Journal of Molecular Catalysis B: Enzymatic 99 (2014) 143–149

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

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

Synthesis of isobutyl propionate using immobilized lipase in a solvent free system: Optimization and kinetic studies

Vishakha V. Kuperkar, Vikesh G. Lade, Arushi Prakash, Virendra K. Rathod *

Department of Chemical Engineering, Institute of Chemical Technology, Matunga (E), Mumbai 400019, India

ARTICLE INFO

Article history:
Received 23 July 2013
Received in revised form 30 October 2013
Accepted 30 October 2013
Available online 8 November 2013

Keywords:
Isobutyl propionate
Novozym® 435
Solvent free system (SFS)
Kinetic models
Ping-Pong Bi–Bi mechanism

ABSTRACT

Isobutyl propionate is widely used in food and beverage industries as a rum flavor. This work presents the optimization and kinetic aspects of synthesis of isobutyl propionate by esterification of propionic acid with isobutyl alcohol using immobilized lipase Novozym® 435 in a solvent free system (SFS). Process parameters such as reaction time, temperature, enzyme loading, speed of agitation, water concentration and acid to alcohol molar ratio were optimized to achieve maximum conversion. Higher conversion of 92.52% was obtained with the reaction conditions such as: temperature 40 °C, enzyme loading 5% w/w, acid to alcohol molar ratio 1:3, time 10 h and stirring speed of 300 rpm. The bisubstrate kinetic models of the enzyme catalyzed reactions namely Ordered Bi–Bi, Random Bi–Bi and Ping-Pong Bi–Bi were applied to determine the initial rates and correlated with the experimental findings. Ping-Pong Bi–Bi model with substrate inhibition by both acid and alcohol gives the best fit with parameter values as V_{max} = 0.5 Mol/min/g catalyst, K_A = 0.631 M, K_B = 0.003 M, K_{iA} = 0.0042 M and K_{iB} = 0.1539 M for the concentration ranges of 2.25–10.21 M for propionic acid and 2.55–9.01 M for iso-butanol. The immobilized lipase could be reused for seven times with the % conversion of acid reaching to 83%; signifies that still it can be reused for several more times. SFS is the added benefit to produce such commercially valuable flavor ester.

1. Introduction

The esterification is a reaction wherein alcohol reacts with acid in presence of catalyst to produce ester with elimination of water. Short-chain esters are the class of compounds that are widely distributed in nature and are major components of cosmetics, food flavor, fragrance, pharmaceutical industries due to its natural aroma. Currently commercial market for food flavor is increasing rapidly. Esters obtained by chemical synthesis suffer from drawbacks like high temperature and pressure, harsh reaction conditions involving strong acid catalyst, hazardous chemicals, longer reaction time and low conversion [1]. Other flaws associated are tedious separation processes, toxicity, and unwanted harmful byproducts [2]. Natural flavors extracted from fruits or plants are too expensive and also incapable of fulfilling growing commercial demands [3]. Therefore it is industrially and economically important to synthesize flavors using cheaper and more broadly available material to meet the consumer demand exploring the alternative methods of the production [1,4]. Use of biocatalyst for the production of flavor is green technology based approach and natural, unlike using chemical catalysts. Application of enzymes as a biocatalyst is the most frequently used technique of biosynthesis to produce the flavors with high specificity, mild reaction conditions and greater efficiency [5]. Lipases (triacylglycerol ester hydrolysis EC 3.1.1.3) are enzymes that can catalyze esterification, transesterification, and hydrolysis reactions [6].

Lipases are important class of enzymes because of their properties like regiospecificity, stereospecificity and substrate specificity [7] and their milder reaction conditions that reduce the energy requirements [8]. Recently, lipases immobilized on various supports like macroporous acrylic resin [9] polyacryclic resin, polyurethane foams [5], Amberlite and Celite [10] and cotton cloth [11] have come up for the industrial production of various specialty esters, aroma compounds and active agents. These immobilized techniques have provided more chances to use biocatalysts for various reactions in wider range of operating conditions in terms of pH, temperature and pressure [12,13]. Novozym® 435 is commercial immobilized lipase preparation supplied by Novozymes, immobilized on macroporous polyacrylic resin beads. It has applications ranging from biodiesel production to fine chemistry [14].

The variant shift has occurred in the lipase catalyzed reactions based on the operating media viz. aqueous → biphasic → non-aqueous media (organic). Since, major emphasis was given to organic solvents to produce short-chain fatty acids ester in middle

* Corresponding author. Tel.: +91 22 33612020; fax: +91 22 33611020.
E-mail address: vk.rathod@ictmumbai.edu.in (V.K. Rathod).

1381-1177/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.molcatb.2013.10.024

decades; lot of literature exists with combinations of substrates with experimental and/or statistical determination of optimum reaction conditions for maximum yield in shortest duration [3,14–18]. However the use of toxic organic solvents is being progressively restricted for many applications due to industrial and social implications. Recently the major shift has occurred in the production of the esters where in the reactions are preferred in the solvent free system; as it facilitates the downstream processing thus reduction in cost and environmental hazards [2]. There are few research studies concerning solvent free system for lipase catalyzed production of flavor ester and it is found that the initial rates are found to increase as compared to organic solvent [18,19].

The kinetic studies can provide better insight of the reaction mechanisms of enzyme catalyzed reactions. The kinetics of the specific reaction can follow the specific kinetic model based on its reaction mechanism. The kinetics information (operating conditions and rate parameters) of the esterification reaction is useful for the designing and scale-up of the reactor. However, kinetic studies of lipase-catalyzed esterification in organic solvents or SFS are remarkably rare; most of these are based on the Michaelis–Menten assumptions [5]. By virtue of the importance of kinetic models, the proper assessment of the dynamics of lipase-catalyzed esterification reactions has been done using several models for different combinations of substrates and enzymes over the years. Most lipases are said to follow the Ping-Pong Bi–Bi mechanism [11,20–22] although Ordered reaction mechanism [23] and Random mechanism [24] have also been reported in the literature.

Isobutyl propionate is an organic ester having an ethereal, rum-like, fruity odor and therefore it is used as rum flavor to beverages, candies, and baked goods [25]. Thus, this ester flavor has a high commercial demand and it is less reported in literature. Therefore, the objective of the present research work is synthesis of isobutyl propionate in SFS using immobilized lipase as biocatalyst. The optimization of process parameters was carried out based on the investigations relating to the influence of reaction temperature, enzyme load, speed of agitation, water concentration and substrate ratio. Three kinetic mechanisms namely Ordered Bi–Bi mechanism, Random Bi–Bi mechanism and Ping-Pong Bi–Bi mechanism were tested for the validation of the experimental data.

2. Materials and methods

2.1. Materials

Novozym® 435 (lipase B from Candida antarctica; immobilized on macroporous polyacrylic resin beads, bead size 0.3–0.9 mm, bulk density 0.430 g/cm^3) was generously gifted by Zytex Biotech Pvt. Ltd., Mumbai (India). Isobutyl alcohol [*B*] and Propionic acid [*A*] used were A.R. grade (with 99% purity) and were supplied by HiMedia Laboratories Private Limited, Mumbai and Thomas Baker (Chemicals) Pvt. Ltd., Mumbai, respectively.

2.2. Experimental method

The experimental set up consisted of 4.5 cm i.d. three necked baffled glass reactor of 50 ml capacity; provided with six-bladed turbine impeller. The entire assembly was immersed in a thermostatic water bath, which was maintained at the desired temperature with an accuracy of ±2 °C. Electric motor with speed controller was provided for agitation. The experiment was performed as: 0.1 mol of each reactant was added to the reactor and mixture was agitated at 200 rpm for 5 min and then 5% w/w enzyme was added to initiate the reaction. The molar concentration of [*A*] and [*B*] for SFS can be expressed in volume as shown in Figs. 1–4. This is because pure [*A*] having 13.36 M and pure [*B*] having 10.83 M were used directly without any solvent. If equimolar (5.98 Mol) mixture of both reactants [*A*] and [*B*] solution has to be made, the reaction mixture should contain [*A*] 7.5 cm^3 and [*B*] 9.3 cm^3. Accordingly all reaction mixtures were made based on predefined molar ratio. Liquid samples free from catalyst particles were withdrawn periodically and further analyzed to determine the extent of reaction. The procedure was repeated based on criterion for the optimization of reaction parameter (reaction time, temperature, enzyme loading, speed of agitation, concentration of water and molar ratio).

2.3. Analytical method

2.3.1. Identification of reaction product

Identification of synthesized isobutyl propionate in liquid samples was carried out by GC (CHEMITO 8610) equipped with flame ionization detector using 3 m × 0.32 mm I.D. stainless steel column packed with 10% OV-17 stationary phase. Nitrogen was used as carrier gas at pressure 0.8 bar. The temperature program was as follow: 60 °C for 1 min; 5 °C/min up to 100 °C; then steady temperature for 1 min. The injector and detector temperatures were both kept at 150 °C. Injection volume of 2 μl was used. After primary identification on GC, titrimetric analysis (method explained below) was used for routine measurements based on the comparison of both analytical methods which gave about ±3% deviation.

2.3.2. Titrimetric analysis

The isobutyl propionate obtained was expressed in terms of percent (%) conversion i.e. percent of propionic acid converted with respect to the total acid in the reaction mixture by titrating reaction mixture with 0.1N NaOH using phenolphthalein indicator and methanol as a quenching agent.

2.3.3. Determination of initial rates of reaction

Initial rates of esterification were determined at various reaction conditions depending on the molar ratio. The molar ratio of acid to alcohol was varied from 4:1 to 1:4 in integral successions. The temperature was maintained at 40 °C with 5% (w/w) enzyme, Novozym® 435, loading. Reactions were carried out for 1 h. Aliquots of the reaction mixture were taken every 15 min and analyzed by titrimetric analysis as discussed above. Conversion data for <10% conversion was used to determine initial reaction rates by plotting conversion-time profiles.

2.4. Kinetics and mechanisms of the esterification reaction

Two substrates i.e. propionic acid [*A*] and isobutyl alcohol [*B*] are bound to the immobilized lipase Novozym® 435 [*E*] in either a specific or random order to form an [AEB] complex, which then reacts to form the products viz. isobutyl propionate [*P*] and water [*Q*]. The reaction scheme for the synthesis of isobutyl propionate in a solvent free system (SFS) can be shown as follows:

Reaction Scheme 1. Synthesis of isobutyl propionate by esterification of propionic acid [*A*] with isobutyl alcohol [*B*] using immobilized lipase Novozym® 435 in SFS.

Based on the experimental data the initial rates of esterification were determined. These initial reaction velocities were then used for identification of maximum velocity and, Michaelis–Menten, inhibition and dissociation constants using three different bisubstrate kinetic models of the enzyme catalyzed reactions viz. Ordered Bi–Bi, Random Bi–Bi and Ping-Pong Bi–Bi. These three generalized two-substrate two-product i.e. Bi–Bi reactions models were selected as they take into account that the product formation occurs only after the formation of an enzyme–two substrate complex [26].