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One-step synthesis of high-yield biodiesel from waste cooking oils by a novel and highly methanol-tolerant immobilized lipase

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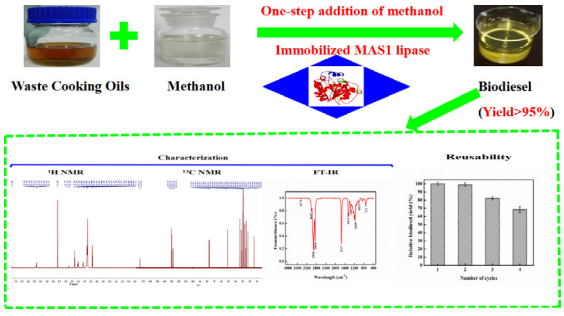
HIGHLIGHTS

- Immobilized MAS1 can be efficiently catalytic waste cooking oils into FAME.
- Immobilized MAS1 showed high tolerance to methanol.
- More than 95% biodiesel yield was obtained with one-step addition of methanol.
- Immobilized MAS1 exhibited good reusability during four batch cycles.
- Characterization of the obtained biodiesel was done.

GRAPHICAL ABSTRACT



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ABSTRACT

This study reported a novel immobilized MAS1 lipase from marine *Streptomyces* sp. strain W007 for synthesizing high-yield biodiesel from waste cooking oils (WCO) with one-step addition of methanol in a solvent-free system. Immobilized MAS1 lipase was selected for the transesterification reactions with one-step addition of methanol due to its much more higher biodiesel yield (89.50%) when compared with the other three commercial immobilized lipases (<10%). The highest biodiesel yield (95.45%) was acquired with one-step addition of methanol under the optimized conditions. Moreover, it was observed that immobilized MAS1 lipase retained approximately 70% of its initial activity after being used for four batch cycles. Finally, the obtained biodiesel was further characterized using FT-IR, ^{1}H and ^{13}C NMR spectroscopy. These findings indicated that immobilized MAS1 lipase is a promising catalyst for biodiesel production from WCO with one-step addition of methanol under high methanol concentration.

1. Introduction

In recent years, biodiesel is becoming more and more attractive as a renewable, non-toxic, and biodegradable fuel (Huang et al., 2015). However, the high production cost limits the development and use of biodiesel, of which the cost of raw materials accounts for >85% (Kuo et al., 2015). Thus, researchers are focusing their attention on minimizing feedstock costs such as using microbial oils or waste cooking oils (WCO). A large amount of WCO available around the world are cheaper than refined oils and can do great harm to environment (Yan et al., 2014; Utlu, 2007). Therefore, production of biodiesel from WCO could help solve environmental pollution and reduce the production cost (Chen et al., 2005; Farag et al., 2011; Meng et al., 2008).

Nowadays, preparation of biodiesel from WCO could be performed by chemical and enzymatic processes. Compared with chemical methods, enzymatic production of biodiesel has drawn

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great attention due to its moderate reaction conditions, easier recovery of products, more simple purification process, insensitive to water content and acidity value (Singh et al., 2015; Lee et al., 2011; Dizge et al., 2009). Moreover, enzymes in immobilized forms can allow their reuse, and be used in continuous operations (Rodrigues et al., 2016; Juan et al., 2011; Halim et al., 2009; Modi et al., 2007). Enzymatic hydroesterification and transesterification reactions are the most widely used methods for biodiesel production(de Araújo et al., 2013). Nevertheless, enzymatic hydroesterification reactions, including two consecutive steps, are relatively more complicated than transesterification reactions (Aguieiras et al., 2015; Haigh et al., 2013). Moreover, the cost of lipases is relatively high and the reaction is slow in the biocatalytic hydroesterification processes (Arumugam and Ponnusami, 2014). Therefore, lipase-catalyzed transesterification reaction is considered to be a promising alternative to produce biodiesel.

In the transesterification reactions, methanol is the most widely used acyl acceptor for biodiesel production due to its economic feasibility and accessibility compared with other alcohols (Deng et al., 2005; Antczak et al., 2009; Zhao et al., 2014). However, small droplets of methanol could result in the denaturation and inactivation of the lipases compared with longer aliphatic alcohols (Romdhane et al., 2013; Salis et al., 2005; Tan et al., 2010). To overcome this drawback, trials using stepwise addition of methanol to reaction mixtures (Duarte et al., 2015; You et al., 2013), co-solvents like *t*-butanol (López et al., 2016; Royon et al., 2007; Li et al., 2006), longer-chain alcohols as acyl acceptors (Iso et al., 2001), and methyl or ethyl acetate as acyl acceptors (Goembira and Saka, 2013; Razack and Duraiarasan, 2016) have been performed in previous studies. Nevertheless, these strategies increased the additional processing steps in the production of biodiesel, resulting in a higher production cost (Véras et al., 2011). Therefore, efforts are made to look for novel lipases with the ability of one-step addition of methanol for biodiesel production under high methanol concentration.

In the present work, lipase MAS1 from marine *Streptomyces* sp. strain W007 (Yuan et al., 2015), immobilized onto XAD1180 resin, was used as catalyst for biodiesel production through transesterification of WCO with methanol in a solvent-free system. Firstly, the catalytic properties of immobilized MAS1 lipase, Novozym 435, Lipozyme RM IM, and Lipozyme TL IM were compared in the production of biodiesel. Then, the effects of oil/methanol molar ratio, enzyme loading and temperature on biodiesel production were separately investigated. Furthermore, the reusability of immobilized MAS1 lipase was evaluated. Finally, the obtained biodiesel was further characterized by FT-IR, ^{1}H and ^{13}C NMR spectroscopy.

2. Materials and methods

2.1. Materials

Waste cooking oils (WCO) were obtained from a local restaurant and were centrifuged before use. The WCO consisted of 92.18% triacylglycerols (TAG), 3.94% free fatty acids (FA), 3.88% diacylglycerols (DAG). The FA composition of WCO was composed of 0.19% lauric acid (C12:0), 1.13% myristic acid (C14:0), 24.3% palmitic acid (C16:0), 2.13% palmitoleic acid (C16:1), 0.33% heptadecanoic acid (C17:0), 6.66% oleic acid (C18:1), 41.11% linoleic acid (C18:2), 21.93% linolenic acid (C18:3n6), 1.48% (eicosatrienoic acid (C20:3n6), 0.21% heneicosanoic acid (C21:0), 0.35% (docosanoic acid C22:0), and 0.18% tetracosanoic acid (C24:0). The oils didn't contain any n-3 polyunsaturated fatty acids with more than four double bonds that were easily oxidized during storage. Based on the FA composition, WCO had an average molecular weight of 900 g/mol.

Lipase MAS1 was produced according to the method described by Lan et al. (2016). Novozym 435, Lipozyme RM IM and Lipozyme TL IM were supplied by Novozymes A/S (Bagsvaerd, Denmark). According to Novozymes propyl laurate unit (PLU) method (Basso et al., 2013), the esterification activities of Novozym 435, Lipozyme RM IM and Lipozyme TL IM were 8247, 3200 and 483 U/g, respectively. Standards of monooleoylglycerol, dioleoylglycerol (15% of 1,2-dioleoylglycerol and 85% of 1,3-dioleoylglycerol), trioleoylglycerol, methyl oleate, and 37-component FAME mix (C_4–C_{24}) were purchased from Sigma-Aldrich. n-Hexane, 2-propanol, formic acid and methanol of chromatographic grade were sourced from Kermel Chemical Reagent Co., Ltd. (Tianjin, China). The Amberlite XAD1180 resin was acquired from Rohm and Haas Company (USA). All other chemicals were of analytical grade unless otherwise stated.

2.2. Preparation of immobilized MAS1 lipase

The preparation of immobilized MAS1 lipase was carried out under the conditions of 75 mg lipase solution/g XAD1180 resin, and an equal volume of sodium phosphate buffer (0.02 M, pH 8.0) at a temperature of 30 °C and a speed of 200 rpm for 8 h. Subsequently, the obtained immobilized MAS1 lipase was rinsed with sodium phosphate buffer (0.02 M, pH 8.0) repeatedly until no protein was detected in the eluate. Finally, the obtained immobilized MAS1 lipase was dried in a vacuum desiccator at 40 °C for 8 h and stored in closed vials at 4 °C until use. The esterification activity of immobilized MAS1 lipase was 1605 ± 30.7 U/g according to Novozymes propyl laurate unit (PLU) method (Basso et al., 2013).

2.3. Transesterification of WCO with methanol

The enzymatic transesterification reactions were performed in a 50 mL-conical flask containing 10 g substrates and were initiated by the addition of immobilized lipases with constant shaking at 200 rpm for 24 h. Novozym 435, Lipozyme RM IM, Lipozyme TL IM and immobilized MAS1 lipase as biocatalysts were compared in the production of biodiesel through transesterification of WCO with methanol under the same conditions in a solvent-free system. Then, the effects of oil/methanol molar ratio (1:1, 1:2, 1:3, 1:4, 1:5, 1:6), enzyme loading (40, 60, 80, 100, 120 U/g substrate) and temperature (25, 30, 35, 40, 45 °C) on biodiesel production were investigated, respectively. Samples were withdrawn periodically for high-performance liquid chromatography (HPLC) analysis.

2.4. Reusability of immobilized MAS1 lipase

The reusability of immobilized MAS1 lipase was assessed in the transesterification of WCO with methanol for biodiesel production. The reactions were performed under the optimized conditions. After each reaction cycle (24 h), immobilized MAS1 lipase was separated from the reaction mixture by filtration, and then washed with n-hexane for three times. After that, the recovered immobilized MAS1 lipase was placed at room temperature to remove residual n-hexane, and then reused in the next cycle under the same reaction conditions with the introduction of fresh substrates. The reusability of immobilized MAS1 lipase was evaluated by measuring relative biodiesel yield in subsequent reactions compared to that of the first reaction.

2.5. Purification of biodiesel

Separation of FAME from other compositions was performed using thin-layer chromatography method in the literature (Qin