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In-situ Biodiesel and Sugar Production from Rice Bran under Subcritical Condition

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Abstract. An integrated method of producing biodiesel and sugar using subcritical water and methanol has been employed as a potential way to reduce the high cost of single biofuel production from rice bran. The effects of temperature, methanol to water ratio and reaction time on the biodiesel yield and purity, and the concentration of sugar in hydrolysate were investigated systematically. Biodiesel with yield and purity of 65.21% and 73.53%, respectively, was obtained from rice bran with initial free fatty acid (FFA) content of 37.64% under the following conditions: T= 200 °C, P= 4.0 MPa (using CO₂ as pressurizing gas), ratio of rice bran/water/methanol of 1/2/6 (g/mL/mL), and 3 h of reaction time. FFAs level was reduced to 10.00% with crude biodiesel recovery of 88.69%. However, the highest biodiesel yield (67.39%) and crude biodiesel recovery (100.00%) were obtained by decreasing the amount of methanol so that the ratio of rice bran/water/methanol became 1/4/4, g/mL/mL. In addition, the highest sugar concentration of 0.98 g/L was obtained at 180 °C and 4.0 MPa with ratio of rice bran/water/methanol of 1/4/4 (g/mL/mL) and reaction time of 3 h. Since no catalyst was employed and the biodiesel and reducing sugar were produced directly from rice bran with high water and FFA contents, the process was simple and environmentally friendly, which would make the production of biofuel more economical and sustainable.

Keywords: Biodiesel; sugars; rice bran; in situ; hydrolysis; subcritical water; subcritical methanol.

INTRODUCTION

The energy demand will be higher in the future due to increase in the world population. One solution to this problem is to develop a sustainable renewable fuel which can be considered carbon neutral. One of renewable fuel which is produced in industrial scale is fatty acid methyl ester (FAME) or also known as biodiesel. Unfortunately, majority of it is currently produced from edible feedstocks [1] which can drive food price higher. Therefore, many researches have been carried out to investigate the production of biodiesel from alternative feedstocks. Ideally, the feedstock should be available in large quantity, relatively cheap and non-edible. One possible candidate for biodiesel feedstock that meets such criteria is rice bran oil [2].

Various methods to produce biodiesel from rice bran oil (RBO) and crude RBO have been investigated. Zullaikah et al. [1] developed two-step acid-catalyzed methanolysis method to produce FAME from dewaxed/degummed RBO with high FFA content (up to 76%). The first step was carried out at 60 °C mainly to convert FFA to FAME while the second step was carried out at 100 °C to convert the remaining triglycerides (TG) to FAME. Both steps were carried out under atmospheric condition. While Lin et al., [3] developed three-step method to produce FAME from crude RBO. The first two steps were carried out at 50 °C using acid catalyst to reduce FFA content to below 1 mg/g while the third step was carried out at 60 °C using base catalyst to convert TG into FAME. Although the FFA contents in the feedstock and the methods were different, both of them reported that more than 98% of FFA and TG were converted into FAME under optimum conditions.

In-situ production of biodiesel from rice bran has been investigated by several authors. Özgül-Yücel and Türkay [4] mixed 50 g of rice bran with 5 ml of sulfuric acid and 200 ml of methanol in a batch reactor and keep the mixture at 65 °C for 30 minutes. They reported that the concentration of FAME increased with increasing FFA content in the rice bran feedstock. However, no information regarding yield was reported. Gunawan et al. [5] carried out similar experiment but with lower sulfuric acid concentration of 1.5 vol. % and longer reaction time of 60 minutes. They reported that this method can produce FAME with high purity of 97% and 50% recovery rate. While Shiu et al. [6] carried out two-step in-situ biodiesel production from rice bran. The first step was carried out using acid catalyst followed by basic catalyst in the second step. They found that the extraction process of lipid using methanol was a slow process that could take 2 – 3 hours to extract most of lipid in rice bran. They reported that this method can convert up to 84% of total FFA and TG in rice bran into FAME under optimum conditions.

Kasim et al. [7] reported production of biodiesel from crude RBO using supercritical methanol method. The operating condition was 300 °C and 30 MPa with CO₂ as the pressuring gas. However, the result was rather disappointing with overall conversion rate of 51.3%. On the other hand, water-methanol mixture at subcritical condition has been successfully employed to produce biodiesel from various feedstocks with high conversion rate. Ju et al. [8] reported a conversion rate of 95% when subcritical water-methanol (175 °C, 3.5 MPa) was employed to produce biodiesel from soybean oil. Tsigie et al. [9] showed that subcritical water-methanol (175 °C, 35 MPa) can be used to produce biodiesel directly from algae (*Chlorella vulgaris*) and reported the yield was 0.29 g biodiesel/g of dry biomass. While Huynh et al. [10] produced FAME from activated sludge using water-methanol subcritical method (175 °C, 3.5 MPa) and a 90% conversion rate was reported.

In this work, the effectiveness of subcritical water-methanol method to produce FAME directly from rice bran oil was investigated. At subcritical condition, the dielectric constant of water is similar to that of organic solvent and therefore can be used to extract the oil from rice bran. Besides that, the ionic constant of water (k_w) at subcritical condition is higher than that at ambient condition, i.e. 10^{-12} at subcritical condition and 10^{-14} at ambient condition. This will enhance the hydrolysis reaction since the concentrations of H⁺ and OH⁻ are higher. The effects of reaction time, temperature, initial water/methanol volume ratio and pressurizing gas type were studied. Furthermore, besides oil, carbohydrates were also extracted in subcritical water which subsequently was broken down into simple sugars. The effects of various operating parameters mentioned previously on carbohydrates extraction were also studied.

EXPERIMENTAL

Materials

Rice bran was obtained from local rice mill (Lamongan, East Java, Indonesia). It is from IR 64 rice variety. The oil content was determined by Soxhlet extraction using n-hexane as solvent for 8 hours. The RBO content found in this work is in a good agreement with those reported in the literature, i.e. between 10 – 26% depending on rice variety and degree of milling [1, 11]. The FFA (determined by using titration method according to AOCS as detailed below) and water contents in the crude RBO were found to be $37.6 \pm 0.2\%$ and $12.9 \pm 0.1\%$, respectively.

The compressing gasses, CO₂ and N₂, were supplied by Genta Prima Gas (Indonesia). Standard of methyl linoleate was obtained from Sigma Chemicals Company (St. Louis, MO). Analytical grades NaOH, sulfuric acid, PP indicator, and glucose were purchased from Merck (Germany). Methanol, n-hexane and 96% ethanol were purchased from Brataco (Indonesia). In all experiments, distilled water was used.

One step (in-situ) biodiesel production

In this method, oil extraction from rice bran and its conversion to biodiesel were carried out simultaneously. Rice bran was mixed with 40 ml of water-methanol mixture in a hydrothermal reactor made of seamless tubing 3/4" OD x 0.083 WT (SS-T12-S-083-6ME, Swagelok). A thermocouple was inserted into the reactor to monitor the temperature inside the reactor. CO₂ was used to increase the operating pressure to 4 MPa and an electric heater was used to heat up the mixture to a certain temperature (160 – 215 °C). After a certain reaction time (1 – 8 hours), the mixture was cooled down by quenching the reactor into iced water bath. The parameters investigated are summarized in Table 1. The liquid phase containing biodiesel was extracted by using n-hexane five times (50 ml each) and the biodiesel was then recovered by evaporating n-hexane in a rotary evaporator. The biodiesel was then stored for further analysis. The aqueous phase was separated from the solid phase by filtration. The solid phase was

washed with distilled water to ensure that all water soluble components have been extracted completely. The aqueous phase was then analyzed for total sugar content.

Table 1. Parameters investigated in one step (in-situ) biodiesel production.

Compressing gas	Temp. (° C)	Reaction time (h)	Methanol/water ratio (ml/ml)
CO ₂	160	1	5/35
	170	3	10/30
	180	5	20/20
	200	7	30/10
	215	8	35/5

Analysis of FFA content

The FFA was determined using titration method according to the modified AOCS Ca 5a-40 [12]. In general, 0.1 g of oil sample was mixed with 0.107 ml of 96% ethanol in a 25 ml Erlenmeyer flask. The mixture was stirred and heated to 50 °C. Titration was carried out using 0.0044 N NaOH solution and 2 drops PP indicator until the solution turned to pink. The FFA concentration was calculated as follows:

$$\%FFA = [(Volume\ NaOH \times Normality\ of\ NaOH \times 28.2) / sample\ weight] \times 100\% \quad (1)$$

Analysis of FAME yield

The biodiesel (FAME) content in oil phase was determined using gas chromatography (GC) (HP 6890, Hewlett-Packard Inc., Avondale, PA, USA) equipped with a FID detector. Separation was carried out on a HP 1 crosslinked methyl siloxane column (60 m x 0.25 mm i.d. x 1 µm film thickness, Hewlett-Packard Inc., Avondale, PA, USA). The sample (0.02 g) was diluted in n-hexane (1 ml) and then was injected (0.5 µL) to the GC column. The injector and detector temperatures were set initially at 125 °C and were increased at a rate of 15 °C/minutes. After reaching 275 °C, the temperatures were held constant for 10 minutes. The flow rate of carrier gas (helium) was set at 28 ml/minutes at 200 °C. External calibration standard curve was obtained using 0.2 – 20 mg methyl ester linoleic acid. The yield of FAME was determined as the weight ratio of FAME to that of oil in rice bran as follows:

$$FAME\ yield\ (\%) = [\{ sample\ weight\ (g) \times FAME\ content\ (\%) \} / oil\ weight\ in\ rice\ bran\ (g)] \times 100\% \quad (2)$$

Analysis of total sugar

The total sugar was determined using phenol-sulfuric acid method [13]. The sulfuric acid will dehydrate carbohydrate to produce furan derivatives. These compounds will react with phenol to produce color that can be detected at a wavelength of 490 nm.

In this method, 2 ml of sample (aqueous phase) was mixed with 1 ml of 5% aqueous solution of phenol in a test tube. Subsequently, 5 ml of 98% sulfuric acid is added rapidly to the mixture. After allowing the test tube to stand for 10 min., they are shaken using vortex mixture for 30 second and then placed in a water bath at room temperature for 20 minutes. The absorbance was measured using spectrophotometer (UV-VIS Genesys 10S, Thermo Scientific) at a wavelength of 490 nm. The total concentration of reduced sugar was calculated using calibration curved based on glucose solution.

RESULTS AND DISCUSSION

Rice bran used in this study has quite high FFA content ($37.6 \pm 0.2\%$). In a conventional method, base-catalyzed transesterification, high FFA and water contents in feedstock are able to impede the reaction due to the formation of soap and emulsion [8]. Therefore, acid-catalyzed esterification and transesterification should be the pathway to convert neutral lipids to biodiesel when using low quality feedstocks. In general, esterification reaction carried out under acidic condition. In this study, subcritical water and methanol with CO₂ atmosphere were employed as a new green method to synthesis biodiesel from low quality rice bran.

Effect of temperature

Temperature plays important roles during extraction, reaction and hydrolysis. The oil recovery and FAME yield increases with temperature as shown in table 2. The experiments were carried out at 4 MPa with methanol/water ratio of 20/20 ml/ml and reaction time of 3 h.

The dielectric constant of both water and methanol decreases with rising temperature [8, 14], so that oil becomes more soluble in water-methanol mixture leading to higher oil recovery. Besides that, the ionization constant of water increases with increasing temperature, i.e. from 10^{-14} at room temperature to 10^{-12} at 200 °C [8]. Therefore, the concentrations of both H⁺ and OH⁻ increase at high temperature. Since transesterification, hydrolysis and esterification reactions are catalyzed by acid, the reactions rates are higher at elevated temperature.

The use of CO₂ as pressurizing gas also increases FAME yield. The FAME yield obtained in this work under CO₂ atmosphere is higher than that reported by Ju et al. [8] under N₂ atmosphere. This is probably due to CO₂ role as co-solvent during reaction [15]. Therefore, FAME content also increases as shown in table 3.

Increasing the temperature up to 180 °C, increases sugar content as shown in table 4. Higher temperature increases the hydrolysis rates of cellulose and hemicellulose. The highest sugar content of 0.98 g/L is obtained at 180 °C. However, increasing the temperature further may degrade sugar into 5-HMF and furfural [9], reducing sugar content. The presence of H⁺ may accelerate this reaction.

Table 2 Effect of temperature on oil recovery and FAME yield

Temp. (°C)	Oil Recovery (%)	FAME Yield (%)
160	77.47 ± 4.35	36.75 ± 2.07
170	80.79 ± 4.93	41.42 ± 2.53
180	95.84 ± 1.23	51.23 ± 0.66
200	102.02 ± 1.40	67.39 ± 0.93
215	97.24 ± 0.35	58.03 ± 0.21

Table 3 Effect of temperature on oil composition

Temp. (°C)	Oil Composition (%)		
	FAME	FFA	Others ^a
160	47.44	13.51	39.05
170	51.28	12.56	36.16
180	53.46	11.49	35.05
200	66.06	10.23	23.71
215	59.68	7.77	32.55

Table 4 Effect of temperature on sugar production

Temp. (°C)	Sugars (g/L)
160	0.70 ± 0.01
170	0.85 ± 0.02
180	0.98 ± 0.00
200	0.72 ± 0.00
215	0.38 ± 0.09

Effect of reaction time

The effect of reaction time on oil recovery and FAME yield is shown in table 5. The experiments were carried out at 200 °C and 4 MPa with 20/20 ml/ml methanol water ratio. The experimental results show that the optimum reaction time is 3 h where 100% oil recovery and 67.39% FAME yield can be achieved. This result shows that oil extraction rate in subcritical water methanol mixture is faster than that using Soxhlet extraction which takes 7 h to extract oil in rice bran completely [16].

As shown in Table 6, FAME content at 7 h is only 5% higher than that at 3 h while the FFA content at 8 h is less than 1% lower than that at 3 h. This suggests that the reactions may close to equilibrium after 3 h. Therefore, longer reaction time will not increase FAME yield. Instead, longer reaction time decreases FAME yield (Table 5) due to denaturation of polyunsaturated fatty acid [17]. The appearance of crude biodiesel obtained after 8 h of reaction time is dark brown and is very different from that obtained after 3 h of reaction time.

The FFA content decreases significantly from 37.6% (FFA content in rice bran oil) to 15.98% within 1 h (Table 6). Gunawan et al. [5] also reported that esterification of FFA under acidic condition occurred quickly within the first 30 minutes of reaction. However, the presence of water prevents complete conversion of FFA into FAME since esterification reaction is in equilibrium with hydrolysis reaction that converts FAME into FFA and methanol.

Table 5 Effect of reaction time on oil recovery and FAME yield

Time (h)	Oil Recovery (%)	FAME Yield (%)
1	55.72 ± 3.07	30.04 ± 1.65
3	102.02 ± 1.40	67.39 ± 0.93
5	67.35 ± 1.81	46.24 ± 1.24
7	60.23 ± 2.07	42.74 ± 1.47
8	58.55 ± 1.58	33.96 ± 0.92

Table 6 Effect of reaction time on oil composition

Time (h)	Oil Composition (%)		
	FAME	FFA	Others ^a
1	53.90	15.98	30.12
3	66.06	10.23	23.72
5	68.65	9.55	21.81
7	70.97	9.49	19.54
8	58.00	9.43	32.57

Table 7 Effect of reaction time on sugar production

Time (h)	Sugars (g/L)
1	0.87 ± 0.01
3	0.72 ± 0.00
5	0.68 ± 0.01
7	0.67 ± 0.00
8	0.63 ± 0.01

The sugar content shows a decreasing trend with reaction time (Table 7). As mentioned previously that sugar degrades into 5-HMF and furfural at high temperature and pressure [16] and therefore, sugar content decreases with reaction time.

Effect of methanol/water ratio

The effect of methanol/water ratio on oil recovery and FAME yield is shown in table 8 while that on FAME and FFA contents is shown in Table 9. The experiments were carried out at 200 °C and 4 MPa for 3 hours. The experimental data show that oil recovery and FAME yield increase with increasing methanol/water ratio up to 20/20 ml/ml. Since the dielectric constant of methanol is lower than that of water, oil is more soluble in methanol than that in water although at room temperature oil is practically insoluble in both methanol and water. Therefore, the extraction rate is faster when the methanol/water ratio is high. The increase in oil recovery will subsequently increase FAME yield since there is more oil to be converted into FAME.

FAME content shows increasing trend with increasing methanol/water ratio although the experimental data show that the maximum FAME content is achieved at 30/10 methanol/water ratio (Table 10). On the other hand, FFA contents shows decreasing trend with increasing methanol/water ratio. Both transesterification of triglyceride and FFA are reversible and excess methanol favors the formation of FAME while the presence of water causes hydrolysis of FAME into FFA and methanol. Ju et al. [8] also reported that the conversion rate of rapeseed oil into FAME also increased with methanol concentration.

Table 8 Effect of methanol/water ratio on oil recovery and FAME yield

Methanol/water ratio (mL/mL)	Oil Recovery (%)	FAME Yield (%)
5/35	63.44 ± 2.82	36.46 ± 1.62
10/30	74.22 ± 2.68	48.72 ± 1.76
20/20	102.02 ± 1.40	67.39 ± 0.93
30/10	88.69 ± 1.57	65.21 ± 1.15
35/5	89.45 ± 1.61	61.01 ± 1.10

Table 9 Effect of methanol/water ratio on oil composition

Methanol/water ratio (mL/mL)	Oil Composition (%)		
	FAME	FFA	Others ^a
5/35	57.46	14.55	27.99
10/30	65.65	13.07	21.28
20/20	66.06	10.23	23.71
30/10	73.53	10.01	16.46
35/5	68.20	8.62	23.18

Table 10 Effect of methanol/water ratio on sugar production

Methanol/water ratio (mL/mL)	Sugars (g/L)
5/35	0.80 ± 0.10
10/30	0.72 ± 0.01
20/20	0.72 ± 0.00
30/10	0.55 ± 0.02
35/5	0.46 ± 0.03

On the contrary, total sugar content decreases with increasing methanol/water ratio as shown in table 10. Water is required to hydrolyze cellulose and hemicellulose into simpler compounds, such as glucose, xylose and some phenolic compounds. Water also provides H⁺ as it dissociates. H⁺ is required as the catalyst during the hydrolysis of cellulose and hemicellulose. Therefore, the sugar content is high when water concentration is high, i.e. low methanol/water ratio.

CONCLUSIONS

Catalyst-free in-situ biodiesel production from low quality rice bran using subcritical water and methanol has been studied. Employing CO₂ as pressurizing gas increased the esterification rate due to CO₂ ability to acidify the medium. By employing CO₂ as a pressurizing gas, high yield and high purity of biodiesel can be achieved in a shorter reaction time (3 h) and at lower temperature (T= 200 °C).

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