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Lipase supported on mesoporous materials as a catalyst in the synthesis of biodiesel from *Persea americana mill oil*

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ABSTRACT

Fatty acid esters (biodiesel) were produced from the enzymatic transesterification of *Persea americana mill oil* (in South America known as Aguacate) with methanol, ethanol, propanol, 2,2-dimethyl-1-propanol, 2-methyl-1-butanol, and 3-methyl-1-butanol were studied. *Candida antarctica B* lipase was immobilised on mesoporous foam synthesised using nonylphenol ethoxylated with $n = 4$ (FC-4), and the enzyme was supported on foam carbon with ethoxylated nonylphenol $n = 6$ (FC-6) for obtaining the biocatalyst. In the conversion of *Persea americana mill oil* to alkyl esters using (FC-6) as a support, 3-methyl-1-butanol gave the highest conversion of 100%, 2-methyl-1-butanol 92%, 2,2-dimethyl-1-propanol 81%, propanol 70% and ethanol 60%, and 48% methyl ester was observed with methanol. With FC-4 used to support the enzyme, 3-methyl-1-butanol gave the highest conversion of 70%, 2-methyl-1-butanol 65%, 2,2-dimethyl-1-propanol 58%, propanol 50% and ethanol 45%, and 37% methyl ester was observed with methanol.

1. Introduction

Biodiesel is composed of a mixture of fatty acid alkyl esters. It is a natural substitute for petroleum-derived diesel fuel and has similar or better specifications concerning density, viscosity, cetane number and flash point, among others. Because biodiesel is formed from renewable resources such as plant oils, it is considered CO_2-neutral, biodegradable and will help conserve fossil fuels. Compared to traditional diesel fuels, its combustion leads to a substantial reduction in polluting emissions [1–4]. Industrially, biodiesel can be produced by the transesterification of vegetable oils and short chain alcohols, usually methanol, with alkaline or acid catalysts. The reaction products are a mixture of the desired esters, mono and diglycerides, glycerol, water and the catalysts. Compared to the process mediated by enzymes, this process is more energy consuming. Due to the presence of soap byproducts, separation and purification of the chemically produced biodiesel requires somewhat more complex steps than enzymatically produced biodiesel. Therefore, the use of biocatalysts could be an interesting alternative because it is more environmentally attractive because biodiesel synthesised enzymatically can be used directly without purification [3–9]. Lipase-catalysed transesterification of vegetable oils has been investigated by many researchers in the last few years [4–12]. For cost reasons, methanol is the alcohol most frequently used for triglyceride transesterification. Nevertheless, other alcohols are also used. In Brazil, one the biggest world plant oil producers, biodiesel is obtained by ethanolysis of triglycerides, since ethanol is a cheap and abundant commodity produced from the fermentation of sucrose from sugarcane. Alternatively, either propanol or butanol can also be used in this process, especially because these two alcohols promote a better miscibility between the alcohol and the oil phases [7]. The use of a triglyceride feedstock for biodiesel production depends on regional availability and economics and many vegetable oils can be used, such as soybean [3,13–15], sunflower [16–18], and rapeseed [3]. The main differences among these oils are their fatty acid compositions, which strongly affects some important properties of the biodiesel (cetane number, heat of combustion, melting point and viscosity) [1,2]. Oxidation of biodiesel is a common problem, depending on the source of vegetable oil.

A large number of heterogeneous catalysts have been reported in the literature, including enzymes, zeolites, clays, guanidines heterogenised 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 optimising the reaction conditions (temperature, alcohol/oil molar

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ratio, type of microorganism which generates the enzyme, enzyme amount and time, among others) to establish the characteristics for industrial applications [19–22]. 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, enzymes seem to prefer ethanol. In the case of chemical catalysis, the high temperature necessary 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 [23–25]. Methanol is also known to cause enzyme inactivation more than ethanol [24,25]. Hence, ethanol is generally preferred for carrying out lipase-catalysed transesterification for the preparation of biodiesel [19,24,25]. 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 [26], sunflower [20,26–28], cottonseed [29], rapeseed [19,30], palm oil [31,32], mango kernel [33], Jatropha oil [22] and beef tallow [32] (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 [33,34]. 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 [35,36] reflects 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 immobilised on mesoporous carbon foams using various non-ionic surfactants from the biocatalyst, of *Persea americana mill oil*. 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

2.1. Samples preparation

Activated carbon foam was successfully prepared from phenolic resin, which was synthesised with phenol and formaldehyde under alkaline conditions. The surfactant template synthesis route used to produce mesoporous carbon foam (STn-CF) is illustrated in Fig. 1, where n is the number of ethylene oxide residues per mole of nonylphenol. Typically, phenol and formaldehyde were dissolved in ethanol (PFE). Nonylphenol ethoxylate was then added to the above solution under stirring. The molar composition of phenol/formaldehyde/ethanol/in the nonylphenol ethoxylate solution was 1:2:60:0.38. NaOH was added to the mixture to initiate polymerisation of formaldehyde and resorcinol. Residual NaOH was then removed from the polymerised phenolic resin by repeated immersions in 70 °C water. Finally, the dried polymer precursor was carbonised at 680 °C, using a heating rate of 1 °C/min under purified nitrogen flow, to decompose the surfactant template, giving mesoporous carbon foams. The carbon foam samples produced in the present study were labelled FC-4 and FC-6, corresponding to carbon foams synthesised using nonylphenol ethoxylated surfactant templates with $n = 4$ and 6 ethylene oxide residues per mole of nonylphenol, respectively.

The commercial *Candida antarctica B* lipase (Novozym 435™) was immobilised on the foams carbon (FC), synthesised in our laboratory, as describe before. Methanol, ethanol, propanol,

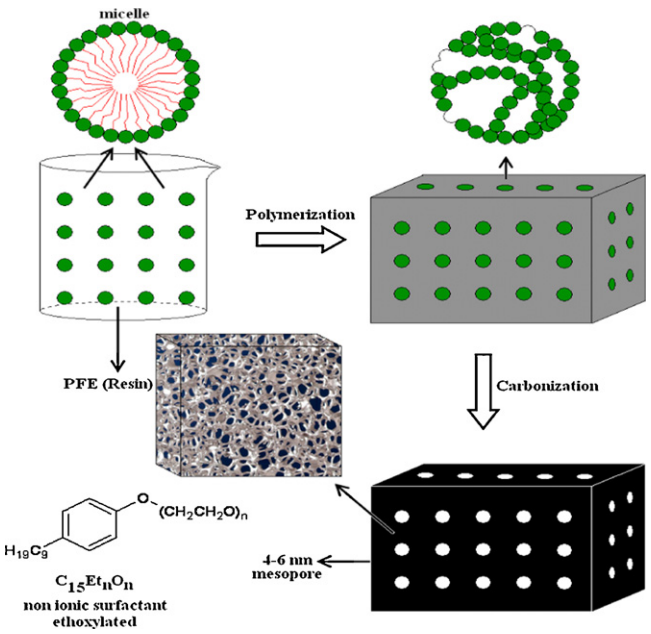

Fig. 1. Preparation of mesoporous carbon foam using a non-ionic surfactant template.

2,2-dimethyl-1-propanol, 2-methyl-1-butanol, and 3-methyl-1-butanol 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 FC-4 and for the enzyme supported on foam carbon synthesised with nonylphenol ethoxylated with $n = 4$, and FC-6 for the enzyme supported on foam carbon with nonylphenol ethoxylated $n = 6$.

2.2. Sample characterisation

The pore structures of the carbon foams synthesised in the present study were examined by SEM. Pore size, porosity, bulk density, and compressive strength were measured by using standard methods. Nitrogen adsorption isotherms were determined at −196 °C using an Autosorb 3B (Quantachrome, Boynton Beach, Miami, FL, US) system in static measurement mode. Prior to sorption experiments, samples were outgassed at 400 °C for 10 h. Pore size distributions were calculated by analysing the desorption branches of the isotherm using the Barrett–Joyner–Halenda (BJH) method [37].

2.3. Preparation of pH-tuned enzyme

Lipase (50 mg) from *Pseudomona Cepacia* was dissolved in 0.5 ml of 0.01 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 lyophilised for 24 h [26]. These were referred to as "pH-tuned" enzyme preparations.

2.4. Enzyme immobilisation

The foams carbon (25 mg) were placed in 5 ml capped vials and moistened with 100 μl of 95% methanol, ethanol, 1-propanol, 1-butanol, isopropanol and isobutanol. This was followed by the addition of 5 ml of *Candida antarctica B* lipase solutions in 10 mM potassium phosphate buffer at pH 7.0. The vials were incubated at