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Production of Xylanase Enzyme from*AspergillusTerreus* SUK-1

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Abstract: Xylanase productionfrom *Aspergillusterreus*SUK-1 was carried out in submerged culture fermentation (SCF). The fermentation process was performed by using 0.30% ofxylan and 0.75% α -cellulose as substrates of carbon source at 30°C, 150 rpm in the shaker for 7 days. Two processes, which were used for xylanaseproduction, were shake flask culture and fermenter. In the shake flask culture, the maximum xylanase activity was 52.18U/mL U/mL with xylan as a substrate, while 63.29U/mL was for a-cellulose as substrate; after 5 days. In 5 L fermenter with 3 L working volume, the xylanase activity reached the maximum value which was 78.65U/mLafter five days. Two conditions were studied to get the optimum conditions for xylanase activities which were pH and temperature by using xylan as substrate. The highest activity was at pH 6.5 and constant temperature 37°C. While the optimum temperature was 50°C with constant pH 5.

1- Introduction

Enzymes are distinct biological polymers that catalyze the chemical reactions and convert substrates to particular products. They arespecific in function and speed up reactions by providing alternative pathways of lower activation energy without being consumed. These are the fundamental elements for biochemical processes and utilized(Javed and Khan, 2006).Most enzymes are proteins, although some are made of RNA.On the surface of the enzyme is usually a small crevice that utilizes as an active site or catalytic site to which one or two specific substrates are able to bind(Yang et al., 2004).Enzymes are critical to life that just one faulty or missing enzyme can have dramatic effects. Lactose intolerance is one example. People whose intestinal cells do not secrete an enzyme called lactase cannot digest milk sugar(Mayer, 1977).The rate of enzymatic reactions is affected by several facts including temperature, pH, enzyme concentration, substrate concentration, and the presence of inhibitors or activators. Therefore, the reaction catalyzed by enzyme must be carried out under an optimum condition in order to obtain high yield of product(Lemmer, 1989).Nowadays there are many industrial enzymes, such as amylase, amyloglucosidase, cellulase, catalase, lipase, glucanase, hemicellulase, phytase, protease, pectinase, tannase, xylanase and glucose oxidase. Most of these enzymes are produced by microorganisms(Ghorai et al., 2009).

Xylanase is produced by diverse genera and species of bacteria, actinomycetes and fungi.While several bacterial species secrete high levels of extra-cellular xylanase, filamentousfungi secrete high amounts of extra-cellular proteins where xylanase secretion oftenaccompanies cellulolytic enzymes (Polizeli et al.,

2005). These enzymes are capable of weakening the lignin bonds in pulp fibers. Xylanases catalyze the hydrolysis of xylan, the main bonding agent between lignin and cellulose. This reaction improves the accessibility of bleaching chemicals to the pulp and enhances the extractability of the dissolved lignin(Chandra et al., 2012). Xylanases are the major hemicelluloses component of wood and constitute as much as 35% of the total dry weight of some plants. In hardwoods O-acetyl-4-Omethylglucuronoxylan is the main xylan, while in softwoods it is arabino-4-O-methylglucuronoxylan(Ramalingam and Harris, 2010). Xylanases have aroused great interest recently due to their potential application in many industrial processes. In the recent years, the biotechnological use of xylans and xylanases has grown remarkably(Subramaniyan and Prema, 2002, Subramaniyan and Prema, 2000). Xylanase began to be used in the 1980s, initially in the preparation of animal feed and later in the food, textile and paper industries. Currently, xylanase and cellulase, together with pectinases, account for 20% of the world enzyme market(Polizeli et al., 2005). In the food industry, xylanase enzymes are used to accelerate the baking of cookies, cakes, crackers, and other foods by helping to break down polysaccharides in the dough. In animal feeds, xylanase aids in the digestibility of wheat by poultry and swine, by decreasing the viscosity of the feed. Most commercial xylanases are produced by Trichoderma, Bacillus, Aspergillus, Penicillium, Aureobasidium, and Talaromycessp(Cowan, 1996, Tegge, 1984). In this work, the Production of xylanse in shake flask culture and fermenter was investigated and the suitable conditions for enzyme activity were examined as well.

2. Materials and Methods:

2.1 Sample Preparation:

The fungi (*A.terreus*) SUK-I were obtained from Biochemical Laboratory, Chemical Engineering Department, University Kebangsaan Malaysia. The fungus (*A. terreus*) was grown on potato dextrose agar (PDA) at 30°C for 3-4 days, and then another four days was at room temperature (27°C) for spore formation before storing it in the fridge. After that, Mendel's media was used for inoculation by adding 20 mL of spores to 200 mL Mendel's media at 150 rpm, 30°C for 3 days.

2.2 Xylanase Production in Shake Flask Culture

25 mL from the inoculated media was infused into 250 mL fresh Mendel's media at 150 rpm, 30°C for 6-7 days. The samples were taken daily to measure pH and enzyme activity. At the end of fermentation, the culture was filtered and the crude enzyme was kept at 4°C.

2.3 Xylanase Production in Fermentation Culture

The production of xylanase was carried out in a 5 L-Biostat-A fermenter. Cultivation was carried out by manually transfer of the inoculum media after 3 days growth in the shaker into the fermenter at 30°C, 0.5 vvm, 150 rpm, and uncontrolled pH for 7 days. Then the culture was flittered (vacuum filter) and the filtrate was used as a crude source for enzyme.

2.4 Xylanse Assay

2.4.1 Crude Enzymes Preparation

Approximately 10mL of the culture medium was taken once a day during 7 days fermentation for determination of enzyme activity. The sample was taken and centrifuged at 13000 rpm for 6 minutes to separate the cells from the culture supernatant containing enzyme. The resulted supernatant was taken for enzyme activity determination

2.4.2 Xylanase Activity

The activity of xylanase enzyme is measured by modified Mendela's method (Mandels et aI., 1976). A mixture of 1.5 mL solution containing 1.0 mL of 0.1M acetate buffer pH5, 0.3mL substrate solution of 2% xylan and 0.2 mL enzyme sample was used for determination of xylanase activity. The assay mixture was incubated for an hour at 37 °C. 3.0 mL DNS reagent was added and heated in boiling water bath at 100 °C for 5 minutes then distilled water was added until reach 16 mL before reading the absorbance value at 550nm.

The amount of xylose produced from degradation by enzyme was determined using xylose standard curve (0-lmM) xylose. One unit of xylanase enzyme was defined as 1 μmol of xylose released or produced per

minute in standard assay. The standard curve was prepared by preparing l0mM stock xylose (0.15g dissolved in 100 mL distilled water). Then each 10mL was dissolved in 90mL distilled water to prepare 1 mM stock xylose.The DNS reagent was used in preparing the stander curve of xylose

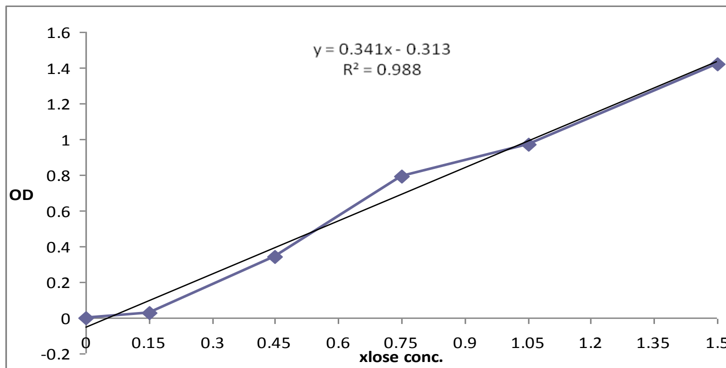

Figure 1: Standard curve xylose

3. Result and Discussion

3.1.Growth in Mandel's Media with Xlan or α -Cellulose in Shake Flask and Fermenter

During growth of *A. terreus*SUK-1 in submerged culture fermentation in the presence of either xylan or α -cellulose as a carbon source, the pH declined from pH 5.5 to 4.5, and from 5 to 4 as shown in Fig. 2 and Fig. 3 respectively. This change in the pH during the growth was probably due to either the production of organic acids or release of ammonia. While the color of the medium turned from the normal color to yellow with the formation of pellets at the bottom with xylan and from the normal whitish color to yellow with the formation of pellets at the bottom with α -cellulose .

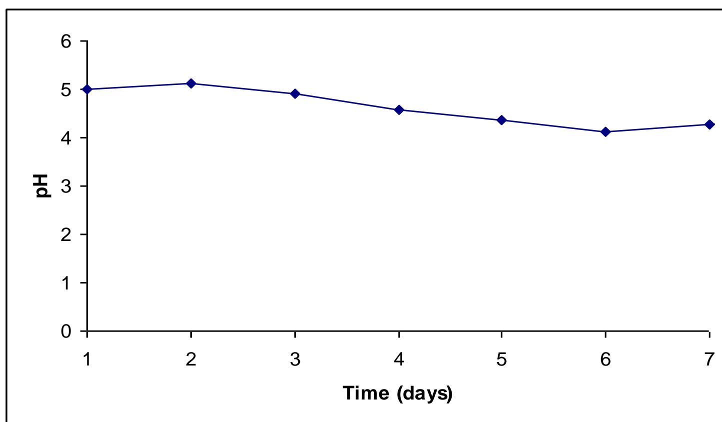

Figure 2: Change of pH during the growth of A. terreuseSUK-I in mendal's medium.

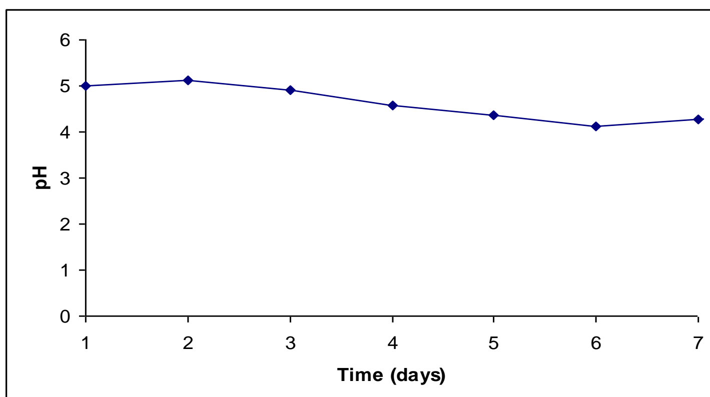

Figure 3: Change of pH during the growth of *A. terreuse*SUK-I in mendal's medium with α -Cellulose

The activity of xylanase increased after second day of growth and reached its peak activity which was 57.32U/mL or 63.29 U/mL in the fifth day for both media with xylanor α -Cellulose respectively, as shown in Fig. 4 and 5. After reaching the maximum value, it reduced for both to in the seventh day. The activity was changed because of the lack of substrate in the media or uncontrolled pH. Another reason was the activity of proteases that degrade protein.

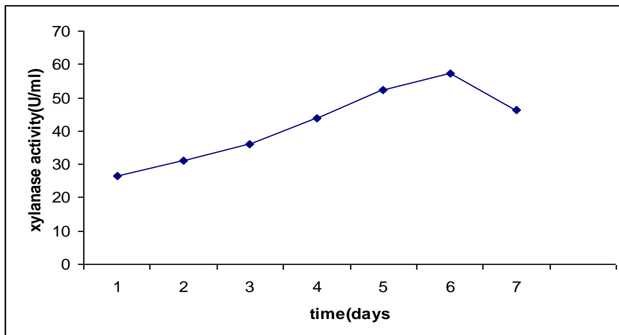

Figure 4: Activity of Xylanase during the growth *of A. terreuse*SUK-l with xylan

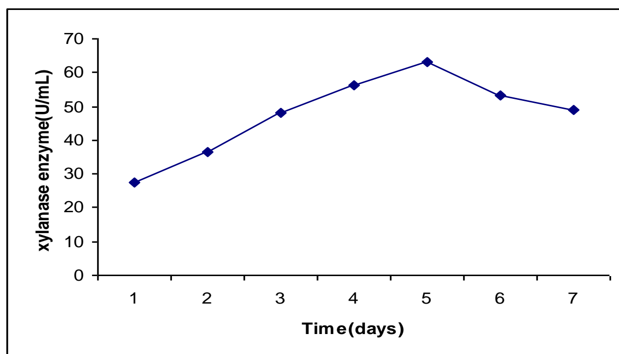

Figure 5: Activity of Xylanase during the growth of *A. terreuse*SUK-l with α -Cellulose.

The production of xylanase enzyme was in small quantity in shake flask so it carried out in 5L batch fermenter with 3L working volume to produce more enzymes in order to study characterization and stability of the enzyme.

The level of enzyme increased steadily from the first day in batch bioreactor fermentation and reached the maximum value which was 78.65U/mLafter five days. Xylanase activity in 5 L batch culture fermentation was 20% higher than that obtained in shaker flask culture(Ang et al., 2013). Sufficient air supply, and flow rate of the air supply were the factors that affecting on the production of the enzyme in fermenter. The sufficient air supply into the fermenter was 0.5 vvm and the flow rate of the air supply increased more than 0.5 vvm thatcause foaming of the media. Foaming was also occurred when using high agitation speed. In contrast, low agitation speed was lower than 150 rpm in shaker has negative influence on the productivity of the enzyme.

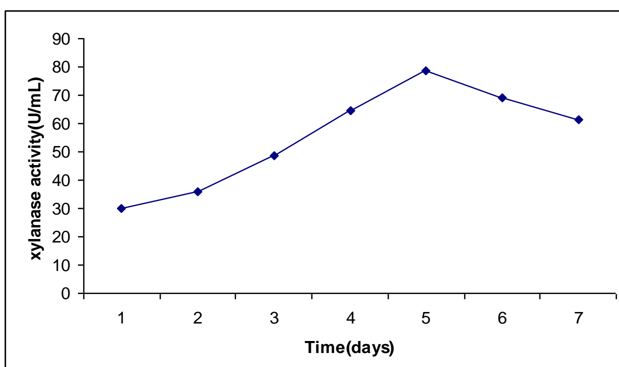

Figure 6: Activity of Xylanase during the growth of A. terre useSUK-l in fermenter with α Cellulose

The activity of the xylanase with α -Cellulose as an organic carbon source in the fermenter was higher than in shake flasks culture with xylan or α -Cellulose as an organic carbon source. This value was due to the high rate and extent of hydrolysis and the amount of substrate surface exposed(Ang et al., 2013). While lowest Xylanase activity was found 26.6 U/mL with xylan and 27.33U/mL with α -Cellulose as a substrate in shake flasks culture and it was 29.82U/mL with α -Cellulose in fermenter. This decrease in xylanase activity was due to the irreversible adsorption of xylose to xylanase, catabolize repression, or decrease in the PH.

3.2. Factors That Effect on Enzyme Activity

1. PH

As shown in figure 7, different pH was used in this study to show the effect of pH on the enzyme activity. The activity of xylanase increased slowly as pH raised from 5 to 6.5. The optimum pH for xylanase activity was 6.5. By using higher value of pH as 9, the activity will decrease accordingly(Saha and Ghosh, 2014).

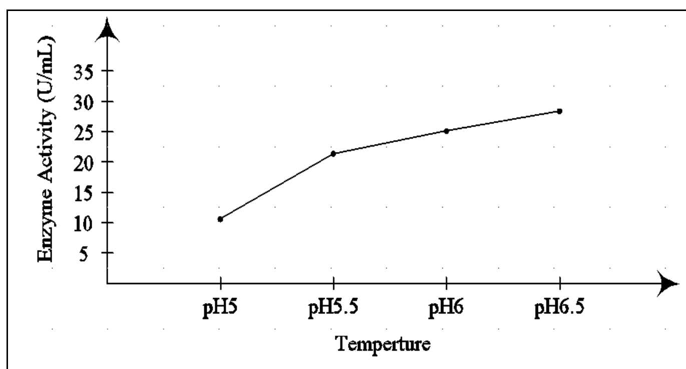

Figure 7: Effect of pH on enzyme activity

2. Temperature

Xylanase activity increased when increasing the temperature from 30°C to 50 °C at pH 5. From figure 8, the activity at 30° C was (27.7) and increased to 32.14 U/mL at 40°C. The optimum temperature for xylanase activity was 50°C when using xylan as substrates(Pal and Khanum, 2010)

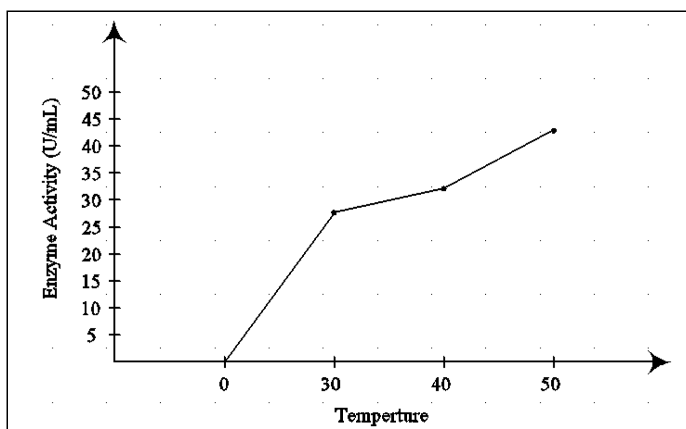

Figure 8: Effect of temperature on enzyme activity

4. Conclusion

In this study, we investigated that the xylanase production from *A.terreus*SUK-I was higher in 5 L fermenter than the shake flask culture which gave 20 % high activity compared to the shake flask culture. Xylanasewhich secreted by *A.terreus* SUK-I showed maximum activityat 50°C m, and pH 6.5 by using xylan as an organic carbon source. In the future studies, other optimization conditions should be studied such as using other substrates rather than xylan or cellulose.

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