Biochemical Engineering Journal 81 (2013) 15–23

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Regular article

Biodiesel production from soybean soapstock acid oil by hydrolysis in subcritical water followed by lipase-catalyzed esterification using a fermented solid in a packed-bed reactor

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ARTICLE INFO

Article history:
Received 19 February 2013
Received in revised form 30 June 2013
Accepted 26 September 2013
Available online 5 October 2013

Keywords:
Biodiesel
Hydroesterification
Lipases
Burkholderia cepacia
Solid-state fermentation
Packed-bed reactor

ABSTRACT

We investigated a new hydroesterification strategy for the production of biodiesel from low-value oil feedstocks: complete hydrolysis of the feedstock to fatty acids in subcritical water, followed by the use of a packed-bed reactor, containing a fermented solid with lipase activity, to convert the fatty acids to their ethyl esters. The fermented solids were produced by cultivating *Burkholderia cepacia* LTEB11 for 72 h on a 1:1 mixture, by mass, of sugarcane bagasse and sunflower seed meal. The esterification of fatty acids obtained from soybean soapstock acid oil was studied in the packed-bed bioreactor, in a solvent-free system, with the best results being a 92% conversion in 31 h, obtained at 50 °C. When the packed-bed reactor was reused in successive 48-h esterification reactions, conversions of over 84% of the fatty acids to esters were maintained for five cycles at 50 °C and for six cycles at 45 °C. Unlike previous hydroesterification processes that have used lipase-catalyzed hydrolysis followed by chemically-catalyzed esterification, our process does not expose the lipases to contaminants present in low quality feedstocks such as soapstocks. This advantage opens the possibility of operating the packed-bed esterification reactor in continuous mode.

1. Introduction

Biodiesel is currently being produced as a substitute for petrodiesel, however, it is not economically competitive, with its use requiring either subsidies or government policies, such as in Brazil, where all petrodiesel is currently required to contain 5% biodiesel [1]. It is composed of esters of short-chain alcohols (methyl or ethyl alcohols) and long-chain fatty acids. The feedstocks used in most commercial biodiesel production processes are derived from triacylglycerols of edible vegetable oils [2]. However, these oils are relatively expensive, and since the feedstock in biodiesel synthesis corresponds to 50–85% of total production costs, it is desirable to use low-cost starting materials, in order to increase the commercial competitiveness of biodiesel [3–5]. Potential low-value feedstocks include animal fat from sewage and residual oil of domestic, industrial or commercial origin.

Current industrial processes for the production of biodiesel from vegetable oils use alkaline transesterification, which gives high yields (98%) in a short reaction time of about 1 h. However, alkaline transesterification requires starting materials with low levels of moisture and free fatty acids and therefore is not appropriate for the production of biodiesel from low-value feedstocks, which contain significant amounts of free fatty acids or water. Free fatty acids react with the alkaline catalyst, producing soaps. This decreases the reaction yield and makes the separation of products difficult. Too much water favors hydrolysis over transesterification. Additionally, the alkaline catalyst contaminates both the glycerol and the biodiesel that are produced. Its removal from the biodiesel requires several washings with water. This not only generates large amounts of wastewater, but also gives rise to residual water in final product [6,7].

Several strategies can be used to overcome problems caused by the presence of fatty acids in low-value feedstocks. It is possible to carry out the process using catalysis with an inorganic acid [8–10], a heterogeneous catalyst [11,12] or lipases [4,13–16], since all can simultaneously catalyze esterification and transesterification. It is also possible to use a two-step process. In the first step, the free fatty acids are converted to esters using an acid catalyst (such as sulfuric acid), with the remaining acylglycerols in the feedstock then being converted to biodiesel esters by alkaline transesterification [17,18].

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1369-703X/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.bej.2013.09.017

All of these processes transesterify the acylglycerols and esterify the free fatty acid, while avoiding the formation of soaps. However, acid catalysts are highly corrosive to equipment, while both heterogeneous catalysts and lipases are expensive and transesterification rates are relatively slow [7]. Also, if there is a significant amount of water in the reaction medium then it will compete with the alcohol, promoting the hydrolysis of the acylglycerols.

Other strategies have also been tried. Haas et al. [8] first carried out an alkali-catalyzed saponification of soybean soapstock, which converts both free fatty acids and the fatty acids of acylglycerols into soaps. The soaps were recovered and then acidified to produce free fatty acids, which were then esterified in an acid-catalyzed process. On the other hand, Wang et al. [19] acidified the soapstock, causing it to separate into an aqueous phase and an acid oil phase containing free fatty acids and acylglycerols. This acid oil phase was then subjected to an acid-catalyzed esterification/transesterification process. However, both of these processes involve homogeneous catalysis with acids, which must be separated from the product by successive washes, generating wastewater contaminated with catalyst and reaction products.

Recently, the production of biodiesel by hydroesterification of oils has been proposed [20–23]. The process involves two steps. In the first step, triacylglycerols are hydrolyzed completely to fatty acids and glycerol. In the second step, the fatty acids recovered from the first step are esterified with an alcohol to give the corresponding ester and water. This process is advantageous for biodiesel production when low-value feedstocks are used. Since the first step is a hydrolysis step, the water content and the free fatty acid content of the feedstock do not interfere with final yields. Additionally, the aqueous glycerol produced in the hydrolysis step is more pure than that obtained in the alkali-catalyzed transesterification process [21,22].

Although both the hydrolysis and esterification steps in a hydroesterification process can be carried out chemically, several authors have investigated the potential of using commercial or plant lipases to catalyze the hydrolysis step [21–23]. The present work takes a different approach. We produce fatty acids by hydrolysis in subcritical water of several low-value feedstocks and we then use lipases, produced by *Burkholderia cepacia* LTEB11, to catalyze esterification with ethanol. Ethanol was selected as the alcohol because it is less toxic than methanol and is abundantly available in Brazil. In order to minimize the costs of the lipases, we use them in the form of "dried fermented solids", obtained by solid-state fermentation.

2. Materials and methods

2.1. Raw materials

Soybean oil, soybean soapstock acid oil, beef tallow and waste cooking oil were donated by the company Ubaldino Rodrigues Soares e Cia. Ltda. (Ponta Grossa, Paraná, Brazil). All other reagents were of analytical grade.

2.2. Hydrolysis of fat feedstocks

Hydrolysis of the different feedstocks (Table 1) was carried out in the pilot plant of the company Ubaldino Rodrigues Soares e Cia. Ltda. (Ponta Grossa, Paraná, Brazil). The process involves continuous hydrolysis of the feedstocks in the presence of subcritical water, in a pressure tower at 60 atm and 250 °C [24]. In this process, fatty material and water react in a countercurrent flow in the absence of catalyst. The free fatty acids produced are discharged from the top of the tower and the water/glycerol mixture is discharged from the bottom of the tower. The free fatty acids from each feedstock were distilled and their compositions (Table 1) were determined by gas chromatography [25]. All free fatty acid preparations contained less than 0.5% (w/w) water. Saponification and acid values were determined according to the methods of the American Oil Chemist's Society (AOCS Official Method Cd 3-25 and Ca 5a-40) [26,27].

2.3. Microorganism

B. cepacia LTEB11 was maintained at −18 °C in Luria Bertani (LB) medium with 50% (w/v) glycerol. A stock culture was transferred to a LB agar plate and incubated for 48 h at 29 °C. Isolated colonies were transferred to 30 mL of LB medium in a 250-mL Erlenmeyer flask and then incubated on a rotary shaker at 29 °C and 200 rpm for 8–10 h, which represents mid-exponential phase. This culture broth was used as inoculum for the solid-state fermentation.

2.4. Solid-state fermentation

The fermented solid was obtained by solid-state fermentation (SSF) of a mixture of sugarcane bagasse and sunflower seed meal (1:1, w/w on a dry basis). Sugarcane bagasse was donated by Usina de Álcool Melhoramentos (Jussara, Paraná, Brazil) and sunflower seed was purchased in the local market. Sugarcane bagasse and sunflower seed were milled, separately, in a knife mill, followed by sieving to obtain particles ranging between 0.85 and 2.36 mm. The SSF was done in 2000-mL Erlenmeyer flasks, each containing 80 g of milled dry substrate. Phosphate buffer solution (0.1 mol L^{-1}, pH 7.0) was added to obtain 75% moisture (w/w, wet basis). The moisture content was determined in an infrared moisture balance (Gehaka IV 2000, São Paulo, Brazil). Flasks were plugged with cotton wool and autoclaved at 121 °C for 20 min. After cooling, the substrates were inoculated with 8 mL of inoculum and incubated at 29 °C. During the fermentation, the hydrolytic and esterification activities of the fermented solids were determined every 24 h. After incubation, the fermented solids were dried (see Section 2.5) to a moisture content of less than 10% (w/w on a wet basis) and stored at 4 °C. The dry fermented solids were then used directly in all esterification reactions.

2.5. Drying and preparation of the fermented solid

Three different drying processes were evaluated. In the first, frozen fermented solids were lyophilized for 24 h at 10^{-1} mbar and −40 °C in a lyophilizer (Jouan LP3, Virginia, USA). In the second, the fresh fermented solids were dried in a column made with two integrated polyvinyl chloride tubes (each 50 cm height and 4.3 cm diameter). The lower tube was filled with activated silica to dry the air and the top contained 200 g of fresh fermented solids. Air at approximately 25 °C was blown at 20 L min^{-1} into the bottom of the column. In the third, 200 g of fresh fermented solids was placed in a fan-forced laboratory oven (Nova Ética 400-3ND, São Paulo, Brazil) at 30 °C. During the drying, the moisture content of the solids was determined with the infrared moisture balance, and the hydrolytic activity of the dried fermented solids was compared with that of the fresh fermented solids. Values are expressed as the means of triplicate analyses ± the standard error of the mean.

In reactions where *n*-hexane was used as the solvent, in order to avoid interferences in the determination of the esterification activity, the fermented solids were delipidated after drying to remove lipids deriving from the fermentation. Delipidation involved three washes with *n*-hexane (10 mL per gram of fermented solids). In each washing, the mixture was agitated vigorously for 10 min at 200 rpm and 25 °C. The solution was then filtered and the retained solids were dried in a vacuum desiccator at room temperature. In the solvent-free reaction system, the delipidation was unnecessary