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Lipase catalyzed methanolysis to produce biodiesel: Optimization of the biodiesel production

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Abstract

A lipase from *Candida* sp., suitable for transesterification of fats and oils to produce fatty acid methyl ester (FAME), was immobilized on a cheap cotton membrane, in this paper. The conversion ratio of salad oil to biodiesel could reach up to 96% with the optimal reaction conditions. Continuous reaction in a fixed bed reactor was also investigated. A three-step transesterification with methanol (methanolysis) of oil was conducted by using a series of nine columns packed with immobilized *Candida* sp. 99–125 lipase. As substrate of the first reaction step, plant or waste oil was used together with 1/3 molar equivalent of methanol against total fatty acids in the oil. Mixtures of the first- and second-step eluates and 1/3 molar equivalent of methanol were used for the second- and third-reaction steps. A hydrocyclone was used in order to on-line separate the by-product glycerol after every 1/3 molar equivalent of methanol was added. Petroleum ether was used as solvent (3/2, v/v of oil) and the pump was operated with a flow rate of 15 L/h giving an annual throughput of 100 t. The final conversion ratio of the FAME from plant oil and waste oil under the optimal condition was 90% and 92%, respectively. The life of the immobilized lipase was more than 10 days. This new technique has many strongpoints such as low pollution, environmentally friendly, and low energy costs.

Keywords: Biodiesel; Methanolysis; Immobilized lipase; *Candida* sp. 99–125 lipase; Fixed bed reactor

1. Introduction

Biodiesel, monoalkyl esters of vegetable oils or animal fats, 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 non-toxic, biodegradable and renewable energy source. Additional environmental benefits include lower exhaust emissions of particulate matter and greenhouse gases such as CO, CO_2 and SO_x.

Conventionally the synthesis of alkyl esters is accomplished by chemical transesterification. Chemical methods give high conversion ratio of triacylglycerols (TAG) to methyl esters (biodiesel) in short times (4–10 h) [1]. However, chemical transesterification are connected with some drawbacks as for example, high energy consumption, difficulty in glycerol recovery, and a high amount of, alkaline waste water from the catalyst. The enzymatic transesterification has been performed in solvent and in solvent-free media by various immobilized lipases [2–8]. For example, Nelson et al. [9] carried out enzymatic alcoholysis of TAGs with the aim of biodiesel production. When alcoholyses of several oils and fats with MeOH and EtOH were conducted using immobilized *Rhizomucor miehei* lipase in the presence of *n*-hexane, >95% of the TAGs were converted to their methyl (ethyl) esters. Methanolysis of beef tallow reached 65% under the similar reaction conditions but in the absence of organic solvent. The stepwise addition of methanol allowed a degummed soybean oil transesterification of 93.8% in a solvent-free system, and the reuse for 25 cycles of immobilized *Candida antarctica* lipase [10]. Deng et al. [11] used *Candida* sp. 99–125 lipase catalyzed esterification produced biodiesel with the petroleum ether as the solvent, the conversion ratio of methyl ester reached 94%.

In this study, a cheap lipase preparation from *Candida* sp. 99–125 was used as the catalyst. The enzymatic transesterification of salad oil and waste oil from Beijing with methanol in solvent system was studied. The reaction conditions for batch and continue reaction were optimized.

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Table 1
Fatty acid composition in salad oil triglycerides

Fatty acid		wt%
Molecular formula	Name of fatty acid	
C14:0	Myristic acid	0.20
C16:0	Palmitic acid	21.66
C16:1	Palmitoleic acid	0.22
C18:0	Stearic acid	2.30
C18:1	Oleic acid	19.15
C18:2	Linoleic acid	55.86
C18:3	Linolenic acid	0.28
C20:1	Erucic acid	0.19
C20:0	Arachidic acid	0.14

Table 2
Components of waste oil

Components	Content (%)
FFA	46.75
MAG	1.44
DAG	5.71
TAG	46.10

2. Materials and methods

2.1. Materials

Salad oil was bought from the local market. It had an average molecular weight on 859 g/mol calculated from the free fatty acid (FFA) composition. See Table 1 for the FFA composition. Waste oil was obtained from Beijing environmental office. It had an average molecular weight on 683 g/mol. See Table 2 for the composition of waste oil, it have the same FFA composition as the salad oil. Lipase from *Candida* sp. 99–125 [12] was immobilized by absorbing onto a textile membrane [11]. Methanol, with a purity of 99% was obtained from Yili Chemical Co. Ltd. (Beijing, China). All the solvents used were of analytical grade and were obtained from Yili Chemical Co. Ltd. (Beijing, China). They were dried by molecular sieves before use.

2.2. Methanolysis

Batch reactions were carried out in 50 mL stoppered flasks with 5 mL of solvent. If not otherwise stated, the reaction was performed with 2 g of oil, 5 mL of *n*-hexane, 20 wt% of salad oil (2 g) immobilized *Candida* sp. 99–125 lipase and 10 wt% of salad oil water. Every 10 h 93 μL methanol (oil:methanol molar ratio is 1:1) were added until the theoretical molar ratio was reached. The mixture was incubated on an orbital shaker at 40 °C and180 rpm.

Continuous methanolysis of oil was conducted using columns (ID 18 cm, high 1 m) packed with immobilized *Candida* sp. 99–125 lipase (activity, 7000 U/g and lipase load, 500 g per coloum). The reactor was heated by a 40 °C circulatory water bath. The overall process of biodiesel production in the fixed bed reactor is seen in Fig. 1. A mixing tank was used before the first reactor. In every reaction step the molar ratio of oil:methanol was 1:1. Petroleum ether was used as the solvent (1:1, petroleum ether/oil, v/v), and 10% water (water/oil, v/v) was added into the reaction system.

2.3. Separation of glycerol

By-product glycerol was separated by a hydrocyclone after every reaction step.

2.3.1. Gas chromatography

Aliquots of the reaction mixture were withdrawn and the concentrations of the product were determined by a gas chromatographic analysis. A GC-2010 gas chromatograph (Shimadzu,

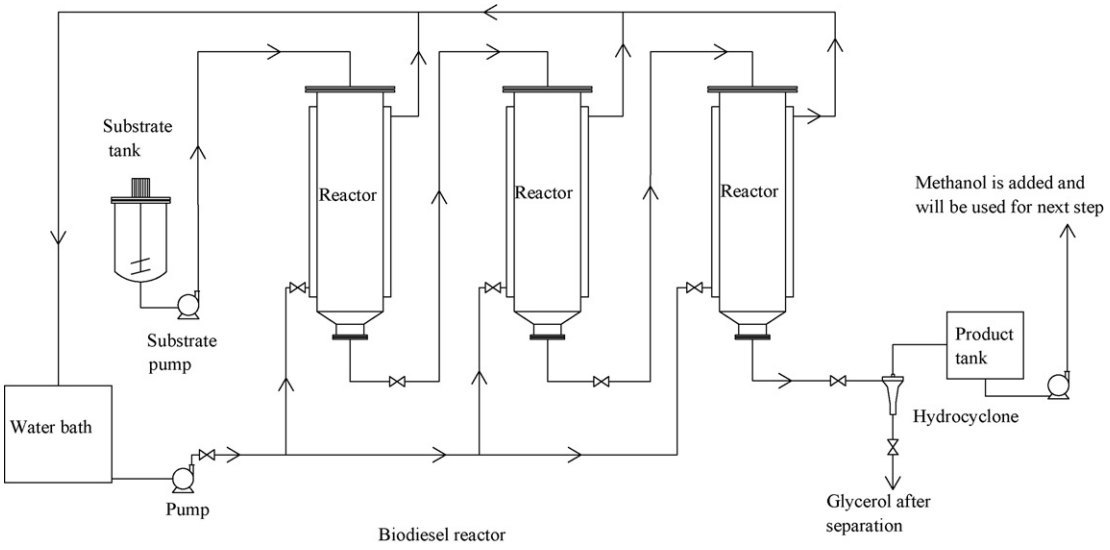

Fig. 1. Process of biodiesel production in fixed bed reactor.