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Effect of chitosan's amino group in adsorption-crosslinking immobilization of lipase enzyme on resin to catalyze biodiesel synthesis

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Abstract

Lipase as biocatalyst is used in biodiesel production but its price is becoming serious problem. Immobilization could improve the ability of enzyme. Immobilization method which gives higher activity and stability is adsorption-crosslinking method. The addition of amino group on supports has proven to stabilize the enzyme. Thus, this research focused on the improvement of lipase immobilization performance by the addition of chitosan which contains amino group. The highest unit activity (24.69 U/g resin) is reached by immobilized lipase on anion-exchange macroporous resin with addition of chitosan on resin directly. This enzyme produces biodiesel with yield of 50.79%.

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Keywords: Adsorption-crosslinking; chitosan; immobilization; lipase; resin

1. Introduction

Lipase is one of the enzymes which used to synthesis biodiesel. However, this commercial lipase is too expensive, difficult to separate and cannot be reused after the reaction. Thus, some immobilization methods were

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developed to put the enzyme in a space physically to retain its catalytic activity [1-3]. The term of space means solid matrix that is called as support. Enzyme immobilization could separate enzyme from medium, so enzyme could reuse in many reaction. One of immobilization methods is adsorption-crosslinking that has higher stability and activity than other crosslinking methods (CLEA and CLEC). Crosslinking reaction is a formation of three dimensional networks between enzyme, support, and reagent. However, this method is not totally perfect due to the lack of amino groups on the support, which led a decrease of enzyme activity due to the conformational changes of enzyme. Some previous researchers [1-6] proposed the addition of chitosan as a source of amino group on the support in enzyme immobilization. The addition of chitosan is expected to increase stability of enzyme immobilization on resin. Chitosan addition on enzyme immobilization should be examined further to get chitosan addition method and types of resin which is resulting excellent enzyme activity. Thus, this research will be focused on the effect of chitosan's amino group that are seen from chitosan addition method in commercial lipase enzyme immobilization on various types of resin on the enzyme activity and immobilized enzyme stability.

2. Methods

2.1. Lipase enzyme preparation

1 g of commercial lipase *Candida rugosa* powder is dissolved in 10 mL phosphate buffer (pH 7) to obtain lipase solution 0.01 g/mL.

2.2. Adsorption-crosslinking immobilization

Commercial lipase enzyme will be adsorbed on resin, and then treated with glutaraldehyde as crosslinking reagent. In the first method (method 1), chitosan solution (0.2 g chitosan powder dissolved in CH_3COOH 5% 25 mL) is adsorbed on resin. Then, the lipase solution is mixed with 0.75 g resin – chitosan and stirred in shaker water bath with conditions of 30 ºC, 150 rpm for 4 hours. After that, 0.5% w/v 5 mL of glutaraldehyde is added and be stirred with conditions 30 ºC, 150 rpm for 20 minutes. In the second method (method 2), lipase solution is mixed with 0.75 g resin and stirred in shaker water bath with conditions 30 ºC, 150 rpm for 24 hours. After that, 1 mL of chitosan solution (0.5 g chitosan powder dissolved in HCl 0.5% 100 mL) parallel with addition of 1% w/v 1 mL of glutaraldehyde, and be stirred with conditions 30 ºC, 150 rpm for 20 minutes. Enzyme concentration is measured by using Lowry method. Enzyme loading is the percentage of lipase concentration of successful immobilized enzyme for adsorption and crosslinking on resin.

2.3. Hydrolysis reaction of enzyme

In this research, lipase is used as biocatalyst in hydrolysis reaction. Substrate in this reaction is triglyceride from olive oil. Free fatty acid (FFA) content is measured by titration with NaOH base. 5 mL of olive oil is added to 5 mL aquades and 0.3 g PVA as emulsifier. Then, 5% wt of immobilized lipase is added to the system. Operating condition of the reaction is 30 ºC, 150 rpm for 30 minutes. The hydrolysis result is added with ethanol and PP indicator.

2.4. Enzyme activity test

Immobilized lipase is used as biocatalyst in synthesis of biodiesel non-alcohol route batch reactor. Reactor in this term is 100 mL Erlenmeyer flask. The reactor contains a mixture of palm oil and methyl acetate with 1:12 mole ratio. Biocatalyst which used in this test is 4% wt of immobilized enzyme. The operating condition of this reaction is 40 ºC on shaker water bath 150 rpm for 50 hours. The sample results from synthesis of biodiesel is analysed by HPLC (High Performance Liquid Chromatography) to obtain methyl oleate (biodiesel) concentration that have been formed. The stability test in this research is using biocatalyst to synthesis the biodiesel repeatedly.