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Short Communication

Biodiesel production by combined fatty acids separation and subsequently enzymatic esterification to improve the low temperature properties

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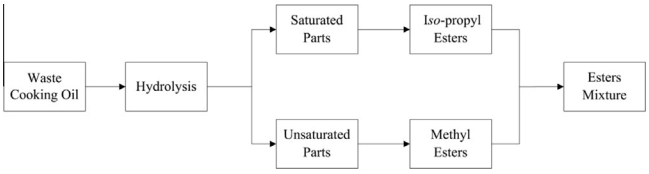
HIGHLIGHTS

- Design of a novel process for enzymatic synthesis of biodiesel.
- Eliminate low temperature crystallization.
- Waste oil hydrolysis, urea complexation and enzymatic esterification are combined.
- Low temperature properties were considerably improved through the novel process.

GRAPHICAL ABSTRACT

Waste oil hydrolysis, urea complexation and enzymatic esterification are combined to produce biodiesel with improved CFPP.



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ABSTRACT

The poor low-temperature properties of biodiesel, which provokes easy crystallization at low temperature, can cause fuel line plugging and limits its blending amount with petro-diesel. This work aimed to study the production of biodiesel with a new process of improving the low temperature performance of biodiesel. Waste cooking oil was first hydrolyzed into fatty acids (FAs) by 60 g immobilized lipase and 240 g RO water in 15 h. Then, urea complexation was used to divide the FAs into saturated and unsaturated components. The conditions for complexation were: FA-to-urea ratio 1:2 (w/w), methanol to FA ratio 5:1 (v/v), duration 2 h. The saturated and unsaturated FAs were then converted to *iso*-propyl and methyl esters by lipase, respectively. Finally, the esters were mixed together. The CFPP of this mixture was decreased from 5 °C to −3 °C. Hydrolysis, urea complexation and enzymic catalyzed esterification processes are discussed in this paper.

1. Introduction

Biodiesel, usually considered as fatty acid methyl esters (FAMEs), is an alternative fuel for diesel engines. It had been tested as an alternative fuel source since the energy crisis in the 1970s. Biodiesel is attractive because it is a biodegradable and renewable energy source with higher flash point and excellent lubricity. The environmental benefits of using biodiesel include lower exhaust emissions of particulate matter, CO and SO_x (Jiang et al., 2014; Jo et al., 2014; Kuo et al., 2013). However, the main disadvantage, limiting the upper fraction of blending biodiesel with petro-diesel at 20% or less, is its relatively poor low-temperature properties (Dunn et al., 1996; Smith et al., 2010). Especially in winter, when the temperature falls below 0 °C, the saturated methyl ester of waste oil will nucleate and form crystals, that propressively plug

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or restrict flow through fuel lines and filters during engine start-up can lead to fuel starvation and engine failure (Knothe et al., 2005). However, the problems of cold plugging are not only experienced at cold start-up, but also during the use of the engine if the temperature suddenly starts to drop.

As was reported, the unsaturated FAMEs have good low-temperature properties, but poor oxidative stability and low cetane numbers (Chastek, 2011; Knothe, 2005; Smith et al., 2010). The saturated FAMEs have good oxidative stability and high cetane numbers, and could increase the cold filter plugging point (CFPP) (ASTM, 2010). However, ethyl and iso-propyl esters have improved low-temperature properties without reducing cetane number or oxidative stability (Knothe, 2008; Lee et al., 1995). Taking a typical saturated fatty acid–palmitic acid as an example, the melting point of its methyl, ethyl- and *i*-propyl esters are 30.5 °C, 24 °C, 13–14 °C, respectively (Knothe et al., 2005). As shown in the results, the melting point of iso-propyl ester of palmitic acid decreased significantly when compared with methyl esters. The sole esterification with ethanol and 1-propanol is however economically prohibitive against the use of cheap methanol.

In this study, waste cooking oil (WCO) was used as raw material to prepare biodiesel. In contrast to previous process, WO was first hydrolyzed into free fatty acid, and then urea complexation was used to separate free fatty acid into saturated and unsaturated fractions. Using *Candida* sp. 99–125 lipase for catalyst, the unsaturated and saturated free fatty acids were converted to methyl and iso-propyl esters respectively (Hama et al., 2013; Lu et al., 2008). The mix of these two esters obviously improved the CFPP of FAMEs from WO. The processes of WO hydrolysis, urea complexation and enzymic catalyzed esterification will be discussed.

2. Methods

2.1. Materials

Waste cooking oil was obtained from Lvming Co. Ltd. Shanghai, China. The waste cooking oil (WCO) contained 83.9% of free fatty acids (FFAs), 0.5% of monoacylglycerols (MAGs), 6.9% of diacylglycerols (DAGs), and 8.7% of triacylglycerols (TAGs). The FFA composition of WCO was as following (wt%): C14:0, 0.8%; C16:0, 21.2%; C16:1, 1.2%; C18:0, 6.6%; C18:1, 34.6%; C18:2, 30.6%; C20:0, 1.0%; C22:1, 0.6%. Based on the FFA composition, the average molecular weight of FFAs was measured at 275.9 g/mol. Free lipase from *Candida* sp. 99–125 was obtained from Kaitai Biochemical Technology Company, Beijing, China; and the activity of the enzyme powder was 50,000 U/g. Other chemicals used in this paper were analytical grade and obtained from Beijing Chemical Factory, Beijing, China.

2.2. Hydrolysis of WO by lipase

The hydrolysis was carried out in a 1 L triple-neck flask with constant agitation at 40 °C. The reaction system contained 200 g WO, 60 g immobilized lipase and 240 g RO water, with 16 h reaction time in total. For analysis, 0.5 ml mixture was taken for acid value determination every hour. For gas chromatography analysis, another 200 μl mixture was taken and centrifuged to harvest the supernatant or upper layer. Then 10 μl of the supernatant was dissolved in *n*-hexane for gas chromatography analysis. All the experiments were replicated at least three times and the results presented were the mean values for the replicated data. The error bars are presented in the figures. Finally, the residual water from the upper layer of the centrifuged reaction mixture was removed by distillation, leaving a 100% oil phase.

2.3. FA separation via urea complexation

Methanol, urea and FAME were mixed into a three neck flask with a reflux and mechanical stirring at a reaction temperature of 50 °C and stirring time 50 min (Bi et al., 2010). Then the mixture was cooled to a specific temperature of 20 °C for a specific time of 2 h. The urea complexes (crystals) and non-urea complexes (filtrate) were separated by filtration with a Buchner funnel. The methanol was recovered from the filtrate by a rotary evaporator under vacuum. The filtrate was washed with saturated sodium chloride solution in 60 °C for two times and then was washed with distilled water in 80–85 °C for one time to remove residual methanol and urea (Stephen et al., 2006). Finally, the unsaturated FA with a low melting point from filtrate was obtained by removing the residue water through a rotary evaporator at 90 °C under vacuum (residue pressure 200 Pa) for 1 h.

The urea complexes were dissolved in distilled water under the condition of 5:8 (w/w) of water to complexes ratio at room temperature, and then the FA in the non-aqueous phase (top layer) was separated from the aqueous phase by a separating funnel. After washing steps as applied for the above filtrate, the FA with a high-melting-point from urea complexes was obtained by removing residual water with a rotary evaporator.

The FA yield of non-urea complexes and urea complexes was calculated as the FA weight of non-complexes and urea complexes divided by the total weight of FA before urea complexation, respectively, as shown in Eqs. (1) and (2) (Bi et al., 2010).

$$\text{Yield of non-urea complexes } (\%) = \frac{\text{Non-urea complexes of FA}}{\text{FA before cystallisation}} \times 100\% \quad (1)$$

$$\text{Yield of urea complexes } (\%) = \frac{\text{Urea complexes of FA}}{\text{FA before crystallization}} \times 100\% \quad (2)$$

where the non-urea complexes of FA is the weight of FA as filtrate, the urea complexes of FA is the weight of FA obtained as crystals, and the FA before crystallization is the weight of FA added into flask.

2.4. Gas chromatography analysis

The free fatty acid and esters contents in the mixture were quantified by a GC-2010 gas chromatography (GC, Shimadzu Japan). The GC analytical method was the same as reported (Liu et al., 2014). Heptadecanoic acid and its methyl ester purchased from Sigma were used as an internal standard. The esters′ yield is defined as esters amount produced divided by the initial amount of oil (g/g).

2.5. Enzyme catalyzed esterification

The methanolysis and iso-propanolysis were carried out in a 1 L triple-neck flask with constant agitation at 40 °C. The reaction system contains 600 g waste oil, 80 g immobilized lipase and 60 g RO water. The reaction time was in total 40 h, and 4.6 g methanol or 8.6 g iso-propanol was added into the reaction system every 2 h (Lu et al., 2010). For analysis, 0.5 ml mixture was taken for acid value determination every hour. The analytical procedure was identical as described in Section 2.2.