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Abstract Submission Form

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TITLE of Abstract

ALGAE DERIVED BIOFUELS

This presentation will focus on the use of algae as a source of biofuel. Biofuels have become increasingly prominent because of their global availability, carbon neutrality, renewability, and higher combustion efficiency (Chisti, 2007). Also economic feasibility appears promising as more research focuses on optimization of algae growth conditions and oil yields. Algae derived biofuel is more beneficial for the environment than cultivation of crops for biodiesel. Oil productivity of algae is much higher than oil production in crops. Algae derived biofuels are becoming the center of attention for a number of reasons as indicated below by Mohan et al. (2010):

- Algae are present globally in abundance.
- Algae can mitigate greenhouse gases by using CO_2 as a source of carbon.
- Algae are photosynthetic which is recognized as an efficient CO_2 sequestration mechanism.
- Algae grow rapidly and have carbon fixing rates that are much higher than those of terrestrial plants.
- Algae can be a powerful source of renewable biodiesel without impacting human food consumption or use of high percentage of cropping areas.
- Algae can use industrial flue gases that contain CO_2.
- Algae are photosynthetic
- Algae can be used for wastewater treatment since they can remove nitrogen and phosphorus from wastewater.
- Algae are also a great resource for other value-added products such as vitamin A, various proteins, and fatty acids.
- Algae are used extensively in aquaculture.
- Algae can reduce dependency on fossil fuels and other nonrenewable resources.

Experiments were conducted with different algae species under various cultivation conditions that included studying the impact of lighting, nutrients, carbon sources and carbon di-oxide delivery. Results of these studies along with the future of algae derived biofuels will be presented.

References:

Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnol. Adv*. 25, *Pages* 294-306.

Mohan, S.V., M. Prathima Devi, G. Mohanakrishna, N. Amarnath, M. Lenin Babu, P.N. Sarma (2010) "Potential of mixed microalgae to harness biodiesel from ecological water-bodies with simultaneous treatment" *Bioresour. Technol*, *102, Pages* 1109-1117.

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Jahan, K., S. Bauer and A. McFarland (2014) Nutrient Removal from Wastewater using Algae, Proceedings of the IWA Annual Conference September 2014, Lisbon, Portugal.

Nutrient Removal by Microalgae from Wastewater Treatment Plants

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Abstract: Global shortages of fossil fuels have driven research into biofuel derived from algal species. Municipal wastewater is rich in nitrogen and phosphorus, which are contaminants removed using expensive treatment processes, and nutrients that stimulate plankton growth. Samples were collected from four local wastewater treatment plants located in New Jersey, USA. *Scenedesmus dimorphus* and *Chlorella vulgaris* were used in this study. Nutrient removal was monitored, and algal lipid yields were measured for each municipal plant and algal species. Microalgae average lipid yield was 13.5% (dcw), with 87% total phosphorus removal, and 84% total nitrogen removal. Results indicate potential for microalgal biofuel production using municipal wastewater as a cost-effective medium, while removing nutrients.

Keywords: algae; nutrient; wastewater

Introduction

The constant demand for diminishing oil resources has driven research into sustainable energy. With global shortages of fossil fuels, a major focus is on sustainable biofuel production derived from microalgae. Chisti (2007) reported that algae produce more oil per U.S. cropping area than many other oil crops. Recent research suggests that microalgae have an areal productivity of 20 to 40 times that of oil crops (Sheehan et al., 1998) and oil content up to 80% by weight of dry biomass (Chisti, 2007). Lipids produced in algae are extracted and used for oil production. Algae produce lipids that are unsaturated, and therefore, require little to no pre-treatment before transformation into biodiesel. Thus algae have the potential to replace current sources for renewable biodiesel production.

The use of algae in wastewater treatment is gaining attention, as algae need nitrogen and phosphorus for growth. Microalgae have been proposed as an alternative for removal of nitrogen and phosphorous from wastewater as early as 1968 (Hemens and Mason, 1968). Algae growth requires nutrients, water, and light, which are the main factors involved in the cost analysis of algal biomass production (Chisti 2007;Parks and Craggs, 2011). Researchers have focused on the use of municipal wastewater as a source of nutrients for promoting algae growth.

Aslan and Kapdan (2006) indicated that *Chlorella vulgaris* removed nitrogen more efficiently compared to phosphorus. Ruiz-Marin et al. (2010) indicated that *Scenedesmus obliquus* showed a higher nitrogen and phosphorus uptake from wastewater than *Chlorella vulgaris*.

Kim et al. (2010) reported the feasibility of the use of *Chlorella vulgaris* in removing ammonia from wastewater treatment plant effluent. Their studies showed that microalgae could be cultivated in wastewater effluent and used as a polishing agent for removal of nutrients in wastewater. Wang et al. (2010) also evaluated the growth of *Chlorella* sp. on wastewaters sampled from five different points of a municipal wastewater treatment plant. Results indicated that the algae were capable of efficient nutrient removal in nutrient rich centrate. Similar results were reported by Li (2012) in a study utilizing *Chlorella* sp. for nutrient removal from centrate.

Zhou et al. (2011) collected 60 algae-like microorganisms from different sampling sites in Minnesota. The researchers used multi-step screening and acclimation procedures to select high-lipid producing facultative heterotrophic microalgae strains capable of growing on concentrated municipal wastewater (CMW) for simultaneous energy crop production and wastewater treatment. Seventeen strains were proved to be tolerant to CMW. These strains were identified as C*hlorella* sp., *Heynigia* sp., *Hindakia* sp., *Micractinium* sp., and *Scenedesmus* sp. Rasoul-Amini et al. (2014) used five strains of microalgae growing as free-cells and compared their ability to remove nitrate-nitrogen (NO_{3-N}) and orthophosphate (PO_3^{-4}-P) in batch cultures of urban wastewater. Results indicated that *Chlorella sp*. (YG01) showed a higher N uptake rate (84.11%) and *Chlamydomonas sp*. (YG04) and *Chlamydomonas sp*. (YG05) showed a higher P uptake rate (100%) in urban wastewater than other species. Most of the N and P removal occurred in the first 4 days of a two week long experiment.

Many experiments have studied algae-aided nutrient removal; however, lipid yield from algae is typically not reported. Zhou et al. (2012) developed a hetero-photoautotrophic algal growth model for improved wastewater treatment and low cost algal biofuel feedstock production. The microalga, *Auxenochlorella protothecoides* UMN280, was grown heterotrophically on concentrated municipal wastewater and then autotrophically with excess carbon dioxide. The maximal biomass concentration and lipid content at the first and second stages reached 1.12g/L and 28.90%, and 1.16g/L and 33.22%, respectively. The nutrient removal efficiencies for total phosphorus, ammonia, nitrogen and chemical oxygen demand at the end of the two-stage cultivation were 98.48%, 100%, 90.60% and 79.10%, respectively. Chinnasamy et al. (2010) conducted a study using a wastewater containing 85-90% carpet industry effluents with 10-15% municipal sewage, to evaluate the feasibility of algal biomass and biodiesel production. Native algal strains were isolated from carpet wastewater and it was concluded that the marine algae showed good growth in the wastewater. Fifteen native algal isolates showed greater than 96% nutrient removal in treated wastewater. About 63.9% of algal oil obtained from the algae consortium could be converted into biodiesel.

Research was conducted to study microalgae growth and lipid production for biofuel using untreated municipal wastewater rich in nitrogen and phosphorous: major nutrients that stimulate plankton growth. These nutrients contaminate wastewater and must be removed by municipal wastewater treatment plants through expensive chemical and biological treatments before being recycled into the environment. Growing microalgae in wastewater serves the dual roles of nutrient reduction and biofuel production. As such this study focuses on nutrient removal from four municipal wastewater treatment plants using two algal species, *Scenedesmus dimorphus* and *Chlorella vulgaris*. These species were chosen due to their common occurrence around the world, easy growth characteristics and significant oil content. The lipid yield of the algae was also determined at the end of the study. Wastewater samples were collected at various treatment stages from four wastewater treatment facilities in the Southern New Jersey area. Nutrient removal was monitored, and percentage lipid yields were calculated for each municipal plant and algal species.

Material and Methods

Scenedesmus dimorphus and *Chlorella vulgaris* were purchased from the UTEX algae collection center. Municipal wastewater samples were collected from: Atlantic County Utilities Authority (ACUA), Landis Sewerage Authority (LSA), Gloucester County Utilities Authority (GCUA), and the Sewage and Treatment Plant of Harrison Township (TPHT), located in southern New Jersey. These facilities were selected based on their variability in treatment technologies. The plant characteristics are presented below in Table 1.

Table 1: WWTP Characteristics

WWTP Plant	Type of Treatment	Type of Outfall	Type of Plant	Flowrate	Primary Effluent		Centrate	
					TN mg/L	PO_4-P mg/L	TN mg/L	PO_4-P mg/L
Landis Sewerage Authority (LSA)	Activated Sludge	Groundwater	Tertiary	5.5 MGD	38.2	46.1	23.6	48.5
Gloucester County Utilities Authority (GCUA)	Activated Sludge	Delaware River	Secondary	26 MGD	31.4	43.5	38.9	42.4
Atlantic County Utilities Authority (ACUA)	Activated Sludge	Atlantic Ocean	Secondary	40 MGD	29.8	45.9	33.6	39.7
Treatment Plant of Harrison Township (MH)	Oxidation Ditch	Racoon Creek	Secondary	0.4 MGD	30.2	10	--	--

The effluent from the primary clarifier and the filtrate from the centrifuge were used in this study. The wastewater was filtered to remove suspended solids and bacteria. Algae was grown in Bold's Basal medium and 300 mL was transferred to 700 mL of the wastewater samples. The samples were kept under light-intensive conditions and were constantly stirred over a growth period of 14 days. Algal growth, measured by optical density, was monitored at a wavelength of 625 nm. Nitrate and total phosphorus concentrations were measured using EPA approved HACH methods. At the end of the experiment, the algae samples were filtered from the wastewater and lipids were extracted using the Bligh and Dyer (1959) method and compared for each algal species and treatment facility.

Results and Conclusions

Select results for nutrient removal by the two select algae species are shown in Figures 1 through 3. It is evident from the figures that both algae species were capable of nutrient removal from the wastewater provided. The two algal species removed an average of 87% total phosphorus and 84% total nitrogen from the wastewater samples. The filtrate wastewater sample from ACUA had the lowest nitrogen content. Studies have indicated that nitrogen deficiency promotes oil yield.

Table 1 shows the percent lipid yield and growth rate for each wastewater treatment plant and algal species studied. Common lipid yields for *S. dimorphus* and *C. vulgaris* are 16-40% and 14-22%, respectively (Chisti, 2007).

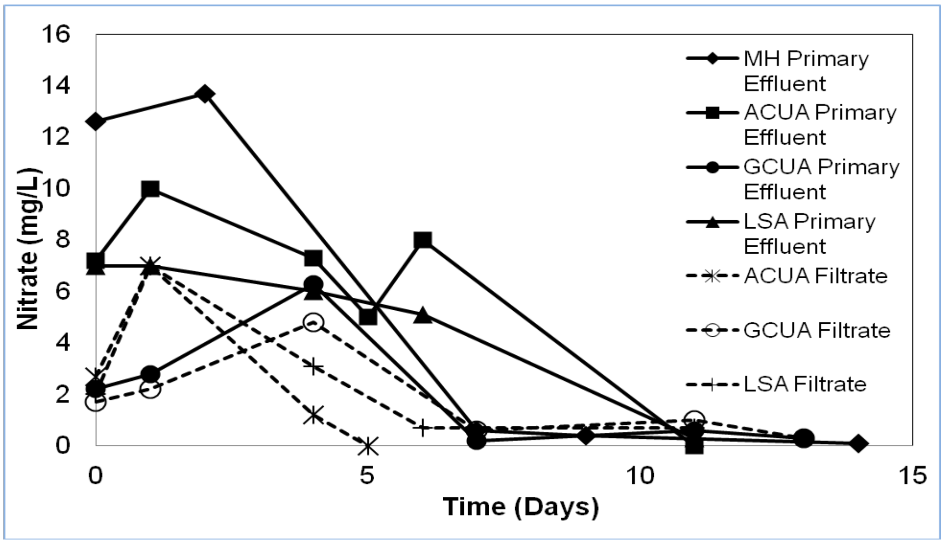

Figure 1. Nitrate Removal by *Scenedesmus dimorphus*

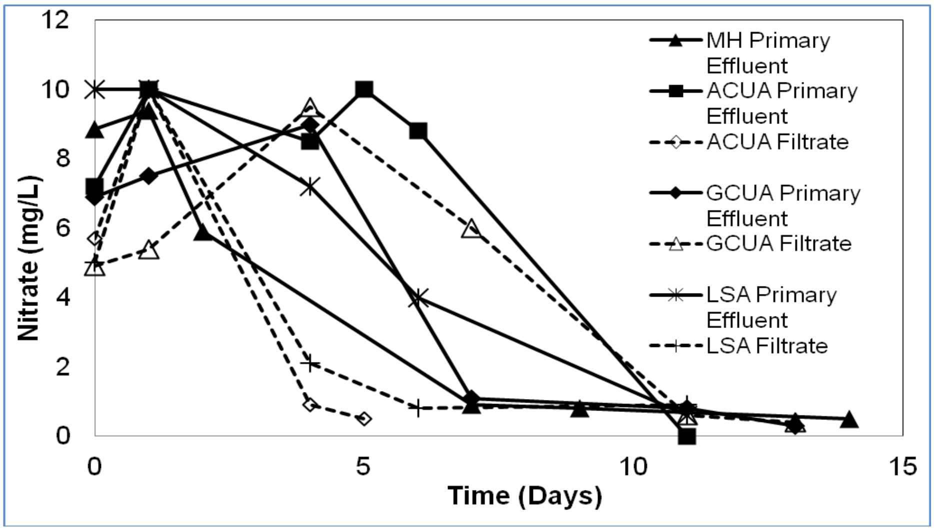

Figure 2. Nitrate Removal by *Chlorella vulgaris*

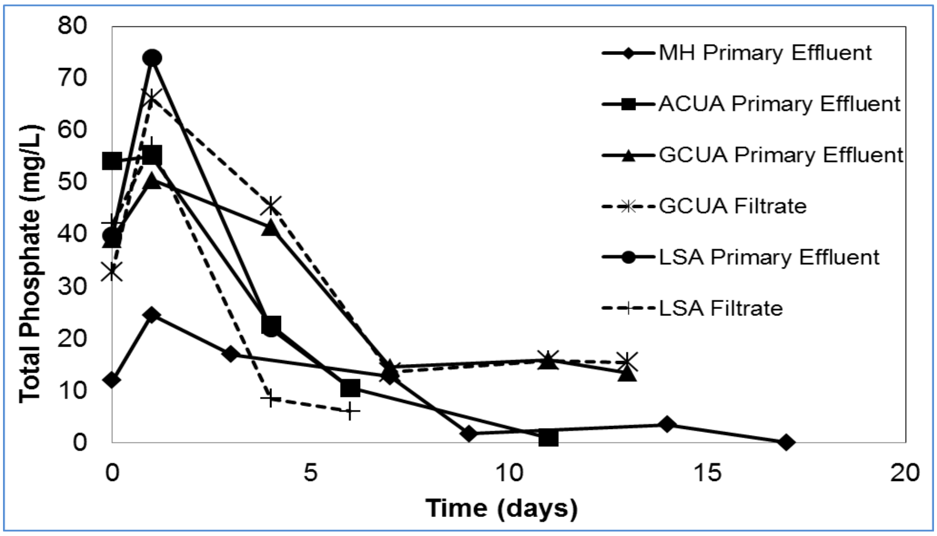

Figure 3. Phosphate Removal by *Chlorella vulgaris*

Table 1: Lipid Yield and Growth Rate for *S. dimorphus* and *C. vulgaris*

Treatment Stage	Lipid Yield (% DCW)	Growth Rate ($days^{-1}$)
Sewage and Treatment Plant of Harrison Township		
SD Effluent	12.8	0.094
CV Effluent	9.29	0.086
Atlantic County Utilities Authority		
SD Effluent	16.7	0.260
CV Effluent	15.7	0.123
SD Filtrate	24.5	0.122
CV Filtrate	25.1	0.076
Landis Sewerage Authority		
SD Effluent	10.8	0.185
CV Effluent	10.4	0.037
SD Filtrate	13.2	0.118
CV Filtrate	11.7	0.142
Gloucester County Utilities Authority		
SD Effluent	12.6	0.279
CV Effluent	15.9	0.100
SD Filtrate	11.5	0.247
CV Filtrate	11.0	0.170

ACUA produced an average of 20.3% DCW. The ACUA filtrate yielded the highest lipid content. The oil yield results are encouraging, because algae have the potential to not only remove nutrients, but also produce lipids for biofuel. Algae-derived biofuel is a renewable energy source that does not negatively impact the environment.

This study indicated that microalgae can be used for nutrient removal from wastewater, as well as serve as a biofuel source. Wastewater with lower nutrient concentrations generally produced more lipids. *S. dimorphus* and *C. vulgaris* performed similarly in nutrient removal; however, lipid yields were slightly higher in studies conducted with *S. dimorphus*. Residual algal biomass after oil harvesting can be used as feed for sludge digesters or by the aquaculture industries. The experiment shows a potential for biofuel production through microalgae growth in municipal wastewater, as well as a mechanism for nutrient removal.

References

Aslan, S., Kapdan, I. K. (2006). Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. *Ecol. Eng.* 28, 64-70.

Bligh, E.G., Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.*, 37, 911-917.

Chinnasamy, S., Bhatnagar, A., Hunt, R. W., Das K.C. (2010). Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. *Bioresour. Techlol.* 101, 3097-3105.

Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnol. Adv*. 25, 294-306.

Hemens, J., Mason, H.M., (1968). Sewage nutrient removal by a shallow algal stream. *Water Res*., 2277-2287.

Kim, J., Bala P. Lingaraju, Rachael Rheaume, Joo-Youp Lee, Kaniz F. Siddiqui (2010). Removal of Ammonia from Wastewater Effluent by *Chlorella Vulgaris, Tsinghua Science & Technology*, 15(4), 391-396.

Li, Y. (2012). Cultivation of algae on highly concentrated municipal wastewater as an energy crop for biodiesel production, Ph.D. Dissertation, University of Minnesota, http://hdl.handle.net/11299/153742

Park, J.B. and Craggs R. J. (2011) Nutrient removal in wastewater treatment high rate algal ponds with carbon dioxide addition. *Water Sci Technol*. 2011;63(8):1758-64.

Rasoul-Amini, S., N. Montazeri-Najafabady, S. Shaker, A. Safari, A. Kazemi, P. Mousavi, M. A. Mobasher, Y. Ghasemi (2014). Removal of nitrogen and phosphorus from wastewater using microalgae free cells in batch culture system, *Biocatalysis and Agricultural Biotechnology* 3(2), 126-131.

Ruiz-Marin, A., Mendoza-Espinosa, L. G., Stephenson, T. (2010). Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. *Bioresour. Technol*. 101, 58-64.

Sheehan, J., Dunahay,T., Benemann, J., Roessler, P. (1998). A look back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from algae. Department of Energy, National Renewable Energy Laboratory.

Wang, L., Min, M., Li, Y., Chen, P., Chen, Y., Liu, Y., Wang, Y., Ruan, R. (2010). Cultivation of green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment plant. *Appl. Biochem. Biotechnol*. 162, 1174-1186.

Zhou, W., M. Min, Y. Li, B. Hu, X. Ma, Y. Cheng, Y. Liu, P. Chen, and R. Ruan (2012). A hetero-photoautotrophic two-stage cultivation process to improve wastewater nutrient removal and enhance algal lipid accumulation, *Bioresour. Technol*. 110(2012):448-455.

Zhou, W., Li, Y., Min, M., Hu, B., Chen, P., Ruan, R. (2011). Local bioprospecting for high-lipid producing microalgal strains to be grown on concentrated municipal wastewater for biofuel production, *Bioresour. Technol*., 102(13), 6909-6919.

Jahan, K., S. Bauer, S. Moore, K. Grimes and A. McFarland (2014), Cultivation of Chlorella vulgaris with Organic Carbon Sources, Proceedings of the 3rd Annual ICDRET Conference, Jan 9-11, Dhaka Bangladesh

Cultivation of *Chlorella vulgaris* with Organic Carbon Sources

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***Abstract*— Experiments were conducted with *Chlorella vulgaris* to compare mixotrophic microalgal growth using two common organic carbon sources: glucose and glycerol. Glycerol is a byproduct of biodiesel production that has potential as an economical source of organic carbon for microalgal growth. *C. vulgaris* was cultivated in Bold Basal medium under mixotrophic conditions. Glucose and glycerol concentrations were varied from 15 to 75 g/L. A higher growth rate was observed for *C. vulgaris* with glucose. The optimum concentration of glucose was determined as 15 g/L, with a growth rate of 0.129 day^{-1}. The optimum concentration of glycerol was also determined as 15 g/L, with a growth rate of 0.056 day^{-1}. Glucose was a more effective carbon source when considering microalgal growth, but results indicate the feasibility of also using glycerol as an organic carbon source.**

***Keywords*—*Chlorella vulgaris*, mixotrophic, glucose, glycerol**

I. INTRODUCTION

Fossil fuels are becoming scarcer as the world's energy demand increases. Attention has been directed to biofuel as a renewable and sustainable source of energy that does not impact the environment negatively [5]. Algae colonies are a plausible source of biofuel, because they require little labour to grow and can be harvested in bulk. Chisti (2007) reported a study proving that algae produce more oil per U.S. cropping area than many other oil crops, such as corn, soy, and jatropa [5].

Microalgae are typically grown in photoautotrophic systems. Photoautotrophic systems attract attention, because the algae are able to use freely available sunlight for photosynthesis and use carbon dioxide, from air as an inorganic carbon source for growth. As light availability throughout the system is required for efficient uptake of CO_2, area to volume ratios of open systems entrench into agricultural land, and the nature of open growth systems generally lead to contamination by bacterial and fungal species which inhibit the growth and oil production of algae [18]. The photoautotrophic growth model is not economically feasible and is difficult to scale to the industrial level required to impact the current oil demand [4].

Difficulties associated with large scale photoautotrophic growth have driven research into alternative growth methods, particularly heterotrophic growth models [1, 2, 9, 12]. Heterotrophic growth conditions are typically created by adding organic carbon sources such as glucose to the system. These conditions tend to promote higher cell density and lipid content than compared to photoautotrophic growth conditions [16, 17]. *Nannochloropsis sp*. growth was enhanced in both phototrophic and mixotrophic conditions under three primary monochromatic light (red, green and blue) [6]. *Nannochloropsis sp.* had a higher growth rate with blue lighting under mixotrophic conditions. Glycerol was used as the external carbon source. The growth and lipid yield of *Chlorella vulgaris* ESP-31 was investigated under phototrophic, mixotrophic and heterotrophic conditions [16]. Glucose was used as the organic carbon source and the tests were conducted in the dark for heterotrophic conditions. The growth on nutrient limiting medium resulted in higher lipid accumulation (20-53%) but slower growth rate. Higher lipid content (40-53%) was obtained under mixotrophic conditions. The impact of various organic carbon sources for photoheterotrophic cultivation of the microalga *Chlorella vulgaris* ESP-31 was also investigated by the same researchers [17]. Carbon sources included glucose, fructose, sucrose, glycerol, sodium acetate and acetic acid. *C. vulgaris* ESP-31 with glucose obtained the highest biomass concentration (3.5g/L) and lipid content (26%) in the absence of pH control. Regardless of the carbon sources used, the fatty acid profile of the microalgal lipid did not change.

Carbon costs for the current autotrophic growth model account for 50% of the cost of the medium [8]. Conversely, it is projected that glucose costs for a heterotrophic growth model would account for 80% of the cost of medium [2]. While the biomass productivity in comparison is favourable, the cost of glucose required to produce these results is too expensive to scale to the industrial level [16].

Opinions in the scientific community tend to assert that heterotrophic growth models for microalgal biofuel production require organic carbon sources that are too expensive. As such current research is exploring the benefits of reusing crude glycerol, a by-product of biodiesel production, as an organic carbon source to create the same growth conditions. It was reported that 0.08 kg of crude glycerol is produced for every one litre of biodiesel using current processes [7]. While glycerol is used widely in the cosmetics, pharmaceutical, and food industries, the glycerol produced is too expensive because of purification and refining needs [7]. Because of its similar chemical composition, many microalgae species are able to use glycerol in the same manner that the species would uptake glucose as an organic carbon source [4]. While this is a key factor in the future of algae biodiesel, it has yet to be determined if the algae are able to grow as effectively using glycerol compared to glucose.

The freshwater microalgae species, *Chlorella* sp., has received attention as these microalgae are dualtrophic, meaning that they can be grown in both autotrophic and heterotrophic environments [4, 10,16-18]. This trait is caused by a symport system referred to as the Hexos/H^+ Symport System. This system is considered "inducible", meaning that, in the presence of organic carbon, the algae will require a short period of time to adjust to the environment, but then will solely uptake organic carbon as the algae's source of carbon [19]. The ability of the *Chlorella* species to quickly adjust to heterotrophic carbon uptake makes it the central species upon which mixotrophic and heterotrophic studies are currently performed.

The objective of this experiment was to determine the growth of *Chlorella vulgaris* on glucose and glycerol under mixotrophic conditions. Mixotrophic conditions are defined when both light, inorganic and an organic are present for growth [4]. It appears that there is a synergistic effect of light and organic carbon that promotes higher growth rates and biomass yield [4]. Photoheterotrophic conditions are rarely mentioned in the literature with regards to algae cultivation.

Chlorella vulgaris was chosen for this study because of the dualtrophic nature of the algae. In addition to this, *C. vulgaris* is a resilient and versatile species of algae found around the world. *C. vulgaris* can be grown under a wide variety of environmental conditions, including those which create heterotrophic growth environments [1]. *C. vulgaris* is a circular shaped algae, 2-5 µm in size.

Current research into heterotrophic growth models are typically conducted using glucose to provide an organic carbon source for the algae [18]. Glycerol is a waste product that has the potential be used to create a similar heterotrophic growth condition [4]. The effect of glycerol and glucose concentrations on the growth of *C. vulgaris* was also investigated.

II. MATERIALS AND METHODS

Chlorella vulgaris, was purchased from the culture collection bank at the University of Texas (UTEX 395) [13]. The culture was grown in Bolds Basal medium [3] in a five gallon glass jar equipped with an external light source (23 W fluorescent light). The light was placed 20 cm from the reactors and the light intensity was adjusted to 400 foot-candles. The reactor was stirred and operated at room temperature (25$\pm$ 2°C) at a pH of 7.2 with filtered air addition (0.22µm Millipore filter).

C. vulgaris was cultivated with the addition of either glucose or glycerol as an organic carbon source. Glucose was purchased from Acros organics while glycerol was purchased from Fisher Scientific. Experiments were conducted in duplicate in 1000 mL flasks. The ratio of Bold's Basal Medium to algae in each flask was 600:400 mL. Glucose and glycerol concentrations were varied as follows: 15 g/L, 30 g/L, 45 g/L, 60 g/L, and 75 g/L. The range of concentrations was selected based on the reported optimal concentrations for glucose (30 g/L) and glycerol (60 g/L) [10, 7]. Organic carbon concentrations were measured at the end of the experiment using the HACH COD test vials (HACH Method 8000). Controls without organic carbon were also maintained.

Flasks were placed on a Phipps and Bird jar test setup that continuously agitated the algae at 90 revolutions per minute and provided the beakers with a constant light source of 400 foot-candles. This allowed mixotrophic conditions for the experiments.

Temperature and pH were monitored and controlled daily throughout the experiment. Algal growth was observed using optical density values measured with a spectrophotometer (HACH DR 4000, HACH, Loveland Colorado, USA) at a wavelength of 684 nm (i.e., OD_{684}). Suspended solids concentrations for dry cell weight were determined using Standard Method #2540 and correlated to OD_{684}. The growth rate $\mu(d^{-1})$ was determined as follows:

$$\mu(d^{-1}) = \frac{\ln(X_t) - \ln(X_o)}{t}$$

where X_t and X_0 are cell density at 684 (OD_{684}) nm of the culture at time *t* and time *0*.

III. RESULTS AND DISCUSSION

Photoautotrophic conditions using CO_2 and light are the most common cultivation conditions for algae growth [15,16]. In recent years many researchers have started investigating heterotrophic and mixotrophic conditions for algae cultivation [4, 8-10, 15,16].

C. vulgaris was grown with two organic carbon sources (glucose and glycerol) with light in this study. The biomass production with time is presented in Figures 1 and 2. The solid lines represent the glucose data, while the dashed lines represent the glycerol data. The calculated growth rates are listed in Table 1.

It is apparent from Figures 1 and 2 that *C. vulgaris* was able to grow on both organic carbon sources. It is also clear that the presence of organic carbon enhanced algae growth. Glucose was a more effective source of organic carbon than glycerol as evidenced by higher biomass concentrations which were observed at all glucose concentrations.

Higher concentrations of each organic carbon did not impact the biomass concentration significantly. In fact biomass growth had a more pronounced lag phase at higher concentrations of both glucose and glycerol. The glycerol data also indicates that stationary conditions were not attained in twelve days.

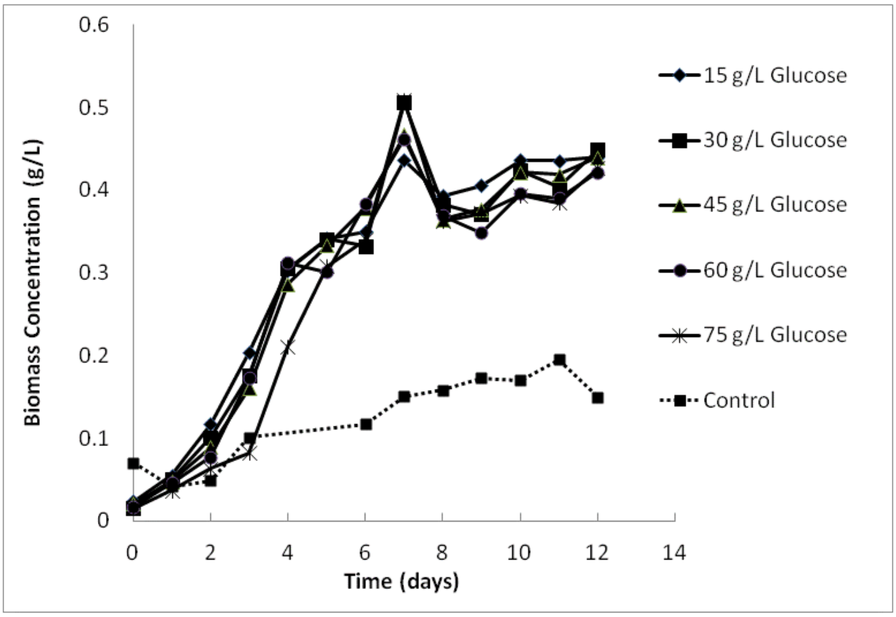

Fig.1 Biomass concentration with time for varying glucose concentrations

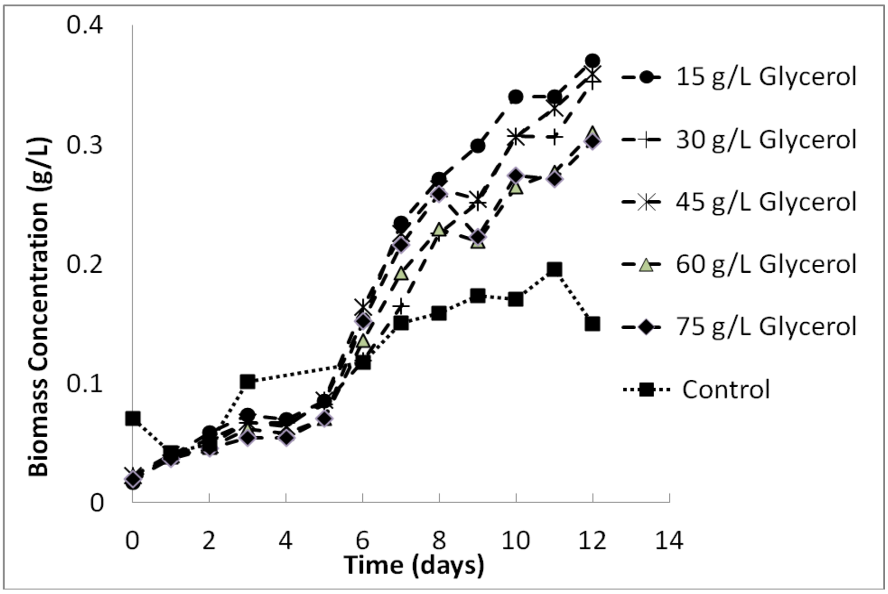

Fig.2 Biomass concentration with time for varying glycerol concentrations

TABLE I
AVERAGE GROWTH RATE OF *C. VULGARIS* FOR VARIOUS GLUCOSE AND GLYCEROL CONCENTRATIONS

Growth Condition	**Growth Rate (1/day)**	**Growth Condition**	**Growth Rate (1/day)**
Control	0.0415		
Glucose 15 g/L	0.129	Glycerol 15 g/L	0.0556
Glucose 30 g/L	0.119	Glycerol 30 g/L	0.0452
Glucose 45 g/L	0.115	Glycerol 45 g/L	0.0522
Glucose 60 g/L	0.115	Glycerol 60 g/L	0.0458

Glycerol studies appeared to have a more pronounced lag phase at all concentrations. This longer adaptation period to glycerol has been reported by a number of researchers and is attributed to a physiological response [4, 16].

The optimum concentration of glucose and glycerol was determined to be 15 g/L, with a growth rate of 0.129 day^{-1} and 0.056 day^{-1} respectively. Growth rates decreased with increasing concentrations of glucose and glycerol. Lower growth rates with glycerol in comparison to growth rates obtained with glucose have been reported for *Chlorella zofingiensis* [10]. In another mixotrophic study sucrose and acetate were deemed to be better than glycerol as an organic carbon source for the growth of *Chlorella minutissima*, *Scenedusmus bijuga* and *Chlamydomonas globosa* [1]. Glyerol was also used to grow *Nannochloropsis sp.* under blue light [6]. COD measurements at the end of the experiment indicated organic carbon removal rates ranging from 89 to 98 % for all the tests. The results of these experiments also indicate that growth of *C. vulgaris* can be enhanced under mixotrophic conditions. Light, CO_2 and organic carbon are essential for mixotrophic cultivation of algae. Evaluation of energy utilization efficiency for various cultivation conditions for algae have indicated that the biomass yield on the supplied energy is the lowest for photoautotrophic cultivation [1, 15] and the highest for mixotrophic cultivation. Results further demonstrated that the glycolytic pathway, TCA cycle and mitochondrial oxidative phosphorylation maintained high activities under illumination [15].

The growth results for glycerol are promising, although the glycerol growth rates were lower than the growth rates of glucose. Glycerol is a by- product of biodiesel production that is too expensive to further process for commercial use. The use of glycerol as an external carbon source to facilitate algae growth would create a desirable "closed-loop system" that eliminates waste from biodiesel production. The fact that *C. vulgaris* can utilize organic carbon with higher growth rates is extremely promising. This finding could be interpreted as an important step to use wastewaters that are rich in organic carbon and nutrients as an algae growth media.

IV. Conclusions

Results indicate that *C. vulgaris* is able to utilize both glucose and glycerol as an organic carbon source under mixotrophic cultivation conditions. The biomass and growth rates were both enhanced in the presence of the organic carbon sources in comparison to the photoautotropic conditions. The optimal concentration of organic carbon in the growth medium was 15 g/L for both glucose and glycerol. Growth rates decreased with increasing organic carbon concentrations. The data also indicates that glucose was a more effective organic carbon source for microalgal growth. However, results indicate that growing microalgae with glycerol is feasible. While it is not the ideal growth condition, using crude glycerol by-product to grow more microalgae can be an economical alternative. Mixotrophic cultivation conditions can also reduce lighting and carbon dioxide needs.

Acknowledgment

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References

[1] O. Perez-Garcia, F.M.E. Escalante, L.E.de-Bashan, L.E. and Y. Bashan, "Heterotrophic cultures of microalgae: metabolism and potential products," *Water Res*. vol.45, pp. 11–36, 2011.

[2] A. Bhatnagar, S. Chinnasamy, M. Singh, and K.C. Das, "Renewable biomass production by mixotrophic algae in the presence of various carbon sources and wastewaters," *Appl. Energy*. vol. 88, pp. 3425-3431, 2011.

[3] H. W. Bischoff and H. C. Bold, "Phycological Studies. IV. Some soil algae from Enchanted Rock and related algal species," University of Texas Publications. vol. 6318, pp.1-95, 1963.

[4] M.C. Cerón-García, M.D.Macías-Sánchez, A. Sánchez-Mirón, F. García-Camacho, and E. Molina-Grima, "A process for biodiesel production involving the heterotrophic fermentation of *Chlorella protothecoides* with glycerol as the carbon source," *Appl. Energy*. vol 103, pp. 341-349, 2013.

[5] Y. Chisti,"Biodiesel from microalgae," *Biotechnol. Adv*., vol. 25, pp. 294-306, 2007.

[6] P. Das, W. Lei, S.S. Aziz, and J.P. Obbard,"Enhanced algae growth in both phototrophic and mixotrophic culture under blue light," *Bioresour. Technol*., vol. 102, pp. 3883-3887, 2011.

[7] S. Ethier, K. Woisard, D.Vaughan, and Z. Wen,"Continuous culture of the microalgae *Schizochytrium limacinum* on biodiesel-derived crude glycerol for producing docosahexaenoic acid," *Bioresour. Technol*. vol. 102, pp. 88-93,2011.

[8] W. Kong, H. Yang, Y.Cao, H.Song, S. Hua, and C. Xia, 2012. Effects of glycerol and glucose on the enhancement of biomass, lipid and soluble carbohydrate production by *Chlorella vulgaris* in mixotrophic culture. Original Scientific paper, uncorrected proof.

[9] Y.N. Liang, N. Sarkany, and Y. Cui, "Biomass and lipid productivities of Chlorella vulgaris under autotrophic, heterotrophic and mixotrophic growth conditions," *Biotechnol. Lett*., vol. 31, pp.1043–1049, 2009.

[10] J. Liu, J.Huang, Z. Sun, Y. Zhong, Y. Jiang, Y. and F. Chen, "Differential lipid and fatty acid profiles of photoautotrophic and heterotrophic *Chlorella zofingiensis*: Assessment of algal oils for biodiesel production," *Bioresour. Technol*., vol.102, pp.106-110, 2011.

[11] Y. Shen, Z. Pei, W.Yuan, and E. Mao, "Effect of nitrogen and extraction method on algae lipid yield," *Int. J. Agric. & Biol. Eng*. vol.2 (1), pp.51-57, 2009.

[12] University of Texas. (2013) *The Culture Collection of Algae*. [Online] Available www.sbs.utexas.edu/utex/ default.

[13] D.Yan, Y. Lu, Y. Chen, Y. and Q. Wu,"Waste molasses alone displaces glucose-based medium for microalgal fermentation towards cost-saving biodiesel production," *Bioresour. Technol.*, vol. 102, pp. 6487-6493, 2011.

[14] C.Yang, Q. Hua, Q. and K. Shimizu,"Energetics and carbon metabolism during growth of microalgal cells under photoautotrophic, mixotrophic and cyclic light-autotrophic/dark-

heterotrophic conditions," *Biochem. Engg. J*., vol.6, pp. 87–102, 2000.

[15] K. Yeh, and J. Chang, "Effects of cultivation conditions and media composition on cell growth and lipid productivity of indigenous microalga *Chlorella vulgaris*," *Bioresour. Technol.* vol.105, pp.120-127, 2012.

[16] K. Yeh, C. Chen, and J. Chang, "pH-stat photoheterotrophic cultivation of indigenous *Chlorella vulgaris* ESP-31for biomass and lipid production using acetic acid as the carbon source," *Biochem. Engg.g J.* vol.64, pp.1–7, 2012.

[17] Y. Zheng, Z. Chen, H. Lu, and W. Zhang,"Optimization of carbon dioxide fixation and starch accumulation by *Tetraselmis subcordiformis* in a rectangular airlift photobioreactor," *Afr. J. Biotechnol.* vol.10 (10), pp.1888-1901, 2011.

[18] Y. Zheng, Z. Chi, B.Lucker, and S. Chen, "Two-stage heterotrophic and phototrophic culture strategy for algal biomass and lipid production," *Bioresour. Technol.* vol.103, pp.484-488, 2012.

Jahan, **K.,** R. Hesketh, A. Cunningham, D. O'Connell, M.J. Savelski, C.S. Slater (2012) "Algae Derived Biofuel," Proceedings of the 2012 Global Conference on Global Warming, Istanbul, Turkey, July 2012.

ALGAE-DERIVED BIOFUEL

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I. ABSTRACT

Increased attention to the harmful effects of greenhouse gases, dangers of offshore drilling, and the depletion of oil reserves has prompted focus on biodiesel as an alternative energy source. Biodiesel is currently produced from plant and animal oils such as soybeans, canola oil, animal fat, palm oil, corn oil, waste cooking oil, and jatropha oil. In 2009, the United States consumed 18,686,000 barrels of oil daily, nearly 22% of the world's total share of oil. In order to displace all transport fuel consumed in the United States, the land area required to grow these oil crops would be unsustainably large and interfere with food production. These downfalls have led research to investigate other sources of biodiesel. Biodiesel derived from algae is one of these sources and is considered a sustainable fuel, but proper algae growth conditions and downstream processing is necessary to minimize the environmental footprint of this process. Previous studies (Xu et al. 2011, Uduman 2010, Danquah 2009) show that the dewatering stages are the major contributors to the overall carbon footprint in algae-derived biodiesel. This is due to the thermal drying needed to remove bound intercellular water (Xu et al. 2011).

This paper focuses on experimental and computational results investigating algae-derived biofuel. Experiments were performed to determine the optimum conditions for growth and lipid production of the algae species, *Scenedesmus dimorphus* and *Chlorella vulgaris*. More specifically, the effect of various light intensities, nitrate concentrations, and carbon dioxide concentrations were investigated. Under laboratory growth conditions, the optimum light intensity was 400 ft-c. The algae experienced a growth rate of 0.095/day with a lipid content of 7.80 % dry cell weight (DCW). The nitrate concentration which promoted the fastest growth was 0.4 g/L with a growth rate of 0.112/day. However, the nitrate deficient sample yielded the highest lipid content with 13.26% DCW. Carbon dioxide studies indicate that higher percent of CO_2 can enhance algae growth, with 5-10% CO_2 being optimum.

Additionally, a complete life cycle assessment (LCA) was conducted to help guide the process design decisions necessary to produce biodiesel with minimum emissions to air, water, and soil. A base case was constructed with the harvesting, dewatering, extraction, reaction, and product purification stages included. The LCA for this base case found 11,050 kg of total emissions with 96% of those attributed to the spray dryer used for dewatering. Alternative cases were developed using a disc stack centrifuge, an Evodos® spiral plate centrifuge, a heat assisted rotary filter press, and Delft University's heat integrated dryer. First, the spray dryer was replaced by Delft University's dryer reducing total emissions by 16%. Next, the heat assisted rotary filter press was used prior to delta drying further reducing the total emissions by 75%. The Evodos® centrifuge was then added prior to the filter, resulting in an additional 52% reduction. Replacing the Evodos® centrifuge with the disc stack centrifuge reduced total emissions by only 1%. Using each aforementioned piece of equipment in series found 1,040 kg of total emissions. This is a 91% reduction when compared to the 11,050 kg of total emissions from the base case. The LCA analysis demonstrated that additional mechanical separation methods resulted in only marginal improvements.

II. INTRODUCTION

II.A. Experimental Studies: Optimizing Algae Oil Yield for *Scenedesmus dimorphus* and *Chlorella vulgaris*

The constant demand for diminishing oil resources has driven research into sustainable energy. Biofuels are particularly attractive, because they are carbon neutral, renewable, and readily available in the environment (Shen et al., 2009). Chisti (2007) reported that algae produce more oil per U.S. cropping area than many other oil crops. Algae reproduce quickly, produce oils more efficiently than crop plants, and require relatively few nutrients for growth. Algae can also be grown on land unsuitable for agricultural purposes, eliminating competition with food sources. The lipids algae produce are unsaturated, and therefore, require little to no pretreatment before transformation into biodiesel (Woertz, 2007).

Two different algae species were evaluated in this study, including *Scenedesmus dimorphus* and *Chlorella vulgaris*. These species were chosen due to their distinct morphologies, frequency around the world, and significant lipid content, as displayed in Table 1 (Chisti, 2007). *Scenedesmus dimorphus* consists of moderately sized cells (10-30 microns) that are elongated, bean shaped and arranged in rows. *Chlorella vulgaris* consists of moderately sized cells (10-30 micron) that are circular and arranged separately. These cells have also been converted to heterotrophic reproduction using organic molecules as a carbon source. The images of the select algae are presented in Figure 1.

Table 1. Algae Species with Lipid Content (Becker, 1994)

Species Name	**% Lipid Yield *(Based on Dry Cell Weight)***
Scenedesmus dimorphus	16-40
Chlorella vulgaris	14-22

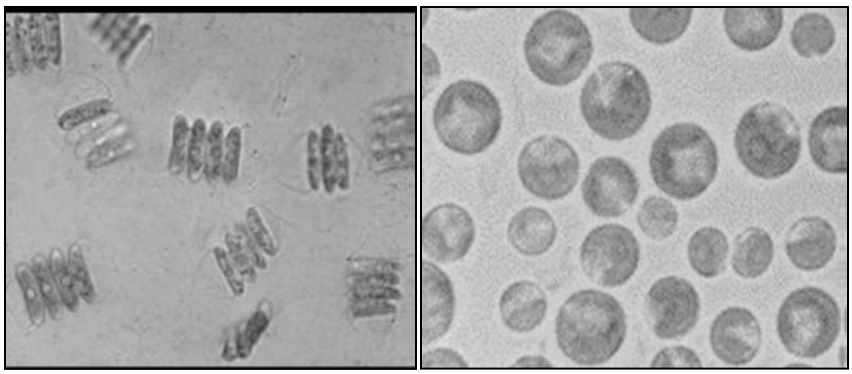

Fig. 1. Images of *Scenedesmus dimorphus* (left) and *Chlorella vulgaris* (right) Cells (Mohan et al. 2010)

Experiments were performed to determine the optimum conditions for growth and lipid production of each algal species. More specifically, the effect of various light intensities, nitrate concentrations, and carbon dioxide concentrations were analyzed. Algae can be grown readily under a variety of environmental conditions. Lipids are produced as a defense mechanism in unfavorable growth conditions. Parameters that affect growth include environmental conditions, such as light exposure and temperature, as well as chemical conditions and nutrients availability. Nutrient concentrations are often changed to produce desired lipid yields and growth rates. Essential nutrients include nitrogen, phosphorous and carbon. Availability of these nutrients affects algal growth and lipid production (Mohapatra, 2002).

II.B. Computational Study: Life Cycle Assessment of Dewatering Routes for Algae Derived Biodiesel Processes

Algae dewatering is one of the major bottlenecks of using algae as a feedstock for biodiesel due to the extensive thermal drying needed to eliminate the intercellular water (Xu et al. 2011). Water removal is required to effectively extract the triacylglycerides (TAGs) from the algae and is most efficient at moisture contents between 5 and 15% (Baligia 2010, Xu et al. 2011). Due to extensive dewatering, a life cycle assessment (LCA) for this stage is required to provide a base reference in comparing other more efficient processing steps. The approaches presented in this paper use viable methods of producing algae derived biodiesel on the commercial scale by adapting and coupling dewatering technologies rather than extrapolating from lab scale techniques. This study expanded on previous findings from Xu et al. (2011) by using a wider range of dewatering equipment. Their research focused on the energy demand required for algae processing, but the work does not address the emissions associated with production of biofuels. For this reason, our work consisted of a rigorous LCA to compare these dewatering technologies when fully integrated into biodiesel production.

Material and energy balances for an industrial scale algae production facility were estimated, and served as the basis to conduct a LCA by evaluating total emissions. This “base case facility” was compared with alternative processing cases, created by implementing potentially scalable dewatering technologies. Total emissions from each stage were quantified, the optimal sequence of dewatering equipment was determined, and the life cycle emissions were compared.

III. METHODS AND MATERIALS

III.A. Experimental Studies: Optimizing Algae Oil Yield for *Scenedesmus dimorphus* and *Chlorella vulgaris*

Algae species, *S. dimorphus* and *C. vulgaris*, were obtained from the algae collection maintained at the University of Texas (UTEX SD# 1237, CV# 2714). The stock cultures were grown in separate 5 gallon glass carboys containing Modified Bold Basal's Medium (Bischoff et al. 1963). The algae were sparged with air at a flow rate of approximately 2 L/min. Hot/Stir plates were used to maintain the cultures at 30 ± 2°C and have well-mixed systems with suspended algae. Illumination was provided by 23 Watt dimmable compact fluorescent bulbs with a color temperature of 6500 Kelvin. The stock cultures were maintained under a photoperiod of 18:6 hours at an approximate light intensity of 400 ft-c.

Temperature, pH, and algal growth were monitored throughout the duration of the experiment. Temperature and pH were measured directly using a Celsius-thermometer and a HACH pH meter, respectively. Growth was monitored using optical density values measured by a HACH DR 4000 Spectrophotometer. Prior to experimentation, samples of *S. dimorphus* and *C. vulgaris* were placed in the spectrophotometer and a wavelength scan was performed to determine what wavelength should be used to measure the optical density of each species. The wavelength of light which was maximally absorbed, 625 nm for both *S. dimorphus* and *C. vulgaris,* was used during experimentation. During experimentation, optical density readings were repeated three times for each sample and then averaged for accuracy. Each sample was refilled with distilled water to its original volume whenever the fluid level dropped over 200 mL. The tests were performed twice per week, over the course of the three to four week growth periods.

Batch experiments were conducted for each growth condition using a modified Phipps and Bird Jar Test Setup. Paddles of the jar test equipment were set to spin at 90 rpm. Each sample throughout the duration of the experiment had a photoperiod of 18 hours of light to 6 hours of darkness. Three experiments were performed to determine how altering growth conditions would affect algal growth and lipid yield. These experiments included: varying nitrate concentrations, varying carbon dioxide concentrations, and varying light intensity.

The first experiment varied light intensity. The experiment included 550 mL samples with an initial optical density of 0.05. Three light intensities were tested, including 400 ft-c, 600 ft-c, and 800 ft-c grown in duplicate.

The second experiment varied nitrate concentrations. The experiment included 800 mL samples under a light intensity of approximately 400 ft-c with an initial optical density of 0.04. Six different nitrate concentrations were tested, including 0.5 g/L, 0.4 g/L, 0.3 g/L, 0.2 g/L, 0.1 g/L, and completely nitrate deficient.

The third experiment varied carbon dioxide (CO_2) concentrations. The experiment included 800 mL samples with attached CO_2 cylinders with a flow rate of approximately 150 mL/min. The light intensity was approximately 400 ft-c with an initial optical density of 0.22. Three different CO_2 concentrations were tested, including 5% CO_2, 10% CO_2, 20% CO_2.

At the end of the experiments, the algae samples were isolated from the medium and lipids were extracted using a modified Bligh and Dyer (1959) method.

III.B. Computational Study: Life Cycle Assessment of Dewatering Routes for Algae Derived Biodiesel Processes

A base case model was developed to compare alternative processes. A capacity of 52,300 t BD/year was chosen based on the typical average production of biodiesel plants (National Biodiesel Board 2010). The plant was designed using scalable technologies and was then converted to the basis of 1 t of BD for comparison. Since no commercial scale algae biodiesel plant is in existence, the facility was designed based on established industrial processes to make an accurate estimation of requirements for a commercial scale biodiesel plant. This is not necessarily the optimal method of producing algae derived biodiesel, but served as a starting point for the LCA. LCA analyses were conducted using alternative cases developed to reduce the emissions associated with algae dewatering. Total mass and energy balances were performed on each stage in the biodiesel process to develop a comprehensive inventory of all inputs and outputs.

The algae biodiesel process begins with algae cultivation, followed by harvesting to separate the algae from the water. The TAGs are then extracted from the biomass and reacted to break down into fatty acid methyl esters (FAMEs), which are high energy content carbon chains with properties similar to those of diesel fuel. The cultivation stage was not included within the LCA boundaries. The following assumptions were made to determine the properties of the algae slurry entering the downstream processing. The microalgae species considered in this process was *Scenedesmus Obliquus*, because of the high lipid yields and wide availability of this algae species (Shovon and Mallick 2009). Goldman et al. (2008) suggested that a harvest concentration of 25 g dry algae per liter of solution is achievable and was used to specify the best case scenario for a harvest density.

The dilute algae solution must be concentrated by removing water before proceeding to the extraction stage. Initial dewatering was performed by a flocculation unit. Aluminum sulfate was added at 250 mg/L and lime was added at 0.73 g/g aluminum sulfate. These flocculants are capable of recovering 95% of the algae within the solution and results in a water-algae slurry of 95% water by weight (Uduman et al. 2010). Spray dryers are commonly used in drying fine slurries; and therefore, the water-algae slurry was fed to a spray dryer where the moisture content was reduced from 95 to 5% (Becker 2008, Lardon et al. 2010). After drying, lipids need to be extracted, for this purpose n-hexane was chosen as the extraction solvent as it is capable of achieving a 95% TAG recovery from dried algae (Xu et al. 2011). The exiting organic phase was assumed to contain only n- hexane and extracted TAGs, while the residual biomass contained the remainder of cell debris and flocculation chemicals. This residual biomass was treated as a waste stream and sent to a landfill. Solvent recovery was performed by a multiple effect forced circulation evaporator such as a rising or falling film system (Perry and Green 2008). Aspen Plus® was used to model the separation of the hexane resulting in 99.5% pure TAGs. Then it is ready for chemical conversion.

The transesterification and purification process was based on an existing model developed by Pokoo-Aikins et al. (2010). This study used an alkali-catalyzed transesterification to convert TAGs and methanol to glycerol and fatty acid methyl esters (FAMEs). Purification of the biodiesel and glycerine was performed in a decanter taking advantages of immiscibility and difference in specific gravity. The excess unreacted methanol was recovered as vapor using a distillation column and the sodium hydroxide catalyst was neutralized with hydrochloric acid. Hydrochloric acid was also used to split any soap formation. The FAMEs were purified by water washing to remove residual catalyst, salts, methanol, free glycerol, and soaps. The water washing resulted in a biodiesel purity of 99.65% by weight (Pokoo-Aikins et al. 2010). This stage includes an environmental credit for producing the co-product glycerine.

IV. RESULTS AND DISCUSSION

IV.A. Experimental Studies: Optimizing Algae Oil Yield for *Scenedesmus dimorphus* and *Chlorella vulgaris*

Experiments were performed to determine the optimum conditions for growth and lipid production of the algae species *S. dimorphus and C. vulgaris*. The three experiments performed included varying light intensities, varying nitrate concentrations, and varying carbon dioxide concentrations. Growth rate and lipid yield were the two factors analyzed in each experiment. The setup with the fastest growth rate and the highest lipid yield was considered most successful in the experiments.

The first experiment performed was the light intensity study. Three different light intensities were tested on species over a 18:6 photoperiod. The first setup used an initial optical density of 0.05 and the second setup used an initial optical density of 1.00. The results were analyzed to attain growth rates and lipid yields for each light intensity. The growth curves are shown in Figure 2. The lipid yields are shown in Table 2.

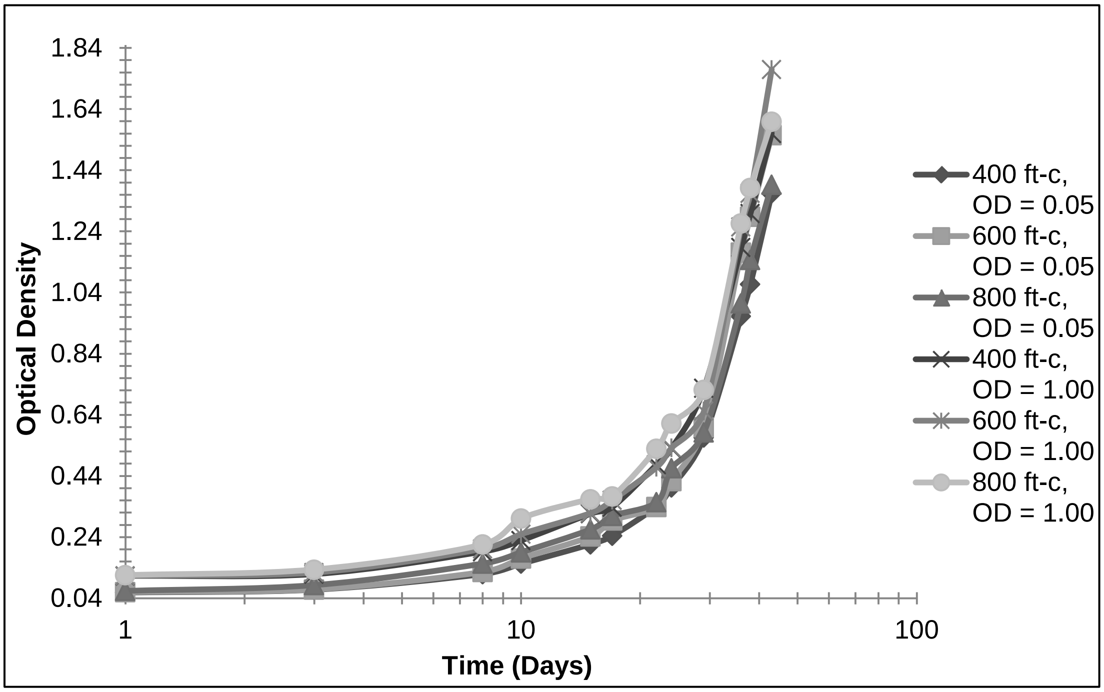

Fig. 2. Growth Rate Comparison of Varying Light Intensities

Table 2. Growth Rate and Lipid Yield Comparison of Varying Light Concentrations

Initial OD	Light Intensity (ft-c)	Growth Rate (doublings/day)	Lipid Content (% DCW)
0.05	400	0.0956	7.799
1.00	400	0.0838	13.263
0.05	600	0.0966	5.777
1.00	600	0.0856	8.001
0.05	800	0.087	4.090
1.00	800	0.0729	10.598

After comparing the growth rates for each light intensity, the results for each setup were not substantially different. Because light intensity did not significantly affect growth, the most economical light intensity would be 400 ft-c. When comparing lipid yield, in both optical density conditions, a light intensity of 400 ft-c proved to maximize lipid yield. Therefore, based on growth rate and lipid yield, a light intensity of 400 ft-c was considered to be the most optimal.

The second experiment performed was the nitrate concentration study. The standard growth medium of Bold's Basal contains 25 g/L of nitrate in the form of sodium nitrate. For this experiment, the concentration of sodium nitrate was varied in each experiment to create a total of six different mediums. Six different nitrate concentrations in the growth medium were tested and then analyzed to attain growth rates and lipid yields. The growth curves are shown in Figure 3. The lipid yields are shown in Table 3.

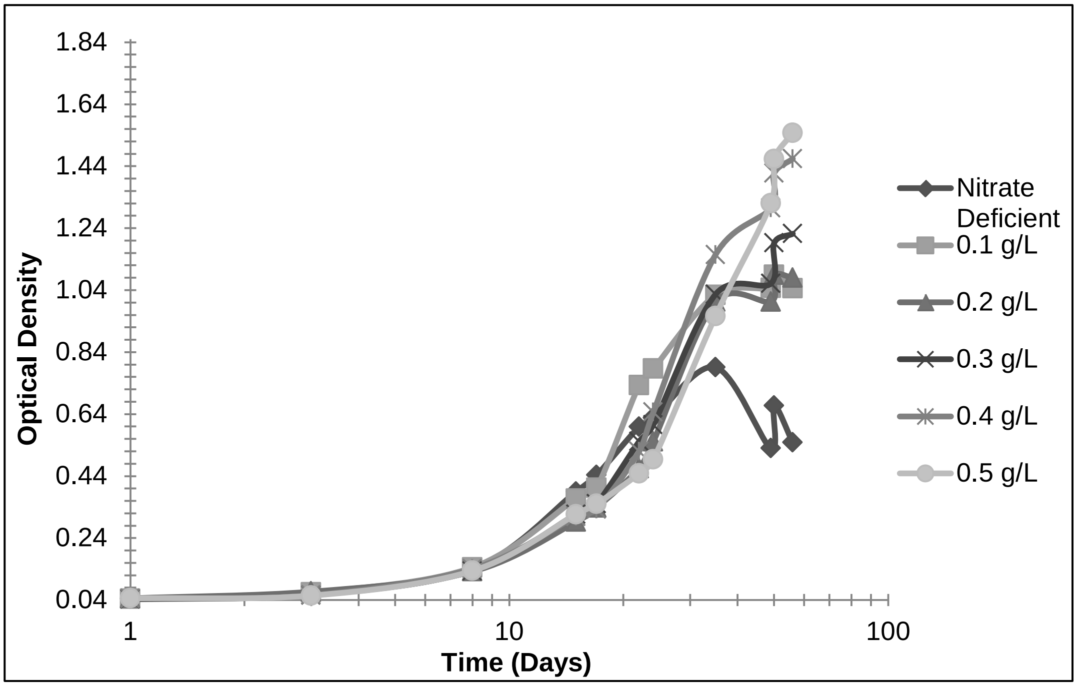

Fig. 3. Growth Rate Comparison of Varying Nitrate Concentrations

Table 3. Growth Rate and Lipid Yield Comparison of Varying Nitrate Concentrations

Nitrate Concentration (g/L)	Growth Rate (doublings/day)	Lipid Content (% DCW)
Deficient	0.0927	13.259
0.1	0.1043	2.938
0.2	0.1082	2.686
0.3	0.1087	7.278
0.4	0.1123	4.185
0.5	0.0820	6.058

The results show that the nitrate deficient sample yielded the highest lipids. As a defense mechanism, algae produce lipids when they are stressed. The competition of nutrient depletion caused the nitrate deficient algae to produce more lipids than in an ideal nutrient condition. Although the lipid yield was maximized with the nitrate deficient sample, the growth rate was minimal. As the growth rates show, a system can be oversaturated with nitrate when it is concentrated at 0.5 g/L, causing a decrease in growth rate. The ideal growth condition for nitrate concentration is 0.4 g/L. All samples experienced an increase in pH as the study progressed. This indicates that CO_2 was a limiting growth factor.

The third experiment performed was the carbon dioxide concentration study.

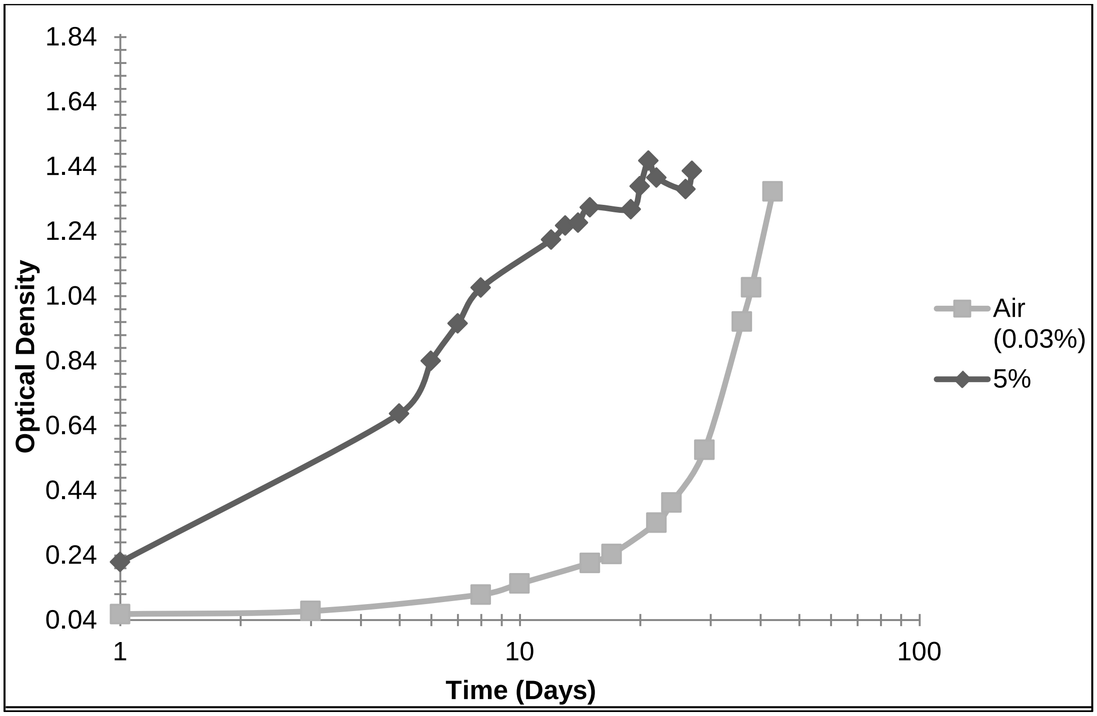

Fig. 4. Growth Rate Comparison of Varying Carbon Dioxide Concentrations

Table 4. Growth Rate and Lipid Yield Comparison of Varying Carbon Dioxide Concentrations

CO_2 Concentration (% v. CO_2/ v. Air)	Growth Rate (Doublings/Day)	Lipid Content (% DCW)
Air (0.03%)	0.0956	7.799
5%	0.1367	30.3
10%	N/A	N/A
20%	N/A	N/A

IV.B. Computational Study: Life Cycle Assessment of Dewatering Routes for Algae Derived Biodiesel Processes

The total life cycle emissions were analyzed for the base case process according to their respective production stages. These emissions are the summation of all emissions to the air, water, and soil. It was determined that the emissions to air contribute to 97% of the total life cycle emissions and CO_2 emissions contribute to 99% of these emissions to air. The extraction step is different from the other stages with the majority of emissions to water. The emissions to water in the extraction step make up 98% of the total emissions due to the landfilling of solid biomass waste. Figure 5 shows the contribution of each processing step to the total life cycle emissions.

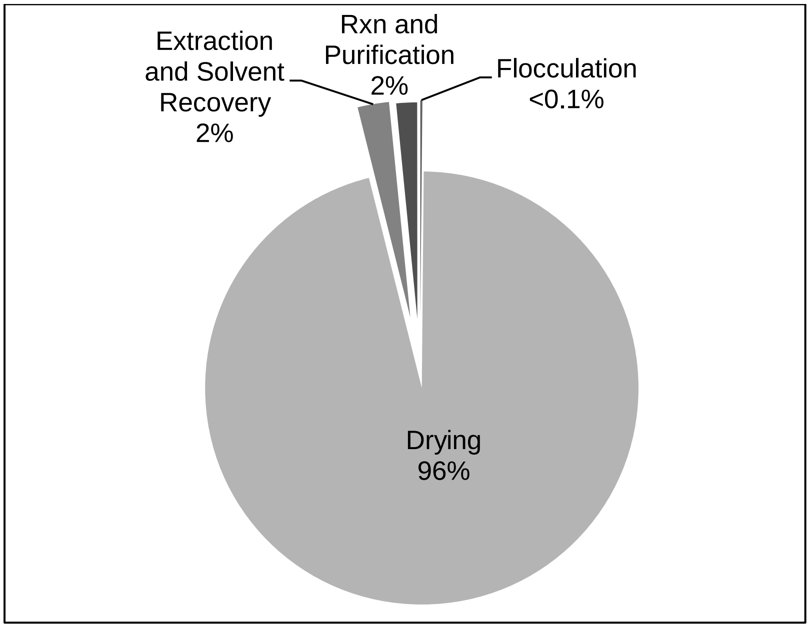

Fig. 5. Base Case Emissions Distribution

The drying step makes up 96% of the total life cycle emissions due to the large quantity of steam required to evaporate the water within the algae. The LCA for the base case shows the drying stage has the greatest opportunity for improvement. Thermal driers can achieve the low moisture contents needed, but the base case demonstrated that this is not energy efficient for high moisture content slurries. These results agree with Lardon et al. (2009) who found that 85% of the process energy came from drying the algae and Xu et al. (2011) who found that approximately 90% of the process energy was due to thermal drying.

Although thermal drying is undesirable, bound intercellular water currently cannot be removed mechanically. Therefore, thermal drying will ultimately be required to achieve 95% dry algae. A steam rotary dryer and a heat integrated dryer were considered as alternatives to spray drying. The steam rotary dryer consumes approximately 3 MJ/ kg of water removed (Fagernas et al. 2010). The heat integrated dryer was developed by Delft University for drying a biomass type sludge and consumes 2 MJ/ kg of water removed (Van Gemert 2009). This dryer uses hot balls to contact the algae slurry under a vacuum, and condenses the water vapor over the metal balls to recover the heat. The production capacity for this technology is currently at 1,000 kg of dried sludge/hr (Hartmann 2004).

In addition, centrifugation and filtration were investigated as means to reduce the energy consumed by thermal-drying operations.

Three different types of centrifuges were considered. The first was a disk-stack centrifuge capable of removing water to approximately 12% dry algae content (Molina Grima 2003). This centrifuge has a processing capacity of 85 m^3/hr and a power consumption of 45 kW. The second centrifuge was a decanter bowl centrifuge. The decanter bowl centrifuge produces a 22 % dry algae slurry, is available at commercial capacities, and consumes 8 kWh/ kg of water removed (Molina Grima 2003). The third centrifuge was a novel centrifuge developed by Evodos® (Breda, The Netherlands) for dewatering algae. It is capable of achieving 31.5% dry algae weight, consumes 0.95 kWh/m^3 of algae slurry processed, and can process up to 40 m^3 of slurry/hr (Algae Industry Magazine 2012).

Tangential flow filtration, chamber filter press, and a heat assisted rotary pressure filter were the filtration methods investigated. The tangential flow filtration was based off a study by Danquah et al. (2009) which found an energy consumption of 2.06 Wh/ kg of water removed and achieved a final dry algae concentration of 8.8%. The chamber filter press consumes 0.88 kWh/kg of water removed and can achieve a 27% dry algae concentration (Molina Grima 2003). The heat assisted rotary pressure filter was used in a study conducted by Mahmood et al. (1998) on biomass type sludge drying. This study used a filter that increased the solids concentration from 33% to 56% while using 60 kWh/dry t sludge. This filter has a capacity of processing 200 tons of sludge/hr. It was assumed that this equipment can handle algae concentrations as low as 22%.

Using these dewatering methods, alternative cases were developed. Material and energy balances were performed for six alternative dewatering configurations, and comprehensive LCA was performed on each case. The best case from this analysis is shown in Figure 6.

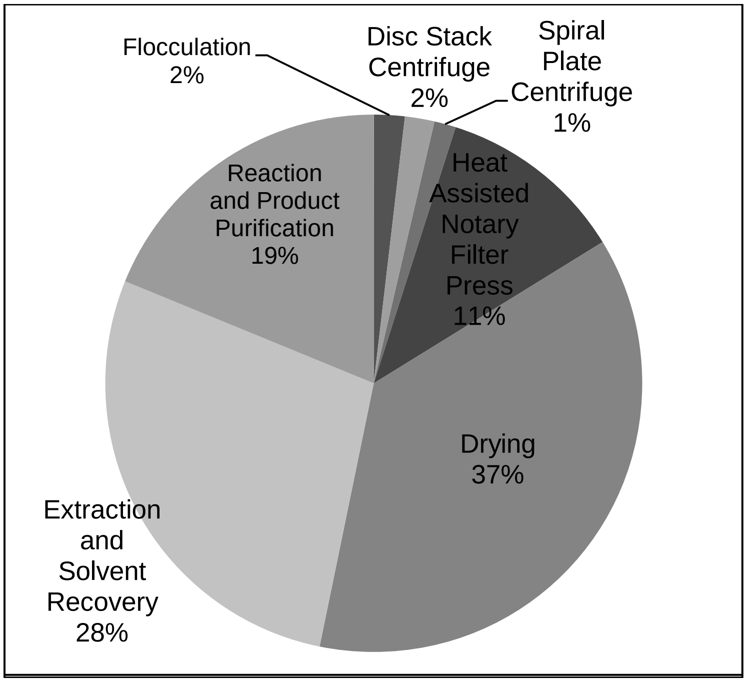

Fig. 6. Total Emissions Distribution for the Best

The disc stack centrifuge was used to dewater the slurry to 12% dry algae, followed by the spiral plate centrifuge to achieve 31.5% dry algae content. This algae-water mixture was sent to a heat assisted rotary filter press to increase the dry algae content to 56% and a heat integrated dryer to attain 95% dry algae. The sequencing of dewatering technologies reduced the total life cycle emissions by 91% compared to the base case. This shows that optimal dewatering sequencing is essential to minimize the environmental impact from the algae biodiesel process.

V. CONCLUSIONS

V.A. Experimental Studies: Optimizing Algae Oil Yield for *Scenedesmus dimorphus* and *Chlorella vulgaris*

Algae samples that were less concentrated experienced a faster growth rate at all light intensities. This is attributed to mutual cell shading which affects light penetration at higher algae concentrations. No significant difference in growth rate was observed at the three different light intensities. Thus, 400 ft-c would be the most economical light intensity for growth and lipid production. As expected, the nitrate deficient sample contained the highest percentage of lipids while higher nitrate concentrations promoted faster growth. All samples experienced an increase in pH as the study progressed. This indicates that CO_2 was a limiting growth factor. It has been observed that sparging with CO_2 has reduced the length of the lag phase and promoted faster growth than previous studies.

Fossil fuel has dangerous effects on the environment, and is not a sustainable source of energy. Algae-derived biofuels are an alternative energy that is renewable and does not have detrimental environmental effects.

V.B. Computational Study: Life Cycle Assessment of Dewatering Routes for Algae Derived Biodiesel Processes

The best case used a disc stack centrifuge, spiral plate centrifuge, heat assisted rotary pressure filter, and heat integrated dryer, which resulted in a 91% reduction in total life cycle emissions compared to the base case, 53% of which are attributed to dewatering. The drying component was responsible for 37% of the total downstream life cycle emissions. Sequencing dewatering technologies resulted in emissions reduction compared to the base case, but the impact of dewatering and drying is still significant. Performing the TAG extraction under considerable water content would avoid thermal drying, which may decrease total emissions. Wet extraction methods could potentially result in a lower environmental footprint by removing the stage with the largest impact (Xu et al. 2011, Halim 2012).

VI. ACKNOWLEDGEMENTS

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VII. REFERENCES

Algae Industry Magazine. 2012. Evodos Expanding Product Line. http://www.algaeindustrymagazine.com/evodos-expanding-product-line/. Accessed 15 February 2012.

Becker, E. W. 2008. *Microalgae: Biotechnology and Microbiology*. Cambridge University Press, Cambridge.

Bligh, E.G. and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*. 37: 911-917.

Bischoff, H.W. and Bold, H.C. 1963. Phycological Studies IV. Some soil algae from Enchanted Rock and related algal species. *University of Texas Publications*. 6318: 1-95.

Chisti, Y. 2007. Biodiesel from microalgae. *Biotechnology Advances*. 25: 294-306.

Danquah, M. K., L. Ang, N. Uduman, N. Moheimani, and G. M. Forde. 2009. Dewatering of microalgal culture for biodiesel production: exploring polymer flocculation and tangential flow filtration. *Journal of Chemical Technology and Biotechnology*. 84: 1078-1083.

Fagernas, L., J. Brammer, C. Wilen, M. Lauer, and F. Verhoeff. 2010. Drying of biomass for second generation synfuel production. *Biomass and Bioenergy*. 34: 1267-1277.

Goldman, A. J., M. Kagan, Y. Kokotov. 2008. High Density Bioreactor System, Devices. Patent Application Publication 20080293132 (Pending), 27 November 2008.

Halim, R., M. K. Danquah, and P. A. Webley. 2012. Extraction of oil from microalgae for biodiesel production: A review. *Biotechnology Advances*. 30: 709-732.

Hartmann, D. 2004. Hot balls dry sludge. Delft Outlook. *Science and Research*. 3: 20-23.

Lardon, L., A. Helias, B. Sialve, J. Steyer, and O. Bernard. 2009. Life-cycle assessment of biodiesel production from microalgae. *Environmental Science and Technology*. 43: 6475-6481.

Mahmood, T., M. Zawadzki, and S. Banerjee. 1998. Pilot study of impulse drying industrial sludge. *Environmental Science Technology*. 32: 1813-1816.

Mohan, L., P. Sharma, and C. Srivastava. 2010. Combination larvicidal action of Solanum xanthocarpum extract and certain synthetic insectidies against filarial vector, Culex quinquefascitatus (Say). *Southeast Asian Journal of Tropical Medicine and Public Health*. 41: 311-319.

Mohapatra, P. K., N. Nayak, and R. C. Mohanty. 2002. Effect of nitrogen and phosphorus limitation on growth of some planktonic green algae. Indian Journal of Microbiology. 42: 309-314.

Molina Grima, E., E. H. Belarbi, F. C. Acien Fernandez, A. R. Redina, and Y. Christi. 2003. Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnology Advances*. 20: 491-515.

National Biodiesel Board. 2010. National Biodiesel Board Member Plants. http://nbb.org/buyingbiodiesel/plants/showall.aspx. Accessed 25 October 2010.

Perry, H. and D. W. Green. 2008. *Perry's Chemical Engineers' Handbook*. McGraw-Hill, New York.

Pokoo-Aikins, G., A. Nadim, M. M. El-Halwagi, and V. Mahalic. 2010. Design and analysis of biodiesel production from algae grown through carbon sequestration. *Clean Technologies and Environmental Policy*. 12: 239-254.

Shen, Y., Z. Pei, W. Yuan, and E. Mao. 2009. Effect of nitrogen and extraction method on algae lipid yield. International Journal of Agricultural and Biological Engineering. 2: 51-57.

Shovon, M. and N. Mallick. 2009. Microalga scenedesmus obliquus as a potential source for biodiesel production. *Applied Microbiology and Biotechnology*. 84: 281-291.

Singh, R. P. and D. R. Heldman. 2009. *Introduction to Food Engineering*. Academic Press, Burlington.

Uduman, N., Y. Qi, M. K. Danquah, G. M. Forde, and A. Hoadley. 2010. Dewatering of microalgal cultures: a major bottleneck to alga-based fuels. *Journal of Renewable and Sustainable Energy*. 2: 1-15.

Van Gemert, G. W. 2009. The delta dryer: theoretical and technological development of an energy-efficient dryer for sludge. Dissertation, Delft University of Technology.

Xu, L., D. W. F. Brilman, J. A. M. Withag, G. Brem, and S. Kersten. 2011. Assessment of a dry and wet route for the production of biofuels from microalgae: Energy balance analysis. *Bioresource Technology*. 102: 5113-5122.

Woertz, I. C. 2007. Lipid productivity of algae grown on dairy wastewater as a possible feedstock for biodiesel. California Polytechnic U., Dept. of CEE. 1-75.

Effect of Nutrients on Oil Yields of Algal Species, *Scenedesmus dimorphus* and *Chlorella vulgaris*

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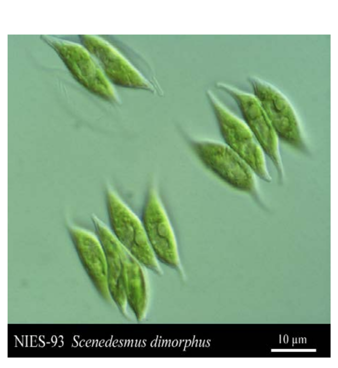
Introduction

- Constant demand for diminishing oil resources is driving research into new energy sources
- Biofuels from microalgal species are a potential new energy source
- Microalgae produce more oil per U.S. cropping area than many other oil crops
- Algae produce oil in the form of unsaturated lipids as a defense mechanism in unfavorable growth conditions
- Growth conditions include nutrient availability, carbon availability, lighting, pH variability, and temperature
- Important nutrients include Nitrogen, Phosphorus, Carbon, Oxygen, and Hydrogen
- Fresh water algae species, *Scenedesmus dimorphus* and *Chlorella vulgaris*, were used
- Effects of nutrients on growth rate and lipid yield were studied in experimental and control conditions

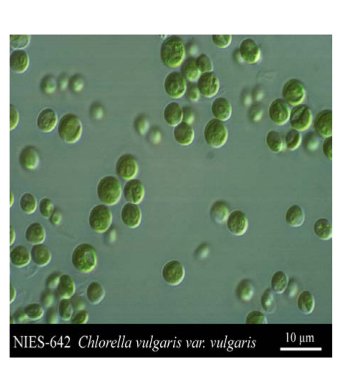
Algal Species

Scenedesmus dimorphus



•Unicellular bean shaped algae, 10 μm in size
•Preferred species for oil yield, particularly for biodiesel
•Lipid Content 16-40% (based on dry cell weight)

Chlorella vulgaris



•Circular shaped algae, 2-5 μm in size
•Widely studied algal species for biodiesel production
•Lipid Content 14-22% (based on dry cell weight)

Experimental Setup

•Modified Jar Test Setup, 1,000 mL samples
•Constantly stirred at ≈ 90 rev/min
•Constant Light Intensity of ≈ 400 ft-candles
•*S. dimorphus* and *C. vulgaris*, each in three different modified Bold's Basal mediums
•Control Bold's Basal medium for each species
•Light intensity, temperature and CO2 concentrations were kept constant for all experiments.

Table 1: Nitrate and Phosphate Concentrations and N:P Used in Each Experimental Setup

Medium	NO_3 Concentration (mg/L)	PO_4 Concentration (mg/L)	N:P
Bold's Basal (Control)	182.4	163.2	3:1
Experiment 1	10.94	163.2	0.05:1
Experiment 2	182.4	749.1	0.15:1
Experiment 3	120.4	326.4	0.20:1

Results

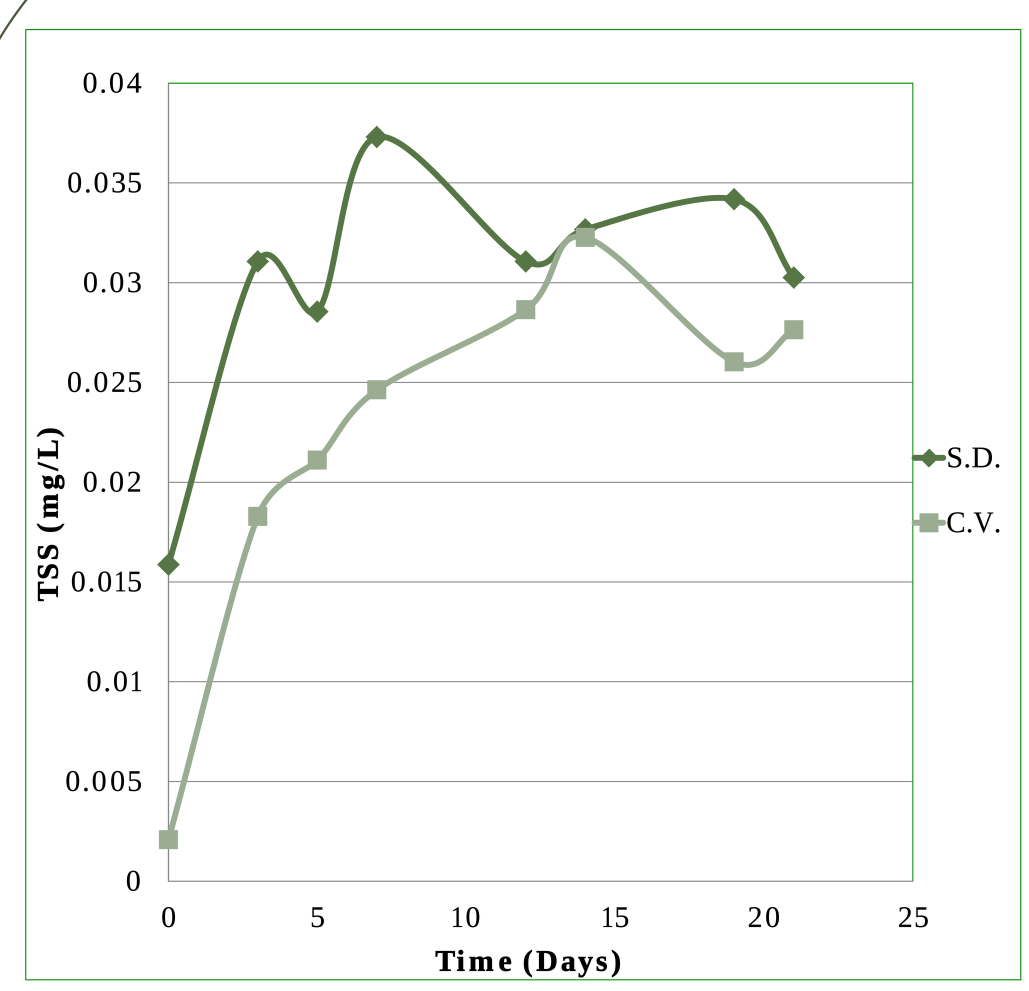


Figure 1: Growth Curve of *S. dimorphus* and *C. vulgaris* Grown in Experiment 1, N:P of 0.05:1

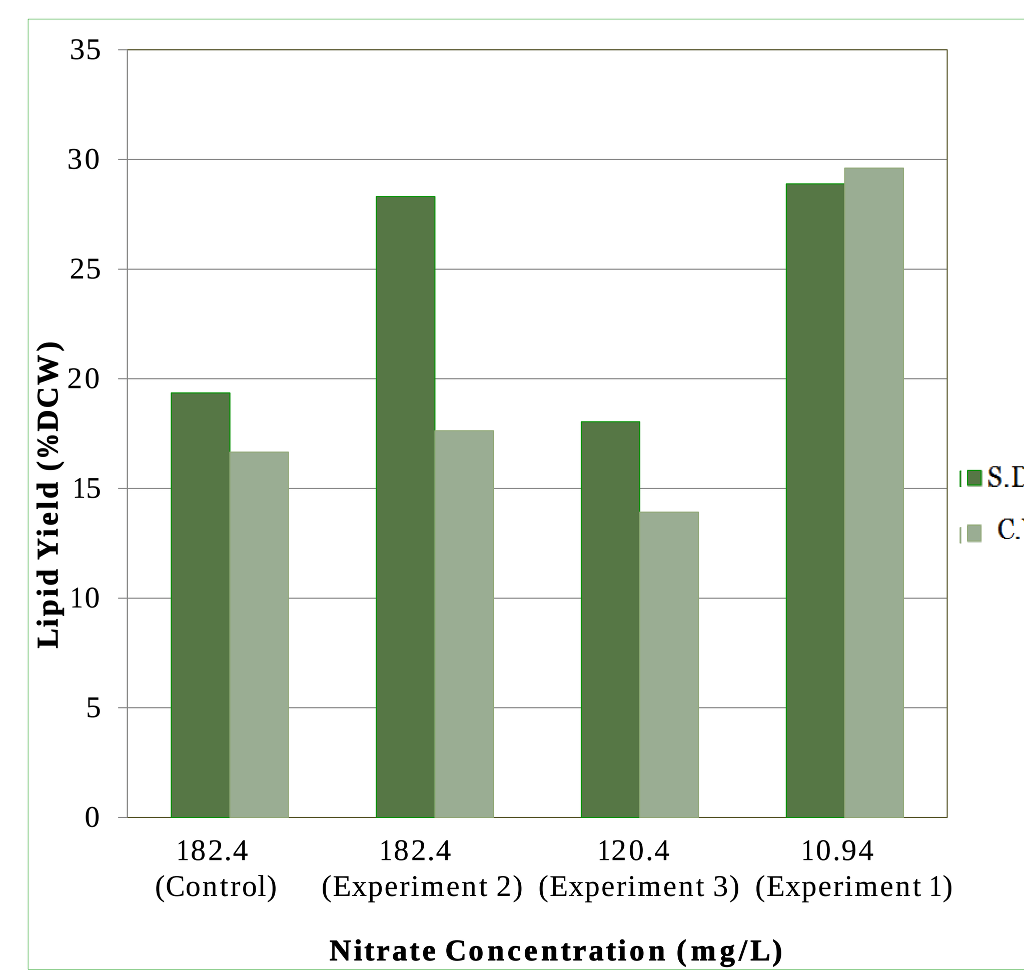


Figure 2: Lipid Yields Resulting from Different Nitrate Concentrations

Table 2: Nitrate Consumption Organized by Species Studied and Medium Used for Cultivation

Species	Medium	Nitrate Consumption (%)
S. dimorphus	Bold's Basal (Control)	97.7
	Modified 1	94.8
	Modified 2	43.0
	Modified 3	51.1
C. vulgaris	Bold's Basal (Control)	68.5
	Modified 1	96.7
	Modified 2	14.8
	Modified 3	47.8

Table 3: Growth Rate and Lipid Yield Results Organized by Species Studied and Medium Used for Cultivation

Species	Medium	Growth Rate (day^{-1})	Lipid Yield (%DCW)
S. dimorphus	Bold's Basal (Control)	0.0158	19.36
	Modified 1	0.0092	28.89
	Modified 2	0.0128	28.31
	Modified 3	0.0061	18.04
C. vulgaris	Bold's Basal (Control)	0.0125	16.66
	Modified 1	0.004	29.61
	Modified 2	0.0041	17.63
	Modified 3	0.0049	13.92

•Table 3 shows that nitrogen deficient conditions decrease algal growth rates and increase lipid yield
•In nitrate deficient experiments, lipid yields were much higher than the control value and growth rates were drastically impacted

Conclusions

- Growth mediums with nitrogen deficiencies produced higher lipid yields; lower growth rates compared to the control medium
- *S. dimorphus* yielded higher growth rates and lipid yields than *C. vulgaris* under nitrogen deficient conditions
- Phosphorus deficiencies did not show a conclusive effect on the growth rate or lipid yield of either algal species

Jahan, K., A. Cunningham, J. Piccillo, S. Bauer and A. McFarland (2011) Optimizing Algae Oil Yield for *Scenedusmus Dimorphus* and *Chlorella Vulgaris*, Algae Biomass Summit, Minneapolis MN.

Optimizing Algae Oil Yield for *Scendusmus dimorphus* and *Chlorella Vulgaris*

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Increased attention to the harmful effects of greenhouse gases, dangers of offshore drilling, and the depletion of oil reserves has prompted focus on biodiesel as an alternative energy source. Biodiesel is currently produced from plant and animal oils such as soybeans, canola oil, animal fat, palm oil, corn oil, waste cooking oil, and jatropha oil. In 2009, the United States consumed 18,686,000 barrels of oil daily, nearly 22% of the world's total share of oil. In order to displace all transport fuel consumed in the United States, the land area required to grow these oil crops would be unsustainably large and interfere with food production. These downfalls have led research to investigate other sources of biodiesel. One of the most promising alternatives is algae biodiesel. Algae reproduce quickly, produce oils more efficiently than crop plants, and require relatively few nutrients for growth. These nutrients can potentially be derived from inexpensive waste sources such as flue gas and wastewater, providing a mutual benefit of helping to mitigate carbon dioxide waste. Algae can also be grown on land unsuitable for agricultural purposes, eliminating competition with food sources.

Experiments were performed to determine the optimum conditions for growth and lipid production of two algae species *Scenedesmus dimorphus and Chlorella vulgaris*. The effect of light intensities, nitrate and carbon dioxide concentrations were investigated. Under laboratory growth conditions, the optimum light intensity was 400 ft-c for *Scenedesmus dimorphus*. The algae experienced a growth rate of 0.095/day with a lipid content of 7.80 % dry cell weight (dcw). The nitrate concentration which promoted the fastest growth was 0.4 g/L with a growth rate of 0.112/day. However, the nitrate deficient sample yielded the highest lipid content with 13.26% dcw. Carbon dioxide studies indicate that higher % of CO_2 can enhance algae growth with 10% CO_2 in air yielding high lipid yield. *Chlorella vulgaris* experiments are currently in progress and will be completed end of May.

The results of the experiments indicate that by optimizing light, nutrient and CO_2 concentrations the lipid yield for algae can be maximized.

- **Topic(s) in which abstract falls: <u>Engineering and Analysis E8</u>**
- **Poster Presentation**