Biochemical Engineering Journal 101 (2015) 23–31

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Regular article

Preparation of highly reusable biocatalysts by immobilization of lipases on epoxy-functionalized silica for production of biodiesel from canola oil

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ARTICLE INFO

Article history:
Received 9 March 2015
Received in revised form 8 April 2015
Accepted 26 April 2015
Available online 28 April 2015

Keywords:
Biocatalysis
Biodiesel
Enzyme
Lipase
Immobilized enzymes
Epoxy-functionalized silica

ABSTRACT

In the present work, lipases from *Candida antarctica* (CALB), *Thermomyces lanuginosus* (TLL) and *Rhizomucor miehei* (RML) were covalently immobilized on epoxy-functionalized silica. The immobilized lipases were used to produce biodiesel by transesterification of canola oil with methanol. It was found that lipases immobilized on silica provided biocatalyst derivatives with lower cost compared with the cost of commercially available Novozym 435. Thermal stability of the immobilized derivatives and the influence of methanol on the catalytic activity were also evaluated. Optimum oil to methanol ratio at 1:3 was observed for CALB and RML in biodiesel production; the corresponding fatty acid methyl ester (FAME) yields obtained after 96 h were 68% and 45% at 50 °C respectively. The lipase from *T. lanuginosus* immobilized on epoxy-functionalized silica displayed particularly high catalytic ability regarding reaction rate and final yield. TLL also gave high FAME contents in the reaction mixture with up to 6 molar equivalents of methanol to oil (98%). The immobilized TLL was quite stable and can be reused for 16 cycles without significant loss in activity (5%). The immobilized preparations of RML and CALB also presented a good reusability, keeping 85% of their initial activities after 16 cycles of the reaction.

1. Introduction

Over the last decades extensive studies have been carried out in different countries for providing appropriate resources of alternative fuels. One strategy is substitution of fossil fuels with fuels of biological origin, such as bioethanol or biodiesel [1]. Biodiesel is defined as fatty acid alkyl ester derived from a variety of vegetable oils, animal fats and waste oil. It can be used in unmodified diesel engines since it has similar physical properties to petroleum light oil [2,3]. The biodiesel combustion generally decreases the level of sulfur oxides, halogens, CO and CO_2. In addition biodiesel fuel is biodegradable, non-toxic and a renewable source of energy [4].

Nowadays, biodiesel is produced from vegetable oils in Europe and North America [5,6] and waste edible oil in Japan [7]. Transesterification of sunflower oil [8], rapeseed oil [9], soybean oil and beef tallow [10] for biodiesel has been reported. Biodiesel are produced by esterification of free fatty acids (FFA) or transesterification of oils and fats with short chain alcohols. For most technical applications methanol is used because it is available as an anhydrous, low-priced and active alcohol. Therefore, biodiesel fatty acid alkyl esters (FAAEs) most commonly refers to fatty acid methyl esters (FAMEs).

As an alternative to the conventional chemically catalyzed process, biodiesel can also be produced using enzymatic catalysis. Chemical processes give high yield in short reaction times but requires vegetable oil of high quality or alternatively a two-step process with acidic esterification of FFA prior to alkaline transesterification. Difficulties in the recovery of the catalyst and glycerol and potential pollution to the environment are other disadvantages in alkali or acid catalyzed processes. Another issue is the energy consumed since the reaction is conducted at high temperatures [1,11]. The method with enzymes as the catalyst has developed quickly in recent years [12–14]. The main advantages of these processes are as follows: milder conditions, lower energy consumption; broader

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http://dx.doi.org/10.1016/j.bej.2015.04.020
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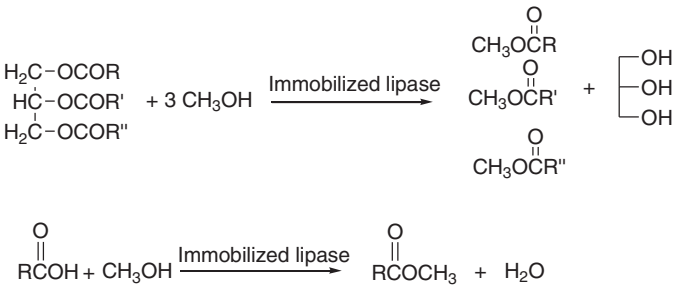


Scheme 1. Lipase-catalysed transesterification of triacylglycerols and esterification of free fatty acids to fatty acid methyl esters.

selection of feedstocks including waste oils with a high acidity; easy separation of catalyst from the reaction mixture – especially if the enzyme is immobilized; easier subsequent separation and purification of biodiesel; and an overall more environment-friendly process [15,16].

Lipases are the enzymes of choice for biodiesel production [17–19]. The most desired characteristic property of the lipases is their activity on both acylglycerols as well as the free fatty acids to produce biodiesel in non-aqueous media (Scheme 1) [20]. An ideal lipase for biodiesel production should result in short reaction times, it should be stable at elevated temperature in the presences of alcohols, and it should be easy to reuse [21]. Whereas promising biodiesel results recently have been reported with free, liquid formulated enzymes [22–24], immobilized enzymes have obvious advantages in terms of stability and ease of recovery. Immobilized enzymes are defined as "enzymes physically confined or localized in a certain defined region of space with retention of their catalytic activities, and which can be used repeatedly and continuously" [25]. Other advantages of enzyme immobilization include the potential of continuous use in a packed-bed reactor, high stability and easy separation from the reaction mixture [26–28].

Various methods for enzyme immobilization have been carried out, such as physical methods (adsorption, entrapment and encapsulation) and chemical methods (covalent bonding and cross-linking). The immobilization method by covalent bonding to the support has the advantage of strong interactions between the enzyme and the support which makes enzyme leaching uncommon [29]. In enzyme immobilization, several carriers are widely employed. Mineral carriers have a high standard of mechanical properties and thermal stability. They are inert toward solvents and do not swell. Consequently, silica gel has drawn considerable attention as a low-cost carrier for enzyme immobilization. Silica gel is an amorphous inorganic polymer displaying siloxane groups (Si—O—Si) and silanol groups (Si—OH) on its surface. Recently, modification of silica gel with inorganic and organic functional groups has been performed [30–32]. Surface modification is usually implemented by silanization with an organosilane compound. Enzyme immobilization by covalent binding will prevent desorption from matrix. Also the strength of bonding reduces enzyme vibration and conformational changes, potentially increasing the stability of the enzyme.

Although enzyme immobilization increases the stability of the enzyme, high concentration of methanol (>1/2 molar equivalent) will typically inactivate the catalyst. Short chain alcohols, especially methanol, have low solubility in oils; therefore a new liquid phase appears in the biodiesel reaction mixture leading to an inactivation of the enzyme. Shimada et al. found that stepwise addition of methanol protected the enzyme from inactivation [7].

During our development of lipase immobilization techniques for organic synthesis applications [33–36], we have investigated silica gel surface treatments with respect to the immobilization efficiency of lipases as well as applicability of that treatment for use in biodiesel synthesis. This paper describes the modification of silica surfaces and immobilization of three lipases *Candida antarctica* lipase B (CALB), *Thermomyces lanuginosus* lipase (TLL) and *Rhizomucor miehei* lipase (RML) onto epoxy functionalized silica. Canola has the highest productivity per hectare in Iran, so in relation to the oil feedstock, canola oil was selected for lipase-catalyzed methanolysis, with reference to the impact of reaction parameters, such as temperature and methanol to oil molar ratio.

2. Materials and methods

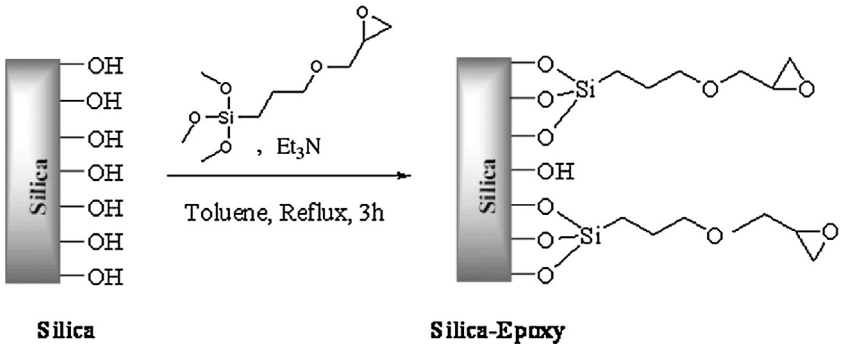
2.1. Materials

Lipases from *T. lanuginosus* and *R. miehei*, methyl ester standards (methyl laurate, methyl stearate, methyl linoleate, methyl oleate, methyl palmitate and methyl myristate), Silica gel 70–230 mesh (distribution particle size of 0.063–0.2 mm) were purchased from Sigma–Aldrich. Lipase B from *C. antarctica* was kindly donated by Novozymes (Bagsvaerd, Denmark). Canola oil was purchased from a local market. Its saponification value and water content were determined to be 190.93 and 0.08% (w/w), respectively. Water content was measured by Karl Fischer titration method [37]. Methanol, triethylamine and silica gel were purchased from Merck. 3-Glycidyloxypropyl trimethoxysilane (3-GPTMS) was purchased from Acros. All other chemicals were obtained commercially and were of analytical reagent grade.

2.2. Methods

2.2.1. Functionalization of silica particles

One gram of dry silica gel was mixed in a dry toluene solution (30 ml) containing 3-GPTMS (1 ml) and triethyamine Et_3N (0.15 ml) (Scheme 2). The resulting mixture was refluxed under argon atmosphere and constant stirring for 4 h. The silica gel was then washed



Scheme 2. A description of the silica gel treatment used for immobilization. The surface was then reacted directly with lipases as described in materials and methods.