

Perdani, Meka Saima; Margaretha, Grace; Sahlan, Muhamad; Hermansyah, Heri

Article

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Solid state fermentation method for production of laccase enzyme with bagasse, cornstalk and rice husk as substrates for adrenaline biosensor

Meka Saima Perdani, Grace Margaretha, Muhamad Sahlan, Heri Hermansyah*

Department of Chemical Engineering, Faculty of Engineering, Universitas Indonesia, Depok, West Java, 16424, Indonesia

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Abstract

Adrenaline is an important neurotransmitter in the body that will be released from the body when someone is in emotional state or in state of physical and emotional stress, such as hypoglycemia, work out, and excessive pain in the body. Early diagnosis based on the determination of adrenaline concentration in plasma and urine can prevent unnecessary drug use (Hollenbach et al., 1998 [1]). In adrenaline, there is an enzyme, called laccase. Laccase has wide application in industry and medical application. Moreover, the producing of laccase is need to be more develop to produce high activity of enzyme. The high activity enzyme will be affected to the determination of adrenaline concentration. Therefore, the aim of this research is to extract extracellular laccase from fermentation of *Trametes versicolor* with solid state fermentation method on lignocellulosic waste in the form of bagasse, corn stalk and rice husk. A utilization of biomass waste had applied in this work. We investigated the best pre-treatment condition to produce laccase. Steam explosion is used for the treatment of raw substrates. The best substrate for producing laccase was cornstalk with additional treatment of steam explosion and nutrient addition. Finally, investigation of substrates selection had been done and obtained 6.885 U/mL of laccase activity.

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Keywords: Adrenaline; Biosensor; Laccase; Solid state fermentation; *Trametes versicolor*

1. Introduction

Enzyme is a catalyst in the form of a protein that can reduce the activation energy of a reaction. If the activation energy of a reaction is successfully reduced, the barrier reaction will decrease which is it will cause the reaction to react faster. In the industrial field, enzymes are widely used as substitutes for chemicals. An analytical method by HPLC was used as a method for determined the final concentration of substrate which was catalyze with the

* Corresponding author.

E-mail address: heri@che.ui.ac.id (H. Hermansyah).

enzyme. The effect of an enzyme in a reaction was able to produce the derivatived structure of substrate [1]. Enzyme has a characteristic that is very active, although its concentration is very low, very selective, and it works in mild conditions. Another advantage of enzymes is that chemical reactions can take place using a small amount of enzymes, the preparation of solid or liquid enzymes requires a small amount of storage space, equipment that can be used more because the operating conditions are not high, and can reduce the negative industrial impact on the environment by reducing use of chemicals, water, energy and can reduce waste [2].

Laccases, EC 1.10.3.2, p-diphenol: dioxygen oxidoreductase, is part of multicopper enzymes. Broadly speaking, laccase enzymes can be divided into two groups, namely those from higher plants and those from fungi [3]. Laccase is the subject of research during this decade because it can be synthesized from various types of substrate, does not require the addition or synthesis of cofactors with low molecular weight because laccase is available in the environment, an extracellular enzyme that facilitates the purification process, have good stability.

The method used in growing Laccase in this study is to use solid state fermentation. Usually, a production of laccase is involve the utilization of bacteria with commercial substrate which is contain high cellulose. In this work, we used lignocellulosic as substrate source. Steam explosion was applied in the pre-treatment of substrates. High temperature and pressure will extract the lignocellulosic. Fermentation is an oxidation–reduction reaction in a biological system that produces energy. Organic compounds such as carbohydrates are donors and receptors in the fermentation process in the research conducted by organic compounds used are cellulose. Solid state fermentation methods are currently more preferred in microbial biotechnology pieces, because they are practical, economical and environmentally friendly because they can utilize agro-industrial waste. Solid fermentation systems are best suited for the fermentation process for microorganisms that require little moisture such as for fungi and other microorganisms [4]. The composition and concentration of the media and fermentation conditions greatly influence the growth and production of extracellular enzymes from microorganisms. Costs and availability of substrates are important factors to consider, and therefore the choice of solid substrate plays an important role in determining efficiency in solid fermentation system processes.

2. Materials and methods

2.1. Microorganism subculture

The enzyme production in this study was carried out through solid state fermentation produced by the *Trametes versicolor* fungus. Before fermenting, a subculture of *Trametes versicolor* isolates was needed. *Trametes versicolor* isolate obtained from LIPI Cibinong, Indonesia. The active subculture of isolates was carried out to increase the number of microorganisms before the fermentation process. *Trametes versicolor* mold subculture is carried out on solid media PDA plate (Potatoes Dextrose Agar).

2.2. Pretreatment fermentation of substrate

In this study, it used 3 types of substrate in agro-industrial waste as the fermented *Trametes versicolor* mold medium, namely corn stalks, bagasse, and rice husk. Before being used as a fermentation medium, the substrate needs to be pre-treated to investigate the best condition for enzyme production. Agro-industrial waste is enumerated to reduce particle size. Then the waste is divided into two parts. The first part is dried and smoothed, while the second part is treated with steam explosion. Agro-industrial waste was put into the explosion chamber for steam explosion. The process was carried out at 200 °C with a pressure of 1.6 MPa for 6 min. The results of the steam explosion are then dried and smoothed. The dried treated waste was filtered by 10 µm micron mesh filter.

2.3. Solid state fermentation

The substrates that used in this method were bagasse, cornstalk and rice husk. All the substrates had been treated with steam explosion. The medium was also designed as two variants, medium with nutrients and without nutrient. The components of nutrients were (g/L sulfate acid pH 5) NH_4NO_3 —3; $(\text{NH}_4)_2\text{HPO}_4$ —3; KH_2PO_4 —5; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ —0.5; NaCl —0.5; CaCO_3 — 0.5; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ —0.35; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ —0.6 [4]. Solid substrates are arranged to cover the entire surface of the bioreactor tray. After the substrate in the container reached the room temperature, *Trametes versicolor* is added to the 1 ml of substrate. Inoculation is done by using needle (covering the entire surface) so that the mold spores are spread evenly. Containers containing substrate and mold are then wrapped and incubated at room temperature for 13 days [5].

2.4. Laccase extraction

After the fermentation process is complete, then the extraction or separation of enzymes is carried out with the fermentation medium. The fermented product is mixed with 50 mL of sodium acetate buffer (50 mM, pH 5.0). The results of the mixture were stirred with a rotary shaker at a speed of 120 rpm for 60 min. Then, the filtered mixing results using muslin cloth and centrifuged at a speed of 9000 rpm for 10 min at 4 °C. The supernatant obtained is a crude Laccase extract, which is partially tested for the activity of the Laccase enzyme and the remainder is dried. Supernatant activity test was carried out using a spectrophotometer UV–VIS at wavelength 240 nm.

2.5. Laccase activity

The laccase activity test was carried out by quantification using a spectrophotometer at 25 °C using a mixture of buffer tartaric acid pH 3.0 with 0.1% (w/v) ABTS as a substrate. The initial concentration of substrate was 0.1 M. The laccase extract was mixed with the substrate solution. Absorbance was measured at a wavelength of 420 nm. One unit of activity was expressed as the amount of laccase needed to oxidize 1 μmol ABTS per minute.

3. Results and discussion

In this study, it used three types of substrate; corn stalks, bagasse, and rice husk. The three types of substrate used are waste from agro-industrial products. The corn stalks obtained after the corn harvest are generally only thrown or burned. Sugarcane pulp is used by the factory to make sugar into a sugar factory fuel by burning it. Rice husk is generally used as animal feed, drum or floor pads, building materials (roofs, walls), and burned as fuel. The three substrates used were given 2 types of treatment, which were given steam explosion and only dried then smoothed. Based on the results of the study, the three steam explosion treated substrates provided a higher activity unit than only dried. Steam explosion helps breakdown the lignocellulose component more effectively. This causes the component to be easily digested by mold so that in the same fermentation time it will produce higher activity units compared to those not through steam explosion.

The substrate used in solid state fermentation is corn stalks, rice husk, and bagasse. The type of substrate will affect the growth of *Trametes versicolor* in the fermentation process which also directly affects the quality of the laccase produced, namely the laccase activity unit. There are two fundamental differences between the two types of substrate. The first difference is the shape of the substrate. According to [6], the shape of the substrate in solid state fermentation will affect oxygen uptake and affect the growth of fungi and the fermentation process in it [6]. The smaller particle size of the substrate provides a greater surface area for microorganism penetration. However, a very small size can cause accumulation of substrate and disrupt the process of respiration and aeration. Conversely, large particles have a large enough area for the efficiency of the aeration process, but the surface area for penetration of microorganisms provided is very limited [3]. The three substrates were given the same treatment, which was dried and mashed, so that the size became homogeneous.

Based on Fig. 1, it is shown the activity units of laccase enzyme with corn stalk as the substrate. The variation of substrate treatments was also obtained in every substrate. The modification of treatment substrate is provided in order to optimize and make comparison between substrate with treatment and without treatment. A kind of treatment that used in this work was explosion method. The grinded substrate is explode with high pressure and temperature. This step was to remove unwanted sources from the substrate. The best result is shown in the treatment with steam explosion and addition of nutrient. The enzyme activity was 6.88 U/ml. the enzyme activity will be very low if there was no steam explosion and no nutrient addition.

The rice husk substrate was also examined with the same treatment. The result is showed best condition from enzyme production by using treatment and nutrient addition in the substrate. The activity unit of this condition is 6.782 U/mL (Fig. 2). Meanwhile, the addition of nutrient is also affected to the activity enzyme. The consumption of substrate when the enzyme is binding each other will depend on the substrate condition.

The activity unit of the three types of substrate is significantly different. In sequential order, the activity units of each substrate that has undergone the steam explosion and addition of nutrients are 6.885 U/mL, 6.782 U/mL, and 6.042 U/mL (Fig. 3). Then followed by rice husk and sugarcane with activities of 3.023 U/mL and 1.935 U/mL, respectively. There was quite significant of the three types of substrate. On the substrate that was not treated but given nutrients, the highest activity unit was obtained by the corn substrate with an activity-value of 4.261 U/mL.

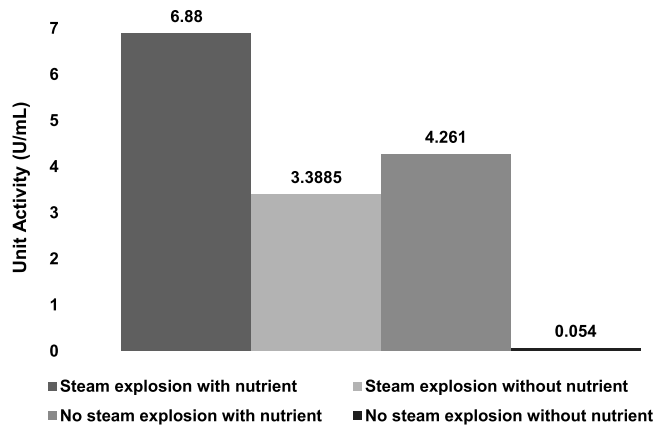


Fig. 1. The effect of corn stalk substrate to the enzyme activity.

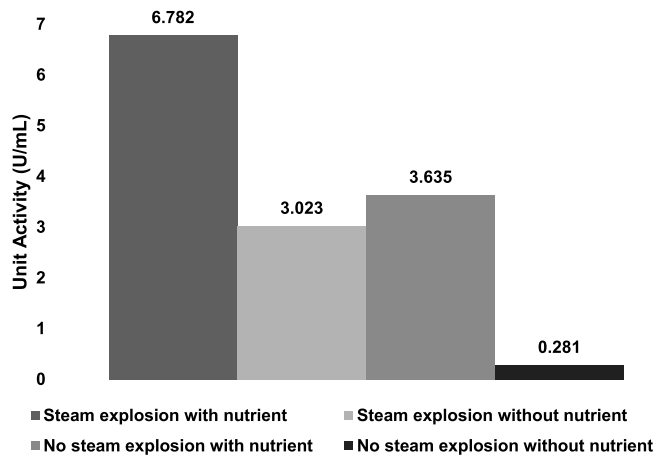


Fig. 2. The effect of rice husk substrate to the enzyme activity.

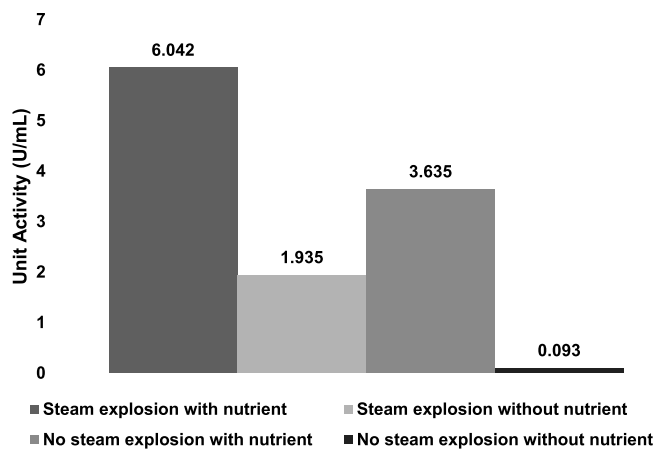


Fig. 3. The effect of bagasse substrate to the enzyme activity.

The rice husk and bagasse substrate each gave an activity value of 3.635 U/mL. The use of substrate without steam explosion treatment and the addition of nutrients provides a greater value of activity compared to the substrate with

steam explosion treatment but without nutrients. When compared between the two, the difference in the value of existing activities is not too large.

The difference in nutrient composition that needs to be considered is the lignocellulose content in each substrate. From the picture above, in general it can be seen that the corn stalk substrate produces a higher activity unit when compared to other substrates. Corn stalks have a higher lignocellulose value when compared to the other two types of substrate. This allows molds to produce enzymes with better activity values compared to other substrates [4]. So the results given here states that if viewed in terms of the nutritional content contained in rice husk it will give a high value of activity units rather than rice husk and bagasse arena, rice bran lipid content is more than rice husk.

Laccase enzyme which is produced by using different substrate and treatment would be stated as a candidate for enzyme production as application in biosensor. The adrenaline itself exhibit laccase enzyme as the active site. The enzyme would be oxidized as well as the decreasing of substrate.

4. Conclusions

The irreversible multilevel reaction model is matched with the experimental data set 3 which is proved by sensitivity analysis. In this study, the kinetic parameter value which is obtained in this study showed k_1 , k_2 and k_3 value; 7.86×10^{-3} , 1.11×10^{-2} and 9.15×10^{-3} , respectively.

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