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Algae Derived Biofuel

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FINAL REPORT

Project Title: Algae to Ethanol Research and Evaluation (NJ)

Award Number: EE0003113

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Dr. Mariano Savelski, Professor Chemical Engineering Dr. C.S. Slater, Professor Chemical Engineering Dr. R.P. Hesketh, Professor Chemical Engineering

Rowan University, Glassboro, New Jersey 08081

SUB-AWARDEES

1. Garden State Ethanol, New Brunswick, New Jersey

2. Algaedyne Corporation Preston, Minnesota

March 2015

Executive Summary:

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 sourcesThis project focuses on a number of innovative research areas where more development is necessary to make algae derived biofuel an attractive option.

The overall goals of this project were to optimize lipid yield for select algae; conduct feasibility studies (lab and pilot scale) for the use of hollow fiber membranes for delivery of $CO₂$ for algae growth and to conduct energy and LCA (Life Cycle Analysis) studies for the algae separation processes.

Research was conducted in three major areas:

- Algae Growth Studies for Optimizing Lipid Yield
- Use of Membrane Technology for $CO₂$ Transfer
- Energy and LCA studies

The overall objectives of the project were to

- Determine algae species and optimize lipid yield under various cultivation conditions
- Evaluate carbon dioxide and oxygen gas transfer characteristics of hollow fiber membrane modules
- Evaluate the design criteria of continuous flow algae reactors
- Evaluate the environmental footprint of the downstream processing for algal feedstocks conversion to biodiesel

Algae Studies: 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, fast growth rate, easy cultivation and significant lipid content. Algae were obtained from UTEX (UTEX 395 and UTEX 1237) and Bold's Basal media was used for algae cultivation. Batch studies were conducted to determine algae growth rates and lipid yield by varying light intensity, carbon dioxide and nutrient concentrations, and cultivation conditions. Cultivation conditions included experiments under dark conditions and

also under mixotrophic conditions where glucose/glycerol was added as an organic carbon source. Results indicated that nitrate deficiency with $5-10\%$ CO₂ under a light intensity of 400 foot candles provided the best oil yield for both algae species. *Chlorella vulgaris* was able to grow under mixotrophic conditions. A higher growth rate was observed for glucose. 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.

Membrane Studies: CO_2 transfer experiments were performed using membrane modules constructed with microporous hydrophobic (polypropylene) hollow fiber membranes (Celgard X40-200, Hoechst-Celanese, Charlotte, NC). Membrane modules were operated in a sealed-end, parallel flow configuration. Both hydrophilic and hydrophobic membranes were evaluated for CO² gas transfer. The membrane modules were evaluated under varying pressure, flowrate and surface area so that the results could provide design data for pilot scale studies. Results indicated that the Sherwood number depended on the Reynolds number. A significant pressure dependence was apparent and it was observed that increasing pressure by 25% increased Sh by factor of 3.

Dewatering Studies by GSE: GSE worked with Algaedyne, its technology company to determine dewatering of harvested algae using various available commercial technologies. GSE directed Algaedyne to conduct several studies of harvesting techniques from its photobioreactors. Chlorella Vulgaris (CV) and Haematococcus Pluvialis (HP) were grown in two different sized bioreactors rated by both volume of working fluid, the growth media, and the length of photon injection into the depths of the tanks. The optical and system technology matched the emission wavelengths of LEDs with absorption wavelengths of primary and accessory pigments in autotrophs to maximize growth, and yield for algal cultivation. Three methods of dewatering were employed and compared: low temperature evaporation, centrifuge, and Phyco BioSciences' Algae ventures HDD capillary action dewatering system. Harvesting and dewatering algal cultures requires taxa specific procedures. Using Ax-10 bioreactors from Algaedyne and the HDD water separation system from Algaeventure, algal biomass was successfully cultivated, harvested and dewatered to a 4-5% dry weight. Cultivating HP (Haematococcus Pluvialis) requires a harvest method that is low energy but effective in transferring samples with the highest densities of suspended alga. A low power pump proved effective to remove settled layers of algae once circulation and aeration was suspended. The HDD system provided a practical solution to dewatering the algae to flake and powder achieving the 4-5% moisture content suitable for vacuum packaging and rail transport.

Algaedyne Studies: In paired pilot-scale studies for each of the experimental strains, hollow fiber gas delivery proved superior to standard sparging. AX-50 and AX-500 systems were used in these pilot scale studies. Average growth for experimental replicates were approximately double for fiber gas delivery systems compared to those with sparge gas delivery. This state is valid for both *Chlorella vulgaris* and *Scenedesmus dimorphus*. Growth rates for the AX-500 experiment was slower than that seen in the AX-50 system. This is attributed to rate limitation of the fiber system being used in the AX-500, specifically the volume of water was an order of magnitude greater and the fiber system and gas flow rate were unmodified. Despite these modifications, post experimental harvest values were just above 1 g/L dry mass (1.13g/L).

LCA Studies: Analysis of the life cycle emissions associated with downstream processing stages for algal biodiesel was performed. A "base case" was developed for comparison using typical commercial technologies, which revealed that the thermal drying component contributed to the majority of life cycle emissions. Alternative cases were evaluated for various sequences of mechanical and thermal dewatering techniques. The best case, consisted of a disc stack centrifuge, followed by the chamber filter press, and a heat integrated dryer. This resulted in 875 kg emissions /t of biodiesel, a 91% reduction from the base case. Significant reductions in life cycle emissions were achieved for all mechanical dewatering alternatives compared to the base case, but further improvements using these existing technologies were limited. Additional improvements will require the development of new techniques for water removal or wet extractions. The following recommendations were made from the LCA study:

- *Minimize use of processes like thermal dryers that consume the most energy/generate most life cycle emissions*
- *Optimize the use of separation technologies*
- *Evaluate energy of novel downstream processes*
- *Model developed to confirm design approach*
- *Outcomes can help guide decision makers in sustainable design of commercial facilities*

Conclusions:

- Algae cultivation experiments indicated that lipid yield is enhanced under nutrient deficient conditions in the presence of an organic substrate
- Not all algae species can use organic carbon
- Membrane delivery of $CO₂$ enhanced algae growth in pilot scale studies
- Harvesting of algae is critical to avoid membrane biofouling
- Minimize use of processes like thermal dryers that consume the most energy/generate most life cycle emissions and optimize the use of separation technologies
- Evaluate energy of novel downstream processes

Provide a comparison of the actual accomplishments with the goals and objectives of the project

The overall objectives of the project were to

• **Determine algae species and optimize lipid yield under various cultivation conditions**

This objective was met successfully (100%). A thorough literature review was conducted to determine algae species that would meet certain criteria for fulfilling growth and lipid yield. The cultures identified included *Scenedusmus dimorphus* and *Chlorella vulgaris.* These species were chosen due to their distinct morphologies, frequency around the world, fast growth rate, easy cultivation and significant lipid content. Algae were obtained from UTEX (UTEX 395 and UTEX 1237) and Bold's Basal media was used for algae cultivation. Batch studies were conducted to determine algae growth rates and lipid yield by varying light intensity, carbon dioxide and nutrient concentrations, and cultivation conditions. Cultivation conditions included experiments under dark conditions and also under mixotrophic conditions where glucose/glycerol was added as an organic carbon source. Results indicated that nitrate deficiency with $5-10\%$ CO₂ under a light intensity of 400 foot candles provided the best oil yield for both algae species. *Chlorella vulgaris* was able to grow under mixotrophic conditions. A higher growth rate was observed for glucose. 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.

• **Evaluate carbon dioxide and oxygen gas transfer characteristics of hollow fiber membrane modules**

This objective was met successfully (100%). Studies on absorption of $CO₂$ into an aqueous solution using hydrophobic microporous hollow fiber membranes (HFM's) were performed. Parameters varied in the experiments included: # of fibers, Membrane Length, Shell Diameter, Flowrate, and Operating Gas Pressure. All of the experiments were performed for $50 < Re <$ 2700. The overall mass transfer coefficients were expressed in their dimensionless form as the Sherwood number.

The use of HFM's for $CO₂$ transfer could significantly improve the biomass growth rate in a photobioreactor for algal biofuel production. The membrane modules were operated in a sealedend, parallel flow configuration. Several modules were constructed that ranged in interfacial surface area from 466 to 1397 m^2/m^3 . The mass transfer coefficients were calculated based on a model of the system that included a prediction of the internal axial gas concentrations within the fiber lumen. This model was validated by measuring bulk gas velocity within fiber lumen. A trend of increasing mass transfer coefficients with internal pressure was observed. A correlation for predicting this effect was developed. The following observations were made

- Sh dependent on Re as expected
- Significant pressure dependence apparent
- Increasing pressure by 25% increases Sh by factor of 3

• **Evaluate the design criteria of continuous flow algae reactors**

This task was carried out by Garden State eEthanol and Algaedyne and was completed successfully. GSE worked with Algaedyne, its technology company to determine dewatering of harvested algae using various available commercial technologies. GSE directed Algaedyne to conduct several studies of harvesting techniques from its photo-bioreactors*. Chlorella vulgaris* (CV) and *Haematococcus pluvialis* (HP) were grown in two different sized bioreactors rated by both volume of working fluid, the growth media, and the length of photon injection into the depths of the tanks. The optical and system technology matched the emission wavelengths of LEDs with absorption wavelengths of primary and accessory pigments in autotrophs to maximize growth, and yield for algal cultivation. Three methods of dewatering were employed and compared: low temperature evaporation, centrifuge, and Phyco BioSciences' Algae ventures HDD capillary action dewatering system. Harvesting and dewatering algal cultures requires taxa specific procedures. Using Ax-10 bioreactors from Algaedyne and the HDD water separation system from Algaeventure, algal biomass was successfully cultivated, harvested and dewatered to a 4-5% dry weight. Cultivating HP (Haematococcus Pluvialis) requires a harvest method that is low energy but effective in transferring samples with the highest densities of suspended alga. A low power pump proved effective to remove settled layers of algae once circulation and aeration was suspended. The HDD system provided a practical solution to dewatering the algae to flake and powder achieving the 4-5% moisture content suitable for vacuum packaging and rail transport.

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• **Evaluate the environmental footprint of the downstream processing for algal feedstocks conversion to biodiesel**

This task was carried out successfully. Analysis of the life cycle emissions associated with downstream processing stages for algal biodiesel was performed. A "base case" was developed for comparison using typical commercial technologies, which revealed that the thermal drying component contributed to the majority of life cycle emissions. Alternative cases were evaluated for various sequences of mechanical and thermal dewatering techniques. The best case, consisted of a disc stack centrifuge, followed by the chamber filter press, and a heat integrated dryer. This resulted in 875 kg emissions /t of biodiesel, a 91% reduction from the base case. Significant reductions in life cycle emissions were achieved for all mechanical dewatering alternatives compared to the base case, but further improvements using these existing technologies were

limited. Additional improvements will require the development of new techniques for water removal or wet extractions. The following recommendations were made from the LCA study:

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- *Optimize the use of separation technologies*
- *Evaluate energy of novel downstream processes*
- *Model developed to confirm design approach*
- *Outcomes can help guide decision makers in sustainable design of commercial facilities*

Summarize project activities for the entire period of funding, including original hypotheses, approaches used, problems encountered and departure from planned methodology, and an assessment of their impact on the project results. Include, if applicable, facts, figures, analyses, and assumptions used during the life of the project to support the conclusions.

Task A: Identification of Algae for Proposed Studies

The literature review allowed identification of two strains for this study. These include *Chlorella vulgaris*, and *Scenedesmus dimorphus*. These species were chosen due to their common occurrence around the world, easy growth characteristics and significant lipid content. Each species has a distinct morphology and high lipid content as shown in Table 1 and Figure 1. They can also be grown readily under a variety of environmental conditions.

Figure 1: Images of*, a)Scenedesmus,* and *b) Chlorella cells (Mohan et al. 2010)*

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 $30 \pm 2^{\circ}$ 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 and 1.0. 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 $CO₂$ 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 $CO₂$ concentrations were tested, including 5% $CO₂$, 10% $CO₂$, 20% $CO₂$.

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.

Experimental data for varying light, $CO₂$ and nitrate concentrations are shown below for the two species tested. The graphs indicate that

- Increased light intensity did not increase growth rates. 400 ft-candle was the optimum.
- The nitrate deficient system produced the highest lipid yield.
- Growth rates were enhanced at higher $CO₂$ percentages with 5 to 10% CO2 being optimum.

Figure 2: Growth of Select Algae Species Under Various Cultivation Conditions

The tables below indicates that lipid yield under varying light, nutrient and $CO₂$ concentrations for the two select species:

			Lipid Content (%dcw)		
Scenedesmus	Chlorella	Nitrate (g/L)	Scenedesmus dimorphus	Chlorella vulgaris	
%co, Air (0.03%)			13.250	3.3	
		0.1	2.938	7.4	
30.31	16.04	0.2	2.686	13.4	
36.46	13.39	0.3	7.278	25.19	
6.467	10.01	0.4	4.185	22.6	
	dimorphus 7.799	Lipid Content (%dcw) vulgaris 6.58	Deficient	-0.000	

Table 2: Lipid Yield of the Select Algae Under Various Growth Conditions.

Chlorella vulgaris was also tested under dark and light conditions with two external sources of carbon. *Chlorella,* is particularly attractive, because these microalgae are dualtrophic, meaning that they can be grown in both autotrophic and heterotrophic environments. 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 [18]. The ability of the *Chlorella* species to quickly adjust to heterotrophic carbon uptake makes it the central species upon which heterotrophic studies are currently performed.

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. 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).

Results of experiments with various cultivation conditions for Chlorella vulgaris are shown below.

Results indicate that *C. vulgaris* is able to utilize both glucose and glycerol as an organic carbon source to facilitate heterotrophic growth. The optimal concentration of organic carbon in the growth medium was 15 g/L for both glucose and glycerol. This means that increasing the concentration of organic carbon in the medium will be more costly and will not improve growth. The data also indicates that when comparing glucose and glycerol, glucose is 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 byproduct to grow more microalgae can be an economical alternative. Experiments with light and dark conditions indicated that *C. vulgaris* grew the best under mixotrophic conditions with light. Lipid analyses below shows nutrient deficient conditions enhanced lipid yield.

LIGHT	DARK				
Cultivation Conditions	μ (d ⁻¹)	Lipid%	Cultivation Conditions	$\mu(d^{-1})$	Lipid%
Photoautotrophic	0.0415	18.627	Autotrophic	0.0128	9.41
Photoautotrophic (N deficient)	0.0187	23.90	Autotrophic (N deficient)	0.0211	8.74
Heterotrophic	0.2003	22.12	Heterotrophic	0.1451	16.19
Heterotrophic (N deficient)	0.1694	34.02	Heterotrophic (N deficient)	0.1204	16.66
Mixotrophic	0.2177	29.81	Mixotrophic	0.1941	14.85
Mixotrophic (N deficient)	0.1821	33.11	Mixotrophic (N deficient)	0.1339	19.06

Table 3: Lipid Yield for *Chlorella vulgaris* **Under Various Cultivation Conditions**

Task B: Conduct tests for delivery of carbon dioxide using hollow fiber membranes

The membrane contactors were designed in a shell and tube configuration, in which a known number of hollow fibers were contained in an external shell. One end of the membrane bundle was sealed, while the other was potted in a Y-connector and exposed, so the inlet to each individual fiber was unobstructed. The module is shown below. The seal was made by using an impulse heat sealer (MP-8, Midwest Pacific 120V, 260W) to melt the polypropylene fibers at one end. An epoxy adhesive (DP-125, 3M, St. Paul, MN) was used as the potting material that immobilized the fiber bundle within the Y-connector.

Figure 4: Details of construction for a sealed end contactor

Carbon dioxide gas from a supply cylinder was pressurized within the fibers and as a result only diffused into the liquid phase through the membrane pores. The use of small diameter fibers resulted in a high specific surface area for mass transfer. Microporous hydrophobic hollow fiber membranes (Celgard X40-200, Hoechst-Celanese, Charlotte, NC) of identical specifications shown in [Table 4](#page-14-0) were used in construction of all membrane contactors.

A total of five different membrane modules were constructed for hydrophobic studies, varying the number of fibers in a bundle of 6, 12, 18, 54, and 60 packed within a glass shell. Two shell sizes were used with internal diameters of 4 and 8 mm. The integrity of each sealed fiber was

checked by first pressurizing the module to 10 psig and then immersing the bundle end in water. A working seal showed no evidence of bubbles. Detailed specifications of the membrane modules used in this study can be found in [Table .](#page-15-0)

Membrane modules for hydrophilic studies were constructed in an identical manner using the same polypropylene membrane fibers. However, before experimentation, each module underwent a treatment to wet the membrane pores. The hydrophobic membranes were submerged in an 80% v/v ethanol solution for 24 hours, followed by a 24-hour distilled water treatment. Treated modules were tested by increasing the feed gas pressure to well above the bubble point. Bubble formation along the length of the fiber bundle would indicate a failed treatment.

A total of three different membrane modules were constructed for gas transfer experiments using the hydrophobic membranes. Design details for each membrane module are shown in [Table .](#page-15-1)

Table 6: Hydrophilic membrane module specifications

The experimental set up shown in

[Figure](#page-16-0) was used for all absorption experiments discussed in this study. Chemically deoxygenated tap water was used as the absorption medium for all experiments. Water was recirculated through the system by a peristaltic pump (Masterflex, Vernon Hills, IL) controlled by a variable frequency drive. A Wheaton MBF-250 Bioreactor (total volume 2.7 L) was used as the Mixing vessel with no gas headspace. The absence of a gas phase was necessary to prevent the diffusion of oxygen into the liquid phase, as discussed previously. To ensure nearinstantaneous mixing, the vessel was stirred at 300 RPM by a shaft equipped with two Rushton impellers. Dissolved CO₂ measurements were performed using an Orbisphere Laboratories Model 3654 Portable Micro Logger (accuracy $\pm 1\%$ of reading). The instrument utilizes a membrane-covered dynamic thermal conductivity sensor. Dissolved concentration values were logged at 15-second intervals over two-hour periods. An identical peristaltic pump was used to continuously circulate water through the sensor. Pure $CO₂$ (Praxair Inc. Danbury CT, 99.5%) purity) gas was used for all studies.

Experimental data for hydrophobic membrane modules operated at three feed pressures (2, 5 and 10 psig) are plotted in **Error! Reference source not found.**. Three mass transfer correlations were obtained by least squares data regression for the three pressures.

Figure 6: Correlation between Sherwood and Reynolds # Under Varying Pressure

The correlations are shown in below for pressures of 2, 5 and 10 psig, respectively. As shown by the data, feed pressure of carbon dioxide supplied to a hollow fiber module operated in a sealed end configuration has a significant effect on the value of the overall mass transfer coefficient. The pressure effect is consistent with previous publications on gas transfer in sealed end hollow fibers. The model was successful at predicting gas velocity and composition within hollow fiber membranes operated in sealed end configuration. However, prediction of the average gas concentration within the fiber lumen did not describe the effect of feed $CO₂$ pressure on the overall mass transfer coefficient and this phenomenon is still not fully understood.

The following conclusions could be drawn from these studies:

- Sh dependent on Re as expected
- Significant pressure dependence apparent
- Increasing pressure by 25% increases Sh by factor of 3
- No significant effect of liquid velocity on mass transfer performance of hydrophilic membrane modules was observed.

Task C: Dewatering of Harvested Algae Using Membranes (100% Completed)

Dewatering Studies by GSE: GSE worked with Algaedyne, its technology company to determine dewatering of harvested algae using various available commercial technologies. GSE directed Algaedyne to conduct several studies of harvesting techniques from its photobioreactors. Chlorella Vulgaris (CV) and Haematococcus Pluvialis (HP) were grown in two different sized bioreactors rated by both volume of working fluid, the growth media, and the length of photon injection into the depths of the tanks. The optical and system technology matched the emission wavelengths of LEDs with absorption wavelengths of primary and accessory pigments in autotrophs to maximize growth, and yield for algal cultivation. Three methods of dewatering were employed and compared: low temperature evaporation, centrifuge, and Phyco BioSciences' Algae ventures HDD capillary action dewatering system. Harvesting and dewatering algal cultures requires taxa specific procedures. Using Ax-10 bioreactors from Algaedyne and the HDD water separation system from Algaeventure, algal biomass was successfully cultivated, harvested and dewatered to a 4-5% dry weight. Cultivating HP (Haematococcus Pluvialis) requires a harvest method that is low energy but effective in transferring samples with the highest densities of suspended alga. A low power pump proved effective to remove settled layers of algae once circulation and aeration was suspended. The HDD system provided a practical solution to dewatering the algae to flake and powder achieving the 4-5% moisture content suitable for vacuum packaging and rail transport.

Algaedyne Studies: Hollow fiber membrane modules were scaled and delivered to Algaedyne for pilot scale studies. These studies were conducted in pilot scale reactors and CO2 delivery via membranes and conventional sparging were compared. AX-50 and AX-500 systems were used in these pilot scale studies.

AX-50 Systems

The AX-50 is a laboratory (non-commercial) scale version of Algaedyne's light injection bioreactor. Two units were constructed and tested for use in the pilot scale study. Light present in the AX-50 systems accompanied by excess carbon (sparge delivered at 5% $CO₂$) and nutrients (5X WC Media, Guillard 1975) have sustained *Chlorella vulgaris* populations at 1 g/ L densities for 30 days. This biomass concentration and duration is sufficient to evaluate the performance of carbon membrane units as a $CO₂$ delivery device.

The AX-50 systems are equipped with an onboard pH system attached to a $CO₂$ cylinder. Carbon dioxide is delivered on an as needed basis using a solenoid hardwired to the pH system. The system can be converted to discharge atmospheric gasses through the membrane delivery system with control regulated by the pH system.

The system used in this study is shown below:

Figure 7: Schematic Drawing of AX-50 system and photograph of operational units dedicated to pilot scale testing.

Graphs are presented below for *Chlorella vulgaris* growth in each of the two AX-50 systems. Both raw chlorophyll A data and log corrected data are presented. Data will serve as a baseline for evaluating the membrane gas delivery system. Log corrected best fit equations were comparable for both AX-50 systems.

Figure 8: Chlorella vulgaris Growth Curves in Tank 1

TANK 2

log(Chlorophyl A)

log(Chlorophyl A)

Figure 9: Chlorella vulgaris Growth Curves in Tank 2

The AX-500 system is equipped with an onboard pH system attached to a $CO₂$ cylinder. Carbon dioxide is delivered on an as needed basis using a solenoid hardwired to the pH system. The system can be converted to discharge atmospheric gasses through the membrane delivery system with control regulated by the pH system.

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Figure 10: Schematic Drawing of AX-500 system and photograph of operational unit dedicated to pilot scale testing.

The modified AX-50 system described above was simultaneously operated with an AX-50 system delivering $CO₂$ via traditional cylinder sparging. Each experiment was conducted for for 3.5 days with systems initiated with similar chlorophyll A inoculates. These paired growth comparisons were conducted three times for each of the two taxa. Results of these experiments is presented below.

Figure 11: Replicate paired growth experiments of *Scenedesmus dimorphus* **in AX-50 Photobioreactors.**

Replicates 1,3, and 5 were equipped with hollow fiber membrane $CO₂$ delivery and replicates 2, 4, and 6 were sparged with $CO₂$ via airstone. The following colnclusions could be drawn:

- In paired experiments for each of the experimental strains, hollow fiber gas delivery proved superior to standard sparging.
- Average growth for experimental replicates were approximately double for fiber gas delivery systems compared to those with sparge gas delivery.
- This state is valid for both *Chlorella vulgaris* and *Scenedesmus dimorphus*.
- Growth rates for the AX-500 experiment was slower than that seen in the AX-50 system. This is attributed to rate limitation of the fiber system being used in the AX-500, specifically the volume of water was an order of magnitude greater and the fiber system and gas flow rate were unmodified. Despite these modifications, post experimental harvest values were just above 1 g/L dry mass (1.13g/L).

Task D: Analysis of Biofuel Life Cycle

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.

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.

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 $CO₂$ 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](#page-26-0) 12 shows the contribution of each processing step to the total life cycle emissions.

Figure 12: 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 $85 \text{ m}^3/\text{hr}$ 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 m³ 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](#page-27-0) 13.

Figure 13: 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.

The summary of the findings are presented in Table 7 and 8 below:

Table 7: Energy Consumption for Various Algae Dewatering Methods

The following conclusions could be drawn:

- Minimize use of processes like thermal dryers that consume the most energy/generate most life cycle emissions
- Optimize the use of separation technologies
- Evaluate energy of novel downstream processes
- Model developed to confirm design approach
- Outcomes can help guide decision makers in sustainable design of commercial facilities

Identify products developed under the award and technology transfer activities, such as: a. Publications (list journal name, volume, issue), conference papers, or other public releases of results.

Journals

- [D. O'Connell,](http://link.springer.com/search?facet-author=%22Daniel+O%E2%80%99Connell%22) [M.J. Savelski,](http://link.springer.com/search?facet-author=%22Mariano+Savelski%22) and [C.S. Slater](http://link.springer.com/search?facet-author=%22C.+Stewart+Slater%22) (2013) "Life cycle assessment of dewatering routes for algae derived biodiesel processes" *[Clean Technologies and](http://link.springer.com/journal/10098) [Environmental Policy,](http://link.springer.com/journal/10098) (Accepted) DOI 10.1007/s10098-012-0537-7*.
- S. Moore, S. Bauer, A. McFarland and K. Grimes (2013), "Algae growth studies under dark and light conditions", Submitted *APCBEE Procedia* (Journal under Elsevier, ISSN: 2212-6708)
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MS Thesis

- Daniel O'Connell (2012) Life Cycle Assessment of Dewatering Routes for Algae Derived Biodiesel Processes, Chemical Engineering, Rowan University.
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Awards

Angela Kinsella (2015)) **NJWEA Daniel E. Bigler Award** for Algae Research

A. MacFarland (2014) **NJWEA Daniel E. Bigler Award** for Algae Research

Kassandra Grimes and Andrea McFarland (2013) "Algae-derived Biofuels: Comparative Algal Yield of Autotrophic, Heterotrophic, and Mixotrophic Growth Conditions, *Best Paper Award*, National Engineers Week, 2013 Delaware Valley Engineers Council, Philadelphia, PA.

Kassandra Grimes and Andrea McFarland (2013) Nutrient Removal from Wastewater using Algae, NJWEA student poster competition, **Second Prize**, May 2013.

Sarah Bauer and Andrea McFarland (2012) Nutrient Removal from Wastewater using Algae, NJAWWA student poster competition, March 12, 2012, **Third Prize**.

Bauer, S., S. Moore, MacFarland, A and K. Grimes (2013**) "Algae Derived Biofuels" Best Civil Engineering Clinic Project, Rowan University.**

McFarland, A., S. Bauer, M. Staehle, Ph.D. and K. Jahan, Ph.D. (2012) "Weaving Sustainability into Undergraduate Engineering Education through Innovative Pedagogical Methods: A Student's Perspective", 2012 Annual ASEE Conference, San Antonio, TX. **Best Paper Award,** 2012 ASEE Annual Conference.

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Bauer, S. (2012) **Bantivoglio Honors Concentration Research Fellowship Recipient** for Algae Derived Biofuels

A. MacFarland (2012) **Bantivoglio Honors Concentration Research Fellowship Recipient** for Mixed Algae Studies for Lipid Yield.

Bauer, S. (2012) **AWWA-NJ Section 2012 Drinking Water Careers Scholarship**

A. MacFarland (2013) **NJWEA Daniel E. Bigler Award** for Algae Research

Bauer, S. (2012) **NJWEA Daniel E. Bigler Award** for Algae Research

Pavlo Kostetskyy (2012) **NJWEA Louis Fontenelli Graduate Student Award** for Algae Research

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Sarah Bauer (2011) Nutrient Removal from Wastewater using Algae, **MAREA Best Paper Award.**

Justin Piccillo (2011) **NJWEA Daniel E. Bigler Award** for Algae Research

Justin Picillo, Algae Derived Biofuel, **1 st Prize**, Annual NJWEA Poster Competition, Atlantic City, May 2011.

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