

Esterification of oil adsorbed on palm decanter cake into methyl ester using sulfonated rice husk ash as heterogeneous acid catalyst

Noor Hindryawati, Erwin, and Gaanty Pragas Maniam

Citation: [AIP Conference Proceedings](#) **1813**, 030002 (2017); doi: 10.1063/1.4975965

View online: <http://dx.doi.org/10.1063/1.4975965>

View Table of Contents: <http://aip.scitation.org/toc/apc/1813/1>

Published by the [American Institute of Physics](#)

Articles you may be interested in

[HLA-DQBI*0402 alleles polymorphisms detected in Javanese HIV patients with positive anti-Toxoplasma gondii IgM](#)

[AIP Conference Proceedings](#) **1813**, 020005020005 (2017); 10.1063/1.4975943

[Sequence conservation, HLA-E-Restricted peptide, and best-defined CTL/CD8+ epitopes in gag P24 \(capsid\) of HIV-1 subtype B](#)

[AIP Conference Proceedings](#) **1813**, 020020020020 (2017); 10.1063/1.4975958

Esterification of Oil Adsorbed on Palm Decanter Cake into Methyl Ester using Sulfonated Rice Husk Ash as Heterogeneous Acid Catalyst

Noor Hindryawati^{1*}, Erwin², Gaanty Pragas Maniam³

¹*Faculty of Mathematic and Natural Sciences, Mulawarman University
Gunung Kelua, 75113 Samarinda, East Kalimantan, Indonesia*

²*Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang
Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang, Malaysia*

³*Central Laboratory, Universiti Malaysia Pahang
Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang, Malaysia*

^{*}Corresponding author: ienwati@yahoo.com

Abstract. Palm Decanter cake (PDC) which is categorized as the waste from palm oil mill has been found to contain residual crude palm oil. The oil adsorbed on the PDC (PDC-oil) can be extracted and potentially used as feedstock for biodiesel production. Feedstock from waste like PDC-oil is burdened with high free fatty acids (FFAs) which make the feedstock difficult to be converted into biodiesel using basic catalyst. Therefore, in this study, a solid acid, RHA-SO₃H catalyst was synthesized by sulfonating rice husk ash (RHA) with concentrated sulfuric acid. The RHA-SO₃H prepared was characterized with TGA, FTIR, BET, XRD, FE-SEM, and Hammett indicators (methyl red, bromophenol blue, and crystal violet). PDC was found to have about 11.3 wt. % oil recovered after 1 hour extraction using ultrasound method. The presence of sulfonate group was observed in IR spectrum, and the surface area of RHA-SO₃H was reduced to 37 m².g⁻¹ after impregnation of sulfonate group. The RHA-SO₃H catalyst showed that it can work for both esterification of free fatty acid which is present in PDC-oil, and transesterification of triglycerides into methyl ester. The results showed highest methyl ester content of 70.2 wt.% at optimal conditions, which was 6 wt.% catalyst amount, methanol to oil molar ratio of 17:1 for 5 hours at 120 °C.

INTRODUCTION

Biodiesel is a potential alternative source to replace petroleum fuels as it provides high product yields, flexible with variety of feedstock, low waste production and less hazardous chemical used in production. However, the needs for large quantity fat oils from vegetable sources as feedstock for biodiesel has causes some problems, especially the deforestation for plantation purpose in some countries. Besides, the major cost associated in the biodiesel production is the feedstock, which it comprises about 80 % of the operating cost [1] Therefore, to overcome the economic barrier and environmental problems, studies attempt to find new feedstock by conversion from waste or new vegetable species.

In Indonesia and Malaysia, palm oil is the common oil used for production of biodiesel as it is beneficially due to locally available and accessible. This is because Indonesia and Malaysia is one of the major producers and exporters of palm oil. Furthermore, non-edible oil such as used frying oil (UFO) and waste oil from palm oil industry can be used for production of biodiesel. Conversion of waste oil into valuable product has add value to the sustainability of biodiesel as the production cause less or no harm to the environment and thus able to replace petroleum fuel and continue for a long time.

Palm decanter cake (PDC) is the waste from the decanting process in palm oil mill where water is used as the solvent for decanting. This process leads to the high moisture content in the oil adsorbed on DC. Due to its high free

fatty acids (FFAs) content, the process involves the esterification of FFAs and transesterification of triglycerides, where acid catalyst is in preference to alkali catalyst. In this study, sulfonated rice husk ash (RHA-SO₃H) will be synthesized and used as strong acid catalyst. The effect of reaction parameters (catalyst amount, methanol to oil molar ratio, and reaction time) on methyl ester content will be studied.

EXPERIMENTAL METHODS

Materials

Fresh palm decanter cake (PDC) was collected from Local oil palm mill (Perkebunan Sawit Nusantara, Tanah Grogot), while Rice Husk (RH) is collected from a rice mill in Lempake. Analytical grade methanol, *n*-hexane, petroleum ether (PE), chloroform (CHCl₃), sulphuric acid (95-97 %), ethanol, and Thin Layer Chromatography (TLC) (20 x 20 cm, silica gel 60 F254) of HPTLC grade were purchased from MERCK (Germany). Phenolphthalein (H_c=8.2), crystal violet (H_c=1.8), bromophenol blue (H_c=4.6), methyl red (H_c=6.2) are purchased from Bendosen. Sodium hydroxide (NaOH), and, potassium hydroxide (KOH), and methyl heptadecanoate as an internal standard GC grades (> 99.1 %) were obtained from Sigma-Aldrich (Switzerland), whereas iso-propanol, glacial acetic acid, potassium iodide, starch, sodium thiosulphate (Na₂S₂O₃), and benzene were from Merck KGaA (Darmstadt, Germany).

Extraction of PDC-Oil via Ultrasound Extraction

The fresh PDC is dried overnight in oven at 110 °C and then grinded into fine particle size. The oil adsorbed on DC is extracted via ultrasound with *n*-hexane (non-polar solvent) according to the method applied in study of Maniam et al. [2]. For optimization of oil extraction, 10 g of DC is extracted with 10 g hexane via ultrasound at temperature of 65 °C for 15 min, 30 min, 45 min, and 60 min respectively. The sonicated mixture is then centrifuge at 7000 rpm for 15 min to obtain clear supernatant layer. The supernatant solution is transferred to pre-weight rotary flask and solvent is recovered via rotary evaporation. The oil content is calculated by dividing mass of oil extracted (M_{oil}) with mass of DC (M_{DC}) in g as expressed in Equation 1

$$\text{Oil wt.\%} = M_{\text{oil}}/M_{\text{DC}} \times 100 \quad (1)$$

Solid Acid Catalyst Preparation

The rice husk (RH) collected is cleaned and then heated in furnace at temperature of 900 °C for 4 hours. The rice husk ash (RHA) produced is washed with deionized water, filtered and dried at 100 °C for 2 hours. The RHA is grinded into fine powder and is treated in furnace at temperature 900 °C for 4 hours. After calcination, 20 g of RHA is weighed and washed with 0.1 N HCl for 3 hours at 60 °C under reflux. The RHA is then neutralized to pH 7 by washing repeatedly with deionized water. The neutralized RHA is dried in oven overnight at 110 °C. After that, sulfonation of RHA by concentrated sulphuric acid is carried out in oil bath at controlled temperature of 90 °C for 3 hours [3]. The mixture is left to cool at room temperature. The mixture is added to distilled water (it is dangerous to add water to mixture containing concentrated sulphuric acid), stirred the mixture and filtered out the precipitate formed. Hot distilled water is used to wash off the sulfate ions. The washed RHA-SO₃H is filtered again and dried overnight in oven at 80 °C.

Catalyst Characterization

The RHA-SO₃H was identified by X-ray diffraction (Rigaku) with Cu Ka X-ray as a source. A FTIR (PerkinElmer Spectrum 100) spectrophotometer was used to characterize the chemical structure of RHA-SO₃H at 400 – 4000 cm⁻¹ range. Surface analysis of the catalyst was examined by using Micromeritics ASAP 2000. Prior to the analysis, the catalyst was degassed at 105 °C and the adsorption of N₂ was measured at -196 °C. The size and morphology of catalyst was observed by FE-SEM (JSM-7800F). The acid strengths of the catalyst (H_c) were determined by using Hammett indicators. The following Hammett indicators were used: crystal violet (H_c= 1.8), bromophenol blue (H_c= 4.6), and methyl red (H_c= 6.0). About 25 mg of catalyst was shaken with 5.0 mL of a

solution of Hammett indicator diluted with methanol, and left to equilibrate for 2 h [4] After the equilibration, the colour change of the solution was noted. The acid value of the oil was determined following the standard EN 14104; and the moisture content was analyzed using Karl Fischer titration method (784 KFP Titrino, Metrohm).

Esterification Reaction

The esterification reaction was carried out in 100 mL round bottom flask contained mixture of 2 g DC-oil, 6 wt% catalyst (0.12, based on weight of oil), methanol: oil molar ratio of 17:1, and magnetic stirrer for mechanical stirring. It is connected with condenser for reflux, equipped with thermometer and heating in an oil bath at 120 °C on a hotplate. Upon completion, the solution is centrifuged to separate methyl ester produced from solution. The esterification reaction is optimized to yield highest methyl ester content (optimal conditions) by repeating the reactions with increments of methanol to oil molar ratio, reactions time, and/or catalyst amount. After the reactions, the mixture formed is centrifuged and methyl ester (ME) formed is collected for qualitative analysis via Thin Layer Chromatography (TLC) and Gas Chromatography – Mass spectroscopy (GC-MS). Gas Chromatography – Flame Ionization Detector (GC-FID) is used to determine the methyl ester content. The GC analysis used capillary column DB-wax (length 30 m, internal diameter of 0.25 mm, and film thickness of 0.25 µm) and heptane as mobile phase. ME content was quantified using the following formula:

$$\text{Methyl ester content (wt. \%)} = \frac{A_{\text{total}} - A_{\text{ISTD}}}{A_{\text{ISTD}}} \times \frac{C_{\text{ISTD}} \times V_{\text{ISTD}}}{W_{\text{sample}}} \times 100\% \quad (2)$$

where A_{total} is the total peak area of methyl esters; A_{ISTD} is the area of methyl heptadecanoate; V_{ISTD} is the volume of methyl heptadecanoate; C_{ISTD} is the concentration of methyl heptadecanoate; W_{sample} is the weight of sample.

In order to examine the potential reutilization of the catalyst, the used catalyst was tested to assess its catalytic activity. For reuse experiments, the used solid catalyst recovered by decanting it after a simple washing using methanol and n-hexane. The dried used catalyst was directly used as a catalyst for the repeated reactions.

RESULTS AND DISCUSSION

Characterization of Sulfonated Rice Husk Ash

Thermogravimetry Analysis

Thermogravimetry analysis is applied to RHA and RHA-SO₃H separately to study their thermal stability [FIGURE !]. From TGA curve of RHA, the steady weight loss of 1.2 % occurred from room temperature to 200 °C might associate with evaporation of adsorbed water absorbed. The steady weight loss after 200 °C is due to the loss of –OH terminal group such as silanol. As for RHA-SO₃H, about 6 % weight loss is observed from room temperature to 130 °C which most probably due to evaporation of water absorbed physically on RHA-SO₃H. The second weight loss of 20 % is observed at range of 130 to 300 °C that might due to decomposition of –SO₃H to SO₂ which was in agreement with Shirini et al. [5]. Finally, started from 300 °C to 900 °C, weight losses of 14 % might associate with decomposition of the remaining organic groups and the dehydration of the silanol groups as in agreement with Noushad [6]

Fourier Transform Infrared Analysis

FTIR analysis is one of the important analyses to confirm formation of RHA-SO₃H catalyst. As shown in FIGURE 2, in RHA spectrum, the broad peak around 3546.71 cm⁻¹ and 1639.56 cm⁻¹ are observed in the functional group region. According to [5], the broad peak at 3546.71 cm⁻¹ corresponded to the -OH stretching of SiOH and symmetry stretching of H₂O, whereas the peak at 1639.56 cm⁻¹ is due to the bending of H₂O adsorbed, respectively. In the fingerprint region, the peaks at 1098.11 cm⁻¹ and 798.58 cm⁻¹ corresponded to asymmetric and symmetric stretching of SiO₂ respectively, whereas the peak at 468.88 cm⁻¹ is corresponded to the bending of SiO₂. In the

spectrum of RHA-SO₃H, the broad peak at 3427.63 cm⁻¹ is due to the -OH stretching of -SO₃H functional group. Besides, the two not fully resolved peaks at 1173.95 cm⁻¹ and 1069.73 cm⁻¹ are attributed to stretching of Si-O and S=O respectively. The adsorption peaks at 885.64 cm⁻¹ and 578.26 cm⁻¹ are attributed to stretching of S-O in -SO₃H group.

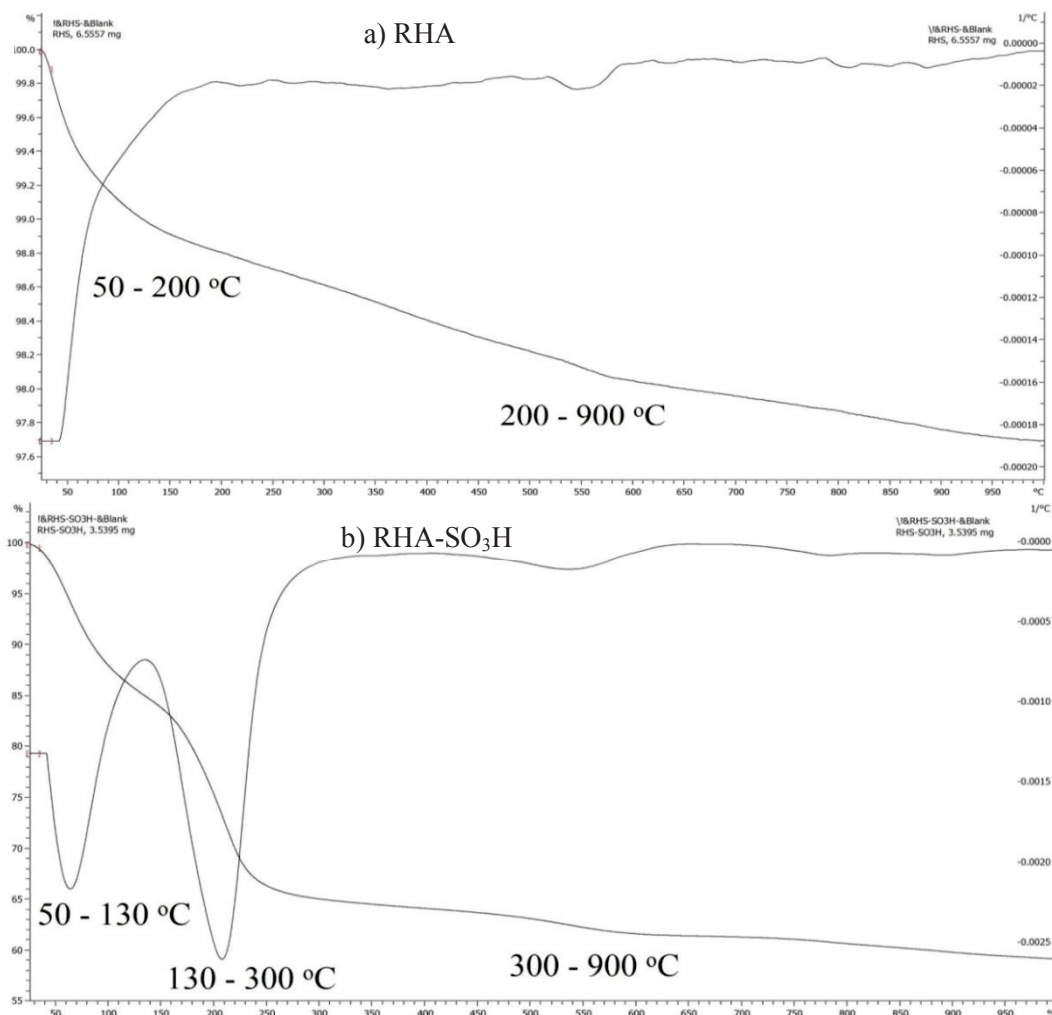


Figure 1: TGA curves for RHA and RHA-SO₃H

Surface Analysis (BET)

From the BET analysis, the surface area of RHA and RHA-SO₃H are 76 m².g⁻¹ and 37 m².g⁻¹ respectively, indicated that the surface area decreased due to the sulfonation of RHA. Correspondingly, the pore volume and pore size are larger for RHA-SO₃H compared to RHA. The pore volume of RHA-SO₃H increased from 0.166584 cm³.g⁻¹ to 0.104097 cm³.g⁻¹ whereas the pore size increased from 84.7618 Å to 107.7659 Å with respect to RHA. This might suggest that the -SO₃H is well supported on the RHA surface [TABLE 1].

TABLE 1. Pore structure parameters of RHA and RHA-SO₃H by BET analysis

	Surface area (m ² .g ⁻¹)	Pore volume (cm ³ .g ⁻¹)	Pore size (Å)
RHA	76	0.166584	84.7618
RHA-SO ₃ H	37	0.104097	107.7659

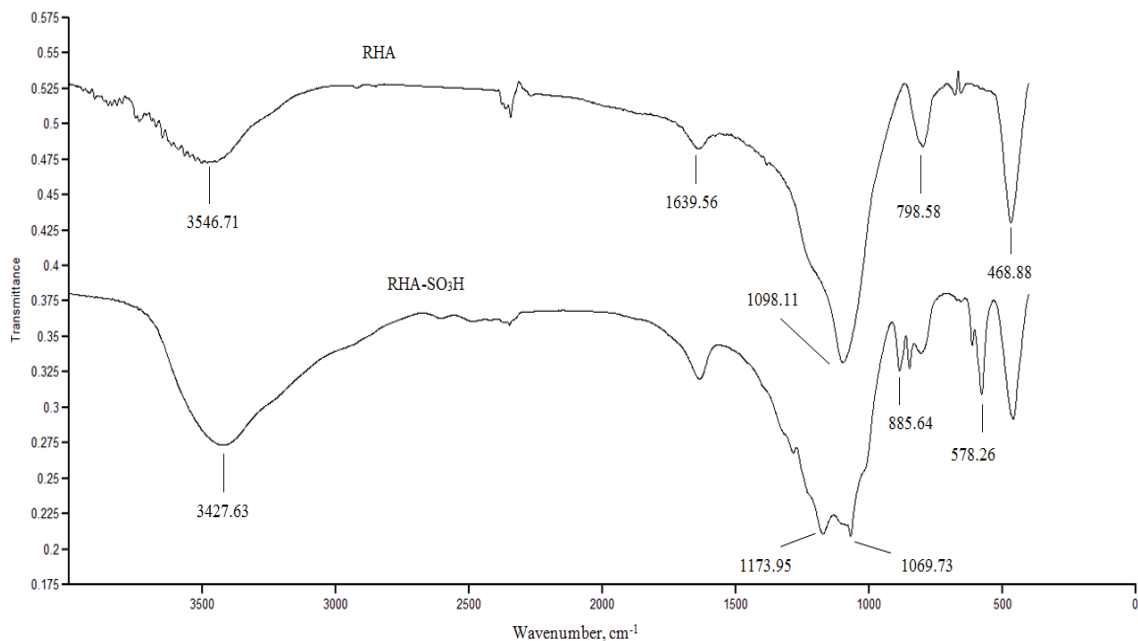


FIGURE 2: FTIR spectra of RHA and RHA-SO₃H

From the N₂ adsorption-desorption isotherm showed in **FIGURE 3**, both RHA and RHA-SO₃H are classified as type IV isotherms, where their pores should be between 1.5 nm to 100 nm [7]. Besides, the slope of type IV isotherm always performs increased adsorption at higher pressure as the pores are filled. Both the hysteresis loop of RHA and RHA-SO₃H appears almost the similar range which is at P/P₀ of 0.4 to 1.0 and 0.45 to 1.0 respectively. It is found that the hysteresis loops are open-ended for both samples. This might due to the incomplete N₂ desorption where the micropores of samples still retaining some of the adsorbed nitrogen [8].

X-ray Diffraction Analysis

FIGURE 4 showed the comparison between the diffractogram of RHA and RHA-SO₃H. A broad diffraction is observed in both diffractogram of RHA and RHA-SO₃H at 2 θ angle of 21.48° and 21.91° respectively. From the XRD analysis, the silica present in the RHA and RHA-SO₃H are similar, which is amorphous silica. The slightly different of RHA-SO₃H peak intensities in comparison to that of RHA indicated an ordered mesoporosity after sulfonation of RHA.

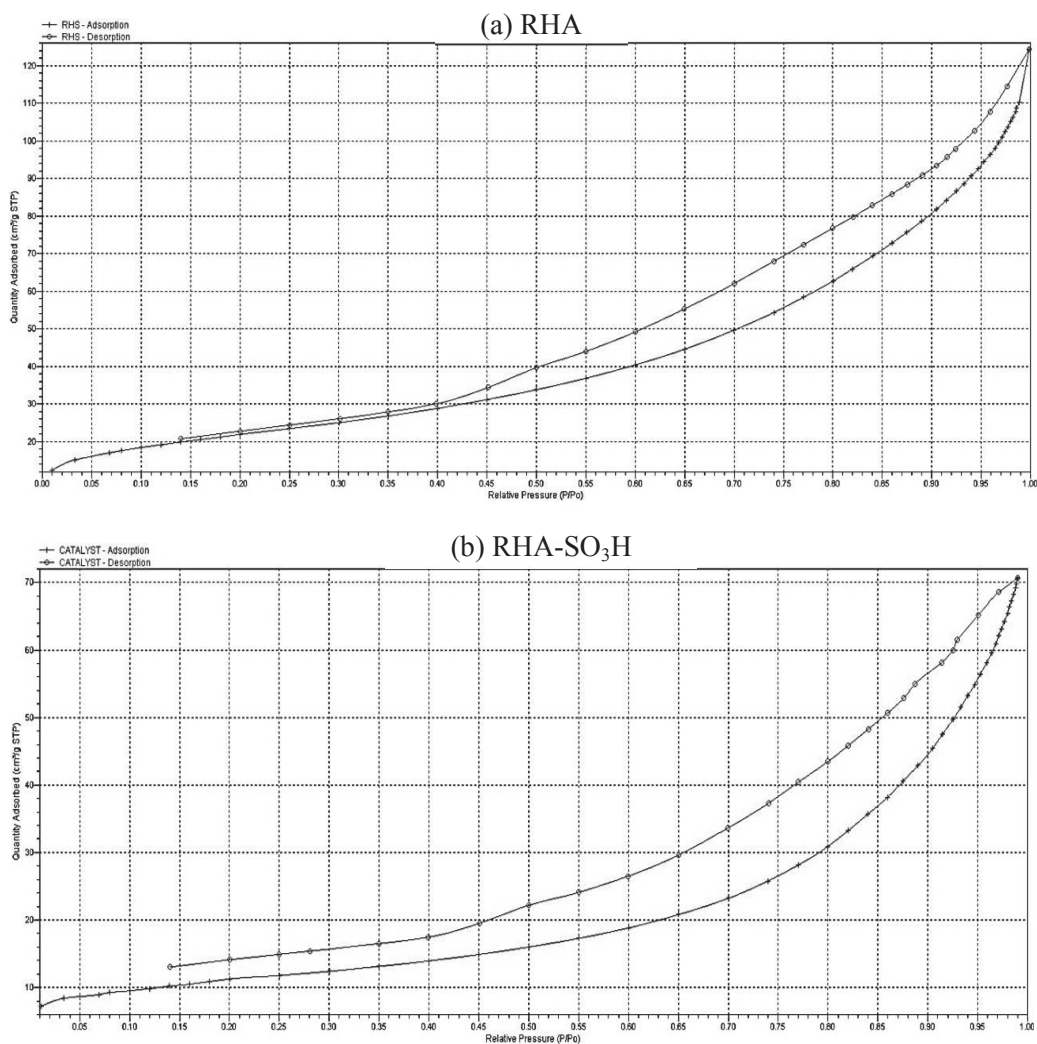


FIGURE 3. N_2 adsorption-desorption isotherms of RHA and RHA-SO₃H

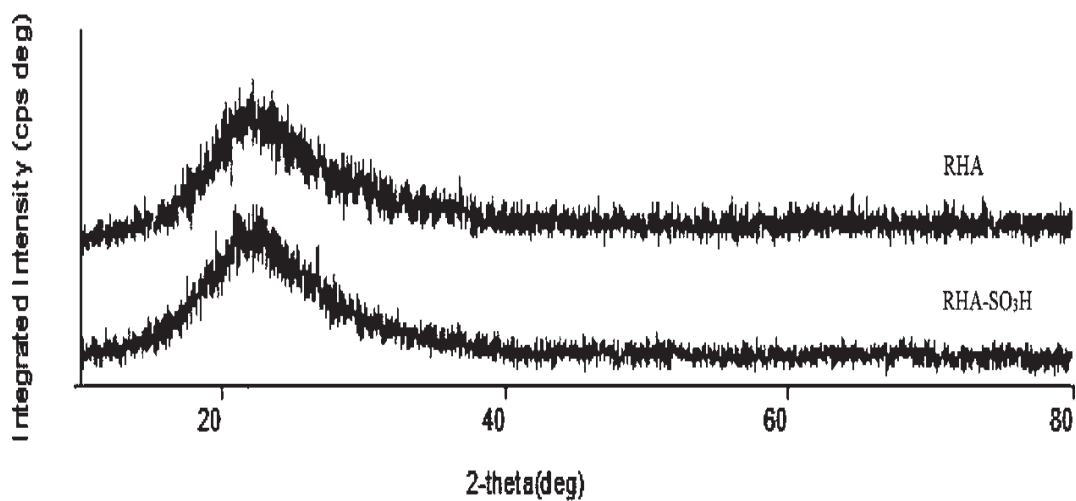
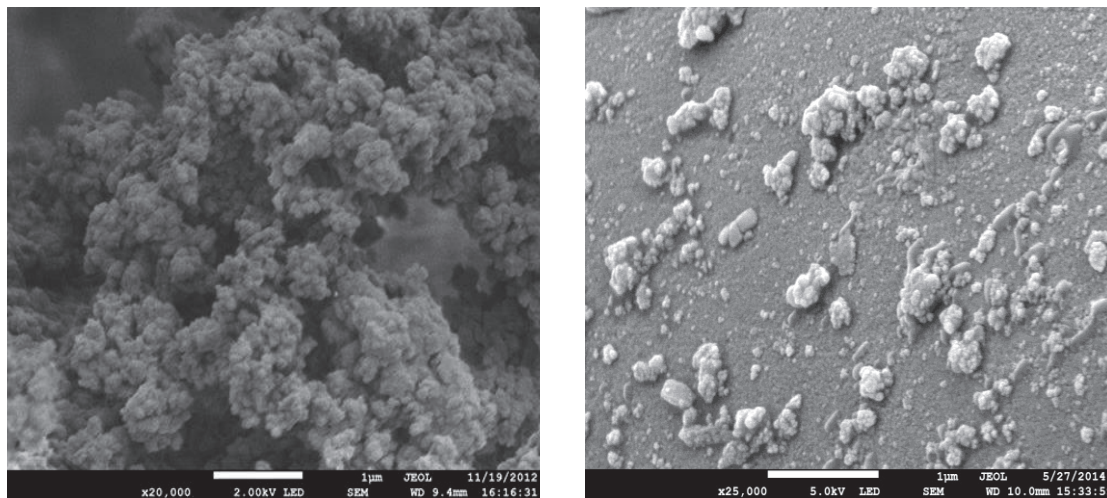


FIGURE 4. XRD diffractogram of RHA and RHA-SO₃H

Field Emission Scanning Electron Microscopy

The FE-SEM image of RHA showed that RHA has porous and irregular shape where this indicated its high specific surface area. FE-SEM image of RHA-SO₃H showed aggregate particles with grain size of micrometer scale as shown in **FIGURE 5** (b), which was similar to the previous studies [5, 8, 9]..



a) RHA at magnification of 20000

b) RHA-SO₃H at magnification of 25000

FIGURE 5. FE-SEM image of RHA and RHA-SO₃H

Hammett Indicator

As shown in **TABLE 2**, the acidity of RHA-SO₃H solid acid catalyst tested by methyl red ($H_{\text{c}} = 6.2$), bromophenol blue ($H_{\text{c}} = 4.6$), and crystal violet ($H_{\text{c}} = 1.8$) showed colour change of red, yellow, and purple, respectively. This indicated that the RHA-SO₃H is a solid acid catalyst with pH range around 0 to 1.8 which is highly acidic.

TABLE 2. Acid strength determination of RHA-SO₃H

Indicators	pH range	Acidic form (<pH)	Colour change
Methyl red	4.4 – 6.2	Red (<4.4)	Red
	Red – Yellow		
Bromophenol blue	3.0 – 4.6	Yellow (<3.0)	Yellow
	Yellow – Purple		
Crystal violet	0.0 – 1.8	Yellow (<0.0)	Purple
	Yellow – Purple	Purple (<1.8)	

Characterization of Methyl Ester

As shown in **FIGURE 6**, the ME-DC produced contained majority of methyl palmitate (C_{16:0}) and methyl oleate (C_{18:1}), while methyl heptadecanoate (C_{17:0}) was internal standard.

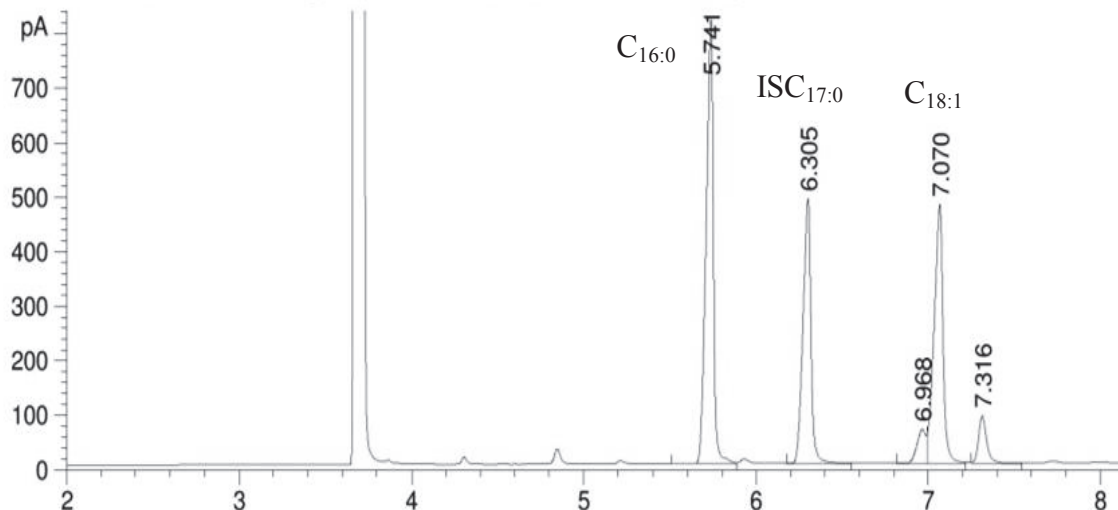


FIGURE 6. Gas Chromatogram of methyl ester from DC-oil

Effect of Catalyst Amount

Esterification is a slow process and required longer reaction time and higher reaction temperature compare to that of transesterification. However, RHA-SO₃H could speed up the reaction. Catalyst amount can significantly affect the ME yield during esterification reaction, thus it is important to optimize the catalyst loading to optimize the ME yield. The catalyst amount is varied from 2 wt% to 12 wt% (based on DC-oil weight). As shown in FIGURE 7, the ME yield increased from 18.1 to 70.2 wt.% with increasing catalyst amount from 2 to 6 wt.%. However, further increased of catalyst amount decreased the ME yield, thus the optimized ME yield is achieved at 6 wt.% catalyst loading. In esterification reaction, the reactants (methanol and oil) encountered mass transfer resistance due to their immiscible properties, thus mechanical stirring is applied to overcome the problem. But at excess catalyst amount, it might disrupted the mixing of reactants and thus lead to poor mixing and higher mass transfer resistance [10]. Besides, at excess catalyst amount, it was observed that some of the catalyst being sidelined from mixture and remain unused throughout the reaction. Furthermore, according to Farooq, et al.[11], some of the ME produced might not desorbed from the surface of catalyst and leading to low ME yield.

Effect of Methanol to DC-oil Molar Ratio

Basically in esterification reaction, excess of methanol to oil molar ratio is necessary to convert free fatty acid (FFA) into methyl ester. The effect of increasing methanol to oil molar ratio was observed by varying from 9:1 to 21:1 molar ratio in FIGURE 8. The highest ME content was observed at 17:1 molar ratio with 70.2 wt.%. The further increased in molar ratio might cause the solution to be too dilute and slowed the rate of reaction, and thus the ME yield is slightly lower [2]

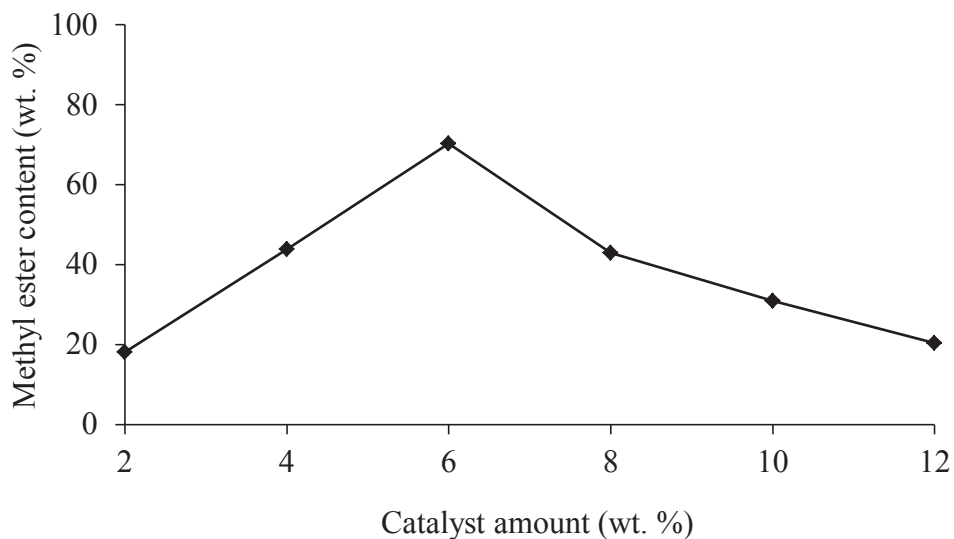


FIGURE 7. Effect of catalyst amount on methyl ester yield at 120 °C, methanol to oil molar ratio of 17:1 for 5 hours

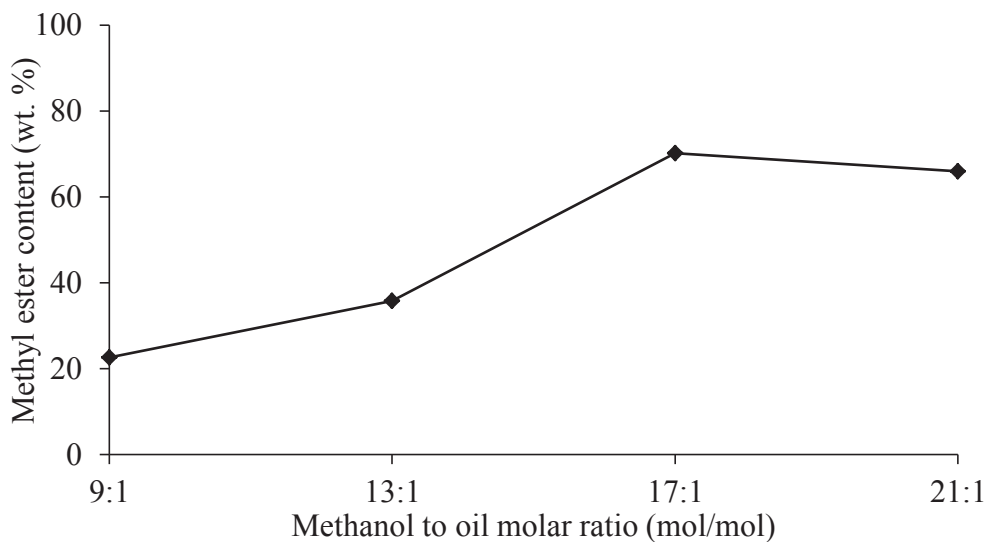


FIGURE 8. Effect of methanol to DC-oil molar ratio (reaction conditions: 6 wt.% catalyst at 120 °C for 5 h)

Effect of Reaction Time

Reaction time is one of the important factors that affect ME yield since the esterification reaction is reversible. Normally, the ME content should remain constant after reached equilibrium where rate of forward reaction equal to that of reverse reaction. As shown in **FIGURE 9**, the ME content initially increased from 47.6 to 70.2 wt.% at 3 to 5 h respectively. However, the ME content decreased to 58.9 and 53.1 wt.% when the reaction time was further to 7 and 9 h. This might due to the decrease in catalytic activity of forward reaction caused by the adsorption of water produced during reactions. According to Li et al. [12], RHA-SO₃H was hydrophilic due to the polar -SO₃H functional group and thus favor the adsorption of water. Besides, as reported in their study on RHA-SO₃H in production of biodiesel using waste cooking oil (WCO), the reactions were carried out with instantaneous removal of water produced, and achieved ME yield of 87.57 wt.% after 15 h with 20:1 methanol to WCO molar ratio at 110 °C.

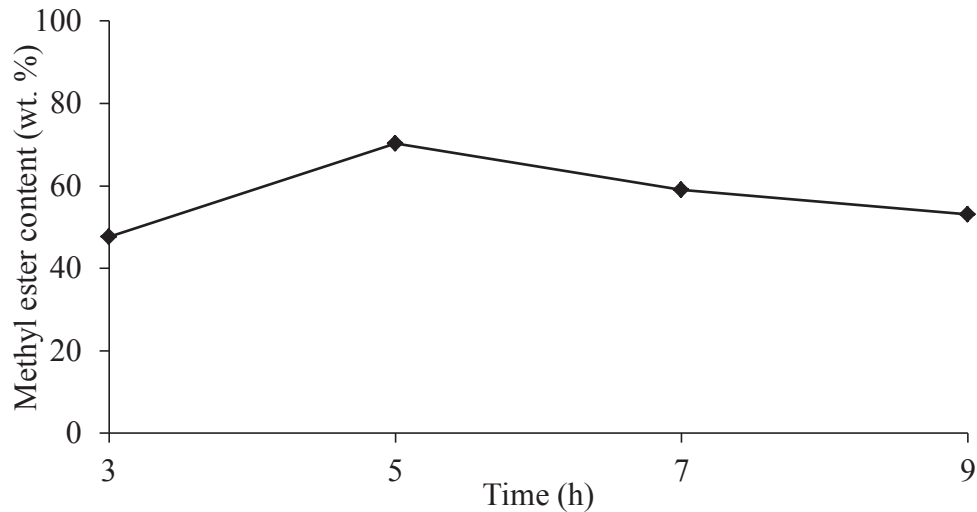


FIGURE 9: Effect of reaction time at 120 °C, methanol to oil molar ratio of 17:1, and 6 wt.% catalyst loading.

Reusability of Catalyst

Reusability of catalyst is important to reduce the production cost of biodiesel. The catalyst was washed and used without any further activation, and it was found not reusable as shown in **FIGURE 10**, where the ME content was decreasing after 4 cycles. It was contrasting with the reusability reported by Li et al. [13] where the catalyst should be able to reuse up to at least 5 cycles. Probably the two samples are chemically different. It might also due to the poor stability of catalyst attributed from method preparation of catalyst and caused the leaching of $-SO_3H$ groups.

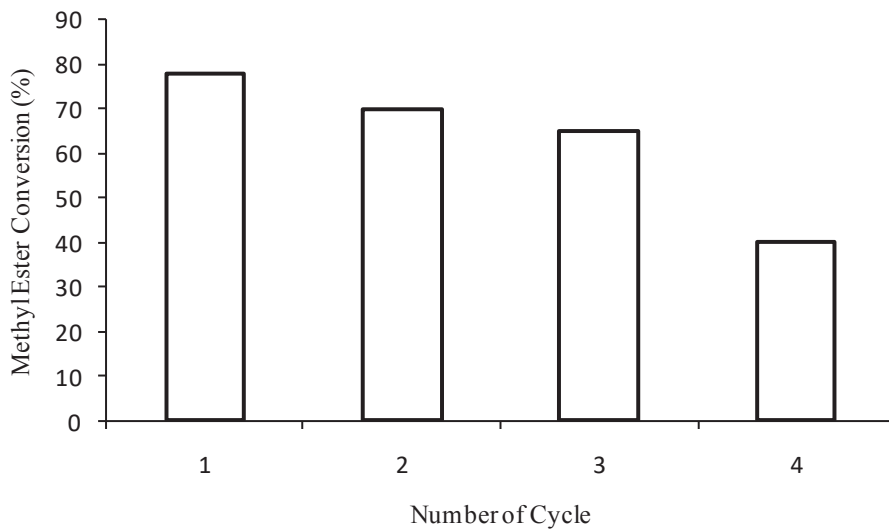


FIGURE 10. Reusability of catalyst (reaction conditions: 6 wt.% catalyst, 17:1 methanol to oil molar ratio, 5 h at 120 °C)

CONCLUSIONS

The solid acid catalyst (RHA-SO₃H) was characterized, where the presence of sulfonate group was observed in FTIR and it has surface area of 37 m² g⁻¹. The RHA-SO₃H was thermally stable below 130 °C and having acid strength between pH 0 – 1.8. On the other hand, the RHA-SO₃H prepared from sulfonation RHA was determined to be able to convert high FFA feedstock (DC-oil) into. The optimal reaction conditions were determined at 6 wt.% catalyst amount and 17:1 methanol to oil molar ratio at 120 °C for 5 h which yield 70.2 wt.% ME content. However, the simple catalyst regeneration method does not recover the catalytic activity of RHA-SO₃H, where the catalyst do not catalyzed the reaction for third time and onward. This indicated the poor stability of the catalyst which contributed to leaching of -SO₃H groups.

ACKNOWLEDGEMENT

The authors acknowledge BOPTN Grant from the Faculty of Mathematic and Natural Sciences, Mulawarman University and Universiti Malaysia Pahang for funding the research project .

REFERENCES

1. Fatih D.M., Balat M., Balat H. Biowastes-To-Biofuels. *Energy Conversion And Management* 2011, 52(4):1815-1828.
2. Maniam Gp, Hindryawati N, Nurfitri I, Jose R, Ab. Rahim Mh, Dahalan Fa, M. Yusoff M: Decanter Cake As A Feedstock For Biodiesel Production: A First Report. *Energy Conversion And Management* 2013, 76(0):527-532.
3. Li J, Yang Wm, An H, Maghbouli A, Chou Sk: Effects Of Piston Bowl Geometry On Combustion And Emission Characteristics Of Biodiesel Fueled Diesel Engines. *Fuel* 2014, 120(0):66-73.
4. Boey P-L, Ganesan S, Maniam Gp, Khairuddean M, Efendi J: A New Heterogeneous Acid Catalyst For Esterification: Optimization Using Response Surface Methodology. *Energy Conversion And Management* 2013, 65(0):392-396.
5. Shirini F, Mamaghani M, Seddighi M: Sulfonated Rice Husk Ash (Rha-So3h): A Highly Powerful And Efficient Solid Acid Catalyst For The Chemoselective Preparation And Deprotection Of 1,1-Diacetates. *Catalysis Communications* 2013, 36:31-37.
6. Noushad M, Rahman Ia, Husein A, Mohammed D, Ismail Ar: A Simple Method Of Obtaining Spherical Nanosilica From Rice Husk. *International Journal On Advanced Science, Engineering And Information Technology* 2012, 2:28-30.
7. Lowell S, Shields Je: Powder Surface And Porosity. . 1984.
8. Adam F, Chew T-S, Andas J: A Simple Template-Free Sol-Gel Synthesis Of Spherical Nanosilica From Agricultural Biomass. *J Sol-Gel Sci Technol* 2011, 59:580-583.
9. Xie W, Yang D: Silica-Bonded N-Propyl Sulfamic Acid Used As A Heterogeneous Catalyst For Transesterification Of Soybean Oil With Methanol. *Bioresource Technology* 2011, 102(20):9818-9822.
10. Hindryawati N, Maniam Gp: Novel Utilization Of Waste Marine Sponge (*Demospongiae*) As A Catalyst In Ultrasound-Assisted Transesterification Of Waste Cooking Oil. *Ultrason Sonochem* 2015, 22(0):454-462.
11. Farooq M, Ramli A, Subbarao D: Biodiesel Production From Waste Cooking Oil Using Bifunctional Heterogeneous Solid Catalysts. *Journal Of Cleaner Production* 2013(0).
12. Li C, Sun J, Fu C, Yu B, Liu Sq, Li T, Huang D: Synthesis And Evaluation Of Odour-Active Methionyl Esters Of Fatty Acids Via Esterification And Transesterification Of Butter Oil. *Food Chem.* 2014, 145: 796-801.
13. Zeng D, Liu S, Gong W, Chen H, Wang G: A Nano-Sized Solid Acid Synthesized From Rice Hull Ash For Biodiesel Production. *Rsc Advances* 2014, 4(39):20535-20539.