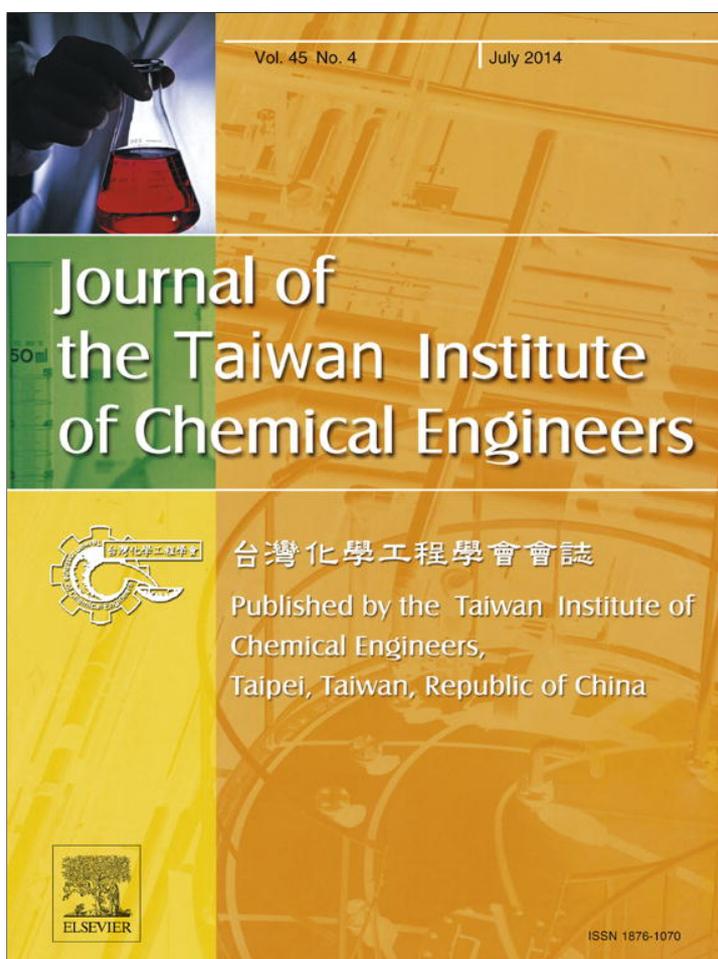


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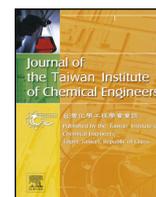
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## In situ transesterification of *Jatropha curcas* L. seeds in subcritical solvent system



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### ABSTRACT

*Jatropha curcas* L. seed is widely studied for the production of biodiesel. A major drawback is the presence of excess free fatty acid in its seeds. The fatty acids make it unsuitable as feedstock oil in the conventional base-catalyzed process for biodiesel production. In this study, in situ transesterification of seed oil was studied with the aim to reduce production steps. A mixture of methanol, acetic acid and water under subcritical conditions was employed for the in situ transesterification of *J. curcas* L. seed kernel to produce biodiesel under less severe operating conditions as compared to supercritical methanol technologies. A yield of 94–98% was obtained, based on extractable lipids (54–56% based on dry kernel). The process investigated is capable of tolerating the presence of moisture (up to 10%) and free fatty acid (up to 5%), eliminating the need for pre-treatment steps.

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## 1. Introduction

Producing biodiesel by supercritical and subcritical solvent technologies has gained interest in the past decade. Most related research was aimed at avoiding the use of a catalyst. The absence of a catalyst lessened downstream processing steps and reduced the amount of wastewater generated.

Among the early works in supercritical alcohol transesterification, Saka and Kusdiana [1] used refined rapeseed oil with oil to methanol molar ratio of 1:42 at 350 °C and 43 MPa and achieved a conversion of over 95% in 4 min. Warabi et al. [2] investigated whether FFAs can be esterified with supercritical alcohols and found that high yield (95%) in less than 15 min at 300 °C was obtained for methanol at 20 MPa and for ethanol at 15 MPa. Their results showed that the presence of FFA in supercritical alcohol (*trans*)esterification will not be a problem if the reaction is carried

at 300 °C for 15 min for methanol or 45 min for other alcohols. Kusdiana and Saka [3] investigated the effects of water on biodiesel production under supercritical methanol condition and found that water up to 50% by weight could be tolerated during esterification. They demonstrated that when waste palm oil containing more than 20% FFA and more than 61% moisture was used as raw material, high yield (95.8%) can be achieved.

Although the supercritical methanol process can tolerate the presence of FFA and water, it requires high methanol loading, high temperature and high pressure. These severe conditions may lead to thermal degradation. To minimize thermal degradation of biodiesel, Imahara et al. [4] suggested that the supercritical methanol method should be carried out at temperature below 300 °C, preferably at 270 °C, with a pressure higher than 8.09 MPa. Shin et al. [5] reported that degradation of unsaturated fatty acids, found in vegetable oils in the presence of water, was observed at temperatures above 250 °C with an operating pressure of 20 MPa. Different co-solvents, propane [6], heptane [7] and carbon dioxide [8] have been used to lower temperature to 280 °C in the supercritical methanol method. In the work by Tan et al. [7], methanol loading was lowered to 30 moles per mole of palm oil by using heptane as co-solvent. However, Imahara et al. [9] concluded that the addition of hexane, CO<sub>2</sub> and N<sub>2</sub> as the third component barely contributed to the improvement in the reaction rate. They found that the addition of N<sub>2</sub> decreased the total glycerol in the

**Abbreviations:** AG, acylglyceride; AOCs, American Oil Chemist Society; FA, fatty acid; FAME, fatty acid methyl ester; FFA, free fatty acid; HTGC, high temperature gas chromatography; JCL, *Jatropha curcas* L.; SSR, solvent to solid ratio.

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biodiesel produced and improved its oxidation stability. Most studies using a co-solvent successfully lowered down process severity, but did not take into consideration the presence of water.

The use of the supercritical alcohol method has an advantage over the conventional method in biodiesel production. Low quality oil such as non-edible and waste oils can be used directly as feedstock without the need of pretreatment. This has the potential of greatly lowering biodiesel production cost. In order to eliminate the step of oil extraction from seeds or microbial cells, in situ transesterification of oil seeds or microbial biomass under sub- and super-critical methanol conditions has been extensively studied [10–16]. Due to the high oil content of *Jatropha curcas* L. (JCL) seeds, the high yield of oil seeds and its easy of cultivation, non-edible oil from JCL seeds has recently been suggested as the next generation feedstock oil for biodiesel production. Lim et al. [10] studied the supercritical fluid reactive extraction of JCL seeds with methanol. They investigated a pretreatment of drying and grinding of JCL seed kernels and found that moisture content of kernel should be reduced to 0.59% at temperature no higher than 75 °C for no more than 12 h prior to in situ (*trans*)esterification reaction [11,13]. The optimum particle size used in their study was between 1 mm and 0.5 mm [10]. They investigated the effects of co-solvents and found that pentane and CO<sub>2</sub> played important roles in reducing methanol loading [12]. Finally an optimized process was developed employing a solvent to solid ratio of 5.9 mL/g at 300 °C and an initial pressure of 2.0 MPa using CO<sub>2</sub> for a reaction time of 12.3 min (a heating up time of 80–90 min was required) [14]. In most of their studies high temperature (300 °C) was used and drying of kernel was required. Moreover high space loadings (30–54 mL/g) were employed [13,14].

In this study the production of biodiesel by in situ transesterification of JCL seed kernels using mixtures of methanol and acetic acid under subcritical condition was investigated with the objective to decrease process severity. The effects of pressurizing gas (CO<sub>2</sub> or N<sub>2</sub>) and moisture on the overall biodiesel yield were studied. In addition, space loading and effective utilization of reactor volume to improve the overall biodiesel productivity were also investigated.

## 2. Materials and methods

### 2.1. Materials

JCL seeds were obtained from Muhammadiyah University (Malang, Indonesia). Seeds obtained were sun dried and kept at –20 °C prior to use. Standards of fatty acid (FA), acylglycerides (AG) such as monoolein, diolein and triolein and fatty acid methyl esters (FAMES) were obtained from Supelco (Bellfonte, PA). All solvents and reagents used were either high performance liquid chromatography (HPLC) or analytical reagent grade, obtained from commercial sources.

### 2.2. Sample preparation and characterization

Sun-dried JCL seeds were de-hulled manually. Kernels were ground to particle size of about 1.0 mm. Ground kernels were divided into 2 portions, the first were kept below –20 °C for later use in transesterification reactions. The second portion was kept at ambient room temperature to study the effect of storage on FFA content in kernel.

Ground kernel (5 g) was loaded in a pre-dried glass tube. The kernel loaded tube was put into a freeze drier (Labconco FreeZone 2.5 L Model: 7670520, Kansas City, MO) operated at –44 °C and 11.0 Pa for 48 h. Water content of the ground kernels was calculated based on difference in weight of kernel sample before and after freeze-drying.

To determine the amount of extractable crude lipid, a seed sample (5 g) was placed into a thimble. The thimble was then

loaded into a Soxhlet extractor and extracted for 8 h using 150 mL of *n*-hexane as the solvent.

The FFA content in the crude lipid was determined by the titrimetric method following AOCS official methods (Method Ca 5a-40) and by high temperature gas chromatography (HTGC). Analyzing known samples of FA with varied concentrations showed that the results of two methods differ from each other by less than 0.5%. Amount of unsaponifiable matter in the lipid sample was analyzed using AOCS official methods (Method 6b-53).

Saponified lipids obtained after determination of unsaponifiable matter were collected and acidified to a pH of 2 with concentrated sulfuric acid and continually stirred at 60 °C until the solution was clear. The solution was then allowed to settle until two phases formed. The upper layer consisting of FAs was extracted with hexane and then reacted with BF<sub>3</sub>-methanol for later analysis of FA profile using HTGC.

Dewaxing and degumming of the extracted lipids was carried out before its AG composition was analyzed using HTGC. In brief, 1 g crude lipid was dissolved in 6 mL acetone and heated in a 60 °C water bath until the solution was clear. The solution was allowed to cool to room temperature and then stored at 4 °C for 3 h. The solution was then filtered (0.22 μm pore size) to remove the precipitated wax and gum. The procedure was repeated twice.

### 2.3. In situ transesterification

Ground seed kernel (3.2 g) containing ~5% moisture was loaded into a glass chamber (190 mL) and placed in a high-pressure reactor (290 mL). The ground kernel was mixed and suspended in a mixture of methanol and acetic acid. A detailed reactor description and configuration is described elsewhere [17]. The reactor is equipped with an external electric heater and a magnetic stirrer. Temperature in the reactor was controlled to within ±2 °C. After the sample was put in the reaction chamber, the reactor was sealed and the chamber was purged with N<sub>2</sub>. Prior to heating, the chamber was pressurized using N<sub>2</sub> or CO<sub>2</sub> to ensure that the solvent was under subcritical state at the temperature carried out in this study.

The reaction was carried out at 250 °C for a predetermined time. Heating rate of the reactor was kept at 5 °C/min with a heating period of 40–45 min. Reaction time was started as the desired reaction temperature was reached. After the reaction, the reactor was rapidly cooled, pressure inside the reactor was released and the product in the reactor was collected at room temperature.

The reaction product was vacuum filtered using a Buchner funnel with Advantec No. 2 filter paper (8 μm pore size) to separate the spent solid from the reaction product. The retained solid was washed three times (each with 30 mL methanol) to recover the FAME produced. Methanol and acetic acid in the filtrate were removed and recovered using a rotary evaporator (BUCHI Labortechnik AG in Flawil, Switzerland). The evaporator was operated at 40 °C and 13.3 kPa. The residual acetic acid and water were further removed by heating the mixture to 80 °C and 13.3 kPa. The concentrated organic extract was then extracted three times (each with 30 mL hexane) to recover the FAME produced and washed three times (each using 20 mL, 5% NaCl solution) in a separation funnel to remove non-lipid products co-extracted by methanol. The solution was allowed to clarify in between washings. The upper hexane phase, which contained FAME, was withdrawn. Hexane from the combined extract was evaporated under a vacuum using a rotary evaporator. The recovered product was weighed and analyzed for its FAME, FA and AG contents.

### 2.4. Gas chromatography analysis

A 20 mg aliquot of the lipid sample was dissolved in ethyl acetate and filtered through 0.2 μm PTFE hydrophobic membrane

to remove moisture. From this prepared solution, a 1.0  $\mu\text{L}$  sample was injected into HTGC for analysis. External calibration curve was generated using 0.2–20 mg of a pure standard dissolved in ethyl acetate. The calibration curve was generated by fitting a straight line with the  $y$ -intercept passing through zero ( $R^2 < 0.99$ ).

Qualitative and quantitative analyses of FAME and un-reacted FFA in each sample were performed using a Shimadzu GC2010 (Kyoto, Japan) equipped with a split-injector and a FID. Separation was carried out on a ZB-5HT (5% phenyl)-methylpolysiloxane nonpolar column (15 m  $\times$  0.32 mm i.d., 0.1 mm film thickness) (Zebron, Phenomenex, Torrance, CA, USA). Both injector and detector temperatures were set at 370  $^{\circ}\text{C}$ . The column temperature was programmed to increase at a rate of 15.0  $^{\circ}\text{C}/\text{min}$  from 80  $^{\circ}\text{C}$  to 365  $^{\circ}\text{C}$  and held at 365  $^{\circ}\text{C}$  for 48 s.  $\text{N}_2$  was used as the carrier gas with a linear velocity of 30 cm/s at 80  $^{\circ}\text{C}$ . Data analyses were carried out by the software “GC Solution version 2.3”, Shimadzu.

### 3. Results and discussion

The moisture content of the ground JCL seed kernels was  $4.63 \pm 0.36\%$ . The crude extractable lipid mass fraction was found to be  $58.1 \pm 0.98\%$ . Most of the extracted lipids were saponifiable with only  $0.84 \pm 0.1\%$  being unsaponifiable. Further analysis showed that  $88.22 \pm 0.61\%$  of the extracted lipid could be converted to FFA. The theoretical maximum overall FAME yield is 92.63% if based on extractable lipid and 53.69% if based on dry kernel. Unlike refined oils, which are primarily triglycerides. The crude lipid extracted from JCL seed kernels contained  $12.98 \pm 0.84\%$  wax and gums,  $2.53 \pm 0.25\%$  FFA and  $85.83 \pm 1.31\%$  AG. The apparent loss in the yield is due to the mass of the glycerol backbone of the AG, phosphate group in phospholipids (gums) and long chain fatty alcohols of wax esters, which do not contribute to formation of FAME and FAME yield.

The presence of moisture and FFA in feedstock oil is a nuisance in biodiesel production. Moisture and FFA contents in feedstock oil are usually kept below 1% to avoid side reactions in conventional processes. Seeds should be kept dry to avoid germination, in which hydrolysis of storage lipids and consumption of lipids in post germination occur causing a decrease in extractable lipids [18]. To avoid this, seeds were dehulled, ground and kept dry in this study. Even with such precaution, the increase of FFA in the ground kernel is unavoidable (Fig. 1). However the amount of extractable crude lipid remained relatively unchanged.

The development of a (*trans*)esterification method capable of tolerating high moisture and FFA contents in feedstock oil is important. Extra energy is required for removing moisture and long storage of oil seeds promotes formation of FFA through hydrolysis of AGs in seeds.

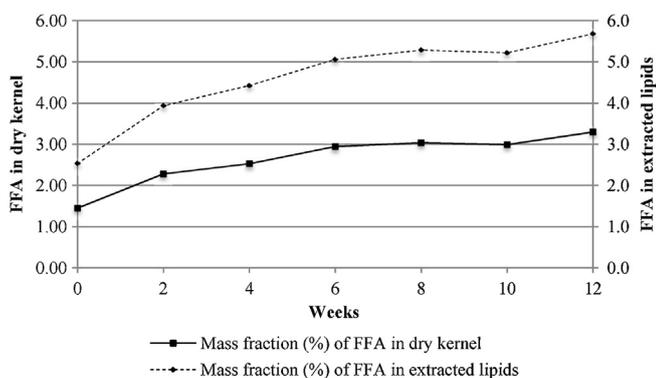


Fig. 1. FFA content in extracted lipids from stored ground JCL seed kernels over a period of 3 months.

Table 1

Effects of methanol to solid ratio on FAME yield. Reactions were carried out at 250  $^{\circ}\text{C}$  for 1 h and initially pressurized to 4 MPa using  $\text{N}_2$ .

| Methanol to kernel ratio (mL/g) | Yield (%) <sup>a</sup> |
|---------------------------------|------------------------|
| 5.0                             | 31.51 $\pm$ 1.29       |
| 6.0                             | 36.48 $\pm$ 1.65       |
| 7.0                             | 42.65 $\pm$ 1.72       |
| 8.0                             | 47.13 $\pm$ 1.64       |
| 10.0                            | 47.86 $\pm$ 1.91       |
| 12.0                            | 45.60 $\pm$ 3.27       |
| 15.0                            | 37.07 $\pm$ 2.07       |

<sup>a</sup> Mass fraction (%) of FAME based on the dry kernel (theoretical maximum  $\sim$  53.69%).

#### 3.1. Effects of methanol loading on FAME yield

A control experiment using only methanol as the solvent and the reactant was carried out at 250  $^{\circ}\text{C}$ . As shown in Table 1, FAME yield increased with increasing methanol loading up to 10 mL per gram of dry kernel. Further increase in methanol loading resulted in lower FAME yields. Similar result was observed at 290  $^{\circ}\text{C}$  by Lim and Lee [12,13]. The decrease in yield was attributed to the improvement in solubility at higher solvent to solid ratio (SSR) resulting in a reverse reaction (glycerolysis of FAME). Glycerolysis was less likely to occur as methanol was present in a large excess and methanol was not continuously removed during reaction, which is often employed during glycerolysis. A possible reason is that dilution of the reaction system by the improved solubility at higher SSR resulted in slower rate of reaction, hence lower FAME yield.

#### 3.2. Effects of acetic acid to methanol ratio on FAME yield

At 250  $^{\circ}\text{C}$  and a SSR of 10 mL/g, a maximum yield of 47.86% was obtained which is only 89.14% of the theoretical yield (53.69%). Mixtures of acetic acid and methanol were tested at different SSRs to achieve higher yields. Table 2 shows that at an acetic acid volume fraction of 0.25 in the solvent and a SSR of 10 mL/g, the highest FAME yield (52.93%) was obtained which is 98.58% of the theoretical yield. Acetic acid fractions higher than 25% were found

Table 2

Effects of SSR and volume fraction of acetic acid in solvent mixture (methanol + acetic acid) on FAME yield. Reactions were carried out at 250  $^{\circ}\text{C}$  for 1 h and initially pressurized to 4 MPa using  $\text{N}_2$ .

| Solvent to kernel ratio (mL/g) | Volume fraction of acetic acid (% AA) | Yield (%) <sup>a</sup> |
|--------------------------------|---------------------------------------|------------------------|
| 5.0                            | 0                                     | 31.51 $\pm$ 1.29       |
|                                | 20                                    | 40.78 $\pm$ 0.49       |
|                                | 25                                    | 41.99 $\pm$ 0.85       |
|                                | 33                                    | 40.21 $\pm$ 1.67       |
| 6.0                            | 0                                     | 36.48 $\pm$ 1.65       |
|                                | 20                                    | 43.37 $\pm$ 0.81       |
|                                | 25                                    | 44.45 $\pm$ 0.51       |
|                                | 33                                    | 43.01 $\pm$ 0.53       |
| 7.0                            | 0                                     | 42.65 $\pm$ 1.72       |
|                                | 20                                    | 46.09 $\pm$ 1.01       |
|                                | 25                                    | 47.39 $\pm$ 0.95       |
|                                | 33                                    | 46.29 $\pm$ 1.20       |
| 8.0                            | 0                                     | 47.13 $\pm$ 1.64       |
|                                | 20                                    | 49.12 $\pm$ 1.27       |
|                                | 25                                    | 50.76 $\pm$ 0.51       |
|                                | 33                                    | 46.74 $\pm$ 0.66       |
| 10.0                           | 0                                     | 47.86 $\pm$ 1.91       |
|                                | 20                                    | 51.03 $\pm$ 0.50       |
|                                | 25                                    | 52.93 $\pm$ 0.03       |
|                                | 33                                    | 46.98 $\pm$ 1.16       |

<sup>a</sup> Mass fraction (%) of FAME based on the dry kernel (theoretical maximum  $\sim$  53.69%).

to be detrimental to FAME yield. At fixed SSR, the use of mixture of acetic acid and methanol enabled the reaction to be carried out at a less severe temperature of 250 °C and resulted in high FAME yield.

Pure methanol was capable of achieving similarly high FAME yields, but required longer reaction time (120 min). The addition of a 25% acetic acid–methanol mixture cut the reaction time in half to reach maximum yield (Fig. 2). At 25% acetic acid, prolonging reaction time to more than 60 min resulted in lower yields due to degradation in acidic environment.

In this study, a SSR of 10 mL/g was required at 250 °C compared to reactions carried out at 300 °C which only required a SSR of 5.9 mL/g [14]. This is probably due to better solubility of oil in methanol at higher temperature. Nevertheless an overall reaction time of 105 min including heating up of the reactor is comparable to those reported in the literature.

The role of acetic acid in the reaction is not clear. This weak acid could act as an acid catalyst at high temperatures. Experiments were also carried out at 65 °C using conventional methods of acid transesterification utilizing crude *Jatropha* oil and acetic acid as the catalyst. This did not result in any detectable FAME even at a reaction time of 24 h (data not shown). In view of its chemical structure, acetic acid is a short chain FFA. It was previously found that the presence of FFA could act as an acid catalyst in the hydrolysis of oils and supercritical esterification [19,20]. This catalytic effect could have been more pronounced at elevated temperatures and due to the presence of water in the reaction system. Apart from the possible catalytic activity provided by acetic acid, its presence has enhanced and accelerated the extraction of oil from JCL seed kernels. It was reported that lipids or oils have better solubility in an acidic environment [21]. From Fig. 3 it can be seen that upon reaching 250 °C, the presence of acetic acid allowed nearly complete extraction of lipids from the solid matrix of kernels. Another possibility is that the esterification of acetic acid to methyl acetate, which could act as a good extracting solvent.

### 3.3. Effects of pressurizing gas and stirring on FAME yield

Both liquid and gas co-solvents have been employed by various researchers to improve the solubility and extraction efficiency. The main disadvantage of using liquid co-solvents is the increase in SSR and may result in decreasing the overall volumetric productivity. The use of gas co-solvents like CO<sub>2</sub> would be favorable as it will not decrease the overall volumetric productivity and is easily separated after reaction. Table 3 shows that upon the addition of CO<sub>2</sub>, yields were improved significantly at lower SSR (5 mL/g). The theoretical maximum yield was achieved at a SSR of 10 mL/g.

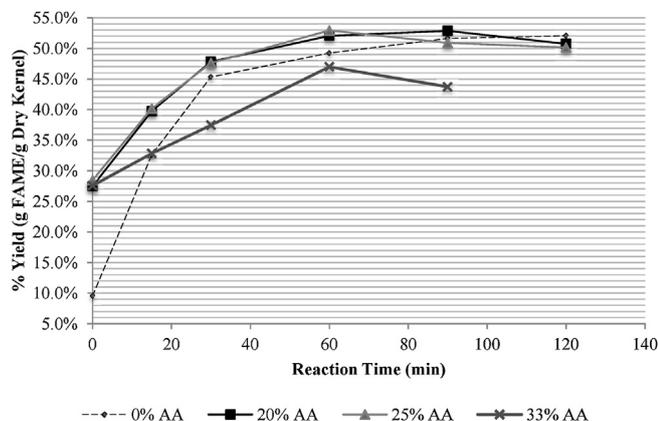


Fig. 2. Effects of volume fraction of acetic acid in solvent on time course of FAME yield. Reactions were carried out at 250 °C, a SSR of 10 mL/g and initially pressurized to 4 MPa using N<sub>2</sub>.

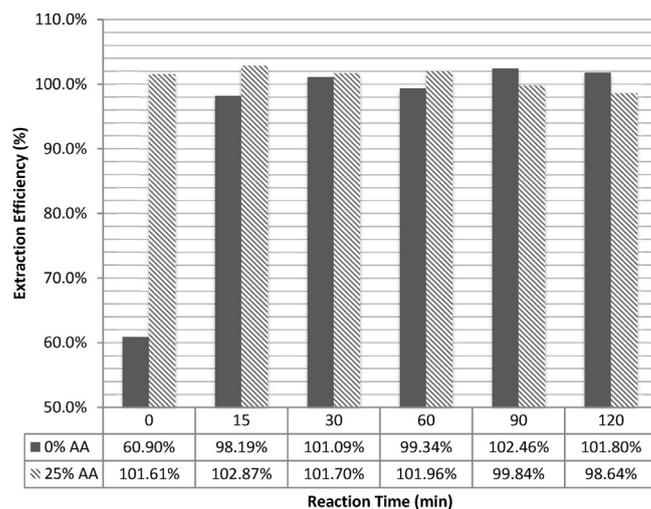


Fig. 3. Extraction efficiency versus reaction time at various acetic acid concentrations. Reactions were carried out at 250 °C, a SSR of 10 mL/g and initially pressurized to 4 MPa using N<sub>2</sub>.

Another important parameter affecting FAME yield is stirring. Stirring would promote better contact between solid and solvent. Fig. 4 shows mixing improved the FAME yield. A FAME yield higher than the theoretical yield was achieved and is probably due to the release of FFA from the hydrolysis of structural lipids in the seed's kernel. The same phenomena were observed in previous investigations [10–14].

Even at a reduced SSR of 7 mL/g, the combined addition of CO<sub>2</sub> and stirring resulted in a FAME of 56.80 ± 1.3% based on dry kernel (98.01 ± 2.3% on hexane extractable lipid). Prolonged reaction time resulted in decreased FAME yield as previously observed except for a SSR of 5 mL/g which achieved a FAME yield of 55.69 ± 0.6% at a reaction time of 90 min. At a SSR of 5 mL/g, prolonged reaction did not result in apparent degradation as the amount of acetic acid present was lesser.

Consider a reactor filled with the reaction mixtures to the same final volume, a lower SSR is preferable as it gives higher overall productivity. In this study, by referring to the results shown in Fig. 5, an overall productivity of 39.9 kg/(m<sup>3</sup> h) and 40.6 kg/(m<sup>3</sup> h) was obtained at a SSR of 5 and 7 mL/g, respectively. The similar productivity obtained is due to the extended time required at lower SSR. In a batch process a shorter batch cycle time is preferable thus a SSR of 7 mL/g was used in the subsequent experiments.

### 3.4. Effects of space loading and pressure on FAME yield

A smaller space loading implies a higher amount of solid is loaded into the reactor [13]. Although the highest loading investigated in this study resulted in a reaction mixture occupying only 68.8% of the effective reactor volume, it occupied ~87% of the

Table 3

Effects of pressurizing gas and SSR (25% acetic acid and 75% methanol) on FAME yield. Reactions were carried out at 250 °C for 1 h and initially pressurized to 4 MPa using N<sub>2</sub> or CO<sub>2</sub>.

| Solvent to kernel ratio (mL/g) | Pressurizing gas | Yield (%) <sup>a</sup> |
|--------------------------------|------------------|------------------------|
| 5.0                            | Nitrogen         | 41.99 ± 0.85           |
|                                | Carbon dioxide   | 49.62 ± 1.41           |
| 7.0                            | Nitrogen         | 47.39 ± 0.95           |
|                                | Carbon dioxide   | 51.37 ± 0.61           |
| 10.0                           | Nitrogen         | 52.93 ± 0.03           |
|                                | Carbon dioxide   | 53.54 ± 0.40           |

<sup>a</sup> Mass fraction (%) of FAME based on the dry kernel (theoretical maximum ~ 53.69%).

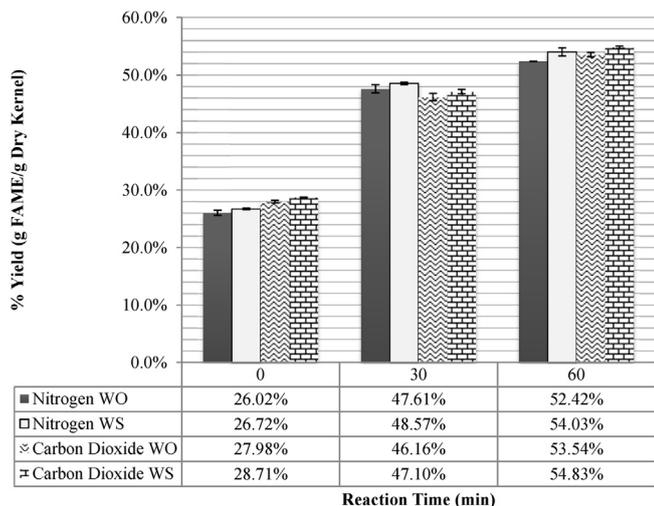


Fig. 4. Effects of stirring on FAME yield. Reactions were carried out at 250 °C for 1 h and initially pressurized to 4 MPa using N<sub>2</sub> or CO<sub>2</sub>. WO: without stirring, WS: with stirring.

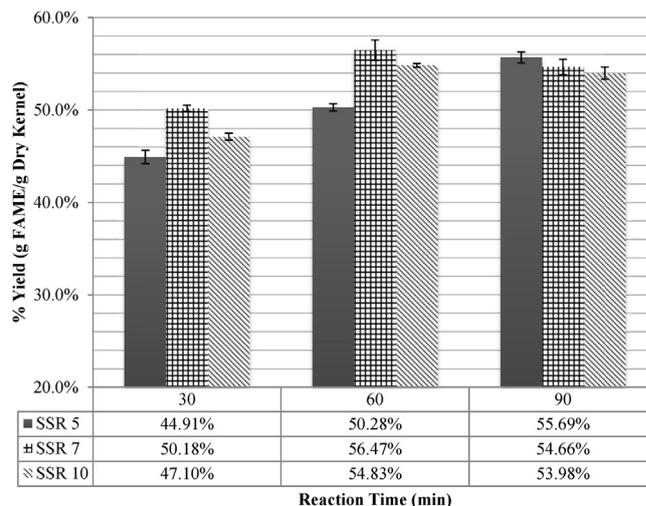


Fig. 5. FAME yield versus time at various SSR. Reactions were carried out with stirring at 250 °C and initially pressurized to 4 MPa using CO<sub>2</sub>.

**Table 4**  
Effect of space loading on FAME yield in in situ transesterification of JCL seed kernel. Reactions were carried out at 250 °C for 60 min with stirring and initially pressurized to 4 MPa using CO<sub>2</sub>.

| Space loading (mL/g) <sup>a</sup> | Utilized reactor volume (mL) | Initial pressure (MPa) | Final pressure (MPa) | Extraction efficiency (%) <sup>c</sup> | Yield (%) <sup>d</sup> (Kernel) <sup>e</sup> |
|-----------------------------------|------------------------------|------------------------|----------------------|--|--|
| 80.0                              | ~25 (10.4%) <sup>b</sup>     | 4.0                    | 12.0                 | 103.72                                 | 98.01 (56.80)                                |
| 48.0                              | ~41 (17.1%)                  | 4.0                    | 13.0                 | 102.26                                 | 92.57 (53.65)                                |
| 24.0                              | ~82 (34.2%)                  | 4.0                    | 14.0                 | 104.69                                 | 90.70 (52.56)                                |
| 12.0                              | ~165 (68.8%)                 | 4.0                    | 21.0                 | 104.26                                 | 94.43 (54.72)                                |
| 12.0                              | ~165 (68.8%)                 | 1.0                    | 14.0                 | 102.98                                 | 88.81 (51.47)                                |
| 12.0                              | ~165 (68.8%)                 | ~0.1                   | 10.0                 | 99.29                                  | 88.24 (51.14)                                |

<sup>a</sup> Space loading = reactors effective volume (240 mL)/g of dry solid.  
<sup>b</sup> Fraction of the reactor volume utilized.  
<sup>c</sup> Mass fraction (%) of hexane soluble product based on the hexane extractable lipid.  
<sup>d</sup> Mass fraction (%) of FAME based on the hexane extractable lipid.  
<sup>e</sup> Mass fraction (%) of FAME based on the dry kernel.

glass chamber and for safety reasons this was taken as the upper limit. Table 4 is a summary of the experimental results with various space loadings and initial pressures. At a fixed initial pressure, final pressure in the reactor increased with decreased space loading due to the smaller effective volume available for solvent and co-solvent expansion. Although a higher final pressure was achieved, FAME yield slightly decreased which is probably due to the lesser amount of CO<sub>2</sub> in the system. A decreased available space also required less CO<sub>2</sub> to be added at a fixed initial pressure. Another possibility is the improved solubility due to higher pressure achieved, which could have resulted in a similar dilution effect observed previously.

Varying the initial pressure of CO<sub>2</sub> added at a fixed space loading resulted in lower final pressure. This further confirms that addition of CO<sub>2</sub> is required to improve the yield of FAME. Higher final pressure did not significantly change FAME yield; the amount of CO<sub>2</sub> added played a more important role. Further investigations is required to better understand the synergic effects on the FAME yield induced by the available space for loading, expansion of solvents, amount of CO<sub>2</sub> and final pressure. This study is the first to obtained high FAME yield with low space loading and high solid loading.

3.5. Effects of moisture content and added water on FAME yield

All oil seeds contain a certain amount of moisture. Methanol and acetic acid are both hygroscopic solvents and thus the presence of water in the reaction system is inevitable. It is

important to develop a process operates at lower temperature (250 °C) that can tolerate moisture so that hydrothermal degradation of products can be avoided.

The presence of water had a positive effect on FAME formation in the process developed in this study (Fig. 6). A water content of 2% in the seed kernel resulted in a slight increase in FAME yield.

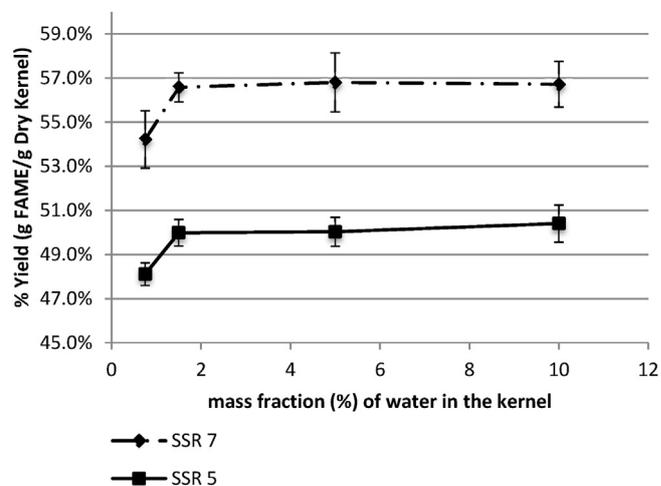


Fig. 6. Effects of moisture content in JCL seed kernel on FAME yield. Reactions were carried out at 250 °C for 60 min with stirring and initially pressurized to 4 MPa using CO<sub>2</sub>.

**Table 5**  
Comparison of conventional and non-conventional catalyzed in situ transesterification methods.

| In situ (trans)esterification method   | Oil content (%) | SSR (mL/g)     | Temp (°C) | Pressure (MPa) | Time (h)               | Space loading <sup>a</sup> (mL/g) | % yield <sup>c</sup> (Kernel) <sup>d</sup> | Ref.       |
|--|-----------------|----------------|-----------|----------------|------------------------|-----------------------------------|--|------------|
| Acid catalyzed (H <sub>2</sub> SO <sub>4</sub> 21.8 wt.%)                          | 54.6            | 10.5           | 60        | ~0.1           | 10                     | 25                                | 98.1 (53.53) <sup>b</sup>                  | [23]       |
| Microwave (110 W) assisted   | 42.5            | Step 1 10.5    | n.s.      | n.s.           | 0.5 (0.8) <sup>b</sup> | 25                                | 90.0 (38.3)                                | [24]       |
| 2-step (ethanolysis)<br>(H <sub>2</sub> SO <sub>4</sub> 7.5 wt.%)<br>(KOH 5 mol/L) |                 | Step 2<br>8.15 |           |                |                        |                                   |  |            |
| Alkaline catalyzed (NaOH 3.94 wt.%)  | 53.7            | 9.8            | 65        | ~0.1           | 1                      | n.s. <sup>e</sup>                 | 98.0 (52.62)                               | [25]       |
| Self-catalyzed methanolysis (germinated seeds)                                     | 51.5            | 2.61           | 35        | ~0.1           | 8                      | n.s.                              | 87.6 (45.1)                                | [18]       |
| Co-solvent (hexane 2.5 mL/g)   |                 |                |           |                |                        |                                   |  |            |
| Alkaline catalyzed methanolysis<br>(NaOH 1.52 wt.%)                                | n.s.            | 7.5            | 38        | ~0.1           | 1.72                   | n.s.                              | 89.0                                       | [26]       |
| PTC (BTMAOH:NaOH 1.42)   |                 |                |           |                |                        |                                   |  |            |
| Alkaline catalyzed ethanolysis<br>(NaOH 1.38 wt.%)                                 | n.s.            | 7.5            | 35        | ~0.1           | 1.54                   | n.s.                              | 99.4                                       | [26]       |
| PTC (BTMAOH:NaOH 1.62)   |                 |                |           |                |                        |                                   |  |            |
| Sub-critical   | 57.9            | 7              | 250       | 12.0           | 1.0 (1.75)             | 80.0                              | 98.01 (56.8)                               | This study |
| Methanol-acetic acid (7.5:2.5)<br>Co-solvent (CO <sub>2</sub> 4 MPa)               |                 |                |           |                |                        |                                   |  |            |

<sup>a</sup> Space loading = reactor effective volume in mL/g of dry solid.

<sup>b</sup> Total reaction time including reactor heat up time.

<sup>c</sup> Mass fraction (%) of FAME based on the hexane extractable lipid.

<sup>d</sup> Mass fraction (%) of FAME based on the dry kernel.

<sup>e</sup> n.s.: not specified.

Further increase in water content did not have significant effect on FAME yield. The results show that this process can use wet kernel with a moisture content up to 10% without any negative effect on FAME yield.

The presence of water may have aided the hydrolysis of lipids thus indirectly lowering the activation energy in FAME production since FFAs can be esterified at lower activation energies [19]. Another possibility was the catalytic activity of water was present at the reaction conditions of this study. Water at 250 °C under a subcritical state releases maximum amounts of hydronium and hydroxide ions, contributing to its catalytic activity [22].

### 3.6. Comparison with other in situ technologies

The result of the in situ transesterification of JCL seed kernels obtained in this study was compared with those of various biodiesel production methods including conventional base-catalyzed methanolysis and supercritical technologies. From Table 5 it can be seen that SSR used in this study is lower than most non-supercritical processes reported in literature utilizing acid and base catalyst, requiring an SSR of between 7.5 ml/g and 10.5 ml/g

[23–26], except for the self-catalyzed methanolysis utilizing indigenous lipase to self-catalyze the reaction. Although the self-catalyzed reaction used a lower SSR, it required a 4-day germination period [18]. The germination period varies depending on seed strain and activity of the indigenous lipase. The method used in this study required much higher temperatures than the conventional acid/base catalyzed reaction. Nevertheless this method needed much shorter time than acid or enzyme catalyzed reactions to achieve comparable FAME yields. The process developed in this study does not require neutralization of the catalyst. Less post treatment is needed and recovery of the acetic acid used can be incorporated into the conventional methanol recovery system.

Table 6 gives comparison of FAME yield obtained by using super and sub critical methanol technologies on in situ transesterification of the JCL seed kernel. When supercritical methanol was employed, high temperature (~300 °C) and high pressure (9.5–20 MPa) were employed. The use of CO<sub>2</sub> as co-solvent was able to lower space loading required from 54 to 30 mL/g. The use of lower initial CO<sub>2</sub> pressure (from 2 to 5 MPa) is offset by the need to use higher SSR (from 5 to 5.9 mL/g). The total time was slightly

**Table 6**  
Comparison of supercritical and subcritical in situ transesterification of JCL seed kernel.

| In situ (trans)esterification method                                 | Oil content (%) | SSR (mL/g) | Temp (°C) | Pressure (MPa)    | Time (h)               | Space loading <sup>a</sup> (mL/g) | % yield <sup>c</sup> (Kernel) <sup>d</sup> | Ref.       |
|--|-----------------|------------|-----------|-------------------|------------------------|-----------------------------------|--|------------|
| Supercritical methanol<br>(Process intensification)                  | 66.8            | 5          | 300       | 9.5               | 0.5 (2.0) <sup>b</sup> | 54                                | 99.67 (66.6)                               | [13]       |
| Supercritical methanol<br>Co-solvent (CO <sub>2</sub> 5 MPa)         | 64.6            | 5          | 300       | 20.0              | 0.5 (2.0)              | 30                                | 102.3 (66.1)                               | [12]       |
| Supercritical methanol<br>Co-solvent (CO <sub>2</sub> 4 MPa)         | 66.8            | 5.8        | 295       | n.s. <sup>e</sup> | 0.125 (1.63)           | 30                                | 87.1 (58.2)                                | [14]       |
| Supercritical Methanol<br>Co-solvent (CO <sub>2</sub> 2 MPa)         | 66.8            | 5.9        | 300       | n.s.              | 0.21 (1.71)            | 30                                | 92.0 (61.5)                                | [14]       |
| Sub-critical   | 57.9            | 7          | 250       | 21.0              | 1.0 (1.75)             | 12.0                              | 94.43 (54.72)                              | This Study |
| Methanol-acetic acid (7.5:2.5)<br>Co-solvent (CO <sub>2</sub> 4 MPa) |                 |            |           |                   |                        |                                   |  |            |

<sup>a</sup> Space loading = reactor effective volume in mL/g of dry solid.

<sup>b</sup> Total reaction time including reactor heat up time.

<sup>c</sup> Mass fraction (%) of FAME based on the hexane extractable lipid.

<sup>d</sup> Mass fraction (%) of FAME based on the dry kernel.

<sup>e</sup> n.s.: not specified.

decreased from 2 h to 1.71 h [12–14]. The subcritical solvent (methanol + acetic acid + water) transesterification approach used in this study was able to obtain high FAME yield that is comparable to that obtained by supercritical methanol methods but at a lower temperature (250 °C) and a much lower space loading (12.0 mL/g). The method used in this study can tolerate the presence of FFA and relatively high moisture content (10%) in the kernel.

Similar to other in situ processes a separate extraction step is not required. Typical extraction method utilizing mechanical expeller requires preheating at 80 °C for 20 min and can only recover ~50 to ~80% of the extractable lipids [27–29]. An optimized screw press design was capable of recovering up to 89.3% of the lipid content but required high pressure (9 MPa) and temperature (140 °C) [28]. Solvent extractions are typically employed as a second step to recover lipids from extruded pressed cakes, but still resulted in a lipid loss of ~10% [27,28]. With a better recovery than mechanical expellers and much shorter time than solvent extraction, supercritical carbon dioxide extraction required very high operating pressure (up to 35.0 MPa) and a long extraction time (5 h) [30]. A three phase partitioning method was used by Vyas et al. [31] which required an overall extraction and separation time of 2 h, but involved several intermediate steps and could only achieve a recovery of 85%. This method on the other hand is tolerant to moisture, eliminating the need to dry the kernels. With this method extraction and reaction can be carried out simultaneously with a total time less than that of any extraction methods reported in literature.

#### 4. Conclusion

In this study an in situ (*trans*)esterification process was developed to produce biodiesel from JCL seed kernel with high FAME yield (94–98% based on extractable lipids). By using a mixture of methanol, acetic acid and water under subcritical condition, the process can be operated under less severe conditions (~250 °C) than that of supercritical methanol methods (above 300 °C). It was shown in this study that the presence of acetic acid has greatly improved the extracting power of methanol and increased the rate of reaction to form FAME. This study successfully employed a high reactor loading utilizing up to 70% of the reactors volume without compromising the yield in FAME. Considerations to the overall reactor loading or volume utilization and the corresponding effects of carbon dioxide need to be investigated in future works, as they would have a big impact on the productivity of the process. Lastly, this newly developed process provides an alternative route in in situ biodiesel production that can use feedstock with high moisture (up to 10%) content and FFA (up to 5%).

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