Journal of Molecular Catalysis B: Enzymatic 132 (2016) 47–53

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

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

Enzymatic methanolysis reaction of canola oil using capillary channel reactor: Determination of the kinetic constants-involved

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ARTICLE INFO

Article history:
Received 29 January 2016
Received in revised form 25 June 2016
Accepted 27 June 2016
Available online 29 June 2016

Keywords:
Biocatalysis kinetics
Micro-mixing
Ping-Pong model
Bioprocess optimization
Micro-reactor

ABSTRACT

Enzymatic methanolysis reaction of canola oil utilizing *Candida rugosa* lipase in a solvent free system was studied in a shake flask as well as capillary channel reactors. The results demonstrated that pre-treatment of the enzyme with the substrate, increased the stability of the enzyme by 21.2% during the methanolysis reaction. Performance of a capillary-channel reactor improved the yield of methanolysis up to 4-fold when compared with the shake flask experiments. Bio-kinetic constants were estimated using the Ping-Pong model by considering the competitive and non-competitive inhibition roles of methanol on enzymatic methanolysis reaction. The results of the kinetics studies showed that the enzymatic methanolysis reaction was best described by the competitive inhibition Ping-Pong model with the maximum enzyme activity of 170368 μmol min^{-1} $g_{enzyme}{}^{-1}$, the inhibition constant of methanol 0.826 mol $g_{enzyme}{}^{-1}$, the dissociation constant of canola oil 0.137 mol $g_{enzyme}{}^{-1}$, and methanol dissociation constant 1.081 mol $g_{enzyme}{}^{-1}$.

1. Introduction

Biodiesel is a mixture of methyl and/or ethyl esters of fatty acids derived from the transesterification of triacylglycerol presence in plant oils with benefits such as nontoxic, biodegradable, and renewable potentials for use as an alternative fuel [1]. Although several processes comprising chemical and biological methods were proposed by researchers for the production of biodiesel, some difficulties were reported in chemical processes such as glycerol recovery from the reaction mixture, release of utilized catalyst and salts to the environment, and high energy consumption rate encouraged the researchers to handle the problems of biological systems as a suitable alternative to the chemicals [1–4].

The biological transesterification of oil was basically catalyzed by lipase enzymes which require an aqueous medium to reveal the catalytic activity [5]. Immiscibility of the aqueous and organic phases that participated in the transesterification reactions usually controls the overall reaction rate. The effect of mixing the reaction medium for increased performance of the biodiesel production processes have been studied [6–13]. In some studies, the application of solvent was suggested as an approach for handling the problem [14,15]. Furthermore, the utilizing of solvent-free systems has important benefits when compared with those of organic solvent in terms of cost reduction, simplicity of interpretation and environmental benefits [16,17]. Currently, the application of micro-channel reactors has been proposed as a novel technique for increasing the conversion efficiency of the biodiesel under continuous condition [18–22]. Simplicity of the process control in the narrow-channel reactor in comparison to the classical mixed reactor, achievement to high surface/volume ratio and short diffusion distance that significantly increased the heat and mass transfer rates, are the main technical advantages for employing them in the industrial process. Yu et al. applied a metal foam narrow size reactor combined with a passive mixer and achieved a 95% biodiesel yield from chemical transesterification of sunflower oil in the presence of NaOH as an alkali catalyst at 3.5 min hydrodynamic retention time (HRT) [19]. Further investigations on the influences of the microchannel geometry on biodiesel synthesis were experimentally evaluated by Arias et al., [21]. Employing a catalyst loading of 1.0 wt% NaOH, yields an ethyl ester conversions of 96.7, 95.3, and 93.5% for Tesla-, omega-, T-shaped micro-reactors, respectively. Recently, Rahimi et al. [22] with the use of a T-shaped junction micro-reactor, statistically studied the chemical transesterification of soybean oil and methanol in the presence of KOH which significantly decreased the reaction time, into a few minutes, for achievement of a high purity methyl ester. To the best of our knowledge, no study has

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http://dx.doi.org/10.1016/j.molcatb.2016.06.014
1381-1177/

Table 1
Fatty acid compositions of canola oil.

Fatty acid	Content (w%)
Palmitic acid	4.8
Stearic acid	2.3
Oleic acid	60.9
Linoleic acid	22.6
Arachidic acid	0.6
Linolenic acid	7.3
Gadoleic acid	1.2
Behenic acid	0.3

been carried out for the enzymatic methanolysis reaction in the narrow-channel reactor. This study aims at the enzymatic production of the biodiesel through methanolysis reaction of canola oil in a solvent free system using the *Candida rugosa* lipase in a capillary-channel reactor equipped by inserted mixers. The kinetics of the reaction was studied based on the Ping-Pong model. The kinetic constants of the methanolysis reaction by considering of the competitive and non-competitive inhibition role of methanol were also determined.

2. Materials and methods

2.1. Materials

Refined canola oil was obtained from Behshahr Industrial Company (Tehran, Iran). The average molecular weight (881.6 g $gmol^{-1}$) of the canola oil was determined from gas chromatography analysis of the fatty acids composition listed in Table 1. Commercial lipase from *C. rugosa* (enzyme activity > 700 IU mg_{enzyme}^{-1}) was purchased from Sigma-Aldrich. The methanol used in this study was of analytical grade (assay ≥ 99.6%) and purchased from Dr. Mojallali Chemical Complex Co.

2.2. Shake flask experiments

In each experiment, 50 mg of crystal lipase enzyme was dissolved in 5 mL of phosphate buffer solution (pH = 7.0, 0.2 M) and then poured in to a 250 mL Erlenmeyer flask. After the addition of 50 g of canola oil and 8.5 mL of methanol (methanol to oil = 4 mol mol^{-1}) to the flask, it was incubated in a shaker incubator at 180 rpm and 42 °C for 4 h. At regular time intervals, the samples were removed from the incubator and the esterified reaction mixture was incubated in a water bath at a temperature of 70 ± 2 °C for 10 min in order to inactivate the enzyme. Thereafter, the mixture was centrifuged at 6000*g* for 10 min and washed with distilled water. Centrifugation separation process was repeated in order to obtain the biodiesel sample with a glycerol impurity lesser than 0.005 mg mL^{-1}.

To evaluate the effect of initial pH of the reaction medium on the enzyme activity, the initial pH was adjusted using the phosphate buffer at 5.6, 6.4, 7.0, 7.6, and 8.2, where other operational conditions were kept constant at a temperature of 42 °C, water to oil = 10 ww^{-1}%, and methanol to oil = 4 mol mol^{-1}.

Temperature effect was studied at 32, 37, and 42 °C, where other operational conditions were kept constant at initial pH = 7.6, water to oil = 10% (ww^{-1}), and methanol to oil = 4 mol mol^{-1}. All experiments were conducted three times.

Substrate pretreatment of the lipase was carried out according to the method described by Lu et al. [23] with slight modifications: 50 mg of crystal enzyme dissolved in 50 g of the canola oil was incubated in 150 oscillations per minute at 25 °C for 15 min.

2.3. Capillary-channel reactor system

The capillary-channel reactor is composed of a micro size T-type conjunction with an internal diameter of 0.8 mm (for proper initial mixing of the aqueous and oily phases) was contacted to a channel with a length of 100 cm, and a dimension of 3 × 1 mm. In order to improve the mixing, nine stainless steel coils with a length of 10 cm and stride length of 0.5 mm were inserted at the beginning of the channel (after T conjunction) and also at all bend sections of the capillary channel. A schematic view from the employed setup is illustrated in Fig. 1. The system contained the syringe pump that injected the following streams in the capillary-channel reactor: the pretreated lipase with oil (as mention above) and a mixture of methanol and the phosphate buffer (with pH = 7.6). The ratio of methanol to oil and buffer to oil were controlled by an adjustable syringe pump at a desired flow rate of each stream according to the test conditions. The temperature of the reaction was maintained at 37 °C using a thermostat chamber, which contained the whole of the system (Fig. 1)

2.4. Fatty acid methyl ester analysis

Fatty acid methyl ester (FAME) in the biodiesel samples was determined by a HP 6890 gas chromatograph with a flame ionization detector (FID) at 230 °C [21]. A BPX-70 high polar column (120 m length) with a film thickness of 0.25 μm and an internal diameter of 0.25 mm was employed as capillary column. Nitrogen was utilized as a carrier gas as well as an auxiliary gas for FID.

In order to analysis the FAME, 0.5 g of the biodiesel sample and 0.05 g of lauric acid methyl ester (as a reference) was dissolved into 10 mL of n-hexane and 1 μL of the mixture was injected at 50 °C means of a 6890 Agilent Series Injector. The biodiesel yield was determined using Eq. (1) i.e. based on the weight percentage of FAME content of the biodiesel sample [24]:

$$\text{Yield of methanolysis}(\%) = \frac{\text{Area of all FAME}}{\text{Area of reference}} \times \frac{\text{Weight of reference}}{\text{Weight of biodiesel sample}} \times \frac{\text{Weight of biodiesel product}}{\text{Weight of oil used}} \times 100 \quad (1)$$

The specific methanolysis reaction rate was determined as follows:

$$\textit{Enzyme activity}\left(\mu\text{molg}^{-1}\text{min}^{-1}\right) = \frac{\textit{Yield}}{100} \times \frac{\text{Weight of oil used}\,(\text{g})}{\text{Molecular weight of oil} \times \text{Weight of enzyme used}\ (\text{g}) \times \text{time}\,(\text{min})} \quad (2)$$

2.5. Determination of kinetic constants

All regression analyses were carried out using the GraphPad Prism software, version 5.00 for Windows. The kinetics constants were determined using Levenburg-Marquardt method available at GraphPad Prism 5.00. Initial values for estimated constants were obtained by considering the Michaelis-Menten model at non-inhibitory concentrations of methanol.

3. Results and discussion

3.1. Biodiesel production in the shaken flask experiments

The performance of the lipase enzyme for the methanolysis reaction of canola oil at 42 °C and 180 rpm is presented in Fig. 2. As illustrated in Fig. 2A, the yield of the methanolysis reaction increases during the reaction time and was up to 56.8% after 240 min from the beginning of the process. The specific rate of the methanolysis reaction in terms of enzyme activity is illustrated in Fig. 2B. The initial reaction rate value was maximized