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Production of dry extract extracellular lipase from *Aspergillus niger* by solid state fermentation method to catalyze biodiesel synthesis

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Abstract

Lipase can be produced from Aspergillus niger using solid state fermentation method. This research aimed to produce lipase in powder form. The highest activity unit (38.67 U/g) was obtained from rice bran substrate at 5th day with inducer concentration of 2%. The best drying method to produce dry extract with activity unit of 566.67 U/g was freeze drying with maltodextrin additive. The best drying method to produce fine powder with activity unit of 333.33 U/g was spray drying with skim powder additive. Supernatant and powder lipase worked optimally at 30 ^{o}C with activity unit of 44.00 U/g and 355.56 U/g respectively.

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Keywords: Additive production; *Aspergillus niger;* drying; extraceluller lipase; solid state fermentation

1. Introduction

Various types of microorganisms, fungi are widely recognized as a good source of extracellular lipase because they can facilitate enzyme recovery from fermentation media [1]. Lipase is one of the enzymes which used to synthesis biodiesel. *Aspergillus* is the best candidate because it can produce more enzymes than other microbes in microbial engineering research developments related to very high lipase [2]. In addition, the use of *Aspergillus niger* as a source of lipase is because it is classified as GRAS (Generally Regarded As Safe) by the FDA (Food and Drug Administration of the United States of America) [3], [4], so that the lipase from *Aspergillus niger* can be used in various fields including food and pharmaceutical industry. Solid state fermentation is a fermentation technique that allows the use

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of simple substrates such as solid waste of agro-industrial products that are induced using oils, such as olive oil. The advantages of solid-state fermentation compared with submerged fermentation include minimized contamination due to the absence of water, the high yield of the product (lipase), and the relatively simple equipment required [5], [6]. To stabilize and store lipase in a long time, drying treatment, the elimination of the water content in the extract of the enzyme lipase, is needed. Spray drying is more efficient and economical, but dehydration by spray drying usually results in changes in physical and chemical properties such as thermal denaturation of enzymes. Denaturation can lead to the loss of enzymatic activity. Freeze-drying combines the benefits of freezing and drying to produce a dry, active, and stable enzyme [7]. However, in some cases, freeze drying results in hygroscopic and sticky products. In addition, this process is not very efficient for large scale production.

In this study, enzyme production process was carried out using *Aspergillus niger* by solid state fermentation method. The substrate to be used was derived from rice bran and rice husk inducer with olive oil as additional inducer. Wet crude extract or supernatant produced from the fermentation process was then dried to increase the activity of lipase units produced and to stabilize lipase storage. The end result of this process was the production of lipase in the form of dry extract or in powder form.

2. Experimental method

2.1. Subculture of Aspergillus niger

Isolates of *Aspergillus niger* derived from InACC will be pre-cultured first on PDA medium (Potatoes Dextrose Agar) for two days at room temperature.

2.2. Preparation of fermentation medium

The medium used was a substrate of solid agricultural waste with two variations: rice bran and rice husk as much as 20 grams each in 250 ml Erlenmeyer flask. The substrate was then added to the nutrient with the following composition: 65% (g/g dss) distilled water, 1.5% (g/g dss) glucose, 0.34% (g/g dss), NH_2CONH_2, 0.75% (g/g dss) $(NH_4)_2SO_4$, 0.3% (g/g dss) KH_2PO_4, 0.0375% (g/g dss) $CaCl_2$, 1.8% (g/g dss) NaH_2PO_4, and 0.045% (g/g dss) $MgSO_4.7H_2O$. Variations made in the preparation of this medium were the concentration of olive oil. The medium was tripled and each was given a different treatment. The first medium was given an inducer of olive oil as much as 2% (g/g dss), while the second medium nourished inducer olive oil as much as 4% (g/g dss). The third medium was given 8% (g/g dss). The medium were then sterilized by autoclaving for 15 minutes at a temperature of 121 ^oC.

2.3. Solid state fermentation

Fermentation was done in 250 ml erlenmeyer with the starter, *Aspergillus niger* which had been in pre-culture, as much as 10% of the total mass of substrate used, ie 2 ml to 20 grams of a solid substrate. Fermentation time was varied from 1 day, 3 days, 5 days, 7 days, and 9 days.

2.4. Lipase extraction

The process of extraction or separation of enzymes by fermentation medium was done by giving 400 ml of distilled water and then magnetic stirring at room temperature for 30 minutes. It was then filtered using muslin cloth and centrifuged at 5000 rpm for 15 minutes at room temperature. The supernatant was collected for enzyme activity assays. Lipase crude extract was stored at 4 °C. The resulting supernatant could be identified as wet lipase was extracted.

2.5. Lipase drying

The supernatant enzyme was dried using freeze drying and spray drying techniques. Before drying, the supernatant was given protective materials or an additives with 0.5% vt maltodextrin and 12% vt skim milk powder.