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Modeling the methanolysis of triglyceride catalyzed by immobilized lipase in a continuous-flow packed-bed reactor

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HIGHLIGHTS

- A *Burkholderia* lipase was immobilized on alkyl-grafted celite carriers.
- Celite-alkyl-lipase catalyzed the methanolysis of triglyceride in packed-bed reactor.
- The kinetics of the enzymatic transesterification follows Ping Pong Bi Bi mechanism.
- Models were developed to discuss the mass transfer and enzyme kinetics in the PBR.

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ABSTRACT

A *Burkholderia* lipase was immobilized on celite grafted with long alkyl groups. The immobilized lipase-catalyzed methanolysis of sunflower oil in a packed-bed reactor (PBR) follows the Ping Pong Bi Bi mechanism. The external mass transfer and enzymatic reaction that simultaneously occurred in the PBR were investigated via the mathematical models. The overall biodiesel production in the PBR was verified to work in an enzymatic reaction-limited regime. Triglyceride conversion and biodiesel yield were higher under a lower reactant feeding rate, while a larger amount of biocatalyst would be required to achieve the designated conversion rate if a higher reactant feeding rate was employed. The PBR can achieve nearly complete conversion of triglyceride at a biocatalyst bed height of 60 cm (ca. 29 g biocatalyst) and a flow rate of 0.1 ml min^{-1}, whereas the biodiesel yield was lower than 67%, probably due to the positional specificity of *Burkholderia* lipase and the accumulation of glycerol.

1. Introduction

Fatty acid alkyl esters (biodiesel) produced from various sources of triglycerides, such as vegetable oils, algae [1,2] and animal fats, have received considerable attention as potential biofuels over the last decade [3,4]. Because it is renewable, biodegradable, and nontoxic, biodiesel has been used as a blending additive with fossil diesel or directive fuel in many diesel engines [3,4]. Biodiesel is conventionally produced via transesterification of triglyceride with alcohol (primarily methanol) catalyzed by various types of catalysts, including homogeneous catalysts (e.g., NaOH, KOH), heterogeneous catalysts (BaO, MgO/SiO_2, SrO/SiO_2, etc.), and biocatalysts (e.g., lipases) [5–9]. Among these, lipases can work well and achieve high conversion rates under mild conditions with less refined oils, which often contain a considerable amount of water and free fatty acids [5,6,10,8]. In contrast, chemical catalysts can be poisoned if the substrate source (either triglyceride or alcohol) contains an excess amount of free fatty acid or water, resulting in lower reaction rate [5,6]. It is thus beneficial to use lipase as the catalyst for the synthesis of biodiesel with less refined oils [11]. In addition, lipases can be immobilized on a variety of carrier materials to allow the reuse and recycling of the valuable enzymes, and to enable continuous biodiesel production with the aid of an appropriate bioreactor design, such as a fixed-bed reactor, for continuous biodiesel production [5,6,10,12].

To scale up the production of biodiesel, the enzymatic transesterification of triglyceride with alcohol is usually carried out in packed-bed reactor (PBR), in which the immobilized lipase is physically trapped inside the PBR column, while reactants are passed

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through the catalyst bed by pumping at a desired flow rate [13–17]. The mass transfer resistance between the immobilized enzyme and substrate is the major factor affecting the global reaction rate of enzyme-catalyzed reaction [5,10], and this issue has been widely addressed in the literature, with, for example, research examining lactose hydrolysis by the β-galactosidase immobilized by cross-linking within the alginate and κ-carrageenan matrix [18]. However, this has been rarely or unsystematically discussed for the case of the enzymatic transesterification of triglyceride and alcohol [15,16]. On the other hand, the kinetics of lipases in the catalytic transesterification of triglyceride and alcohol has recently been studied [15,19]. It was reported in such works that the alcoholysis triggered by lipase includes two substrates (alcohol and lipids), and that the alcohol may inhibit the activity of lipase during the enzymatic reactions. The Ping Pong Bi Bi mechanism is thus often used to describe the enzymatic transesterification of triglyceride with alcohol [10]. However, the validity of the related kinetic model of the effects of immobilized lipase on continuous biodiesel production in a fixed-bed reactor still needs to be verified, and this is one aim of the current work.

Lipase produced from the *Burkholderia* sp. C20 strain isolated from food waste has been shown to successfully catalyze the alcoholysis of triglyceride with alcohol to form biodiesel [20–22]. In our previous work, the *Burkholderia* lipase was immobilized onto a cellulose nitrate (CN) membrane via filtration [21], or onto a celite carrier functionally modified with 3-amino-propyltriethoxysilane via covalent bonding [22]. While the immobilized lipases showed great thermostability, their specific activity was still low, thus making it impossible to achieve a high conversion rate of biodiesel in the transesterification of oil with methanol [21,22]. Recently, it was found that *Burkholderia* lipase has a high affinity with regard to hydrophobic surfaces, leading to the development of hybrid nanocomposite alkyl-grafted Fe_3O_4–SiO_2 as new immobilization carrier for the lipase [12,23]. The resulting Fe_3O_4–SiO_2-alkyl-lipase had a specific activity of up to 1281 U g^{-1} [23], which is significantly higher than that of celite-lipase (273.5 U g^{-1}) created by covalent bonding [22]. The immobilized lipase exhibits high tolerance to methanol and water, and has great potential in catalyzing the alcoholysis of various oil sources, such as olive oil [23] and microalgae oil [24,25], to produce biodiesel. However, due to the small particle size of the nanocomposite carriers, it is difficult to physically trap the immobilized lipase in the packed-bed reactor for continuous biodiesel production [23–25].

In this work, a new immobilization system was developed by immobilizing the *Burkholderia* lipase on micro-size celite 545 grafted with the hydrophobic functional group. The resulting immobilized lipase has a suitable particle size (ca. 48.2 μm) for the use in continuous biodiesel production via a PBR. The kinetics of the celite-alkyl-lipase on catalyzing methanolysis of sunflower oil was examined in a PBR to validate the Ping Pong Bi Bi mechanism. In addition, the enzymatic transesterification in the PBR was mathematically modeled by simultaneously taking into account the substrate diffusion, external mass transfer of the substrate to the non-porous surface of the biocatalyst, and the enzymatic kinetics in order to evaluate the performance of the PBR. The mathematical model developed in this work is a useful tool to identify the required amount of biocatalyst by weight, the effective size of the PBR, as well as the flow rate of the reactant, in order to achieve the desired conversion of triglyceride and biodiesel yield.

2. Materials and methods

2.1. Chemicals

n-Hexadecane (99%) was obtained from Alfa Aesar (Ward Hill, USA). [3-(Trimethoxysilyl)propyl] octadecyl dimethyl ammonium chloride (72%), sunflower oil (99%), gum arabic (99%), methyl palmitate (99%), methyl oleate (99%), and methyl linoleate (99%) were purchased from Sigma-Aldrich (St. Louis, USA). Methanol (99.9%) was obtained from Mallinckrodt (St. Louis, USA). Bacto™ Yeast extract was obtained from Difco (Lawrence, USA). HEPES (99%) was obtained from Across (New Jersey, USA). Potassium chloride (99%) was obtained from Wako (Osaka, Japan). Ammonium sulfate (99%), magnesium (II) chloride hexahydrate (99%). Celite 545 was purchased from Showa Chemical Co., Ltd. (Tokyo, Japan). Ethanol (95%) was obtained from Taiwan Tobacco and Liquor Co. Inc. (Taipei, Taiwan).

2.2. Synthesis of alkyl-grafted celite

One hundred grams of celite 545 was added into 1 l ethanol (95%) and 20 ml de-ionized water with vigorous mixing. The mixture was heated up to 40 °C. Ten milliliters of [3-(Trimethoxysilyl)propyl] octadecyl dimethyl ammonium chloride was added to the mixture with vigorous mixing, and the reaction was carried out at 40 °C and 400 rpm for 8 h. The particles were filtered and washed with water several times to completely remove unadsorbed materials, and then dried at 100 °C for 24 h.

2.3. Microorganism

The lipase-producing bacterium was isolated from the food waste obtained from a food processing plant located in central Taiwan [20]. The strain was identified as *Burkholderia* sp. C20 by a 16S rDNA sequence comparison, with a NCBI accession number of AY845053 [14]. The strain was stored in 1 ml mixture of 0.5 ml glycerol (30%) and 0.5 ml LB (25 g l^{-1}) (Difco) medium under −80 °C as a stock source [20–22]. The stock microorganism was pre-cultured in 4 ml of LB medium at 30 °C, with a shaking speed of 200 rpm for 12 h. The pre-cultured bacterium was then transferred to a fermentor containing 2 l of optimal sterilized medium consisting of 2 g l^{-1} yeast extract, 4.8 g l^{-1} HEPES, 0.2 g l^{-1} $MgCl_2.6H_2O$, 9.9 g l^{-1} KCl, 6 g l^{-1} $(NH_4)_2SO_4$, 5.4 ml l^{-1} sunflower oil, 5 ml l^{-1} hexadecane [26]. Fermentative lipase production with *Burkholderia* sp. C20 was conducted in a 5 l fermentor at a controlled pH of 6.5, a temperature of 30 °C, an aeration rate of 1 vvm, and a stirring speed of 400 rpm during 30 h fermentation time [26]. After fermentation was complete, the fermentation broth was centrifuged at 9050*g* for 10 min to harvest the supernatant as crude lipase. After determining the lipase activity and protein concentration, the crude lipase was subsequently bound onto alkyl-grafted celite to prepare the immobilized lipase.

2.4. Lipase immobilization

In brief, 100 g of the alkyl group grafted celite was added into a beaker containing 3 L harvested crude lipase as described in Section 2.3 under vigorous mechanical stirring at 600 rpm. The immobilization was conducted at a stable temperature of 25 °C, stirring rate of 600 rpm, pH of 6.67, and adsorption time of 24 h. The residual activity and adsorbed protein concentration on the microsize particles were regularly monitored at designated time intervals until the immobilization reached equilibrium state, which was defined as when the residual activity of lipase remained unchanged. The immobilized lipase was filtered and washed with de-ionized water several times until complete removal of the unbounded lipase. The resulting immobilized lipase (denoted as alkyl-celite-lipase) was then subjected to activity assay and used for further experiments.