Increasing Free Fatty Acid in Wet *Spirulina* sp. Residue by Pretreatment with Chemicals

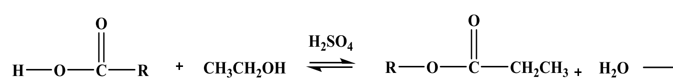
T. Saejung and S. Chaiklangmuang

***Abstract*—** This study focused on the free fatty acid increasing by pretreatment with the chemicals. The variable studies including chemical types (H_2SO_4 and NaOH), chemical concentration and pretreatment temperature and time were investigated. Canola oil was the preliminary study for screening type of chemical. It was found that H_2SO_4 acid gave the amount of free fatty acid (FFA) higher than basic chemical as NaOH and the highest FFA at 1M was H_2SO_4. By SPSS and Box-Behnken design analysis programs in case of H_2SO_4, it was found that the optimal conditions were 3M at 100°C and 6 hours and these conditions gave the highest free fatty acid around 72%.

***Keywords*—**Free fatty acid, Sulfuric acid, *Spirulina* sp., Pretreatment

I. Introduction

Nowadays, finding renewable energy sources for liquid fuels are also needed so much [1]. The depletion of fossil fuel reserves and the pollution caused by the continuously increasing energy demands [2]. Biodiesel is a liquid fuel that is comparable to diesel as an alternative energy source of interest and produced from renewable resources such as vegetable oil, animal fats, oil from small algae waste products and biomass [3]. Biomass fuels for heat and power generation are very interesting subjects for analysis because they can be considered environmental and climatic due to their carbon dioxide cycle, low sulphur dioxide emissions, good opportunities of ash utilization, and the potential to save fossil energy resources [4]. In the present the most of biofuels raw materials are used sugarcane, cassava, palm oil, sunflower, castor, coconut and jatropha and microalgae. But microalgae have the oil more than other plants when the same of cultivation areas [5]. The process of algae for biodiesel production can produce wide verity processes such as pyrolysis, co-solvent process, supercritical process, hydrolysis, transesterification etc [6]. Some process wants to dry raw materials which loss of energy costs for drying and high production costs. So esterification is a very interesting process because it can apply to wet algae, saves time and costs in process. Esterification is the reaction for preparing ester, which the reaction come from carboxylic acid and alcohol show in equation 1. Esterification reaction often used in producing biodiesel, when the sample high moisture and free fatty acid. Free fatty acid is important substrate in biodiesel production. Wet *Spirulina* sp. residue can produce biodiesel because it high of HC and 14 to 22 atom of carbon which it is the range of diesel [7]. Biodiesel production from wet *Spirulina* sp. residue should pretreatment for eliminating the encapsulated cell structures because *Spirulina* sp. is a prokaryotic bacteria that is none nuclear membrane organism and has ability for photosynthesis, so it is called cyanobacteria or blue-green algae. *Spirulina* sp. has a line shape that is called trichome which is generated from the concatenation of numerous cells, coils to left hand opened helix like a spring, does not have branching and not found heterocyst. *Spirulina* sp. has multi-layer cell wall that is composed by mucoprotein and pectin, the outer cell wall is a polysaccharide [8], so pretreatment can increase the amount of free fatty acid which is useful for biodiesel substrate.



(1)

free fatty acid (FFA) ethanol fatty acid ethyl ester (FAEE) water

II. Materials And Methods

2.1. Materials

Canola oil produced by Lam Sung company in Thailand under natural brand which bought from the supermarket and the wet *Spirulina* sp. residue extracted phycocyanin received from the private company in Chiang Mai, Thailand. Sulfuric acid 96% assay, commercial grade ethanol and sodium hydroxide were used in pretreatment process and FFA analysis. Hexane was for FFA extraction in our laboratory experiments.

2.2. Preliminary pretreatment

To compare chemical type, the sulfuric acid or sodium hydroxide was added in 20 g of canola oil. Reactions were preformed at 100 °C for 3 hours. After the reaction, hexane was added to the mixture. Each experiment was centrifuged at relative centrifugal force of 2095 xg for 10 min to accelerate phase separation (lipid and aqueous phases). The FFA contained in lipid phase which was in hexane was evaporated and analyzed by AOCS CA 5a-40 standard method.

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2.3. Pretreatment of wet Spirulina sp. Residue with sulfuric acid

Wet *Spirulina* sp. residue (20g dry) was mixed with various concentrations (1, 2, 3, 4 and 5M) of sulfuric acid. Reactions were preformed at room temperture, 60, 80 and 100 ° C for 2-7 hours. After the pretreatment, hexane was added to the treated mixture to separate the FFA from the mixtrue. The separation and analysis methods of FFA were similar as 2.2.

2.4. Experimental design and analysis

To achieve higher free fatty acid, subsequent experiments were designed with Design-expert software version 7. Values of three process variables based on single-factor experiment were further optimized and analyzed using Box-Behnken design (BBD). The range and levels of independent variables and coded values were presented in Table 1. Seventeen experiments consisting of five central points were designed with three variables, and free fatty acid amount was selected as the response, shown as Table 2.

TABLE I
RANGE AND LEVELS OF INDEPENDENT VARIABLES AND CODED VALUES IN BBD.

Independent variables	Symbols	Range and levels		
		-1	0	1
Concentration (M)	A	3	3.5	4
Temperature (°C)	B	90	95	100
Time (hr)	C	4	5	6

2.5. Calculation

The free fatty acids measured by standard methods (AOCS CA 5a-40) were calculated as the equation 2 (V is volume of NaOH in ml unit, C is concentration of NaOH in normality unit, m is weight of sample in g unit and Mw is molecular mass in g/mole unit).

$$\%FFA = \frac{V \times C \times Mw \times 100}{m} \tag{2}$$

III. RESULT AND DISCUSSION

3.1. Preliminary pretreatment

Figure1 shows the effect of the added H_2SO_4 and NaOH on FFA amount in term of acidic and basic pretreatments. In the same process, the H_2SO_4 pretreatment gave the FFA higher than NaOH pretreatment. FFA amounts in the treated mixtures were 0.34% and 0.22% when added H_2SO_4 and NaOH, respectively. This confirmed that the acidic chemical as H_2SO_4 can be applied to increase FFA in canola oil.

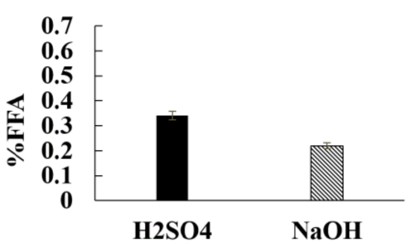

Fig 1 Effect of H_2SO_4 and NaOH on FFA in canola oil

Next, H_2SO_4 was studied on the concentration in canola oil for screening the concentration range before using with wet *Spirulina* sp. residue. Figure 2 shows the effect of H_2SO_4 concentration. With H_2SO_4 concentration increase, the FFA amounts were 0.11%, 0.34%, 0.56% and 0.52% when the added concentration of H_2SO_4 were 0, 0.5, 1 and 2M, respectively. The 1M H_2SO_4 showed the amount of FFA more than other concentrations. From this preliminary screening, the H_2SO_4 was selected to study in case of wet *Spirulina* sp. residue and the experiments determined the range of H_2SO_4 concentration around 1 to 5M

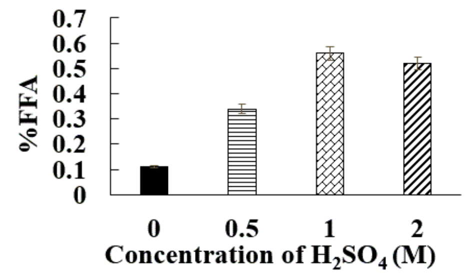

Fig. 2 Effect of H_2SO_4 concentration on FFA in canola oil

3.2. Pretreatment of wet Spirulina sp. residue with sulfuric acid

3.2.1 Effect of H_2SO_4 concentration on wet Spirulina sp. residue

To see the effect of sulfuric acid concentration on the FFA amount, the concentration was varied from 1 to 5 M while the following conditions were maintained; 100 °C and 3 hours. The results of pretreatment on wet *Spirulina* sp. residue show in Figure 3. Increasing of H_2SO_4 concentration the FFA amounts were 45.48%, 52.93%, 67.92%, 64.97% and 44% when the added concentration of H_2SO_4 were 1, 2, 3, 4 and 5M, respectively. The result were analyzed by SPSS program, it was found that the results were divided into two groups. In concentrations of 3M and 4M of group b, they are the high free fatty acid contents. There fore, the 3M of H_2SO_4 was selected for studying on temperature in the next experiment.

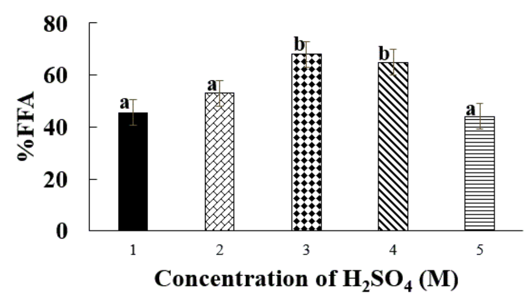

Fig 3 Effect of H_2SO_4 concentration on FFA in wet *Spirulina* sp. residue

Figure 4 indicates the effect of H_2SO_4 concentration on lipid color after the pretreatment. The orange was found at 5M, this color may be come from some of structure that was destroyed. The orange color may cause from the remain carotenoids in *Spirulina* sp. residue and it was associated with a decrease of FFA at 5M.

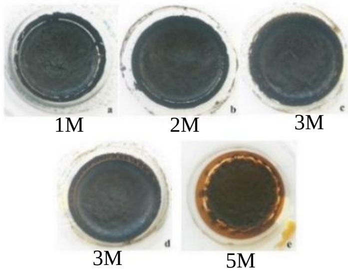

Fig. 4 Effect of H_2SO_4 concentration on lipid color of pretreatment

3.2.2 Effect of temperature on wet Spirulina sp. residue

To see the effect of temperature on the FFA amount, the temperature was varied from room temperature (30°C) to 100°C while the following conditions were maintained; 3M and 3 hours. The result illustrates in Figure 5. The FFA amounts were 45.48%, 51.34%, 55.77% and 67.92% when the reaction temperatures were 30, 60, 80 and 100 °C, respectively.

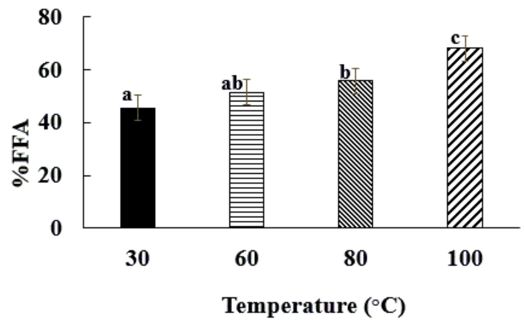

Fig 5 Effect of temperature on FFA in wet *Spirulina* sp. residue

By SPSS program, it was found that 100°C gave the highest free fatty acid, so the reaction temperature at 100°C was selected to study on the reaction time in the next experiment.

3.2.3 Effect of time on FFA in wet Spirulina sp. residue

Figure 6 shows the effect of time on FFA amount. The reaction time was varied from 2 to 7 hours, while the following conditions were maintained; 3M and 100 °C. The FFA amounts in lipids were 64.45%, 67.92%, 72.00%, 73.63%, 75.74% and 75.95% when the reaction times were 2, 3, 4, 5, 6 and 7 hours, respectively.

TABLE II
RESULTS BASED ON BOX-BEHNKEN DESIGN

Run	Concentration (M)	Temperture (°C)	Time (hr)	FFA (%wt of crude bio oil)
1	3	95	4	67.94
2	3	95	6	69.99
3	4	95	4	57.59
4	3.5	90	6	57.06
5	3.5	95	5	61.77
6	4	90	5	49.79
7	3.5	95	5	64.19
8	4	100	5	64.41
9	4	95	6	60.87
10	3.5	100	4	68.77
11	3.5	90	4	53.06
12	3.5	95	5	63.41
13	3.5	95	5	62.14
14	3.5	95	5	62.52
15	3	90	5	61.82
16	3.5	100	6	68.04
17	3	100	5	70.60

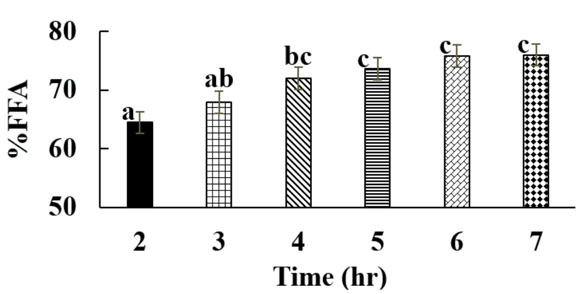

Fig 6 Effect of time on FFA in wet *Spirulina* sp. residue

3.2.4 Optimization of H_2SO_4 pretreatment with response surface methodology (RSM)

From 3.3.1-3.2.3, we can determine the range and levels of independent variables and coded values in BBD as indicating in Table 1. To examine the combined effects of the three independent variables (concentration (A), temperature (B), time (C)) on Free fatty acid amount of pretreatment processes, RSM analysis was performed to optimize and determined the optimal values, the results were shown in Table 2. According to the numerical data of tests, the quadratic polynomial regression model for free fatty acid was given by Design-expert software version 7, and expressed as follows:

$$FFA = -532.73 - 84.97A + 13.75B + 14.54C + 0.58AB + 0.61AC - 0.24C + 2.43A^2 - 0.07B^2 + 0.68C^2$$

In addition, statistical significance of the model was checked with F-test analysis of variance (ANOVA) (Table 3). The F-value of the model was 69.28 ($p < 0.0001$), which implied the model was highly significant. While F-value for the Lack of Fit of 0.71 ($p > 0.05$) implied, the Lack of Fit was not significant relative to pure error. If the p-value for Lack of Fit for model is significant ($p < 0.05$), a more complicated model would be required to fit the data [9, 10]. Moreover, F-value can represent the effects of three factors on pretreatment, the larger the F-value, the greater the effect. According to the F-values, the order of significance for the three variables during H_2SO_4 pretreatment was temperature > concentration > time. In addition, the 3-D response surface plots could graphically show the effects of individual and combined relationships on the free fatty acid amount. Interactions among the three variables were represented in Figure 7, it could be seen the effects of the coupling variables were obvious. When a single factor was fixed, with the increase of the other two factors, free fatty acid amount would increase but higher concentration could lead to more inhibition of free fatty acid. The optimal conditions were predicted by Design-expert software version 7 as follows: 3M,100°C and 6 hr. The predicted optimal free fatty acid amount was 72%

TABLE III

RESULTS OF ANOVA ANALYSIS ON H_2SO_4 PRETREATMENT.

Source	Sum of squares	df	Mean square	F-value	p-value	Significance
Model	530.70	9	58.97	69.28	<0.0001	**
A	177.57	1	177.57	208.63	<0.0001	**
B	313.63	1	313.63	368.50	<0.0001	**
C	9.25	1	9.25	10.86	0.0132	*
AB	8.53	1	8.53	10.02	0.0158	*
AC	0.38	1	0.38	0.44	0.5264	-
BC	5.59	1	5.59	6.57	0.0374	*
A^2	1.55	1	1.55	1.82	0.2190	-
B^2	13.01	1	13.01	15.29	0.0058	**
C^2	1.97	1	1.97	2.32	0.1717	-
Residual	5.96	7	0.85			
Lack of Fit	2.08	3	0.69	0.71	0.5926	-
Pure Error	3.88	4	0.97			

Value of p less than 0.05 indicated the term was significant (*), less than 0.01, highly significant (**), more than 0.1, insignificant (–).

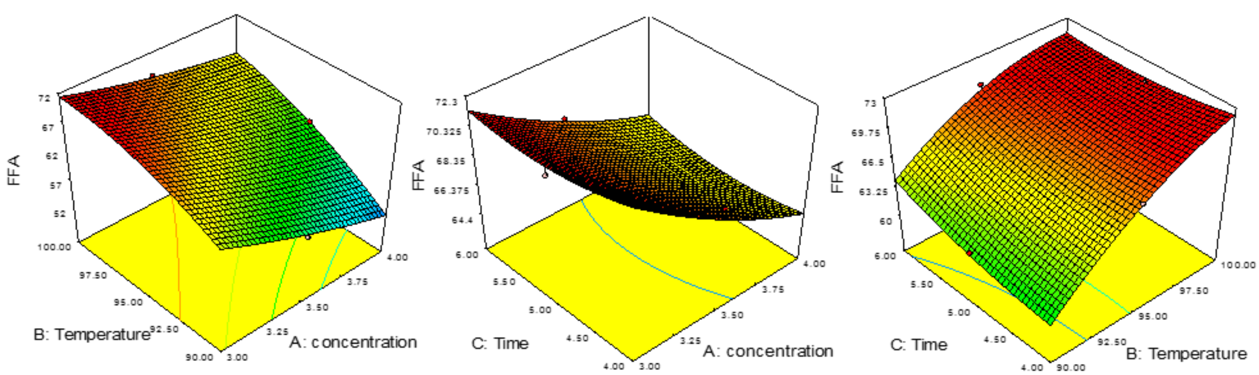

Fig. 7. 3-D response surface plots of three factors by response surface methodology. (A) temperature and concentration, (B) time and concentration, (C) time and temperature

IV. CONCLUSION

Pretreatment with sulfuric acid can increase the amount of free fatty acids. The concentration of sulfuric acid, temperature and time can affect the amount of free fatty acids. A model has been developed to predict the H_2SO_4 pretreatment process of wet *Spirulina* sp. residue, the optimal conditions were determined following: a concentration of 3M, a reaction temperature of 100°C and a reaction time of 6 hours. At these conditions, the amount of FFA could attain to 72%. Thus, the pretreatment with H_2SO_4 can be regarded as an ideal for FFA increase in wet *Spirulina* sp. residue.

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