IN SITU TRANSESTERIFICATION OF MICROALGAL OIL TO PRODUCE ALGAL BIODIESEL

Final Report

KLK768

N12-10

National Institute for Advanced Transportation Technology

University of Idaho

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June 2012

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1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
4. Title and Subtitle In situ *Transesterification of Microalgal Oil to Produce Algal Biodiesel*		5. Report Date *June 2012*
		6. Performing Organization Code *KLK768*
7. Author(s) *He, B. Brian*		8. Performing Organization Report No. *N12-10*
9. Performing Organization Name and Address National Institute for Advanced Transportation Technology University of Idaho PO Box 440901; 115 Engineering Physics Building Moscow, ID 83844-0901		10. Work Unit No. (TRAIS) 11. Contract or Grant No. DTRT07-G-0056
12. Sponsoring Agency Name and Address US Department of Transportation Research and Special Programs Administration 400 7th Street SW Washington, DC 20509-0001		13. Type of Report and Period Covered *Final Report: August 2011-June 2012* 14. Sponsoring Agency Code *USDOT/RSPA/DIR-1*

15. Supplementary Notes:

16. Abstract

This research was to process whole microalgae cells for biodiesel production without first extracting lipids. The ultimate goal is develop a novel process for algal biodiesel production directly from microalgae cells in a single step, i.e., *in situ* transesterification, to lower the processing costs. In this stage, we conducted research on (1) characterizing the selected microalgae strains and (2) screening the influential process parameters.

Characterization was performed on microalgae through proximate and ultimate analyses, fatty acid profiles, ash content and mineral components of the microalgae samples, Thermogravimetric analysis and scanning electron microscopy examination on the samples. It was found that green microalgae are suitable for algal biodiesel production due to their relatively high lipid content, while brown microalgae may be good for fermentable sugar production due to their high carbohydrate content. Microalgae tested in this study have relatively high ash content (up to 25%wt), which is somewhat unexpected. Fatty acid profiles varied widely among the microalgae tested and need validation through further investigation. Reaction time affected the microalgal lipid conversion considerably at 300°C, and extending the reaction would lead to satisfactory process efficiency.

Findings from this study provide necessary information for the continued systematic investigation on algal biodiesel production.

17. Key Words Biodiesel fuels; microalgae; fuel processing	18. Distribution Statement Unrestricted; Document is available to the public through the National Technical Information Service; Springfield, VT.

19. Security Classif. (of this report) Unclassified	20. Security Classif. (of this page) Unclassified	21. No. of Pages 21	22. Price …

Form DOT F 1700.7 (8-72) Reproduction of completed page authorized

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EXECUTIVE SUMMARY

Microalgae are promising candidate biomass resources as potential feedstocks for renewable energy production. Microalgae have high biomass productivity, high lipid content, low cultivation cost, and no competition with agricultural land. Biofuels from microalgae are renewable and carbon neutral, and can use non-arable land. The most advantageous benefit of using microalgae as the feedstock for biofuels production is its high oil productivity per unit land, 1,000–6,500 gallons of oil per acre per year.

Tremendous efforts have been made worldwide to advance the technologies of cultivating microalgae for algal biofuels, especially the algal biology, screening for robust microalgae strains, and maximization of the productivity and oil content. To get the algal oil out of the microalgae cells, oil extraction, typically by an organic solvent, is needed. This step is expensive because it involves cell rupture, solvent extraction, oil/solvent separation and reuse, etc. The next step is the algal oil transesterification.

In this research, we proposed to explore the combination the algal oil extraction and transesterification into one through an *in situ* transesterification process. The ultimate goal is develop a novel process for algal biodiesel production directly from microalgae cells in a single step, i.e., in situ transesterification, to lower the processing costs. In the first stage of this research, we conducted studies on (1) characterizing and determining the selected microalgae strains and (2) screening and investigating the process parameters that affect the lipid esterification and transesterification for algal biodiesel production. This is to prepare the needed information for next stage research on investigating systematically the process efficiency as affected by all process parameters.

Research found that the content of volatile matter among the eight microalgae samples was approximately in the range of 68% to 78%. The fixed carbon content were low (1~4%) except the *N. Salina* samples (approx. 15%wt), which is considerably different from that of the others. It was also found that microalgae has a relatively high ash content, 14~16% for green microalgae and up to 25%wt for brown microalgae. There were 15 minerals/non-mineral components detected in the ash samples. Among them, calcium (Ca), magnesium

(Mg), potassium (K), and sodium (Na) are in large quantities at 10^6 ppm level. Ultimate analysis provided the information from elemental aspects, including carbon, nitrogen, oxygen and sulfur. It is seen from the experimental results that the carbon contents (in %) of brown microalgae are much lower compared to those of green microalgae. This is caused by the high ash content in the brown microalgae.

Algal lipids data show that brown microalgae contain high carbohydrate content, up to 75%, and might be a suitable source for sugar production. Therefore, the green microalgae tested in this study are more suitable feedstocks for biodiesel production, due to their higher lipid contents, approximately 25%wt. Scanning electron microscopy (SEM) images of the microalgae samples showed that the physical structure did not change significantly after the lipids were solvent extracted. However, the algal cell structure changed considerably from different drying procedures. Fatty acid compositions of the microalgae samples were tested after obtaining the algal lipids using solvent extractions. Preliminary data showed that, although not in significant quantities, there are fatty acids of odd-numbered carbon chain (i.e., C15 and C17) which are rarely seen in seed oils such as soybean oil and canola oil. It is also seen that the fatty acid profiles of the algal oil samples vary widely, not only between the samples of different microalgae, but also between the lipid samples of the same type of microalga by different extraction methods. Further investigation is needed to verify the results.

Preliminary results indicated that the reaction time significantly affects the *in situ* transesterification efficiency. By extending the reaction from 10 to 20 minutes after the set point was met, the lipid conversion rate increased from approximately 50% to 64% (area %). It is expected that increasing reaction time would considerably improve the process and achieve satisfactory conversion efficiency.

The project is on-going. In addition to the reaction time, other parameters including microalgal cell loading rate, necessity of catalysis application as well as the range of operating temperatures and corresponding pressures, will also be further systematically investigated.

DESCRIPTION OF PROBLEM

Among the suitable biomass resources as potential feedstock for renewable energy production, microalgae is a promising candidate because of its high biomass productivity, high lipid content, low cultivation cost, and no competition with agricultural land. In addition, some strains of microalgae can fix CO_2 very efficiently thus contributing greatly to the reduction of greenhouse gas emission.

Microalgae are among the simple microorganisms on our planet. Microalgae grow in various natural aqueous and terrestrial habitats. Most microalgae convert CO_2 from the atmosphere and sunlight into biomass through photosynthesis. Some of the lipid-bearing microalgae have high levels of oil in their bodies. Shown in the picture is an example of a microalgae cell. It accumulates clusters of "green oils" in its capsule, as indicated by the green in the image.

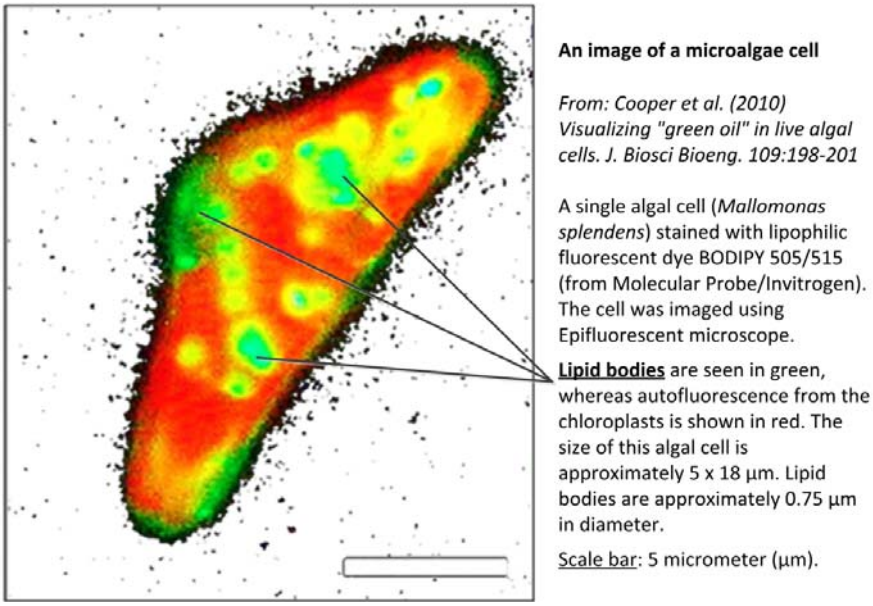


Figure 1: Image of a microalgae cell.

Depending on the specific strains, some microalgae can have as much as 75% oil in their cells. Table 1 shows examples of the oil level in some microalgae strains. The high oil content and its carbon sequestration capability make microalgae the perfect candidates for environmentally friendly biofuel production.

Biofuels from microalgae are renewable and carbon neutral, and can use non-agricultural land. Microalgae potentially can be produced industrially. Producing algal biofuels has minimal effects on our freshwater systems and environment. Cultivating microalgae would greatly reduce CO_2 emissions to the atmosphere by converting the CO_2 from fossil power plants into algal biomass. Microalgae can grow in freshwater and a wide variety of water sources containing salts and other nutrients, such as seawater and wastewater.

Table 1: Oil Content of Some Strains of Microalgae [1]

No.	Microalga	Oil content (% dry wt)
1	*Botryococcus braunii*	25 - 75
2	*Chlorella* sp.	28 - 32
3	*Crypthecodinium ochnii*	20
4	*Cylindrotheca* sp.	16 - 37
5	*Dunaliella primolecta*	23
6	*Isochrysis* sp.	25 - 33
7	*Monallanthus salina*	> 20
8	*Nannochloris* sp.	20 – 35
9	*Nannochloropsis* sp.	31 – 68
10	*Neochloris oleoabundans*	35 – 54
11	*Nitzschia* sp.	45 – 47
12	*Phaeodactylum tricornutum*	20 – 30
13	*Schizochytrium* sp.	20 – 77
14	*Tetraselmis sueica*	15 – 23

The most advantageous benefit of using microalgae as the feedstock for biofuels production is its high oil productivity per unit land. The potential yield of algal oil is much higher than that of conventional oilseed crops. Algae can produce 1,000–6,500 gallons of oil per acre per year[2], compared to approximately 45–55 gallons for soybean and 70–150 gallons for canola.

During the past two decades, tremendous efforts have been made worldwide to advance the technologies of cultivating microalgae for algal biofuels, especially the algal biology, screening for robust microalgae strains, and maximization of the productivity and oil content.

[1] Chisti, Y. 2007. Biodiesel from microalgae. *Biotechnology Advances* 25(3):294-306.

[2] US DOE, Energy Efficiency & Renewable Energy. National Algal Biofuels Technology Road Map. May 2010.

Means to cultivate microalgae include photoautotrophic cultivation (requiring light but absorbing CO_2 as a carbon source) in open or closed ponds, and heterotrophic cultivation (does not require light but requires added nutrients as a carbon source) in bioreactors.

After cultivation, microalgae need to be harvested, dewatered, and dried before oil extraction and conversion for biofuels. These are mainly the steps of process engineering. Some of these processes are very cumbersome in unit operations and energy intensive. In the commercialization stage, scaling up these harvesting, dewatering, and drying processes can be very challenging.

The specific steps in converting algal oils are dependent on the targeted final biofuel types. The conversion technologies can be chemical, biochemical, or thermochemical, or a combination of these processes. Various decisions have to be made at each stage of the chosen process. Illustrated below is a schematic process that targets the diesel-replacement biofuels.

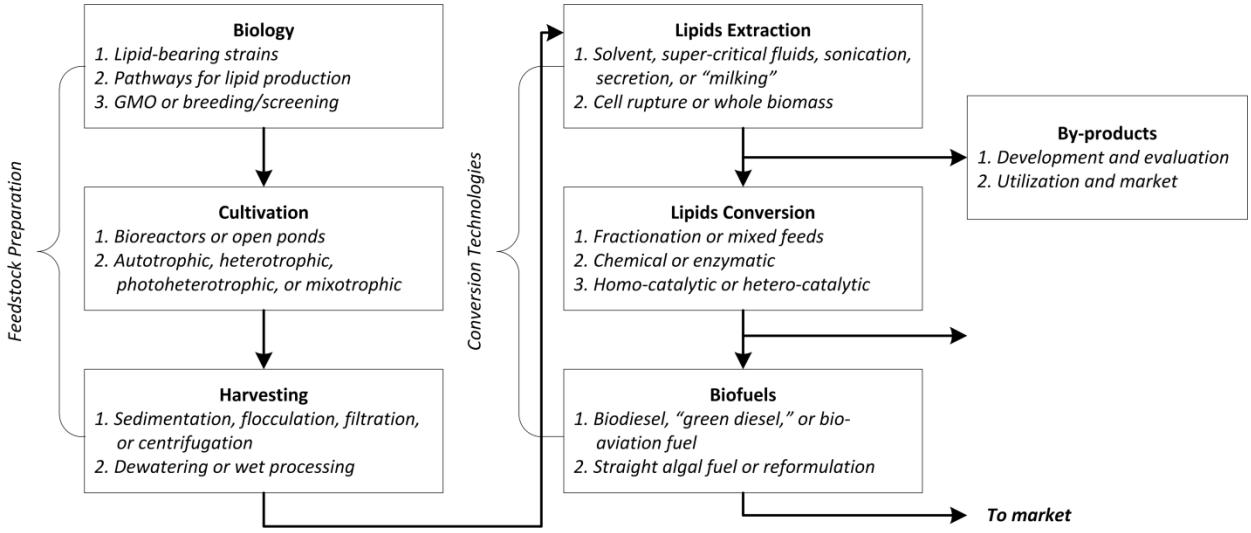


Figure 2: Considerations of lipid-bearing microalgae for biofuels production.

Steps involved with producing biodiesel from algal lipids are shown in Figure 3. The selected strain of microalgae (e.g., blue-green algae) is cultivated in bioreactors or ponds. When the biomass is mature enough to be harvested, it is first flocculated to increase critical mass before being effectively separated from the spent medium by either centrifugal sedimentation

or filtration. The harvested microalgae still contains a large quantity of water, up to 99%, and dewatering, typically by mechanical means, is necessary. Drying is necessary to further reduce water content in the microalgae for suitable lipid extraction. This is a step that could be very energy intensive and challenging. The algal cells may be ruptured and oil may leak out, thus lowering yield if too much thermal stress is applied.

The steps mentioned above are typically considered as the feedstock preparation and harvesting. The step of dewatering/drying is at the boundary of feedstock preparation and post-harvest processing.

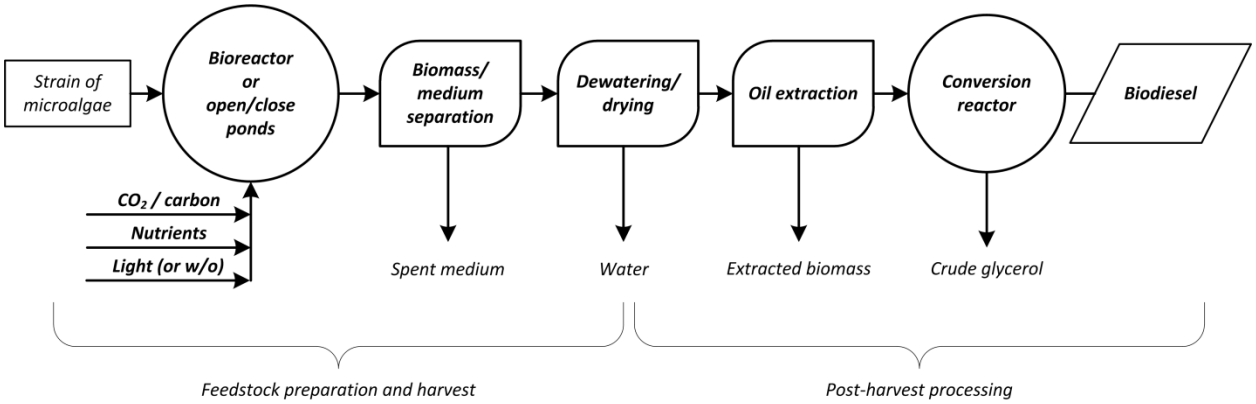


Figure 3: Illustrative scheme of conventional biodiesel production from microalgal oil.

To get the algal oil out of the microalgae cells, oil extraction, typically by an organic solvent, is needed. This step involves microalgae cell rupture, solvent extraction, and oil/solvent separation from the cell debris. The algal oil extracted contains free fatty acids and triglycerides. The fatty acid profiles of algal oils vary widely, depending on the strains of microalgae, the level of maturity of the microalgal biomass harvested, and the conditions of oil extraction. Table 2 lists examples of fatty acid profiles from various literatures, where C16:0, C18:1, and C18:2 are the dominants and odd-numbered fatty acids present as well.

Table 2: Fatty Acid Profiles of Microalgal Oils

No.	Fatty acids compositions (%wt)											Ref.
	C12:0	C14:0	C16:0	C16:1	C16:2	C17:0	C18:0	C18:1	C18:2	C18:3	C18+	
1			1.37	0.44				61.81	19.92	12.22	1.22	[3]
2			15			8.4	11	36	7.4			[4]
3	0.21	1.25	22.49	0.64		0.19	3.15	19.36	26.9	17.04	1.96	[5]
4		0.6	19.1	6.7	2.6	0.8	2.2	62.8	3.8		1.4	[6]
5			26.6	2.2	6.6		3.7	17.9	20.8	12.2	1.1	[7]
6			22.2	1.7	8.3	2.3	1.2	35.2	20.2	8.3	0.4	[7]

In this proposed research, we will focus on the post-harvest processing of microalgae to produce algal biodiesel. We will combine the two steps of oil extraction and transesterification into one through an *in situ* transesterification process in sub- or supercritical methanol, as shown in Figure 4.

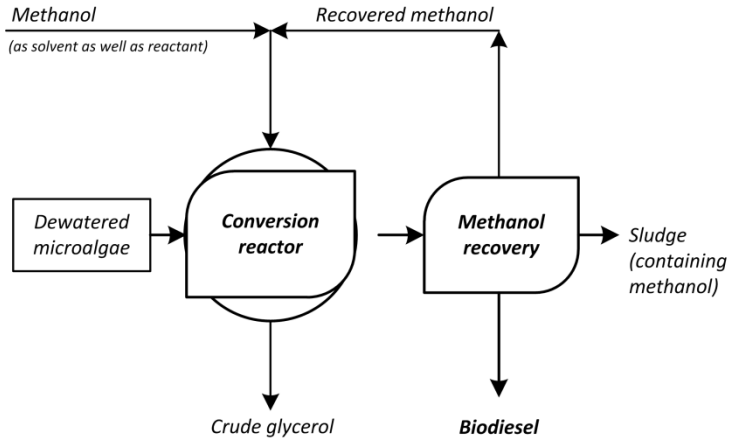


Figure 4: Proposed biodiesel production from microalgal oil.

Specifically, we will use dewatered microalgae as the feedstock. The dewatered microalgae may still contain a certain amount of water and are considered "wet." We will dry the wet microalgae and use both dried and wet microalgae in our processing process.

[3] Ehimen, E. Z. Fan, and C. Carrington. 2010. Variables affecting the *in situ* transesterification of microalgae lipids. *Fuel* 89(3):677-684

[4] Demirbas, A. 2009. Production of biodiesel from algae oils. *Energy Sources, Part A: Recovery, Utilization and Environmental Effects* 31(2):163-168.

[5] Damiani, M., Cecilia A. Popovich, D. Constenla, P. Leonardi. 2010. Lipid analysis in *Haematococcus pluvialis* to assess its potential use as a biodiesel feedstock. *Bioresource Technology* 101(11): 3801-3807.

[6] Halim, R., B. Gladman, M. Danquah, P. Webley 2011. Oil extraction from microalgae for biodiesel production. Bioresource Technology 102(1):178-185.

[7] Liu, J., J. Huang, Z. Sun, Y. Zhong, Y. Jiang, and F. Chen 2011. Differential lipid and fatty acid profiles of photoautotrophic and heterotrophic Chlorella zofingiensis: Assessment of algal oils for biodiesel production. *Bioresource Technology* 102(1):106-110.

Methanol is the preferred alcohol in transesterifying triglycerides and esterifying free fatty acids into fatty methyl esters, or biodiesel. Under supercritical conditions, methanol is also a very strong solvent. Therefore, methanol in our process serves as a reactant and also as a solvent for oil extraction *in situ*. The critical point of methanol is at 239.5°C and 8.14 MPa (1,180 psi). Under this condition, the triglycerides and free fatty acids can be converted to methyl esters with the potential of using no catalysts, homogenous or heterogeneous.

After the reaction, the methanol can be easily separated from the biodiesel because, at room temperature, methanol has a very limited solubility in biodiesel and is thus immiscible. The methanol will be cycled back and reused. The small quantity of methanol in biodiesel can be removed using established technologies and will not be further researched in this project. The leftover microalgae debris will be separated from the liquids via simple filtration.

APPROACH AND METHODOLOGY

The ultimate goal of this research is to develop a novel process to produce algal biodiesel directly from microalgae with oil extraction and esterification/transesterification in a single step, i.e., *in situ* transesterification. This ultimate goal is to be realized with the specific objectives of (1) characterizing and determining the selected microalgae strains, (2) screening and investigating the process parameters that affect the lipid esterification and transesterification for algal biodiesel production, and (3) investigating systematically the process efficiency as affected by all process parameters including feeding wet and pre-dried microalgae to the system.

This study was planned to be conducted in two stages with the specific objectives (1) and (2) in stage 1 and objective (3) in stage 2. This report is for studies conducted in stage 1.

Characterization of Microalgae for Processing

The chemical and physical properties of microalgae vary widely from one strain to another. These properties include the composition of free fatty acids and triglycerides, fatty acid profiles, cell wall rupture conditions, presence of volatile organic compounds, biomass glass transition points, presence of inorganic elements, etc. These parameters will affect the choice of process operating conditions. Moisture content of the microalgae is another parameter that affects the optimal process efficiency.

In this study, a non-isothermal thermo-gravimetric analysis (TGA) was utilized for determining the approximate thermal profiles of eight different microalgae. A Q50 TGA by TA Instruments (New Castle, DE) with a pair of platinum weight pans was used. The samples were heated up to 900°C, and the completed thermal profile of each microalga was obtained from the automated recording mechanism.

Karl Fischer tests were performed on the algae samples for moisture determination. Furnace combustion tests were used for ash content determination. The mineral contents in the algae samples were determined by the Analytical Sciences Laboratory at the University of Idaho by the devices of Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES),

Inductively Coupled Plasma Mass Spectrometer (ICP-MS), and Inductively Coupled Plasma Mass Spectrometer Collision/Reaction (ICP-MScx) following the American Society of Biological and Agricultural Engineers (ASABE) standard methods of E871, E872, and D1102. According to ASABE standard E870, the fixed carbon of samples is the resultant of the difference of percentage summation of moisture, ash and volatile matter subtracted from 100. Collectively, the proximate analysis is obtained by combining the above results.

The ultimate analysis on elemental content was conducted by two external laboratories, Analytical Sciences Laboratory at the University of Idaho and the Nutrition Analysis Center Eurofins Scientific, Inc. (Des Moines, IA). In determining carbon (C), sulfur (S), and nitrogen (N), a complete combustion process converts the elemental carbon, sulfur, and nitrogen into CO_2, SO_2, N_2, and NOx. These gases are then passed through IR (infra-red) cells to determine the carbon and sulfur contents and a TC (thermal conductivity) cell to determine nitrogen content.

In order to analyze the fatty acid profiles of the algal oils, extraction of the lipids out of the algae samples were performed by Soxhlet extraction and the modified Bligh & Dyer extraction. Lipids extracted were analyzed for their fatty acid profiles as described by Hammond [8].

SEM images were taken for analyzing the microalgal cell structures before and after algal lipids extractions at three different resolutions, i.e., 500×, 1000×, and 1500×.

Prescreening of Operating Variables

Preliminary experiments were carried out to explore a wide range of reaction conditions to identify the key influential parameters. The operating temperature and residence time were reported as the major parameters in supercritical processing of vegetable oils for biodiesel production. The operating pressure is coupled with the boiling temperature of methanol at the

[8] Hammond E.G. 1991. Organization of rapid analysis of lipids in many individual plants. Modern Methods of Plant Analysis Vol. 12: Essential Oils and Waxes p. 321-330. H.F. Linskens and J. F. Jackson (editors). Springer-Verlag, Berlin, Germany.

critical point, thus not an independent variable. Solid biomass, i.e., microalgae cells, suspends in methanol in a slurry form.

Experiments were conducted in the existing reactor system. The high-temperature, high-pressure reactor (300 mL Parr 4560 Pressure Reactor) was acquired through the financial support from the National Institute for Advanced Transportation Technology (NIATT). The reactor system is installed at the Biofuels Research Laboratory of the Department of Biological and Agricultural Engineering (BAE) at the University of Idaho (UI). The reactor system can handle up to 20 MPa (3000 psig) of pressure and 350°C of temperature. It is controlled by the 4857 Reactor Controller that has PC-based software for the control of temperature and agitation speed. The controller measures temperature, pressure, and motor speed using a dual thermocouple, pressure transducer, and tachometer, respectively. The reactor system was hosted/isolated in a metal-framed chamber to vent any possible gas/vapor leakage and to ensure safe operation of the system.

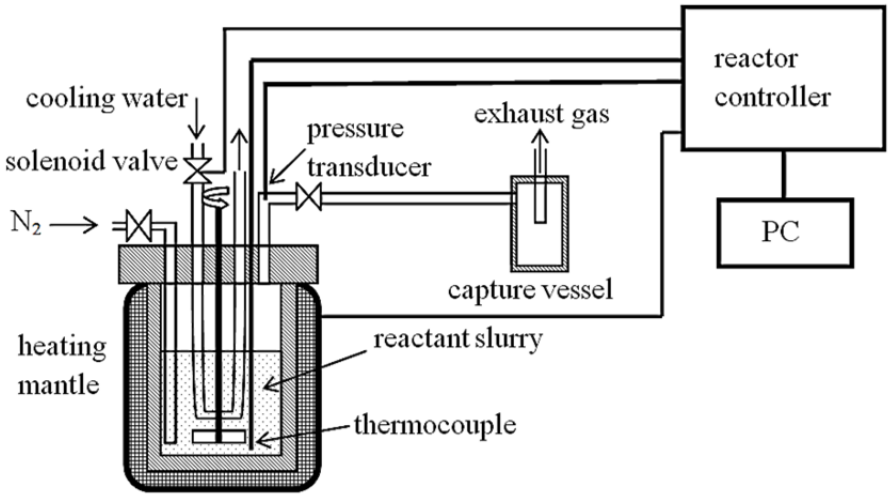


Figure 5: Schematic diagram of the reactor system for this project.

Figure 6: Chamber for hosting the pressure reactor unit.

FINDINGS; CONCLUSIONS; RECOMMENDATIONS

Characterization of Microalgae for Processing

In this study, eight microalgae samples of green algae and brown algae were tested. Five of the samples were green algae, which were freezer dried (FD green), spread dried (SD green), Refractaned Window dried (RW) MCD#TD 1440, freezer dried *N. Salina* (FD *N. Salina*), oven dried *N. Salina* (SD *N. Salina*). Three were brown algae, i.e., freezer dried (FD brown), spread dried (SD brown), and Refractaned Window dried (RW) MCD#TD 1427. FD green algae and SD green algae were purchased from Algal Technology (Cumming, GA), FD brown algae and SD brown algae were from Algae Bioscience (Scottsdale, AZ), and MCD # TD 1427 and MCD # TD 1440 were from MCD Technologies Incorporated (Tacoma, WA).

Thermo-gravimetric analysis (TGA) is a good method for determining moisture content (MC), volatile matter, fixed carbon and ash content[9]. Volatile matter is the organic matter lost between 110 and 700˚C, and the ash content is the weight left after 900˚C. The fix carbon content (FC) is determined by the difference of total sample and the moisture content, volatile matter (VM) and ash contents. A proximate analysis on the eight microalgae samples were obtained as summarized in Table 3.

Table 3: Proximate Analysis of Microalgae Samples from TGA Analysis

No.	Microalgae species	MC (%wt)	VM (%wt)	FC (%wt)	Ash (%wt)
1	FD Green algae	4.5±0.14	76.7±0.02	4.0	14.8±0.41
2	SD Green algae	3.7±0.23	78.3±0.27	4.3	13.7±0.28
3	RW MCD#TD 1440 (Green)	4.5±0.16	75.7±0.12	3.6	16.2±0.37
4	FD *N. Salina*	2.9±0.26	67.7±0.24	15.9	13.5±0.42
5	OD *N. Salina*	0.7±0.08	63.5±0.18	14.5	21.3±0.36
6	FD Brown algae	5.3±0.15	69.0±0.11	1.4	24.3±0.13
7	SD Brown algae	6.0±0.62	67.9±0.15	1.1	25.1±0.26
8	RW MCD#TD 1427 (Brown)	6.2±0.32	67.7±0.67	1.0	25.0±0.19

Note: MC is the moisture content, VM is the volatile matter, and FC is the fix carbon content.

[9] Silva, Vilma Mota da et al. 2008. Determination of moisture content and water activity in algae and fish by thermoanalytical techniques. *Quím. Nova* [online]. 31(4):901-905.

Since the samples were essentially very well dried, the moisture contents are low and not statistically different. The volatile matters among the samples are approximately in the range of 68% to 78%. However, the fixed carbon content of the *N. Salina* samples, i.e., approx. 15%wt, is considerably different from that of the others. This indicates that the biomass structure of *N. Salina* may be significantly different from that of the others. Another important piece of information can be revealed after carefully examining the data. All microalgae samples characterized in this study have very high ash content, up to 25%wt for brown microalgae.

To further explore the individual composition of the ash, a mineral test was performed, as summarized in Table 4.

Table 4: Mineral Contents in Ash of Eight Different Microalgae (µg/g)

No.	**Microalgae species**	1	2	3	4	5	6	7	8
		Ba	Ca	Cr	Co	Cu	Fe	Mg	Mn
1	FD Green Algae	5.1	19000	4.3	25	62	3300	15000	850
2	SD Green Algae	4.5	16000	4.2	22	56	3000	13000	780
3	MCD#TD 1440	9	27000	9.2	31	120	4400	21000	1200
4	FD *N. Salina*	24	66000	4.9	2.6	23	630	29000	100
5	OD *N. Silina*	25	57000	3.8	2.3	16	560	24000	88
6	FD Brown Algae	250	8200	3.2	0.32	22	540	22000	17
7	SD Brown Algae	210	7500	4	0.2	11	370	19000	14
8	MCD#TD 1427	230	7700	3.2	0.39	19	480	21000	17

Table 4 (cont'd).

No.	Microalgae species	9	10	11	12	13		14	15
		Mo	Ni	K	Na	Zn		P	S
1	FD Green Algae	6	22	120000	79000	210		67000	11000
2	SD Green Algae	5.8	18	110000	72000	180		60000	9400
3	MCD#TD 1440	< 8	< 8	160000	110000	290		90000	15000
4	FD *N. Salina*	2.3	2.8	42000	100000	43		21000	46000
5	OD *N. Silina*	3.1	2.3	34000	84000	36		18000	40000
6	FD Brown Algae	3.4	< 2	15000	180000	40		6600	8500
7	SD Brown Algae	< 2	< 2	14000	170000	27		6200	7500
8	MCD#TD 1427	2.1	< 2	16000	160000	39		6300	7500

Among the minerals detected from the microalgae samples, a few of them are in large quantities, including calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na). Additionally, the non-metal components of phosphorous (P) and sulfur (S) are also in large quantities. These high ash contents were unexpected. This high ash/mineral content will affect the decision on determining the operating procedures and possibly the process efficiency of *in situ* algal biofuel production as well.

Ultimate analysis provides the information from element aspect, including carbon, nitrogen, oxygen, and sulfur. Table 5 below summaries the ultimate analysis on eight microalgae samples. It is seen from Table 5 that the carbon contents of brown microalgae are much lower compared to those of green microalgae, although the oxygen content (the other major component of the samples) is similar for both. This is caused by the high ash contents in the brown microalgae (Table 4) and implies that brown microalgae tested in this study may not be good choices for algal biofuel production. This assumption is, in fact, confirmed by the low lipid content in brown microalgae tested in this study (see Table 6).

Table 5: Ultimate Analysis of Microalgae Samples

No.	Microalgae species	Carbon (%wt)	Nitrogen (%wt)	Oxygen (%wt)	Sulphur (%wt)
1	FD Green algae	58	6.8	27.5	0.40
2	SD Green algae	58	6.8	27.8	0.47
3	RW MCD#TD 1440 (Green)	56	6.7	27.0	0.49
4	Freezer Dried *N.Salina*	51	1.6	37.1	0.90
5	Oven Dried *N.Salina*	49	1.6	35.7	0.98
6	FD Brown algae	25	3.6	22.5	1.00
7	SD Brown algae	25	3.6	23.2	0.92
8	RW MCD#TD 1427(Brown)	24	3.5	22.8	0.93

Table 6: Contents of Carbohydrate, Protein, and Crude Fat

No.	Microalgae specie	Carbohydrate (%wt)	Protein [a] (%wt)	Crude Fat [b] (%wt)
1	FD Green algae	34.19	42.50	23.31
2	SD Green algae	29.69	42.50	27.81
3	RW MCD#TD 1440 (Green)	32.65	41.88	25.47
4	Freezer Dried *N. Salina*	64.55	10.00	25.45
5	Oven Dried *N. Salina*	72.86	10.00	17.14
6	FD Brown algae	74.45	22.50	3.05
7	SD Brown algae	75.45	22.50	2.05
8	RW MCD#TD 1427 (Brown)	75.48	21.88	2.64

[a] Protein content by nitrogen conversion (N × 6.25).
[b] Crude fat content by acid hydrolysis.

Data in Table 6 show that brown microalgae contain high carbohydrate content, up to 75%, and might be a suitable source for sugar production. However, their crude fat (or lipids) contents are considerably low, in the range of 2~3%, about ten times lower than those of green microalgae. Therefore, for algal biodiesel production, the green microalgae tested in this study are more suitable feedstocks due to their high lipid content.

Due to the relatively low lipid content, the physical structure of the microalgal samples did not change significantly after the lipids were solvent extracted, as shown by the SEM images (Figure 7). However, the algal cells show extremely different structures from different drying procedures. For example, the structure of green algae cells from freezer dry (i.e., FD green algae in Figure 7) collapses the cells into pieces but the spray dry (i.e., SD green algae in

Figure 7) keep the typical cell form. This phenomenon may affect the lipid extraction efficiency and should be explored in the algal biofuel processing design.

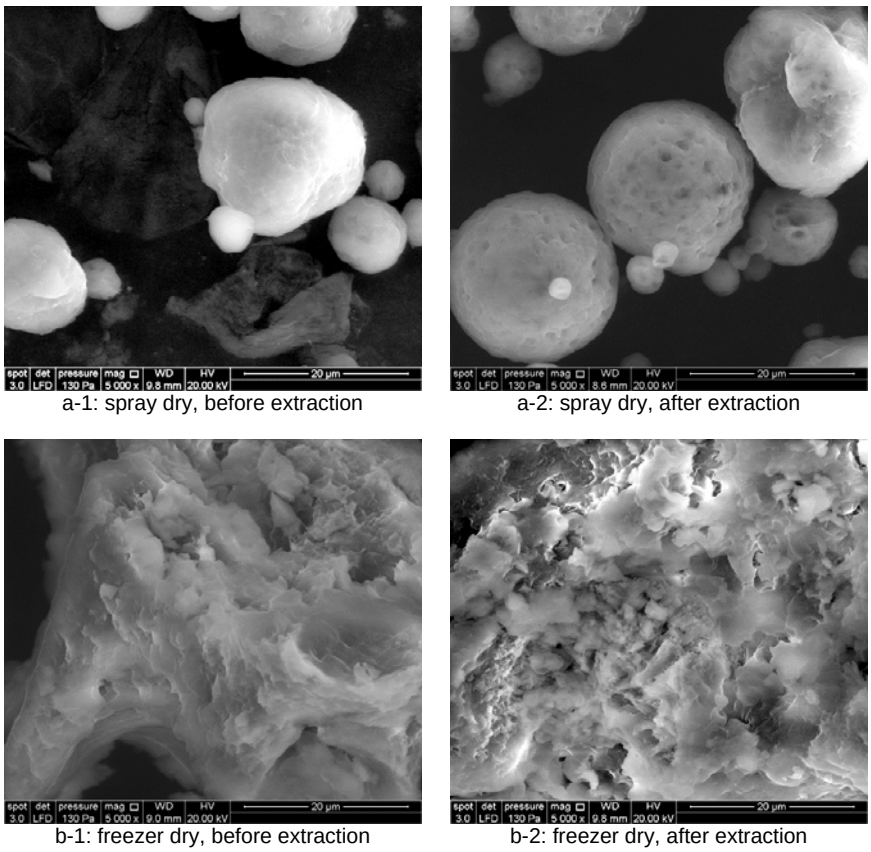


Figure 7: Examples of SEM images of microalgae samples.

In order to analyze the fatty acid compositions of the microalgae samples, two types of solvent extraction were used to obtain algal lipids for gas chromatography (GC) analysis, the Soxhlet extraction and the modified Bligh & Dyer extraction. Preliminary data show that, although not significant, there are fatty acids of odd-numbered carbon chain (i.e., C15 and C17) which are rarely seen in seed oils such as soybean oil and canola oil. Table 7 also shows that the fatty acid profiles of the algal oil samples vary widely, not only between the samples of different microalgae, but also between the lipid samples of the same type. The reason is not clear and needs further investigation.

Table 7: Major Fatty Acid Components in Microalgae Lipid Samples from Different Drying Treatments

ID	Samples	Extraction method	Fatty acids (%wt)									Total (%wt)
			14:0	15:0	16:0	16:1	17:0	18:0	18:1	18:2	22:0	
1	FD Green Algae	Soxhlet	2.86	2.88	18.19	1.45	1.03	6.64	16.24	23.26	1.28	73.83
2	FD Green Algae	Modified Bligh & Dyer	3.33	0.41	38.57	20.96	0.56	1.02	4.73	2.98	16.10	88.66
3	SD Green Algae	Soxhlet	3.52	0.49	35.52	14.63	0.54	1.42	3.87	2.29	12.78	75.06
4	SD Green Algae	Modified Bligh & Dyer	3.60	0.44	32.30	19.42	-	0.90	5.59	3.65	21.79	87.69
5	FD *N. Salina*	Soxhlet	0.83	-	29.97	3.87	0.78	1.84	39.50	5.00	1.36	83.15
6	FD *N. Salina*	Modified Bligh & Dyer	1.72	0.23	32.40	16.43	0.93	1.88	27.21	3.30	3.08	87.18
7	OD *N. Salina*	Soxhlet	1.33	-	26.45	11.59	1.08	0.94	32.90	4.03	2.89	81.21
8	OD *N. Salina*	Modified Bligh & Dyer	1.44	0.26	32.51	11.94	0.93	1.28	30.00	3.60	2.22	84.18
9	RW MCD #TD 1427	Modified Bligh & Dyer	18.10	0.93	11.43	14.95	1.77	14.60	2.84	2.16	4.50	71.28
10	RW MCD #TD 1427	Soxhlet	3.99	0.38	15.97	6.71	0.65	5.78	28.09	22.29	1.48	85.34
11	RW MCD #TD 1440	Soxhlet	3.17	0.53	34.04	16.66	0.49	0.96	4.06	2.31	14.79	77.01
12	SD Brown Algae	Modified Bligh & Dyer	12.96	0.80	10.65	15.50	2.62	0.52	4.89	0.95	9.67	58.56

In above table, the short notations of the fatty acid names are:

Short notation	14:0	15:0	16:0	16:1	17:0	18:0	18:1	18:2	22:0
Systematic Name	Tetradecanoic	Pentadecanoic	Hexadecanoic	Palmitoleic	Heptadecanoic	Octadecanoic	Oleic	Linoleic	Docosanoic

Another phenomenon observed from the data in Table 7 is that the summations of the fatty acids in all microalgal samples are all far below 100%. The GC spectra show quite a number of unknown peaks, which, to our knowledge, are the non-fatty components extracted from the microalgae. This may be one of the interferences and/or contributors to the largely varied fatty acid profiles. Effort will be made in our on-going study to investigate the extraction effects and attempt to identify the unknown components in the extracted algal lipid samples.

Prescreening of Operating Variables

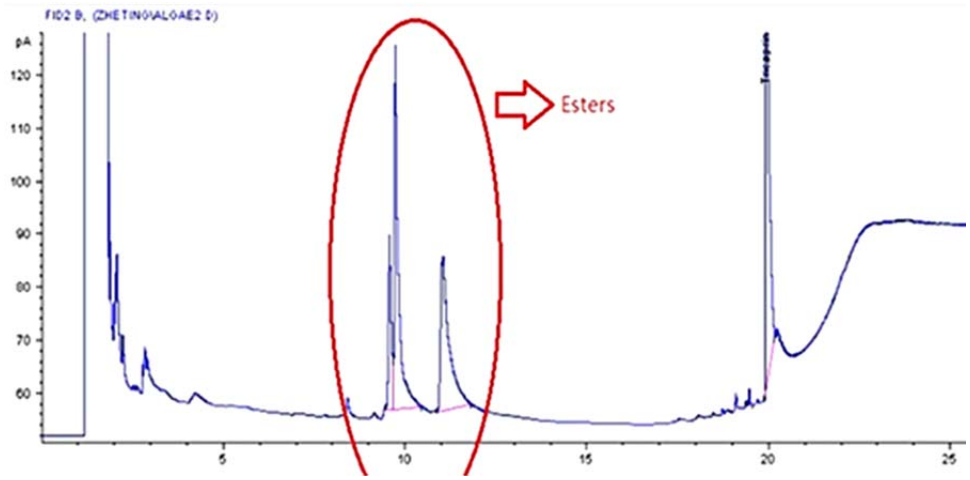
[Note: this portion of the research is behind schedule. The project now continues and is on-going to achieve the objectives set.]

To identify the most influential operating parameters, preliminary experiments were first conducted on operating temperature and residence time. Considering the solid microalgal cells present, the reaction temperature was set at 300˚C to ensure the system to be operated into the supercritical fluid region of methanol (c.p. 239.5 °C and 8.14 MPa). The corresponding pressure was approximately 17.4 MPa (2000 psi). Sampling was taken at 10 and 20 minutes after reaching the set temperature. In these preliminary experiments, *N. Salina* microalgae were used.

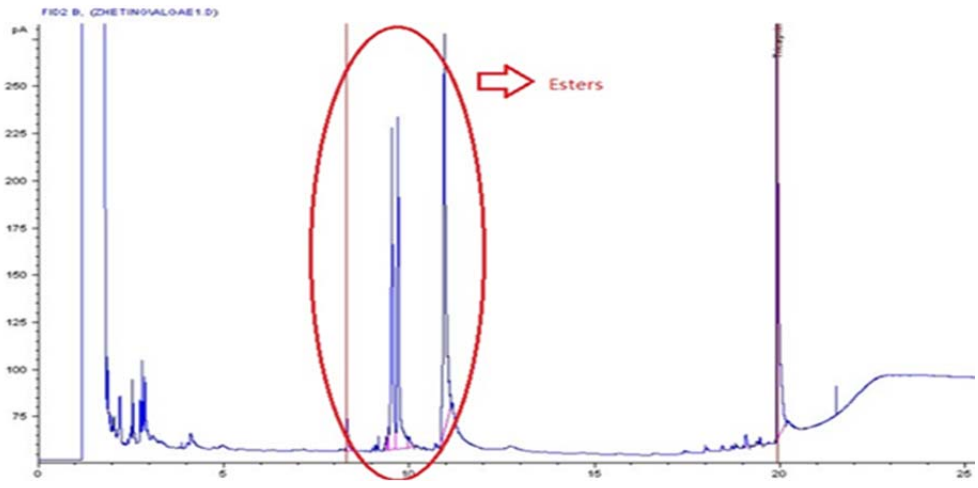
Table 8 and Figure 8 show the examples of preliminary experimental results. It is seen that the system works well in converting the algal lipids in microalgae cells directly into fatty acid esters or biodiesel without lipid extraction first. This validates the concept of *in situ* transesterification.

The other important notice from the preliminary results is that the resection time significantly affects the *in situ* transesterification efficiency. When the reaction time was held for 10 minutes after the set operating temperature was met, the lipid conversion rate was approximately 50% (area percentage, which is proportional to the mass percentage for the purpose of comparing the same set of experiments); if the operating time was extended to 20 minutes, the lipid conversion rate increased to approximately 64% (Table 8(b)). It is expected that increasing reaction time would considerably improve the process and achieve satisfactory conversion efficiency.

The project is on-going. In addition to the reaction time, other parameters including microalgal cell loading rate, necessity of catalysis application as well as the range of operating temperatures and corresponding pressures, will be further systematically investigated.



(a) 10 minutes after set temperature was met



(b) 20 minutes after set temperature was met

Figure 8: GC spectrum of in situ transesterification of N. Salina microalgae.

Table 8: GC Test Results of In Situ Transesterification of N. Salina Microalgae

(a) 10 minutes after set temperature was met.

#	Time	Area	Height	Width	Area%
1	9.552	172.3	32.2	0.0664	7.498
2	9.716	525.7	68.5	0.092	22.884
3	11.03	459.1	29	0.186	19.985
4	19.455	5.5	3	0.026	0.239
5	19.923	1134.7	514.3	0.0297	49.393

(b) 20 minutes after set temperature was met.

#	Time	Area	Height	Width	Area%
1	8.322	50.6	16.2	0.0409	1.397
2	9.094	3.4	2.1	0.0255	0.093
3	9.377	26.2	7.4	0.0446	0.723
4	9.433	25.8	12	0.031	0.712
5	9.528	565.6	170.9	0.047	15.628
6	9.69	629.1	175.2	0.0499	17.383
7	9.986	28.6	5.7	0.0648	0.791
8	10.942	955.7	210.8	0.0613	26.406
9	19.071	23.3	6.8	0.0454	0.642
10	19.453	6.7	3.8	0.0256	0.186
11	19.925	1304.2	615.1	0.0299	36.037