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Biodiesel production from woody oil catalyzed by *Candida rugosa* lipase in ionic liquid

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ABSTRACT

In this study, biodiesel production from Chinese tallow kernel oil catalyzed by *Candida rugosa* lipase (CRL) in ionic liquid was investigated. Biodiesel was prepared from Chinese tallow kernel oil. Nineteen kinds of ionic liquids were screened for CRL to enhance its catalysis ability for biodiesel synthesis. Interestingly, [Hmim][PF_6], not only help to simplify the downstream processing, but also boost the efficiency of CRL from about 35% to 95.4%. In addition, the biodiesel mixed with various blending ratios of fossil diesel was used to fuel the conventional diesel engine to examine fuel and emission properties. Most of fuel characteristics of the biodiesel met the standards set by EN and ASTM. Its output power was found to be close to pure diesel. The emission products, including hydrocarbons (HC, 52.7% lower) and carbon monoxide (CO, 48.6% lower), decreased substantially with the exception of oxynitride (NOx) emission, which displayed a 12.8% increase. The results suggest that biodiesel production from woody oil catalyzed by CRL in [Hmim][PF_6], is feasible in future application with a promising prospect.

1. Introduction

Although biodiesel has been under development for several decades, it cannot at present completely replace fossil fuels, which is to some degree due to the high production cost involved, as about 70–95% of the total biodiesel production cost comes from the raw materials [1]. Cheap feedstock, such as inedible woody oils may be a good solution in reducing production costs. In fact, biodiesel production from woody oils has been reported in many previous studies [2,3]. Chinese tallow kernel (CTK) oil is a typical non-edible woody oil from Chinese tallow seeds that contains 40–70% fatty acids. More than 100,000 metric tons are produced annually in China [4,5]. Moreover, due to the limited cultivating land area and large consumption of edible oil, Chinese government has regulated laws to prohibit edible oil from being used for biodiesel production. Therefore, in China, woody oil is the first choice for biodiesel feedstock.

As known, lipases are regarded as the most attractive catalysts for biodiesel production because of their green and mild catalysis properties. In addition, rapid development of genetic engineering and fermentation engineering makes large-scale production of cheap lipases available. For example, in our laboratory condition, the cost of *Candida rugosa* lipase can be controlled to less than 150 USD/kg, which is one-tenth of that of Novozym 435, a most widely used commercial lipase in biodiesel preparation [6–8].

However, although lipases have many advantages in environment protection, they are easily inactivated by reaction substrates and byproduct. To solve these bottlenecks, researchers turned to the aid of organic solvents which are toxic, inflammable, and highly volatile [9–11]. As an alternative to these dangerous solvents, ionic liquids (ILs) are particularly intriguing. ILs are organic salts that are entirely composed of ions including bulky asymmetric cations and small inorganic anions. ILs have some attractive features, such as negligible vapor pressure, recyclability, low combustibility, high thermal stability, and the possibility of existence in the liquid state at room temperature. Actually, these features have made them as potential green solvents for biodiesel production. Most noteworthy is that ILs can actively influence the activity of lipases by the interactions between protein and cations/anions in ILs [12]. Pan et al. [13] reported there was a decrease tendency of a-helix content for lipase protein in ILs. So, choosing proper combination of cations and anions has feasibility to boost the activity of lipase. Taking *C. rugosa* lipase (CRL) as an example, which will be used in the following experiments, it is often regarded as an inappropriate

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catalyst for biodiesel catalysis because of its generally low transesterification efficiency. Lee et al. reported that CRL immobilized with silica gel was used for biodiesel production, and only about a 30% biodiesel yield was obtained after 30 h [14]. Shah et al. [15] also demonstrated that the free CRL and immobilized CRL almost had no yields in transesterification of *Jatropha* oil. However, when ILs is applied for the CRL-catalyzed transesterification reaction, incredible things have happened. Kaar et al. [16] discovered that the transesterification of methyl methacrylate with 2-ethylhexanol in 1-butyl-3-methylimidazolium hexafluorophosphate (one type of IL) proceeded at a rate that was 1.5-fold greater than that in organic solvent. Meanwhile, other researchers also proved that ILs of [OmPy][BF_4] were able to replace organic solvents and got 82.2% conversion yield [17]. Therefore, we attempted to find out a novel way to boost the activity of CRL via choosing the most appropriate IL. If this succeeds, it may provide a possibility to thoroughly change the unfavorable situation of certain lipases that used to be regarded as unsuitable for biodiesel production. It also may mean wider kinds of lipases can be used and exhibit equal or even better performance than those expensive commercial lipases. However, so far, there has been no systematic research on the effects of ILs on CRL. Hence, in this study, we first tried to sieve the most suitable IL for CRL from 19 ILs, and then systematically optimize the procedure of biodiesel preparation in the ILs.

In addition, especially in the field of enzymatic catalysis, most efforts have been devoted to the synthesis technologies of biodiesel itself, but few studies have shown any concerns with the integrated process from production to application, particularly in downstream practical applications such as fuel characteristics, adaptability in conventional diesel engines, and emission profiles. Therefore, in this study, we employed an integrated process for biodiesel production with Chinese tallow kernel oil (CTK oil) as raw material to further explore its fuel and emission properties. To fulfill it, we tested the practical performance of biodiesel synthesized from CTK oil in a conventional diesel engine, and examined its output power, fuel combustion, and emission properties. To the best of our knowledge, no previous work has been done on this integrated process.

Therefore, based on the above analysis, the main purposes of this study were to: (1) reduce the cost of biodiesel production by utilization of woody oils; (2) systematically explore on the effects of ILs on CRL to fish out the most proper ILs to enrich the selection range of catalysts; and (3) test the practical performance of biodiesel synthesized from CTK oil in a conventional diesel engine.

2. Materials and methods

2.1. Materials

CTK oil was purchased from a local oil process company from Dawu County, Hubei, China. The physicochemical properties of CTK oil were listed in Tables 1 and 2. Chromatographical purity of methyl heptadecanoate standard substance was bought from Sigma–Aldrich (USA). Other chemicals, including acetone, ethanol, *tert*-amyl alcohol, *tert*-butanol, cyclohexane, petroleum ether, *n*-hexane and isooctane, were of analytical grade and obtained from Sinopharm Chemical Reagent Co. Ltd., Shanghai, China. CRL was commercially available from Sigma–Aldrich (St. Louis, Missouri, USA). Nineteen ILs (99% purity) were got from Shanghai Cheng Jie Chemical Co. Ltd. (Shanghai, China).

Table 1
Physico-chemical properties of CTK oil.

Item	Value
Density (g/cm^3, 25 °C)	0.92
Moisture and volatile matter content (%)	0.12
Phospholipid content (mg/g)	0.011
Dynamic viscosity (mPa·s, 25 °C)	47.87
Acid value (mgKOH/g)	1.36
Saponification value (mgKOH/g)	201.28
Peroxide value (meq/kg)	4.17
Iodine value (g/100 g)	186.75
Average molecular weight (g/mol)	841.99

2.2. Methods for biodiesel synthesis

The reactions for screening ILs and organic solvents were conducted in a 50-mL screw-capped flask, which contained CTK oil (2.5 mmol), lipase (0.22 g, 10 wt % based on oil weight), methanol (10 mmol), and ILs or corresponding organic solvents (2.2 mL, 1:1 v/v based on oil volume). The reaction mixture was stirred at 40 °C with an agitation rate of 200 rpm in a thermostat shaking bed for 24 h. The experiments were carried out in triplicates.

For the CRL-catalyzed biodiesel preparation, the method of GC analysis was according to our previous work [17]. The biodiesel yield (%) was calculated by Equations (A) and (B) [17].

$$\text{Biodiesel yield }(\%) = \frac{Asample\, f0}{Ainternal\ /Winternal} \tag{A}$$

$$f0 = \frac{Wsample\ Ainternal}{\text{Winternal}\ Asample} \tag{B}$$

where, A_{sample} is the peak area of free fatty acids in the sample, f_0 is the response factor, $A_{internal}$ is the peak area of the internal standard, $W_{internal}$ is the weight of the internal standard, and W_{sample} is the weight (g) of the sample.

2.3. CTK oil fatty acids profiles

Methanolysis of oil was conducted to obtain fatty acid methyl esters. Sixty micrograms of oil sample was taken in a test tube, with addition of 4 mL of isooctane to dissolve the oil. Two hundred microliters of potassium hydroxide methanol solution was then added. After 30 s of shaking, the solution was then kept static for 5 min. Subsequently, 1 g of sodium bisulfate was added to neutralize the solution. After another 3 min of standing, the methyl esters in the upper layer of the solution were taken out for GC analysis.

2.4. The specifications of instruments

The experiments were conducted on a direct-injection, four-stroke, single cylinder diesel engine. All the specifications were list in Table 3.

Emissions were detected by utilizing a Gasboard-5020 exhaust gas analyzer, which could take measurements continuously. The average concentrations of CO, CO_2, NOx, and HC were recorded. The

Table 2
Proximate fatty acid profile of CTK oil.

Item	Value
2, 4-decadienoic acid (C10:2)	4.4%
Palmitic acid (C16:0)	6.96%
Stearic acid (C18:0)	2.0%
Oleinic acid (C18:1)	14.08%
Linoleic acid (C18:2)	29.75%
Linolenic acid (C18:3)	42.8%