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BIODIESEL SYNTHESIS FROM ALGAE (*Chlorella* SP.) IN CONDITION OF SUBCRITICAL METHANOL

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ABSTRACT

In this study, transesterification reaction under subcritical condition of methanol (or called subcritical methanol method) was employed to directly produce biodiesel from Chlorella sp. instead of conventional method that was based on acid/base catalysts. The effects of reaction parameters on FAME (biodiesel) conversion such as temperature (165, 175 and 185°C), methanol to biomass ratio (10/1, 15/1, 20/1 and 25/1 g/g), water content (5, 15, 30, 50 and 80%), and reaction time (1, 4, 6, 8, and 12 h) were studied. According to the experimental results, FAME conversion increased with the increase of reaction time and highest FAME conversion 95% at 175°C, 20/1 (g/g) methanol to biomass ratio and 50% water content in biomass.

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1 INTRODUCTION

Rapid development of industries in the world is main reason for the depletion of fossil fuel resources and environmental pollution due to harmful gas emission such as CO_x, NO_x, and SO_x. Therefore, finding new and safety materials replaced for fossil fuels are becoming more important and challenging toward researchers. Biodiesel is renewable, biodegradable, nontoxic and safety material that can be considered as alternative material for petroleum diesel (Ju *et al.*, 2013). Currently, biodiesel were mainly produced from edible oils such as soybean, palm, peanut, olive, rice bran and catfish that might affected to food security in the future (Mata *et al.*, 2010). Some researches have been proved that microalgae a potential feedstock due to its advantages such as high oil content, high photosynthesis, ability of living in severe environment, utilizing nitrogen and phosphor from industrial and agricultural waste water, using green house emission CO_2 (Zhou *et al.*, 2013) and especially more proliferation from 7 to 10 days to harvest (Hsieh and Wu, 2009; Mata *et al.*, 2010) and its oil can be used to convert to biodiesel.

Besides feedstocks, producing technologies are very important to synthesize and commercialize biodiesel product. Conventional methods used to synthesize biodiesel from microalgae are basically acid and base catalytic transesterification. These methods have limitations in term of complicated purification steps, leading to high producing cost for biodiesel (Ju *et al.*, 2013). To overcome these problems, some studies were carried out under the condition of supercritical methanol, however this method has existed obstacles due to severe reaction conditions. Therefore, the non-catalyst *in-situ* transesterification under subcritical methanol can be considered as a comparable method since its reaction conditions were not as strict as supercritical but high biodiesel yield still can be obtained and microalgae biomass can be used directly instead of extracted oil (Huynh *et al.*, 2012). The objective of this study is to investigate the synthesis of biodiesel from microalgae under subcritical methanol method (SBCM).

2 MATERIALS AND METHODS

2.1 Materials

Source of *Chlorella* sp. was provided from College of Aquaculture & Fisheries, Can Tho University. Biomass of the algae used to convert to biodiesel were produced following the process that was presented in previous work (Ho *et al.*, 2014). Standard of fatty acid methyl esters (FAMEs) mixture (47885-U, 37 components FAME Mix) was purchased from Supelco (Bellefonte, PA, USA). All solvents and reagents were either high performance liquid chromatography (HPLC) or analytical reagent grade, obtained from commercial sources.

Table 1: Oil composition of *Chlorella* sp

Composition	%wt.
Free fatty acid	54.56
Monoglyceride	9.97
Diglyceride	10.43
Triglyceride	18.59
Others	6.46

2.2 Biodiesel production based on SBCM method

Biodiesel was directly synthesized from *Chlorella* sp. based on the process that was described in Figure 1. Dry biomass of *Chlorella* sp. was mixed with methanol and distilled water with different ratio in a high pressure reactor that was equipped with an external electrical furnace. The reactor was made of stainless steel and can withstand an estimated maximum pressure of 30 MPa. Eight M8 screws which can afford 12.8 tons of tensile force were used for tightening the reactor with its cap. Figure 2 is the schematic diagram of the reaction system set up. Temperature in the reactor was measured by a thermocouple and controlled by a temperature controller. The applied pressure (3.5 MPa) which is higher than the saturated vapor pressure of water and methanol by using nitrogen to ensure all experiments was carried out under subcritical condition of water and methanol. Parameters that affected FAME yield such as water content, reaction time and temperature and amount of methanol will be investigated.

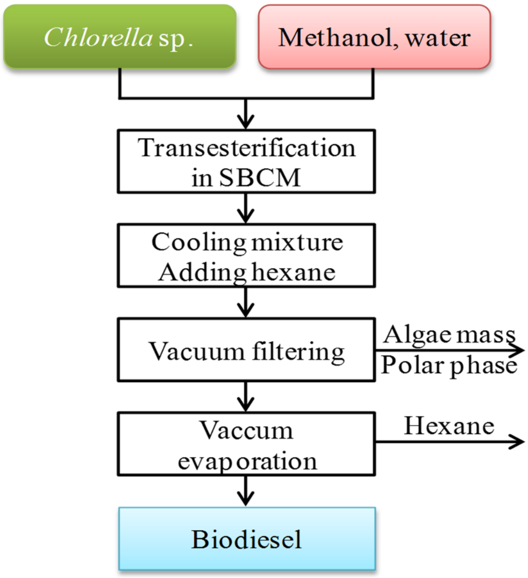

Fig. 1: Diagram of biodiesel production from *Chlorella* sp. using SBCM

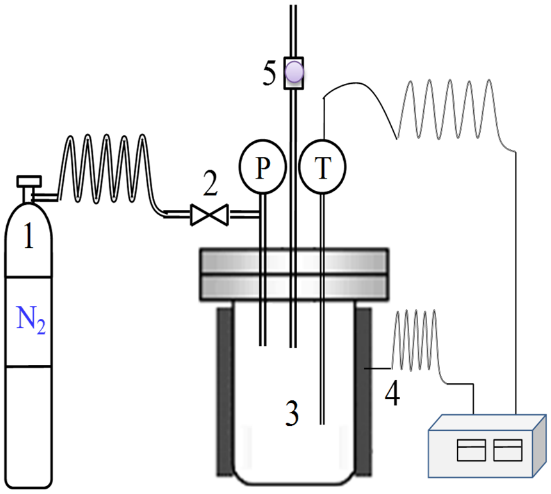

Fig. 2: Schematic diagram of reaction system: (1) nitrogen cylinder, (2) needle valve, (3) reactor, (4) electric heater, (5) rupture disc, (P) pressure gauge, (T) thermocouple

After finishing reaction, temperature of the reactor was quickly reduced by using cold water. Pressure inside the reactor was reduced by releasing the gas phase in the reactor. Reaction in the reactor stopped as soon as the pressure dropped below the vapor pressure of methanol. The released vapor was collected and condensed in a container submerged in ice water bath. Liquid in the reactor was collected and combined with liquid from the condensed vapor. Hexane was then added to the mix ture to extract FAME and the supernatants were separated from unreacted biomass by vacuum filtration. Hexane was then removed by rotary evaporator and mass of the product was determined. Ester conversion was estimated from the area percentage of FAME, free fatty acid (FFA), triglyceride (TG), diglyceride (DG) and monoglyceride (MG) by high temperature gas chromatography (HTGC) analysis of the products.

2.3 Gas chromatography analysis

Qualitative and quantitative analysis of un-reacted FFA, AG (MG, DG and TG) in each sample was performed on a Shimadzu GC2010 (Japan) equipped with a split-split injector and a FID. Separation was carried out on a ZB-5HT (5% phenyl)-methylpolysiloxane nonpolar column (15 m x 0.32 mm i.d., 0.1 µm film thickness) (Agilent technologies). Both injector and detector temperatures were 370°C. The temperature program was set at 80°C, increase to 365°C at 15°C/min and hold for 8 min. Total analysis time was 29 min. Nitrogen was used as the carrier gas with a linear velocity of 30 cm/s at 80°C. Data analysis was carried out by software ‘‘GC Solution version 2.3’’, Shimadzu. Biodiesel (or FAME) conversion was calculated by formula 1 (Tsigie *et al.*, 2012):

$$\%\text{FAME} = \frac{\text{Peak area of FAME}}{\text{Total peak area}} \qquad (1)$$

3 RESULTS AND DISCUSSION

3.1 Effect of reaction temperature on FAME conversion

Temperature plays an important role not only in conventional method of acid/base catalysts but also in subcritical methanol method in term of biodiesel conversion. To study effect of temperature on FAME conversion, different temperature ranging from 165, 175 and 185°C was applied. All experiments were conducted by using 1 g biomass of microalgae with 5 wt.% water content, 10 g methanol and 1 hour reaction time. The result was presented in Table 2 and highest FAME conversion (20.5%) was obtained at 175°C.

Table 2: Effect of temperature on FAME conversion

Temperature (°C)	165	175	185
FAME (%)	13.2±0.8	20.5±1.1	14.5±0.6
FFA (%)	37.3±2.3	20.2±1.0	36.3±1.1
Monoglyceride (%)	28.8±1.8	42.5±2.4	31.7±1.3
Diglyceride (%)	13.1±0.9	10.2±0.5	7.3±0.2
Triglyceride (%)	7.0±0.3	5.0±0.1	8.0±0.1
Others (%)	0.6±0.0	1.6±0.0	2.2±0.0

Reaction condition: 1 g biomass; 5 wt.% water content, 10 g methanol, 1h

3.2 Effect of methanol content on FAME conversion

In transesterification reaction, stoichiometric ratio requires three molecules of methanol to react with one molecule of triglyceride and the transesterification reaction is an equilibrium reaction, therefore the amount of methanol reactant used is usually higher than that in theory in order to shift the reaction to the product side (FAME). In this study, 1 g of biomass contain 5 wt.% water was reacted with methanol at 175°C, 1 hour and the biomass ratio to methanol ranged from 10/1, 15/1, 20/1 and 25/1 g/g.

Table 3: Effect of methanol content on FAME conversion

Methanol (g)	10	15	20	25
FAME (%)	20.5±1.1	23.4±0.7	44.1±2.0	43.1±0.7
FFA (%)	20.2±1.0	22.3±0.6	30.3±1.3	29.3±1.5
Monoglyceride (%)	42.5±2.4	46.4±1.7	21.1±1.2	17.8±2.0
Diglyceride (%)	10.2±0.5	2.2±0.1	1.7±0.0	7.5±0.4
Triglyceride (%)	5.0±0.1	0.0	1.3±0.0	0.7±0.0
Others (%)	1.6±0.0	2.8±1.4	1.5±0.0	1.7±0.0

Reaction condition: 1 g biomass, 5 wt.% water content, 175°C and 1 h

According to experimental results (Table 3), FAME conversion increased with increasing amount of methanol from 10-20 g and then slightly reduced when higher methanol amount (i.e. 25 g) was used. Obviously, highest FAME conversion of 44.1% was obtained at 20 g of methanol. This could be explained that 10-15 g methanol was not enough to perform both simultaneous process of oil extraction from algae mass and transesterification reaction. However, use of larger amount of methanol (25 g) resulted in high polarity of the reaction mixture, leading to the extraction of other polar compounds such as carbohydrates, chlorophyll, and proteins and then reduced the interaction between nonpolar oil and methanol, hence reduce FAME conversion (Huynh *et al.*, 2012). In addition, large amount of method is not good in term of producing cost; therefore, 20 g of methanol/1 g microalgae is a suitable ratio for the transesterification reaction of microalgae under subcritical condition of methanol.

3.3 Effect of water content on FAME conversion

In conventional transesterification of edible or non-edible oils for biodiesel production, the presence of free fatty acids and water will lead to the formation of soap, reducing catalyst effectiveness, and therefore low conversion. Catalyst-free subcritical methanol was introduced to be an efficient method to overcome the negative effects of FFA and water (Kusdiana and Saka, 2004). In this work, different water content varied from 5 wt.% up to 80 wt.% was applied to investigate the effect of water on FAME conversion. All experiments were carried out by using 1 g of biomass and 20 g of methanol at 175°C and 1 hour. Experiment results showed that FAME conversion is not different at 5-30% water content and strongly increased with increasing amount of water to 50%. Highest FAME conversion (52.4%) was obtained at 50 wt.% water content. However, when the amount of water reached to 80 wt.%, FAME yield significantly drop to 27.7%. It can be explained that in subcritical condition, water plays a role as a co-solvent as well as an acid/base catalyst, helping to accelerate the extraction of lipid and to catalyze for the tranesterification reaction. The same result was obtained when transesterification reaction was performed at supercritical condition: water is considered as a catalyst to enhance FAME conversion (Leung *et al.*, 2010; Tsigie *et al.*, 2013). However, the transesterification reaction is an equilibrium reaction, if water content in the reaction was too high (80 wt.%), the FAME yield would be reduced since water is the product of this reaction. According to the results, 50 wt.% of water is chosen for the producing of biodiesel in this case.

Table 4: Effect of water content on FAME conversion

Water content (%)	**5**	**15**	**30**	**50**	**80**
FAME (%)	44.1±2.0	45.4±0.4	43.1±2.4	52.4±0.4	27.7±1.5
FFA (%)	30.3±1.3	28.8±1.7	22.3±1.3	23.7±0.8	36.7±1.7
Monoglyceride (%)	21.1±1.2	13.6±2.0	14.9±1.3	14.3±1.3	25.2±2.1
Diglyceride (%)	1.7±0.0	9.5±0.9	7.0±0.7	7.5±1.5	8.8±1.9
Triglyceride (%)	1.3±0.0	0.4±0.0	11.4±0.0	0.0	0.0
Others (%)	1.5±0.0	2.5±0.3	1.3±0.1	2.1±0.1	1.7±0.0

Reaction condition: 1 g biomass; 20 g methanol, 175°C, 1h

3.4 Effect of reaction time on FAME conversion

To study the effect of reaction time on FAME conversion, the experiments were conducted with different reaction time (1-12 h). All the experimental conditions was fixed at 1 g biomass, 20 g methanol, 50 wt.% water content, 175°C. The experimental results showed that FAME conversion increased with the increase of reaction time and 95.1% of FAME was obtained after 12 h. This suggests that longer reaction time is necessary to ensure an efficient extraction and conversion of oil in algae into biodiesel.

Table 5: Effect of reaction time on FAME conversion

Reaction time (h)	**1**	**4**	**6**	**8**	**12**
FAME (%)	52.4±0.4	73.4±1.7	79.8±1.3	79.0±0.8	95.1±0.0
FFA (%)	23.7±0.8	12.0±0.2	10.7±2.0	10.7±1.0	2.5±0.0
Monoglyceride (%)	14.3±0.3	11.1±0.9	3.5±0.6	10.1±0.0	2.4±0.0
Diglyceride (%)	7.5±4.5	2.1±0.1	5.7±0.1	0.0	0.0
Triglyceride (%)	0.0	0.0	0.0	0.0	0.0
Others (%)	2.1±0.1	1.6±0.7	0.3±0.1	0.3±0.0	0.0

Reaction condition: 1 g biomass; 20 g methanol, 50 wt.% water content, 175°C

3.5 Composition of FAME from microalgae

After purification, the obtained FAME was analyzed by GC and FAME composition was presented in Table 6. Linoleic acid methyl ester is the most abundant component in the product obtained, which was accounted for 47.4 wt.%, followed by palmitic acid methyl ester (38.3 wt.%). Other fatty acid methyl esters were presented in minor amount such as 7,10-hexadecadioneic acid methyl ester (6.9 wt.%), stearic acid methyl ester (2.0 wt.%) and arachidic acid methyl ester (5.5 wt.%). FAME profile in this study was consistent with other similar result that was published by Zhou (Zhou *et al.*, 2013).

Table 6: Composition of FAME from microalgae

Composition	%wt
Palmitic acid methyl ester (C16:0)	38.3
7,10-Hexadecadioneic acid methyl ester (C16:2)	6.9
Stearic acid methyl ester (C18:0)	2.0
Linoleic acid methyl ester (C18:2)	47.4
Arachidic acid methyl ester (C20:0)	5.5

Reaction condition: 1g microalgae; 50 wt.% water, 15g methanol, 175°C, 12 h

3.6 Physical properties of biodiesel from *Chlorella* sp. biomass

Physical properties of the synthesized biodiesel from *Chlorella* sp. biomass under subcritical condition was measured and compared to specific criteria such as: TCVN 7717: 07 and ASTM D6751. The results given in Table 7 showed that biodiesel from microalgae *Chlorella sp.* have met the required standards.

Table 7: Physical properties of biodiesel from *Chlorella* sp. biomass

Properties	Unit	Biodiesel	Evaluation standard	
			TCVN 7717:07	ASTM D6751
Specific weight, 15°C	g/cm^3	890	860 - 900	-
Kinematic viscosity, 40°C	mm^2/s	5.3	1.9 - 6.0	1.9 - 6.0
Acid value	mg KOH/g	0.35	0.5	0.5
Water content	% vol	0.04	0.05	0.05

4 CONCLUSIONS

This study has demonstrated that biodiesel can be directly and effectively synthesized from microalgae biomass under the condition of subcritical methanol. FAME conversion can reach to 95% when 1 g microalgae was implemented with reaction condition of 50% water content, methanol to biomass ratio of 20 g/g, at 175°C and 12 hours. Although the reaction time was quite long, this method possess advantages over traditional and supercritical methods in term of water content, reaction temperature, pressure and purification steps.

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