Journal of Molecular Catalysis B: Enzymatic 66 (2010) 166–171

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Lipase supported on granular activated carbon and activated carbon cloth as a catalyst in the synthesis of biodiesel fuel

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ARTICLE INFO

Article history:
Received 21 January 2010
Received in revised form 22 April 2010
Accepted 6 May 2010
Available online 13 May 2010

Keywords:
Enzymes
Biodiesel fuel
Lipase
Granular activated carbon
Activated carbon cloth

ABSTRACT

Fatty acid esters (biodiesel) were produced from the enzymatic transesterification of palm oil with methanol, ethanol, 1-propanol and 1-butanol. Isopropanol and isobutanol were also studied. *Candida antarctica B* lipase was immobilized on granular activated carbon (ACG-E) and activated carbon cloth (ACC-E) and used as a catalyst (biocatalyst). In the conversion of palm oil to alkyl esters using granular activated carbon as a support, isobutanol gave the highest conversion of 100%, isopropanol 86%, 1-butanol 77%, 1-propanol 68% and ethanol 57%, while only 48% methyl ester was observed with methanol. With activated carbon cloth used to support the enzyme, isobutanol gave the highest conversion of 82%, isobutanol 72%, isopropanol 59%, 1-butanol 45% and propanol 40%, while only 28% methyl ester was observed with methanol.

1. Introduction

The limited reserves of fossil fuels, the increasing prices of crude oils, and environmental concerns have spurred the development of alternative renewable energy sources. Biodiesel, which is a mixture of monoalkyl ester produced by transesterification of vegetable oils, has attracted considerable attention in the recent past as a renewable, biodegradable, and nonpolluting fuel. For industrial biodiesel production, homogeneous basic catalysts, including potassium hydroxide, sodium hydroxide, as well as potassium and sodium alkoxides, are commonly used for the transesterification of vegetable oils with methanol to produce fatty acid methyl esters [1,2]. In addition, biodiesel is better than diesel fuel in terms of sulphur content, flash point, aromatic content and biodegradability [2]. Several types of vegetable oils (soybean, rapeseed, sunflower and palm oils are the most studied), with diverse compositions of fatty acids, can be used for the preparation of biodiesel. Among the raw materials with potential for obtaining biodiesel, palm oil stands out for being the second most abundant oil in the world, as well as for the palm being characterized as having superior productivity among all other crops [3]. The conventional chemical route has several drawbacks: it is energy intensive, recovery of glycerol is difficult, the alkaline catalyst must be removed from the product, alkaline wastewater requires treatment and free fatty acids and water interfere with the reaction [4]. To minimize homogeneous process problems, attempts have been made to use heterogeneous catalyst systems in the alcoholysis of triglycerides [5–8]. These catalysts greatly simplify the post-treatment of the products (separation and purification). They can be easily separated from the system at the end of the reaction and may also be reused. Besides, the use of heterogeneous catalysts does not produce soaps through free fatty acid neutralization or triglyceride saponification. A large number of heterogeneous catalysts have been reported in the literature, including enzymes, zeolites, clays, guanidines heterogenized on organic polymers, ion-exchange resins and oxides, among others. Although the enzymatic process is still not commercially developed, a number of articles have shown that enzymes hold promise as catalysts. These studies mainly consist of optimizing the reaction conditions (temperature, alcohol/oil molar ratio, type of microorganism which generates the enzyme, enzyme amount and time, among others) to establish the characteristics for industrial applications [7–9]. The reaction is carried out under moderate temperatures, thus the catalyst and process temperature do not degrade the reactor material. Also, unlike chemical catalysis which works better with methanol [10], enzymes seem to prefer ethanol. In the case of chemical catalysis, the high temperature necessary

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doi:10.1016/j.molcatb.2010.05.002

in the process improves the miscibility between oil and methanol, while in the case of biocatalysis, the reaction is carried out at lower temperatures at which the miscibility of methanol in oil is very poor [1,11–13]. Methanol is also known to cause enzyme inactivation more than ethanol [12,13]. Hence, ethanol is generally preferred for carrying out lipase-catalysed transesterification for the preparation of biodiesel [7,12,13]. Ethanol as such is a renewable starting material for plant feedstock. Thus, an enzyme-based route fits better as a part of developing sustainable technology for biofuels. All of this has generated immense interest in using lipase for the production of biodiesel from a variety of oils/fats such as soybean [14], sunflower [8,15,16], cottonseed [17], rapeseed [7,18], palm oil [19,20], mango kernel [21], Jatropha oil [9] and beef tallow [22] (lipase-catalysed transesterification of mahua oil has not been attempted thus far). This interest exists despite the current high cost of the biocatalyst. It is hoped that efficient downstream processing techniques would make enzyme production costs much cheaper [23,24]. Also, if the enzyme-based transesterification is adopted on a large scale, a high demand would induce large-scale production of the enzyme and would result in the lowering of the market price of lipases. The existing usage of enzymes in several areas such as detergents, dairy products and textile and leather processing [25,26] reflect the validity of such a strategic approach. The present work shows the effect of different alcohols on biodiesel production when *Candida antarctica B* lipase was immobilized on granular activated carbon and activated carbon cloth to form the biocatalyst. The results were compared with systems that use enzymes without support. Additionally, different alcohols were used to study their influence in the production of biodiesel.

2. Materials and methods

The commercial *Candida antarctica B* lipase (Novozym 435®) was immobilized on the granular activated carbon, synthesized in our laboratory from coconut shell, and on the commercial activated carbon cloth (Zorflex®). Methanol, ethanol, 1-propanol, 1-butanol, isopropanol and isobutanol from Merck (purity > 99.8%, Merck, Germany) were used as reactants in the enzymatic reaction. Methyl myristate was purchased from Sigma Aldrich (St. Louis, US) and used as an internal standard. All other chemicals were reagent grade. The biocatalysts were labelled as AGG-E for the enzyme supported on granular activated carbon and ACC-E for the enzyme supported on activated carbon cloth.

2.1. Characterization of the prepared activated carbons used as supports

Coconut shell was cut into small pieces (1.0–2.0 mm), washed with distilled water and oven dried at 120 °C overnight. A series of 10 g of the sample were mixed with 100 ml of $ZnCl_2$ solution of various concentrations (5–40%, w/w). The impregnation process was performed at 80 °C in an oil bath until the excess water had evaporated. The impregnated sample was dried in an oven at 150 °C overnight. The sample was placed inside a quartz tube and inserted horizontally into the middle of a tubular electric furnace. The carbonization and activation were carried out at 800 °C under N_2 gas flow for 5 h followed by CO_2 gas flow for 1 h. The resulting activated carbon was washed with 0.05 M HCl followed by distilled water until traces of chloride ions were no longer detected. The textural characteristics of the resulting activated carbons were determined by nitrogen adsorption at 77 K using an automatic adsorption instrument (Quantachrome, Autosorb-3B). Prior to gas adsorption measurements, the samples were degassed at 300 °C in a vacuum condition for 3 h. Adsorption data were obtained over a relative pressure, P/P_0, ranging from approximately 10^{-6} to 1. The surface area, pore volume and pore size distribution of the activated carbons were determined by the application of the Brunauer–Emmett–Teller (BET) and *t*-plot analysis software available with the instrument, respectively. The BET surface areas were assessed by applying relative pressures ranging from 0.01 to 0.15. The total pore volumes (V_t, cm^3/g) were estimated to be the liquid volumes of N_2 at a high relative pressure near unity (~0.99). The *t*-plot method was applied to the micropore volume and mesopore surface area, and the mesopore volume was obtained by deducting the micropore volume from the total pore volume. Pore size distribution of the activated carbons was obtained by applying the micromeritics density functional theory (DFT) method to the nitrogen adsorption isotherms using the software supplied by Autosorb-3B. The microstructures of activated carbons produced from coconut shell and commercial cloth with the enzyme were examined using scanning electron microscopy (JEOL JSM-5600 LV Model SEM).

2.2. Preparation of pH-tuned enzyme

Lipase (50 mg) from *P. cepacia* was dissolved in 0.5 ml of 0.05 M phosphate buffer at pH 7.0 (this was the optimum pH for the lipases as reported by the vendors and other workers [5–8]). The enzyme solution was immediately frozen at −20 °C and lyophilized for 24 h [26]. These were referred to as "pH-tuned" enzyme preparations.

2.3. Enzyme immobilization

The activated carbon and activated carbon cloth (50 mg) were placed in 5 ml capped vials and moistened with 150 μl of 95% methanol, ethanol, 1-propanol, 1-butanol, isopropanol and isobutanol. This was followed by the addition of 4 ml of *Candida antarctica B* lipase solutions in 20 mM potassium phosphate buffer at pH 7.0. The vials were incubated at 25 °C with constant shaking at 300 rpm overnight. The solutions were then withdrawn from each vial and stored, while the solid porous particles were washed twice with 1 ml of phosphate buffer. The lipase activity and protein were determined in immobilization solution and washings. The immobilized lipase preparations were dried using a speed vacuum system (UVS4004 Universal Vacuum system, Thermo Savant). In this study was fixed of pH and temperature for study of activity of enzyme on alcohols.

2.4. Enzymatic transesterification reaction

Palm oil (0.5 g) with methanol, ethanol, 1-propanol, 1-butanol, isopropanol or isobutanol were each placed into different screw-capped vials at a molar ratio of 1:6. The pH-tuned lipase preparations (50 mg) were added to these vials and the mixtures were incubated at 40 °C with a constant shaking at 300 rpm [9]. The progress of the reaction was monitored using aliquots (40 μl) removed at various time intervals. The alkyl esters formed were analyzed using Gas Chromatograph (GC).

2.5. GC Analysis

At the end of the reaction, the enzyme was separated out by filtration and the filtrate was washed with distilled water and hexane after transferring it to a separating funnel. The ethyl esters phase, diluted with hexane, was mixed with methyl myristate, which served as the internal standard. The ethyl ester content in the reaction mixture was quantified by gas-chromatography using a GS Varian 3400, equipped with a fused silica capillary column (30 m × 0.32 mm × 0.1 μm). The column temperature was held at 150 °C for 2 min, then heated to 190 °C at 4 °C/min and held at that temperature for 3 min, heated again to 250 °C at 5 °C/min and