

Original Article

Image analysis of glycerol effecting on transesterification

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Abstract

As known, glycerol, one of the products from reversible transesterification of alcohol with glycerides, drives the reaction backward. This work focuses on the effects of glycerol in the reaction with image analysis. The experiments were done by using various alcohols and refined palm oil (molar ratio of 6:1) with sodium methoxide catalyst (0.6% w/w oil) at 60 °C for 10 minutes. Amounts of glycerol and order of mixing glycerol with oil or alcohol were studied. Results show that the glycerol amount estimated from image analysis is mostly compatible with titration determinations. Image analysis shows that glycerol dissolves well in methanol and yields less generated glycerol. The different alcohols give different amounts of glycerol. Longer reaction time gives more and larger glycerol drops, which slows down the mass transfer rate of transesterification and induces the reaction to pseudo-equilibrium.

Keywords: refined palm oil, transesterification, image analysis, glycerol, confocal laser microscope**1. Introduction**

Biodiesel is an interesting alternative energy source to be in place of petroleum diesel. It is a product from triglycerides (oil or fat from animals or plants with low free fatty acids, <1%) and alcohol, mostly reacting with a base catalyst in transesterification reaction. For decades, transesterification for biodiesel production has become known to be reversible: glycerol, a byproduct from biodiesel, can drive the reaction backward (Darnoko & Cheryan, 2000; Noureddini & Zhu, 1997). Therefore, glycerol needs to be continuously removed to increase reaction rate and extent by either reactive distillation (Mueanmas, Prasertsit, & Tongurai, 2010; Prasertsit, Mueanmas, & Tongurai, 2013) or deglycerol reactor (Nikhom & Tongurai, 2014).

Many previous works focused on effect of glycerol in area of phase separation for biodiesel production (Hama *et al.*, 2011; Ye, Sha, Zhang, Yuan, & Wu, 2011; Zhou & Boocock, 2006). Abeynaik *et al.* (2012) studied sedimentation and

creaming for glycerol droplet dispersion from biodiesel product by optical techniques and magnetic resonance imaging. It showed that smaller droplets of glycerol flowed counter to the predominant droplets flows as a dispersion phase.

Boer and Bahri (2015) used computational fluid dynamics to explain liquid-liquid reaction on producing biodiesel or ester. One of the two liquid phases is the continuous non-polar phase (oil and ester) and the other is the dispersed polar phase (alcohol, glycerol; and catalyst). However, pseudo-single phase emulsions were simulated.

Additionally, the kinetics of transesterification relate to three main factors: mass transfer, chemical kinetics and chemical equilibrium. Narvaez, Sanchez, and Godoy-Silva (2009) showed that transesterification of palm oil in liquid-liquid film reactor has an initial mass transfer controlled stage followed by reaction kinetics controlled stage, while Likoza and Levec (2014) showed that initial diglyceride content of the oil affects the mass transfer controlled stage.

The first order reaction kinetic rate for the heterogeneous model presented by Allain *et al.* (2016) showed that in their tubular reactor for transesterification, the overall reaction rate was limited by the diffusion of triglyceride. In order to support that the increasing in glycerol reduces the transesterification rate, the graphical effects of glycerol on

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transesterification process at low operating conditions, which was almost not explained intentionally, were studied by image processing of glycerol-alcohol-biodiesel emulsions.

2. Materials and Methods

2.1 Materials

Commercial refined palm oil (RPO) with less than 0.05% free fatty acid and less than 1% moisture was used with commercial grade alcohols (methanol, ethanol, isopropanol, or butanol) as reactants and sodium methoxide catalyst purchased from Merck.

2.2 Method

Effects of alcohol type, distribution of glycerol in the reactants (RPO or alcohol), and amount of glycerol on esterification at low conditions were examined experimentally. From stoichiometry, three moles of ester are equivalent to one mole of generated glycerol. Generated glycerol was calculated from the total glycerol measured by titration method (ASTM D7637) minus the initial added glycerol. This represents ester content from transesterification. Also, the percentage of generated glycerol was determined by the weight of generated glycerol per total product weight multiplied by 100.

2.2.1 Effect of alcohols

Transesterification which was from methanol, ethanol, isopropanol or butanol were examined in this work. In each case, RPO 100 g and alcohol (molar ratio of alcohol to oil 6:1) were mixed with sodium methoxide catalyst (0.6 % w/w RPO). The reaction was performed in 100 ml Duran flask at 60°C and 500 rpm. After 10 minutes, 0.1 g product was analyzed for glycerol by titration with phenolphthalein indicator.

2.2.2 Distribution of glycerol in reactant phases (oil and alcohol)

To observe the distribution of glycerol in RPO or alcohol phase, fresh glycerol was mixed with either RPO or alcohol at molar ratio 1:1 in Duran flask. The mixture was stirred at 500 rpm for 5 minutes at room temperature. Samples were taken microphotography by LCD digital microscope (Model NLCD 307, Novel, 4 x) to observe the glycerol distribution.

2.2.3 Effect of glycerol amount on transesterification

To evaluate effect of glycerol on transesterification, the situation that the system contained more glycerol were simulated by first adding fresh glycerol at 0, 3 or 5 % (w/w RPO) into 100 mg RPO (first-RPO-case) and then mixed them with methanol (molar ratio of methanol to RPO 6:1) with 0.6% (w/w RPO) sodium methoxide catalyst, at 60 °C in 100 ml Duran flask for 10 minutes and mixing speed of 500 rpm. After two and 10 minutes, samples from each condition were analyzed in order to determine the total glycerol by titration and image processing. Similar amounts of reactants and procedures were applied with first mixing with methanol instead of the RPO (first-methanol-case).

For image processing, samples were taken from each condition at 2 and 10 minutes. Two drops of each sample were placed on slide and stained with Nile blue A (5 µg of dye per 1 mL of sample) to allow the visualization of glycerol. Ten pictures, 512×512 pixels or (1.42×1.42) mm², of each condition were taken by a confocal laser scanning microscope (Olympus Fluoview FV300 with a XY mode, a laser combiner featuring a red helium-neon at 543 nm). The pictures were taken using a 10X objective with additional zoom factor 2 and 1 µm axial step. Each picture was analyzed by MATLAB Academic version (40483416). The three main steps of the image processing after loading original picture file were thresholding (change color picture to gray scale and determine threshold value on gray scale), noise reduction (choosing a value below the threshold, or <100), and image segmentation shown in Figure 1. Image segmentation can be based on thresholding or on edge detection (Canny, Laplacian of Gaussian, Prewitt, Roberts or Sobel methods) (Karnpracha, Seanton, & Kaitwanidvilai, 2007). The results from image processing in all cases are areas or pixel counts satisfying the criterion to approximate the target area. Further, glycerol drop sizes can be classified to small (<3,000 pixels), medium (3,000-10,000 pixels) and large (>10,000 pixels).

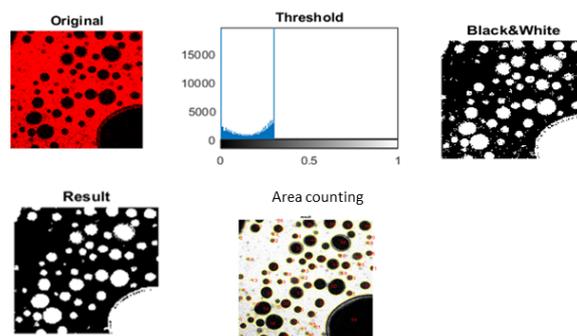


Figure 1. Steps of image processing for pictures from a confocal laser microscope.

3. Results and Discussion

3.1 Effect of alcohols

The amounts of the generated glycerol from transesterification of RPO and different alcohols (methanol, ethanol, isopropanol and butanol) are shown in Table 1. Methanol yielded the highest amount of the glycerol followed by ethanol, butanol and isopropanol, because methanol is the smallest molecule but has the highest polarity while isopropanol has the lowest polarity and its branch of CH₃ being difficult to attach to oil (Reichardt, 2003).

Table 1. Effects of alcohols on generated glycerol from transesterification of RPO with alcohol at molar ratio 1:6 and 60°C for 10 minutes.

Alcohol	Generated glycerol (%)
Methanol	8.17
Ethanol	1.96
Isopropanol	0.12
Butanol	0.35

3.2 Glycerol distribution between reactant phases

The pictures of glycerol in RPO and in methanol from LCD microscope show that the amount and size of glycerol drops (dispersed phase) occurring in the continuous RPO phase were much more and bigger than those in the methanol phase, as seen in Figure 2. These were caused by the polarity of glycerol which was closer to methanol than RPO (Reichardt, 2003). Therefore, glycerol dissolved in methanol better than in RPO and left lesser amount with small drops size of glycerol in methanol. The dissolve of glycerol in methanol resulted in dilution of methanol.

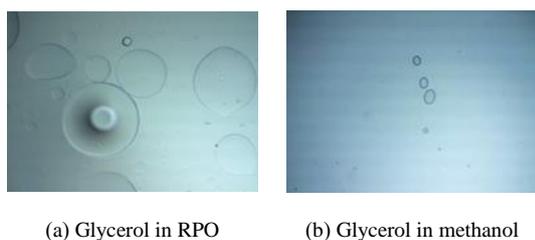


Figure 2. Glycerol distribution.

3.3 Amount of added glycerol

The effect of added glycerol on transesterification was shown in Figure 3. The amounts of the generated glycerol obtained by titration show that adding glycerol either in first-RPO- cases or in first-methanol-cases decreased the reaction as represented by decreasing in generated glycerol. From the stoichiometry of transesterification, the increment of net glycerol refers to the increment of ester content.

Additionally, when glycerol was not initially added to reactants, the amount of the generated glycerol at 2 minutes and 10 minutes were similar and higher than those in added glycerol cases. The reaction occurred rapidly in less than 2 minutes and then slowed down because of no glycerol to dilute methanol and catalyst at the beginning of transesterification. Therefore, transesterification is a fast reaction. A dilution effect of glycerol occurs when the reaction had glycerol as mention in part 3.2 that glycerol can dissolve in methanol better than in RPO and it lowered the reaction rate. As in a somewhat ana-

logue study on protein production, yield and productivity of the desired protein were strongly affected by glycerol-methanol dilution effects (Canales, Altamirano, & Berrios, 2015).

Figure 4 shows a picture taken from one of all cases for an example. It showed that the strained RPO presented as a continuous phase while glycerol-alcohol (black drops) was the disperse phase. This agrees with the work of Boer and Bahri (2015): transesterification is a liquid-liquid two-phase reaction, which oil is the continuous phase and glycerol-alcohol is the disperse phase. Figure 5 shows the amounts of total glycerol analyzed by image processing. They agree with those measured by titration in Figure 3, except for Figure 5(a) because of no initial added glycerol.

In addition, the effect of glycerol drop size on the reaction presents in Figures 5(b) and Figure 5(c). The amount of large drops increased with time because the generated glycerol may formed over old drops, or smaller drops of glycerol merged together to form larger drops. The large drop size can slow down reaction rate. This is confirmed by the same amounts of added glycerol in first-RPO cases and first-methanol-cases. In first-RPO cases, more glycerol generated in 2 minutes with large and medium size while, in first-methanol case, most drops were small in size. After 10 minutes, the increase of glycerol in first-methanol cases was greater than in the first-RPO cases. Figure 5 also shows the incident indicating that the proposed reaction may occur at the interface between methanol-catalyst and RPO and the generated glycerol cover around its interface. The larger area possibly represented the thickness of the cover and retarded mass transfer between methanol-catalyst and RPO to react together.

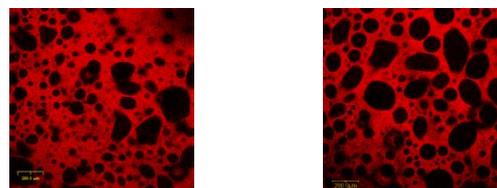


Figure 4. Glycerol drops (black) of 10 minute-transesterification from confocal microscopy.

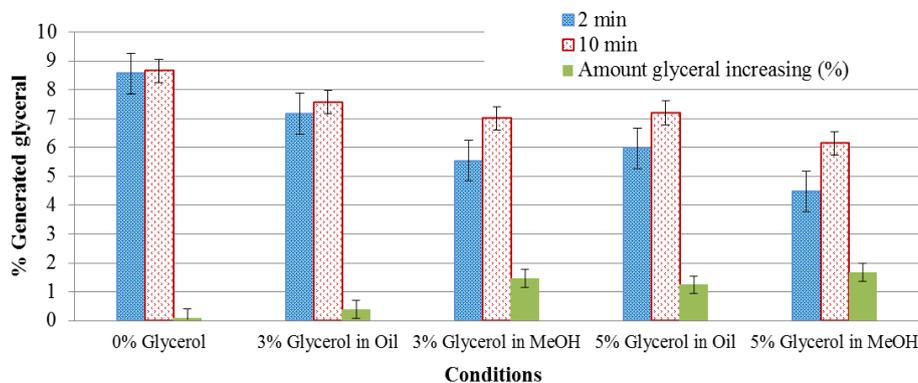
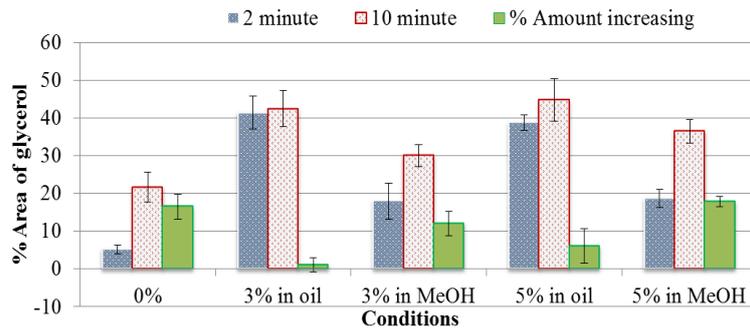
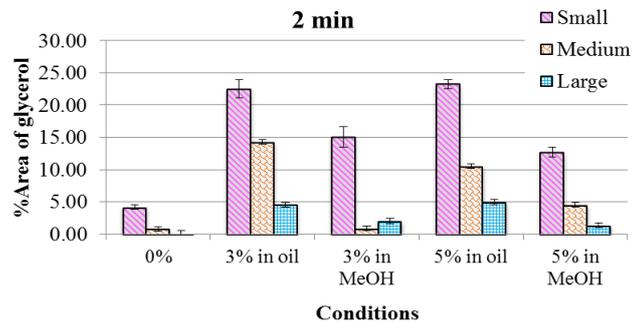


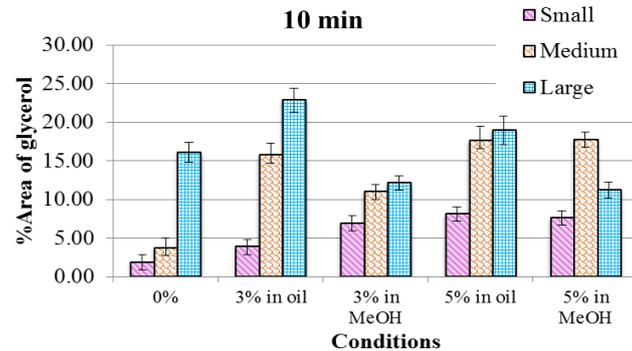
Figure 3. Effect of added glycerol on the amount of generated glycerol from transesterification reaction (100 g refined palm oil and oil to alcohol molar ratio of 1:6 at 60°C for 10 minutes) determined by titration method.



(a) Total area of glycerol



(b) Total area of glycerol at 2 minutes by size category



(c) Total area of glycerol at 10 minutes by size category

Figure 5. Effects of added glycerol on the total glycerol from transesterification (100 g refined palm oil and oil to methanol ratio of 1:6 at 60°C) determined by image analysis.

Therefore, after reaction occurred, glycerol resulted in dilution effect followed by drop size effect or mass transfer which slowed the reaction until reaching pseudo equilibrium. These effects support the researches that the removal of glycerol during the process gives better conversion.

4. Conclusions

Alcohol which has high polarity and small molecule gave higher transesterification. Analysis of glycerol amounts by titration and image analysis showed that the transesterification decreased with amount of glycerol increasing. Transesterification reaction, itself, was a fast reaction but the overall re-

action rate was limited by the dilution of glycerol in alcohol, and by hindered mass transfer of the alcohol-catalyst through a glycerol layer separating it from the oil at the interface. The image analysis can confirm that longer reaction times gave more and larger glycerol drops which slowed down reaction rate and pushed reaction to pseudo equilibrium.

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