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Kinetics of transesterification of olive oil with methanol catalyzed by immobilized lipase derived from an isolated *Burkholderia* sp. strain

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HIGHLIGHTS

- Transesterification was catalyzed by immobilized 1,3-positional *Burkholderia* lipase.
- Kinetics of acyl migration from 2-sn to 1- or 3-sn position was studied.
- Kinetic model for the lipase-catalyzed transesterification was developed.
- Kinetic parameters and equilibrium state were estimated for the kinetic model.

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ABSTRACT

This work was carried out to investigate the acyl migration phenomena which has been considered as the factor having significant impact on kinetics of transesterification of oils catalyzed by a *Burkholderia* lipase with 1,3-regioselectivity. Transesterification of olive oil with methanol catalyzed by the immobilized lipase produces various intermediates, including 1-monoglyceride, 2-monoglyceride, 1,2-diglyceride, and 1, 3-diglyceride. Migration kinetics of fatty acid groups from sn-2 of 2-monoglyceride and 1,2-diglyceride to 1-monoglyceride and 1,3-diglyceride were investigated for the temperature range of 25–65 °C. The kinetics of transesterification of olive oil with methanol involving acyl migration in the presence of water was also systematically studied at 25, 40, and 65 °C. Increasing temperature could increase the acyl migration rate. The overall biodiesel conversion was improved from 73.4% (at 25 °C) to 90.0% and 92.4% when conducting at 40 and 65 °C, respectively. Thermodynamics aspects of equilibrium state of the immobilized lipase-catalyzed transesterification were also discussed.

1. Introduction

Lipases (triacylglycerol acyl hydrolases, EC 3.1.1.3) are a class of enzymes that catalyze the hydrolysis of long chain triglycerides (Contesinia et al., 2010). They preferentially act on the carboxylic ester bonds in the interface between lipid and water in heterogeneous systems (Reis et al., 2009). Lipases can be classified according to their regioselectivity with regards to the acyl chains of triglycerides as 1,3-positional lipases which catalyze at sn-1 and sn-3 position of triglyceride backbone and as non-specific positional lipases which catalyze at un-selected position of the bonding of acyl group with glycerol backbone (Reis et al., 2009). Microbial lipases are currently receiving much attention with the rapid development of enzyme technology (Saxena et al., 2003; Hasan et al., 2006; Contesinia et al., 2010). Lipase is a biocatalyst with broad spectrum of industrial applications, such as detergent, food, flavour industry, cosmetics and perfumery, biocatalytic resolution of pharmaceuticals, esters and amino acid derivatives, biosensor (Saxena et al., 2003; Hasan et al., 2006; Contesinia et al., 2010), biofuels (Véras et al., 2011), and production of ferulyl oleins as an antioxidant (Xin et al., 2011). Commercial preparations of microbial lipases are often produced by fermentation of different fungi, yeast and bacteria such as *Rhizopus delemar*, *Aspergillus niger*, *Geotrichum candidum*, *Candida rugosa*, *Chromobacterium viscosum*, and *Burkholderia* sp., etc. (Houde et al., 2004).

Homogeneous catalysts including acid (ethanesulfonic) (Hayyan et al., 2011) and base (sodium ethoxide) (Shahla et al., 2012) have been successfully used for biodiesel production from palm oil. Lipases can also serve as biocatalyst to trigger transesterification of oil with methanol for the synthesis of fatty acid methyl ester (FAME) (Li et al., 2007; Tran et al., 2012). The enzymatic

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biodiesel synthesis process has many advantages over conventional methods, but commercialization of the enzymatic process is still restricted by the high cost of lipase (Demirbas, 2007; Lam et al., 2010). Immobilization of lipase on supporters is considered as a vital solution for the cost issue, as the immobilized lipase can reduce the operation cost via repeated uses of the enzyme and via improving the enzyme stability (Demirbas, 2007; Lam et al., 2010). Therefore, it is of great importance to develop effective, reusable, and durable immobilized lipase to make lipase-catalyzed biodiesel production commercially feasible.

It was reported that many immobilized lipases and whole cell catalysts possess 1,3-positional specific activity towards triglycerides, which are used as substrates in alcoholysis for biodiesel synthesis (Du et al., 2005; Oda et al., 2005; Li et al., 2007; Tamalampudi et al., 2008; Caballero et al., 2009). The immobilized lipases were also applied as a biocatalyst to minimize the kinematic viscosity and maximize heat transfer coefficient of oil blends containing rice bran oil and refined, bleached, deodorized palm olein via transesterification (Debnath et al., 2011). In general, methanolysis of triglyceride catalyzed by 1,3-positional specificity would generate partially-converted intermediates of triglyceride such as 1,2-diglyceride (1,2-DG), 1,3-diglyceride (1,3-DG), 1-monoglyceride (1-MG), and 2-monoglyceride (2-MG). As a result, usually less than 66% FAME conversion could be achieved when using 1,3 specific lipase as the biocatalyst for transesterification (Du et al., 2005). The lipase produced from *Burkholderia* sp. C20 was reported to have 1,3-positional specificity for the transesterification of olive oil with methanol (Wang, 2010). Using the *Burkholderia* lipase, the FAME conversion rapidly reached nearly 60% (at 25 °C) for first 12 h reaction. After that, the FAME conversion increased very slowly due probably to a slow acyl migration process. The same phenomenon was also found when using other 1,3-positional specific lipases as the catalyst for biodiesel production (Du et al., 2005; Caballero et al., 2009). Thus, acyl migration plays a key role in governing the efficiency of FAME formation while using 1,3-positional specific lipase as the catalyst (Wang et al., 2008; Li et al., 2010a).

In the alcoholysis process, acyl groups at sn-2 position of 1,2-DG and 2-MG could migrate to the sn-1 or sn-3 positions to form corresponding isomer 1,3-DG and 1-MG, respectively. Based on this theory, the rate of biodiesel conversion can be improved through optimizing the key factors (e.g., temperature, solvent, etc.) to accelerate acyl migration to form 1,3-DG and 1-MG, which are preferably catalyzed by the 1,3-specific lipase to eventually produce FAME (reaction (1) and (2)). The acyl migration phenomenon in glycerols has been studied extensively in the stereochemical research (Kodali et al., 1990; Millqvist Fureby et al., 1996; Du et al., 2005; Compton et al., 2007; Laszlo et al., 2008; Li et al., 2010a). ^{1}H NMR spectroscopy was recently used as a useful technique for rapidly and accurately determining and characterizing triglyceride, fatty acid methyl esters, as well as intermediate products (1,2-diglyceride, 1,3-diglyceride, 1-monoglyceride, 2-monoglyceride, free fatty acids) of transesterification of sunflower oil with ethanol in acyl migration studies (Compton et al., 2007; Laszlo et al., 2008).

Alcoholysis kinectics of triglyceride using sodium ethoxide as homogeneously chemical catalyst has been simply modeled and well validated with irreversible second order kinetics (Shahla et al., 2012). Moreover, immobilized *Candida antarctica* (Novozym 435) was used as a biocatalyst in catalyzing simultaneous esterification of free fatty acids and transesterification of triacylglycerols originating from highly acidic distilling oil palm or chicken residual oil to validate the first order kinetics (Véras et al., 2011). These alcoholysis reactions were either catalyzed by homogeneous catalysts or non-specific lipases; thus the side reaction and acyl migration were not considered. Recently, Li et al., 2010a,b,c carried out a series of methanolysis of triglyceride under catalysis of immobilized 1,3-positional lipase *Rhizopus oryzae*. The acyl migration phenomena were addressed in the kinetics model, but in their model, the countable amount of water involved in reacting system that causes hydrolysis of triglyceride to intermediate components and free fatty acids as side reactions was not taken into account.

In our recent work, the lipase obtained from an isolated strain *Burkholderia* sp. C20 was immobilized on alkyl-grafted silica magnetite composite Fe_3O_4–SiO_2, which was synthesized by sol gel method (Tran et al., 2012). The immobilized lipase was also determined as 1,3-specific lipase towards olive oil in transesterification with methanol (Wang, 2010); however, the immobilized lipase was unable to catalyze esterification of oleic acid with methanol. In addition, the activity of the immobilized lipase on transesterification of olive oil was prohibited in the absence of water. Therefore, in the present study, kinetics of transesterification of olive oil with methanol as well as acyl migration was studied systematically to understand the lipase-catalyzed transesterification in a quantitative manner. The hydrolysis occurs due to the presence of water in the reaction system was clearly addressed in the kinetics model. The second order, third order, and fourth order reactions were simultaneously modeled to describe the kinetics of consecutive reactions that generates fatty acid methyl esters (E) and free fatty acids (FFA). Thermodynamics aspects of the transesterification and acyl migration were also discussed. ^{13}C NMR spectroscopy technique was used to determining the profile of each component during the time course of the reaction to assist interpretation of the kinetic data for the acyl migration and transesterification.

2. Methods

2.1. Materials

Magnetic nanoparticles and n-hexadecane (99%) were obtained from Alfa Aesar (Ward Hill, USA). [3-(Trimethoxysilyl)propyl]octadecyldimethylammonium chloride (72%), olive oil (99%), gum arabic (99%), methyl palmitate (99%), methyl oleate (99%), and methyl linoleate (99%), glycerol (99%) were purchased from Sigma–Aldrich (St. Louis, USA). Methanol (99.9%), acetone (99.9%), hexane (99.9%), and 2-propanol (99.9%) were 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%), and tetraethyl orthosilicate (TEOS) (98%) were purchased from Showa (Saitama, Japan). Ammonium (29%) was purchased from Fisher Chemicals (Waltham, USA).

2.2. Preparation of alkyl-grafted core–shell Fe_3O_4–SiO_2

Nanocomposite supporters (Fe_3O_4–SiO_2) were prepared and grafted to [3-(trimethoxysilyl) propyl] octadecyl dimethyl ammonium chloride by the method described in Tran et al. (2012). The alkyl-grafted Fe_3O_4–SiO_2 was decanted using a magnetic device and washed with ethanol several times to completely remove unadsorbed components and dried at 100 °C for 24 h.

2.3. Lipase production and lipase immobilization

The lipase-producing bacterium was isolated from natural environment and identified as *Burkholderia* sp. C20 following procedures described in Liu et al. (2007). The stock bacterium was pre-cultured and was then cultivated on the optimal medium under optimal conditions for lipase production following the procedures described in Liu et al. (2012). The resulting fermentation broth was centrifuged at 9050×*g* for 10 min and the supernatant