Characterization extrinsic factors of immobilized cells thermoxilanolytic bacteria in producing xylanase

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Abstract. Enzyme is one of important commodity in industrial world. The majority of industrial enzymes are produced by microorganisms through fermentation. The use of enzymes is largely as a biocatalyst in chemical transformations. Currently there are about 400 known enzymes and about 200 are used commercially. The enzyme industry has grown rapidly and occupies an important position in the field of industry one of which is xylanase enzyme. Xylanase can be produced by molds and bacteria through a fermentation process. Groups of bacteria that are known to produce xylanase are Bacillus and Clostridium. In today's microbial use of thermophilic bacteria has commercial value because it is able to produce stability of heat-resistant enzymes. In producing enzymes cell immobilization techniques are used to facilitate the purification of products, increase productivity and ease in controlling cell stability. The aim of this study was to Characterization Extrinsic Factors of Immobilized Cells Thermoxilanolytic Bacteria in Producing Xylanase. This research was conducted in June 2017-June 2018 in Laboratory of Microbiology FMIPA UNP. This research was using descriptive method. This research conclusion that temperature effect on xylanase activity with optimum temperature was 70°C and pH affects the xylanase activity with the highest pH was 7.5.

1. Introduction
Enzymes are one of the important commodities in the industry. The majority of industrial enzymes are produced by microorganisms through fermentation. The use of enzymes is mostly as a biocatalyst in chemical transformation. At present, there are around 400 known types of enzymes and around 200 are used commercially [1]. The enzyme industry has developed rapidly and occupies an important position in the industry, one of which is the xylanase enzyme [2]. The types of microorganisms that commonly produce xylanase are from mold and bacterial groups.

Xylanase can be produced by mold and bacteria through a fermentation process. Examples of molds that can produce xylanase are from the Aspergillus and Trichoderma groups, then examples of xylanase-producing bacteria are from the Bacillus and Clostridium [3] groups of the bacterium Geobacillus sp. [4] and from the group Acinetobacter baumannii [5]. In addition to the fungi and bacteria other microorganisms that can produce xylanase are algae, fungi, and actinomycetes [6] then gastropods and arthropods [7] hereinafter are groups of yeast, protozoa, insects and snails [8].

Xylanase is an extracellular enzyme that has the ability to hydrolyze xylan (hemicellulose) to xylo-oligosaccharides and xylose. Bacterial xylanase production requires a substrate as an inducer, xylan, because the presence of xylan in the fermentation medium will trigger bacteria to secrete extracellular enzymes that can convert xylan into simple molecules as carbon sources. This carbon source is used...
by bacteria as an energy source to produce xylanase enzyme and degrade xylan found in the fermentation medium [9].

Application of xylanase in industry is found in the beverage industry [10] as well as the food, animal feed, paper or pulp bleach and lignocellulose bioconversion for fuel [11]. Some industries require enzymes that are active and stable at high temperatures so as to facilitate mixing, the substrate easily dissolves, speeds up the reaction, and prevents contamination. One of them is a thermostable enzyme produced by thermophilic bacteria [12]. Thermophilic and thermotolerant microorganisms are capable of producing xylanase enzyme [13]. Besides being able to produce thermostable enzymes, thermophilic bacteria can also be used for bioremediation processes [14].

In this day and age the use of microbes from thermophilic bacteria has commercial value, this is because thermophilic bacteria are able to produce stability of enzymes that are resistant to heat. As an environment that has a high temperature, hot water is a place of life for microorganisms [15]. One of the hot springs is the SapanAroSolok Selatan hot spring. Of the several types of isolates obtained in these hot springs, SSA2 isolates were chosen because these isolates have the most optimum growth.

The results of the study from Habibie, et al [16] showed that C211 bacterial isolates were able to grow at 55°C so that these isolates were able to produce xylanase enzymes which had optimum activity at a temperature of 50°C with pH 7.0. Thermostable enzymes can be obtained from microorganisms that can grow above a temperature of 45°C where thermophilic isolates can grow at temperatures of 45-70°C. High temperatures are good for selecting thermophilic and hyperthermophilic microorganisms.

In producing enzymes, cell immobilization techniques are used to facilitate product purification, increase productivity and ease the control of cell stability. [17]. When compared with free cells, immobilized cells have the advantage that they can be used repeatedly because they can be made in immobilized form [18]. Enzymes produced by free cells are easily denatured so that they are not economical because they can only be used in one reaction [18]. Because cell immobilization techniques can be used repeatedly, it can save the budget for enzyme use. Based on the results of research conducted by Wuryanti [19] the use of immobilized enzymes can be used several times, this indicates that immobilized enzymes are relatively more stable when compared to free enzymes. In addition Meryandini [20] also confirmed that immobilized xylanase is more stable than free xylanase.

Cell or immobilization of enzymes is a process in which the movement of enzyme molecules is retained in such a way that an active enzyme system that is insoluble in water is formed Kusumaningtias [21] The immobilization method used in this study is a entrapment technique using sodium alginate matrix. Cell immobilization techniques with Na alginate are chosen because of their non-toxic nature, high stability and porosity mechanisms, simple procedures for immobilization and low prices to be applied in the food or pharmaceutical industry. Isya [22] also emphasized that the benefits of the immobilization method can improve stability.

Kusumaningtias [21] explained that in his research using immobilization techniques, he did the determination of the optimum temperature and pH which aims to determine the optimum temperature conditions of the enzyme after immobilization and the determination of optimum pH to determine the optimum conditions of the enzyme in reacting with the substrate. Based on research conducted by Saparianti [23] using bacteria Pediococcusacidilactici with cell immobilization technique using 3% sodium alginate showed that pH affects the fermentation process. Likewise with research conducted by Awwaly, [24] with entrapment method using alginate matrix shows that temperature can affect enzyme immobilization. Ratnasari [25] also emphasized that using sodium alginate as a cell trapping with different concentrations can affect temperature, pH and variations of bead.

Based on research Irdawati et al [10] SSA 2 was selected as isolate in fermentation because it had higher ability to produce xylanase.

Therefore, the author needs to do research on bacteria that are in the Sapan Sungai Aro at Solok Selatan hot springs which is capable of producing xylanase from thermophilic immobilized bacteria so that it can be used as needed.
2. Research Methods
Temperature parameters that will be observed with 7 variations: 50°C, 55°C, 60°C, 65°C, 70°C, 75°C, 80°C. Meanwhile the pH parameters that will be observed with 6 variations: 7.5; 8.0; 8.5; 9.0; 9.5; 10.

2.1. Tools and Materials
The tools used in this study include: test tubes, test tube shelves, beaker, tube flask, beakers, Bunsen lamp, spatula, electric stove (hot plate), vortex, stirrer, digital scales, the needle inoculation, drill glass, spatula glass, stir bar, incubators, ovens, filter paper, measuring cups, pH meter, drop pipette, autoclave, centrifuge, shaker incubator, spectrophotometer, cooling, refrigerator, petri dish, label, eppendorf, volumetric, blue type, white type, micropipette and syringe.

2.2. Research Procedure

2.2.1. Preparation of Research
a. Sterilization of Apparatus
All the tools that will be used first sterilized. Sterilization and medium performed by autoclaving at 121°C and a pressure of 15 psi for 15 minutes.

b. Medium Formation
Medium used that medium to regenerate NA thermophilic bacteria. A total of 10 g of NA was dissolved in 500 mL plus Gellan Gum 3 g in 1 litre of distilled water and heated until homogeneous, then sterilized in an autoclave at 121°C at a pressure of 15 psi for 15 minutes.

2.2.2. Research Implementation
a. Xylanase Enzyme Activity Test
Testing of xylanase enzyme activity was carried out by centrifuging 1 ml of sample and after that 0.25 ml of supernatant was taken and added with 0.5 ml xylan in phosphate buffer pH 8.5 then incubated at 60°C for 10 minutes. Then the reaction was stopped by adding 1 ml of dinitrosalicylic acid (DNS) reagent and incubated at 90°C for 15 minutes and absorbance was measured using a spectrophotometer with a wavelength of 540 nm.

The amount of reducing sugar is determined using the standard xylose curve. Unit (U) xylanase activity is defined as the amount of enzyme needed to free 1 mol of xylose / minute under test conditions. Inactive substrate and enzymes (deactivated at 100 °C for 30 minutes) are used as blanks. The xylose standard curve is made in the range of 20, 40, 60, 80, and 100 μg / ml. A total of 0.5 ml of each standard solution mixed with 0.5 ml of aquadest, then added 1 ml of DNS reagent. The tube is inserted in a boiling water bath for 15 minutes then cooled and absorbance measured at a wavelength of 540 nm.

b. Enzyme Production with Cell Immobilization on Temperature Variation
The variations of bead optimum many as 500 grains were inserted into erlenmeyer 100 ml containing 50 ml of medium beechwoodxylan. Enzyme production is carried out by making temperature variations of 50, 55, 60, 65, 70, 75 and 80 °C. Then fermented during harvest. Then the enzyme activity was measured using a spectrophotometer with a wavelength of 540 nm.

c. Enzyme Production with Cell Immobilization on pH Variations
125 grains of alginate were inserted into 100 ml erlenmeyer containing 50 ml of medium *xylanbeechwood* with the pH of the optimization results. Enzyme production is carried out by variations the pH of 7.5, 8.0, 8.5, 9.0, 9.5 and 10. Then incubation during the harvest is 6 hours. Then the enzyme activity was determined using a spectrophotometer with a wavelength of 540 nm.

Isolation of Xylanase Crude Extract Xylanase enzyme was isolated and purified where xylanase enzyme in medium was *beechwoodxylan* separated from the cell by centrifugation at 5000 rpm for 15 minutes at room temperature between 26°C-27°C. Supernatant formed was crude xylanase enzyme extract.

### 3. Results and Discussion

#### 3.1. Effect of Enzyme Production with Cell Immobilization on Temperature Variations

Results of the xylanase characterization of immobilized thermophilic bacteria on cell immobilization in immobilized temperature variations obtained an average value of xylanase activity as Table 1.

| No. | Variation of Temperature | Average (U/ml) |
|-----|--------------------------|----------------|
| 1.  | 50°C                     | 7.831          |
| 2.  | 65°C                     | 7.942          |
| 3.  | 80°C                     | 8.323          |
| 4.  | 75°C                     | 8.518          |
| 5.  | 55°C                     | 8.581          |
| 6.  | 60°C                     | 8.725          |
| 7.  | 70°C                     | 8.992          |

Treatment of temperature variations of 70°C is the most optimum condition in producing xylanase enzyme compared to other treatments. But the tendency is seen in the treatment temperature variation of 70°C with a value of 8.992 U/ml compared to other treatments. The lowest temperature variation of enzyme activity was at a temperature of 50°C which was 7.831 U/ml. From this study the temperature of 70°C is the optimum temperature of several other temperature variations.

Based on the results of research by Bai *et al.* [26] which states that immobilization using sodium alginate produces higher xylanase compared to gelatin, agar and polyacrylamide with an optimum fermentation temperature of 55°C. Xylanase activity above 55°C decreases due to denatured immobilized cell enzymes.

Graph of immobilization of thermophilic bacterial cells in temperature variation can be seen in Figure 1.
Figure 1. Enzyme activity in immobilization of immobilized temperature variation cells

Based on the results of research from Fawzya et. al [27] regarding the production and characterization of xylanase produces optimum temperature at 70°C. Similar to the research conducted by Inayah, (2016) the characterization of xylanase from xylanolytic bacteria also showed optimum results at 70°C.

The results of enzyme production by cell immobilization at temperature variations produce optimum temperature at 70°C because it is in accordance with the environment of the thermophilic bacteria themselves which are resistant to heat and capable of producing thermostable enzymes. Xylanase activity tends to decrease above 70°C due to enzyme denaturation. Habibie, et al. [28] also confirmed that thermostable enzymes can be obtained from microorganisms that can grow above a temperature of 45°C.

3.2. Effect of Enzyme Production with Cell Immobilization on pH Variations.

| No | Variations of pH | Average(U/ml) |
|----|------------------|---------------|
| 1. | pH 9             | 8,327         |
| 2. | pH 9.5           | 8,577         |
| 3. | pH 8.5           | 8,628         |
| 4. | pH 8             | 8,950         |
| 5. | pH 10            | 9,115         |
| 6. | pH 7.5           | 9,789         |

Treatment of variation of pH 7.5 is the most optimum condition in producing xylanase enzyme compared to other treatments. This is due to the optimum conditions in the metabolism of immobilized cells caused by its oxidative properties. Zur et. al [29] concludes that immobilization can maintain the oxidative properties of several species by adjusting the optimum pH so that immobilization is thought to stabilize the capacity of protein synthesis of microorganisms.
Based on Figure 4, the optimum pH was 7.5 and for pH 8, 8.5, 9 and 10 it decreased below pH 7.5 with the lowest yield at pH 9. This is also in accordance with the results of Nyoman's study et al. (2015) which produced the optimum pH of the enzyme thermostable immobilization at 7.5.

The advantage of using immobilized cells in enzyme production is that it can reduce the time of enzyme production (Quintana and Dalton, 1999) and can increase enzyme activity [31]. Characterization was carried out to determine the optimum conditions for the xylanase enzyme fermentation process. The characterization of immobilized xylanase shows optimum pH at 7.5. Immobilized xylanase can be used repeatedly but there is a decrease in enzyme activity after 4 times of use. When compared with several other studies that do not use alginate matrix, repeated use is only up to 3 repetitions [20].

Zur et al., [29] confirmed that immobilized cells with alginate matrix have high resistance to toxic pollutants or other compounds. Bead or beads are able to maintain environmental conditions so that they become stable against cells that have been trapped. Because it has high resistance ability, the beads or beads are able to maintain oxidative properties with optimum pH and temperature. Granules/beads are not only able to maintain the environmental conditions but also against other bacteria because of the size of small pores which can inhibit the entrance of other bacteria. In addition, cell immobilization can also stabilize the capacity of protein synthesis of microorganisms. The effect of immobilization on physiological bacteria can obtain high productivity and cell density. Cells that are immobilized can increase metabolic activity, increase cell growth rate and stability.

4. Conclusion
Based on the research that has been done it can be concluded that xylanase activity varies in optimum immobilized at temperature 70°C. Cell immobilization in temperature variation does not affect xylanase enzyme activity. 3. Xylanase activity variations optimum immobilized at and pH 7.5.

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