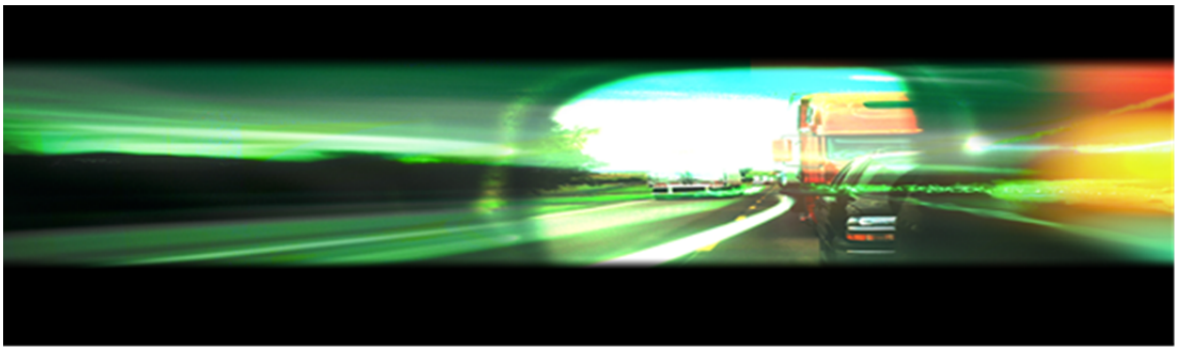
IN SITU TRANSESTERIFICATION OF MICROALGAL OIL TO PRODUCE ALGAL BIODIESEL

Final Report



TranLIVE

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May 2014

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16. Abstract

Research on processing microalgae into algal biofuels is a needed step for commercialization. We aimed at exploring a one-step processing technology that combines lipid extraction and transesterification in a single step or *in situ* transesterification. Specifically, the effects of process parameters on the process efficiency were investigated using whole microalgae as the feedstock. With the model microalga *Schizochytrium limacinum*, experiments were performed on the effects of lipid-to-methanol molar ratio (sRatio), operating temperature, reaction time, and CO_2 as a co-solvent based on a factorial experimental design. It was found that the operating temperature and the reaction time are the most influential process parameters. The effects on the product yield and selectivity were collectively contributed by the combinations of these two parameters. A product yield of 68.7% was achieved after 60 minutes at 210°C with a selectivity of 35%. Similar product yield of 68.6% but higher selectivity of 46.8% were obtained after 30 minutes at 250°C. The sRatio did not affect the product yield significantly due to the higher ratios tested. Despite a noticeable influence found in preliminary investigations, the addition of CO_2 did not display significant effects in low pressure operations. The conditions for optimum product yield and/or product selectivity were inconclusive. Further investigation is needed to gain a thorough understanding of the process.

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EXECUTIVE SUMMARY

Microalgae are considered as one of the most promising feedstocks for biofuel production for their environmental and social benefits. Challenges exist in converting microalgal lipids into algal biofuels due to the unique characteristics of microalgae and the technologies for processing them. In this study, we aimed at exploring an alternative technology that combines lipid extraction from whole microalgae and lipid esterification/transesterification in a single step or *in situ* transesterification. Specifically, the effects of process parameters on the process efficiency were investigated using whole microalgae biomass as the feedstock to obtain microalgal methyl esters or algal biodiesel. The advantages of the proposed one-step, *in situ* transesterification include the simplified process for the conversion of microalgal lipids to algal biodiesel and the reduction of some technological challenges in traditional processes.

Microalga *Schizochytrium limacinum*, a well-known lipid-rich strain of green microalgae, was used as the model algae in this study for its high lipid content (50%). Experiments were performed on the lipid-to-methanol molar ratio (sRatio; 1:50, 1:75 and 1:100), operating temperature (170°C, 210°C, 250°C, and 290°C), reaction time (30, 60, 90, and 120 minutes), and initial pressure of CO_2 (0 psig and 200 psig of CO_2) based on a factorial design. All experiments were carried out in a batch reactor system with precise temperature control. The content of microalgal methyl esters, the targeted product in the mixture after reaction, was analyzed by a gas chromatography-mass spectrometry (GC-MS). The product yield and the product selectivity were used as the indicators for process efficiency.

Experimental findings indicated that the operating temperature and the reaction time are the most influential process parameters in the *in situ* transesterification of the microalga in sub-/supercritical methanol with no catalyst application. The effects on the process efficiency were collectively contributed by the combinations of these two parameters. A product yield of 68.7% was achieved after a 60 minute reaction time at 210°C with a corresponding product selectivity of 35%. A similar product yield of 68.6%, with a corresponding product selectivity of 46.8%, was obtained after 30 minutes when the operating temperature increased

to 250°C. The lipid-to-methanol molar ratio (sRatio) is an important parameter for lipid extraction, but its effects on product yield were found insignificant between levels of sRatio due to the higher ratios tested in this study. The addition of carbon dioxide (CO_2) as a co-solvent showed a noticeable influence on the processing in preliminary investigations; however, further investigation did not reveal significant effects on the overall *in situ* transesterification process. This is likely because not all operating conditions reached the supercritical pressure of CO_2 (7.4 MPa or 1,073 psig), thus a lack of the supercritical fluid effect from the CO_2.

The conditions for optimum product yield and/or product selectivity were inconclusive. Preliminary statistical analysis on the experimental data did not show an optimum point; instead, multiple points of higher yield and/or selectivity can be found under the interactive effect of the process parameters.

This study is a work in progress. We continue experimenting on the process and exploring further to gain better understanding. The recommended future work includes further investigation on the process parameters and statistical analyses on the data, thermodynamic and/or reaction kinetic analysis of the process, potential technologies for separating the targeted algal methyl esters from the product mixture, and a conceptual process design for performing the *in situ* transesterification of microalgae in a continuous-flow mode. An engineering economic analysis on the process is also under consideration to develop estimates on the energy and operating costs.

DESCRIPTION OF PROBLEM

Microalgae are considered one of the most promising feedstocks for biofuel production. Biofuels from microalgae bear many environmental and social benefits including renewability, reduced net CO_2 emissions, and minimal effect on environment (Christi, 2007). The most advantageous benefit of using microalgae as the feedstock for biofuel production is attributable to its high oil productivity per land unit (US DOE, 2010). During the past two decades, tremendous efforts have been made worldwide to advance the technologies of cultivating microalgae of various strains and producing algal biofuels for transportation purposes. However, the research community still faces tremendous challenges in microalgae processing, such as harvesting, dewatering, lipid extraction and conversion due to the unique properties of microalgae.

Traditionally, biodiesel from algal lipids is produced by a two-step approach. Solvent extraction is first used to obtain the algal lipids from microalgae biomass, which involves cell rupture, lipid extraction, and lipids/solvent separation from the biomass residues. Next, the extracted microalgal lipids are transesterified and esterified to convert the triglycerides and free fatty acids, respectively, in the lipids to algal methyl esters or biodiesel. This approach works in general and most technologies involved in this traditional process are readily available and proven. However, due to the complexity of the chemical and physical properties and the unique characteristics of microalgae, such as low algae concentrations in cultivation media and high water content in microalgal biomass, traditional processes for algal biodiesel production are inefficient and have high processing costs.

The ultimate goal of this research is to develop a novel process to produce algal biodiesel directly from microalgae with lipid extraction and esterification/transesterification in a single step or *in situ* transesterification. In this study, we specifically aimed to explore the effects of process parameters on process efficiency in using whole microalgae biomass as the feedstock to obtain microalgal methyl esters. The advantages of the *in situ* transesterification include the simplified process for converting microalgal lipids to algal biodiesel and the reduction of some technological challenges encountered in traditional processes.

APPROACH AND METHODOLOGY

Microalga *Schizochytrium limacinum*, a well-known lipid-rich strain of green microalgae, was used as the model algae in this study. To examine the lipid content and the fatty acid (FA) profile of algal lipids, the microalga was extracted by three types of solvents (hexane, methanol, and a mixture of chloroform – methanol and water) to obtain the microalgal lipids. Chemical analysis of the extracted algal lipids by gas chromatography (GC) revealed similar FA profiles (Table 1). The lipid content was averaged for 50% on dry-matter mass basis and the average molecular weights of the triglycerides and fatty acids were 284 g/mol and 890 g/mol, respectively. An analysis on the extracted microalgal lipids according to the American Oil Chemists' Society (AOCS) official method Ca 5a-40 indicated a content of 16.6%wt free fatty acids (FFA).

Table 1: Average FA Profile of the Microalgal Lipids Used in This Study

FA	Short notation	Composition (wt%)
Myristic	C14:0	2.67
Palmitic	C16:0	40.57
Stearic	C18:0	1.20
Oleic	C18:1	5.40
Linoleic	C18:2	13.13
Linolenic	C18:3	1.90
Docosapentaenoic	C22:5	6.50
Docosahexaenoic	C22:6	27.37

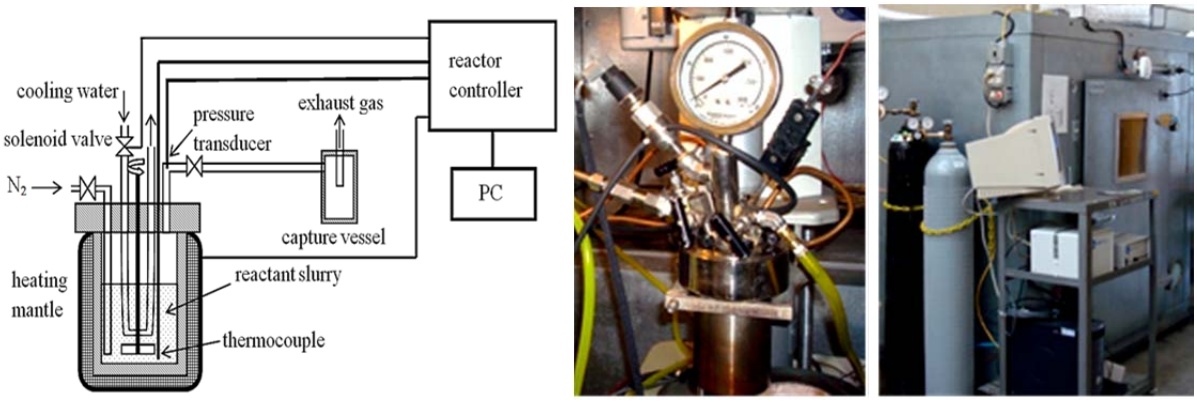
In the *in situ* transesterification, methanol at elevated temperatures was used as the solvent to extract the microalgal lipids also as the reactant for transesterifying/esterifying the microalgal lipids into esters.

Preliminary experiments were carried out first to identify the influential process parameters. These parameters include the reactant (algal lipids) to methanol molar ratio, operating temperature, reaction time, and application of CO_2 as a co-solvent. Preliminary experiments were conducted under two temperatures, 210˚C and 250˚C, which are in the vicinity of the

supercritical point of methanol (240 and 8.1 MPa), and with five levels of lipid-to-methanol molar ratios (abbreviated as sRatio) for 30 and 60 minutes. The stirring was set constant at a speed of 500 rpm and was not considered as a process parameter.

Following the preliminary investigation, a systematic investigation on process parameters was conducted. The objective was to understand the effects of process parameters on the process efficiency as indicated by the product yield and product selectivity. Experiments were performed based on a factorial experimental design, designated as 3×4×4×2, which included three levels of sRatio (1:50, 1:75 and 1:100), four levels of operating temperature (170°C, 210°C, 250°C, and 290°C), four levels of reaction time (30, 60, 90 and 120 minutes), and two levels of initial co-solvent pressure (0 psig and 200 psig of CO_2). According to this experimental design, 288 experiments in 96 sets were conducted successfully and each experiment was implemented in triplicates.

All experiments were carried out in a batch reactor system (Pressure Reactor 4560, 300-mL; Parr Instrument Co., Moline, Illinois). The reactor can handle temperatures up to 350°C and pressures up to 20 MPa or 3,000 psig. The operating temperature is controlled by a PC-based 4857 Reactor Controller. The controller displays the operating temperature, pressure, and agitation speed. The reactor system was hosted in a metal-framed chamber with an exhaust vent to ensure safe operations (Figure 1).



(a) schematic diagram (b) reactor assembly (c) the chamber

Figure 1: The batch reactor system for this study.

The content of the targeted product microalgal methyl esters was determined by a gas chromatography-mass spectrometry (GC-MS) (PolarisQ instrument, ThermoFinnigan, West Palm Beach, Florida) in the electron impact mode. Gas separation in the GC-MS analysis was achieved by a ZB-1 capillary column (15 m x 0.25 mm dia.) with helium as the carrier gas. The temperature was programed at 5°C/minute from 40°C to 250°C while the injector and MS-transfer line were kept at 250°C.

FINDINGS; CONCLUSIONS; RECOMMENDATIONS

Findings

The objective of this study was to investigate the process of converting the microalgal lipids to fatty acid methyl esters which are commonly abbreviated as FAMEs and commercially known as biodiesel. Microalgal lipids are mainly composed of triglycerides, free fatty acids (FFA), and phospholipids. In this study, the microalgal lipids were extracted by methanol at elevated temperatures and sequentially converted to microalgal methyl esters via transesterification with methanol but without adding a catalysts. This process of *in situ* transesterification needs to be carefully controlled so that only the microalgal lipids are extracted and converted to FAMEs while the microalgal cellulosic biomass is kept intact as much as possible. If the process is operated in such a way that the whole microalga, including the lipids and cellulosic biomass, is converted into liquefied products, the process is then referred as the thermochemical liquefaction, which is not within the scope of investigation in this study.

Before investigating the effects of process parameters, the microalga was first characterized for its fatty acid (FA) profile. This strain of microalga is quite unique. It not only contains much higher lipid content (50%wt on dry matter basis) but also a different FA profile as compared to other strains previously studied by the authors (Bi and He, 2013).

Prescreening of Operating Parameters

Based on the understanding of transesterifying vegetable oils, the process parameters in this study included processing temperature, reaction time, and quantity of solvent which is represented by the lipid-to-methanol molar ratio (sRatio).

The processing temperature and reaction time determine the reaction environment. Higher temperature and longer reaction time would enhance the reaction rate and increase the conversion of the lipids, respectively. However, too high of a temperature or too long of a reaction time would also convert the microalgal biomass to other products, which is undesirable for the purpose of this study. Operating temperatures of 210˚C and 250˚C were chosen in the preliminary investigation, since they are in the vicinity of the critical

temperature of methanol (240˚C) and would presumably lead to favorable fluid properties of methanol as a solvent.

Two indicators were used to evaluate the process efficiency, the yield and the selectivity of the FAMEs in the product mixture. The product yield is defined as the percentage of the molar quantity of FAMEs in the product mixture to the theoretical molar quantity of fatty acids in the feedstock. The selectivity of FAMEs is defined as the mass percentage of targeted product FAMEs to the overall products in the product mixture.

Preliminary experimental results showed that the product yield depends on the interactive effect of the operating temperature and the reaction time at high sRatio. At the lower temperature (210˚C), longer reaction time is needed to achieve a good product yield. At the higher temperature (250˚C), extended reaction time may not be beneficial for the same purpose. The possible cause of this phenomenon is that some decomposition reactions of FAMEs may occur at higher temperatures and extended reaction time. A general observation is that a combination of lower temperature and longer reaction time led to a significantly higher lipid conversion yields (Figure 2). This finding was used as a guideline in the following stage of investigations.

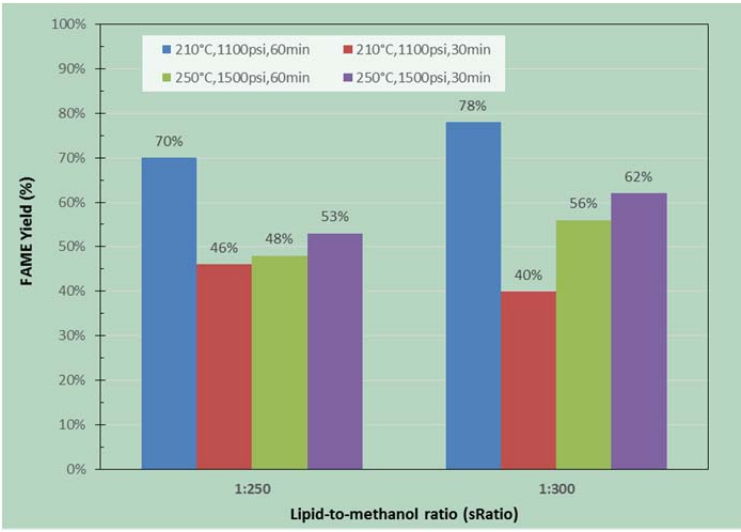


Figure 2: Product yields of microalgal lipids at two levels of sRatio.

Carbon dioxide (CO_2) at its supercritical conditions (304K or 31°C and 7.4 MPa or 1,073 psig) is an excellent fluid for supercritical extraction. It is often used with methanol or ethanol as the co-solvent in supercritical processing (Wyatt and Haas, 2009; Soh and Zimmerman, 2011). In this study, the application of supercritical carbon dioxide as the co-solvent was also explored for possibly enhancing lipid extraction. Experimental results showed that the addition of CO_2 promoted the reaction enhanced lipid extraction and product yields (Figure 3). However, the significance of its enhancement requires further investigation.

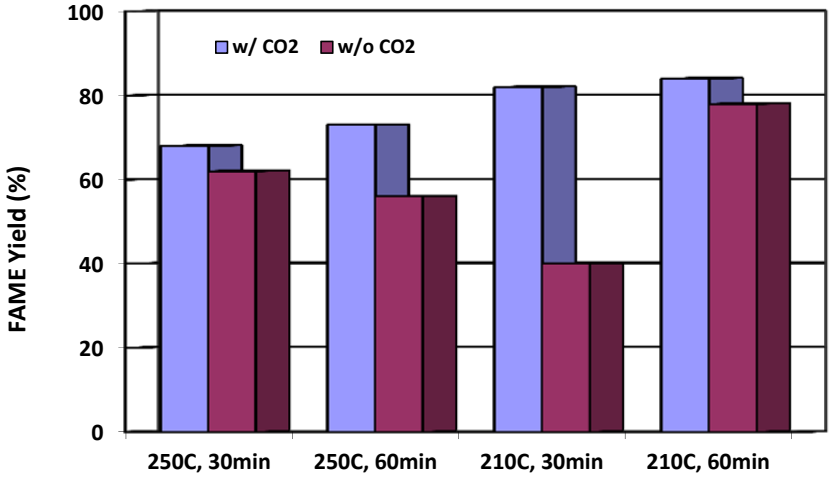


Figure 3: Product yields of FAME with/without co-solvent CO_2 at sRatio 1:300.

Stoichiometrically, one mole of microalgal lipids as expressed in triglycerides require three moles of methanol to react. Meanwhile, methanol is also a solvent for lipid extraction from the microalga. Higher amounts of methanol promote lipid extraction during *in situ* transesterification, but also increase the processing cost. To examine the sRatio effect, six sRatio levels were explored: 1:50, 1:75, 1:100, 1:250, 1:300, and 1:350; which correspond to mass ratios of 1:1.8, 1:2.7, 1:3.6, 1:4.5, 1:10.8, and 1:12.6, respectively.

Experimental results indicated that the sRatio does not clearly show the effect on product FAME yield (Figure 4). As the sRatio increased, the product yield fluctuated and reached the lowest point with sRatio 1:100. This phenomenon suggests that from the transesterification

point of view, sRatio of 1:50 is adequate; from lipid extraction and physical operation point of view, sRatio of 1:50 to 1:100 are still preferable. A reason for this is that a low methanol application would not make a good microalgae-methanol suspension slurry and would limit the mass transfer of microalgal lipids from biomass structure into the liquid phase for reaction. Therefore, in the second stage of this study, sRatio of 1:50-1:100 were used.

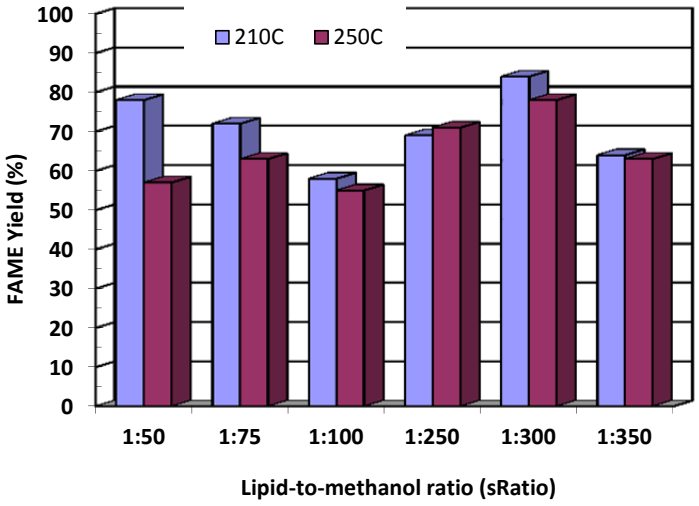
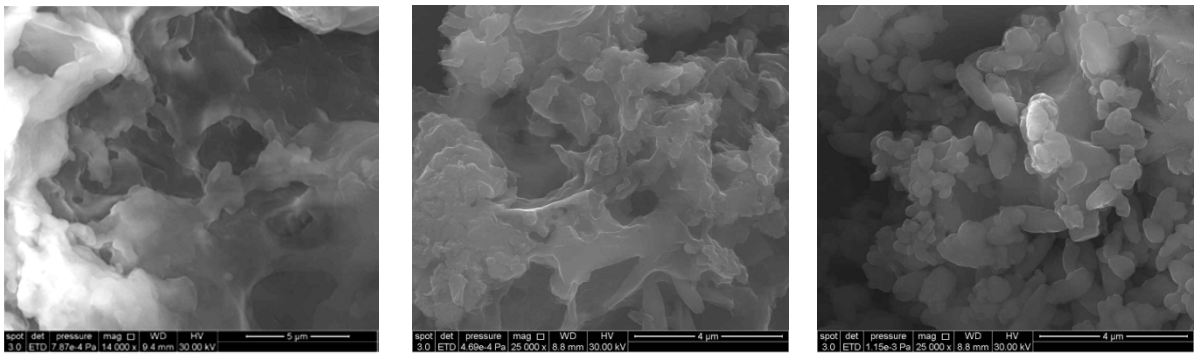


Figure 4: Product FAME yields vs. sRatio under with 500 psig carbon dioxide for 60 minutes.

Another possible barrier in *in situ* transesterification of microalgal lipids is the physical structure of the microalgal cells. Lipids need to overcome the resistance of the algal cell structure and the membrane to migrate to the methanol phase for reaction. Methanol at an elevated temperature possesses strong solvent properties and may break the cell structure. To observe solvent effect on the microalgal biomass structure in the *in situ* transesterification process, images of scanning electron microscope (SEM) were taken to help understand how methanol works under different thermal conditions (Figure 5).

Before processing, the cell structure of microalgal biomass was intact with visible pores on the surface and in a spherical structure. As the processing temperature increased, the cell structure gradually collapsed. At 290˚C, the cell structure was completely destroyed. Microalgal biomass no longer held its spherical structure and disintegrated into smaller

pieces. This suggests that methanol at elevated temperatures breaks the cell structure, which leads to biomass decomposition. Under extreme thermal conditions, therefore, the strong supercritical fluid effect of methanol was enhanced to such a level that it can physically destroy the cell structure and react with lignocellulosic matters. This was an undesirable result in this study.



(a) before (b) after (210˚C) (c) after (290˚C)

Figure 5: SEM images of microalgal biomass before and after *in situ* transesterification.

Systematic Investigation of Process Parameters

Preliminary experimental results revealed that *in situ* transesterification of the microalga is achievable in methanol in the vicinity of its critical point (240˚C and 8.1 MPa). However, the complexity of the microalgal properties, namely physical and chemical properties of the microalgal lipids versus the microalgal cellulosic biomass, implies that the process operating parameters need to be systematically investigated in order to achieve satisfactory yields and selectivities of the targeted product FAMEs.

The next stage of this study was to conduct a systematical investigation on the effects of the influential process parameters (i.e., operating temperature, reaction time, sRatio, and co-solvent CO_2 addition). The goal was to find an optimal reaction condition at which the highest product yield and selectivity could be achieved within the selected ranges of operating parameters. Experiments were performed based on a factorial experimental design that includes three levels of sRatio (1:50, 1:75 and 1:100), four levels of operating temperatures (170°C, 210°C, 250°C, and 290°C), four levels of reaction time (30, 60, 90 and 120 minutes), and two levels of CO_2 initial pressure (0 psig and 200 psig). The factorial

design is designated as 3×4×4×2. Experiments were conducted in triplicate to ensure the repeatability and reliability of the data. Tables 2 and 3 summarize the results.

Closely examining the results leads to some preliminary conclusions. First, the product yield ranged widely from as low as 12% to as high as 75% in these sets of experiments and were highly dependent on the operating conditions. Second, the operating temperature significantly affected the product yield. Product yields generally increased as the temperature increased in the range 170-250°C. At 290°C, the product yields were sometimes lower than yields at lower temperatures. For example, under the operating temperature of 290°C, sRatio 1:100 and 200 psig initial CO_2 addition, the product yields were 30-54% lower than those at 250°C (50-68%). A possible reason might be the decomposition of FAMEs at high temperatures.

The reaction time showed effects on the product yield that correlated with the operating temperatures with a few exceptions. At low temperatures (170°C and 210°C), the product yield showed an increasing trend as the reaction time extended in the range of 30-120 minutes. Once the operating temperature was higher than 210°C, especially at 290°C, the product yield changed inversely as the reaction time increased. Similar to the reason discussed above, this decrease in product yield might be due to the decomposition of the FAMEs at the high temperature and extended reaction time.

The sRatio did not exhibit a clear effect on the product yield. This finding confirms what was observed in preliminary investigations (Figure 4). Under the same operating temperatures, the product yields were approximately in the same ranges among the three sRatio.

The possible reasons may include that (1) all three sRatios were far beyond their limiting quantities for the transesterification reaction then their effects on lipid conversion efficiency are minimized; (2) the quantity of methanol as the solvent was adequate for the purpose of lipid extraction and not a limiting factor. Therefore, the optimum sRatio in this range (i.e., 1:50- 1:100) is not based on the requirement for lipid extraction or transesterification, rather on the operability of the methanol-microalgal biomass slurry system. From this point of view, the lower sRatio of 1:50 seemed adequate.

Table 2: Yield (mol%) of Microalgal Esters (FAMEs) via I*n situ* Transesterification

Time	sRatio 1:50 & 0 psig initial CO_2				sRatio 1:75 & 0 psig initial CO_2				sRatio 1:100 & 0 psig initial CO_2			
(min)	170°C	210°C	250°C	290°C	170°C	210°C	250°C	290°C	170°C	210°C	250°C	290°C
30	13.9±0.8	41.1±1.1	38.9±2.3	37.2±2.7	15.7±2.0	31.0±2.3	61.7±1.4	42.7±3.6	17.0±1.5	51.3±2.9	63.0±2.6	63.0±2.9
60	15.1±0.7	42.3±2.1	47.1±2.1	45.0±1.3	11.1±1.3	56.5±2.5	52.1±1.2	36.0±1.4	21.0±1.7	51.8±3.1	58.9±3.5	33.2±3.1
90	20.7±1.3	43.0±1.8	48.1±1.1	27.5±1.4	37.4±1.5	44.4±1.6	18.6±2.3	36.0±3.4	2.6±0.9	41.5±1.9	41.7±3.1	29.6±2.4
120	53.7±1.7	63.6±2.3	75.8±1.9	30.5±1.3	34.6±2.6	62.0±3.0	30.2±3.4	32.0±2.6	39.8±2.6	38.2±2.1	37.0±2.9	36.7±2.8
Time	sRatio 1:50 & 200 psig initial CO_2				sRatio 1:75 & 200 psig initial CO_2				sRatio 1:100 & 200 psig initial CO_2			
(min)	170°C	210°C	250°C	290°C	170°C	210°C	250°C	290°C	170°C	210°C	250°C	290°C
30	12.1±0.7	55.6±1.2	49.6±1.7	56.2±1.7	10.6±3.1	27.2±1.9	48.0±2.1	58.9±2.4	10.9±2.1	37.4±2.6	68.6±3.1	53.9±2.3
60	13.7±1.2	68.7±1.5	49.2±1.8	68.8±2.1	20.6±3.4	67.7±2.0	60.2±1.7	38.4±3.1	42.7±1.8	42.7±2.5	63.7±2.6	30.6±3.3
90	26.3±1.7	66.4±1.9	43.1±2.0	46.7±1.5	34.7±2.1	46.0±2.1	52.7±1.5	28.9±3.5	37.3±2.4	33.3±3.0	52.5±2.4	33.2±2.4
120	35.9±1.8	68.8±1.7	37.3±1.9	48.1±1.1	38.1±1.6	54.3±1.3	47.1±2.6	31.6±2.7	32.4±2.6	53.2±1.6	50.4±3.0	29.9±3.1

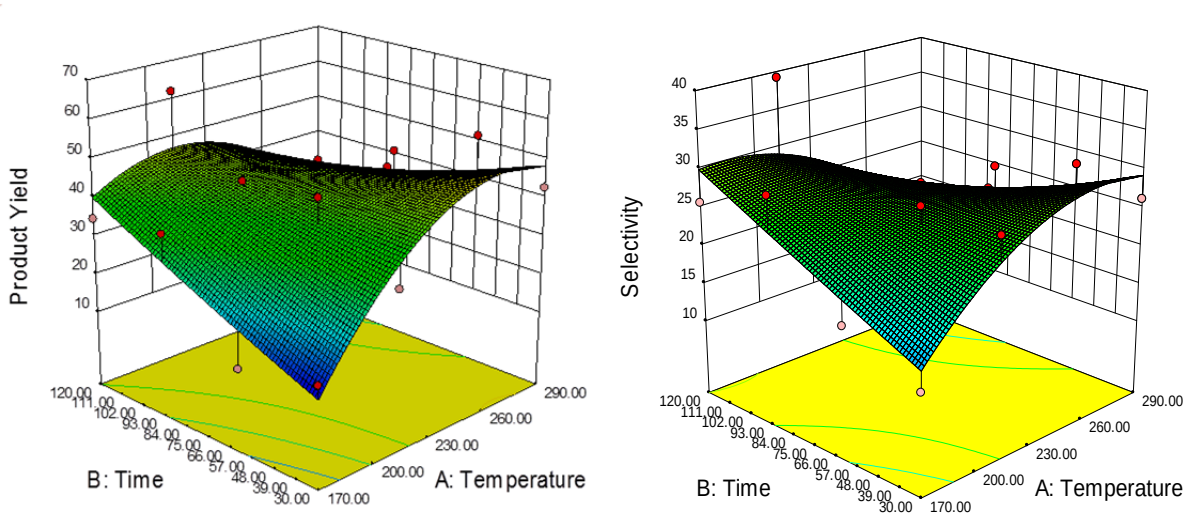
Table 3: Selectivity (%wt) of Microalgal Esters (FAMEs) via I*n situ* Transesterification

Time	sRatio 1:50 & 0 psig initial CO_2				sRatio 1:75 & 0 psig initial CO_2				sRatio 1:100 & 0 psig initial CO_2			
(min)	170°C	210°C	250°C	290°C	170°C	210°C	250°C	290°C	170°C	210°C	250°C	290°C
30	12.1±1.9	27.4±2.4	29.8±1.9	19.8±2.3	13.1±0.9	28.2±0.9	33.6±0.3	26.2±1.0	17.1±1.3	35.2±0.9	36.7±0.3	17.4±1.9
60	18.8±1.1	29.0±1.9	24.9±1.7	22.9±3.0	17.1±0.9	28.4±0.8	30.4±1.0	22.8±1.0	18.8±2.1	35.6±0.8	34.5±2.8	19.4±2.1
90	20.7±2.1	28.8±1.1	27.4±2.7	14.1±1.4	29.8±0.7	27.5±0.9	10.5±0.8	21.4±0.4	21.0±1.5	47.4±0.9	25.0±2.8	19.6±2.4
120	35.9±1.8	36.3±1.6	39.8±2.5	16.3±1.2	25.7±0.9	39.2±0.6	17.0±0.8	19.2±0.7	29.3±1.9	41.2±0.6	20.8±3.1	22.7±1.7
Time	sRatio 1:50 & 200 psig initial CO_2				sRatio 1:75 & 200 psig initial CO_2				sRatio 1:100 & 200 psig initial CO_2			
(min)	170°C	210°C	250°C	290°C	170°C	210°C	250°C	290°C	170°C	210°C	250°C	290°C
30	16.6±2.0	24.4±2.6	30.0±1.6	32.3±2.0	15.1±0.8	21.7±1.1	25.4±0.3	40.6±1.0	10.9±2.1	26.6±1.1	46.8±2.3	38.5±2.1
60	16.7±1.6	35.0±2.1	29.8±2.1	41.0±1.6	20.4±0.7	30.6±2.1	35.2±1.0	24.1±1.0	39.0±2.3	28.3±2.1	37.6±1.5	19.3±1.9
90	22.1±2.2	35.8±1.6	25.9±2.0	26.6±2.7	28.7±1.3	29.9±1.8	36.7±0.8	20.5±0.4	25.7±3.1	21.6±1.8	38.7±1.8	23.5±2.5
120	28.2±1.3	36.8±1.5	21.6±1.7	25.6±1.9	29.3±1.7	33.1±2.3	24.9±0.8	24.0±0.7	24.2±1.2	34.7±2.3	37.7±3.4	25.4±3.4

Comparing the results between experiments with and without CO_2 addition showed that the addition of CO_2 as a co-solvent did not show a significant influence on the product yield. This observation is in agreement with the statistical ANOVA analysis. However, this conclusion does not agree with our initial observations in preliminary investigations when it was observed that the addition of CO_2 did show a significant effect on the product yield (Figures 2 and 3). The possible reason is that not every operating condition reached the supercritical condition of CO_2 (31°C and 7.4 MPa or 1,073 psig due to the lower temperatures tested. For example, when the system was run at 170°C, the operating pressure was about 880 psig which is below the supercritical pressure of CO_2. As a result, the effect of this subcritical CO_2 was not significant. It may be concluded that the addition of CO_2 as a co-solvent could help the lipid extraction in some degree but its significance is limited. From operating cost point of view, addition of CO_2 for enhanced *in situ* transesterification may not be justified.

To summarize the findings of process parameter effects on the product yield, the operating temperature and the reaction time are the most influential process parameters in the *in situ* transesterification of the microalga in sub-/supercritical methanol with no catalyst application. Observable effects on product yield are collectively contributed by the operating temperature and the reaction time. Figure 6(a) is a graphical presentation of a selected set of data that shows the high product yields can be achieved by a combination of the operating temperature and the reaction time.

Another indicator for process efficiency is the selectivity of the targeted product microalgal methyl esters or FAMEs. Close examination of the data on product selectivities reveals similar conclusions as those observed on product yields. Generally, the product selectivity increased as the operating temperature increased in the range of 170-250˚C. A clear trend is when the operating temperature was at 290˚C, the selectivities were generally lower than those at lower temperatures (Figure 6(b)).



(a) product yield (b) product selectivity

Figure 6: Surface response analysis of the product yield and selectivity of microalgal esters via *in situ* transesterification with sRatio 1:75.

Figure 6 shows that to obtain satisfactory product yields and/or selectivities, the temperature-time combinations are either lower operating temperature (210˚C) and longer reaction time (120 minutes) or higher operating temperature (290˚C) and short reaction time (30 minutes). The decision on choosing the optimum conditions for practical applications, therefore, depends also on other parameters such as the productivity (short reaction time) or the requirement for utility availability, such as high temperature media for heating. The final decision should be made ultimately by optimizing the system based on the overall capital and operational costs.

Conclusions

The experimental results obtained in this study suggest that direct conversion of algal lipids of microalga *Schizochytrium limacinum* can be achieved in one step, i.e., lipid extraction and conversion, via *in situ* transesterification in methanol at elevated temperatures. The most influential process parameters are the operating temperature and reaction time. The lipids-to-methanol molar ratio or sRatio is an important factor in this process, but its effect is insignificant in the range explored. The addition of CO_2 as a co-solvent does not indicate significant effect on the overall *in situ* transesterification process, despite positive findings in

preliminary investigations, if the operating condition does not reach the supercritical condition of CO_2, i.e., 304K (31°C) and 7.4 MPa (1,073 psig).

The conditions for optimum product yield and/or product selectivity were inconclusive. Preliminary statistical analysis on the experimental data does not show an optimum point or area; instead, multiple points of higher yields and/or selectivities can be found with the combinations of process parameters.

Recommendations for Future Work

This study is considered a research on-going. We are continuously experimenting on the process and exploring further to answer questions that are unclear to us at this stage. The following are the recommendations for future work.

1) Further investigation on the process parameters and conduction of statistical analyses on the data obtained for better understanding of the process,

2) Interpretation of the experimental results from the thermodynamics and reaction kinetics point of view,

3) Exploration on the means of separating the targeted product, i.e., the algal methyl esters or biodiesel, from the rest of the products, including the unreacted algal biomass and other non-ester products in the mixture,

4) A conceptual process design for performing the *in situ* transesterification of microalgae in a continuous-flow mode, and

5) An engineering economic analysis on the process to estimate the energy and operating costs.

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APPENDIX

Publications:

Bi, Z., and B. He. 2013. Characterization of Microalgae for the Purpose of Biofuels Production. *Trans. ASABE* 56(4):1529-1539.