Enzyme and Microbial Technology 58–59 (2014) 60–67

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

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

Enhancing trimethylolpropane esters synthesis through lipase immobilized on surface hydrophobic modified support and appropriate substrate feeding methods

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ARTICLE INFO

Article history:
Received 21 September 2013
Received in revised form
29 December 2013
Accepted 10 February 2014
Available online 19 February 2014

Keywords:
Esterification
Immobilized lipase
Substrate feeding
Surface hydrophobic treatment
Biodegradable lubricants

ABSTRACT

Candida sp. 99–125 lipase immobilized on surface hydrophobic modified support and appropriate substrate feeding methods were used to improve the synthesis of tri-substituted trimethylolpropane (TMP) esters, which can be used as raw materials for biodegradable lubricants. The proposed novel production method is environmentally friendly. Lipase was adsorbed on surface hydrophobic silk fibers that were pretreated by amino-modified polydimethylsiloxane. A 5-level-4-factors central composite model, including reaction time, temperature, enzyme amount, and molar ratio of fatty acid to TMP, was designed to evaluate the interaction of process variables in the enzymatic esterification. The water activity was kept constant using a LiCl-saturated salt solution. Under the optimum conditions with 30% enzyme amount and substrates molar ratio 8.4 at 45 °C for 47 h, the total conversion of caprylic acid is 97.3% and the yield of tri-substituted TMP esters is 95.5%. The surface hydrophobic treatment resulted in less cluster water accumulated on the surface immobilized lipase, which was demonstrated by near-infrared spectra. Consequently, the optimum temperature and water tolerance of immobilized lipase were increased. Two TMP-feeding methods were used to maintain high molar ratio of fatty acid to TMP, and increase the final tri-substituted TMP esters content exceeding 85% (w/w) in reactant.

1. Introduction

Lubricants and their production need to be environmentally adapted [1–4] and a higher level of performance is required [2,3]. The environment must be protected against pollution caused by the current petroleum-based lubricants, with approximately 50% of all lubricants sold worldwide ending up in the environment via volatilization, spills, or total loss applications [5,6]. In addition, high-quality lubricants should be developed to satisfy the extreme conditions required. After World War II, the development of high performing ester-based fluids was closely linked to the development of the aviation gas turbine. Synthetic esters (SEs) were hence increasingly popular as lubricant base oils due to superior technical properties, their high quality, the possibility to achieve tailor-made properties, the lack of toxicity, their excellent biodegradation and their non-petroleum based synthesis [7]. Trimethylolpropane (TMP) or pentaerythritol comprises a neopentyl carbon that determines the high performance of SEs if used as an alcohol substrate.

Traditional methods of producing SEs were alkali-based reactions [7,8]. A relative new and promising development in the production of biodegradable lubricant oils and additives making use of enzymatic esterification with lipase as catalyst, and has been suggested since the late 1990s [9,10]. Unlike alkali-based reactions, the products can be easily collected and separated. The particular benefits offered by enzymes are significant: mild synthesis conditions and reduced waste. The structure of TMP is quite similar with glycerin, but it is non-natural substrate for biocatalysts. Synthesis of TMP esters in solvent-free system by transesterification [9–11] or direct esterification [12,13] with immobilized lipase were reported. Direct esterification could avoid release of toxic methanol, and was proven to be more effective than transesterification, although the direct esterification took a long time (>72 h) at relative low temperature (30–70 °C) [12,13]. The reaction time could be reduced to less than 36 h when the reaction temperature was increased to 100 °C, but most lipase cannot keep activity under such high temperature. It almost took about 10 days for the reaction between oleic acid (OA) and TMP (at a molar ratio of 3:1) at 70 °C

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http://dx.doi.org/10.1016/j.enzmictec.2014.02.006
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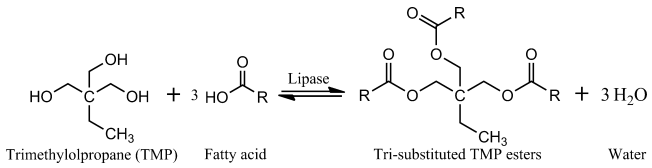

Fig. 1. The reaction scheme of esterification of TMP with fatty acid.

to reach equilibrium at 70% conversion of OA in a closed system [12].

A particular structural property of many lipases is the presence of a mobile subdomain, called lid, whose conformational changes control the access of substrate molecules to the active site. The lid has two distinct conformations: (1) a closed form in which the subdomain covers the active site considering lipase inactive; and (2) an open active form with an accessible active site [14–16]. These conformational rearrangements are generally assumed to be induced either by the adsorption of the enzyme to a hydrophobic interface [17] or by substrate binding [15]. Namely, these two conformations of lipase are in an equilibrium affected by the environmental conditions. Taking advantage of the catalytic mechanism, two strategies for lipase immobilization [18] were developed with an improved activity: (1) lipase adsorbed on hydrophobic supports; (2) lipase immobilized on the presence of detergents that was thought to permit the hyperactivation of lipases. Prof. Guisan et al. [19] and Prof. Fernandez-Lafuente et al. [20–22] explained that during adsorption lipase is interfacially activated versus hydrophobic supports, and the hydrophobic areas surround the active centre and stabilize the open form of the lipase. These are the reasons for lipase immobilized on hydrophobic supports usually exhibited higher activity than the free enzyme or other immobilized enzymes preparation. In addition, different supports with different surface hydrophilicity/hydrophobicity will greatly alter enzyme properties such as stability [23–25], activity [23–25], selectivity [26], or specificity [27]. Hence, the interfacial adsorption of lipases on hydrophobic support becomes a popular strategy to prepare immobilized lipase with high catalytic activity. Due to the three-dimensional symmetric structure of hydroxymethyl of TMP, the esterification of TMP with sole fatty acid (See Fig. 1) resulted in unique mono-, di-, tri-substituted TMP esters. The goal of this work was to enhance the synthesis of tri-substituted TMP esters because the free hydroxyl group of mono-, di-substitued TMP esters leads to pour point reversion of lubricant. Despite the reports on many methods involving immobilized lipase property modification [28–30] and reactor design [31] to improve the efficiency of esterification, enhancements of the synthesis of polyol esters like tri-substituted TMP esters have not been reported.

In this work, lipase immobilized on surface hydrophobic modified silk fibers was prepared to improve the synthesis of tri-substituted trimethylolpropane esters. Hydrophobic silk fibers functionalized with methyl groups were prepared by treatment with amino-functional polydimethylsiloxane (PDMS). Lipase was immobilized on PDMS-treated (hydrophobic) and native (hydrophilic) silk fibers to compare the effect of different support surface properties on synthesis of TMP esters. Meanwhile, comparisons of three different kind lipase forms were carried out via investigation of the hydrolysis ability to tri-substituted TMP esters under relative low water content (<17.5%, w/w) system and characterization of the water molecular state by near-infrared spectra. In order to assess the interaction of process variables in the enzymatic esterification by lipase immobilized on surface hydrophobic modified silk fibers, a 5-level-4-factors central composite model according to the principle of response surface methodology (RSM) [31] was used, including reaction time, temperature, enzyme amount and molar ratio of fatty acid to TMP. Caprylic acid, used as acyl donor in this work, is the common name for the eight-carbon saturated fatty acid, which can be found in coconut oil; caprylic acid with a lower flammability and explosion hazard is more benign and environmentally friendly than other common acyl donors (e.g., vinyl acetate) [32]. The water activity (aw) was kept constant value using a LiCl-saturated salt solution (aw = 0.12).

The 2nd principle of Green Chemistry [33] requires as follows: synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final products. For polyol reactions when saturated substituted esters are the final product, it requires high molar ratio of fatty acid to polyol in order to reach the highest yield of polyol. However, the excess fatty acid will decrease the final maximum content of product in reactant and make the separation more costly and complex. In our case, the RSM method suggested the optimum molar ratio between TMP and caprylic acid to be 1:8.4, so the theoretical maximum content of tri-substituted TMP esters in the reactant was 39.7% (w/w). Therefore, the excess caprylic acid should be consumed. Hence, two different TMP-feeding methods keeping relative high process molar ratio of caprylic acid to TMP were designed to improve the final content of tri-substituted TMP esters in reactants.

2. Materials and methods

2.1. Chemicals

Trimethylolpropane (2-ethyl-2-(hydroxymethyl)-1, 3-propanediol) with a melting range of 56–59 °C and caprylic acid with a purity of 98% were obtained from Fuchen Chemical Co. Ltd. (Tianjin, China). The other solvents and salts of analytical grade were obtained from Beijing Chemical Factory. They were dried by molecular sieves before using.

2.2. Lipase immobilization

Lipase (EC.3.1.1.3) was obtained from *Candida* sp. 99–125. Its characterization and catalytic properties were described in previous works [34–36]. The method of immobilization was developed in our laboratory, and the procedure was previously published [37]. Prior to immobilization, dried fabric films were treated by dipping in 0.5% (w/v) solution of amino-modified polydimethylsiloxane in hexane at 35 °C for 1 h with slight shaking. Then, the treated fabric films were rinsed with fresh hexane and dried in an oven at 70 °C for 1 h to remove the residual solvents. The crude lipase powders were dissolved in phosphate buffer (25 mmol/L, pH 8.0). The concentration of lipase solution was 10 mg/mL. Subsequently, the solution was centrifuged at 8085 g for 10 min to remove the insoluble residues and the supernatant was used for the immobilization of lipases. Adsorption of lipase was carried out by immersing each fiber film (native or PDMS-treated) into 10 ml lipase solution in a 50 ml flask and then incubating overnight in a water bath at 15 °C with slight shaking. Finally, the films were taken out and washed three times with the phosphate buffer (50 mmol/L, pH 8.0), and then dried under reduced pressure. The immobilized lipase fiber films were stored at 4 °C before use. Esterification activity of immobilized lipase was estimated through a model esterification reaction of dodecanoic acid and lauryl alcohol. A standard esterification procedure was described in previous papers [23]. The esterification activity of lipase immobilized on PDMS-treated silk fibers was 620 μmol/(min cm^2). The static water contact angle of this immobilized lipase was 117° and results in a lower accumulation of water on the surface compared with lipase immobilized on native fibers [23].

2.3. Experiments design and statistical analysis of RSM

A 5-level-4-factors central composite design with three center-points was used according to the principle of RSM. The four factors were enzyme amount (*En*, wt.% based on the weight of fatty acid), reaction time (*Ti*, h), reaction temperature (*Te*, °C), and substrate molar ratio (*Mr*, caprylic acid/TMP, mol/mol). The total conversion of caprylic acid (mol%) and the formation of tri-substituted TMP esters (TRI, mol%) were the response. The ranges of settings for factors were determined on the basis of the primary investigation of single factors and chosen as follows: *En* 10, 15, 20, 25, 30%; *Ti*, 12, 24, 36, 48, 60 h; *Te*, 30, 35, 40, 45, 50 °C; *Mr*, 6, 7, 8, 9, 10 mol%. The variables and the applied ranges are presented in Table 1.

The experiments of RSM design took place in a 50 ml flask connected with another 50 ml flask filled with LiCl-saturated salt solution (aw = 0.12) in order to keep the water activity at a constant value. 2.02 g caprylic acid was mixed with different amounts of TMP, corresponding to different substrate molar ratios generated by RSM. About 25 min after addition of TMP to caprylic acid at 40 °C, a clear, homogeneous solution was observed. Heating to 60 °C accelerated the dissolution of TMP. Subsequently, different amounts of lipase were added. The reaction was carried out