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Endoglucanase, exo-glucanase and β-glucosidase activity thecombination of crude enzyme from Trichoderma reesei and Aspergillus niger at different temperatures and pH

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ABSTRACT

The purpose of this study was to compare the activity of the crude cellulase enzyme produced by Trichoderma reesei and Aspergillus niger and the various combination of two crude enzyme with a ratio of Trichoderma reesei and Aspergillus niger (0/1, 1/0; 1/1; 1/2, 2 / 1; 1/3; 3/1 v / v) at different pH and temperature. The results showed that the highest exo-glucanase activity (FP ase), endo-glucanase activity (CMC ase) and β-glucosidase activity of combined enzyme of Trichoderma reesei : Aspergillus niger in sequence (2 : 1) at pH 5 is 1.002 IU / ml; (1 : 3) at pH 5 is 2.90 IU/ml and (1 : 3) at pH 4.5, generated 0.316 IU/ml and three of those activity reached the highest level at temperatures of 50°C.

KEYWORDS

Cellulase enzyme
FPAse;
CMCAse;
β-glucosidase;
Trichoderma reesei;
Aspergillus niger.

INTRODUCTION

The use of enzymes as catalysts of biological reactions has provided benefits and advantages for humans, one for the hydrolysis process with the material content of cellulose by cellulase enzymes used for ethanol production. This was driven by a decline in oil reserves of fossil energy and the energy requirement increases with the increase in population. The use of enzymes in industry is constrained by the high cost of commercial enzymes. It is felt necessary information extracted enzyme activity produced by the enzyme cellulase-producing microorganisms.

The rate and extent of cellulose conversion into glucose is dependent upon the amount of active β-glucosidase enzyme present in the cellulase preparation used for saccharification. This is because cellobiose produced during cellulolysis is inhibitory to both expand endocellulases and, hence, retards saccharification.

The highly cellulolytic fungus Trichoderma reesei Have potential for use in the practical saccharification of cellulosic materials. Although cellulase preparations derived from this fungus contain a very active cellulose complement of enzymes (i.e. both exo- and endo-cellulases) they are, nevertheless, deficient in β-glucosidase activity.' Cellulase preparations of Trichoderma reesei containing higher levels of p-glucosidase activity can be obtained by supplementing with exogenous β-glucosidase preparations derived from another microorganism to increase the rate and extent of saccharification of cellulose. Fungi belonging to the genus Aspergillus niger produce β-D-glucosidases in high yields which are compatible with Trichoderma reesei cellu-

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lases[9]. The key of enzyme activity is pH optimum, temperature, interaction with other protein[7] and activities of combined crude enzyme from Trichoderma reesei and Aspergillus niger is limited to some of pH and temperature properties of this enzyme.

MATERIAL AND METHOD

Spore suspension preparation

Trichoderma reesei and Aspergillus niger from Microbiology laboratory PAU Food and Nutrition Department in Gadjah Mada University Indonesia, was grown on potatoes dextrose agar for 7 days. From 7 days old cultured was used to inoculated for enzyme production. The spores were resuspend in saline water, 2 ml spore suspend was inoculated on 50 ml medium was contains yeast extract 4 g/l, malt extract 10 g/l, and glucose 4 g/l. incubation for 3 days on waterbath shaker at 120 rpm and 30°C. count spore by haemocytometer.

Enzyme production

5 grams rice straw was mixed with 25 ml nutrition solutions, per 1000 ml contains 1,0 g yeast extract, 1.5 g peptone, 1,4 g $(NH_4)_2SO_4$; 2,0 g KH_2PO_4, 0,005 g $FeSO_4 \cdot 7H_2O$, 5 ml solution CMC 1%. The flasks were sterilized for 15 minute at 121°C. Two milliliters of spores (10^7-10^8 spores/ml) was inoculated and incubates at room temperature and static condition, for 6 days for Trichoderma reesei and 8 days for Aspergillus niger.

Enzyme extraction

The solid state culture were prepared by adding 100 ml solution 1% tween 80 and shaking at 180 rpm at 30°C for 60 minutes. The solid material and fungal biomass were separated by filtration by cotton. Filtrate was centrifugation at 5000 rpm, 4°C for 30 minutes. The clear supernatant used for enzyme assay.

Experimental design

The first experiment, effect of initial pH (4,5; 5; 5,5) for activities mixed crude enzyme from Trichoderma reesei and Aspergillus niger (0/1; 1/0; 1/1; 1 / 2; 2/1; 1/3; 3/1 (v/v)). The second is effect of incubation temperature (45 and 50°C) for enzyme activities. The pH optimum was determined by measuring enzymes activity in the pH range 4,5-5,5 incubated at 50°C, and temperature optimum was determined by assay at activity and temperature of 45 and 50°C in presence each substrate dissolved in acetate buffer (pH 5,0).

Activities enzyme assay

CMCase activity in the culture filtrate was determined by incubating the 0.5 ml of crude enzyme sample with 0.5 ml of 1% CMC (0.05M Citrate buffer pH 4,5; 5; 5,5) at 50°C for 30 min. After incubation, the reaction was stopped by the addition of 1.5 ml of DNS and then boiled for 5 min in boiling waterbath. The reaction mixture was allowed to cool and the reducing sugars released were estimated by Miller's method (1959).

For the estimation of FPase activity, 500 µl of culture filtrate was added to test tube containing Whatman No.1 filter paper strip (1x 6 cm) incubated at 45 and 50°C for 10 min. After that 1.5 ml DNS were added to test tube and boiled for 5 minutes and absorbance was taken spectrophotometrically at 540 nm. The reducing ends liberated were then measured with DNS.

The β-glucosidase activity was determined using Bergem' method, one milliliter of 5 mM p-nitrophenyl-β-glucopyranoside substrate was dissolved in citrate buffer (pH 4,5; 5; 5,5) and 0,1 ml crude enzyme was incubated at 50°C for 10 minutes. The reaction was terminated by addition of 2 ml of 1 M sodium carbonate solution. After cooling down to room temperature, 10 ml of distilled water was added. The absorbance was measured at 400 nm. The unit of p-glucosidase activity is defined as the number of µmoles of p-nitrophenol produced/min/ml enzyme under the assay conditions.

RESULT AND DISCUSSION

The effect of different substrate pH on cellulase enzyme activities of combined crude enzyme obtained from both types of fungus which are combined and the activity of endo-glucanase, exo-glucanase and β-glucosidase analyzed at different pH and temperature.

The result of exo-glucanase activity analyzed the combination of the two enzymes, as shown in Figure 1, exo-glucanase activity increased in the crude enzyme combined of Trichoderma reesei and Aspergillus niger higher than exo-glucanase activity of each crude enzyme, where the highest exo-glucanase which obtained

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from the combined of Trichoderma reesei and Aspergllus niger 2: 1 at pH 5 as high as 1.002 IU/ml while the value of exo-glucanase activity of crude enzyme from Trichoderma reesei is 0.408 IU/ml dan exo-glucanase activity from Aspergllus niger is 0.338 IU/ml at pH 5.5.

The analyzed of endo-glucanase activity which produced from Tricoderma reesei was 2.21 IU/ml at pH 4.5, while the endo-glucanase activity which produced from Aspergillus niger was 1.34 IU/ml at pH 5.5. Endo-

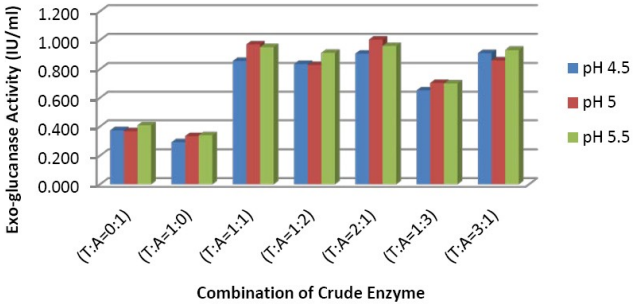

Figure 1 : The effect of different pH levels on exo-glucanase activity of combined crude enzyme from Tricoderma reesei and Aspergillus niger

glucanase activity increased to 2.90 IU/ml at pH 5 on a ratio of combined crude enzyme cellulase 1:3 as shown in Figure 2.

The highest β-glucosidase activity obtained from Aspergillus niger as high as 0.369 IU/ml at pH 4.5 while the lowest β-glucosidase activity obtained from Trichoderma reesei which was 0.074 IU/ml at pH 5.5. As

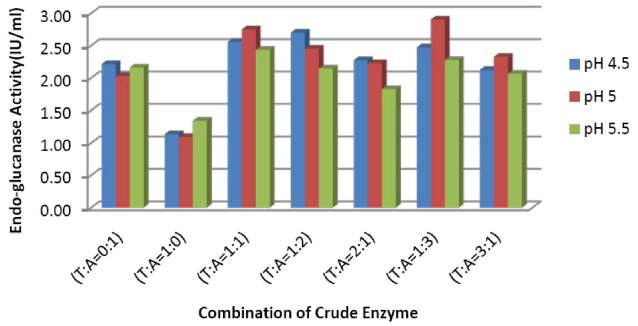

Figure 2 : The effect of different pH levels on endo-glucanase activity of combined crude enzyme from Tricoderma reesei and Aspergillus niger

shown in Figure 3, β-glucosidase activity tended to decrease in both crude enzyme which had been combined. B-glucosidase activity of the combined of crude enzyme produced from Trichoderma reesei and Aspergillus niger (1:3) obtained a high activity of 0,316 IU/ml at pH 4.5. This is consistent with the statement of Dekker[2] that the Aspergillus niger Showed that β-glucosidase activity optimum at pH 4.5.

From the three analyzes of enzyme activity which had been done, can been seen that the optimum pH of the crude enzyme cellulose activity from Trichoderma reesei and Aspergillus niger or the combine of enzyme cellulose from both fungus is in ranged of pH 4.5 to pH 5.5. It is also be expressed by Stewart[1] that the extra-

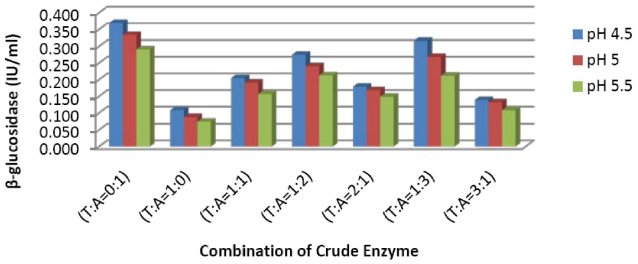

Figure 3 : The effect of different pH levels on β-glucosidase activity of combined crude enzyme from Tricoderma reesei and Aspergillus niger

cellular enzymes were most efficient in the range pH 4.5 to pH 5.5.

The effect of temperature to the enzyme activities of the combination of crude cellulose enzyme

This study establishes the optimum temperature to produce high enzyme activity was about 45 °C to 50 °C, it is useful to regulate the temperature of cellulose hydrolysis by the enzyme which is combination of the two fungus (Trichoderma reesei and Aspergillus niger). This statement refers to the Sun[8], that the optimal temperature hydrolysis using the fungus Trichoderma reesei is in the range of 45-50 ° C. In the Figure 4-6 shows that the highest enzyme activity at 50 °C in the hydrolysis of cellulose using enzymes from the fungi Trichoderma reesei, sellulase enzymes from Aspergillus niger, or a combination of enzymes sellulase of the two types of fungus, but the endo-glucanase activity of the crude enzyme produced from Aspergillus niger was higher at

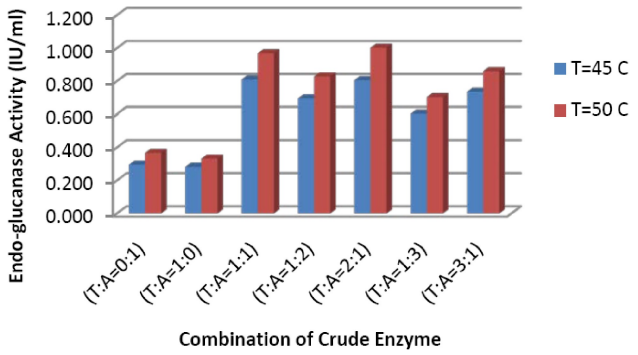

Figure 4 : The effect of temperature on exo-glucanase activity of the combination of crude enzyme from Tricoderma reesei and Aspergillus niger

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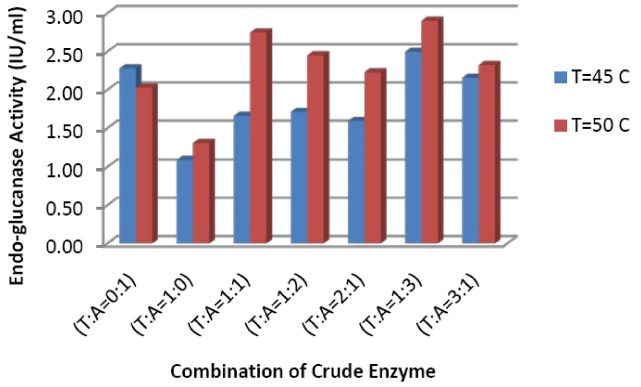

Figure 5 : The effect of temperature on endo-glucanase activity of the combination of crude enzyme from Tricoderma reesei and Aspergillus niger

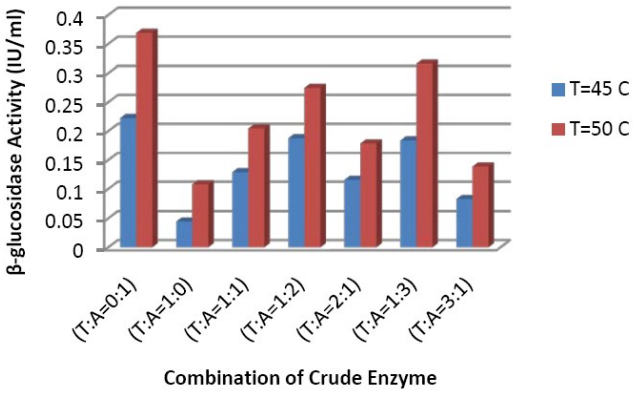

Figure 6 : The effect of temperature on β-glucosidase activity of the combination of crude enzyme from Tricoderma reesei and Aspergillus niger

45 ° C than at 50 °C.

CONCLUSION

Combined crude cellulase enzymes derived from Trichoderma reesei and Aspergillus niger will produce different activities at different pH and temperature as well. The highest value of exo-glucanase activity and endo-glucanase activity of the two types of fungus are achieved at pH 5 with a ratio of Trichoderma reesei enzyme sellulase bigger than Aspergillus niger. Whereas for β-glucosidase activity, optimum activity at pH 4.5. The highest activity of crude cellulase enzyme combinations can be achieved at a temperature of 50 °C.

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