

Enzymatic Conversion of Potato Starch into Glucose using The purified α -Amylase Enzyme from Locale Isolate Bacteria *Bacillus subtilis* ITBCCB148

S Karlinasari¹, T Suhartati², H Satria², S Hadi² and Yandri^{2*}

¹ Master Program in Chemistry, Departement of Chemistry, Faculty of Mathematics and Natural Science, University of Lampung, Bandar Lampung 35145 (Indonesia).

² Departement of Chemistry, Faculty of Mathematics and Natural Science, University of Lampung, Bandar Lampung 35145 (Indonesia).

* Email: yandri.as@fmipa.unila.ac.id

Abstract. The objective of this study was to determine the ability of the purified α -amylase enzyme to convert potato starch into glucose. For this objective, the researcher isolated and purified the α -amylase from locale isolate bacteria *Bacillus subtilis* ITBCCB148. The isolation of the extracellular α -amylase was conducted by using cold centrifuge method done to separate the enzyme from the cells. The purification of the α -amylase was examined by fractionation using ammonium sulphate salt followed by dialysis. The activity of the α -amylase enzyme was determined by using the Fuwa method and Mandels while the protein content was determined by using the Lowry method. Purified α -amylase obtained from 20–80% of ammonium sulphate saturation has specific activity 11312.64 U.mg⁻¹. Regarding the purity, it was increased to 6.41 times compared with the crude one (1765.25 U.mg⁻¹). On the other hand, the purification in dialysis step could increase the specific activity at 28834.13 U.mg⁻¹, which the purity rose to 16.33 times higher than crude. The purified enzyme had an optimum pH at 5 and optimum temperature at 65°C. The purified amylase from dialysis step was then applied for enzymatic conversion with various concentrations of potato starch 0.1; 0.2; 0.4; 0.6 and 0.8 % respectively to produce glucose. These starch concentration could respectively produce glucose at 0.14; 0.33; 0.49; 0.72 and 0.71 mg.mL⁻¹. The activity of α -amylase since it converted the starch potato were respectively 26.54; 60.51; 91.18; 133.33 and 131.72 U.mL⁻¹. In brief, The purified α -amylase enzyme is able to convert potato starch to glucose with an optimum concentration of potato starch 0.6%.

1. Introduction

Many microorganisms detected as sources of α -amylase including bacteria and fungi. It also have already studied widely to apply α -amylase in industrial field that connected into large scale production of α -amylase [1]. Bacterial α -amylases which have novel properties, have been the major extent of recent research. [2]. *Bacillus subtilis* is a rod-shaped gram-positive bacterium, members of the genus *Bacillus*. It can form endospore, to persist dangerous ecological surroundings of radiation, solvents, temperature and extreme pH, the endospore is considered at times of nutritional stress, allowing the organism to persist in the environment until the conditions become favorable [3]. In the field of biotechnology, *Bacillus subtilis* is microorganism which could produce some kind of enzyme such as proteases and amylase that high product yields (20 to 25 gram per litre) with excellent fermentation properties [4].

Amylase a starch degrading enzyme, is an important enzymes that used in industry and accounts for high proportion of the enzyme market [5]. The α -amylase (E.C. 3.2.1.1) has been applied as catalysts for enzymatic hydrolysis of materials with high content of starch [6]. α -amylases act on starch (polysaccharide) as the main substrate and yield small units of glucose (monosaccharide) and maltose (disaccharide). Starch is made of two glucose polymers, amylose and amylopectin, which comprise glucose molecules that are connected by glycosidic bonds. Both polymers have different structures and properties. A linear polymer of amylose has a

maximum of 6000 glucose units linked by α -1,4 glycosidic bonds, whereas amylopectin is composed of α -1,4-linked chains of 10–60 glucose units with α -1,6-linked side chains of 15–45 glucose units [7]. Enzymatic hydrolysis generates a glucose solution which can be used without further treatments [8].

Glucose, an important industrial product of starch hydrolysis finds application as sweetener in the food and pharmaceutical industries [9]. Starchy substrates are good source of sugar, this study was designed to develop a suitable technology (by optimizing an enzymatic process) for conversion of locally available and cheaper starchy substrates. Starch hydrolysates containing glucose can be obtained from potato, sweet potato, corn, wheat, sorghum, sugarcane, sugar beet and cassava starch into fermentable sugars and bio-ethanol [10,11]. Carbohydrates based agricultural products like starch from potato occur abundantly and can be used as a raw material for the production of energy. Interestingly, it is reported that after completion of saccharification process, glucose is found to be the main hydrolysis product of potato and sweet potato starch [12].

Several industrial processes are carried out using whole cells as the source of enzymes but the efficiency can be improved using isolated and purified enzymes. However, the criteria for selection of a particular method of isolation and purification depend on its end use. Various steps of ammonium sulphate precipitation are followed by dialysis for partial purification of crude enzyme. The partial purification and characterization of the enzyme from Local Isolate Bacteria *Bacillus subtilis* ITBCCB148 are presently reported. Furthermore, The objective of this study is to determine the characteristics of the purified α -amylase enzyme to convert potato starch into glucose.

2. Research Methods

2.1 α -Amylase Production

2.1.1 Inoculum Preparation. The stock culture of *Bacillus subtilis* ITBCCB148 was grown in Nutrient agar (NA) medium with 0.5 % soluble starch in order to obtain fresh isolates. The inoculum preparation was then inoculated in the production medium with the inoculum medium used by (Yandri *et al.*, 2010) [13] consisting of 0.5% soluble starch, 0.5% yeast extract, 0.01% CaCl_2 , 0.02% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 0.08% KH_2PO_4 . The pH of medium was set up to 6.5. Then the medium was sterilized at 121 °C, pressure 1 atm for 15 minute by using autoclave.

2.1.2 Production of α -amylase Enzymes. The composition of the production medium was equal to the composition of the inoculum medium. The active inoculum (2%) was inoculated into an Erlenmeyer containing the production medium, incubated in a shaker incubator with an agitation speed at 200 rpm for 72 hours at room temperature [13]. The centrifugation was then conducted at 6000 rpm for 20 minutes. The supernatant obtained was a crude extract of α -amylase enzyme.

2.2. Activity test of α -amylase and determination of protein content

2.2.1. α -Amylase assay

α -amylase activity was measured by determination the liberated reducing sugars as end products according to Fuwa [14] and Mandels method [15].

2.2.2. Protein determination

Lowry [16] method was used to determine the protein content.

2.3. Purification of α -amylase Enzymes

2.3.1 Fractionation. The crude extract enzyme obtained was purified by fractionation using ammonium sulphate. The crude enzyme was added by solid ammonium sulphate with continuous overnight stirring and separation into the following saturation ranges: (0-20)%; (20-40)%; (40-60)%; (60-80)%; and (80-100)%

respectively [17]. The precipitates collected by centrifugation (10,000 g for 20 min) were dissolved in 0.1M phosphate buffer, pH 6.0.

2.3.2. Dialysis

The fractionated ammonium sulphate enzyme with the highest activity was purified by dialysis in the cellophane pouch with the extraction buffer for 24 h at 4 °C. Dialysis of the α -amylase enzyme was used to partially hydrolyze the potato starch into glucose.

2.4. Effect of pH on α -amylase activity and stability

Effect of pH on α -amylase activity was determined by performing enzyme assay at various pH value (4.5; 5.0; 5.5; 6.0; 6.5; 7.0; 7.5 and 8.0) using 0.1 M phosphate buffer.

2.5. Effect of Temperature on α -amylase activity and stability

To determine the optimum incubation temperature for maximum α -amylase activity, the reaction was carried out at various incubation temperatures (55; 60; 65; 70; 75 and 80°C) for 30 min. The reaction mix comprising the purified amylase and 0.1% of potato starch solution was incubated at different temperatures at pH optimum.

2.6. Enzymatic Conversion of Potato Starch into Glucose

The purified amylase from dialysis step with the optimum pH and Temperature was then applied for enzymatic conversion with various concentrations of potato starch 0.1; 0.2; 0.4; 0.6 and 0.8 % respectively to produce glucose.

3. Result and Discussion

3.1. Purification of α -amylase Enzymes

3.1.1 Fractionation. The crude extract enzyme obtained was purified by fractionation using ammonium sulphate. The crude sample was fractionated into five, (0-20)%; (20-40)%; (40-60)%; (60-80)%; and (80-100)% based on the saturation of ammonium sulphate (Figure 1).

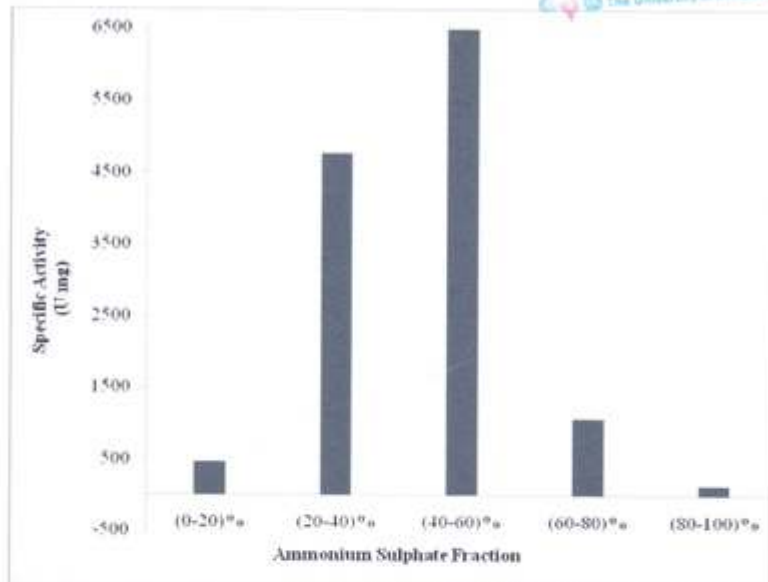


Figure 1 Effect of enzyme fraction at various levels of ammonium sulphate saturation on specific activity of the precipitates.

Figure 1 shows that α -amylase enzyme has the highest specific activity at fraction 40-60%, of ammonium sulphate saturation has specific activity $6519.51 \text{ U.mg}^{-1}$. Whereas in the fraction 0-20; 20-40; 60-80; and 80-100% respectively of ammonium sulphate saturation have specific activity 465.213; 4769.22; 1071.76; and 144.32 U.mg^{-1} . This indicates the presence of enzymes deposited on these fractions. For the next process, fractionation was carried out in 2 levels of saturation of ammonium sulphate, i.e. 0-20 and 20-80%. The division of fractions into 2 levels was aimed to increase the yield of enzyme proteins so that there is no loss of many enzymes when the fractionation process takes place and to increase the acquisition of considerable enzyme activity.

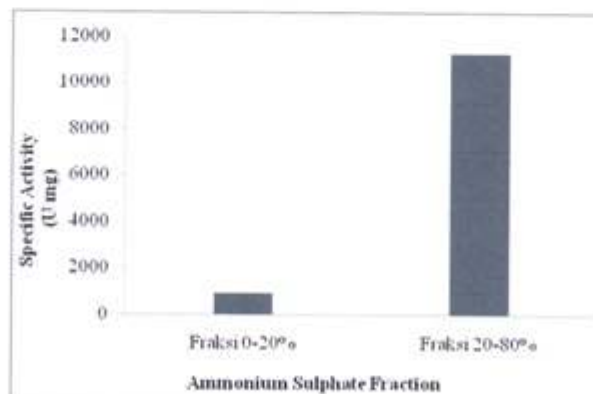


Figure 2 Effect of enzyme fraction at 2 levels of ammonium sulphate saturation on specific activity of the precipitates.

The specific activity showed a purity level of amylase enzyme. The more pure amylase enzyme obtained, the more value of specific activity increased. Figure 2 shows that purified α -amylase obtained from 20–80% of ammonium sulphate saturation has specific activity 11312.64 U.mg⁻¹. Regarding the purity, it was increased to 6.41 times compared with the crude one (1765.25 U.mg⁻¹). The results of this fraction were used for the next purification stage, namely dialysis.

3.1.2. Dialysis

The membrane has pores that will permit small molecules such as ammonium and sulphate ions to cross, and hence equilibrate in the larger volume of buffer outside, while not permitting large protein molecules to cross. If the buffer is changed several times, allowing several hours each time for the ammonium sulphate to equilibrate, more or less all of it will be removed from the protein solution. Dialysis will increase the volume of the enzyme solution [18].

Table 1 The α -amylase enzyme purification scheme from *Bacillus subtilis* ITBCCB148

Steps	Enzyme Volume (mL)	Unit Activity (U.mL ⁻¹)	Protein Levels (mg.mL ⁻¹)	Specific Activity (U.mg ⁻¹)	Total Activity (U)	Yield (%)	Fold Purification
Crude Extract	3500	138.65	0.07854	1765.25	485272.20	100	1
Fractionation (20-80)% ammonium sulphate salt	165	1358.25	0.12007	11312.64	224111.99	46.18	6.41
Dialysis	203	1096.53	0.03803	28834.13	222596.16	45.87	16.33

From the table 1, it can be clearly seen that the specific activity of the amylase enzyme crude extract unit is 1765.25 U.mL⁻¹ with a protein content of 0.07854 mg.mL⁻¹. Ammonium sulphate fractionated amylase enzyme from dialysis results has a specific activity of 28834.13 U.mL⁻¹ with a protein content of 0.03803 mg.mL⁻¹. Ammonium sulphate fractionation enzyme from dialysis results has 16.33 times the purity of crude extract enzymes with 45.87 %. The purification of the enzyme was carried out in two stages namely fractionation with ammonium sulphate and dialysis.

3.2. Effect of pH on α -amylase activity and stability

Effects of pH on amylase activity were observed by conducting amylase assay with phosphate buffer of different pH (4.5–8.0) in which 0.1% soluble starch solutions were prepared. The pH stability of amylase was incubated at 60°C, as previously described in Table 2.

Table 2 Effect of pH of reaction on amylase activity

pH	Unit Activity (U.mL ⁻¹)	Relative Activity (%)
4.5	34.96	82.64
5.0	42.31	100
5.5	37.37	88.34
6.0	37.20	87.92
6.5	34.65	81.90
7.0	33.86	80.03
7.5	26.99	63.80
8.0	33.36	78.87

It was found that the purified α -amylase produced by *Bacillus subtilis* ITBCCB148 has an optimum pH at 5.0 with unit activity 42.31 U.mL⁻¹ and stable at 4.5-8.0 of pH. Activity of α -amylase at low pH range is very important for industrial applications. The application of liquefying amylases that are active and stable around the saccharification pH is attractive to avoid or reduce the use of acid to lower the pH from liquefying to saccharifying range and also to simplify the procedures during downstream processing [17].

3.3. Effect of Temperature on α -amylase activity and stability

The effect of temperature on enzyme activity was measured by incubating the enzyme at different temperatures. The Effect of Temperature of reaction on amylase activity is shown in Table 3.

Table 3 Effect of Temperature of reaction on amylase activity

Temperature (°C)	Unit Activity (U/mL)	Relative Activity (%)
55	34.43	78
60	37.19	84
65	44.13	100
70	41.74	95
75	38.39	87
80	25.75	58

The purified enzyme has an optimum temperature at 65°C with unit activity 44.13 U.mL⁻¹. The purified amylase also still have high activity between 55-75°C.

3.4. Enzymatic Conversion of Potato Starch into Glucose

The purified amylolytic preparation was observed to exhibit α -amylase activities on potato starch. The α -amylase enzyme activity in various of substrat concentration is shown in Figure 3. The purified amylase from dialysis was able to convert potato starch to glucose with an optimum concentration of potato starch 0.6% and could produce glucose at 0.72 mg.mL⁻¹ with unit activity 133.33 U.mL⁻¹. Enzymatic conversion with various concentrations of potato starch at 0.1; 0.2; 0.4 and 0.8% respectively, could produce glucose at 0.14; 0.33; 0.49; and 0.71 mg.mL⁻¹ respectively with unit activity of α -amylase 26.54; 60.51; 91.18 and 131.72 U.mL⁻¹ respectively.

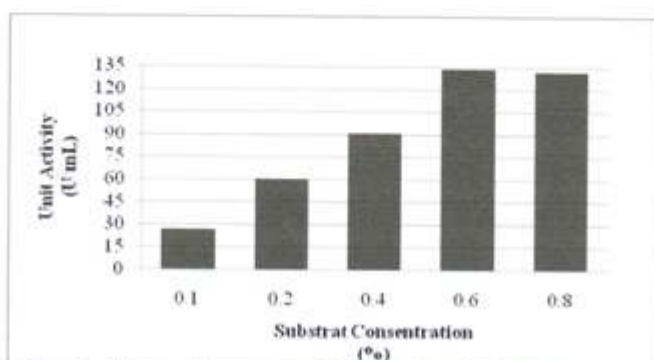


Figure 3 The α -amylase enzyme activity in various of substrat concentration

4. Conclusion

In conclusion, the locale isolate of bacteria (*Bacillus subtilis* ITBCCB148) could produce α -amylase, which is not only stable at low pH, but also still active in a wide pH range (4.5-8.0). The purified amylase also still have high activity between 55-75°C. The examination indicated that the enzymatic Conversion of potato starch optimum at pH and temperature, 5.0 and 65° C respectively. On the other hand, the best concentration of substrate state at 0.6% that could produce the highest glucose. These properties are considered to be very important for industrial starch liquefaction.

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