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Article

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Optimization of *Aspergillus niger* lipase production by solid state fermentation of agro-industrial waste

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Abstract

Lipase is one of the most used industrial enzymes, which can be produced by *Aspergillus niger*. Large scale production of *Aspergillus niger* lipase is more profitable by using solid state fermentation method and utilizing agro-industrial waste as the substrate. Optimization of *Aspergillus niger* lipase production has been performed in this study. Optimization was performed on solid state fermentation of rice bran and Jathropa seed cake for 5 days with variations of inducer and extractant. Fermentation cake produced was extracted, filtered using muslin cloth, and centrifuged. The supernatant was spray-dried and assayed using olive oil hydrolysis titrimetry. Inducer optimization results showed that 1% of olive oil was the best inducer, yielding dry lipase extract with highest activity unit (176 U/ml enzyme). Extractant optimization results showed that 1% of NaCl – 0.5% of Tween 80 was the best extractant, yielding dry lipase extract with highest activity unit (282 U/ml enzyme).

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Keywords: Agro-industrial waste; *Aspergillus niger*; Lipase; Solid-state fermentation

1. Introduction

Enzyme demand in Indonesia is increases every year. However, 99% of the supplies are comes from imports. Therefore, development of enzyme industry in Indonesia is necessary in order to fulfill national enzyme demand and reduce the amount of imports. As one of the enzymes in high demand, lipase plays an important role in a lot of industries such as food, pharmaceutical, and biofuel production [1]. One of the best lipase producer is *Aspergillus niger*, a species of a filamentous fungi enlisted as GRAS (generally recognized as safe) by Food and Drug Administration (FDA) in the United States. Through solid state fermentation, *A. niger* could utilize low cost solid agro-industrial waste [2,3] to reduce production cost [4] and overcome environmental issues. Several potential solid agro-industrial wastes that can be utilized are rice bran and Jatropa seed cake, which are abundant

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in Indonesia, and has been studied as the substrate to produce lipase from *A. niger* [5,6]. In order to produce lipase in large scale, therefore the objective of this study was to optimize the production of dry *A. niger* lipase by solid state fermentation using rice bran and Jatropha seed cake as the substrates in order to obtain higher lipase activity.

2. Materials

2.1. Materials

Aspergillus niger isolate was obtained from Indonesian Culture Collection (InaCC), Research Center for Biology, Indonesian Institute of Sciences (LIPI, Bogor, Indonesia). Rice bran and Jatropha seeds were purchased from local suppliers (Cilacap, Indonesia). The Jatropha seeds were dried and pressed to obtain seed cakes, then powdered before use. Olive oil, sesame oil, and Jatropha oil were purchased from the supermarket and local supplier. All chemicals used were analytical grade.

2.2. *Aspergillus niger* culture and solid state fermentation

Aspergillus niger cultures were maintained in potatoes dextrose agar media (3.9%, w/v) inside petri dishes, stored at 4 °C and subcultured every week. Inoculation of *A. niger* spores was done through hyphal tip inoculation. Subcultures were done under room temperature and incubated at 30 °C for 4 days.

Fermentation media were prepared using substrates of rice bran and Jatropha seed cake supplemented with different nutrients formulation. Rice bran was supplemented with (w/w substrate) 65% distilled water; 1.5% glucose; 0.34% NH_2CONH_2 ; 0.75% $(\text{NH}_4)_2\text{SO}_4$; 0.3% KH_2PO_4 ; 0.0375% CaCl_2 ; 1.8% NaH_2PO_4 ; and 0.045% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ [7]. Jatropha seed cake was supplemented with (w/w substrate) 40% distilled water; 2% maltose; and 2% peptone [8].

5 g of substrate were sterilized and mixed with nutrients and inducer. The fermentation media was put into tray bioreactor with the maximum height of 3.2 cm from the base [9]. To optimize the fermentation, different substrates (rice bran and Jatropha seed cake) were used and different inducers (olive oil, sesame oil, and Jatropha oil; with concentrations of 1% and 2% each, w/w substrate) were supplemented (the control was not supplemented with inducer). *A. niger* was then inoculated into the fermentation media and incubated under anaerobic conditions and room temperature for 5 days.

2.3. Enzyme extraction and drying

Extraction was carried out by mixing the fermented cake with 20:1 (v/w substrate) distilled water and filtered using muslin cloth. The filtrate obtained was centrifuged at 4000 rpm for 20 min. The supernatant was then filtered using muslin cloth and assayed for lipase activity. The supernatant were spray dried with the addition of 12% (w/v supernatant) skim milk powder; with inlet temperature of 70 °C and 130 °C. The dry extract was also assayed for lipase activity. Extraction optimization was done after obtaining the optimum fermentation condition. To optimize the extraction, different extractants were used (10:1, v/w substrate) such as distilled water, NaCl (1% and 2%, w/v), NaCl-Tween 80 (1%–0.5%; 1%–1%; 2%–0.5%, 2%–1%, w/v). The following steps were performed under similar conditions.

2.4. Lipase activity assay

Lipase activity assay was carried out by olive oil titrimetric assay. To determine the lipase activity, 2.5 ml of distilled water, 1 ml of 0.2 M Tris–HCl buffer (pH 7.7 at 37 °C), and 3 ml of olive oil was taken and pre-incubated at 37 °C, then mixed with 1 ml enzyme extract. The solution was stirred for 30 min with the temperature maintained at 37 °C. After 30 min, 3 ml of ethanol (95%) was added to stop the reaction and 4 drops of thymolphthalein indicator was added. Titration was then carried out using standardized 0.05 N NaOH to obtain a light blue colored solution. To determine the lipase activity of dry extracts, 0.1 g of dry enzyme extract was first dissolved in 10 ml of distilled water prior the assay. One unit of activity is defined as the amount of enzyme that hydrolyzes 1 micro equivalent of fatty acid from a triglyceride in one hour at pH 7.7 at 37 °C.

3. Results and discussion

3.1. Effect of substrate type on lipase activity unit

The effect of substrate type on lipase activity unit is shown in Fig. 1. Based on results, rice bran as the fermentation substrate for *A. niger* generally yields higher lipase activity unit than Jatropha seed cake. This might be the result of different characteristics between the substrates. Rice bran has smaller particle size visually, higher carbohydrate content, lower protein content, higher lipid content and higher water content [10,11] compared to Jatropha seed cake [12]. Smaller particle size provides larger surface area for microorganism to grow on and consume the substrate [13]. Higher carbohydrate content provides more carbon source for microorganism to grow. Lower protein content hinders the production of protease that can degrade lipase, other proteins and enzymes [14]. Higher lipid content induces the microorganism to produce more lipase with higher activity. Higher water content supports the microorganism's growth since solid state fermentation depends highly on the substrate's water content [4] and increases oxygen transfer efficiency in the fermentation cake [13]. Similar results also reported by [3], whereas particle size and chemical content of the fermentation substrate affects the lipase activity.

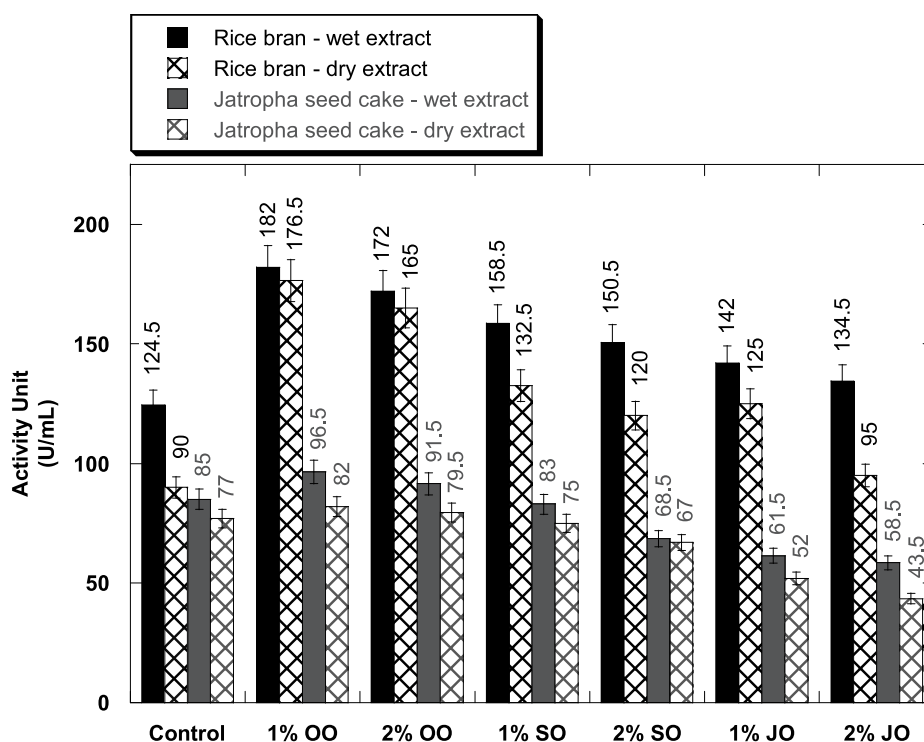


Fig. 1. Effect of substrate type, inducer type and concentration on lipase activity unit. (OO: olive oil; SO: sesame oil; JO: jatropha oil).

3.2. Effect of inducer type and concentration on lipase activity unit

The effect of inducer type and concentration on lipase activity unit is also shown in Fig. 1. Based on results, it is also shows that olive oil generally yields higher lipase activity unit compared to other inducers. This might be due to the difference of lipid composition in each oils. Lipase production as an inducible extracellular enzyme is highly influenced by the type and concentration of lipid sources in the fermentation media, such as fatty acids [14]. Olive oil has highest oleic acid content [15] compared to sesame oil [16] and Jatropha oil [17], which most lipase are specific towards [13]. Olive oil also has the lowest linoleic acid content [15] compared to the other oils [16,17].

Linoleic acid has been reported having negative impact on lipase activity [13]. High concentration of inducers might inhibit growth and oxygen transfer [3,13]. Similar results are reported by several studies [3,18–20]; whereas supplementation of olive oil as an inducer yields high lipase activity unit, especially on low concentrations.

3.3. Effect of extractant type and concentration on lipase activity unit

The effect of extractant type and concentration on lipase activity unit is shown in Fig. 2. Based on results, NaCl-Tween 80 mixture as an extractant yields higher lipase activity unit than distilled water. This might be due to the physical and chemical changes in the extraction process caused by the compounds.

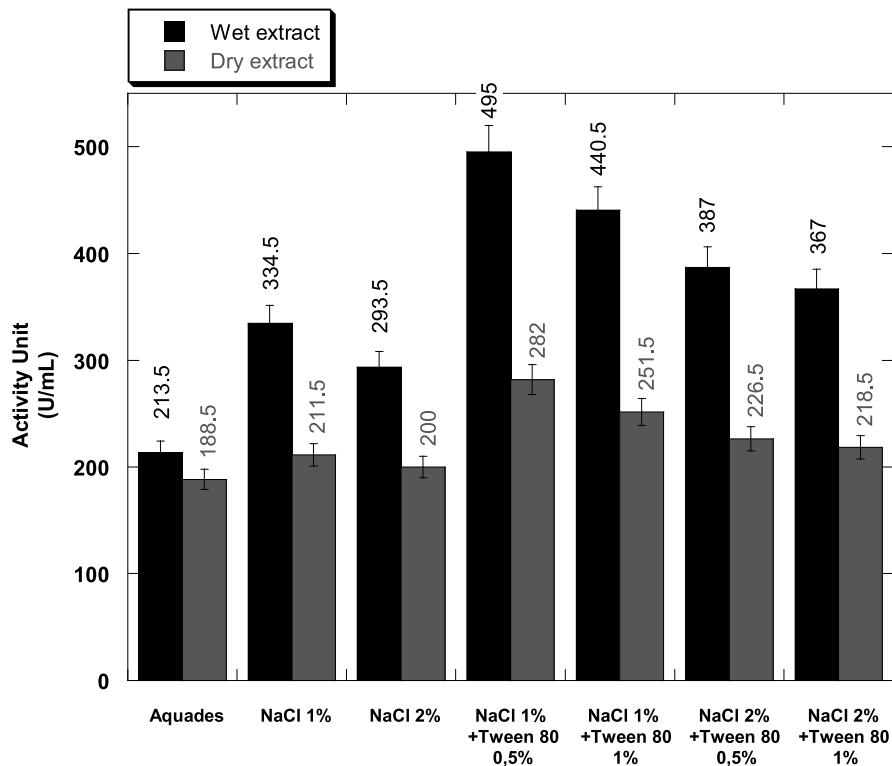


Fig. 2. Effect of extractant type and concentration on lipase activity unit.

Generally, lower concentrations of NaCl and Tween 80 yields higher lipase activity unit. This might be due to the enzyme denaturation or inhibition that could occur on higher concentrations. NaCl binds more water molecules thus extracting more lipase on the water phase and enhances the ionic strength of the enzyme solution which stimulates higher activity. Enzyme extraction with NaCl also reportedly increases the enzyme activity [21] and maximum reaction rate [22], both reports stating that high concentrations exhibit inhibitory effects. Tween 80 reduces the surface tension between water and oil; increasing the enzyme solubility and expands the access to the active site. Tween 80 and other cationic surfactant such as Triton X-100 reportedly increases lipase activity [23]. Similar results were reported by several studies [6,19,20], whereas enzyme extraction using NaCl and/or Tween 80 enhances lipase activity unit, especially on low concentrations.

4. Conclusion

Based on results in this study, it can be concluded that the optimum condition for the production of *Aspergillus niger* dry lipase was obtained using rice bran as the substrate with 1% of olive oil as the inducer; which yields the

activity unit of 176 U/ml enzyme and 1% of NaCl-0.5% of Tween 80 solution as the extractant which yields the higher activity unit of 282 U/ml enzyme.

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