Biochemical Engineering Journal 137 (2018) 116–124

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Enzymatic kinetics of cetyl palmitate synthesis in a solvent-free system

Natália Allana Cirillo, Carolina Gomes Quirrenbach, Marcos Lúcio Corazza, Fernando Augusto Pedersen Voll*

Department of Chemical Engineering, Paraná Federal University (UFPR), Polytechnic Center, Jardim das Américas, Curitiba, PR, 82530-990, Brazil

ARTICLE INFO

Article history:
Received 13 January 2018
Received in revised form 18 May 2018
Accepted 22 May 2018
Available online 24 May 2018

Keywords:
Esterification
Wax ester
Cetyl palmitate
Immobilized lipase
Solvent free synthesis
Kinetic modeling

ABSTRACT

Cetyl palmitate was synthetized by esterification the cetyl alcohol with palmitic acid in a solvent-free system. Reactions were performed in batch mode using commercial lipase *Lipozyme RM IM* as catalyst. Stirring speed (180, 480 and 720 rpm), temperature (60, 70, 80 and 87 °C), amount of catalyst (0.5, 1.0 and 1.5% wt% related to the mass of substrates) and alcohol:acid molar ratio (0.5:1, 1:1 and 2:1) were evaluated and best reaction conditions obtained were 480 rpm, 70 °C, 1.0% of enzyme and 1:1 M ratio. A novel kinetic model based on random-sequential bi-bi mechanism was proposed and showed a good agreement with experimental data. Furthermore, this model was proved to be suitable for kinetics predictions with a small uncertainty. The catalyst was recycled and showed good stability since reaction conversion decreased only 6.8% after 15 reuses.

1. Introduction

Wax esters of fatty acids, or waxes, are long-chain esters with chain lengths of 12 or more carbons with high added value and high degree of biodegradability. Among the industrial applications of waxes are the production of lubricants, cosmetics, personal care products, pharmaceuticals, wood coatings, antifoaming agents, printing inks and varnishes [1]. They are found in nature on the surface of fruits, on the cranium cavity of sperm whales (*Physeter macrocephalus*), on hives and on the leaves of carnauba (*Copernicia cerifera*) [2]. Despite its high availability from natural sources, the extraction and exploitation from natural sources is not advantageous in technical and economic aspects, which motivates the synthetic production of waxes.

Cetyl palmitate ester is one of the waxes with large application in the cosmetics industry due to its emollient characteristics. It is used as a surfactant in shampoos, as an emulsifying agent and thickener in creams and adds texture to various make-up products, usually in those that are stick-shaped [3].

The most common method of cetyl palmitate synthesis is the enzyme-catalyzed esterification. Although enzymes have a high cost, they are able to catalyze reactions under milder conditions, with medium temperatures, moderate pH and near atmospheric pressure, which avoids unnecessary expenses with fuels, steam and robust reactors (which would be necessary to withstand higher pressures). The products also have a higher degree of purity and a lower degree of degradation, so they are easier to purify [4]. Additionally, there is a continuing interest in immobilized enzymes since they are heterogeneous catalysts, which facilitates their application in continuous reaction processes.

Many enzymes have good catalytic activity in organic solvents, and their applications have been studied by many researchers [5–8]. However, there is a growing concern that chemical processes are not only efficient, but also environmentally friendly, economically viable and free from product contamination. Therefore, when there is miscibility between the reagents to promote sufficient contact between the molecules and when the enzyme is active in that condition, the use of solvent-free systems is preferable. Numerous researchers applied commercial immobilized lipases *Novozym 435* [9–15], *Lipozyme RM IM* [15] and *Lipozyme TL IM* [16,17] in esterification reactions in solvent-free systems and reached conversions above 86%, indicating their good activity in this condition.

Kinetic modeling of enzymatic reactions have been proposed based in bi-bi mechanisms [8,15,18] and King-Altman mechanism [10]. However, there is a lack of description on how the temperature influences the reactions rate near the optimum temperature for enzyme activity and how the uncertainty on the kinetic parameters affects the uncertainty on the predictions of the model.

* Corresponding author.
E-mail address: fernando.voll@ufpr.br (F.A.P. Voll).

https://doi.org/10.1016/j.bej.2018.05.021
1369-703X/

Nomenclature

Ca	Cetyl alcohol
Pa	Palmitic acid
Cp	Cetyl palmitate
E	Immobilized lipase *Lipozyme RM IM*
$FFA_{t=0}$	Free fatty acid in the beginning of reaction (%)
FFA_t	Free fatty acid at specific time*t* (%)
[*Ca*E*Pa*]	Cetyl alcohol × enzyme × palmitic acid complex concentration (mmol $g_{substrate}^{-1}$)
[H_2O*E*Cp]	Water × enzyme × cetyl palmitate complex concentration (mmol $g_{substrate}^{-1}$)
[*Ca*E*Ca*]	Cetyl alcohol × enzyme × cetyl alcohol complex concentration (mmol $g_{substrate}^{-1}$)
[*Pa*E*Pa*]	Palmitic acid × enzyme × palmitic acid complex concentration (mmol $g_{substrate}^{-1}$)
[H_2O*E*H_2O]	Water × enzyme × water complex concentration (mmol $g_{substrate}^{-1}$)
[*Cp*E*Cp*]	Cetyl palmitate × enzyme × cetyl palmitate complex concentration (mmol $g_{substrate}^{-1}$)
[*Ca*]	Concentration of cetyl alcohol (mmol $g_{substrate}^{-1}$)
[*Pa*]	Concentration of palmitic acid (mmol $g_{substrate}^{-1}$)
[*Cp*]	Concentration of cetyl palmitate (mmol $g_{substrate}^{-1}$)
[*E*]	Concentration of free enzyme *Lipozyme RM IM* (mmol $g_{substrate}^{-1}$)
[E_t]	Total concentration of enzyme *Lipozyme RM IM* (mmol $g_{substrate}^{-1}$)
[E_t^m]	Concentration of enzyme *Lipozyme RM IM* (mg_{enzyme} $g_{substrate}^{-1}$)
[E_t^d]	Concentration of enzyme *Lipozyme RM IM* available for reaction (mg_{enzyme} $g_{substrate}^{-1}$)
k_i (i = 1, 6, 7, 9, 11, 13)	Rate constants ($g_{substrate}^2$ $mmol^{-2}$ h^{-1})
k_i (i = 2, 3, 4, 5, 8, 10, 12, 14)	Rate constants (h^{-1})
K_i (i = 1,. . ., 6)	Model constants ($g_{substrate}^2$ $mmol^{-2}$)
M_1	Apparent rate constant of esterification ($g_{substrate}^2$ $mmol_{enzyme}^{-1}$ $mmol^{-1}$ h^{-1})
M_2	Apparent rate constant of hydrolysis ($g_{substrate}^2$ $mmol_{enzyme}^{-1}$ $mmol^{-1}$ h^{-1})
V_1	Apparent rate constant of esterification ($g_{substrate}^2$ mg_{enzyme}^{-1} $mmol^{-1}$ h^{-1})
V_2	Apparent rate constant of hydrolysis ($g_{substrate}^2$ mg_{enzyme}^{-1} $mmol^{-1}$ h^{-1})
k_c	Mass transfer limitation constant ($g_{substrate}/mg_{enzyme}$)
P_i (i = 1, 2)	Constants of rate reaction equation in function of temperature ($g_{substrate}^2$ mg_{enzyme}^{-1} $mmol^{-1}$ h^{-1})
Q_i (i = 1, 2)	Constants of rate reaction equation n in function of temperature (K^{-1})
T_{ref}	Reference temperature (K)
EMM	Theoretical enzyme molar mass ($mg_{enzyme}/mmol_{enzyme}$)
obf	Objective function
EN	Total number of experimental data
$Conversion_k^{exp}$	Experimental reaction conversion (%)
$Conversion_k^{model}$	Reaction conversion calculated by model (%)
$rmsd_r$	Residual root mean square deviation
$rmsd_p$	Prediction root mean square deviation

This work is focused on experimental and theoretical kinetics of esterification of palmitic acid with cetyl alcohol catalyzed by *Lipozyme RM IM* in solvent free system for cetyl palmitate ester synthesis. A kinetic model was proposed for describing the reaction on different conditions of temperature, catalyst amount and reagents molar ratios with a suitable uncertainty.

2. Materials and methods

2.1. Materials

Cetyl alcohol (99%) was purchased from Alphatech (São José dos Pinhais – Brazil) and palmitic acid (≥98%) was purchased from Sigma Aldrich (Germany). The commercial enzyme *Lipozyme RM IM* (from *Rhizomucor miehei* immobilized on a macroporous ion-exchange resin) was purchased from Sigma Aldrich (Denmark). Ethanol (99%) and *n*-hexane (99%) were purchased from Neon (São Paulo – Brazil). Other chemicals were of analytical grade and used as received.

2.2. Lipase catalyzed esterification system

Batch reactions were conducted in a jacketed glass vessel (25 ml) closed with stopper to avoid evaporation of the water product (see Fig. A of Supplementary material). Temperature was set by a circulation thermostatic bath (*Nova Ética 521 – 5D)*. When the temperature reached the desired value cetyl alcohol and palmitic acid (3.5 g total mass of substrates) was added to reactor and melted before adding the *Lipozyme RM IM*. All the experiments were performed in a solvent-free system and the agitation was provides by a magnetic stirrer (*IKA C-MAG HS 4)*. Four parameters were analyzed being speed agitation (180, 480 and 720 rpm), temperature (60, 70, 80 and 87 °C), amount of catalyst (0.5, 1.0 and 1.5 wt% related to the mass of substrates) and molar ratio (0.5:1, 1:1 and 2:1 alcohol:acid). At the end of the reaction time, the stirring was interrupted for enzyme decantation and the reaction was stopped by adding room temperature ethanol. Samples were collected in triplicate for quantification of remaining free fatty acids.

2.3. Analytical methods

The esterification progress was monitored by determining the residual palmitic acid in the reactor. Samples were titrated in triplicate with NaOH 0.1 M using alcoholic phenolphthalein solution (95%) as indicator. The percentage conversion was calculated from the amount of acid consumed in the reaction:

$$Conversion(\%) = \frac{(FFA_{t=0} - FFA_t)100}{FFA_{t=0}} \quad (1)$$

Identification of palmitic acid, cetyl alcohol and cetyl palmitate in samples was made using gas chromatography in a Shimadzu® 2010 Plus chromatograph equipped with an Agilent Select Biodiesel capillary column (0.32 mm × 0.10 mm, 15 m), an autosampler, a split/splitless injector at 1:10 split ratio and a flame ionization detector (FID). The injection and detector temperatures were 380 °C and 400 °C, respectively. The carrier gas was helium at a 29.2 cm^3/min flow rate and the injected volume was 1.0 μl. The column temperature program was: 50 °C, 15 °C/min up to 180 °C, 7 °C/min up to 230 °C, and 10 °C/min up to 380 °C, remaining for 6 min.

2.4. Kinetic modeling

In this work, a random-sequential bi-bi based model with additional empirical terms for temperature dependence, mass transfer limitation and second-order inhibition by substrate was proposed to model the Lipozyme RM IM catalyzed esterification of the cetyl alcohol with palmitic acid in a solvent-free system.