



Development of Clay Foam Ceramic as a Support for Fungi Immobilization for Biodiesel Production

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ABSTRACT

Biodiesel is an attractive alternative fuel because of its nontoxicity and biodegradability. Biodiesel is produced through transesterification of vegetable oils' triglyceride. It is obtained from vegetable oils or fats either by chemical or enzyme-catalyzed transesterification with methanol or ethanol. Use of whole-cell biocatalyst immobilized within biomass support particles (BSPs) can overcome the obstacle of high cost of enzymatic catalyst. The objective of this research is to produce clay foam ceramic as BSP by replica method from raw materials such as clay, sodium silicate and sodium tripolyphosphate. To prepare the whole cell biocatalyst *Rhizopus oryzae* fungi (PTCC 5174) was immobilized on the ceramic foam with bulk density and porosity of 0.3 g/cm³ and 88.8%, respectively. The deposited biomass on clay foam particles can be observed through scanning electronic microscopy (SEM). A packed-bed reactor (PBR) system using whole-cell biocatalyst was developed for biodiesel production by pretreated used cooking oil (UCO). Clay foam ceramic seems to be more suitable compared to polyurethane foam for supporting fungi immobilization because it shows high mechanical strength and reduces damaged microorganisms.

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1. INTRODUCTION¹

Biodiesel is a kind of renewable biofuel that can be synthesized from edible, nonedible and waste oils. Due to diminishing of petroleum reserves, vegetable oils have been known as an alternative fuel for petroleum-based diesel fuel. There are several methods for transesterification of vegetable oil such as using chemical or enzymatic catalysis or supercritical alcohol treatment [1, 2].

The current technology for biodiesel production is use of alkali catalysts that suffers from several important limitations. First, it cannot be used for feedstocks with free fatty acid (FFA) content greater than a few percent. Second, feedstocks must be free of water. These two limitations mean that some waste oils cannot be processed without pretreatment to remove FFAs and water. The use of acid catalysts have shown

promise as successful approach for esterification of FFAs prior to reaction with base catalyst, but still requires feedstock free of water [3]. Both alkali and acid catalytic methods require use of excess methanol, which must be recovered, produce salts that must be removed from the product, and generate glycerol as a low-grade by-product. Compared to chemical catalysis, the lipase-catalyzed synthesis of biodiesel is more compatible with variation in the quality of raw materials. By using lipase catalysis the process needs simple purification steps, also it is capable to be done under moderate reaction condition. The main obstacle for commercialization of lipase-catalyzed process is the cost of the enzyme [4-6]. An effective way to compensate the high cost of the enzyme is to extend the operational life of the lipase, which would significantly increase productivity of a given amount of enzyme and reduces the biodiesel production price. This can be achieved by immobilized whole-cell biocatalyst within biomass support particles [7-9].

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Ceramic foam is a type of porous material with porosity between 70 to 90% and volume density between 0.3 to 0.6 g/cm³. It has been widely used as membrane for filtration, heat insulation, sound insulation, catalyst support, absorbents, kiln furniture, biomedical devices, molten metals, hot gases, thermal protection system and heat exchangers due to their several advantages such as low density, high porosity, large specific surface area, low heat transfer rate, high temperature resistance, corrosion resistance and excellent acoustic properties [10-17].

There is great interest in inorganic supports for enzyme and cell immobilization due to their durability and high mechanical strength for usage in PBR and fluidized-bed reactors and relatively low cost. In the case of inorganic supports with honeycomb macrostructure and foam-like materials, diffusion limitation of substrate transport towards biocatalyst is obviously minimized and efficiency of process significantly increases [18].

Ceramic foam can be produced using various methods including replication method, starch consolidation, foaming method and gel-casting [19-21]. The polymeric foam replication method is the most popular method that was patented in 1963. In this process, the open-cell polymeric foam is coated by ceramic slurry, and then burning of polymeric foam by sintering process is done.

In the present study, a clay ceramic foam by means of replica method was fabricated. With the immobilization of *Rhizopus oryzae* on it, clay whole cell biocatalyst was prepared and it was used for biodiesel production from pretreated UCO.

2. MATERIALS AND METHOD

2.1. Preparation of Clay Ceramic Foam Open cell polyurethane foam of 25 ppi (pores per inch) (Safoam Company, Iran) was used to prepare ceramic foam. Polyurethane foam samples were cut into 6 mm × 6 mm × 3 mm pieces. The natural clay used consisted of a mixture of calcite (CaCO₃), quartz (SiO₂), feldspar, clay, mica, chlorite group, gypsum (CaSO₄, 2H₂O) and other compounds. Table 1 shows

the bulk chemical compositions of the natural clay. Sodium tripolyphosphate powder and 4 wt. % sodium silicate solution also were used. Specific values of clay as precursor and lubricants consisting of sodium tripolyphosphate and sodium silicate solution were mixed in ball mill at speed of 200 rpm for 30 min to obtain a well dispersed slurry. Polyurethane sponge particles were coated with the ceramic slurry. After removal of the excess slurry by squeezing and drying foam in air for 24 h, the polymer was burned out and sintered in an auto-controlled furnace (B180, Nabertherm, Germany). The sintering program was defined as follows: increasing temperature to 1200°C with rate of 5°C/min, holding it at 1200°C for 1 h, and then cooling down the sample in the furnace.

X-ray diffraction (XRD) was carried out on clay ceramic foam after sintering to identify the crystalline phase of sample. The porous sample was pulverized and pressed in a glass sample holder and measured by Philips PW 3710 X-ray diffractometer with filtered CuK_α radiation at a wavelength of 0.154 nm. The scan angle ranged from 5-95° with a 2θ scanning speed of 2.42θ°.min⁻¹ and a step size of 0.022θ°. Cell geometry, struts, surface fracture and macrostructure of ceramic foams were observed using an optical camera (Olympus DP72).

2.2. Immobilization of *Rhizopus Oryzae* on Clay Ceramic Foam

Filamentous fungus *R. oryzae* PTCC 5174 was used as a lipase catalyst in all experiments. A specific medium consisting of polypepton, NaNO₃, KH₂PO₄, MgSO₄.7H₂O and olive oil: 70, 1.0, 1.0, 0.5 and 30 g/l, respectively were prepared for *R. oryzae* growth. Erlenmeyer containing 100 ml of the basal medium with clay ceramic foams was inoculated by aseptically transferring spores from a fresh agar slant using 4% potato dextrose agar and 2% potato dextrose agar, and incubated at 30°C for 90 h on a reciprocal shaker (150 oscillations/min, amplitude 70 mm) [22]. The *R. oryzae* cells immobilized completely within the clay foams as a natural consequence of their growth during shake-flask cultivation. Immobilization was done by placing 150 particles inside an Erlenmeyer that contained the prepared medium, subjected to prior sterilization.

TABLE 1. The clay bulk chemical composition

Comp.	SiO ₂	CaO	Al ₂ O ₃	MgO	Na ₂ O	K ₂ O	LOI	Fe ₂ O ₃	TiO ₂	P ₂ O ₅	MnO	Ag ₂ O
Wt.%	43.7	10.3	11.1	12.9	1.3	2.5	13.27	3	0.34	0.1	0.04	0.02
Comp.	CdO	MoO ₃	Cs ₂ O	SO ₃	Rh	BaO	TeO ₂	SnO ₂	SrO	CuO	Sb ₂ O ₃	ZrO ₂
Wt.%	0.055	0.01	0.095	0.09	0.01	0.03	0.02	0.03	0.02	0.03	0.02	0.03

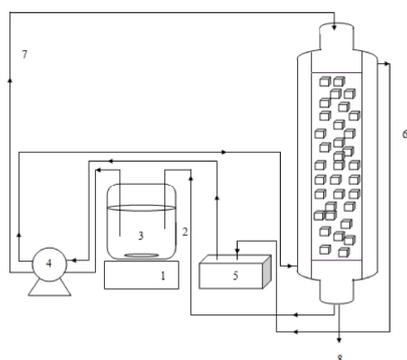


Figure 1. Schematic diagram of PBR system. (1) Bath sonicator; (2) magnetic stirrer; (3) reaction mixture reservoir; (4) peristaltic pump; (5) water bath; (6) Plexiglas column packed with 6 mm × 6 mm × 3 mm cuboid clay biocatalyst containing dry *Rhizopus oryzae* cells; (7) TYGON tube and (8) product.

The initial pH of the medium was adjusted to 5.6 and then allowed to follow its natural course. After cultivation, the immobilized cells were separated from the culture broth by filtration, washed with tap water, and dried at room temperature for about 24 h. To stabilize the lipase activity, dried cells were treated with a 0.1% (v/v) glutaraldehyde solution at 25°C for 1 h, washed with tap water, dried at room temperature for more than 24 h, and used as whole-cell biocatalyst for methanolysis reaction [23, 24].

2. 3. Measurement of Cell Mass Immobilized within Clay Ceramic Foams The immobilized cell within clay ceramic foam was measured as follow: 20 g of clay foam ceramic particles was weighed before immobilization. Specified clay ceramic foams after immobilization process were washed with acetone to remove unwanted materials and dried for 2 h at 105°C. The particles plus dried cells were weighed and the cell mass was calculated from the difference between the weights.

2. 4. Scanning Electron Microscopy (SEM) of whole Cell Biocatalyst Whole cell biocatalysts were soaked in pure water, 50% ethanol, 75% ethanol, 95% ethanol, 100% ethanol and 100% acetone for 5, 15, 15, 15, 30 and 15 min, respectively. After that, samples were dried and retained in desiccators and the immobilized *Rhizopus oryzae* fungi on the clay ceramic foams were observed through VEGA/TESCAN scanning electron microscopy (SEM).

2. 5. Experimental Apparatus A schematic diagram of the PBR system used for methanolysis reaction is shown in Figure 1. The PBR vessel

consisted of two wall Plexiglas column (25 mm in internal diameter and 200 mm in height) and was equipped with the TYGON tube and a peristaltic pump. The reaction mixture was continuously circulated through desired whole cell biocatalysts packed into the PBR.

2. 6. Methanolysis Reaction Raw UCO was filtered by a filter paper (Whatman 42) to eliminate undesirable impurities, after that it was heated for 15 min at temperature of 90-110°C to remove extra water that has an influence on the methanolysis reactions yield [25]. Methanolysis reaction was carried out in the PBR which contained 100 whole cell biocatalysts, the reaction mixture consisted of 9.65 g pretreatment UCO, 0.35 g of methanol (one molar equivalent to 9.65 g of pretreatment UCO), and 1.5 ml of 0.1 M phosphate buffer (pH 6.8) [26, 27]. The process was performed at 35°C by a water jacket connected to the water bath in the PBR, where the reaction mixture was continuously circulated at a fixed flow rate of 40 ml/min with a peristaltic pump. To fully convert the UCO to its corresponding methyl esters, at least three molar equivalents of methanol was required. In order to reduce the toxicity of lipase activity from methanol, the UCO/methanol molar ratio was kept 1:1 in the reaction step. Consequently, 0.35 g of methanol was successively added to the reaction mixture at 24 and 48 h reaction time. At the end of each batch reaction cycle (72 h), the glycerol rich-phase was separated from the methyl ester layer in a decantation funnel. 200 µl methyl ester (biodiesel) was analyzed by capillary gas chromatography. Afterwards, the reaction mixture was replaced with fresh material before starting the next batch.

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

2. 8. Calculation of Methyl ester Content The result for the fatty acid methyl ester content is expressed as a mass fraction in percent using methyl

nonadecanoate (C19) as the internal standard. The following formula is used: Equation (1)

$$C = \frac{\sum A - A_{IS}}{A_{IS}} \times \frac{M_{IS}}{M} \times 100$$

where:

$\sum A$ = total peak area C14:0 – C24:1

A_{IS} = internal standard (methyl nonadecanoate) peak area

M_{IS} = concentration of the internal standard solution, in mg/mL

M = mass of the sample, in mg

3. RESULT AND DISCUSSION

3. 1. The Porosity of Clay Ceramic Foam Biocatalyst

Morphology, cell geometry, struts, surface fracture, presence of triangular voids inside struts, presence of internal pores and macrostructure of the clay ceramic foam with 25 ppi can be seen in Figure 2. The porosity of fired product was calculated by the following equation: porosity = $(\rho_r - \rho_b) / \rho_r$; where ρ_r and ρ_b are the real and bulk densities, respectively. Bulk density of discs was simply calculated from their weight and volume because they are rather uniform and flat. As for the real densities, the disc was first ground to powder and then measured with a Densitometer (AccuPyc 1330 V3.00). The porosity of sample sintered at 1200°C is 88.8%. All open-cells and small pores make this porosity [28].

3. 2. XRD on Clay Ceramic Foam Biocatalyst

Figure 3 shows the X-ray diffraction pattern of clay ceramic foam. It can be noted that the main phase of ceramic foam is predominantly composed of Calcite (CaCO_3). The presence of minor phases such as Feldspar and Quartz (SiO_2) was also identified.

3. 3. SEM of Clay Ceramic Foam Biocatalyst The weight of 20 clay ceramic foam particles before immobilization was 2.1027 g and after immobilization increased to 2.5163 g. Therefore, it was concluded that the weight of the clay ceramic foam particles after immobilization increased almost 1.2 times. Figure 4 (a-d) shows the SEM micrographs of clay ceramic foam surface with 4x magnifications after immobilization.

3. 4. Methanolysis of UCO by Clay Ceramic Foam Biocatalyst

The efficiency of the process was checked using GC-MS analysis of biodiesel product. The result of GC-MS analysis indicated that the main components in the UCO-derived biodiesel were methyl octadecenoate, methyl hexadecanoate, methyl octadecadienoate, methyl octadecanoate, methyl tetradecanoate, methyl heptadecanoate, methyl dodecanoate and methyl eicosanoate. Figure 5 shows the GC-MS analysis results. These components account for 89.98% of the fatty acid methyl ester. In order to confirm the GC-MS results, the kinematic viscosity was measured in the purified biodiesel samples. Viscosity can be used as a control of transesterification reaction, confirming the formation of methyl esters from UCO, through the viscosity reduction of the feedstock. Results showed that the measured viscosity for purified product had a substantial reduction in comparison with UCO. The kinematic viscosity of product was 6.5 mm²/s however for UCO it was 35 mm²/s. This result is in agreement with the attained transesterification yields.



Figure 2. Clay ceramic foam with a pore size of 25 ppi

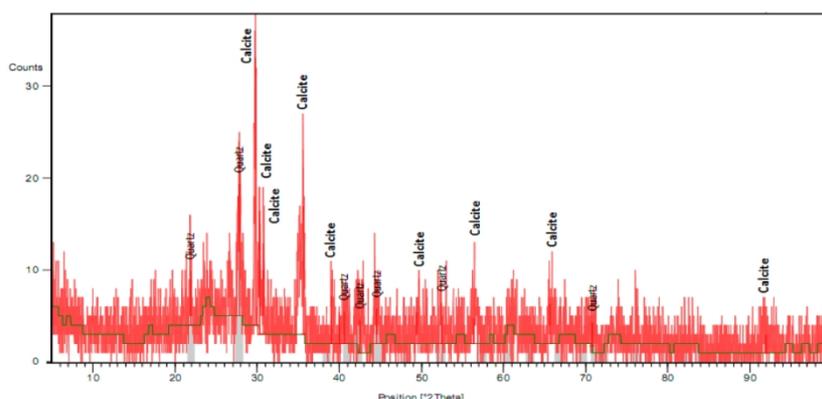


Figure 3. XRD pattern of prepared porous clay ceramic

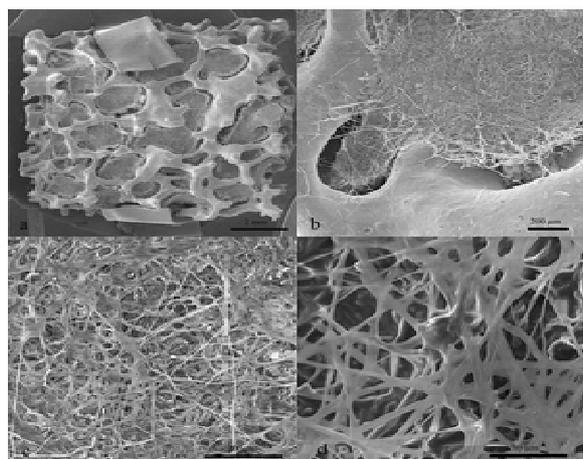


Figure 4. SEM micrograph of immobilization of *Rhizopus oryzae* on clay ceramic foam: (a) 15 X; (b) 100 X; (c) 400 X and (d) 800 X

3. 5. Durability of Ceramic Foam Biocatalyst in Repeated Use for Methanolysis

Results are satisfactory compared with data already reported in the literature and demonstrated that *R. oryzae* whole cells on clay ceramic foam is a cheaper biocatalyst that can be used in the biodiesel synthesis. The whole cells from *R. oryzae* immobilized in clay ceramic particles express high transesterification activity in the methanolysis of UCO. The high operational stability of the clay ceramic foam biocatalyst is rather promising for the application of lipase catalyzed transesterification in a packed bed reactor. In industrial applications, recycling of lipase is required to reduce the production cost. Thus, use of whole cell clay ceramic foam as biocatalyst can be very suitable because of physical stability.

4. CONCLUSION

This paper presents the preparation method of clay ceramic foam as a support for fungi immobilization. Experiments show that UCO can be effectively converted to biodiesel fuel with a maximum fatty acid methyl ester (FAME) content of 89.98% in a three-step PBR methanolysis process using immobilized *Rhizopus oryzae* on clay ceramic foams. The images show successful immobilization of *R. oryzae* cells on the clay ceramic foam. The weight of ceramic foam particle after immobilization was increased almost 1.2 times which shows the weight of dry cells. The kinematic viscosity of product decreased dramatically against UCO. It seems that the whole cell clay ceramic foam is a suitable biocatalyst for biodiesel production from UCO in PBR because of its high mechanical strength and reduction of damaged cells.

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بیودیزل به دلیل دارا بودن خواص غیرسمی و زیست تخریب پذیری، قابلیت جایگزینی سوخت فسیلی را دارد. بیو دیزل از ترانس استریفیکاسیون تری گلیسرید روغن های گیاهی و الکل های نظیر اتانول و متانول در حضور کاتالیزورهای شیمیایی و آنزیمی تولید می شود. با استفاده از بیوکاتالیست تمام سلولی تثبیت شده درون ذرات حامل توده زیستی می توان بر هزینه بالای تولید بیودیزل توسط کاتالیست آنزیمی غلبه کرد. هدف از این پژوهش، تولید سرامیک های متخلخل رسی به عنوان ذرات حامل توده زیستی با استفاده از روش رلیکا از مواد اولیه رسی، سیلیکات سدیم و تری پلی فسفات سدیم است. برای تهیه بیوکاتالیست تمام سلولی، فارچ رایزوپوس اوریزایی (PTCC 5174) بر روی سرامیک های متخلخل با دانسیته حجمی 0.3 g/cm^3 و تخلخل 78% تثبیت شد. توده زیستی ایجاد شده بر سرامیک متخلخل به وسیله میکروسکوپ الکترونی روبشی قابل مشاهده است. راکتور بستر پر شده با استفاده از بیوکاتالیست تمام سلولی برای تولید بیودیزل از روغن پسماند خوراکی توسعه داده شد. استفاده از سرامیک های متخلخل رسی در مقایسه با فوم های پلی یورتانی به عنوان پایه تثبیت فارچ به دلیل مقاومت بالای مکانیکی و کاهش در تخریب میکرواورگانیزم ها ارجح است.

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