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Measurement of α-Amilase Enzymes in *Bacillus subtilis* Bacteria

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Abstract

The bacterium Bacillus subtilis is able to produce amylase enzymes which are able to hydrolyze various types of starch sources into simple compounds such as maltose, glucose. Amylase converts carbohydrates which are polysaccharides into maltose. Amylase enzyme is a group of enzymes that are needed in the industrial field. The purpose of this study was to determine the level of the amylase enzyme produced by the bacterium Bacillus subtilis. The method used in this research is a quantitative method using an amylase enzyme measurement experiment. The results of this study are from two types of enzyme sources, namely Bacillus subtilis + Starch + NB and Bacillus subtilis + NB. Both samples showed enzyme activity. The conclusion of this study is that Bacillus subtilis + Starch + NB produces 63.186 units of the α-amylase enzyme used to produce 1 ppm / 1 µmol maltose per minute. Whereas the Bacillus subtilis + NB sample was 19.474 units of the α-amylase enzyme used to produce 1 ppm / 1 µmol maltose per minute.

1. INTRODUCTION

Biochemical reactions can take place quickly with the help of enzymes, enzymes can be obtained from living things, namely in microorganisms. Microorganisms have more potential to be exploited, because in addition to being cultured, the types of enzymes produced are also varied, one of the enzymes producing is bacteria, such as the termophilic bacteria can produce amylase enzymes. Amylase enzymes can be used in the textile industry, detergents, medicines, foodstuffs and beverages. The bacterium *Bacillus subtilis* is able to produce amylase enzymes which are able to hydrolyze various types of starch sources into simple compounds such as maltose, glucose. Amylase converts carbohydrates, which are polysaccharides, to maltose (alpha and beta) or glucose (gluko amylase) (Ningsih, *et al.*, 2012). Amylase enzyme is a group of enzymes that are needed in industry to reach nearly 25% of the world's enzyme market (Craigen, *et al.*, 2011).

The production of amylase enzymes can use various carbon sources. like molasses, corn flour, tapioca flour. Amylase enzyme production using microbes can be influenced by various factors. For example control of environmental factors is very important. Because, in the production of microbes affected by various things such as temperature, incubation time, initial pH, the number of inoculums and other influential factors is the treatment process (Kumaunang & Kamu, 2011). The amount of enzyme produced is also influenced by the type of microbes (Dini & Munifah, 2014; Angraini *et al.*, 2019). Therefore, to produce amylase enzyme products with satisfactory quality and quantity, it is necessary to optimize conditions and high-potential bacteria in producing amylase enzymes. The purpose of this study was to determine the level of the amylase enzyme produced by the bacterium *Bacillus subtilis*.

2. RESEARCH METHODS

a. *DNS Creation.*

Preparation of DNS was done by adding 10 g of NaOH, 182 g of potassium sodium tartrate and then dissolved in 400 mL of distilled water (Hardianti & Aziz, 2019). Subsequently added DNS 10 g and stirred until homogeneous. Then added 10 g of Na2SO3, then added distilled water until the volume reaches 1000 mL.

b. ***Manufacture of 0.2 M phosphate buffer pH 7.***

Solution A (NaH2PO4 or KH2PO4) as much as 39 mL and solution B (Na2HPO4 or K2HPO4) as much as 61 mL in 100 mL with a concentration of 0.2 M. How to dissolve starch 1%. The starch was weighed as much as 1.5 g then dissolved in a 0.05 M concentration phosphate buffer with a volume of 150 mL.

c. ***Making Blanks***

1 mL of distilled water mixed with 1 mL of starch. Preparation of standard maltose solution. Created starting from a concentration of 0-600 (Table 1).

Table 1. Standard maltose solution

Concentration	Maltose Stock (mL)	Distilled water (mL)
0	0	1
100	0.1	0.9
200	0.2	0.8
300	0.3	0.7
400	0.4	0.6
500	0.5	0.5
600	0.6	0.4

d. ***Measurement of α-amylase from Bacillus subtilis bacteria.***

Samples of 1 mL of bacterial suspension (*Bacillus subtilis* + 1% starch substrate + NB) were added 1 mL of dissolved starch as much as 1 mL then incubated at 40°C for 15 minutes. Then added 2 mL of DNS, then homogenize it with vortex. After the vortex is boiled for 5 minutes, then cooled for 5-10 minutes. Then the absorbance value is measured with λ 550 ηm. A sample of 1 mL of bacterial suspension (*Bacillus subtilis* + NB), blank, standard solution of maltose was carried out the same procedure with the treatment of bacterial suspension sample of *Bacillus subtilis* + 1% starch substrate + NB. The formula for calculating amylase activity is as follows:

$$\alpha\text{-amylase unit activity} = \frac{[\text{maltose}] \times df}{MW\ \text{maltose} \times V \times t}$$

where [maltose]: concentration / level of maltose (ppm), df: dilution factor (1x), MW: molecular weight of maltose (360.31 dalton), V: enzyme volume used (1 mL), t: incubation time (15 minute). To get the value of [maltose] the absorbance of the sample is calculated using the standard equation for the maltose curve.

3. RESULTS AND DISCUSSION

Determination of the enzyme produced by the bacterium *Bacillus subtilis*, before measuring the enzyme level, it is necessary to make a standard curve as a reference **(**Figure 1).

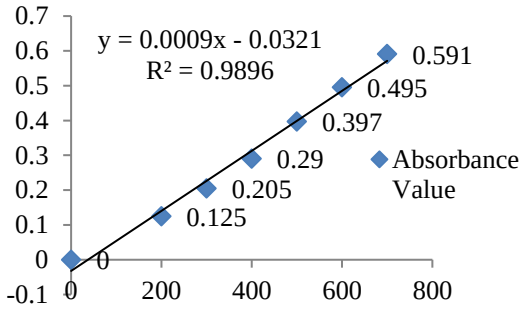

Figure 1. Standard curve of maltose solution

The results of the measurement of amylase unit activity values derived from samples of *B. subtilis* + starch + NB and *B. subtilis* + NB (Table 2).

Table 2. Activity of *B. subtilis* + starch + NB + NB and *B. subtilis* + NB

	Sampel	
	B+S+N	**B+N**
Ay	1,248	0,387
[M]	1517,768	467,7683
Aua	63,186	19,474
t	15	15
V	1	1
DF	1	1
MW	360,31	360,31
x	0,00082	0,00082
z	0,00343	0,00343

Note:
S: sample, B + S + N: *B. subtilis* + Starch 1% + NB, B + N: *B. subtilis* + NB, Ay: absorbance of the sample as a value of y, [M]: maltose concentration (results of the calculation of Ay on the formula maltose standard solution equation, Aua: Amylase unit activity (unit / mL), t: incubation time, V: enzyme volume, DF: dilution factor, MW: Maltose molecular weight, x and z: formula value of the standard maltose solution solution (y = 0,00082x + 0.00343).

The results of this research are producing amylase enzyme, the substrate used is starch. The use of substrates aims to induce bacteria to produce amylase enzymes. Because the substrate is needed in the process of producing enzymes, the amylase enzyme produced from the bacterium type *B. subtilis* can be inducible enzyme.

In this study, there are two types of enzyme sources, namely *Bacillus subtilis* + Starch + NB and *Bacillus subtilis* + NB. Both of these samples showed the presence of enzyme activity can be seen in table 2. The highest activity in the sample *Bacillus subtilis* + Starch + NB is 63,186 units of the α-amylase enzyme used to produce 1 ppm / 1 µmol maltose per minute. Whereas in the *Bacillus subtilis* + NB sample, 19,474 units of the α-amylase enzyme were used to produce 1 ppm / 1 µmol maltose per minute. It can be seen from these results that the *Bacillus subtilis* + Starch + NB sample has higher amylase enzyme activity than the *Bacillus subtilis* + NB sample. This happens because the use of the substrate aims to induce bacteria to produce amylase enzymes.

The confidence level of the α-amylase unit activity data for each sample shows a confidence level of% 95% where the regression value of the standard maltose solution equation is $R^2 = 0.9896$. This shows that the absorbance value of the maltose standard curve is in accordance with the standard, which is a continuous increase from 0 to 600 ppm concentration.

Enzyme production is influenced by various factors such as temperature, incubation time, initial pH, the amount of inoculum and other influential factors are the treatment process. the amount of enzyme produced is also influenced by the type of microbes (Sukmawati, 2018; Pangesti, *et al.*, 2012; Sukmawati & Rosalina, 2020). The effect of incubation time has been shown in the data (figure 1.), which starts from a concentration of 0 - 600 ppm, where the value of maltose absorbance increases continuously.

Amylase enzyme production is influenced by several factors and in general, amylase enzymes are optimally generated at a certain point in the logarithmic phase of the microbial growth curve, but there are certain microbes where the logarithmic phase is longer so that the amylase enzyme is not produced because the point has not been reached so long incubation is influential in this matter (Gunam, *et al.*, 2012; Sukmawati & Badaruddin, 2019). The temperature can affect because there are some thermophilic bacteria that are more optimal bacteria in the production of amylase enzymes in higher temperature conditions (Supriyanti & Heryanto, 2013).

4. CONCLUSIONS AND RECOMMENDATIONS

Bacillus subtilis + Starch + NB produces 63.186 units *of* the α-amylase enzyme used to produce 1 ppm / 1 µmol maltose per minute. Whereas in the *Bacillus subtilis* + NB sample, 19,474 units of the α-amylase enzyme were used to produce 1 ppm / 1 µmol maltose per minute.

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