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Application of the ZIF-8 towards Immobilizing *Aspergillus ni*ger Lipase (ANL@ZIP-8) in Biodiesel Preparation

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Abstract: ***Biodiesel was produced from waste soybean oil, it is renewable source, used to transesterification catalyzed by lipase immobilized (ANL@ZIF-8). The optimal conditions for methanolysis are 1:4 (mol/mol) ethanol to oil molar ratio, 9 wt% water content, 35°C and lipase dosage 6 wt% in 15 wt% isooctane reaction system.The conversion yield was 90% a maximal yield in 15 h reaction time under these optimal conditions. Immobilized enzyme could be used for 6 cycles without appreciable loss in activity. These results indicate that soybean oil production is a suitable raw material to produce biodiesel via in situ transesterification using ANL@ZIF-8.***

Keywords: Aspergillus niger lipase (ANL); transesterification; Zeolitic imidazolate framework; Biodiesel

1. Introduction

The depletion of fossil fuel resources and the environmental impacts of fossil fuel use are the main motives for investigating and developing renewable fuel sources [1]. Furthermore, the term biodiesel implies a fuel that is biodegradable, and such fuels are being increasingly used in compression-combustion engines [2]. Thus, according to global Oil and Gas Industry Magazines, global biodiesel production in 2016 increased by up to 3280 million tons, with a yearly increase of 11%, or 310–320 million tons [3].

The biodiesel or known as fatty acid alkyl esters have been considerably investigated. It is a clean source with low exhaust emissions, such as CO, SOx, and HC [1]. Also, biodiesel is usually produced by the transesterification of oils or lipids or the esterification of fatty acids with short-chain alcohols. Primary routes are chemical, physical, and enzymatic routes [4]. In comparison with chemical and physical routes, enzymatic routes, such as lipase catalysis, have advantages because of their low energy consumption, simple post-processing, environmentally friendliness, and compatibility with all kinds of raw oils, especially those with high free fatty acid contents [3]. The Lipase-catalyzed biodiesel production has been reported. Various lipases include *Burkholderia cepacia* lipase (BCL), *Candida antarctica* Lipase B (CALB), *Rhizomucor miehei* lipase (RML), *Candida rugosa* lipase (CRL), *Rhizopus oryzae* lipase (ROL), *Aspergillus niger* Lipase (ANL) and *Thermomyces lanuginosus* lipase (TLL) [5].

However, the BCL lipase can maintain good transesterification catalytic performance in anhydrous or slightly aqueous systems, but CALB in an organic phase can maintain a high transesterification catalytic activity. PCL and porcine pancreatic lipase are more sensitive to short-chain fatty acid. *Rhizopus* lipaseand *Aspergillus niger* lipases are specific to long-chain fatty acids. The free lipase is unstable. As such, it is readily affected by the environment and not easily recovered. It is rarely used directly in industrial production [6]. At present, lipases used in biodiesel preparation are mostly immobilized and commercially available immobilized lipases include non-location-specific Novozym 435, 1, 3-specific Lipozyme TLIM, and Lipozyme RM IM. Immobilized lipases can relieve the toxic effect of methanol on enzymes to a certain extent and improve their stability [7]. Although biodiesel production by enzymes has many advantages, few industrial chemical factories produce biodiesel by using enzymes because the high cost of raw oil and lipase leads to the high price of biodiesel. In comparison with edible oil, non-edible oil, such as jatropha oil [4], yeast oil [5], castor oil, natural palm oil [6], algal oil [7], and household waste oil [8], show potential for biodiesel production. Immobilization can effectively improve the stability and availability of lipases [9], thereby providing a new way for the industrial application of biodiesel technology. To this study the industrial application of the ANL@ZIP-8 of immobilized enzymes, we utilized soybean oil as a raw material and immobilized enzyme as a biocatalyst for biodiesel preparation. The following factors influencing the biodiesel preparation were optimized: amount of added enzyme, water content, reaction temperature, alcohol-to-oil molar ratio, reaction time, and amount of added isooctane.

2. Materials and methods

2.1 Materials

ANL@ZIF-8 was prepared in a laboratory. Soybean oil was obtained from local supermarkets. The standards of fatty acid methyl/ethyl esters were purchased from Aladdin Industrial Corporation (Shanghai, China). Other analytical-grade reagents, including isooctane and hexane, were procured from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2 Biodiesel production via enzymatic catalysis

Transesterification for biodiesel production was conducted in 50 mL capped flasks with stirring at 200 rpm. The mixture was composed of 2.19 g of soybean oil, immobilized lipase (ANL@ZIF-8), isooctane, water, and ethanol. At regular intervals, ethanol was added in three steps to avoid its inhibitory influence on the immobilized enzyme. All of the dosage percentages were based on the oil weight. The effects of biodiesel production conditions, including amount of isooctane, amount of immobilized enzyme, water content, alcohol-to-oil molar ratio, reaction temperature, and reaction duration, were methodically determined. After a specified reaction time, the supernatant was collected through centrifugal separation at 12,000 rpm for 5 min. Thereafter, 10 µL of the supernatant was withdrawn and mixed with 290 µL of n-hexane and 300 µL of 1.0 mg/mL heptadecanoic acid methyl ester as the interior standard. The mixture was then completely agitated for gas chromatography (GC) to determine biodiesel yield. All of the experiments were repeated thrice. (**Fig 2.1**)

2.3 GC analysis of biodiesel yield

The content of fatty acid ethyl esters (FAEEs) was analyzed via GC through a previously reported method [9]. The biodiesel yield was specified using a GC-9790 gas chromatography system (Fuli Analytical Instrument Co., Wenling, China) furnished with an Agilent HP-INNOWax capillary column (30 m × 0.25 mm × 0.25 µm; Agilent Technologies, Folsom, CA, USA). The initial temperature of the column was set at 180 °C, raised to 230 °C at a rate of 3 °C/min, and maintained at 230 °C for 3 min. The temperatures of the injector and the hydrogen flame ionization detector were set to 230 °C and 280 °C, respectively. Nitrogen was used as a carrier gas at a flow rate of 3 mL/min. The biodiesel yield (%) is described as the total FAEE content in the conversion oil. The product was calculated using Equations (1)–(3) [10].

$$\text{Weight of FAEE} = \frac{A_{sample} f_0}{A_{internal} W_{internal}} \quad (1)$$

$$f_0 = \frac{W_{sample} A_{internl}}{W_{internal} A_{sample}} \quad (2)$$

$$\text{Biodiesel yield (\%)} = \frac{W_e}{W_t} \times 100\% \quad (3)$$

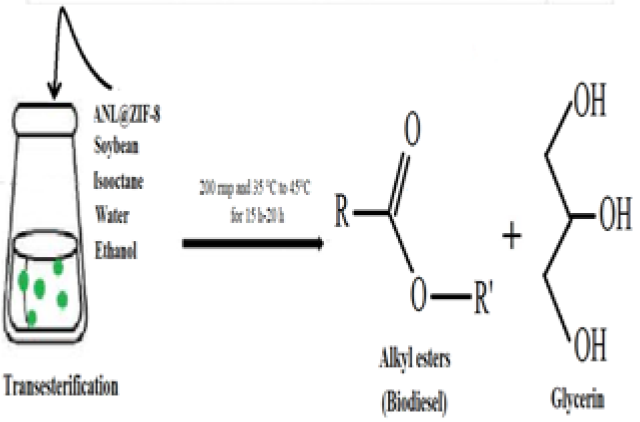

Figure 2.1: The scheme of the path of this research

Where, A sample is the peak area of FAEE in the sample, f_0 is the response factor, Ainternal is the peak area of the internal standard, Winternal is the weight of the internal standard, Wstandard is the weight of standard substance of FAEE, W′internal is the weight of the internal standard, Astandard is the peak area of standard substance of FAEE, We is the experimental value of all FAEEs identified by the GC equipment, and Wt is the theoretical value of all FAEEs.

3. Results and Analysis

3.1 Influence of transesterification parameters on the biodiesel yield

The immobilized lipase ANL@ZIF-8 was used to catalyze biodiesel preparation. Various factors, including the amount of enzyme added, organic solvents, water addition, alcohol-to-oil molar ratio, reaction time, reaction temperature and alcohol addition strategies, influencing the production of biodiesel from soybean oil were optimized.

3.1.1 Influence of the amount of added isooctane on the biodiesel yield

The characteristics of organic solvents affect not only the mass transfer in the reaction system but also the enzyme structure and activity. Enzymes perform different catalytic activities, substrate selectivities, operating stabilities, and kinetic properties in various solvents. Li et al.[11] investigated the performance of *Candida* sp. 99-125 catalyzing the methanolysis of glycerol trioleate in 12 different solvents and observed that the fundamental effect of organic solvents on enzymatic catalysis is hydrophobicity (log *P*, logarithm of the partition coefficient (P) of a solvent in a two-phase 1-octanol and water system). Therefore, the ANL@ZIF-8-catalyzed transesterification of soybean oil with ethanol in the isooctane system was investigated. In (**Fig. 3.1**) the biodiesel product considerably increased to 82.5 % with the addition of 15% isooctane to the reaction system. At above 15% isooctane, the biodiesel yield gradually decreased.

Figure 3.1: The effect of isooctane amount on biodiesel yield catalyzed by ANL@ZIF-8

3.1.2 Influence of the water content on the biodiesel yield

The moisture content of reaction systems is an important factor that determines the lipase-catalyzed transesterification reaction rate and the output of biodiesel synthesis. Enzymes should require the addition of a small aliquot of water to maintain their activities[12]. For the immobilized enzyme ANL in the isooctane system, the highest biodiesel yield in the isooctane system was 86.5% when 9 wt% water was added. At concentrations larger than these values, the

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biodiesel yield decreased continuously (**Fig.3.2**).

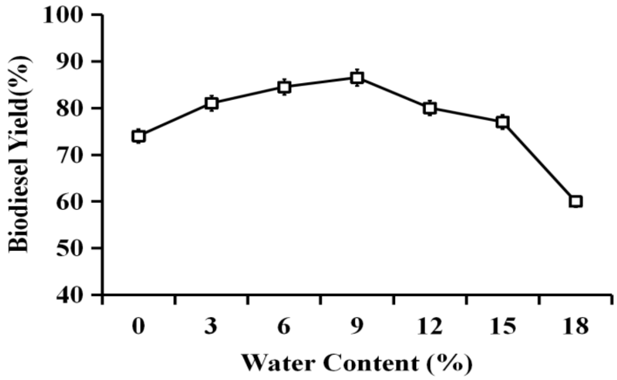

Figure 3.2: The effect of water content on biodiesel yield catalyzed by ANL@ZIF-8.

3.1.3 Influence of alcohol-to-oil ratioon the biodiesel yield

In **Fig. 3.3** illustrates the effect of alcohol-to-oil ratio on the yield of biodiesel catalyzed by immobilized enzymes. As the molar ratio increased from 1:2 to 1:4, the biodiesel conversion yield of ANL gradually increased and then declined consistently when the amount of ethanol further increased. Therefore, a molar ratio of oil to ethanolof 1:4 was appropriate for FAEE production.

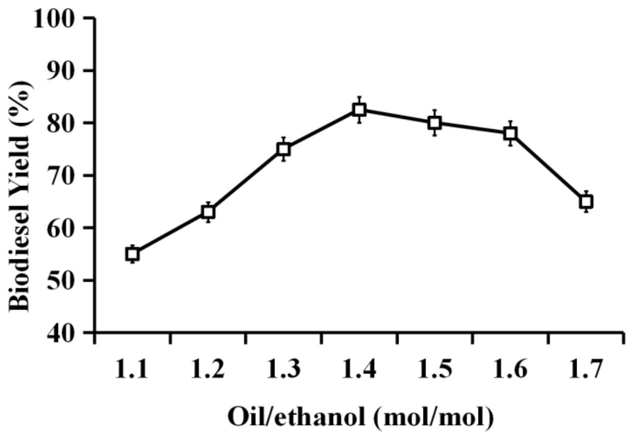

Figure 3.3: The effect of the molar ratio of ethanol to oil on yield catalyzed by ANL@ZIF-8.

3.1.4 Influence of reaction temperature on the biodiesel yield

Transesterification is an endothermic reaction, that is, when temperature increases, the final output increases. However, enzymes show the best activity at a suitable temperature based on their kind and immobilization technique. Hence, the effect of temperature on enzymatic reactions should not be disregarded. The optimum reaction temperatures for biodiesel catalyzed by this immobilized enzymes are different, and the highest product yield of biodiesel was obtained at 35 °C (**Fig. 3.4**). A further increase in temperatures induced a notable decrease in biodiesel product yield.

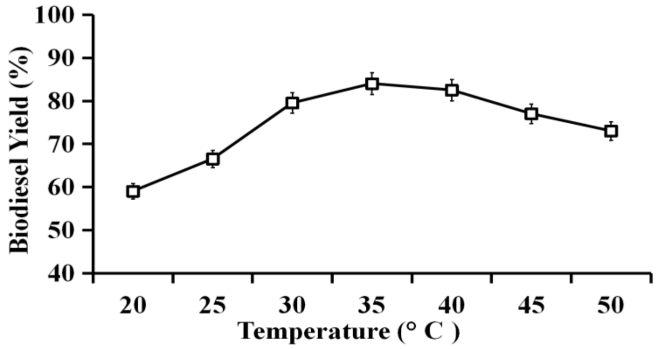

Figure 3.4: The effect of temperature on biodiesel yield catalyzed by ANL@ZIF-8

3.1.5 Influence of time interval of ethanol addition on the biodiesel yield

The time factor and the addition period of alcohol are important in biodiesel production. The addition of more than half the amount of alcohol required to the reaction system at the beginning will obstruct the enzyme activity and reduce biodiesel synthesis [13]. Therefore, in the reaction catalyzed by immobilized enzymes, a three-step addition method was adopted. ANL were subjected to the addition of alcohol at reaction temperatures of 35 °C.For the immobilized ANL, the time interval of ethanol addition significantly influenced the yield of biodiesel; that is, as the time interval of the addition was extended, the final product yield of biodiesel also increased. Therefore, was obtained on the highest yield in 24 h reaction time with intervals 8 h for added ethanol. However, the prolongation of the time of ethanol addition did not evidently affect the final yield of biodiesel production catalyzed by immobilized ANL. Thus, was reached on a maximal yield in 15 h reaction time with intervals 4 h for added ethanol (**Fig. 3.5**).

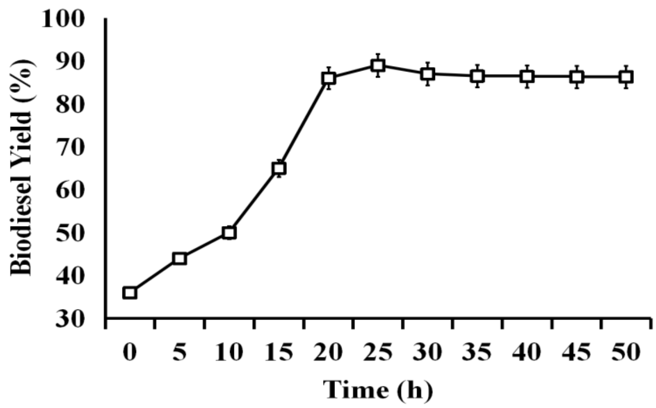

Figure 3.5: The effect of time interval of ethanol addition on biodiesel yield catalyzed by ANL@ZIF-8

3.1.6 Influence of immobilized enzyme addition on the biodiesel yield

The amount of the immobilized lipase in the reaction mixture was optimized to reduce the cost of biodiesel production and obtain the maximum yield. The biodiesel yield increased as the quantity of the immobilized enzyme increased to 6 wt % of ANL@ZIF-8 (**Fig. 3.6**). As the amount of the enzyme further increased, the biodiesel yield almost did not improve.

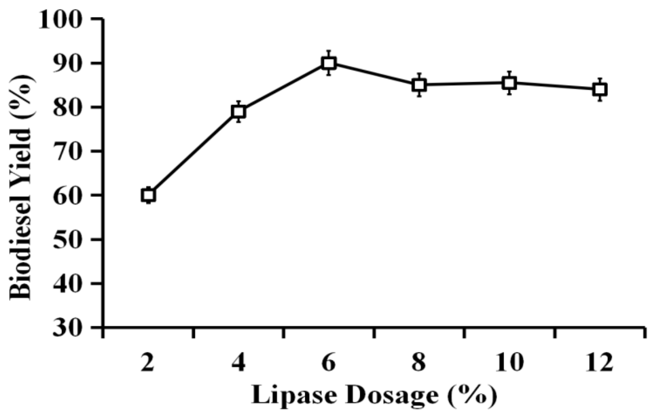

Figure 3.6: The effect of ANL@ZIF-8 dosage on biodiesel yield

3.2 Operational stability of immobilized enzymes

One of the objectives of using an immobilized enzyme is to design an efficient bio-catalyst that can be quickly recovered and reused. Reusability is an essential reference for the evaluation of the influence of immobilized enzymes. To study the reusability of the immobilized enzymes in biodiesel applications, we separated and recovered the immobilized enzymes by using a centrifuge after each batch of reaction was completed. The reaction times of ANL in each batch was 15 h. Other reaction conditions were maintained and the reusability of the immobilized enzyme was investigated (**Fig. 3.7**).

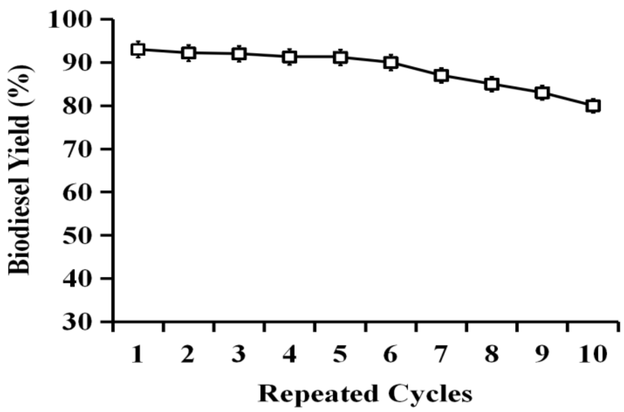

Figure 5.7: The reusability of ANL@ZIF-8 for biodiesel production

4. Discussion

ANL@ZIF-8 was used as catalysts for the preparation of biodiesel from soybean oil via transesterification. During transesterification, the biodiesel yield could be affected by various reaction parameters.

In transesterification catalyzed by lipases, an organic solvent noticeable affects a reaction system. In this study, the above biodiesel yield was obtained in the isooctane with 15 wt % concentration probably the reason returned to isooctane solvent molecules can interact with hydrophobic amino acid residues in the lid that covers the catalytic site of an enzyme,Subsequently keeping the enzyme in its open conformation and leading to a high catalytic activity[14]. A biodiesel yield gradually decreased when the amount of added isooctane was above 15 wt % possibly because the poor solubility of ethanol and the byproduct glycerol in isooctane as a solvent; consequently, mass transfer and diffusion constraints, as well as inactivating lipase, increase [15].

A Water is regarded as one of the main factors in a mixture of transesterification reaction because of a lipase exhibits a unique characteristic of acting as an interface between an aqueous phase and an organic phase, so the lipase activity generally depends on the interfacial area[16]. Nevertheless, the moisture content in a transesterification reaction mixture can positively or negatively affect the lipase catalytic activity. Excess moisture in a reaction mixture may cause lipase to become more flexible and can induce undesirable reactions, such as hydrolysis. For example, Khan et al. [17].

The catalytic activity of each enzyme at the optimal temperature directly influences the rate and velocity of a reaction. High reaction temperatures may inactivate immobilized lipase and favor hydrolysis, and this observation is consistent with the trends in previousreports [18, 15]. Cui et al. [19] obtained the maximum yield of biodiesel at 35 °C when they used immobilized BCL in biodiesel production. Fan et al.[20] observed the highest conversion at 50 °C when they utilized immobilized RML in biodiesel production.

Alcohols have double roles in transesterification, an excess amount of alcohols enhances the reaction rate and shifts transesterification toward an increased production of FAEEs [21]. Second, a high alcohol concentration negatively affects the activity and stability of enzymes by binding immobilized enzymes to insoluble alcohol present in a reaction system, leading to a decrease in the production yield of biodiesel [22, 23]. Thus, in our experiment, we determined the maximum amount of added ethanol that caused the least damage to immobilized lipase and resulted in a maximal yield of fatty acid ethyl esters.

In terms of the effect of the time interval of ethanol addition on biodiesel production, the biodiesel yield maintained a dynamic equilibrium as the reaction time was prolonged and did not significantly increase when the reaction time passed 15 h to 24 h. This phenomenon may be ascribed to the increasing water content as transesterification reaction progresses and the larger water content induced biodiesel hydrolysis [20, 24].

The amount of immobilized lipase is a key factor that affects biodiesel production. When the amount of enzyme continuously increased, the transesterification efficiency did not improve significantly because the reaction rate was restricted by the number of active sites that the complex enzyme could provide when the amount of substrate was constant. When the amount of the substrate was much higher than that of the active site, increasing the amount of enzyme could remarkably improve the transesterification efficiency. However, when the amount of added enzyme exceeds a specified amount, the amount of a substrate molecule becomes a limiting factor [21, 23]. ANL@ZIF-8 can retain a 78.3% conversion yield after a continuous running of 10 cycles.

5. Conclusion

ANL@ZIF-8 was used as catalysts and soybean oil as substrates to synthesize the preparation of biodiesel. Its preparation conditions were also optimized. Under the optimal conditions, immobilized ANL@ZIF-8 showed good catalytic effect on soybean oil in biodiesel production to reach 90%.

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