Synthesis of biodiesel from vegetable oils wastewater sludge by *in-situ* subcritical methanol transesterification: Process evaluation and optimization

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ABSTRACT

Biodiesel are gaining increased public and scientific attention as an alternative to petroleum diesel fuel, driven by factors such as oil price spikes, energy security and environmental concerns. In this study, low grade wastewater sludge originated from wastewater treatment unit of vegetable oil factory as a viable alternative lipid source for biodiesel production was evaluated. The lipid mass fraction of the dry and ash-free sludge was $12.44 \pm 0.87\%$, which mainly comprised of C16–C18 fatty acids. The *in-situ* transesterification process under subcritical water and methanol conditions was applied as a green pathway to convert lipids into fatty acid methyl esters (FAMEs). The reaction parameters investigated were temperatures (155–215 °C), pressures (5.5–6.5 MPa) and methanol to lipid mass ratios (1:1, 5:1 and 9:1). The highest FAME yield of $92.67 \pm 3.23\%$ was obtained at 215 °C, 6.5 MPa and methanol to lipid mass ratio of 5:1. Statistical analysis based on response surface methodology in 3-factor-3-level central composite designed experiments and analysis of variance were applied to examine the relation between input parameters and the response and to locate the optimum condition. Results showed that 98% of the variability in the response could be adequately explained by the second-order polynomial model. The optimum FAME yield (90.37%) was obtained at 215 °C, 6.5 MPa and methanol to lipid mass ratio of 5.12:1. Experimental validation ($N = 3$) demonstrated satisfactory agreement between the observed and predicted values with an error of at most 3.3%.

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1. Introduction

The development of sustainable biofuels is vitally crucial to global transportation and many industrial operations to generate electricity and heat. Such today's concern is driven by long-term supply issue associated with fossil fuels and their environmental impacts due to the release of carbon dioxide, SO_x and NO_x pollutants during fuel combustion. One of potential alternative and sustainable biofuels which have been extensively studied and now commercially produced is biodiesel. Although biodiesel produces less air pollutants and is fast becoming a currently recognized substitute and/or blending agent for petroleum diesel, yet it still possesses key limitations include a heavy price, life-cycle assessment and the necessity of a vast land to produce enough biofuel without threatening food supplies and biodiversity.

There are a number of factors affecting high variable costs in the production of biodiesel, limiting the fuel's widespread use. In this light, the cost of biodiesel feedstock is the most burdensome, which accounts for 70–85% of total cost of production [1]. Palm oil [2], soybean oil [3], corn oil [4], rapeseed oil [5], sunflower oil [6], sesame oil [7], rice bran oil [8] and a number of other food crops have all served as feedstock for biodiesel, however they all suffer from the same problems including threatening the food chain, increasing carbon emissions when planted outside traditional agricultural settings and intense growth requirements. Animal fats such as beef tallow [9], poultry fat [10], fish oil [11] and pork lard [12] have been investigated to produce biodiesel. Although much of animal fats are not considered edible by humans and their cost is substantially lower than the cost of vegetable oils, yet their applications can be challenging because they frequently contain contaminants (e.g., phospholipids and gums) that should be removed before the fuel is used in an engine and also animal fat-based biodiesel typically has higher viscosity and sulfur content. Therefore, it is necessary to gain a new perspective on the production of biodiesel by seeking an alternative feedstock, which is non-food crops and easily obtainable in large quantities. Recently, municipal wastewater sludge is being explored for its untapped potential as a cheap and readily available lipid feedstock for sustainable biodiesel production. The sewage sludge is a by-product generated after wastewater treatment and usually a blend of thickened primary and secondary sludges. This sludge contains considerable mass fraction of lipids (up to 30%) either as a composite organic matrix (oils, greases, sterols, fats and long-chain fatty acids) originated from direct adsorption of lipids in municipal and industrial wastewater or from phospholipids in the cell membrane of microorganisms, their metabolites and by-products of cell lysis [13]. The conventional technology to produce biodiesel from sewage sludge is by a two-step process involving lipid extraction using organic solvents and then alcoholysis of extracted lipids. However, this usual approach poses a great challenge for industrial practice. The lipid recovery process from sewage sludge is tedious and costly because it requires huge amounts of organic solvents and large vessels with stirring and heating systems. Also, most of organic solvents used for extracting lipids are not environmentally acceptable. In addition, the alcoholysis either by esterification or transesterification using liquid or solid catalysts often creates limitations in the catalyst recovery, yield and purity of biodiesel and treatment of wastewater. Kwon et al. [14] had demonstrated a thermochemical approach to transform lipids to biodiesel employing a catalyst-free, continuous flow system. However, non-catalytic thermochemical process suffers from the drawback of intense energy consumption due to the use of high temperatures (350–500 °C) although nearly complete conversion reaction is achieved within a short period of time.

Subcritical water (SubCW), that is, pressurized water at temperatures above the boiling point at ambient pressure and below critical point (T_C = 374 °C), is considered as a unique and green reaction medium for various applications including catalytic/non-catalytic reactions, biomass transformation to chemicals and materials and extraction of bioactive compounds from natural matrices. Several relevant properties of SubCW as a reaction medium are miscibility, ionic product, electrolytic solvent power, dielectric constant and transport properties (e.g., viscosity, diffusivity and ion mobility). The physicochemical properties of interest of SubCW can be tuned through changes in pressure and temperature. With the increase of temperature, there is a marked and systematic decrease in permittivity, viscosity and surface tension while the diffusion rate increases [15]. In the extraction of lipids by SubCW, the process is feasible at mild temperatures (typically 150–200 °C) due to reduced dielectric constant of water, making it capable to extract weakly polar to non-polar compounds. A successful implementation of SubCW as a green alternative solvent to recover lipids from wet algae [16] and dewatered activated sludge [17] has been reported and the possibility of producing biodiesel from wet activated sludge without any catalyst under subcritical water and methanol condition has been investigated by Huynh et al. [18]. In their recent paper, dried activated sludge was used instead of wet activated sludge as the lipid feedstock and water was added prior to methanolysis. Drying of wet activated sludge is a time-consuming as well labor- and energy-intensive process and for large-scale operation, this process is not economically feasible due to large fuel consumption in drying machine or huge land area for sun drying process.

To date, limited studies on the production of biodiesel from wet activated sludge can be found in literature. In most cases, transesterification is performed under catalytic action of acidic or base homogeneous/heterogeneous catalysts, either in single or two-step processes. In contrast to such method, the *in-situ* transesterification procedure under subcritical alcohol condition is an ongoing area of intense research. Therefore, the aims of this study are to evaluate *in-situ* transesterification of wet wastewater sludge to fatty esters under subcritical water and methanol conditions along with the influencing parameters (temperature, pressure and methanol to lipid mass ratio) and to determine the optimum reaction condition by employing response surface methodological approach (RSM) and analysis of variance (ANOVA). Several key properties of biodiesel is also investigated and compared to ASTM D6751 standard.

2. Materials and methods

2.1. Materials

Fresh wastewater sludge was collected from a vegetable oil wastewater treatment unit located at Surabaya city, East Java. Distilled water was obtained from a local supplier. Anhydrous methanol (99.8%) and *n*-hexane (95%) was supplied from Merck, Germany while ultra pure N_2 gas (99.9%) was supplied from a local company. The fatty acid methyl esters (FAMEs) reference standard mix (Supelco) consisting of C14–C22 fatty acids was purchased from Sigma Aldrich Co., Singapore and used without any further purification.

2.2. In-situ *subcritical methanol transesterification*

The experimental setup of a lab-scale high pressure batch reactor in this work (150 cm^3, temperature limit: 273–473 K, pressure range: 0–10 MPa) is shown in Fig. 1. The reactor was made of Type 316 stainless steel and equipped with an external heater, a pressure gauge, a Type K thermocouple and M8 screws for tightening the reactor with its cap. The reaction vessel was charged with a given quantity of wet sludge and methanol (methanol to sludge mass ratios of 1:1, 5:1 and 9:1). Here, mass ratio was used instead of molar ratio to represent methanol loading due to the difficulty in calculating exact molecular weight of the sludge containing numerous components. N_2 was then flowed into the reactor to remove air and build a bit of pressure prior to heating. The reactor was heated at 20 K min^{-1} from room to desired temperatures (155–215 °C) and kept at final temperatures for 12 h. To maintain an isothermal and isobaric condition, temperature was controlled by a PID-type controller with uncertainty of ±1 °C and pressure was monitored in real time by a pressure gauge. During the reaction period, the mixture was magnetically stirred at a constant rate of 13.33 Hz. When final temperature was reached, the pressure inside the reaction vessel ranged between 5.5 and 6.5 MPa.

After completion of the reaction, the reactor was immersed in a cold water bath for immediate cooling and the vapor

mixture was vented to the condenser inlet. The condensate was collected in a conical flask and extracted with *n*-hexane. The liquid contents were placed in the separatory funnel and after addition of *n*-hexane and shaking; the mixture was allowed to settle for 24 h to ensure complete separation between ester-rich (organic) and glycerol-rich (aqueous) phases. The aqueous phase (bottom phase) was removed and left in a separate container. The organic phase was evaporated in vacuum to remove *n*-hexane. The solid fraction was removed and a given amount of *n*-hexane was added to extract fatty esters and unreacted lipids. The extraction process was conducted in a stirred borosilicate cylindrical glass vessel for 2 h with a constant stirring rate of 13.33 Hz. The solid was removed using a Buchner filtering funnel. The retained solid was washed three times with 50 cm^3 *n*-hexane. The removal of *n*-hexane was conducted in vacuum using a rotary evaporator flask and the mass of biodiesel (FAMEs) was weighed using an analytical balance (Mettler Toledo).

2.3. Analysis of FAME mass fraction

The FAME mass fraction was assayed by gas chromatography, in a Shimadzu GC 2014 equipped with a flame ionization detector (FID) and an Agilent DB-WAX capillary column (30 m length, 0.25 mm i.d., 0.25 μm film thickness). Highly pure helium (99.9%) was used as the carrier gas at a linear velocity of 40 cm s^{-1}. The injector temperature was set at 250 °C running in splitless mode and the detector temperature was kept at 300 °C for the duration of analysis. The oven temperature program was started at 50 °C with a holding time of 2 min, then ramped to 250 °C at 10 K min^{-1} and held constant at 250 °C for 8 min. The injection volume of the samples was 1 mm^3. Calibration of the instrument was conducted using a 10-component FAMEs standard mix with methyl heptadecanoate as internal standard. All calibration curves were linear with a correlation coefficient of 0.98 or better. Peaks of FAMEs were identified by comparing retention time with those of FAMEs standard mix. FAMEs not included in this standard were compared with the peaks of well-recognized materials under similar analytical conditions. The FAME mass fraction was determined by the following equation:

$$F_c(\%) = 100 \left(\frac{\sum A_{FAME} - A_{int}}{A_{int}} \times \frac{V_{int} C_{int}}{m} \right) \tag{1}$$

where F_c is the FAME mass fraction, $\sum A_{FAME}$ is total peak area of FAMEs, A_{int} is the peak area of methyl heptadecanoate, V_{int} is the volume of methyl heptadecanoate internal standard solution added to sample (cm^3), C_{int} is the concentration of methyl heptadecanoate internal standard solution (g cm^{-3}) and m is the mass of sample (g). The percent yields of FAMEs based on lipid mass fraction of the dry and ash-free sludge were calculated as follows:

$$\text{Yield}(\%) = \frac{100 \times m_p \times F_c}{m_l} \tag{2}$$

where m_p is the mass of biodiesel (g), m_l is the mass of lipids in the dry and ash-free sludge (g) and F_c is the FAME mass fraction obtained from Eq. (1).

Thin-layer chromatography (TLC) was carried out for qualitative analysis of fatty esters, employing silica gel TLC
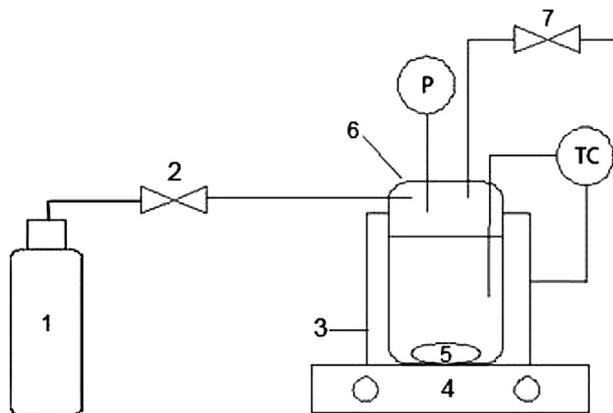

Fig. 1 – Schematic diagram of the subcritical reactor system: N_2 cylinder (1); relief valve (2); electric heating element (3); RPM controller (4); magnetic bar (5); high-pressure reactor (6); release valve (7).

plates (Polygram® Sil G) on polyester support (20 cm × 20 cm, 0.20 mm). The product was dissolved in *n*-hexane and spotted on a TLC plate which was then developed using a solvent mixture of *n*-hexane, ethyl acetate and acetic acid (volume ratios of 90:10:1) in a covered glass vessel until the solvent front reached the top of the plate. After a brief drying, the plate was dipped in a water bath for 1−2 min. The wet plate was stained in 100 cm³ of 1% $KMnO_4$ solution in 4% NaOH for approximately 20 s with constant agitation for visualization [19]. Then, the stained plate was extensively washed with water (3−4 changes for 3−4 min) and dried. The individual fractions as spots on the plate were identified by comparing R_f values with authentic standards of known masses.

2.4. Biodiesel fuel properties

For commercial applications in the compression-ignition engines, biodiesel should meet ASTM D6751 standard specification. The key properties of biodiesel were analyzed as per standard methods described in ASTM D613 for cetane number, ASTM D93 for closed-cup flash point, ASTM D445 for kinematic viscosity, ASTM D1480 for relative density, ASTM D664 for acid number, ASTM D2500 for cloud point and ASTM D97 for pour point.

2.5. Statistical experimental design and optimization

Statistical experimental design was applied to optimize the reaction conditions for maximum FAME production. Here, a statistical method called response surface methodology (RSM) coupled with a 3-factor-3-level central composite design (CCD) was adopted to obtain maximum response by optimizing three independent variables namely temperature (°C), pressure (MPa) and methanol to lipid mass ratio. Table 1 shows the coded and actual values of reaction parameters used in the design of experiments. The selection of pressure, temperature and methanol to lipid mass ratio as reaction parameters to be optimized was based on the fact that these parameters are directly relevant to process economics and safety concerns. The experiments were conducted in random order and after finishing the experiments, a suitable mathematical model was developed for prediction of the response based on experimental factors. A 95% confidence level was applied for model development and analysis of variance (ANOVA). The analysis of variance was performed using Minitab software (Version 16.2.1) and the response surface plots were obtained using MatLab R2013b software. All experiments were repeated three times and the results are averaged. The central data point (0, 0, 0) was replicated five times.

3. Results and discussion

3.1. Characteristics of vegetable oil wastewater sludge

The characteristics of as-received (wet) vegetable oil wastewater sludge are given in Table 2. The dry and ash-free sludge contains quite significant amounts of lipid fractions (12.44 ± 0.87%) include triglycerides, diglycerides, monoglycerides, sterols, phospholipids and free fatty acids. The fatty acids in the sludge based on gas chromatography analysis consist of myristic acid (C14:0, 5.7%), palmitic acid (C16:0, 31.6%), palmitoleic acid (C16:1, 24.8%), stearic acid (C18:0, 10.4%), oleic acid (C18:1, 16.3%), linoleic acid (C18:2, 7.4%), eicosanoic/arachidic acid (C20:0, 2.2%) and docosanoic/behenic acid (C22:0, 1.6%). Similar fatty acid compositions had been reported by Olkiewicz et al. [20] and Mondala et al. [21] for primary and secondary sludges originated from municipal wastewater treatment facilities (Table 3). The distribution of fatty acids is also in good agreement with sludges originated from sunflower oil [24] and palm oil [25] industries, which mainly comprised of C16−C18 fatty acids (~90%). High moisture content of the sludge is necessary to conduct *in-situ* transesterification process where fatty acids are simultaneously extracted and transesterified to fatty esters. Compared to conventional process employing solid or liquid catalysts, water content should be taken into consideration because water can hydrolyze fats to form free fatty acids, which then form soap and consequently decrease biodiesel yield and cause difficulty in product separation (for base-catalyzed transesterification).

3.2. Process optimization

RSM is a collection of mathematical and statistical techniques for empirical model building and optimization, which examines the relation between one or more response parameters and a set of experimental input parameters. The design procedure of RSM to locate the optimum value of response (output parameter) from a set of experimental input parameters can be divided into four steps [26]: (i) designing a series of experiments for reliable and adequate measurements of the response of interest, (ii) constructing a mathematical model of the second order response surface with best fittings, (iii) finding the optimal set of experimental

Table 1 − 3-factor-3-level experimental design.

Parameters	Coded values		
	−1	0	1
Temperature (°C)	155	185	215
Pressure (MPa)	5.5	6.0	6.5
Methanol to lipid mass ratio	1	5	9

Table 2 − The characteristics of as-received (wet) wastewater sludge.

Parameters	Mass fraction, %
Water content[a]	85.51 ± 2.38
Dry matters	14.49 ± 2.38
Lipidic fractions[a] (dry basis)	10.78 ± 1.22
Wax and gum	62.13 ± 4.61
Oil components	37.87 ± 2.35
- Fatty acids	82.05 ± 3.18
- Acylglycerols	10.73 ± 1.64
- Unsaponifiable matters	7.22 ± 0.51

[a] The values represent the averages of the results for three replicate runs.

Table 3 – Distributions of fatty acids in various industrial wastewater sludges.

Origin of sludge	Type of sludge	Fatty acids											
		C12:0	C14:0	C15:0	C16:0	C16:1	C18:0	C18:1	C18:2	C20:0	C20:1	C22:0	C24:0
Municipal WWTP [20,21]	Primary	trace[a]	□	trace	□	□	□	□	□	−	trace	−	−
		−	□	−	□	−	□	□	□	NR[a]	NR	NR	□
	Secondary	□	□	−	□	□	□	□	□	trace	□	□	−
		trace	□	trace	□	□	□	□	□	NR	NR	NR	−
	Blended	−	□	−	□	□	□	□	□	NR	NR	NR	□
	Stabilized	−	−	−	□	□	□	□	□	NR	NR	NR	−
Pulp & paper mill WWTP [22]	Blended	−	trace	−	□	□	□	□	□	trace	−	−	□
Swine manure WWTP [23]	Secondary	□	□	−	□	trace	□	□	□	−	−	−	−
Sunflower oil WWTP [24]	Composite	NR	trace	NR	□	NR	□	□	□	□	NR	NR	NR
Palm oil WWTP [25]	Secondary	□	□	−	□	trace	□	□	□	trace	−	−	−
Vegetable oil WWTP (this work)	Secondary	−	□	−	□	□	□	□	□	□	−	□	−

Fatty acids: C12:0 (lauric acid), C14:0 (myristic acid), C15:0 (pentadecanoic acid), C16:0 (palmitic acid), C16:1 (palmitoleic acid), C18:0 (stearic acid), C18:1 (oleic acid), C18:2 (linoleic acid), C20:0 (eicosanoic acid), C20:1 (gadoleic acid), C22:0 (behenic acid), C24:0 (lignoceric acid).

[a] Trace (concentration below 1%), NR (not reported), □ (detected), − (not detected or zero).

parameters that give a maximum or minimum response and (iv) representing direct and interactive effects between input parameters and response as 2D or 3D surface or contour plots. It is realized that the energy requirement of *in-situ* transesterification process under subcritical water and methanol conditions is higher compared to that of acidic- or base-catalyzed transesterification. However, the *in-situ* technique offers ancillary advantages than the conventional ones since this technique does not require any solid or liquid catalyst to achieve relatively high FAME yield and eliminating the need for pretreatment steps. In order to implement this method for large-scale operation, optimization of reaction condition is important to give maximum benefit from an economic viewpoint. In this light, RSM was applied to obtain the optimum condition for production of fatty esters by integrating temperature, pressure and methanol to lipid mass ratio simultaneously. The designed experiments based on 3-factor-3-level CCD model are presented in Table 4.

The fitting model, as a function of independent variables, was expressed as a quadratic (second-order) polynomial regression form using least squares analysis:

$$Y = k_0 + \sum_{i=1}^{3} k_i X_i + \sum_{i=1}^{3}\sum_{j=1}^{3} k_{ij}X_iX_j \tag{3}$$

where Y is the predicted response, k_0, k_i, k_{ii} and k_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively and X_i and X_j are the coded levels of independent variables. The second term of Eq. (3) represents linear effect of single process variables while the cross-product term represents an interaction effect between two variables X_i and X_j. After developing the response surface plots, the multiple regression coefficients of the model was estimated using the method of least squares at a significance level of 0.05 and the results are listed in Table 5. As shown in Table 5, it is evident that the main linear effects of

temperature (T) and pressure (P) are significant factors toward FAME production. Results also suggested that the square interaction of methanol to lipid mass ratio (M^2) and an interaction effect between temperature and pressure ($T{*}P$) significantly affects FAME yield. The main linear effect of methanol to lipid mass ratio (M), square interaction of pressure (P^2) and temperature (T^2), interaction effects between pressure and methanol to lipid mass ratio ($P{*}M$) and temperature and methanol to lipid mass ratio ($T{*}M$) are not significant toward the dependent variable (Y) therefore all these terms except the linear term of methanol to lipid mass ratio (M) and quadratic term of temperature (T^2) could be removed from the model without affecting the accuracy of predictions. The variables M and T^2 still preserved in the model because after elimination of the insignificant terms, the *p*-value of linear term M becomes approximately same to the α-level (0.05) thus methanol to lipid mass ratio has significance toward FAME yield. Similarly, the *p*-value of quadratic temperature term (T^2) satisfies 95% confidence level. By inserting the values of significant regression coefficients into Eq. (3), the following second-order polynomial coded model for prediction of FAME yield is obtained:

$$\text{Yield}(\%) = 67.58 + 9.89(T) + 5.12(P) + 1.64(M) + 4.41(T^2)$$
$$- 30.87(M^2) + 3.36(T\cdot P) \tag{4}$$

The above coded model fits the data very well with coefficient of determination (R^2) of 0.98. This indicates that 98% of the variability in the response could be adequately interpreted by the developed second-order polynomial prediction model. Additionally, all linear, quadratic and interaction terms in the model are significant at 95% confidence level (Table 5).

The response surface plots of the interaction effects between two independent variables are represented in Fig. 2a–c. As displayed in Fig. 2a, the positive linear influence for both temperature and pressure indicates that FAME yield increases

Table 4 – The designed experiments based on 3-factor-3-level central composite design.

Run	Input parameters			Response (FAME yield, %)	
	P/MPa (X_1)	T/°C (X_2)	M (X_3)	Experimental (N = 3)	Prediction
1	−1 (5.5)	1 (215)	1 (9:1)	42.35 ± 2.28	44.17
2	0 (6.0)	0 (185)	0 (5:1)	67.92 ± 2.58	67.58
3	1 (6.5)	1 (215)	−1 (1:1)	58.12 ± 4.33	57.85
4	−1 (5.5)	−1 155)	−1 (1:1)	27.77 ± 2.02	27.83
5	1 (6.5)	−1 (155)	1 (9:1)	36.12 ± 0.89	34.63
6	0 (6.0)	0 (185)	0 (5:1)	68.15 ± 1.24	67.58
7	0 (6.0)	−1 (155)	0 (5:1)	56.33 ± 3.65	62.10
8	0 (6.0)	0 (185)	0 (5:1)	67.32 ± 2.09	67.58
9	1 (6.5)	0 (185)	0 (5:1)	70.96 ± 4.12	72.70
10	0 (6.0)	0 (185)	1 (9:1)	37.12 ± 2.23	38.35
11	0 (6.0)	1 (215)	0 (5:1)	84.02 ± 4.54	81.88
12	0 (6.0)	0 (185)	−1 (1:1)	32.67 ± 1.81	35.07
13	−1 (5.5)	0 (185)	0 (5:1)	65.77 ± 2.15	62.46
14	1 (6.5)	0 (185)	1 (9:1)	40.38 ± 2.57	43.47
15	1 (6.5)	−1 (155)	−1 (1:1)	34.02 ± 1.12	31.35
16	0 (6.0)	0 (185)	0 (5:1)	68.25 ± 4.68	67.58
17	−1 (5.5)	1 (215)	−1 (1:1)	40.35 ± 2.54	40.89
18	1 (6.5)	−1 (155)	0 (5:1)	58.24 ± 2.88	63.86
19	1 (6.5)	1 (215)	1 (9:1)	61.02 ± 3.63	61.13
20	1 (6.5)	1 (215)	0 (5:1)	92.67 ± 3.23	90.37
21	1 (6.5)	0 (185)	−1 (1:1)	38.49 ± 3.28	40.19
22	0 (6.0)	0 (185)	0 (5:1)	67.44 ± 3.51	67.58
23	−1 (5.5)	0 (185)	−1 (1:1)	31.21 ± 1.65	29.95
24	0 (6.0)	1 (215)	−1 (1:1)	43.72 ± 3.25	49.37
25	−1 (5.5)	−1 (155)	1 (9:1)	32.77 ± 1.53	31.11
26	0 (6.0)	1 (215)	1 (9:1)	47.11 ± 1.33	52.65
27	−1 (5.5)	1 (215)	0 (5:1)	83.11 ± 1.92	73.40
28	0 (6.0)	−1 (155)	−1 (1:1)	30.11 ± 2.47	29.59
29	−1 (5.5)	−1 (155)	0 (5:1)	55.15 ± 3.08	60.34
30	−1 (5.5)	0 (185)	1 (9:1)	35.21 ± 2.42	33.23
31	0 (6.0)	−1 (155)	1 (9:1)	33.21 ± 1.79	32.87

as the level of both factors increases. It was observed that the curve line of pressure vs. temperature vs. FAME yield rose sharply by changing the levels of one independent variable from lowest (−1) to highest (1) while keeping another independent variable in the highest level. It is expected that the increase in FAME yield would be greater for temperature rather than pressure since the coded coefficient of

Table 5 – Results of significance test on the multiple regression coefficients of the quadratic polynomial model for estimation of the response (coded form).

No	Full			Partial[a]	
	Term	p-Value	Coefficient	p-Value	Coefficient
1	Intercept	0.00	67.78	0.00	67.58
2	X_1	0.00	5.12	0.00	5.12
3	X_2	0.00	9.89	0.00	9.89
4	X_3	0.09	1.65	0.05	1.65
5	X_1^2	0.07	1.65	0.01	–
6	X_2^2	0.35	3.46	–	4.41
7	X_3^2	0.00	−31.82	0.00	−30.87
8	$X_1 \cdot X_2$	0.01	3.36	0.00	3.36
9	$X_2 \cdot X_3$	0.78	−0.28	–	–
10	$X_1 \cdot X_3$	0.80	−0.25	–	–

[a] Partial means the insignificant terms (p-values > 0.05) of the model are eliminated.

temperature is higher than that of pressure. This is also evident on the main effect plots, showing a steeper slope for temperature than for pressure (Fig. 3). Response surface plots in Fig. 2b and c shows that FAME yield tends to have a maximum level for interaction effects between temperature and methanol to lipid mass ratio and pressure and methanol to lipid mass ratio. Maximum FAME production was noticed in the middle levels of both factors while further increase in the factor levels resulted in a gradual decrease in yield. A consistent result was verified on the main effect plot of methanol to lipid mass ratio in Fig. 3. It can be seen the contribution of each parameter investigated has significant effect on FAME yield in the order of temperature > pressure > methanol to lipid mass ratio. After constructing variable design, the optimal levels of process variables were determined. The optimal levels of process variables for *in-situ* transesterification of wet sludge under subcritical water and methanol conditions are temperature of 215 °C, pressure of 6.5 MPa and methanol to lipid mass ratio of 5.12:1. The predicted FAME yield under this condition was 90.37% with a model desirability of 0.97. To further assess the reliability of prediction, three replicate experiments were performed under the condition predicted by the model. Results show that FAME yield of 91.63 ± 1.72% was obtained; giving an error of at most 3.3% and evidencing that the model prediction is highly reliable.
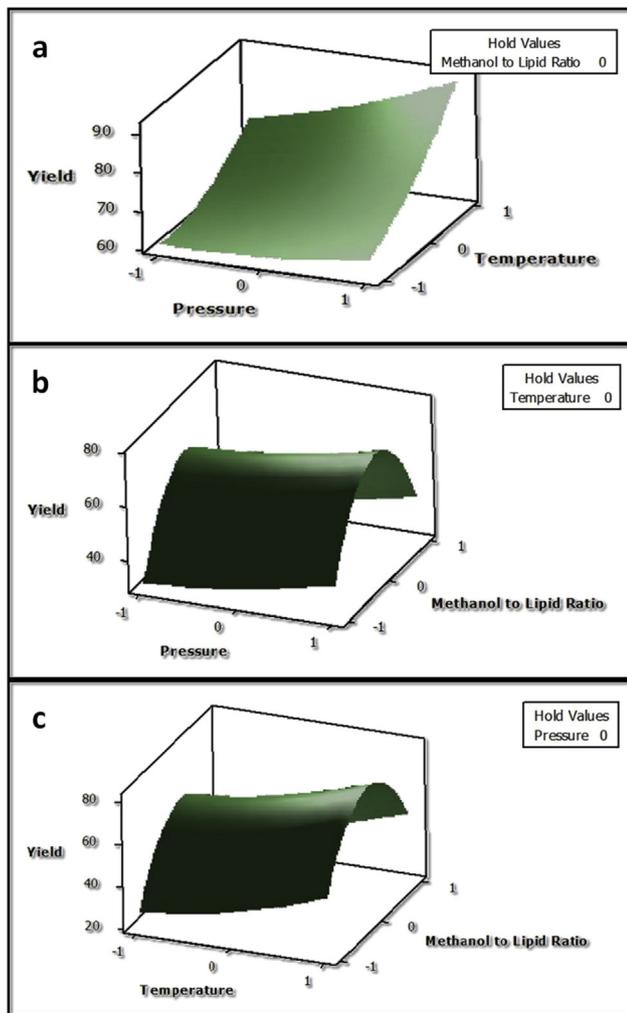
Fig. 2 − 3D response surface plots of FAME yield, showing interaction between pressure and temperature (a), pressure and methanol to lipid mass ratio (b) and temperature and methanol to lipid mass ratio (c).

3.3. Effect of methanol to lipid mass ratio on FAME yields

Methanol is the most widely used alcoholic reactant in the production of methyl esters from oils or fats. Stoichiometrically, three moles of methanol are required to produce three moles of methyl esters and one mole of glycerol. Since transesterification is a reversible reaction, excess amounts of methanol are required to shift the reaction to the right-hand side. Excess methanol to lipid molar ratio, to a certain extent, seems favor the equilibrium position moving to the right-hand side. However, the addition of highly excess methanol has a tendency to give negative effects on FAME yields. According to the results obtained, the addition of methanol greater than five times the mass of wet sludge lowers biodiesel yield. A reasonable explanation is that a further increase in methanol amount (9:1), aside from shifting the equilibrium to the right-hand side, would promote the extraction of more polar compounds in the sludge, such as

phenols, pigments, carbohydrates and proteins [18], which retards the formation of fatty esters. Excess methanol in the reaction mixture would also interfere with glycerol separation due to increased solubility. Encinar et al. [27] showed there is a slight recombination of fatty esters and glycerol to monoglycerides because their concentration keeps increasing during the course of reaction when an excess alcohol is present. From the equilibrium point of view, excess glycerol concentration could drive the equilibrium back to the left, lowering FAME yields. In addition, increasing methanol amount above the optimal value not only decreases yield but also raises cost for excess reactant recovery.

3.4. Effect of reaction temperature on FAME yields

Reaction temperature is the major factor influencing neutral lipids extraction from the sludge and transesterification processes. In the extraction of neutral lipids, temperature changes under isobaric condition vary the polarity and thus solvation properties of water. With increasing temperature, the dielectric constant of water drastically decreased due to weakening of hydrogen bonding between water molecules, allowing greater miscibility between lipids and water [28]. This property of water also makes separation between lipid and water very easy when temperature is reduced to room temperature. The presence of water in the reaction system also initiates hydrolysis of triglycerides to form free fatty acids due to triglycerides−water reactive system. As the hydrolysis proceeds, more free fatty acids are formed which increases the solubility of water in the oil-rich phase and thus the reaction rate. The resulting free fatty acids are esterified by methanol to form methyl esters and glycerol, increasing the product yield.

The effect of reaction temperature on FAME yield is depicted in Fig. 4. The results show that increasing reaction temperature causes an increase in FAME yield. At room temperature, methanol and oil do not mix well and poor methanol and oil miscibility means the reaction rate is very slow. From a kinetic viewpoint, an increase in FAME yield was attributed to enhanced reaction rate at higher temperatures as a result of increased solubility of methanol in the oil-rich phase and higher energy state of the molecules to undergo useful collisions. Also, the time for the mass transfer-controlled region is shortened as temperature is increased [29]. The percent of FAME yields ranged between 34.02% and 92.67% at temperatures of 155−215 °C. It is well-known that transesterification between triglycerides and methanol is a reversible and high activation energy reaction. Thus, increasing temperature would favor transesterification of triglycerides to diglycerides, diglycerides to monoglycerides and monoglycerides to methyl esters [29]. In addition, the formation of H_3O^+ and OH^- ionic products from dissociation of water molecules increased with increasing temperature and these ionic products could act either as acid or base catalysts to promote hydrolysis and methanolysis reactions. Below the critical point of mixture, both H_3O^+ and OH^- ionic products promote transesterification reaction under the same catalytic mechanisms using Bronsted acids and alkali catalysts [30]. The increase in temperature also causes the polarity of methanol to decrease by the same phenomenon of that water and it leads to increasing amount
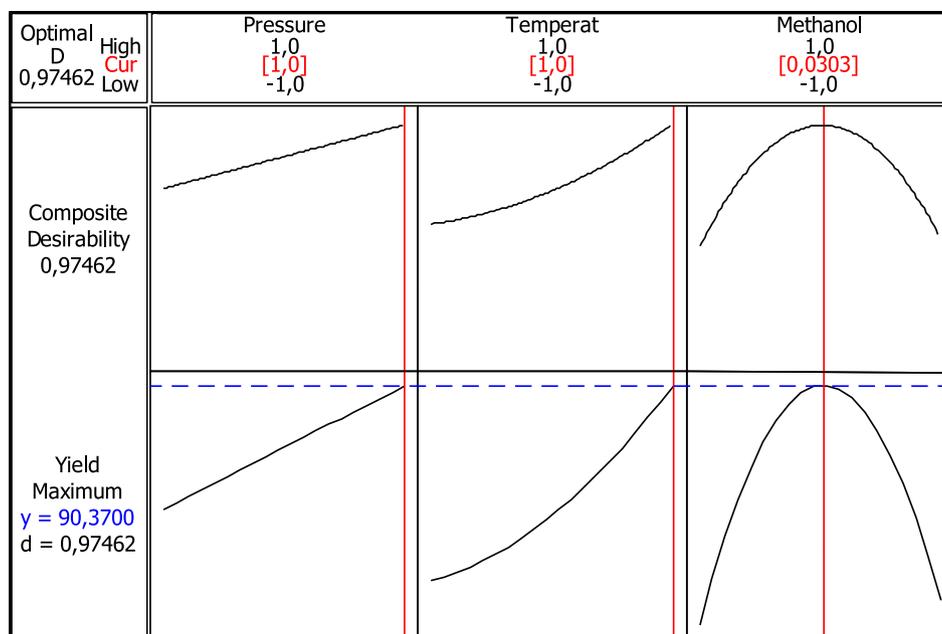
Fig. 3 – **Main effect plots of reaction parameters investigated and the response at optimal levels (D – composite desirability; y – predicted response; d – desirability).**

of fatty acids that are soluble in the methanol phase. As the system continues to rise in temperature, the initial two-phase oil and methanol mixtures become more homogeneous (single phase) thus facilitates the conversion of triglycerides to fatty esters.

3.5.   Effect of reaction pressure on FAME yields

Since transesterification and esterification reactions occur in the liquid phase, the pressure inside the reaction vessel should be sufficient to keep methanol and water both in the liquid state at all temperatures. The applied pressures of the *in-situ* transesterification process under subcritical water and methanol conditions are 5.5, 6.0 and 6.5 MPa. Generally speaking, pressure has only minor effects on the solvent strength of liquid water and methanol. By increasing pressure, the yields of FAME slightly increased as shown in Fig. 4. In the wastewater sludge, the lipids are attached to protein, mineral or carbohydrate structures [18]. When pressure and temperature of the system are increased to subcritical condition, water could enter more easily into the solid matrices where the lipids are trapped in the complex-bonded structures. Chen et al. [31] had also reported similar results in their recent paper for extraction of lipids from wet *Nannochloropsis* sp. micro-algae paste. The ability of subcritical water to extract lipids was due to that the solubility parameter of water becomes closer to the solubility parameter of lipids at elevated pressure and temperature. Also, subcritical water provides enhanced mass-transfer properties of solutes compared to liquid water at room temperature and pressure [15].

In the vicinity of critical point of methanol at 239 °C (T_C) and 8.09 MPa (P_C), methanol is highly compressible and the density is a strong function of pressure. The solubility of

methanol in the oil-rich phase becomes higher at elevated pressure under critical temperature while only minor amount of oil present in the vapor methanol-rich phase. According to vapor–liquid phase equilibria data reported by Glisic et al. [32], the mole fraction of methanol in the liquid phase at 503 K and 3.0 MPa was 0.82 and this value increases to 0.98 when pressure is increased to 5.6 MPa. Meanwhile, the mole fraction of methanol in the vapor phase remains constant at values close to one, indicating pure methanol in the vapor phase [32]. The higher solubility of methanol in the oil-rich phase with increasing pressure could overcome the interphase mass-transfer resistance arising from dissimilarity in size and polarity between lipids and methanol. Thus, this phenomenon can enhance reaction rate of transesterification. The presence of vapor phase in the reaction mixture also contributes on the enhanced chemical kinetics at pressures and temperatures correspond to lower densities of subcritical methanol [32,33]. The highest FAME yield (92.67 ± 3.23%) was obtained at a methanol to lipid mass ratio of 5:1 and under condition in the vicinity of critical point of methanol (6.5 MPa and 215 °C).

3.6.   Fuel properties of biodiesel

The fuel properties of biodiesel are varied quite widely, depending upon the quantity and types of fatty acids in the lipid sources and also the refining method. In this regard, the quality of biodiesel produced from wastewater sludge was judged according to ASTM D6751 specification and the results are given in Table 6. Several parameters directly depend upon the fatty ester composition are cetane number, oxidative stability, kinematic viscosity and cold-flow properties in form of the cloud point, pour point and cold filter plugging point (CFPP) [34]. Density is one of the properties of biodiesel that
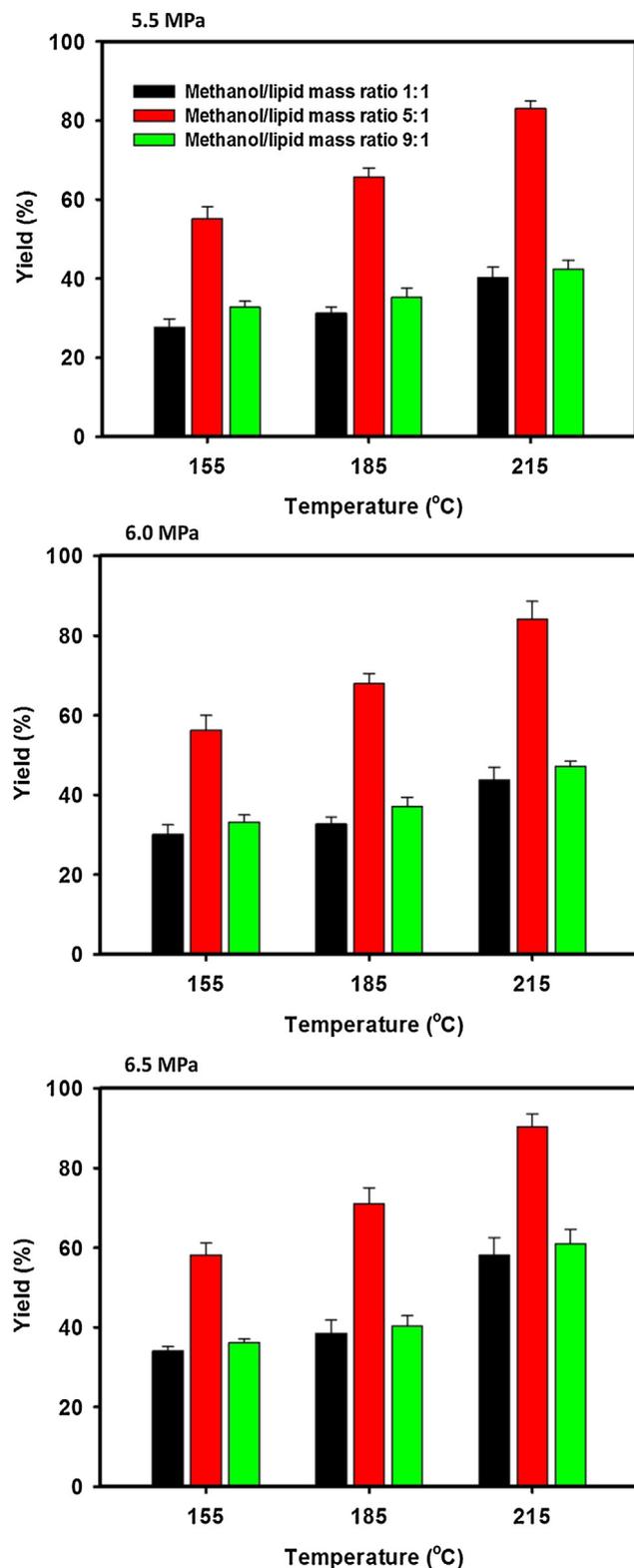
Fig. 4 – **Variation of FAME yields with reaction temperatures at different mass ratios of methanol to lipid (1:1, 5:1 and 9:1) and pressures (5.5, 6.0 and 6.5 MPa).**

can be used to indicate contamination by unwanted components such as residual alcohol, water and sediment (refers to any substance that is higher in density than biodiesel, such as unreacted monoglycerides and diglycerides). The presence of

these contaminants, particularly water could make the fuel becomes rancid, corrode metal parts in fuel lines, accelerate fuel gelling in cold weather and reduce the heat and power of combustion. The density of biodiesel produced in this study is about 885 kg m^{-3}. Although the density requirement is not specified in ASTM D6751, the value satisfies EN 14214 standard. Kinematic viscosity is associated with the injection and atomization of the fuel in the combustion chamber of the engine and is also known to impact the engine life and the rate of injector fouling. The sludge-based biodiesel has kinematic viscosity of 2.91–3.17 mm^2 s^{-1}, which is an acceptable viscosity range based on ASTM D6751 standard specification.

The flash point of biodiesel samples is generally over 150 °C, which is much higher than ASTM specification requires. High flash point of biodiesel confirms that this fuel is safer to handle than regular diesel fuel in high temperature environment. Cetane number indicates how well a fuel will combust inside a compression engine and is an important quality parameter for engine performance. Knothe [34] reported that cetane number of biodiesel depends upon the nature of fatty esters comprising the fuel and this combustion-related parameter increases with an increasing fatty acid carbon chain and increasing saturation of components. The cetane number of biodiesel samples in this study exceeds the minimum standard value specified in ASTM D6751, characterizing good ignition and combustion quality. Moreover, high cetane number minimizes soot (carbon) particles and white smoke emissions from the engine during cold start operation. The cetane number obtained in this study agreed well with those of vegetable oils-based biodiesel such as peanut oil methyl ester [35], sunflower oil methyl ester [6], soybean oil methyl ester [3], cottonseed oil methyl ester [6] and rapeseed oil methyl ester [6]. This result may be ascribed to similar distribution of fatty acids in the feedstock from which biodiesel fuel was made.

Acid value is a crucial property for biodiesel quality check due to its relevancy with oxidative stability of the fuel during long-term storage. This parameter is commonly used to indicate free fatty acid content of finished biodiesel. During long-term storage, biodiesel can absorb water which can lead to the formation of free fatty acids from hydrolysis of triglycerides. Biodiesel with an acid value as KOH higher than 500 mg kg^{-1} has a greater tendency to corrode fuel tank, linings and pipelines. The vegetable oil sludge methyl esters have acid values as KOH in the acceptable range of 280–410 mg kg^{-1}. The cloud point is a measure of low-temperature operability of the fuel and is well-correlated with filter plugging point. This cold-flow property depends mostly on the fatty acid composition and also the type and quantity of impurities in the fuel. Biodiesel made from saturated fatty acid chains has a higher cloud point compared to biodiesel made from mono- or poly-unsaturated fatty acid chains. From Table 6, it can be seen that vegetable oil sludge methyl esters which mainly comprised of methyl palmitate (29.8%), methyl palmitoleate (23.4%) and methyl oleate (15.4%) have a moderate cloud point about 8 °C. The pour point, a temperature at which the fuel contains many agglomerated crystals and will no longer flow was about 7 °C. The cloud point and pour point of biodiesel can be further lowered by a winterization technique [36], using a branched-chain alcohol

Table 6 – Fuel properties of biodiesel produced from vegetable oil wastewater sludge. (D – density; KV – kinematic viscosity; FP – flash point; PP – pour point; CP – cloud point; CN – cetane number; AN – acid number).

Sample[a]	D @ 15 °C (kg m^{-3})	KV @ 40 °C (mm^2 s^{-1})	FP (°C)	PP (°C)	CP (°C)	CN	AN as KOH (mg kg^{-1})
155/1/5.5	885.7	2.97	163	7.2	8.2	52.8	342.5
185/1/5.5	886.4	2.96	155	7.1	8.0	51.7	378.3
215/1/5.5	886.2	3.04	158	6.8	7.6	52.7	322.6
155/5/5.5	886.2	3.08	161	6.9	7.8	52.6	335.2
185/5/5.5	885.9	3.02	157	7.1	7.9	51.7	364.1
215/5/5.5	885.9	3.06	156	7.0	8.1	51.1	391.2
155/9/5.5	884.9	2.94	165	6.8	8.0	54.0	285.4
185/9/5.5	885.0	2.92	162	7.2	7.8	53.7	314.8
215/9/5.5	884.9	2.91	165	7.1	8.0	53.5	348.6
155/1/6.0	885.9	2.98	160	6.9	7.7	52.4	363.0
185/1/6.0	886.4	2.97	155	7.0	7.9	51.6	412.7
215/1/6.0	886.1	3.02	157	6.8	8.2	52.5	357.8
155/5/6.0	885.8	3.07	159	6.7	8.1	53.0	310.6
185/5/6.0	885.7	3.02	157	7.2	7.9	51.9	282.5
215/5/6.0	885.9	3.07	154	6.9	7.8	51.0	347.2
155/9/6.0	884.9	2.97	170	6.6	7.6	53.9	354.4
185/9/6.0	885.1	2.99	165	7.0	8.1	53.4	339.1
215/9/6.0	884.8	2.93	162	7.1	8.0	53.5	307.3
155/1/6.5	885.9	2.98	158	6.9	7.7	52.4	344.9
185/1/6.5	886.5	3.03	157	6.6	7.6	51.8	305.8
215/1/6.5	886.1	3.02	160	6.8	7.9	52.5	338.5
155/5/6.5	886.3	3.17	163	6.7	7.5	53.1	319.8
185/5/6.5	885.7	3.02	160	7.1	7.7	51.9	340.2
215/5/6.5	885.9	3.10	156	6.9	7.6	51.0	371.3
155/9/6.5	885.1	2.95	165	6.9	8.1	53.9	356.6
185/9/6.5	885.2	3.00	168	7.2	8.3	53.7	323.5
215/9/6.5	884.9	2.96	172	7.0	8.0	53.8	388.1
ASTM D6751	na	1.9–6.0	min 93	report	report	>47	max 500

[a] Biodiesel samples codes: T (°C)/methanol to lipid mass ratio/P (MPa).

instead of methanol during transesterification [37], adding polymeric cold-flow improvers [38] or blending with other methyl esters having a lower cloud point or No.1/No.2 diesel fuels [39].

4. Conclusions

This study demonstrated the synthesis of biodiesel from low grade vegetable oil wastewater sludge with lipid mass fraction of 12.44 ± 0.87% (dry and ash-free basis) by a catalyst-free transesterification method. Effects of temperature and pressure show that increasing these parameters contributed positively on FAME yields. The highest FAME yield of 92.67 ± 3.23% was obtained at 215 °C, 6.5 MPa and methanol to lipid mass ratio of 5:1. The optimum reaction condition for *in-situ* subcritical methanol transesterification of sludge to fatty esters was 215 °C, 6.5 MPa and 5.12:1 of methanol to lipid mass ratio with a predicted FAME yield of 90.37%. The predicted FAME yield was in good agreement with experimental results under the optimum reaction condition.

Acknowledgments

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