Study on Reaction Conditions in Whole Cell Biocatalyst Methanolysis of Pretreated Used Cooking Oil

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Abstract: Biodiesel fuel (fatty acid methyl esters; FAMEs) can be produced by methanolysis of waste edible oil with a whole cell biocatalyst which is an attractive alternative to fossil fuel because it is produced from renewable resources. Utilizing whole cell biocatalyst instead of free or immobilized enzyme is a potential approach to reduce the cost of catalyst in lipase-catalyzed biodiesel production. *Rhizopus oryzae* (*R. oryzae*) PTCC 5174 cells were cultured with polyurethane foam biomass support particles (BSPs) and the cells immobilized within BSPs were used for the methanolysis of pretreated used cooking oil (UCO) for biodiesel production in this research. UCO is the residue from the kitchen, restaurant and food industries which promotes environmental pollution and human health risks. The inhibitory effect of undissolved methanol on lipase activity was eliminated by stepwise addition of methanol to the reaction mixture. The optimum conditions for the reaction were as follows: 50 BSPs, molar ratio of methanol to UCO 3:1, 15.54% (wt) water (in the form of buffer phosphate with pH= 6.8) based on UCO weight and temperature 35°C in three-step addition of methanol. The maximum methyl ester yield of 98.4% was obtained after 72 h of reaction in a shaken Erlenmeyer at mentioned conditions.

Keywords: *Biodiesel, Whole-cell biocatalyst, Methanolysis, Pretreated UCO*

1. Introduction

With the reduction of energy sources from fossil fuels, increase of the crude petroleum price and public awareness on impacts of its emissions on environment and their potential health hazards have created an interest for alternative fuel sources. Biodiesel is renewable, biodegradable, non-inflammable and non-toxic and it also has a favorable combustion emission profile, producing much less carbon monoxide, sulfur dioxide and unburned hydrocarbons than petroleum based diesel. The biodiesel fuel (fatty acid methyl esters), is defined as the mono-alkyl esters of fatty acids produced by transesterification of triglycerides [1-6] obtained from vegetable oils like soybean oil, jatropha oil, rapeseed oil, palm oil, sunflower oil, corn oil, peanut oil, canola oil and cottonseed oil [7]. Apart from vegetable oils, biodiesel can also be produced from other sources like animal fat (beef tallow, lard), waste cooking oil, greases (trap grease, float grease) and algae [8]. Because of the high price of high-quality virgin oils, the cost of biodiesel from these resources is higher than petroleum-based diesel [9]. The increasing of production of UCO from household, restaurants and industrial sources and to pour down it into drain has resulted in problems. The production of biodiesel from waste cooking oil to partially substitute petroleum diesel is one of the measures for solving the twin problems of environment pollution and energy shortage [10].

A number of processes have been developed for biodiesel-Production involving chemical or enzyme catalysis or Critical alcohol treatment [11-14]. Presently, industrial production of biodiesel from waste cooking oil is performed by chemical alkaline or acidic processes. Chemical catalysts including alkaline have been employed most widely since they give a high conversion of triglycerides to methyl esters in a short reaction time. However, chemical transesterification has some unavoidable drawbacks such as high energy and methanol

consumption, difficulty in glycerol recovery, the need to eliminate the catalyst and salt and a large amount of alkaline wastewater from the catalyst [15-18]. In the case where supercritical alcohol was used, higher rates of reaction were observed when it was compared to conventional transesterification. However, the requirements of high temperature, high pressure and high molar ratio of alcohol to oil make the process costly for industrial scale [8].

In recent times, there has been a growing interest in the use of enzymes such as lipases as biocatalyst for biodiesel production. Some advantages of lipase biocatalyst over the chemical-catalyzed reactions include the generation of no by-products, easy product removal mild reaction conditions (reaction temperature of 35-45°C) and catalyst recycling [19]. It has been reported that enzymatic reactions are insensitive to FFA and water content in waste cooking oil [19-21]. Hence, enzymatic reactions can be used in transesterification of used cooking oil [22]. But the cost of enzyme remains as a challenge for its industrial implementation. In order to enhance the cost effectiveness of the process, the enzyme (both intracellular and extracellular) is reused by immobilizing in a suitable biomass support particles of polyurethane that has resulted in considerable improvements in process efficiency [18].

In this work, waste edible oils obtained from MERC restaurant was used to produce biodiesel employing immobilized *R. oryzae* cells within biomass support particles of polyurethane foam. Furthermore, The effect of several parameters such as the molar ratio of methanol to UCO, water content, the enzyme content with the BSPs content and reaction temperature in three-step addition of methanol was determined on enzymatic methanolysis of pretreated UCO in a shaken Erlenmeyer for 72 h.

2. Methodology

2.1. Procedure of immobilization

All lipase catalyzed experiments were carried out using the filamentous fungus *R. oryzae* PTCC5174. Basal medium for growth of *R. Oryzae* which contained of polypepton 70 g; NaNO3 1.0 g; KH2PO4 1.0 g; MgSO4·7H2O 0.5 g and olive oil 30 g in 1 l of distilled water). Erlenmeyer (500 ml) containing 100 ml of the basal medium with biomass support particles (BSPs) were inoculated by aseptically transferring spores from a fresh agar slant using from 4% potato dextrose agar and 2% potato dextrose agar, and incubated at 30°C for 90h on a reciprocal shaker (150 oscillations/min, amplitude 70 mm). The *R. oryzae* cells became well immobilized within the BSPs as a natural consequence of their growth during shake-flask cultivation. Immobilization was effected by placing 150 particles inside an Erlenmeyer together with the medium, subjected to prior sterilization. The pH of the medium was initially adjusted to 5.6 and then allowed to follow its natural course. Reticulated polyurethane foam with a particle voidage of more than 97% and a pore size of 50 pores per linear inch (ppi) was cut into 6mm×6mm×3mm cuboids. After cultivation, the BSP-immobilized cells were separated from the culture broth by filtration, washed with tap water, and dried at room temperature for around 24h. To stabilize the lipase activity, the dried cells were treated with a 0.1% (v/v) glutaraldehyde solution at 25°C for 1h, washed with tap water, dried at room temperature for more than 24h, and then used as whole-cell biocatalyst for methanolysis reaction [23-24].

2.2. Methanolysis reaction

Methanolysis reaction was carried out in a 50 ml Erlenmeyer flask while incubated on a reciprocal shaker (150 oscillations/ min, amplitude 70 mm) at the temperature range of 25°C

to 45°C for 72 h. First, raw UCO was filtered by applying a reduced pressure system using a filter paper (Whatman42) to eliminate the indiscerptible impurities and was heated for 15 min at temperature of 90-110°C to eliminate extra water that has an influence on the transestrification reactions yield with methanol as an alcohol. The reaction mixture UCO 9.65 g, 0.1M phosphate buffer (pH 6.8) 0.5-3.5 ml and molar ratio of methanol to UCO 2-9:1 (one molar methanol (0.35 g) equivalent to 9.65 g UCO) was dispensed with 30-90 BSPs into an Erlenmeyer. The total of methanol was equally added to the reaction mixture at 0, 24 and 48 h reaction time. After the reaction, the whole cell biocatalyst was separated from the reaction mixture by filtration. Samples (200 µl) were withdrawn from the reaction mixture at specified time, centrifuged at 12,000 rpm for 5 min, to obtain the upper layer and were analyzed by capillary gas chromatography.

2.3. Gas chromatography (GC) analysis

The methyl esters content in the reaction mixture were quantified by using a gas chromatography/mass spectrometer (GC–MS) which was equipped with a HP-5 column with 30 meter long, internal diameter 0.25 millimeter. The column temperature was held at 160°C for 2 min, heated to 300°C with rate 8ºC/min and then maintained for 5min. The temperatures of the injector and detector were set at 280 and 230ºC; respectively. The total time of the process is 29.5 minute. For GC-MS analysis, 5µl of the aforementioned mixture and 300 µl of 1.4 mmol/l heptadecanoic acid methyl ester (hexane as the solvent) which is served as the internal standard were precisely measured and mixed thoroughly. A 1.0 µl of the treated sample was injected into a gas chromatograph column.

3. Results

Figure 1(a, b) and (c, d) shows the SEM micrographs of polyurethane foam particles surface with 2 magnifications before and after cell immobilization. The images are shown that the immobilization process was successfully preformed. Also, the weight of polyurethane foam particle after immobilization has increased almost twice. The efficiency of the process was checked by employing GC-Mass analysis of biodiesel product.

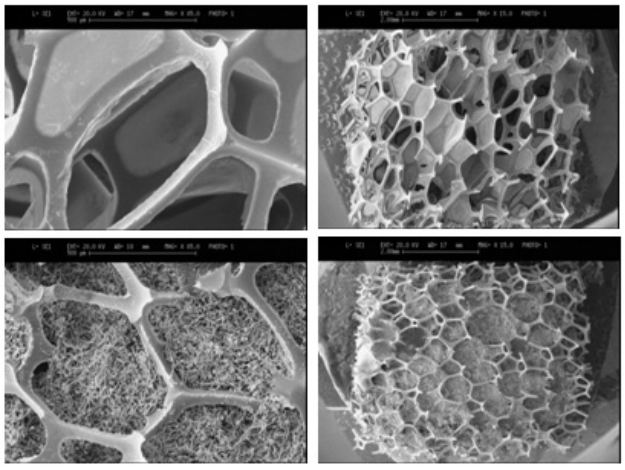

Fig. 1. SEM micrograph of foam particles: a. low magnification and b. high magnification, before immobilization; c. low magnification and d. high magnification, after immobilization.

3.1. Effect of temperature on the reaction

The reaction temperature is an important parameter in enzymatic catalysis. Higher temperatures can give a faster transformation, but too high temperature will lead to enzyme denaturing [25]. The effect of temperature on the lipase activity was examined by using a

temperature range of 25°C to 45°C as shown in Fig. 2 with a constant content of 50 BSPs, molar ratio of methanol to UCO 3:1, 15.54% (wt) water based on UCO weight, the reaction time of 72 h and in three-step addition of methanol. The lipase activity increased sharply when temperature enhanced from 25°C to 35°C, The highest yield was observed at 35°C. However, a further increase above 35°C in temperature leads to decrease in the reaction yield. This decrease in methyl ester yield can be explained by enzyme thermal denaturation.

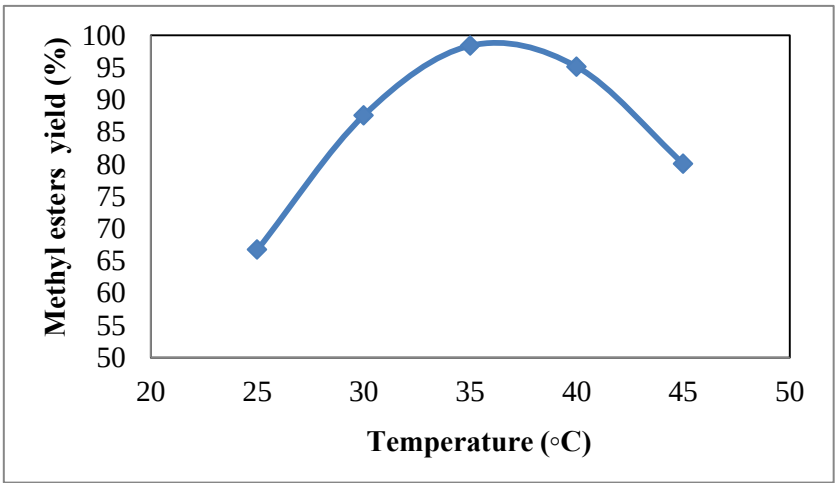

Fig.2. Effect of temperature on lipase-catalyzed methanolysis reaction. Reaction conditions: 50 BSPs, molar ratio of methanol to UCO 3:1, temperature of 25°C to 45°C,15.54% (wt) water based on UCO weight, the time of 72 h and in three-step addition of methanol.

3.2. Effect of water content on the reaction

It is well known that lipase, as a form of protein, requires the presence of water to maintain its live structure, and the activity of the enzyme in non-aqueous media is affected by the water content [10]. The reaction was examined in cases of water content (in the form of phosphate buffer with pH= 6.8) ranging from 5.18% to 36.27% (wt) water based on UCO weight, 50 BSPs, temperature 35°C, molar ratio of methanol to UCO 3:1, the reaction time of 72 h and in three-step addition of methanol. The result is shown in Fig. 3. As indicated in Fig. 3, the FAME content rose gradually as water content increased from 5.18% to 15.54% (wt) water based on UCO weight, and then declined as water content rose from 15.54% to 36.27%. This result indicates that the excessive water content affects the mass transfer of the oil phase of the reaction product, and inhibits esterification. It was observed that the FAME content reached its maximum at a water content of 15.54 wt% which was about 32.4% higher than water content of 5.18 wt%.

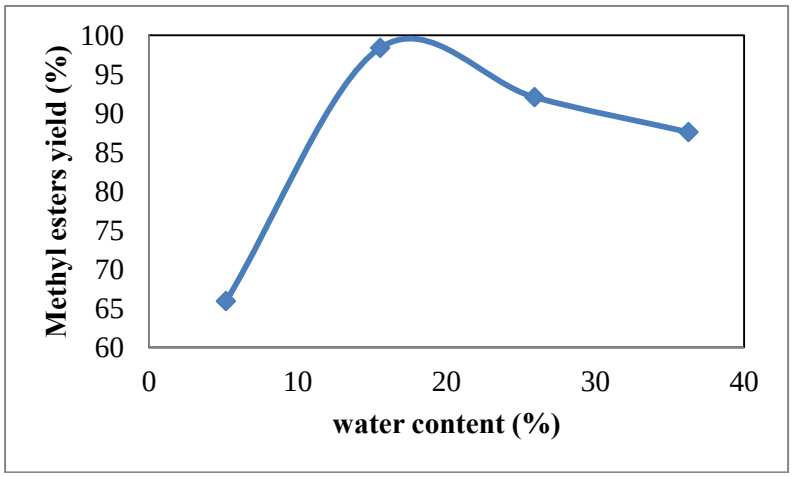

Fig. 3. Effect of water content on lipase-catalyzed methanolysis reaction. Reaction conditions: 50 BSPs, temperature 35°C, molar ratio of methanol to UCO 3:1, 5.18% to 36.27% (wt) water based on UCO weight, the time of 72 h and in three-step addition of methanol.

3.3. Effect of immobilized microorganism on the reaction

The enzyme activity produced of immobilized microorganism within BSPs increases with the immobilized microorganism content [10]. The effect of enzyme content on the transesterification reaction was examined with the BSPs content range from 30 to 90, temperature 35°C, molar ratio of methanol to UCO 3:1, 15.54% (wt) water based on UCO weight, the reaction time of 72 h and in three-step addition of methanol. As shown in Fig. 4, the FAME content increased along with the increase in enzyme content, because the more lipase available, the more substrate molecules were absorbed into the active center of the lipase, but the increase rate of FAME content declined as BSPs rose from 50 to 90. This phenomenon can be explained that the UCO content was excessive when enzyme content was under 50, and that as the enzyme content rose to become sufficient from 50 to 90, the FAME content increased only 0.88%. From an economic point of view, 50 BSPs is the most feasible content level for lipase in reaction of biodiesel synthesis from UCO.

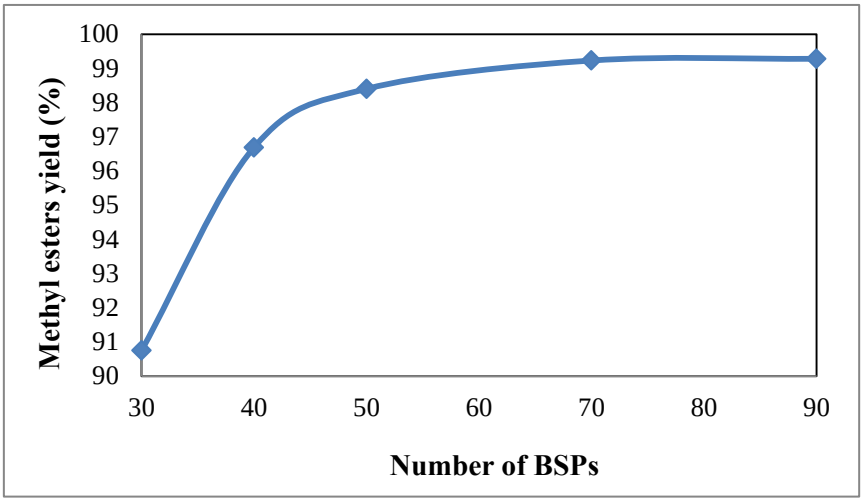

Fig. 4. Effect of BSPs content on lipase-catalyzed methanolysis reaction. Reaction conditions: BSPs content range from 30 to 90, temperature 35°C, molar ratio of methanol to UCO 3:1, 15.54% (wt) water, the reaction time of 72 h and in three-step addition of methanol.

3.4. Effect of Molar Ratio of Substrates on the Reaction

Methanolysis reaction was performed by using different substrate molar ratios of methanol to oil varying in the range of 2–9. The results (Fig.5) demonstrate that the methyl esters yield initially increases with increasing the methanol to oil molar ratio from 2 to 3 and reaches to its maximum at 3. However, a further increase in the methanol to oil molar ratio leads to decrease in the reaction yield. The highest methyl ester yield of 98.4% was achieved at a methanol to oil molar ratio of 3:1, and decreased to 50.68% when the molar ratio of 9:1 was utilized. This is in agreement with the earlier observation that excessive methanol concentration lead to lipase enzyme inactivation. The addition of methanol more than stoichiometric amounts exerts an inhibitory effect on enzyme performance. This could be due to the fact that the immiscible methanol was accumulated around the lipase structure including its active sites, reaching a concentration level sufficient to cause a denaturation of the protein. This phenomenon might lead to enzyme inactivation [24].

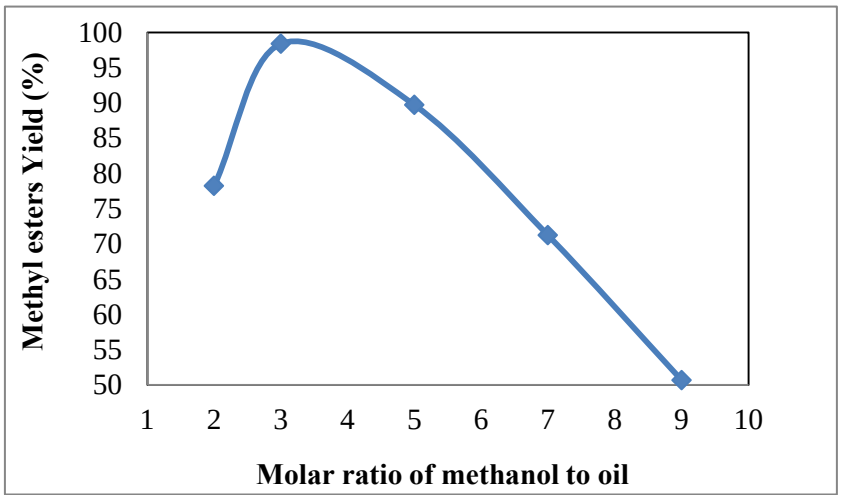

Fig. 5. Effect of methanol to oil molar ratio on lipase-catalyzed methanolysis reaction. Reaction conditions: 30 BSPs, temperature 35°C, molar ratio of methanol to UCO 2-9:1, 15.54% (wt) water, the reaction time of 72 h and in three-step addition of methanol.

3.5. Product analysis

The result of GC-Mass analysis indicated that the main components In the UCO-derived biodiesel were methyl octadecenoate, methyl hexadecenoate, methyl octadecadienoate, methyl octadecanoate, methyl tetradecanoate, methyl heptadecanoate, methyl dodecanoate and methyl pentadecanoate. These components account for 98.4% of the FAME.

3.6. Conclusions

The study shows that pretreated used cooking oil (UCO) can be efficiently converted to biodiesel fuel in a shaking Erlenmeyer methanolysis reaction using immobilized *Rhizopus Oryzae* (PTCC 5174) on polyurethane foam biomass support particles (BSPs). Therefore, this is an effective approach to reduce the cost of biodiesel feedstock and pollution problems.The optimum reaction conditions for the reaction were as follows: 50 BSPs, molar ratio of methanol to UCO 3:1, 15.54% (wt) water (in the form of buffer phosphate with pH= 6.8) based on UCO weight and temperature 35°C in three-step addition of methanol. The maximum methyl ester yield of 98.4% was obtained after 72 h of reaction in a shaken Erlenmeyer at mentioned conditions.

Based on GC-Mass analysis, the main components in UCO-derived biodiesel are methyl octadecenoate, methyl hexadecenoate, methyl octadecadienoate, methyl octadecanoate,

methyl tetradecanoate, methyl heptadecanoate, methyl dodecanoate and methyl pentadecanoate, which are the most compositions of FAME.

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