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Enzymatic production of biodiesel from Jatropha oil: A comparative study of immobilized-whole cell and commercial lipases as a biocatalyst

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Abstract

The large percentage of biodiesel fuel (BDF) cost associated with feedstock oil and enzyme. In order to reduce the cost of BDF production, the lipase producing whole cells of *Rhizopus oryzae* (ROL) immobilized onto biomass support particles (BSPs) was used for the production of BDF from relatively low cost non-edible oil from the seeds of *Jatropha curcas*. The activity of ROL was compared with that of commercially available most effective lipase (Novozym 435). Different alcohols as a hydroxyl donor are tested, and methanolysis of Jatropha oil progresses faster than other alcoholysis regardless of lipases used. The maximum methyl esters content in the reaction mixture reaches 80 wt.% after 60 h using ROL, whereas it is 76% after 90 h using Novozym 435. Both the lipases can be used for repeated batches and both lipases exhibit more than 90% of their initial activities after five cycles. Our results suggest that whole-cell ROL immobilized on BSP is a promising biocatalyst for producing BDF from oil.

Keywords: Lipase; Transesterification; Filamentous fungi; Immobilized cells

1. Introduction

Biodiesel (BDF) produced by alcoholysis of vegetables oils or animal fats is viewed as promising renewable resources of fuel. The use of BDF is becoming increasingly important due to diminishing petroleum reserves and environmental regulations. BDF is expensive in comparison with petroleum-based fuel and 60–75% of the cost is associated with feedstock oil [1]. Therefore, the exploring ways to reduce the cost of BDF with respect to enzyme and substrate oils are of prime interest in the recent BDF research.

Jatropha curcas, an agro-forestry crop is a genus comprising 70 species growing in topical and sub-tropical countries. Jatropha grows as a natural habitat across sub-Sahara Africa, India, South East Asia and China. It grows rapidly, takes approximately 2–3 years to reach maturity and generate economic yields. It has a productive lifespan in excess of 30 years. The fatty acid composition of Jatropha oil is similar to other edible oils but the presence of some anti-nutritional factors such as toxic phorbol esters renders this oil unsuitable for cooking purposes [2]. Jatropha oil is thus a promising candidate for BDF production in terms of availability and cost.

BDF is industrially produced via chemical catalysis using strong bases as a catalyst. The strong base process suffers

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from several drawbacks such as difficulty in recovery of glycerol, removal of base catalyst from product and the treatment of alkaline wastewater. The utilization of lipases for the production of BDF has been reported as an effective means of circumventing the aforementioned problems [3,4]. The first difficulty of using lipase is that it is more expensive than the base catalyst like NaOH. Immobilized lipase is distinguished from free lipase because of its easy recovery from the reaction mixture facilitating its repeated use. Several researchers [5–7] have reported that the commercially available Novozym 435 (*Candida antarctica* lipase B immobilized on acrylic resin) was the most effective catalyst among tested lipases for the production of BDF. However, laborious and expensive purification processes of this lipase from culture broth are restricting its application for BDF production in industrial scale.

Here, we report that whole cells of lipase producing *Rhizopus oryzae* (ROL) immobilized onto biomass support particles (BSPs) made of reticulated polyurethane foam can catalyze the alcoholysis of Jatropha oil more effectively than Novozym 435. The advantage of using whole cells of *R. oryzae* immobilized onto BSP over Novozym 435 is that no labor intensive and cost associated lipase purification steps prior to the immobilization is required because the whole cells of *R. oryzae* can spontaneously immobilized onto BSPs during cultivation. Moreover, Jatropha oil makes the biodiesel fuel production more feasible for industrial applications than the other edible vegetable oils.

2. Materials and methods

2.1. Materials

Jatropha oil was obtained as a gift from Dr. Jayaveera, Jawaharlal Technological University (JNTU), Oil Technological Research Institute, Anantapur, India. The saponification value of Jatropha oil is 210. The water content in Jatropha oil is 1.5 wt.%. *Candida antarctica* lipase B immobilized on macro-porous acrylic resin (Novozym 435) was purchased from Sigma–Aldrich Japan K.K, Tokyo, Japan. According to the manufacturer, the enzyme belongs to the class of triacylglycerol hydrolases (EC 3.1.3.3), with a declared activity of ≥10,000 U/g (propyl laurate units per gram). All other chemicals are of analytical grade.

2.2. Microorganism and culture medium

R. oryzae IFO 4697 which has 1,3-positional specificity lipase was used as the whole-cell biocatalyst. The organism was maintained on 4% potato dextrose agar (Difco, Sparks, MD, USA) slants. *R. oryzae* was grown in basal medium containing 1% glucose/olive oil, polypepton 70 g, $NaNO_3$ 1.0 g, KH_2PO_4 1.0 g and $MgSO_4{\cdot}7H_2O$ 0.5 g in 1 l distilled water. Reticulated polyurethane foam particles (Bridge Stone Co. Ltd., Osaka, Japan) with a particle voidage of more than 97% and a pore size of 50 pores per linear inch were used for the immobilization of *R. oryzae*. In all the methanolysis experiments, 0.2 g of BSPs containing immobilized *R. oryzae* and Novozym 435 were used.

2.3. Air-lift bioreactor cultivation

Seed culture of *R. oryzae* was grown in 500 ml Sakaguchi flask containing 100 ml basal medium with 1% glucose. After cultivation for 24 h, the seed culture was transferred to air-lift bioreactor containing 10 l basal medium with 30 g/l olive oil and 12,000 BSPs. The bioreactor was maintained at 2.5 vvm at 30 °C. During the growth, *R. oryzae* cells were naturally immobilized in BSPs during the cultivation in air-lift bioreactor. After cultivation, the BSP-immobilized cells were separated from the culture medium by filtration, washed with tap water and dried at room temperature for around 24 h followed by cross linking with glutaraldehyde.

2.4. Glutaraldehyde treatment of immobilized cells

To stabilize the lipase activity, separated whole-cell biocatalysts were incubated with 0.1 vol.% glutaraldehyde (GA) solution at 25 °C for 1 h. BSPs were separated from the GA solution and were shaken in phosphate buffer at 4 °C for few minutes, washed with tap water for 1 min followed by drying at room temperature for 1 day.

2.5. Alcoholysis

Alcoholysis was carried out at 30 °C in 50 ml screw-capped vessel with reciprocal shaking at 150 rpm. A typical reaction mixture consisted of Jatropha oil (5 g), alcohol–oil molar ratio (3:1) and lipases (0.2 g) for the complete conversion of triglycerides to methyl esters. Reaction was started by adding lipase into pre-incubated reaction mixture. The alkyl ester contents were analyzed by capillary gas chromatography (GC) as described below [8]. The activity of lipases is expressed as amount of methyl esters (ME) produced per hour per gram lipase.

2.6. Analysis

Samples (150 μl) were taken from the reaction mixture at specified time and centrifuged at 12,000 rpm for 5 min to obtain the upper layer. The upper layer (80 μl) and tricaprylin (20 μl) were precisely weighed into a 10 ml bottle, to which a small amount of anhydrous sodium sulfate and hexane (3 ml) were added. Tricaprylin and sodium sulfate served as the internal standard and dehydrating agent, respectively. A 1.0 μl aliquot of the treated sample was injected into GC-18A gas chromatograph (Shimadzu Corp., Kyoto, Japan) connected to a DB-5 capillary column (0.25 mm × 10 m, J&W Scientific, Folsom, CA, USA). The column temperature was held at 150 °C for 0.5 min, raised to 300 °C at 10 °C/min, and maintained at this temperature for 3 min. The temperature for injector and flame ionization detector (FID) were set at 245 and 250 °C, respectively.