

TEMPERATURE AND PH OPTIMIZATION OF CAR THERMOPHILIC BACTERIA FOR THE PRODUCTION OF XILANASE THE BLEACHING OF ENVIRONMENTALLY FRIENDLY INKED PAPER

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ABSTRACT

Xylanase is an extracellular enzyme capable of hydrolyzing hemicellulose so that it can convert xylan into xylose. Xylanase enzymes can be used in the pulp and paper industry. This study aims to determine the effect of temperature, pH on enzyme production by immobilized thermophilic bacteria using rice straw xylan extract as a substrate and to see the effect of xylanase administration on the whiteness level of inked paper waste. This study was an experimental study and used a completely randomized design (CRD). Enzyme activity was measured using a spectrophotometer at a wavelength of 540 nm. Enzyme activity data were analyzed using the ANOVA test and continued with the DMRT (Duncan Multiple Range Test) further test with a level of 5% and the whiteness of the paper was determined by testing the kappa number. The results obtained were that the temperature of immobilize thermophilic bacteria using rice straw xylan extract as a substrate affected the xylanase enzyme activity with an optimum temperature of 75°C having the highest average value of enzyme activity 4.668 U/mL. Meanwhile, the optimum pH for immobilized thermophilic bacteria to produce xylanase was pH 8.5 with the highest average value of enzyme activity 4.854 U/mL. The addition of xylanase enzyme in the fermentation process of inked paper waste was able to increase the brightness of the inked paper with a lower average value of kappa number of 2.762 compared to the higher control of 5.525.

Keywords: Thermophilic Bacteria, Inked Paper, pH, Temperature, Xylanase

1. INTRODUCTION

Paper is a thin and flat material which is usually made of wood with a fiber content of 39% (Asngad et al., 2016). According to the Ministry of Industry of the Republic of Indonesia (2015) the amount of paper production in Indonesia reaches 10.4 million tons per year and is ranked sixth in the world. The increase in paper production has an unfavorable impact on the environment. Bahri's research (2015) states that until now the main raw material for pulp that is widely used is wood. As a result, deforestation is becoming more widespread. An alternative to overcome this problem is to reuse used paper as paper raw material. Waste paper that has undergone processing is a fiber raw material known as secondary fiber (Rismijana et al., 2003).

To make white writing paper by obtaining fibers from the type of waste paper that contains ink, it is usually done through a deinking process. Deinking is the process of removing ink from fibers (Rismijana et al., 2006). Conventional deinking is done by adding chemicals such as sodium hydroxide, sodium silicate and hydrogen peroxide to help the ink release (Wirawan et al., 2008). Using these chemicals will produce chemical waste that has an impact on environmental pollution (Rismijana et al., 2003). To avoid environmental damage caused by paper bleaching chemicals, enzymes can be used as substitutes in environmentally friendly paper bleaching, the process of releasing ink on paper using enzymes is also called Biodeinking (Hader et al., 2013). One of the enzymes that is widely used is the xylanase enzyme (Takhur et al., 2012).

Xylanase is an enzyme capable of hydrolyzing xylan into xylooligosaccharides and xylose (Susilowati et al., 2012). Xylanase has a major role in industry, one of which is in the pulp and paper industry (Sharma et al. 2014). In the pulp and paper industry, xylanase enzymes can change the fiber structure by breaking the xylose-xylose bonds in the xylan chain (Beg et al., 2001).

Xylan is commonly found in lignocellulosic agricultural wastes (hemicellulose, cellulose, and lignin) such as rice straw, rice bran, wheat bran, corn cobs and bagasse (Utarti and Siswanto, 2018). Soffiyyana's research (2020) stated that the enzyme activity contained in the xylan substrate extracted from rice straw was 6.033 U/mL. When compared with other alternative substrates, using rice straw xylan extract as a substrate has a higher activity than rice husk xylan extract with an enzyme activity of 5.677 U/mL and corn cobs with an enzyme activity of 5.785 U/mL.

2. LITERATURE REVIEW

Enzyme assay:

Xylanase activity was assayed by measuring the release of reducing sugar from beechwood xylan following the dinitrosalicylic acid (DNS) method (Miller 1959). To 1.8 mL of substrate in phosphate buffer, pH 6.5, 0.4 mL of culture supernatant was added and incubated at 90°. After 10 min, 2.0 mL of DNS solution was added to the reaction mixture and boiled for 10 min. Absorbance was measured at 540 nm against a reagent blank. One unit of xylanase activity was defined as the amount of enzyme that released 1 µmol reducing sugar equivalent to xylose per min under the above assay conditions.

Effect of pH on activity and stability of xylanase:

Effect of pH on the activity of xylanases was measured by incubating 0.4 mL of enzyme and 1.8 mL of buffers, adjusted to pH of 5.5 to 8.5, containing beechwood xylan (0.5%). The buffers used were: sodium acetate buffer, pH 5.5; phosphate buffer, pH 6.0 – 8.0; Tris-HCl buffer, pH 8.5. Stability of the enzyme at different pH values was also studied by incubating the enzyme at various pH values ranging from 5.5 – 8.5 for 24 hours at 25° C and then estimating the residual activity.

Effect of temperature on activity and stability of xylanase:

The effect of temperature on the enzyme activity was determined by performing the standard assay procedure as mentioned earlier for 10 min at pH 6.5 within a temperature range of 40 – 100° C. Thermostability was determined by incubation of crude enzyme at temperatures ranging from 40-100° C for 2 hours. After treatment the residual enzyme activities were assayed.

Effect of inhibitors

Various metals (1 mM) and others reagents (1 mM) were added to the standard enzymatic reaction mixture in order to study their effect on xylanase and β -xylosidase activities.

Method**1. Type of Research**

This type of research is experimental research.

2. Time and Place of Research

This research was conducted in May 2021 - August 2021 at the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padang State University.

3. Tools and Materials

The tools used in this research are: test tube, test tube rack, beaker glass, Erlenmeyer tube, measuring cup, Bunsen lamp, spatula, electric stove (hot plate), vortex, stirrer, digital scale, inoculation needle, drill glass, spatula, incubator, oven, filter paper, pH meter, dropper pipette, autoclave, centrifuge, shaker incubator, spectrophotometer, cooling, refrigerator, petridish.

The materials used in this study were SSA2 Sapan Sungai Aro Solok Selatan bacterial isolate (Irdawati et al., 2018) from the Microbiology Laboratory, inked paper waste, Medium Nutrient Agar (NA), Gellum gum, xylan, Dinitrosalicylic Acid (DNS), Medium Beechwood Xylan, 0.3% straw extract, Bactereological Peptone, gauze, cotton, aquades, tissue, 70% alcohol, Yeast Extract, K₂HPO₄, MgSO₄·7H₂O, CaCl 0.2 M, H₂SO₄, Na₂S₂O₃, KMNO₄, 1% starch and KI 10%.

4. Research Design

The research design used was a randomized block design (CRD) with the following parameters variation of temperature with 7 treatments and 3 replications and variation of pH with 6 treatments and 3 replications. Application of xylanase with optimum

temperature and pH on inked paper waste with 2 treatments and 2 replications, control (inked paper waste without xylanase enzyme), and waste paper is inked with xylanase enzymes produced at optimum temperature and pH

5. Research Procedure

Tools and materials were sterilized using an autoclave at a temperature of 121°C and a pressure of 15 psi for 15 minutes. The straw was washed and dried, after being half dry, then it was oven-dried at 50°C for 7 days. After the straw is dry, it is broken, then it is blended and the powder is obtained which will be used as a substrate. The powder from the prepared rice straw was weighed 50 g and then put into a glass beaker and soaked with 1% NaOCl for 5 hours at 28°C. After that, it was rinsed and filtered and then immersed in 10% NaOH solution at 28°C for 24 hours. Centrifugation of the obtained filtrate at 4000 rpm for 30 minutes. The liquid (supernatant) from the centrifugation was neutralized with 6N HCL to be centrifuged again at 4000 rpm for 30 minutes. The resulting supernatant already contains xylan, to separate the soluble xylan by adding 95% ethanol and centrifuging at 4000 rpm for 30 minutes (Richana, 2007).

For the regeneration of bacterial isolates, a medium of NA was made by weighing 20 grams of NA (plus 3 g of Gellan gum in 1000 mL of NA) then put into a beaker glass and added with distilled water until the volume became 1000 mL. The mixture is heated to boiling and then put into an Erlenmeyer and tightly covered with cotton and aluminum foil. The medium was sterilized using an autoclave at 121°C at 15 psi pressure for 15 minutes.

The medium for growing xylanase-producing bacteria is using Beechwood medium with a composition of 0.5% polypeptone, 0.1% yeast extract, K₂HPO₄, 0.02% MgSO₄·7H₂O, and 0.3% straw extract (Sofiyana, 2020). Dissolved in 1000 mL of distilled water with a pH of 8. Then heated until homogeneous and sterilized in an autoclave at a temperature of 121°C and a pressure of 15 psi for 15 minutes.

3. EXPERIMENTAL

Tabel 1. Average of xylanase activity at some temperature of bacteria thermofilik amobil isolat SSA₂

NO	Temperature (°C)	Average Xylanase (U/mL)
1	50	4,222 ^{ab}
2	55	4,109 ^a
3	60	4,442 ^{bc}
4	65	4,651 ^c
5	70	4,629 ^c
6	75	4,668 ^c
7	80	4,566 ^c

Note: Numbers followed by the same letter are not significantly different at =5% according to the DMRT test.

Table 2. The average value of xylanase activity in several pH . treatments

NO	pH	Average Xylanase (U/mL)
1	7,5	3,708
2	8,0	3,872
3	8,5	4,854
4	9,0	4,114
5	9,5	3,759
6	10,0	3,747

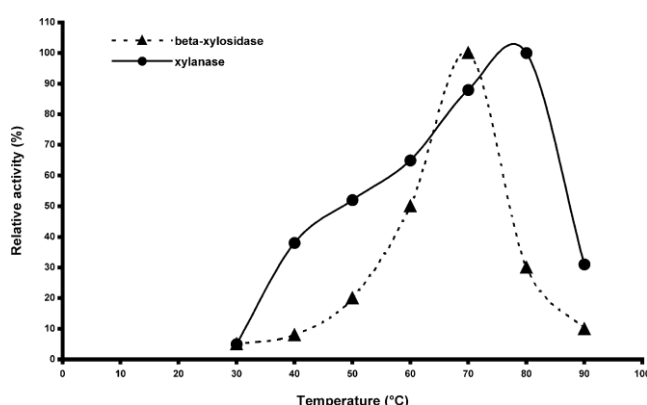


Figure 2. Effect of temperature (a) and pH (b) on purified xylanase and β -xylosidase activities from *B. thermantarcticus*. The values were obtained from at least three determinations in standard assay conditions.

4. RESULTS AND DISCUSSION

Temperature Optimization of Immobilized Thermophilic Bacteria for Xylanase Enzyme Production. Table 1 shows the results of the analysis that the temperature treatment of immobilized thermophilic bacteria 50°C, 55°C, 60°C, 65°C, 70°C, 75°C and 80°C showed significant differences with the highest average value of xylanase enzyme activity at 75°C treatment, which was 4,668 U/mL. The temperature treatment of 55°C resulted in the lowest average xylanase enzyme activity, which was 4.109 U/mL. Based on the results of the ANOVA, the results obtained were calculated $F(6.907) > F_{table}(3.00)$. This shows that there is a significant effect on the activity of the xylanase enzyme. Therefore, further test of DMRT was carried out.

pH Optimization of Immobilized Thermophilic Bacteria for Xylanase Enzyme Production

Based on the results of this study, pH 7.5 produced the lowest average enzyme activity, which was 3.708 U/mL. Enzyme activity continued to increase up to pH 8.5 which resulted in the highest average activity value of 4.854 U/mL. However, the enzyme activity again decreased at pH 9.0 and continued to decrease until pH 10.0 which had an enzyme activity value of 3.747 U/mL as can be seen in Table 2. The results of the

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ANOVA test obtained F count (2,591) < F table (3.11). This shows that there is no significant difference from the results of the tests carried out at pH 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0. Therefore, no further DMRT test was conducted.

Kappa Number Test on Inked Paper Waste

Based on Table 3, when viewed from the kappa number value, paper plus xylanase enzyme produced by immobilized thermophilic bacteria isolate SSA2 had a lower kappa number value of 2.762 compared to the control treatment (paper without enzymes) with a kappa number value of 5.525. Therefore, the xylanase enzyme can be used as a bleaching agent for inked paper waste to reduce the use of chlorine which is harmful to the environment, this is due to the ability of the xylanase enzyme to reduce the kappa number so that it can increase the brightness of the inked paper waste.

Xylanase enzymes play a role in breaking the xylose-xylose bonds in the xylan chain, breaking the bonds between the remaining lignin and carbohydrates. This means that xylanase acts as an enzyme that promotes lignin transfer during the paper bleaching process (Bajpai et al., 2004). The ability of xylanase to break the xylose-silose bonds in the xylan chain will also help to release the bonds between the ink and the paper fibers so as to increase the whiteness of the inked paper waste.

Maximum enzyme activity will increase the whiteness of the paper and reduce the kappa number. According to Irdawati et al., (2020) the use of optimum temperature is able to produce the highest enzyme activity which causes maximum xylanase enzyme production so that the brightness of the pulp in pulp fermentation is also higher as evidenced by the low kappa number obtained, which is 1.031 compared to the temperature treatment (50°C, 55°C). , 60°C, 65°C) with a kappa number value of 1.842; 2,799; 1,031; 2,394.

The use of xylanase is generally carried out before the bleaching process with bleaching chemicals (Martin et al., 2012). Xylanase will reduce the consumption of bleaching chemicals, so it can reduce the release of organochlorine compounds into the environment (Cheng et al., 2013). Research by Khonzue et al., (2011) states that xylanase can reduce the consumption of bleach by 20% to get the same brightness value compared to without the enzyme.

5. CONCLUSION

The temperature of thermophilic bacteria using rice straw xylan extract as a substrate affects the activity of the xylanase enzyme. With the highest average value of enzyme activity that is 4,668 U/mL at 75°C and the lowest concentration at 55°C is 4,109 U/mL. The optimum pH of immobilized thermophilic bacteria SSA2 isolate in producing xylanase enzyme was pH 8.5 with the highest enzyme activity of 4.854 U/mL. The application of maximum xylanase enzyme in the fermentation of inked paper waste can increase the whiteness level of inked paper waste as evidenced by the low average value of kappa number, which is 2,762 compared to control which has an average value of 5,525 kappa number.

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