Specific Thermophilic Bacterial Xylanase Enzyme Activity Using Rice Straw as Substrate and Its Possibility as an Eco-friendly Fabric Bleach

Irdawati Irdawati[1(✉)], Zul Hidayati[1,2], Linda Advinda[1,2], Mades Fifendy[1,2], and Salvia Salvia[1,2]

[1] Department of Biology, Faculty of Mathematics and Sciences, Universitas Negeri Padang, Padang, West Sumatra, Indonesia
irdawati.amor40@gmail.com

[2] Biology Department, Faculty of Mathematics and Sciences, Andalas University Padang, Padang, Indonesia

Abstract. Extracellular enzyme xylanase has a wide range of industrial uses. The xylanase enzymes made by thermophilic bacteria can hydrolyze xylan into xylose and xylooligosaccharides. Rice straw, for example, is a natural carbon source that can be utilized to create xylan carbon sources. One of xylanase's uses in industry is as an environmentally friendly bleaching agent (biobleaching). This study's objectives were to ascertain the xylanase enzyme's specific activity at varied substrate concentrations and to evaluate the enzyme's potential impact on fabric brightness. This investigation is experimental. This is an experimental study using RAL that includes three replications and six treatments. The xylanase enzyme's contribution to the fabric's whitening will be evaluated using the kappa number. At a substrate concentration of, the xylanase enzyme's specific activity peaked 4%, or 0.419 U/mg protein, based on the results. The kappa number results show that the xylanase enzyme from agricultural waste substrates can reduce the kappa number (2.21) whereas the kappa number without xylanase is higher. This is supported by the potential activity of the xylanase enzyme on fabric brightness (3.42).

Keywords: Xylanase Enzyme · Bio-bleaching · Thermophilic Bacteria · Agricultural Waste

1 Introduction

Enzymes are being used more frequently in the industry as a result of advances in biotechnology. Because of the widespread public awareness of environmental pollution, enzyme technology is being used to explore a variety of chemical processes [1]. Industry applications for enzymes include textiles, food, detergents, paper, cosmetics, and biofuels, to name a few [2]. One of the enzymes that is most frequently used is xylanase [3].

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An extracellular enzyme called xylanase can break down xylan into xylooligosaccharides and xylose. Microbes produce xylanase through a fermentation process. Microorganisms can use xylan as a carbon source, substrate, and inducer in growth media to manufacture the enzymes needed to break down xylan [4]. Xylanase is a biodenking agent that can be employed in the industrial sector. Xylanase is able to between xylan in cellulose which binds to lignin so that xylan will become the monomer and can release lignin from cellulose to produce white pulp and is more environmentally friendly. In addition, the xylanase enzyme can also be used in the textile industry for fabrics [5].

Xylanase can reduce the use of bleaching chemicals, it helps to limit the release of organochlorine compounds into the environment [6]. According to Khonzue's research, xylanase can cut bleaching chemical usage by 20% to achieve the same brightness value as controls without xylanase [7]. Garg's research also revealed that xylanase was more successful in removing hemicellulose from bio-scouring programs. After the bleaching procedure, bio-scouring of hemp cloth increases a variety of physical qualities. When compared to chemically treated bleach, the whiteness and brightness of the treated enzyme rose by 10.96% and 15.64%, respectively [5].

Xylanase enzyme is one of the thermostable enzymes that can be produced by thermophilic bacteria [8]. Thermophilic bacteria contain heat-resistant protein, resistant to denaturation and proteolysis so they are able to adapt to extreme temperature environmental conditions [9]. Bacteria need xylan as a substrate in order to produce xylanase because the presence of xylan in the fermentation medium stimulates bacteria to secrete extracellular enzymes that can break down xylan into simple molecules as a carbon source. Because using pure xylan on a large basis costs too much, agricultural waste can be used as a medium for alternative carbon sources with lignocellulose as the primary component [10].

According to Fachry, lignocellulosic waste is agricultural waste containing hemicellulose, cellulose and lignin [11]. Xylan components can be found in agricultural wastes, one of which is rice straw [12]. Because rice straw contains a high (20%) xylan concentration, it has the potential to replace xylan in some applications [13].

2 Materials and Methods

2.1 Making a Flour Powder Substance from Agricultural Waste

The straw is washed and dried after sorting. Place it in the oven at 50 °C for 7 days when it is half dry. The materials are combined to create a powder that will be utilized as a substrate after they are entirely dry and can be broken.

2.2 Xylan Extraction from Agricultural Waste

Soaked 50 g of rice straw in a 1 percent NaOCl solution at 28 °C for 5 h. After that, rinse and filter before immersing in a 10% NaOH solution for another 24 h at 28 °C. After getting the filtrate, it was centrifuged for 30 min at 4000 rpm. Then, using 6N HCl to neutralize the centrifuged supernatant, centrifuge it again for 30 min at 4000 rpm. The resultant supernatant already contains xylan, so add 95 percent ethanol and centrifuge for 30 min at 4000 rpm to separate the dissolved xylan.

2.3 Xylanase Enzyme Activity Test

The material was centrifuged for up to 1 mL, and the pellet and supernatant were separated. Then, in phosphate buffer (pH 8.5), combine 0.25 mL of sample and 0.5 mL of Xylan and incubate at 60 °C for 10 min. Then 0.5 mL of Dinitrosalycilic Acid (DNS) was added and incubated for 15 min at 90 °C. After that, by measuring the absorbance at a wavelength of 540 nm, the enzyme's activity is ascertained. Based on the results of the absorbance measurement, the xylose content is calculated using the linear regression equation shown below:

$$Y = ax + b$$

Information:
y = wavelength 540 nm, which is the absorbance value.
a and b = calculation of the standard sugar for xylose
x = *content of xylose*

Using the conventional xylose curve, the amount of reducing sugar freed was calculated. Under the test circumstances, one unit of xylanase activity was defined as the amount of enzyme required to liberate 1 mol of xylose per minute. At the time of testing with aquadest, the sample was blank. Heat the substrate for 10 min at 100 °C to deactivate it.

There were created standard xylose solutions with concentrations of 20, 40, 60, 80, and 100 g/mL. After mixing 0.5 mL of each standard solution with 0.5 mL of distilled water, 1 mL of DNS reagent was added. The tube was cooled after 15 min in a boiling water bath, and the absorbance was calculated at a wavelength of 540 nm.

2.4 Measurement of Protein Content

The protein content was measured using the Lowry (1976) method. By combining 0.1 ml of the enzyme sample with 0.5 ml of the D reagent, stirring for 10 min, adding 0.05 ml of the Folin-Ciocalteu solution, and waiting 30 min, the protein content was ascertained. Take a lengthy measurement of the absorbance using a spectrophotometer with a 750 nm wavelength after that. By comparing the absorbance data to the standard bovine serum albumin (BSA) curve, the amounts of enzyme protein were determined.

2.5 Fabric Cooking Using 5% NaOH

The fabric was weighed up to 1.7 g, and it was cooked in 34 ml of 5 percent NaOH in a 100 ml Erlenmeyer at 100 °C for 60 min. The cloth is then filtered and washed with distilled water until the pH is neutral. The rinsed cloth was combined with 50 ml of distilled water, and then it was autoclaved at 121 °C for 15 min to sterilize it.

2.6 Effects of Xylanase Addition to the Bleaching Process

The pieces of cloth that have been decomposed are added to each of them with 20 ml of xylanase enzyme. Then it is allowed to react for 60 min at a temperature of 60 °C

and pH 7. While maintaining a stable temperature and pH conditions, the enzymes work to degrade the fiber and weaken the bond between the fiber and the color to remain stable, namely by stirring the cloth pieces. The purpose of the stirrer is to create friction between the fibers (swelling) which helps the particle release process. After the bleaching process with xylanase is complete, then the cloth pieces are cooled at 5ºC to deactivate (xylanase inactive). Then the cloth pieces were added with 2% NaOH and 2% H2O2, then separated and filtered the cloth pieces that were ready to be used to measure the white level by looking at the kappa number.

2.7 Kappa Number Test on Fabric (S1I 0530-8, Baristan)

By titration, the kappa number test is carried out. The sample of the test cloth was first dried at a temperature of 100 °C in an oven. The process of drying is continued until the amount of water in the fabric equals zero, or the weight of the fabric. The sample of cloth pieces was then cut into smaller pieces with scissors and weighed up to 0.5 g. Add 350 ml of distilled water after placing in a 1000 ml Erlenmeyer. Then swirled for 60 min till the cloth fragments broke down at 25 °C room temperature. Then, after waiting for three minutes, added KMNO4 and H2SO4 in amounts of up to 12.5 ml each (as KMNO4 rinse). After adding 3–4 drops of starch to create a blue–black tint, 5 ml of 10% KI was added until the color changed to light brown. Next, the solution was titrated with Na2S2O31N (Thiosulfate) until the color turned purple. Once the purple color has been achieved, count how much Na2S2O3 solution was used, and then enter the results into the kappa number formula. However, a blank has already been made using the same procedures as above without using cloth. The National Standardization Research Institute provides the following formula for computing the kappa number:

$$\text{Kappa} = \frac{(b-a)Mx10}{W}$$

Information:
b = Blank titration volume (ml)
a = Sample titration volume (ml)
W = Typical Weight (gr)
N = Na2S2O3 standardization (0,11053).

3 Result and Discussion

3.1 Result

3.1.1 The Specific Activity of the Xylanase Enzyme

In this research, rice straw was used as a growth medium with several different concentrations, namely 0.1%, 0.2%, 0.3%, 0.4% and 0.5%. The results of statistical analysis revealed that giving varying quantities of xylan extract from straw substrate had no effect on the activity of the xylanase enzyme. The enzyme activity peaked at 3.185 Unit/mL at a concentration of 0.1 percent, then began to decline at a substrate concentration of 0.2%, then continued to decline at substrate concentrations of 0.3%, 0.4%, and 0.5%.

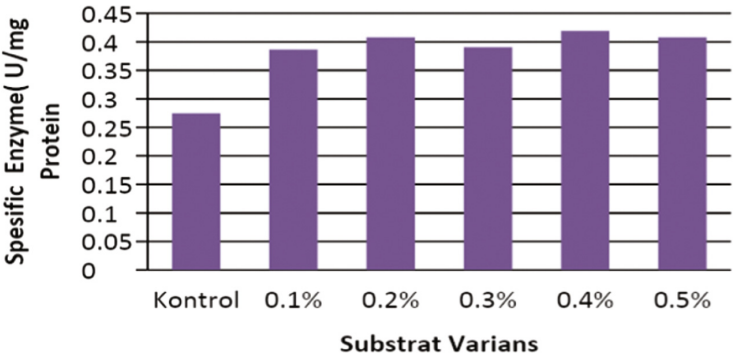

Fig. 1. Xylanase-specific enzymes with several different concentration

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Determination of protein content was carried out using the Lowry method. The results obtained on the specific enzyme activity of thermophilic bacteria xylanase MS 18 ranged from 0.275 - 0.419 U/mg protein. Based on the MS 18 thermophilic bacteria's particular enzyme activity measurements, as shown in Fig. 1. At a substrate concentration of 0.4%, it was discovered that the particular enzyme activity had a greatest average tendency of 0.419 U/mg protein. Meanwhile, the lowest average specific enzyme activity was found in the control, which was 0.275 U/mg protein.

3.1.2 Xylanase Enzyme's Potential Impact on Fabric Brightness

The kappa number produced by the bleaching agent, xylanase enzyme from MS 18 Solok Selatan isolate, is shown in Fig. 2. The results of the kappa number in the control (fabric without xylanase enzyme) were higher than the cloth added with xylanase enzyme. This shows that the xylanase enzyme has the opportunity as an environmentally friendly fabric bleaching agent because it can lighten fabrics by reducing the kappa number. The fact that xylanase was able to break the connections between xylan and lignin is what causes the low kappa number, which represents the fabric's whiteness (brightness). The outcomes demonstrated that the xylanase enzyme from agricultural waste substrates could lower the kappa number by 2.21 whereas the kappa number was greater in the absence of xylanase, namely 3.42.

3.2 Discussion

3.2.1 The Specific Activity of the Xylanase Enzyme

An essential component in the manufacture of xylanase enzymes is the choice of substrate type and the make-up of the appropriate medium. According to the study's findings, rice straw was the best substrate for the growth of the SSA 2 isolate as a replacement for pure xylan [14]. Jacobsen et al. stated that the hemicellulose content in rice straw was 24.5% [15]. The content of hemicellulose in the substrate is very influential on enzyme

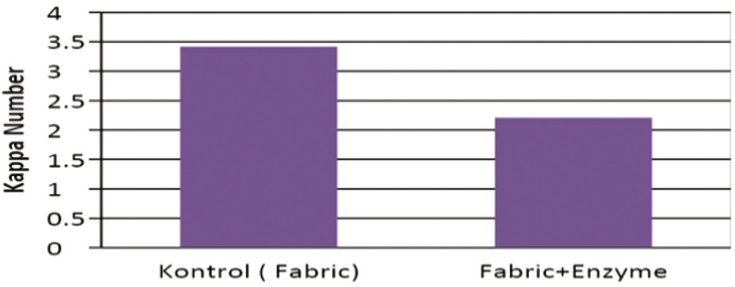

Fig. 2. Result of kappa number

activity because xylan is the main component of hemicellulose so that in the selection of substrates it is necessary to pay attention to the hemicellulose content.

In this research, rice straw was used as a growth medium with several different concentrations, namely 0.1%, 0.2%, 0.3%, 0.4% and 0.5%. At a concentration of 0.1%, the enzyme activity tends to have the highest value, which is 3.185 Unit/mL then the enzyme activity begins to decrease at a substrate concentration of 0.2% and continues to decrease at a substrate concentration of 0.3%, 0.4%, and 0.5%. However, there is no significant difference in statistical tests. The findings of this study show that the enzyme activity decreases with increasing substrate concentration.

Substrate concentration affects enzyme production and enzyme activity. The need for the enzyme to the substrate has a certain limit, if the enzyme is already at the optimum substrate concentration, the enzyme activity will be constant. So the enzyme activity will increase to a certain substrate concentration according to these needs, after that the enzyme activity will remain even though the substrate concentration continues to increase [16].

The specific activity of the enzyme is directly proportional to the level of purity of the enzyme, the higher the specific activity of an enzyme, the purity of the enzyme will also increase [17]. High specific enzyme activity is not always based on high enzyme activity and vice versa [18]. According to the results of measuring the enzyme's specific activity on rice straw substrate, the level of specific activity that was highest was 0.4 percent substrate concentration, or 0.419 U/mg protein. These results can indicate that most of the protein at 0.4% substrate concentration is xylanase. This indicates that the level of purity of xylanase at a concentration of 0.4% is higher than the concentration of other substrates.

3.2.2 Potential of Xylanase Enzyme on Fabric Brightness

The results showed that the fabric that was added with the xylanase enzyme had a lower kappa number (2,21) than the cloth that was not added with the enzyme (3,42). For chemical and semi-chemical pulps, whether they are semi-white or not, the kappa number is a metric used to determine the level of maturity, whitening power, or degree of delignification. The lower the kappa number, the easier it is to bleach the pulp [19]. The role of xylanase is to act as an enzyme that facilitates the transfer of lignin in an environmentally friendly fabric bleaching process.

The kappa number of pulp treated with xylanase enzyme from immobilized bacterial cells was lower (6.71) compared to a pulp without xylanase enzyme (8.01). In order to facilitate the release of lignin and increase the degree of fabric whiteness, the inclusion of enzymes can affect how hydrogen bonds occur in the cellulose chains [20]. The findings of Baipai's study, which clarify that one of the processes in the pulp industry's bleaching process is the removal of lignin from the pulp, support this by showing that doing so will produce paper pulp with a more precise brightness level. [21].

Greater porosity, swelling, and loss of density in the pulp microfibrils are effects of the xylanase enzyme addition. The walls of the pulp fiber are thinner, the surface is smoother, and the diameter has greatly risen. This occurs because the presence of enzymes can lead to the creation of hydrogen bonds in the cellulose chain, facilitating the release of lignin and resulting in a whiter pulp color [22]. The decrease in active chlorine used in xylanase-treated fabrics means that the consumption of chlorine used will be more efficient and ultimately result in the bleaching process becoming more environmentally friendly. This is in accordance with the research of Garg and Vicuna in Beg et al., which stated that the use of xylanase in this pre-bleaching stage can reduce the use of chemicals based on chlorine/toxic oxidizing agents by as much as 20–40% [23].

4 Conclusion

We come to the conclusion that the rice straw substrate had the maximum specific activity of the xylanase enzyme at 0.4 percent substrate concentration of 0.419 U/mg protein. The activity of xylanase enzymes from agricultural waste substrates can lighten the fabric by reducing the kappa number by 2.21 while without using xylanase the kappa number is higher, which is 3.42.

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