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Lipase coated clusters of iron oxide nanoparticles for biodiesel synthesis in a solvent free medium

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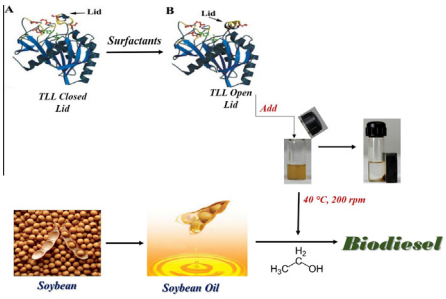
HIGHLIGHTS

- TLL precipitated over the clusters of Fe_3O_4 was used for obtaining biodiesel from soybean oil.
- Complete conversion to biodiesel was obtained in 3 h.
- Interfacial activation of the lipase resulted in more active enzyme preparation.
- The biocatalyst design enables one to use an economical amount of lipase.

GRAPHICAL ABSTRACT



Thermomyces lanuginosus lipase (TLL) in 'open lid' form precipitated over clusters of Fe_3O_4 nanoparticles in an organic solvent catalyzed biodiesel synthesis at very high rates. The substrate soybean oil itself was used as the reaction medium.

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ABSTRACT

Methyl or ethyl esters of long chain fatty acids are called biodiesel. Biodiesel is synthesized by the alcoholysis of oils/fats. In this work, lipase from *Thermomyces lanuginosus* was precipitated over the clusters of Fe_3O_4 nanoparticles. This biocatalyst preparation was used for obtaining biodiesel from soybean oil. After optimization of both immobilization conditions and process parameters, complete conversion to biodiesel was obtained in 3 h and on lowering the enzyme amount, as little as 1.7 U of lipase gave 96% conversion in 7 h. The solvent free media with oil:ethanol (w/w) of 1:4 and 40 °C with 2% (w/w) water along with 20% (w/w) silica (for facilitating acyl migration) were employed for reaching this high % of conversion. The biocatalyst design enables one to use a rather small amount of lipase. This should help in switching over to a biobased production of biodiesel.

1. Introduction

Methyl or ethyl esters of long chain fatty acids are called biodiesel (Nordblad et al., 2014; Xie and Wang, 2014). Biodiesel is synthesized by the alcoholysis of oils/fats (Banerjee et al., 2013; Xie and Wang, 2014; Xie and Zang, 2016). While most of the industrial processes employ chemical catalysts, few plants have started using lipases as the catalysts (Fjerbaek et al., 2009). The enzyme based approaches in general offer greener options and various applications of lipases in organic synthesis are already available (Singh et al., 2013; Malhotra et al., 2015). The major issue in adoption of the use of lipases in the production of biodiesel is the cost of the biocatalyst (Fjerbaek et al., 2009). One solution to this problem is to enhance the catalytic efficiency of the lipases. There is a good deal of scope there as enzymes in low water media tend to display

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[1] An Indian patent application [Prov. Pat. No. 1585/DEL/2015 dated 02.06.2015] has been filed describing this application of the biocatalyst design.

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low catalytic activity (Shah and Gupta, 2007a; Mukherjee and Gupta, 2012; Mukherjee et al., 2015). Preparation of biodiesel using lipase employs either nearly anhydrous organic solvents or even solvent free media (Kumari et al., 2007; Antczak et al., 2009; Shah and Gupta, 2007b).

In the present work, we used *Thermomyces lanuginosus* lipase (TLL) for the synthesis of biodiesel from soybean oil. Soybean oil is composed of approximately 16% saturated fatty acids (palmitic [C16:0] and stearic [C18:0]), 24% monounsaturated fatty acids (oleic [C18:1]), and 60% polyunsaturated fatty acids (linoleic [C18:2] and linolenic [C18:3]) (Neff and List, 1999). We describe a biocatalyst design in which an inexpensive lipase [TLL] has been precipitated over a suspension of ~5 nm (diameter) Fe_3O_4 nanoparticles in an organic solvent. This approach was developed for immobilizing alpha chymotrypsin and was found to give high catalytic efficiency for the biocatalyst in low water media (Mukherjee and Gupta, 2012). It has also been shown earlier that enzymes (including lipases) precipitated with an organic solvent (called enzyme precipitated and rinsed with organic solvents or EPROS) show much better activity than enzyme powders obtained by drying with freeze drying (Roy and Gupta, 2004; Shah et al., 2006; Solanki and Gupta, 2008; Mukherjee and Gupta, 2015). We show here that the lipase deposited over clusters of Fe_3O_4 nanoparticles (called enzyme coated clusters of nanoparticles or ECCNs) had higher catalytic activity than simple EPROS of the lipase. The biodiesel synthesis was carried out in solvent free media.

2. Methods

2.1. Materials

T. lanuginosus lipase (TLL) was a kind gift from Novozyme, Denmark. Solvents like *n*-propanol was obtained from Sigma–Aldrich Co. (St. Louis, MO, USA). Ethanol was procured from Merck, Hohenbrunn, Germany. *N*-octyl-β-D-glucopyranoside (OG) was obtained from Calbiochem, Massachusetts, USA. Refined soybean oil was purchased from the grocery store. All other reagents and solvents were obtained from Fisher Scientific and were of the highest grade commercially available.

2.2. Methods

2.2.1. Synthesis of Fe_3O_4 nanoparticles

The magnetic Fe_3O_4 nanoparticles were prepared by the hydrothermal co-precipitation method (Solanki and Gupta, 2011). Solution (45 mL) containing 0.32 M $FeCl_3{\cdot}6H_2O$ and 0.16 M $FeSO_4{\cdot}4H_2O$ were prepared in deoxygenated milli-Q water and NaOH solution (5 mL, 10 g NaOH in 5 mL) was added to the above mentioned solution drop wise so that the final concentration of NaOH in the solution becomes 5 M over a period of 5 min at 60 °C. This resulted in the formation of black precipitate of Fe_3O_4 nanoparticles. The nanoparticles formed were repeatedly washed with milli-Q water (at least 6–7 times) to remove the excess NaOH till a neutral pH is reached. Thereafter they were air dried using a vacuum pump which resulted in free flowing magnetic nanoparticles which were stored for further use throughout the work.

2.2.2. Preparation of lipase coated clusters of nanoparticles

A suspension of nanoparticles in acetone was prepared (dry weight of the nanoparticles in mg in 2 mL of acetone) and they were extensively sonicated for 40 min at 88 W power and a frequency of 40 Hz in a sonicator bath [Model No. Elma D-78224] containing chilled water so that the temperature of the solution was maintained at 25 °C during sonication. A uniform suspension of nanoparticles was obtained. This suspension was then added in aliquots of 0.4 mL to the lipase solution (0.5 mL in 200 mM Tris HCl buffer, pH 7.5 containing 40 mM OG wherever mentioned) placed in a vial at 4 °C on a shaker at constant shaking of 250 rpm. The suspension was shaken for 30 min and then separated using a magnetic separator. The supernatant was removed and the precipitate was rinsed three times with dry chilled acetone (2 mL), and then twice with dry chilled ethanol (2 mL) which is the reaction medium for the transesterification reaction.

The control in this case was called enzyme precipitated and rinsed in organic solvent (EPROS) of TLL.

2.2.3. Synthesis of biodiesel using different biocatalyst preparations of lipase

Soybean oil (0.5 g) and ethanol were taken in the molar ratio of 1:4 in a vial. Optimum amount of water and silica (w/w of the oil) was added to the reaction mixture. The lipase preparation was added to this and incubated at the optimum temperature with a constant shaking at 200 rpm. Reactions were carried out in duplicate, and the yields between duplicates were found to be within 3%. The progress of reaction was monitored by taking aliquots (40 μL) from the reaction mixture at different time intervals and analyzed by gas chromatography.

2.2.4. GC analysis of alkyl esters

The fatty acid ethyl esters (biodiesel) formed were analyzed with methyl heptadecanoate as internal standard by GC on an Agilent 6890N system fitted with a capillary column EQUITY TM – 5 (30 m × 0.32 mm × 0.25 μm film thickness) from Supelco (Bellefonte, USA) with flame ionization detection. The programme used was: initial oven temperature 100 °C, ramp at 15 °C/min up to 380 °C. The detector temperature was maintained at 300 °C. Peak areas of fatty acid esters and internal standard were obtained. Result for the fatty acid ester content was expressed as a mass fraction in percent using methyl heptadecanoate C17 as the internal standard by using the following formula:

$$c = \frac{\Sigma A - A'}{A'} \times \frac{C' \times V'}{m} \times 100\% \quad (1)$$

where ΣA = total peak area C14:0–C24:1; A' = internal standard peak area (methyl heptadecanoate); C' = concentration of internal standard solution in mg mL^{-1}; V' = volume of internal standard solution used in mL; m = mass of the sample in mg.

Reactions were carried out in duplicate, and the conversions between duplicates were found to be within 3%.

3. Results and discussion

ECCNs were prepared by adding a suspension of Fe_3O_4 nanoparticles in acetone to an aqueous solution of *T. lanuginosus* lipase (TLL). TLL is commercially available and is a relatively inexpensive lipase. It has been frequently used in the synthesis of biodiesel (Rodriguez et al., 2010; Mangas-Sánchez and Adlercreutz, 2015). ECCNs of TLL were employed for synthesizing biodiesel from soybean oil in this work. It has been seen earlier that the ratio of nanoparticles to alpha chymotrypsin plays an important role in the performance of the ECCNs. Thus, the ratio was optimized. Keeping the concentration of Fe_3O_4 nanoparticles in the suspension at 2 mg mL^{-1} of organic solvent, the concentration of the enzyme was varied in the range of 7–28 U in 0.5 mL of the aqueous buffer. The preparation obtained with 14 U of the enzyme seemed to be the best in terms of both initial rates and % conversion (Fig. 1). Thus, the ratio of the nanoparticles to the enzyme (4 mg:14 U) was used for the further experiments.

The ratio of alcohol:oil (the two substrates) is known to affect the % conversion in the biodiesel synthesis (Antczak et al., 2009).