XYLANASE ACTIVITY TEST WITH DNS METHOD TO KNOW HIGH ACTIVITIES OF XYLANASE FOR THE QUALITY OF DISSOLVING PULP

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Abstract

This research is propose to know the high activity of xylanase for the alternative in pulp industry as replacement of the bleaching process that is not environmentally friendly. This is partly due to the great potential of an environmentally safe method. And the background of this writing is because paper is very necessary in our daily life and also because the industry of paper use trees to making pulp as raw materials to produce paper which cut much trees with much waste to the environment, so we have to minimalized the use of that chemical process. Xylanase is extracellular enzim which can hydrolyze Xylan (hemicellulose) become Xylo-oligosaccharides and Xylose. Every plant has hemicelluose and we can find the xylanase from this research. Xylanase has potential to decomposed the hemicellulose content in pulp while in chemical process use clorint agent to decomposed hemicellulose that is not more efficient and environmentally friendly. As we known the characteristic of enzyme is work specific to their substrate so the uses of xylanase to decompose hemicellulose is more effective than chemical procces. Xylanases act mainly on the relocated, reprecipitated xylan on the surface of the pulp fibres. We separate the sample by pH and temperature. DNS (Dinitro Salysilic acid) has function to activating the enzyme so we can use DNS to know activity of the enzyme in specific pH and temperature. The result of the research show that the enzyme with high temperature and normal pH has high activity than others. Therefore, the enzyme with high activity, it can be more effective to decomposed the hemicellulose.

Keywords : xylanase, DNS method, hemicellulose, bleaching

INTRODUCTION

Nowadays, the use of enzymes as alternative compound which use to ease some process very much do and searched. Enzym is a biomolecul in the form protein dan has a circle shape. Enzym consist one or more chain of polypeptides. This enzyme will changing compounds and speed up the reaction by changing the known initial molecule and tied specifically by Enzym (substrate) become the other molecule (product). The ability of enzyme to activated the other compounds with specifically ways called biocatalysator.

In this research, the enzyme that we will test is Xylanase. Xylanase is enzyme from the hydrolases class (EC 3.2.1.8) which has a role to degrades the linear of polysaccharides β-1,4, xylan become Xylose and decomposed the hemicellulose, that one of the main component from the wall of plant cell. Xylanase has many uses, one of them is for bioconvertion the remains of the plants that consist lignocellulose become glucose and ethanol. Xylanase has to many benefits that we can apply in industry, such as an whitening agents in pulp industry, and as an clarification agents in making of juice and wine.

In industry, xylanase use in paper industry. Paper industry use pulp as raw material to produce papers that we use in everyday life. The raw materials of pulp derived from plant that still have lignin and hemicellulose, so require stage to eliminate the lignin which still consist in raw materials of pulp with paper industry process. Pulp that has been through the paper industry process still have characteristic that is has lightly browned colour caused by the presence of lignin residues and derivatives, so the next stage is required bleaching process use the clorin agent. This clorin agent that is not friendly with the environment and add the cost of pulp production because waste management is pretty complex.

The alternative in making pulp can use biological materials that is biocatalysator in the form of Xylanase . The uses of Xylanase in paper industry process required specific characteristic to adjust with the paper industry process which

the process goes in high pH and temperature. If the Xylanase that use in paper industry process has no high stability against high pH an temperature, then the use of Xylanase in process of making paper can not be done effectively, because if pH and the temperature adjust with optimum pH and temperature of enzyme then process in paper industry do not start completely finish. Therefore, the research has done to xylanase activities test and from this research we have to find which xylanase has high enzyme activities to use in process.

Enzym came from word in + zyme that means something in yeast. Based one research can be concluded that enzyme is a protein in form big molecules and have thousand of molecular weight. As example catalase enzyme have 248.000 of molecular weight while enzim urese has 438.000 of molecular weight. In enzymes there are part of protein that can stand the heat called apoenzyme, while part that instead of protein are active parts and called prosthetic group. Usually a metal like iron, copper, zinc or something organic compound that consist of metal. Apoenzyme and prosthetic cluster is the unity that called holoenzyme, but there are part of enzyme that has apoenzyme and the prosthetic cluster is not fused. Example coenzyme are vitamin or part of vitamin (for example : B1 vitamin, B2, B6, Niacin and Biotin) (Kartasapoetra, 1994).

The enzymes work is influenced by several factor, mainly the substrate, the acidity, cofactor and inhibitor. Every enzymes need the different optimum temperature and pH (the level of acidity) because enzymes are protein which can experience the transformation, if the temperature and the pH changes outside of the appropriate temperature and pH, causing enzymes can not work optimal or the structure of enzymes will be broken. This thing caused enzyme loses the functionality. In an enzymatic reaction , the enzyme will bind the substrate to form form the enzyme-substrate group, and then will break into enzyme and product. Inhibitor is molecule that decreased the activities of enzyme, while activator is increased the activities of enzyme. There are many medicine and poison are enzymes inhibitor (Soewoto, 2000).

Xylanase is extracellular enzyme which can hydrolyze xylan (hemicellulose) become xilo-oligosaccharydes and xylose. Xylanase can produced by microbes through fermentation process. The industry application of xylanase among them are food, feed, and bleach whitening pulp. The replacement of the use of chlorine with xylanase for bleaching pulp has given chance for biotechnology application an has been used on several paper industry. Xylanase has the ability to increase the bleachability of kraft pulp process. The enzymes has a specific work with their substrate and the substrate of xylanase is xylan. Enzymatic hydrolysis of this specific type of xylan renders the structure of the fibres more permeable, allowing enhanced extraction of residual lignin from the fibres. The hydrolysis of hemicelluloses in the inner fibre layers may also enhance the bleachability.

DNS is a reagent which can be use to do quantitative assay of glucose. This reagent is function to give colour to the solution, so that can read by spectrophotometer vis which read the colour from the solution. Quantitative analysis of glucose dinitrosalicylic acid method.

This method is used to measure reducing sugar with Colorycmetri technic. It can detect one reducing sugar, such as glucose. Glucose has aldehyde compound, so it can be oxidized become carboxil compound. Aldehyde compound that glucose has will oxidized by 3,5-Dinitrosalycilic acid become carboxil compound and produce 3-amino-5-salycilic acid.

Reaction with DNS occurs oxidation and reduction reaction in aldehyde form of glucose and oxidized become carboxil form. Meanwhile the DNS as oxidizing agent will reduced formed 3-amino and 5-nitrosalicylic acid. This reaction works on base condition. If there are reducing sugar on the sample, then DNS solution initially yellow will react with reducing sugar so cause reddish orange colour (Lehninger, 1997).

Making of DNS reagent, we need to add NaOH to the solution which aim to give base condition. Because the reaction from DNS are working at base condition. In addition to adding NaOH, also added Calium Natrium Tartrat 0.4% (Rochelle Salt). The function of adding the solution is stabilized the colour that formed while the reaction works that is browning. Beside them, sometimes we need to do is heat the solution to speed up the reaction. Because the later will be measured from the colour which formed with spectrophotometer at length of wave 512 nm (Lehninger, 1997).

After the several step about what the xylanase is, how to make a DNS solution and the DNS test to these samples we know the activity of xylanase which higher than the others xylanase based on different pH and the temperature, The main goals in the enzyme-aided bleaching of kraft pulps have been the reduction of consumption of chlorine chemicals in the

bleaching process, and consequent lowering of the chlorine of the effluents. In the production of totally clorine-free pulps, enzymes have also been successfully used for increasing the brightness of pulp. Other suggested enzymatic modifications are improvement of fibre properties or production of dissolving pulps.

METHOD

This research is done by experiment with the design of the treatment till get substrate from enzyme that will be tested. After that create stock solution of xylose 1000 ppm for test. Then standard solution from xylose stock solution 100, 150, 200, 250, 300, 400 ppm for test, and then we make DNS solution that is dinitrosalicylic acid 2N and Xylan solution 0,5% for test. Then do enzyme activities test with xylan substrat 0,5% and adding DNS solution into it, then heat at 50^{o}C and 60^{o}C temperature, then measure the value of absorbance at length of wave 512 nm from sample solution that will we test with spectrophotometer. Flow diagram xylanase activities test is shown in Figure 1.

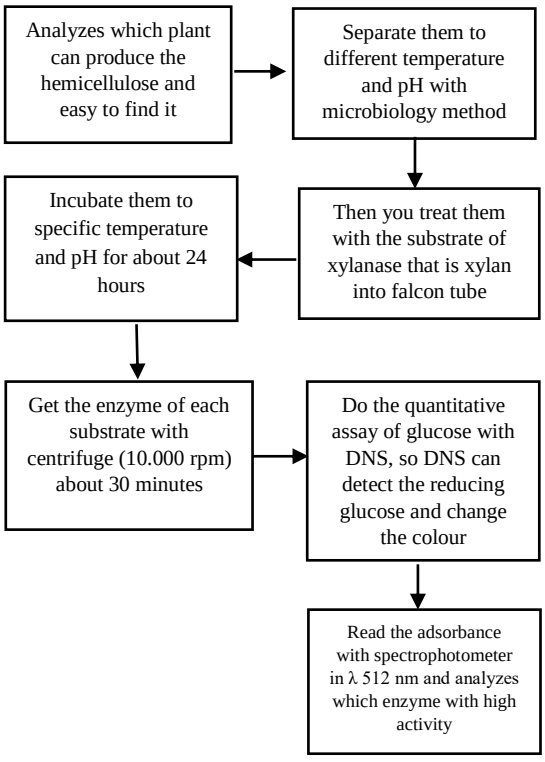

Figure 1. Flow Diagram Xylanase Activities Test

RESULT AND DISCUSSION

Observation data of Xylanase is shown at Figure 2 and Table 1-3.

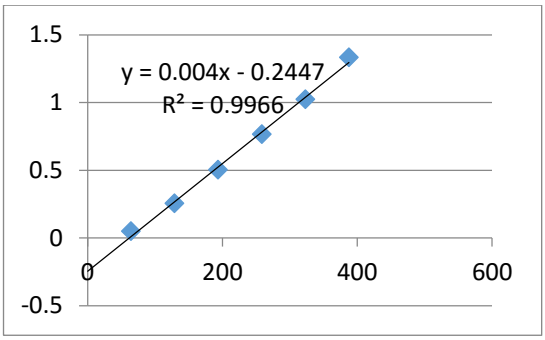

Figure 2. Graphic Value of Xylose Adsorbance

Table 1. Value of Adsorbance Xylose

Constanta. (mg/L)			Absorbance
0	0	0	0
64.64	0.023	0.073	0.048
129.28	0.245	0.264	0.2545
193.9	0.489	0.514	0.5015
258.6	0.753	0.779	0.766
323.2	1.029	1.015	1.022
387.8	1.329	1.335	1.332

Tabel 2. The Average Value of Xylanase Activities Enzym at 50 °C

50 ^{o}C	Average activities (nmol/mL/menit)		Average
ph 7	Experiment 1	Experiment 2	
A1	5.444444	0	2.722222
A2	0	0.166667	0.083333
A3	2.944444	6.77778	4.861112
ph 8	Experiment 1	Experiment 2	Average
B1	0	3.055556	1.527778
B2	0	0	0
B3	7.203704	0	3.601852

Tabel 3. The Average Value of Xylanase Activities Enzym at 60 °C

60 °C	Average Activites (nmol/mL/min)		Average
pH 7	Experiment 1	Experiment 2	
B_1	0	8.62963	4.314815
B_2	0.092593	4.44444	2.2685165
B_3	2.12963	9.166667	5.6481485
pH 8	Experiment 1	Experiment 2	Average
B_1	0	0	0
B_2	2.814815	0.611111	1.712963
B_3	0	0	0

In Xylanase 50^{o}C there are 3 bacteries, we decide them by variating the temperature and pH, if the bacteries grow on that pH and temperature and we choose that to get enzyme from that

bacteries. But, basically those are still with no substrate so after that we grow those bacteries in their substrate with the temperature and pH that we already known before. we test te value of activity same with 60^{o}C there are 3. So, then we got 6 bacteries which have potential to produce the enzyme with high activity divided into 3 xylanase 50^{o}C and 3 xylanase 60^{o}C. The bacteries that produce xylanase at 50^{o}C we give a code to those bacteries are A_1, A_2, A_3 and 60^{o}C there is B_1, $_{B2,}$ B_3.

In 7 pH the A_1 bactery the average value of it test is 2.72222 nmol/ml/min. While A_2 bactery on 7 pH, the average value of the enzyme activity is 0.08333 nmol/ml/min. And in A_3 bactery on 7 pH it get the value is 4.86111 nmol/ml/min. While in 8 pH the A_1 value of activity is 1.52778 nmol/ml/min, A_2 nothing activity, A_3 is 3.60185 nmol/ml/min. On 60^{o}C in 7 pH the value of the activity ini B_1 is 4.31482 nmol/ml/min, B_2 is 2.26842 nmol/ml/min, and in B_3 the value of the activity is 5.64815 nmol/ml/min. While for 8 pH the value of the enzyme activities B_1, B_2, B_3 are 0, 1.71296, and 0.

Based on the result of the experiments in enzyme activity test then I choose three bacteries from each endoglucanase and xylanase producing bacteries. *For* xylanase 7 pH and 50^{o}C the chosen bacteries are A3, and then B1 and B2 with 60^{o}C 7 pH, why did not choose the temperature above that, because when we try to vary the temperature. The enzyme can not work that mean it is not the appropriate temperataur of the enzymes, because the characteristic of enzyme is work in specific temperature and pH.

CONCLUSION

Based on the research has been done then you can see that: (1) we know how to improve the quality of pulp in industry that environmentally friendly, (2) we can decrease the chemical material use in paper industry and replace them with the enzyme which can cut the hemicellulose chains more effectively, (3) enzyme with high activity that can cut hemicellulose chains and increase the level of cellulose in dissolving pulp, (4) the enzyme with high activity has a potential to replace the chemical material in bleaching process in pulp industry that is xylanase 60^{o}C with pH 7 (B_3), (5) based on result of this enzyme activity test that we have done , then will be chosen 3 bactery from each endoglucanse producing bactery. The chosen bacteries of xylanase producing bactery are A_3 with 7 pH 50^{o}C, B_1 and B_3 with 60^{o}C 7 pH.

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