Conference Paper

Effect of Surfactant Type Modifications, Glucose Inducer Concentrations, and Mineral Sources towards Lipase Enzyme Activity of *Aspergillus niger* ITBCC L74 on Rice Bran Substrate

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ABSTRACT

A surface-active agent is a substance added to a liquid to increase its spreading properties by lowering the surface tension. On the other hand, an inducer is a substance to triggers the production of lipase enzymes from bacteria. This study aims to formulate the microbial growth medium to produce high activity of lipase through modification of surfactant type, glucose inducer concentrations, and mineral sources using *Aspergillus niger* as a microbial starter and rice bran as substrate. A solid fermentation was built and prepared in this study for lipase production. The titrimetric method using NaOH solution was used to determine mol of free fatty acids per minute, type of surfactant, the optimal glucose concentration, and mineral sources on lipase enzyme activity. Tween 80 and Tween 20 are lipid-based surfactants used in this experiment. The best type of surfactant was Tween 80 with 1% of glucose inducer. It produced a lipase activity of 2.53 U/mL. In contrast, Tween 20 with 0.75% glucose inducer produced a lipase activity of 2.27 U/mL. FeSO₄ and CaCl₂ were the inorganic mineral sources used in this study. The best result was found at 0.6% of FeSO₄, it can generate enzyme activity of 2.13 U/mL.

Keywords: Lipase enzyme activity, types of surfactants, nutrient sources, *Aspergillus niger*, rice bran substrate, glucose inducer

Introduction

Enzymes are widely used in various food and non-food industrial processes (Indah et al., 2017). Enzymes play an important role in the fields of biotechnology and industry. According to data from the trade ministry of Indonesia, Indonesia still imported enzymes with a nominal value of 514.1 million dollars in 2018, increased by 48.45% from the previous year. This condition shows that the demand for enzymes is still high in Indonesia. Innovative strategies are needed to enhance the production of enzymes domestically. Therefore, it is expected to reduce the number of the imported enzyme (Kementerian Perdagangan, 2018). According to the Ministry of Research Technology and Higher Education, almost 99% of enzymes for industrial activity are imported from abroad, such as China, India, Japan, and Europe. The need for enzymes tends to increase every year. It is estimated that global market demand for enzymes increased by 7% (2015 - 2020) per year (Kemenristekdikti, 2020). Temperature, pH, enzyme concentration, the concentration of substrates, inhibitors, and activators are primary factors for enzyme activity (Risnawati, 2013). Lipase enzyme cannot work optimally at a pH that is low (acidic) or pH is high (alkaline) (Safaria et al., 2013).

Production of lipase enzymes using *Aspergillus niger* is important. It is a microorganism that can produce lipase enzymes with high activity (Aulia et al., 2020). *Aspergillus niger* lives and grows at temperatures around 6°C to 47°C. The optimal pH for *Aspergillus niger* growth is between 1.4 to 9.8 (Wang et al., 2019). The abundant biodiversity in Indonesia makes it possible to develop enzyme production from local microbes through exploration (Kurnia, 2010). The production of

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lipase enzyme is interesting to be researched due to the high demand for this enzyme for industrial activity. Lipase enzymes could hydrolyze lipids, triglyceride esters, fatty acids, and glycerol (Haedar et al., 2017). Lipase enzymes also act as biocatalysts for hydrolysis, esterification, alcoholysis, acidolysis, and aminolysis reactions (Murni et al., 2011).

Solid fermentation is the easiest and often-used technique for producing lipase enzymes. Agricultural waste materials can be utilized as a medium or substrate for the growth of microbial starters in solid fermentation (Bardant et al., 2013). The selection of suitable substrates and microorganisms are important aspects for the development of solid fermentation to produce certain enzymes, in this study is lipase. The best substrate for microbial growth in solid fermentation is the dense and moist substrate. Due to this reason, the solid fermentation technique is more suitable for lipase production than the submerged fermentation technique (Behera & Ray, 2016).

Aspergillus niger is a type of microbe to produces extracellular enzymes with high activity and easy maintenance. Rice bran is solid media to grow *Aspergillus niger*. Rice bran was chosen as the best substrate to grow *Aspergillus niger* because it contains 27.60% of lipid 28.78% of carbohydrates, and 12.53% of protein (Costa et al., 2017). In addition, Nema et al. (2019) used agro-industrial residues as a substrate to produce lipase enzymes using *Aspergillus niger* MTCC 872.

The addition of surfactants, glucose inducers, and mineral sources are factors to produce lipase enzymes. Inducers are useful to stimulate the production of the enzyme that contain lipids or monosaccharides. Tween 80 and Tween 20 are lipid-based surfactants (Moentamaria et al., 2016). Tween 80 is a surfactant based on hexahydrate alcohol, alkylene oxide/oxyethylene, and fatty acids. The difference between Tween 20 and Tween 80 is the number of hydrocarbon chains. The hydrocarbon chain in tween 80 is longer than Tween 20 (Zhang, 2003). Tween 80 is widely used because it has non-toxic properties and is stable against the influence of pH (Sanaji et al., 2019). The addition of mineral sources is also supported by research Abdillah et al. (2020) found that the best inorganic mineral source is 0.6% FeSO₄ to produce the highest lipase enzyme activity of 3.47 U/mL and 2 mmol of MgSO₄ could produce lipase enzyme activity of 0.67 U/mL. The addition of mineral sources affects enzyme activity. Therefore, a study to formulate the best growth media to produce high lipase enzyme activity by modifying surfactant type, glucose inducer concentration, and optimal mineral sources was conducted.

Material and Methods

Aspergillus niger ITBCC L74, rice bran from rice mills in Sragen, Central Java, Tween 80, Tween 20, glucose, and distilled water were used in this study. MgSO₄, NaCl, FeSO₄, NH₄NO₃, CaCl₂, Na₂B₄O₇, CuSO₄, FeCl₂, MnSO₄, NaMO₄, ZnSO₄ phosphate buffer pH 7, acetone and ethanol were used for chemicals analysis.

Culture Preparation of *Aspergillus niger* (ITBCC L74)

Potato dextrose agar (PDA) was prepared in a petri dish. *Aspergillus niger* IT-BCC L74 was grown on PDA with the aseptically in the laminar airflow. The petri dish was wrapped in aluminum foil and incubated for three days at 35°C in the incubator. Then, it was stored in the refrigerator at a temperature of 4°C. Figure 1 shows *Aspergillus niger* ITBCC L74 culture that has been grown in PDA media.

Figure 1. Culture of *Aspergillus niger* ITBCC L74

Pre-treatment of rice bran substrate

The rice bran was soaked in distilled water and the pH was measured. Then, phosphate buffer pH 7 was added to maintain the pH of the medium. Rice bran was ground and sieved through 40 mesh after 24 hours.

Microbial inoculum preparation

Inoculum preparation was conducted by preparing microbial growth medium containing 50 mL of glucose solution (0.5 %), 5 mL of Mandel's solution, and 1 mL of metal solution (Na₂B₄O₇ 40 mg/0.25 l, CuSO₄ 400 mg/0.25 l, FeCl₂ 800 mg/0.25 l, MnSO₄ 800 mg/0.25 l, NaMoO₄ 800 mg/0.25 l, ZnSO₄ 8 mg/0.25 l) in a 250 mL Erlenmeyer. Then, the medium was then sterilized at 121°C using an autoclave. After cooling, the medium was inoculated with *Aspergillus niger* ITBCC L74 colony with a size of 1 cm² from a petri dish in laminar airflow. Then, it was incubated for three days.

Lipase enzyme production

A total of 10 grams of dried rice bran pH 7 was put into an Erlenmeyer as a substrate. Then, 50% water was added based on the weight of the media. Glucose inducer was added with different concentrations (0.5%, 0.75%, 1%, 1.5%, 2%). Different surfactants (Tween 80 or tween 20) were also added. Then, FeSO₄ with varied concentration (0.2%; 0.4%; 0.6% and 0.8%) and CaCl₂ (0.008%, 0.02%, 0.08%) were added as the mineral sources, then sterilized at 121°C. The solution was cooled to a temperature of 30°C after sterilization. Furthermore, 1 mL of inoculum solution was added to the cold medium. The Erlenmeyer was plugged using cotton and covered with aluminum foil, then incubated for six days at 35°C. Figure 2 shows the production of lipase enzymes in an incubator after six days of incubation at 35°C.

Figure 2. Lipase enzyme production

Lipase enzyme screening process

The media was added with sterile distilled water in a ratio of 1:5. Ten grams of media were added to 50 mL of sterile distilled water and stirred until homogeneous for 30 minutes. After that, it was filtered using a vacuum filter. Therefore, the crude enzymes and biomass were separated.

Determination of lipase enzyme activity

The mixture was incubated at 200 rpm of shaker incubator at 37°C for 1 hour. After the incubation time was reached, 2 ml of acetone-ethanol (1:1) was added to inactivate the enzyme. The mixture was stirred homogeneously and added with three drops of phenolphthalein (PP) indicator. Titration was conducted with 0.01N of NaOH solution until the mixture color turns pink. The lipase enzyme activity was expressed in mol Free Fatty Acid (FFA) per ml of enzyme per minute. It was calculated using equation (1):

Lipase Enzyme Activity:

$$\frac{(A - B) \times N \times \text{NaOH} \times 1000}{V \times t} \quad (1)$$

A	= Volume of NaOH sample (mL)
B	= Volume of blank NaOH (mL)
NNaOH	= 0.01 N
1000	= Conversion value of mmol from mol
V	= Enzyme volume (mL)
t	= Incubation time

Results and Discussion

Analysis method using the titrimetric method required high accuracy to determine the end-point of the titration of a solution. The water-oil interface became more stable insolubility after the addition of surfactants, namely Tween 80 and 20.

Effect of modification of glucose inducer concentration and type of surfactant (Tween 20 and Tween 80)

The relationship between lipase enzyme activity with the use of different types of surfactants (tween 80 and tween 20) and the use of various concentrations of glucose inducers can be seen in Figure 3.

Figure 3. The effect of modifying glucose inducer concentration and surfactant types in lipase activity

As shown in Figure 3, the highest enzyme activity for the surfactant type Tween 20 is at a glucose inducer concentration of 0.75%, with an enzyme activity of 2.27 U/mL. At the concentration of 0.5%, the medium was still adapted to glucose. Therefore, the enzyme production was not optimum. Meanwhile, the highest lipase enzyme activity was obtained at 0.75% concentration. Under optimal conditions between the media and the concentration of glucose inducer, the lipase enzyme reached an optimum production. The inducer concentration exceeds the optimal conditions resulted in suboptimal enzyme production. There was a gradual decrease in enzyme activity with an increase in the concentration of glucose inducers.

The highest enzyme activity for Tween 80 was at 1% of glucose inducer concentration with an enzyme activity of 2.53 U/mL. The optimal condition for lipase enzyme production was at a concentration of 1% glucose inducer. When the inducer concentration was high and reached optimal conditions, the lipase enzyme production decreased. Meanwhile, before reaching the optimal condition, enzyme production increased, marked by increasing lipase enzyme activity.

The addition of a glucose inducer serves as a carbon source. Increasing the concentration of carbon sources in solid fermentation media resulted in higher enzyme activity until the optimum concentration. Then, the decrease in enzyme activity can be caused by substrate inhibition (Marasmius, & Kultur, 2018).

Effect of modification of FeSO_4 and CaCl_2 concentrations

The relationship between lipase enzyme activity and the use of FeSO_4 can be seen in Figure 4.

Figure 4. The effect of modifying FeSO_4 concentration in lipase activity

As shown in Figure 4, the most optimum lipase activity was 2.13 U/mL with the addition of 0.6% levels of FeSO_4 . It indicated that FeSO_4 was needed for the growth of enzymes as a source of minerals. Our results demonstrated that the addition of 0.2% to 0.6% FeSO_4 increased the lipase enzyme activity; however, the addition of 0.8% FeSO_4 decreased the lipase enzyme activity. It might be due to the concentration of FeSO_4 was too high for *Aspergillus niger*.

The relationship between lipase activity and the use of CaCl_2 mineral sources can be seen in Figure 5.

Figure 5. The effect of modifying CaCl₂ concentration in lipase activity

The effect of CaCl₂ (%) concentration on the activity of the lipase enzyme is shown in Figure 5. Rice bran media was given additional CaCl₂ with a concentration of 0.008%; 0.02%, and 0.08%. The most optimum lipase enzyme activity was found at the addition of 0.02% CaCl₂. The lipase enzyme activity increased with the addition of 0.008%-0.02% of CaCl₂; however, the lipase enzyme activity decreased at the addition of 0.08% CaCl₂. It could be due to the CaCl₂ concentration was too high for *Aspergillus niger*. The growth inhibition makes Ca ions toxic to *Aspergillus niger*. While in research (Satriana et al., 2014) showed that Ca²⁺ ions at concentrations of 2, 4, 6, 8, and 10 mM increase the pectinase activity of *Bacillus firmus*.

CaCl₂ ion inhibits lipase from coconut, and the lipase enzyme activity is not too high (Lipase et al., 2004). It is due to the crude lipase enzyme in liquid form. The lipase enzyme must be concentrated to increase its activity. The enzyme activity increases several times compared to the dilute state. In this study, enzyme concentration has not been measured. Therefore, measuring enzyme concentration should be done in further research.

Conclusion

The optimal activity of the Lipase enzyme can be obtained from certain conditions of the medium according to the surfactant, glucose inducer concentration, and mineral source used. If the inducer concentration exceeds optimal conditions, the production of enzymes will not be optimal. The addition of Tween 80 and Tween 20 caused a decrease in surface tension and increased the solubility rate of the enzyme. Thus, the addition of Tween 80 gave higher lipase enzyme activity than the addition of Tween 20. FeSO₄ became toxic to *Aspergillus niger* if the concentration was too high while the high concentration of CaCl₂ will be an inhibitor and toxic too.

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