Journal of the Taiwan Institute of Chemical Engineers 45 (2014) 2233–2237

Contents lists available at ScienceDirect

Journal of the Taiwan Institute of Chemical Engineers

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

Lipase-catalyzed synthesis of butyl propionate in solvent-free system: Optimization by response surface methodology

Wei-Chen Dai [a], Shwu-Jer Chiu [a], Dao-Yi Huang [b], Horng-Yi Juan [a], Cheng-Yu Chen [a], Shang-Shian Chen [a], Chia-Hung Su [a,*], Sing-Ying Li [a]

[a] Department of Chemical Engineering, Ming-Chi University of Technology, No. 84 Gungjuan Rd., Taishan, Taipei 24301, Taiwan
[b] Department of Mechanical Engineering, Ming-Chi University of Technology, No. 84 Gungjuan Rd., Taishan, Taipei 24301, Taiwan

ARTICLE INFO

Article history:
Received 19 December 2013
Received in revised form 6 May 2014
Accepted 11 May 2014
Available online 23 June 2014

Keywords:
Solvent-free
Esterification
Butyl propionate
Lipase

ABSTRACT

This paper presents the lipase-catalyzed synthesis of butyl propionate from butanol and propionic acid in solvent-free system. Commercially available lipases from different sources were evaluated for their catalytic activity in the reaction. Among the tested biocatalysts, Novozym 435, which is an immobilized lipase, was found to be the most active. The reaction conditions affecting the lipase-catalyzed reaction were systematically optimized using a response surface methodology. An empirical model was developed to correlate the reaction factors to the reaction yield. A maximal reaction yield of 93.76% was obtained at 42.77 °C, at a butanol/propionic acid molar ratio of 9, at an enzyme loading of 2.35%, and at a reaction time of 24.87 h. The correspondence of the predicted and experimental yields indicated that the model was reliable in predicting the reaction yield. The reusability of Novozym 435 was also studied. Under the optimal reaction conditions, the biocatalyst could be reused at least 20 times to drive the reaction to a conversion yield of 92%.

1. Introduction

Butyl propionate (BP) is a colorless and fruit-flavor liquid that can be used as a solvent for dissolving nitrocellulose and natural and synthetic resins [1]. Furthermore, it is also used in daily essence and lacquer manufacturing [1]. The usage of BP as a volatile solvent alternative has recently received a considerable amount of attention because of its relatively low volatility and high electrical resistance [2–4]. In addition, BP is a sustainable solvent because its feedstocks (butanol and propionic acid) can be produced using microbial fermentation technology [5,6]. Because of these merits, several studies have focused on developing more efficient, more economical, and safer processes for BP production.

Regarding the commercial production of BP, the esterification of butanol and propionic acid can be catalyzed using homogeneous acid catalysts, such as sulfuric and hydrochloric acids [1]. However, the quality of the product is adversely affected by the homogeneous catalysts [7]. Additional purification processes are required to remove the nonrecyclable catalysts from the product, thus increasing the production cost and leading to wastewater pollution. To circumvent the mentioned problems, the homogeneous catalysts can be replaced by using solid acid catalysts, such as functionalized molecular sieves [1,7] and commercial cation-exchange resins [2–4]. Although the synthesis of BP, which is catalyzed by the solid catalysts, is efficient because of its fast reaction rate and high yield, it involves several drawbacks, such as the requirement of high reaction temperature, the high cost involved in complex catalyst synthesis, and the leaching of catalyst active sites that might cause catalyst deactivation and product contamination [8].

Enzyme-catalyzed esterification shows considerable promise for eliminating the inherent problems associated with the use of chemical catalysts because it is an energy-saving, eco-friendly, and highly selective process [9–11]. Swarts *et al.* [12] investigated the lipase-catalyzed esterification of butanol and propionic acid in the presence of an organic solvent, which facilitates product extraction from the reaction mixtures. However, eliminating solvent usage in the process is technically feasible and offers substantial advantages. Reactions performed under solvent-free conditions can enhance the volumetric productivity, reduce environmental hazards, and allow for the recovery of products by using simple purification steps [13–15]. Although the lipase-catalyzed biotransformation in the presence or absence of organic solvents have been widely studied [16], based on thorough research, few studies

* Corresponding author. Tel.: +886 2 29089899x4665/933732616; fax: +886 2 29083072.
E-mail addresses: chsu@mail.mcut.edu.tw, d913612@alumni.nthu.edu.tw (C.-H. Su).

http://dx.doi.org/10.1016/j.jtice.2014.05.007
1876-1070/

address lipase-catalyzed esterification for BP synthesis [12,17], particularly in solvent-free systems.

In this work, the esterification of butanol and propionic acid catalyzed by various lipases in a solvent-free system was studied. To effectively analyze the factors affecting the reaction, response surface methodology (RSM) was used to optimize the esterification reaction by conducting few experiments and maintaining a high level of statistical significance among the results [18]. Under the optimal reaction conditions, the reusability of the biocatalyst was also examined.

2. Materials and methods

2.1. Materials

Two immobilized lipases containing Novozym 435 (*Candida antarctica* lipase) and Lipozyme RM–IM (*Rhizomucor miehei* lipase) were purchased from Novozymes A/S (Bagsvaerd, Denmark). Amano lipase PS (*Burkholderia cepacia*) and lipase G (*Penicillium camemberti*) were obtained from Amano Enzyme Inc. (Nagoya, Japan). Lipases from *Rhizopus oryzae*, *Candida rugosa*, and porcine pancreas were purchased from Sigma–Aldrich Corporation (Missouri, USA). Propionic acid, butanol, and other reagents were provided by ECHO Chemical Co. Ltd. (Miaoli, Taiwan).

2.2. Optimization of esterification using the RSM

Butanol was added to propionic acid at different molar ratios in 150-mL Erlenmeyer flasks, followed by the addition of Novozym 435. The mixtures of butanol, propionic acid, and the enzyme were stirred in an orbital shaker (130 rpm). To optimize the reaction conditions, a 5-level and 4-factorial central composite design was employed to investigate the reaction factors affecting the synthesis yield of BP. Experiments were conducted under various reaction temperatures (30–50 °C), molar ratios of butanol to propionic acid (1:1–9:1), enzyme loadings (0.5–2.5%, relative to the weight of propionic acid), and reaction times (12–36 h). The BP yield was determined using the sample withdrawn from the reaction mixtures.

The response function of interest was the BP yield. The function was approximated using the following quadratic equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{11} X_1^2 + \beta_{22} {X_2}^2 + \beta_{33} X_3^2 + \beta_{44} X_4^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 + \beta_{23} X_2 X_3 + \beta_{24} X_2 X_4 + \beta_{34} X_3 X_4 \quad (1)$$

where Y is the BP yield; X_1 is the reaction temperature; X_2 is the butanol/propionic acid molar ratio; X_3 is the enzyme loading; X_4 is the reaction time; β_0 is the offset term; β_1 to β_4 are linear parameters; β_{12}, β_{13}, β_{14}, β_{23}, β_{24}, and β_{34} are interaction parameters; and β_{11}, β_{22}, β_{33}, and β_{44} are quadratic parameters. The model parameters were determined using the method of least squares [19]. After the parameters were determined, the empirical model was used to plot surfaces for all factors and to determine the optimal reaction conditions for obtaining a maximal yield using a canonical method [19]. Design-Expert 7 was used to establish the model parameters, conduct an analysis of variance (ANOVA), and determine the optimal reaction conditions.

2.3. Reuse experiments

The esterification of butanol and propionic acid was initiated by adding Novozym 435. The reaction was conducted under the optimal conditions determined using the RSM. After the reaction was completed, Novozym 435 was separated from the reaction medium through filtration. The recovered enzyme was re-mixed with fresh reactants to initiate a new reaction. In each experimental cycle, the BP yield was determined from the withdrawn sample.

2.4. Determination of BP

The BP yield during the esterification was determined using the modified method of Su *et al.* [20]. A sample was withdrawn from the reaction medium and weighed, then dissolved in 20 mL diethyl ether–ethanol (1:1 volume ratio) solution. The sample mixture was then titrated against 0.1 M KOH until the phenolphthalein endpoint was reached. The BP yield was calculated as being equivalent to the consumed acid. The reliability of the method was verified according to an appropriate calibration curve. A similar method has been successfully applied in determining the ester yield during other esterification reactions [19,21].

3. Results and discussion

3.1. Enzyme screening

Seven commercial lipases from different sources were screened by incubating 3% lipase with a 1:1 molar ratio of butanol to propionic acid at 40 °C for 12 h. Table 1 shows the lipase activity in the esterification of butanol and propionic acid. The BP yield varied significantly according to the types of lipase. The highest yield of BP was 56.41% when using Novozym 435; however, Amano lipase PS displayed low activity. Novozym 435 was the most active biocatalyst in the reaction. A previous study also reported that Novozym 435 can drive the synthesis of butyl esters to a high yield [22]. Furthermore, Novozym 435 is a thermostable lipase and can be used repeatedly [23]. Therefore, Novozym 435 was chosen for further study.

3.2. Development of the RSM model

Novozym 435 was used to catalyze the esterification of butanol and propionic acid. The effects of the reaction temperature, molar ratio of butanol to propionic acid, enzyme loading, and reaction time on the BP yield were examined using a central composite RSM design involving 7 central replicates. Table 2 lists the variables and their coded and uncoded values. The reaction temperature, butanol/propionic acid molar ratio, enzyme loading, and reaction time were input variables, whereas the BP yield was set as a measured response. The experimental results using the central composite RSM design are shown in Table 3. Based on the central runs of the experiments (runs 25–31), a relatively low value of the coefficient of variance (0.55%) on the repeated experiments indicated the reproducibility of the experiments. The measured responses can be demonstrated using an empirical model. The coded variables were correlated to the BP yield by using a quadratic

Table 1
Effect of various lipases on reaction yield of butyl propionate.

Lipase	Yield[a] (%)
Novozym 435	56.41 ± 4.13
Porcine pancreas lipase	13.46 ± 1.52
Candida rugosa lipase	12.82 ± 2.21
Rhizopus oryzae lipase	11.56 ± 3.34
Lipozyme RM–IM	7.69 ± 1.52
Amano lipase G	3.85 ± 0.88
Amano lipase PS	2.56 ± 1.11

[a] Data are the mean values ± standard deviation of three repeated experiments.