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The increasement of rice straw hydrolysis using blend crude cellulose enzyme from Trichoderma reesei and Aspergillus niger

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ABSTRACT

The crude enzyme from Trichoderma reesei and Aspergillus niger have different activities. The use of two crude enzyme separately only able to produce a low levels of glucose, but after the two crude enzyme being mixed, it is able to improve the yield of glucose on cellulose hydrolysis process by using rice straw as substrate which has undergone pretreatment by alkali microwave. The combination of crude enzyme from Trichoderma reesei and Aspergillus niger (2:1) has the FP-ase activity of 1,002 IU/ml, CMC-ase activity of 2.23 IU/ml and β-glucosidase activity of 0.168 IU/ml. The activity of the crude enzyme cellulose combination are able to generate sugar at 12.89 mg/ml in the process of hydrolysis for 72 hours.

KEYWORDS

Cellulose;
Microwave pretreatment;
Hydrolysis;
Glucose.

INTRODUCTION

Fuel prices continue to rise and world oil reserves are more limited which by that has prompted efforts to obtain alternative fuels. Bio-ethanol (C_2H_5OH) is one of the present biofuel as an alternative fuel that is more environmentally friendly and renewable nature.

Lignocellulosic materials are the most economical and highly renewable natural resources. In Indonesia are approximately produced 180 million tons of rice straw every year[12]. Traditionally, rice straw was incinerated to produce fertilizer in agricultural field after harvest resulting in large amount of environmental pollution. To overcome this problem some states have implemented state law to restrict the burning of rice straw[5].

Fuels derived from lignocellulosic biomass also hold the potential for clean and renewable transportation energy[2,4]. The conversion lignocellulosic to ethanol includes two sub-processes: hydrolysis of cellulose in the lignocellulosic materials to fermentable reducing sugars, and then fermentation of reducing sugars to target products. The hydrolysis is usually catalyzed by cellulase enzyme.

The rate and extent of cellulose conversion into glucose is dependent upon the amount of active β-glucosidase enzyme which present in the cellulase preparation used for saccharification. This is because cellobiose which produced during cellulolysis is inhibitory to both exo and endocellulases and, hence, retards saccharification. The highly cellulolytic fungus is Trichoderma reesei have potential for use in the practical saccharification of cellulosic materials. Although cellulase preparations derived from this fungus contain a very active cellulase complement of enzymes (i.e. both exo- and endo-cellulases) they are, nevertheless, deficient in β-glucosidase activity.' Cellulase preparations of Tricho-

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derma reesei containing higher levels of β-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 such as fungi which belong to the genus Aspergillus. niger[8].

MATERIALS AND METHODS

Raw rice straw was obtained from local farmers in Pakis area in Malang city, east java province, Indonesia. Before any pretreatment. The cellulase enzyme used in this study was a crude cellulose enzyme which produced from Trichoderma reesei and Apergillus niger. All experiment were performed in triplicates and average values represented. Compotitions straw or its residues was expressed on wet basis.

Microwave pretreatment of rice straw

10 gr samples of rice straw after milled to 100 mesh were suspended in 100 ml of 0,5 N NaOH and heated by microwave irradiation for 40 minutes. After microwave pretreatment of rice straw, its residues were collected and washed extensively with water until the pH level became neutral, then dried at 50°C for later usage.

Enzyme production

5 grams of rice straw was mixed with 25 ml nutrition solutions, which every 1000 ml contains 1,0 gr yeast ekstract, 1.5 gr peptone, 1.4 gr $(NH_4)_2SO_4$; 2.0 gr KH_2PO_4, 0.005 gr $FeSO_4 \cdot 7H_2O$, 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 of 1% tween 80 solution and shaked 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 was then used for hydrolysis.

Enzymatic hydrolysis

The hydrolysis experiments were carried out in 300 ml flasks with a total reaction volume of 100 ml at a substrate concentration of 5% (w/v). 5 gr rice straw was add with 50 ml acetat buffer (pH 5) and 50 ml mixed crude enzyme (Trichoderma reesei : Aspergillus niger (0/1; 1/0; 1/1; 1 / 2; 2/1; 1/3; 3/1 (v/v)). Each experiment was run for 72 hours in a water bath thermostat at 50°C. Liquid samples (1 ml) were taken at every 8 hours for analysis of reducing sugar in the solution formed by the hydrolysis.

Analytical methods

Cellulose, hemicelluloses and lignin content was estimated by Chesson Method in Daffa[3]. FPA for total cellulase activity, endo-glucanase activity (CMC ase) using method of IUPAC. and β-glukodidase activity using Berghem's method. Reducing sugar was determined by DNS method.

RESULT AND DISCUSSION

Effect of β-glucosidase

Trichoderma reesei has long been used in industry widely for its highly productive and powerful destroyers of crystalline cellulose. However, the level of BG activity of Trichoderma reesei is low, leading to incomplete conversion of cellobiose, which inhibits the cellulose conversion to glucose in the cellulose hydrolysis process. TABLE 1 showed the effect of supplementation of β-glucosidase from crude enzyme Aspergillus niger. The combination of crude cellulose enzyme from Trichoderma reesei and Aspergillus niger have different activities.

Mixed enzyme added into hydrolysis rice straw. Rice straw after pretreatment have 21,28% hemicelluloses, 66,31% cellulose and 8,49 % lignin. Result of hidrolysis rice straw showed on Figure 1.

TABLE 1 : Activities of combined crude enzyme produced by Trichoderma reesei and Aspergillus niger

Trichoderma reesei : Aspergillus niger (v/v)	FP ase (IU/ml)	CMC ase (IU/ml)	B-Gukosidase (IU/ml)
0:1	0.367	2.03	0.333
1:0	0.332	1.09	0.088
1:1	0.969	2.75	0.191
1:2	0.828	2.45	0.240
2:1	1.002	2.23	0.168
1:3	0.703	2.90	0.268
3:1	0.860	2.32	0.132

Figure 1 shows that the highest rice straw hydroly-

sis is the combination of two crude enzyme with ratio of 2: 1 which has exo-glucanase activity of 1,002 IU/ml,

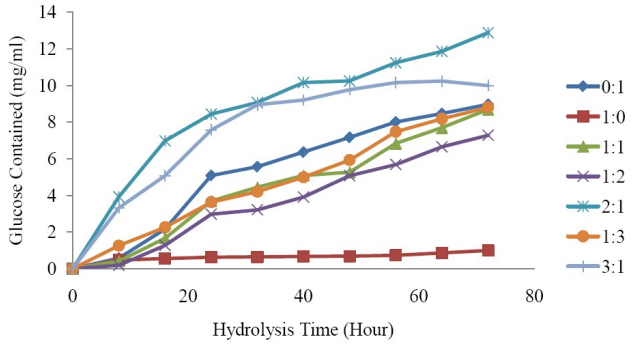

Figure 1 : Enzymatic hydrolysis of cellulose using crude enzymes isolation of Trichoderma reesei and Aspergillus niger and combination of both

endo-glucanase activity of 2.23 IU/ml and β-glucosidase activity of 0.17 IU/ml. The activity of crude cellulose enzyme combination are able to generate sugar at 12.89 mg/ml in the process of hydrolysis for 72 hours. The combination form of crude cellulose enzyme produces glucose higher than the results of hydrolysis using crudecellulase enzyme which work separately. Hydrolysis using crude enzyme from Trichoderma reesei produced the lowest glucose level which is 0.99 mg/ml with exo-glucanase activity, endo-glucanase and β-glucosidase in a sequence of 0.33 IU/ml, 1.09 IU/ml and 0.09 IU/ml. This proves that the cellulase enzyme's ability to hydrolyze rude not optimal because the cellulose content a low β-glucosidase. This is consistent with the statement Taherzadeh et al[13] that the loss of the use of Trichoderma reesei cellulase for hydrolysis still be at suboptimal levels and not optimal due to the content of a low β-glucosidase.

Increased hydrolysis results in the combined use of both crude enzyme suggesting that the addition of crude enzyme from A.niger to crude enzyme from Trichoderma reesei endoglucanase can improve composition, and β-glucosidase eksoglukanase be better able to degrade cellulose in the straw to glucose compared with single crude enzyme composition according Xin et al., (1993) the addition of β-glucosidase in the cellulase from Trichoderma can produce a saccharification process better than without the addition of β-glucosidase.

Hydrolysis yield is increased by using a combination of both crude enzyme suggesting that the addition of crude enzymes from Aspergillus niger to crude enzymes from Trichoderma reesei to improve the composition of endo-glucanase, exo-glucanase and β-glucosidase for the better, so as to degrade cellulose in the straw to glucose compared with the composition of the crude enzyme separately. By Xin et al., (1993) the addition of β-glucosidase in the cellulase from Trichoderma reesei is able to produce a saccharification process better than without the addition of β-glucosidase.

The low glucose produced in this study due to the low activity of crude enzymes used for hydrolysis process, in accordance with the statement of Jorgensen[7] factors that affect the enzymatic hydrolysis, such as performance enzyme (enzyme activity). The results Roslan et al[11] showed that the use of cellulase enzymes with activity at 6 FPU per gram of rice straw was able to produce glucose from 0.221 to 0.245 gr/gr of rice straw.

CONCLUSION

The combination of crude cellulase enzymes from Trichoderma reesei and Aspergillus niger with different comparisons will have a different activity that will produce cellulose hydrolysis process differently.

Hydrolysis of cellulose pretreated rice straw using microwave alkali can be enhanced with a mixture of cellulase from Trichoderma reesei and Aspergillus niger with a ratio of 2: 1 (v/v).

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