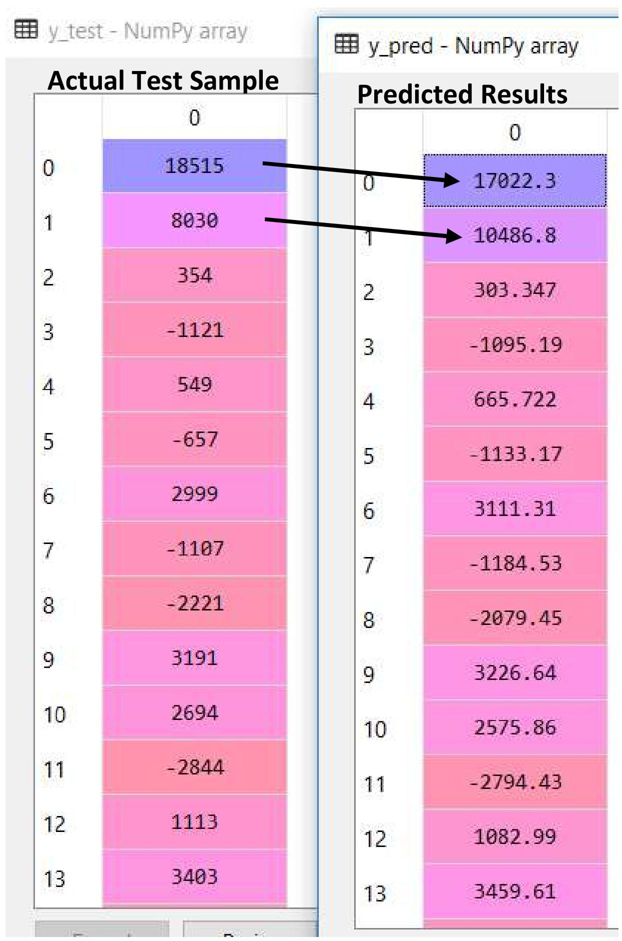

Figure 39. Prediction of Y based on the test sample

Further analysis of the results indicated that for different accuracy scenarios the model can predict good level of accuracy for the results. For example, considering the results with 75%

accuracy (the absolute error between prediction and actual data is less than 25%), our machine learning model accurately predicted in 1,822 cases. These results are summarized in table 22.

Table 20. Results of the model in predicting different level of information

Accuracy level	Accuracy > 75%	Accuracy > 90%	Accuracy > 95%
Total No. of accurate prediction	1822	1391	923
% of total actual data	73.1%	55.4%	37.1%

We considered removing the parameters that have less effect by calculating the p-value. A low p-value (< 0.05) indicates that we can reject the null hypothesis. In other words, a predictor that has a low p-value is likely to be a meaningful addition to the model because changes in the predictor's value are related to changes in the response variable. Conversely, a larger (insignificant) p-value suggests that changes in the predictor are not associated with changes in the response (Minitab 2013). The results for our model is presented in the following tables.

So further analysis to remove these variables. However, we have to do it one by one, and I started from the very first one that has the maximum p-value. In this case, it is variable X6 which is Total energy output from biomass (MJ/year). After removing this I re-ran the model and see what the next variable that is less important in predicting is Y. After continuing this for the following parameters (X13, X12, and X5) need to remove because the p-value in the rerun model is still > 0.05. The final table would have X1, X2, X3, X4, X5, X7, X8, X9, X10, and X11 as the parameters that affect the results. However even based on this modification the final prediction does not improve or even reduce the accurate prediction. Table 25 shows the results for running the model after this modification.

Table 21. Results of the model in predicting after some modifications

Accuracy level	Accuracy > 75%	Accuracy > 90%	Accuracy > 95%
Total No. of accurate prediction	1819	1386	920
% of total actual data	73.0%	55.3%	36.9%

4.3. Summary

In this chapter, I developed a life cycle optimization tools for optimizing the life cycle model in effects of them in algal wastewater treatment process with CO_2 sequestration. The method here is totally hypothetical and many parameters such as pH, toxic gas in the emission etc. should be evaluated for a real model.

Besides that, I have developed a machine learning model for life cycle assessment analysis that can predict the results of a model based on some independent variable. Our model can predict the 73% of the data with a level of accuracy of 75%.

CHAPTER 5 CONCLUSION AND FUTURE WORK

5.1. Summary and conclusion

Growing algae in wastewater have some potential for sustainable algal biofuel production. On the one hand, wastewater can provide free nutrient and water supply for algae cultivation; on the other hand, combining wastewater treatment with algae can remove nutrients such as nitrogen and phosphorus, which usually requires tertiary treatments with intensive energy use and high cost. However, there existing knowledge gaps that prohibit the utilization of wastewater-based algae systems.

In this proposed work, both modeling and experimental approaches are utilized to address these knowledge gaps to improve the sustainability of wastewater-algae systems. This research encompasses several disciplines and creates the following contributions:

- The realistic potential of wastewater-based algal biofuel and the corresponding environmental impacts were assessed by a high-resolution spatially explicit LCA.
- A new wastewater-based algae cultivation strategy was established to improve the stability, productivity, and cost-efficiency of algae biofuel production.
- A new LCO and machine learning tool was developed for the general application of wastewater-algae systems for system design, modeling, and optimization.

5.2. Future works

Based on this research, I have found some area of work that needs to be continued in the future.

- A complete LCA studies for a HRAP wastewater treatment site. This research gave real data for a more precise LCA study.

- Use the attached growth model in Detroit wastewater treatment plant. I have used wastewater lab conditions. Further research is needed to evaluate mixotrophic growth in WWTPs.
- Machine learning provided a promising result in our research. This indicates that the integration of machine learning and LCA could provide a new tool for predictive LCA analysis. Further work can be conducted to develop more advanced machine learning tools for LCA.

5.3. Publications and presentation

Some of the research results have been published in journals, and some are under review. In the following section, some of the publications are described.

5.3.1. Journal papers

- **Roostaei, Javad**; Zhang, Yongli; Spatially Explicit Life Cycle Assessment: Opportunities and Challenges of Wastewater-Based Algal Biofuels in the United States**,** 2017, Algal Research Journal
- Gopalakrishnana, K,. **Roostaei, Javad**.; Zhang, Y,; Mixed Culture of Chlorella sp. and Wastewater Wild Algae for Enhanced Biomass and Lipid Accumulation in Artificial Wastewater Medium, Frontiers of Environmental Science and Engineering, Accepted.
- **Roostaei, Javad**; Zhang, Yongli, Gopalakrishnan, Kishore, Ochocki, Alexander; Mixotrophic Microalgae Biofilm for Improved Productivity and Cost-efficiency of Biofuel Feedstock Production, under review, Nature - Scientific Report.
-

5.3.2. Journal papers submitted under the second review

- Resurreccion, Eleazer; Martin, Mason; Kumar, Sandeep; Jeffrey, Paul; Maglinao, Randy; Rice, Benjamin; **Roostaei, Javad**; Zhang, Yongli. “A Multifaceted Approach in Analyzing

Advanced Aviation Fuels Production from Camelina Oil." Under review. Environmental Science & Technology Journal,

5.3.2. Journal papers under preparation

- Life Cycle Optimization for CO2 Sequestration in Wastewater Algae Cultivation
- Applications of Machine learning for predictive life cycle assessment

5.3.4. Some of the Conference Presentation

- Stratified Multilayer Algal-biofilm Reclamation Technology (SMART) Coupled with Internet of Things (IoT): A Novel Wastewater-Algae System for Efficient Wastewater Treatment and Sustainable Bioenergy Production, EPA P3, Washington DC, 6-7 April 2018
- Using Internet of Things (IoT) to Optimize Algae Yield for Wastewater-based Algae Cultivation, Roostaei, J., Ochocki, A., Zhang, Y., June 20-22, 2017, AEESP 2017, Ann Arbor, Michigan
- Comparing the Removal Efficiency of 4-Nonylphenol by UV, Chlorination and Algae Cultivation, Roostaei, J., Zhang, Y., Pitts, D.K., and McElmurry, S.P., AEESP 2017, June 20-22, 2017, University of Michigan, Ann Arbor, Michigan
- Optimization for Wastewater Treatment Efficiency and Biofuel Productivity by Chlorella sp. and Mixed Wastewater Algae (MWWA) Using Response Surface Methodology (RSM), Gopalakrishnan, K., Zhang, Y., and Roostaei, J., June 20-22, 2017, AEESP 2017, Ann Arbor, Michigan
- Spatially explicit life cycle assessment of algae cultivation integrated with wastewater for biofuel production: understanding the realistic potential of wastewater-based algal

biofuel in the U.S., Roostaei, J.; Zhang, Y.; 6th International Conference on Algal Biomass, Biofuels and Bioproducts, 26-29 June 2016, San Diego, USA

- Integrating Algal-Bacterial Mixed Cultures with Wastewater Treatment for Cost-efficient Production of Algae Feedstock, Javad Roostaei, Yongli Zhang, Algae Biomass Summit, Washington DC, 2015

APPENDIX

Some of the tables and scripts for machine learning has been presented in the following section.

Table 22. Data which is used in the machine learning model

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	Y
2	189.3	2097.4	4.4	275.0	15217.8	-283690.0	15000.0	24.5	146523.4	57066.8	15674.3	11.7	7030.0	1704.0
3	189.3	2097.4	4.6	275.0	15765.0	-293890.6	1000.0	20.3	140676.3	59118.7	16237.9	12.1	7282.7	82.0
4	189.3	2097.4	4.4	275.0	15365.7	-286447.6	1000.0	20.3	137723.6	57621.5	15826.7	11.8	7098.3	196.0
5	189.3	2097.4	4.7	275.0	16016.2	-298573.7	1000.0	20.3	142534.2	60060.8	16496.7	12.3	7398.8	13.0
6	189.3	2097.4	4.4	275.0	15217.8	-283690.0	15000.0	24.5	136629.7	57066.8	15674.3	11.7	7030.0	1142.0
7	189.3	2097.4	4.5	275.0	15689.9	-292490.1	1000.0	20.3	140120.7	58837.0	16160.6	12.0	7248.0	103.0
8	189.3	2097.4	4.5	275.0	15648.0	-291709.5	1000.0	20.3	139811.1	58680.0	16117.4	12.0	7228.7	115.0
9	189.3	2097.4	4.6	275.0	15886.9	-296162.8	1000.0	20.3	141577.7	59575.8	16363.5	12.2	7339.0	48.0
10	189.3	2097.4	5.1	153.0	10489.3	-195541.7	1000.0	20.3	56560.1	39335.0	10804.0	8.0	4845.6	-3377.0
11	189.3	2097.4	5.4	214.0	15375.7	-286633.5	1000.0	20.3	107231.4	57658.9	15837.0	11.8	7102.9	-2190.0
12	189.3	2097.4	4.4	214.0	13028.2	-242872.0	1000.0	20.3	93721.7	48855.9	13419.1	10.0	6018.5	-1554.0
12451	...	...	...	...	...	...	...	...	...	...	...	...	...	...
12452	1.748E+06	1.937E+07	5.0	365.0	1.901E+08	-3.543E+09	1.800E+05	149.3	2.161E+09	7.128E+08	1.958E+08	1.456E+05	8.781E+07	21062.0
12453	2.500E+06	2.771E+07	3.8	184.0	1.361E+08	-2.538E+09	1.500E+05	111.7	8.865E+08	5.105E+08	1.402E+08	1.043E+05	6.288E+07	11584.0
12454	3.074E+06	3.406E+07	3.9	214.0	1.942E+08	-3.620E+09	1.800E+05	120.1	1.422E+09	7.282E+08	2.000E+08	1.488E+05	8.970E+07	13539.0

Figure 39 presenting the part of the code for predicting the results and the table 21 shows how data is indexed after running the python code.

```python
# Set the work directory
import os
os.chdir("D:\\Wayne Works\\1. PhD Dissertation\\ML Model on LCA Data")

# Multiple Linear Regression

# Importing the libraries
import numpy as np
import matplotlib.pyplot as plt
import pandas as pd

# Importing the dataset
dataset = pd.read_csv('DataLCAV5.csv')
X = dataset.iloc[:, :-1].values
y = dataset.iloc[:, 13].values

# Splitting the dataset into the Training set and Test set
from sklearn.cross_validation import train_test_split
X_train, X_test, y_train, y_test = train_test_split(X, y, test_size = 0.2, random_state = 0)

# Fitting Multiple Linear Regression to the Training set
from sklearn.linear_model import LinearRegression
regressor = LinearRegression()
regressor.fit(X_train, y_train)

# Predicting the Test set results
y_pred = regressor.predict(X_test)
```

Figure 40. Python Code for the multilinear regression model

Table 23. Data preview in the python machine learning code

Index	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	Y
0	189.27	2097.4	4.4	275	15217.8	-283690	15000	24.4525	146523	57066.8	15674.3	11.6581	7029.97	1704
1	189.27	2097.4	4.6	275	15765	-293891	1000	20.2968	140676	59118.7	16237.9	12.0772	7282.74	82
2	189.27	2097.4	4.4	275	15365.7	-286448	1000	20.2968	137724	57621.5	15826.7	11.7714	7098.3	196
3	189.27	2097.4	4.7	275	16016.2	-298574	1000	20.2968	142534	60060.8	16496.7	12.2697	7398.79	13
4	189.27	2097.4	4.4	275	15217.8	-283690	15000	24.4525	136630	57066.8	15674.3	11.6581	7029.97	1142
5	189.27	2097.4	4.5	275	15689.9	-292490	1000	20.2968	140121	58837	16160.6	12.0197	7248.04	103
6	189.27	2097.4	4.5	275	15648	-291710	1000	20.2968	139811	58680	16117.4	11.9876	7228.69	115
7	189.27	2097.4	4.6	275	15886.9	-296163	1000	20.2968	141578	59575.8	16363.5	12.1706	7339.05	48
8	189.27	2097.4	5.1	153	10489.3	-195542	1000	20.2968	56560…	39335	10804	8.03566	4845.61	-3377
9	189.27	2097.4	5.4	214	15375.7	-286634	1000	20.2968	107231	57658.9	15837	11.779	7102.91	-2190
10	189.27	2097.4	4.4	214	13028.2	-242872	1000	20.2968	93721…	48855.9	13419.1	9.98067	6018.48	-1554
11	189.27	2097.4	4.4	214	13151.8	-245176	1000	20.2968	94432…	49319.3	13546.4	10.0753	6075.56	-1593
12	189.27	2097.4	4.5	275	15659.7	-291928	5000	21.4842	139898	58723.9	16129.5	11.9966	7234.1	362

Table 23 shows the ordinary least squares (OLS) results in the Regression model.

Table 24. Ordinary Least Squares for the regression model

Dep. Variable:	y	R-squared:	0.988
Model:	OLS	Adj. R-squared:	0.988
Method:	Least Squares	F-statistic:	8.103e+04
Date:	Sun, 01 Jul 2018	Prob (F-statistic):	0.00
Time:	12:06:18	Log-Likelihood:	-87687.
No. Observations:	12452	AIC:	1.754e+05
Df Residuals:	12438	BIC:	1.755e+05
Df Model:	13		
Covariance Type:	nonrobust		

Table 24 shows the results for p-value analysis for each of the parameters.

Table 25. The P-Value results analysis

	coef	std err	t	P>|t|	[0.025	0.975]
const	-6588.5246	25.822	-255.149	0.000	-6639.140	-6537.909
x1	-2460.0248	939.460	-2.619	0.009	-4301.511	-618.539
x2	221.9994	84.779	2.619	0.009	55.819	388.180
x3	-1011.1459	7.340	-137.766	0.000	-1025.533	-996.759
x4	30.2868	0.053	568.123	0.000	30.182	30.391
x5	109.9868	86.486	1.272	0.203	-59.540	279.513
x6	14.8500	81.406	0.182	0.855	-144.719	174.419
x7	0.0057	0.001	6.123	0.000	0.004	0.008
x8	143.7065	0.343	419.445	0.000	143.035	144.378
x9	1.013e-05	1.09e-06	9.297	0.000	7.99e-06	1.23e-05
x10	3511.7644	1221.366	2.875	0.004	1117.698	5905.831
x11	-1.134e+04	3875.396	-2.927	0.003	-1.89e+04	-3745.437
x12	-7.293e+06	5.3e+06	-1.376	0.169	-1.77e+07	3.1e+06
x13	9236.5473	1.06e+04	0.871	0.384	-1.15e+04	3e+04

Omnibus:	27607.717	Durbin-Watson:	1.889
Prob(Omnibus):	0.000	Jarque-Bera (JB):	399786719.838
Skew:	20.097	Prob(JB):	0.00
Kurtosis:	879.889	Cond. No.	2.04e+14

Based on table 22 variables X5, X6, X12, and X13 have the p-value higher than 0.05.

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ABSTRACT

INTEGRATED STRATEGIES FOR SUSTAINABLE WASTEWATER-BASED ALGAL BIOFUEL PRODUCTION AND ENVIRONMENTAL MITIGATION IN THE US

by

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The integration of algae cultivation with wastewater treatment has received increasing interest as a cost-effective strategy for biofuel production. However, there has been no full assessment of algal biofuel production with wastewater on macro-scale by taking into account wastewater resources, land availability, CO_2 emission resources, and geographic variation. This research addressed and evaluated the use of wastewater for algae cultivation, in terms of modeling and laboratory experiments. <u>The first purpose</u> of this research was to develop a spatially explicit lifecycle model, by integrating life cycle assessment (LCA), tech-economic analysis (TEA), and Geographic Information Systems (GIS) analysis, for the evaluation of environmental and economic performance of algal biofuel production with wastewater across the continental U.S. The environmental impacts of the process were minimized by the Life Cycle Optimization (LCO) model.

Integration of LCA and GIS has helped to produce a spatially explicit estimation of algal biofuel production with wastewater. For that I calculated for each municipal wastewater treatment plant (WWTP) across the continental U.S (total 12,455 WWTPs). Wastewater resources, land availability, and meteorological variation were included for algae cultivation. Three downstream

process pathways, namely lipid extraction, hydrothermal liquefaction, and microwave pyrolysis, were modeled for biofuel conversion from the algal feedstock. Crystal Ball is used to automate the Monte Carlo simulation for the characterization of input and output uncertainty.

Results indicate that growing algae in wastewater for biofuel production would be both environmentally and economically sustainable. The potential production of algal crude oil is 0.98 billion gallons/yr (nearly 20% of advanced biofuel projection as outlined in the U.S Energy Independence and Security Act (EISA) of 2007). However, the spatial analysis shows that only 61% of the total wastewater could be used, based on current land use efficiency for algae cultivation and land availability around each WWTP, in a radius where algal biofuel production is energy positive (energy output > energy input). This result indicates that land availability or land use efficiency are limiting factors for algal cultivation that have not been considered in previous studies. It also suggests that improvement should be made in cultivation technologies and system design to increase land use efficiency or land availability for the full potential of wastewater as a resource for algal biofuel production. This research is the first spatially explicit LCA of algae biofuel production with wastewater by including analyses of resources availability and geographic variation. Although focusing on the U.S. as the case study, the developed methodology could be used for spatially explicit analysis of algal biofuel integrated with wastewater on macro-scale in other regions as well.

Currently, most of the algal wastewater systems are open pond raceways, in which algae grows in an autotrophic mood by utilizing the energy from the sun. However, other methods such as heterotrophic, mixotrophic, and biofilm growth cultivation, all of which use organic carbon as sources of energy, have not been used in wastewater treatment effectively. <u>The second purpose</u> I performed some laboratory experiments for a better understanding of the potential of using the heterotrophic and mixotrophic methods (mixotrophic uses both light and carbon sources for energy), in addition to biofilm attached growth in algal wastewater. Because one of the main issue in algae cultivation is the harvesting process which is energy intensive, the attached growth can help reduce the cost. In this research, I evaluated the results of growing attached algae in different material and surface roughness.

This novel algae cultivation strategy, mixotrophic microalgae biofilm, can help improve the productivity and cost-efficiency of algal biofuel production. In contrast to previous methods, this improved approach can achieve high productivity at low cost by harnessing the benefits of mixotrophic growth's high efficiency, i.e., capable of subsisting on inorganic and organic carbons thus unaffected by limited light, and microalgae biofilm's low harvesting cost. Our results, as one of the first studies of this type, proved that microalgae biofilms under mixotrophic condition exhibited significantly higher productivity and quality of biofuel feedstock: 2-3 times higher of biomass yield, 2-10 times higher of lipid accumulation, and 40 – 60 % lower of ash content when compared to microalgae biofilms under autotrophic condition. In addition, I investigated the impact of cell-surface properties (hydrophobicity and roughness) on the growth activities of microalgae biofilms and found that the productivity of mixotrophic biofilms was significantly correlated with the surface hydrophobicity. Finally, our work demonstrated the applicability of integrating this novel cultivation method with wastewater for maximum efficiency. This study opens a new possibility to solve the long-lasting challenges of algal biofuel feedstock production, i.e., low productivity and high cost of algal cultivation.

Finally, I have evaluated the potential of using life cycle optimization (LCO) and machine learning in the sustainability analysis and LCA studies. Availability of CO_2 emission from coal and natural gas power plants has been evaluated to be used in HRAP. Previous studies have shown that CO_2 is one of the main limiting factors in growing algae with wastewater. In the spatial model I analyzed the CO_2 resources based on distance to the nearby WWTP, the capacity of emission, and the capacity of demand for CO_2. For LCO study I considered a hypothetical model of providing CO_2 gases to the nearby HRAP system. I have optimized the conditions and decisions based on minimizing function 1) energy needed for pumping wastewater and CO_2 function 2) water needed to produce pipes, and finally function 3) eutrophication potential. The primary results indicate that a HRAP close to the WWTP is the optimized condition in a distributed system on CO_2 pipe. The second challenge that I have evaluated in our research is the application of machine learning. I collected some of those independent parameters that affect the CO_2 generation divided by functional unit. The independent variables are Exist total flow m^3/d, Area needed (m^2), Solar annual ($kWh/m^2/day$), Day more than 10 °C in Annual, Biomass yield annual (kg/year), Total energy output from biomass(MJ/year), Total pumping Distance (m), Total head static and dynamic (m), Total energy needed for operation (MJ/year), Pretreatment heat required (MJ/year), HTL heat required (MJ/year), Extraction heat required (MJ/year), Extraction electricity required (MJ/year). The dependent variable is Total CO_2 Generation / FU in HTL Scenario (kg CO_2 / year). The results indicate that for different accuracy scenarios the model can predict very well. For example, considering the results with 75% accuracy (the absolute error between prediction and actual data is less than 25%), our machine learning model can accurately predict in 1,822 cases.

AUTOBIOGRAPHICAL STATEMENT

Javad Roostaei is a doctoral candidate in Civil and Environmental Engineering and a Master student in Computer Science at Wayne State University in Detroit, Michigan. His area of research is spatial data analysis, sustainability, and algae biofuel and using drone for environmental engineering applications. Besides that, he is doing research on using Internet of Things (IoT), Edge Computing, Cloud Computing, and their applications in Environmental Engineering.

He has a bachelor's degree in Civil Engineering (Water and Wastewater Engineering) and a master's degree in Civil Engineering (River Engineering Specialty) both from Shahid Beheshti University (SBU), Tehran and he received my second master's degree in Environmental Engineering from Wayne State University.

He has five years of professional experience in the consulting engineering companies as a senior Hydraulic Engineer and Project Manager in water network design, ArcGIS, and AutoCAD mapping. In addition, he has published 16 papers in national and international conferences, one journal article (4 journal articles under review), two joint registered patents, and another patent under review for sending to US patents.

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