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Using Packed-Bed Bioreactor for Loofa-Immobilized Rhizopusoryzae as a Whole-Cell Biocatalyst

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ABSTRACT

Biodiesel is a sustainable fuel made from transesterification reaction of vegetable oils with short chain alcohols. Use of loofa-immobilized Rhizopusoryzae as a biocatalyst for transesterification reaction of canola oil and the effect of some parameters on biodiesel production were studied. The highest methyl ester yield of 85.3% at optimum conditions, containing 15wt% of water and methanol to oil molar ratio of 3:1, was achieved. The performance of the reaction in a packed bed reactor was able to produce biodiesel with a yield of 92%.

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1. Introduction

Biodiesel (fatty acid methyl ester) which is a clean burning diesel fuel derived from vegetable oils or animal fats by transesterification with short chain alcohols that is represented in Figure 1, has attracted considerable attention as a biodegradable, renewable and non-toxic fuel [10].

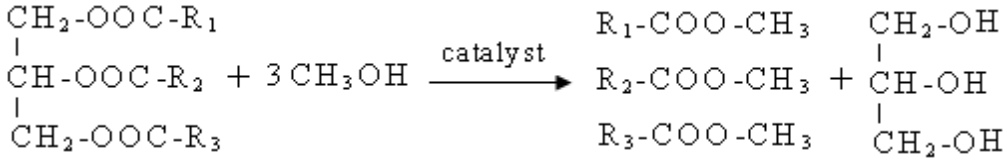

Fig. 1. Transestrification of triglyceride with alcohol: general equation

Enzymatic production of biodiesel using lipase as a catalyst can be carried out in the mild reaction conditions and the glycerol can be easily recovered without complex processing, but the high cost of lipase preparation due to complicated recovery, purification, and immobilization is the main hurdle of this process. Alternatively, enzymatic production of biodiesel using whole-cell biocatalyst has attracted considerable attention because it helps to overcome the afore-mentioned problems and enables the direct use of lipase-producing microorganisms. The previous studies show that the immobilized Rhizopusoryzae cells have an acceptable potential to catalyze the biodiesel production process [11,3]. Loofa as a vegetable sponge is a natural lignocellosic material that grows widely in the northern part of Iran. It can be used for cell immobilization because of its high degree of porosity, high specific pore volume, stable physical properties, biodegradability, non-toxicity and low cost [8,9].Packed bed reactor (PBR) is used to develop the scale of methyl ester production because of its high efficiency, low cost and ease of construction, operation and maintenance [6,7]. The objective of the present work is to achieve immobilized RhizopusOryzae within loofa sponge and their utilization as biocatalyst for biodiesel production. In this study, the effect of some important parameters in the methanolysis reaction of canola oil such as water content, methanol to oil molar ratio and catalyst amount on methyl ester production were investigated. After determination of the optimized condition, methanolysis reaction was conducted in the PBR and the result was compared with shake-flask scale.

2. Materials and Methods

Materials and microorganism

The strain of RhizopusOryzae (PTCC 5174) was purchased from Persian Type Culture Collection. Refined olive oil and canola oil sources were obtained locally. Polypepton was purchased from Liofilchem and all other chemicals were obtained commercially from Merck Chemical Co. and were of analytical grade.

3. Biomass Support Particles (BSPs) and Cells Cultivation

The carriers used in this study for cell immobilization (BSPs) were loofa pieces that cut into disc form having 1.5 cm diameter. The pieces were placed in a beaker of boiling water for 10 min followed by removing and drying them at 70 °C in an oven. Erlenmeyer flasks of 250 ml, containing 50 ml of medium (70 g polypeptone, 1.0 g $NaNO_3$, 1.0 g KH_2PO_4, 0.5 g $MgSO_4$-$7H_2O$ in 1 L distilled water with 30 g/l olive oil as carbon source). The pH of the medium was initially adjusted to 5.6. These flasks with 6 pieces of loofa were inoculated from a fresh potato dextrose agar slant and placed in a shaker incubator (150 rpm) for 48 hrs.

4. Preparation the Whole-Cell Biocatalysts and Methanolysis Reaction

The loofa-immobilized fungal cells were separated from the broth by filtration through a strainer. After washing with tap water and acetone they were dried at room temperature. Methanolysis reaction was carried out in a 50 ml screw-capped bottle at 30°C with incubation on an orbital shaker (150 rpm) for 72 hrs. The composition of the reaction mixture containing various amount of canola oil, methanol, and phosphate buffer 0.1 M (pH 6.8). Two pieces of loofa-immobilized R. oryzae were used as the biocatalyst. Stepwise addition manner of methanol was used to prevent the inactivation of lipase enzyme in immobilized cells. The esterified mixture was centrifuged at 8000 rpm for 10 min to obtain the upper layer, and analyzed to determine the yield of methanolysis reaction.

5. Methanolysis Reaction in the Packed Bed Reactor

Packed-bed reactor that was used for methanolysis of canola oil in a solvent-free medium consisted of a glass column with dimensions of 10 cm height and 1.5 cm in internal diameter and an external water jacket for temperature control (Fig.2). This reactor was filled with 18 pieces of loofa-immobilized R. oryzae. The reaction mixture, contained 50 g canola oil, 7.8 ml phosphate buffer 0.1 M, was circulated at constant flow rate at 10 ml/min with a peristaltic pump for 72 hrs. The appropriate volume of methanol (2.6 ml methanol according to equivalent molar ratio) was added in stepwise manner to the reaction mixture.

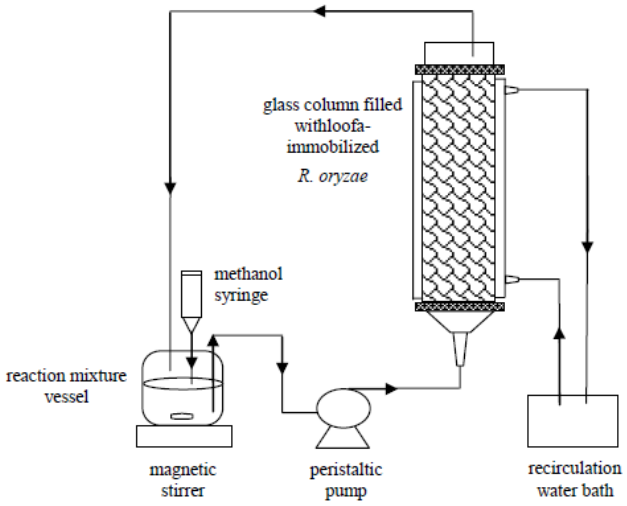

Fig. 2.A schematic diagram of the packed-bed reactor used in the present study

6. Analysis

Identification and quantification of methyl esters in the reaction mixture were carried out using 1H nuclear magnetic resonance (NMR) spectroscopy (UltraShield Plus, Bruker) [7,4]. 1H-NMR spectra were obtained at a frequency of 500MHz with $CDCl_3$ as solvent. The result of refractive index was determined by using Abbe refractometer at 30°C. There was a direct relationship between conversions determined by 1H-NMR spectra data and those determined by the refractive index [13]. This method could be used for rapid determination of methanolysis reaction yield.

7. Results and Discussion

Effect of water content in the reaction mixture on methyl ester yield: In order to examine the effect of water content in the reaction mixture on methyl ester production, methanolysis reaction in the mixture of 1.93 g canola oil and methanol to oil molar ratio of 3:1, was carried out with 0.10–0.50 ml Phosphate buffer (5–25 wt.% water by substrate weight). High amount of water can promote the hydrolysis reaction of the esters instead of methanolysis reaction, thus product yield was decreased. On the other hand, with low water addition, the lipase can be denatured and inactive with methanol and the methyl ester yield was decreased [2,12]. The maximum yield of methyl ester in this study was achieved with addition of 15% water (Fig.3).

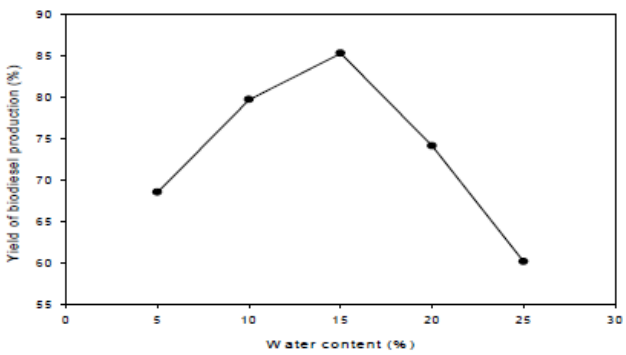

Fig. 3. Effect of water content in the reaction mixture on biodiesel yield

Effect of methanol content on methyl ester yield: To demonstrate the effect of the methanol concentration in the reaction mixture on the methyl ester yield, 0.1- 0.24 ml methanol was added three-step every 24 hrs to the reaction mixture containing 1.93 g canola oil and 0.3 ml phosphate buffer solution. The results (Fig.4) show that the yield of methanolysis reaction significantly decreased with increase of methanol to oil molar ratio. The stoichiometric molar ratio of methanol to triglyceride for transesterification reaction is 3:1. Addition of methanol more than the stoichiometric amount has an inhibitory effect on enzyme performance, since the excess methanol in the reaction mixture can surround the active sites of the lipase enzyme and can finally degrade its structure [1,6]. The highest methyl ester yield of 85.3% was achieved at a methanol to oil ratio of a 3:1, and decreased to 40.4% when the molar ratio of 7:1 was utilized.

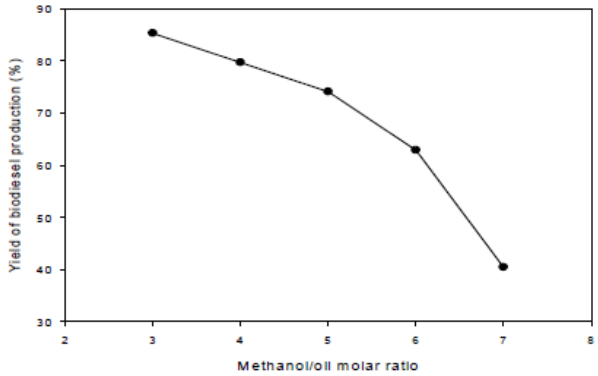

Fig. 4. Effect of methanol to oil molar ratio on biodiesel yield

Effect of canola oil to biocatalyst proportion on methyl ester yield: The influence of canola oil quantity in the presence of constant level of biocatalyst on methanolysis reaction was investigated. Reaction mixture involved 0.3 ml phosphate buffer and methanol to oil molar ratio of 3:1. According to results (Fig. 5) the methyl ester production decreased as the amount of substrate increased.

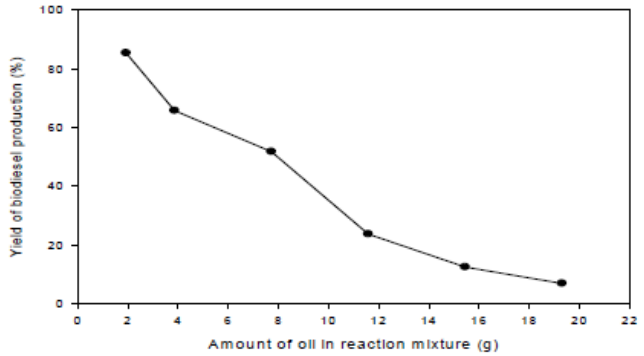

Fig. 5. Effect of oil to catalyst ratio on biodiesel yield

Methanolysis reaction in the PBR:A packed bed reactor system by the use of loofa-immobilized fungal cells of R. oryzae was utilized for methanolysis reaction of canola oil. This reactor was able to convert more amount of substrate at the same reaction time up to 92%. This may be due to the protection of immobilized cells from damaging shear stress [6].

8. Conclusions

An excellent immobilization of R. oryzae fungal cells was achieved by loofa-sponge as a natural porous carrier. The cells were entrapped within the sponge matrix during cultivation. The loofa-immobilized cells were used as catalyst in biodiesel production reaction from canola oil and some important reaction parameters such as water content, methanol to oil molar ratio and catalyst amount were investigated. The highest methyl ester yield of 85.3% was obtained in shake flask study. The methanolysis was also carried out in a packed-bed reactor and the conversion was promoted to 92%.

References

[1]. Ban, K., Hama, S., Nishizuka, K., Kaieda, M., Matsumoto, T., Kondo, A., Noda, H., Fukuda, H., 2002, Repeated use of whole-cell biocatalysts immobilized within biomass support particles for biodiesel fuel production, J. Mol. Catal. B: Enzyme. 17 , 157-165.

[2]. Ban, K., Kaieda, M., Matsumoto, T., Kondo, A., Fukuda, H., 2001, Whole cell biocatalyst for biodiesel fuel production utilizing Rhizopusoryzae cells immobilized within biomass support particles, Biochem. Eng. J. 8 , 39-43.

[3]. Fukuda, H., Kondo, A., Noda, H., 2001, Biodiesel fuel production by transesterification of oils, J. Biosci. Bioeng. 92, 405-416.

[4]. Gelbard, G., Bres, O., Vargas, R.M., Vielfaure, F., Schuchardt, U. F., 1995, 1H nuclear magnetic resonance determination of the yield of the transesterification of rapeseed oil with methanol, J. Am. Oil Chem. Soc. 72, 1239-1241.

[5]. Hajar, M., Shokrollahzadeh, S., Vahabzadeh, F., Monazzami, A., 2009, Solvent-free methanolysis of canola oil in a packed-bed reactor with use of Novozym 435 plus loofa, Enzyme Microb. Technol. 45, 188-194.

[6]. Hajar, M., Vahabzadeh, F., Shokrollahzadeh, S., 2008, Study on reaction parameters in lipase-catalyzed methanolysis of plant oil, Chem. Eng. Trans. 14, 295-300.

[7]. Hama, S., Yamaji, H., Fukumizu, T., Numata, T., Tamalampudi, S., Kondo, A., Noda, H., Fukuda, H., 2007, Biodiesel-fuel production in a packed-bed reactor using lipase-producing Rhizopusoryzae cells immobilized within biomass support particles, Biochem. Eng. J. 34, 273-278.

[8]. Liu, Y., Seki, M., Tanaka, H., Furusaki, S., 1998, Characteristics of Loofa (Luffacylindrica) sponge as a carrier for plant cell immobilization, J. Ferment. Bioeng. 85 416-421.

[9]. Ogbonna, J.C., Tomiyama, S., Tanaka, H., 1996, Development of a method for immobilization of non-flocculating cells in Loofa (Luffacylindrica) sponge, Process. Biochem. 31, 737-744.

[10]. Singh, S.P., Singh, D., 2010, Biodiesel production through the use of different sources and characterization of oils and their esters as the substitute of diesel: A review, Renew. Sust. Energy Rev. 14 200-216.

[11]. Tan, T., Lu, J., Nie, K., Deng, L., Wang, F., 2010, Biodiesel production with immobilized lipase: A review, Biotechnol. Adv. 28, 628-634.

[12]. Verdugo, C., Luque, R., Luna, D., Hidalgo, J.M., Posadillo, A., Sancho, E.D., Rodriguez, S., Ferreira-Dias, S., Bautista, F., Romero, A. A., 2010, A comprehensive study of reaction parameters in the enzymatic production of novel biofuels integrating glycerol into their composition, Bioresour. Technol. 101, 6657-6662.

[13]. Xie, W., H. Hydroxyl, Li, 2006, content and refractive index determinations on transesterified soybean oil, J. Am. Oil Chem. Soc. 10, 869-872.