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The Effect of Microwave-NaOH Pretreatment and Hydrolysis Enzyme Using *Trichoderma reesei-Aspergillus niger* on Rice Straw Bioethanol Production

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***Abstract*— The process of bioethanol production from rice straw consists of two steps: (1) conversion of cellulose into simple sugars which is conducted by using microwave-NaOH pretreatment and straw hydrolysis using mold catalyst *T.reesei* and *A. niger*; and (2) fermentation of simple sugars into ethanol. In a microwave-NaOH pretreatment process has been obtained the best value content of cellulose in straw size of 100 mesh and a long exposure of 40 minutes for 72.70±1:10%. Crude cellulase enzyme activity of *T.reesei* isolation, *A.niger* and it mixtures were optimum at temperature of 50°C. The addition of crude enzyme from *A.niger* and *T.reesei* on a comparison of 1: 2 (v/v) was able to increase the yield of the rice straw cellulose hydrolysis which is produces sugar at 12.89 mg/ml (1.29% w/v) or 0.15% (w/v) when converted into ethanol. The glucose yield from rice straw was 25.47% with 3% ethanol.**

***Keywords*— bioethanol, pretreatment, *T.reesei*, *A. niger*.**

I. Introduction

World oil reserves are getting limited and the price of fuel continues to increase, therefore it needed efforts to obtain alternative fuels such as bio-ethanol which is more environmentally friendly and renewable. The raw material for bioethanol production is having a cellulose abundant biomass such as rice straw. The processing of straw into ethanol includes the stages of the conversion of cellulose into simple sugars and fermentation of simple sugars into ethanol.

The processing of rice straw into lignocellulosic polymer (cellulose, lignin and hemicellulose) is more difficult. Thus requiring a pretreatment process to dismantle the structure of straw lignin, cellulose crystalline structure damage and increase the porosity of the material so that the acid or enzyme hydrolysis of cellulose easy to enter [1] [2] [3] [4] [5] [6]. Pretreatment process which is carried out by a combination of wave and microwave irradiation using NaOH alkaline peroxide, effectively reduce the lignin and hemicellulose [7] [8] [9] [10] [11] [12] [13] [14] [15] [16].

The conversion process of cellulose into simple sugars, carried out by the hydrolysis process which consists of two stages: the degradation of cellulose into cellobiose by endoglukanase and subsequent breakage exoglukanase cellobiose to glucose by β-glucosidase. The best conditions for rice straw enzymatic hydrolysis, may obtained by used catalysts from combination of *Trichoderma reesei* and *Aspergillus niger* as a producer of cellulases [17] [18]. The results obtained from the hydrolysis of glucose can be used for fermentation.

The purpose of this study was to obtain bioethanol from rice straw through a pretreatment process (the size of the old straw and microwave) on the content of cellulose, hemicellulose and lignin; hydrolysis process (*Trichoderma reesei* enzyme volume ratio and *Aspergillus niger*, pH and incubation time) and ethanol fermentation process.

II. Materials and Methods

The main material used for this study is rice straw (Ciherang IR64, Indonesia) obtained from Pakis District Malang City, Indonesia. Chemicals used had purity of pro analysis (pa). The material used is a PDA (Potato Dextose Agar), yeast extract, malt extract, glucose, peptone, KH_2PO_4, $FeSO_47H_2O$, CMC (carboxyl methyl cellulose), ethanol 70%, NaOH, 2.5 Dinitrosalisilic acid, K-Tartat, Na-metabisulfite, Phenol, sodium citrat, citric acid and pure culture of *A. niger* and *T. reesei*.

The instruments which were used in this study i.e: disc mill, blender (Philips), Analytical balance Precisa XT 120 A, 950 watt Panasonic microwave oven, sieves 20, 50 and 100 mesh, waterbath shaker Max Q 7000, Vortex Maxi Mix II type 37600, and centrifuge Heraeus Stratos Biofuge Pico 17, spectrophotometer Smartspec Plus Bio Rad, pH meter Consort C861, Memmert waterbath, vacuum pump and filter, and Iwaki Pyrex glassware, Laminar Air Flow, glassware, autoclave ALP KT-30L / -30 LDP, test tubes, needles ose, micropipette, magnetic stirrer, Bunsen, microscope Olympus CX-21, and a haemocytometer. The schematic diagram in present study can be seen in Fig. 1

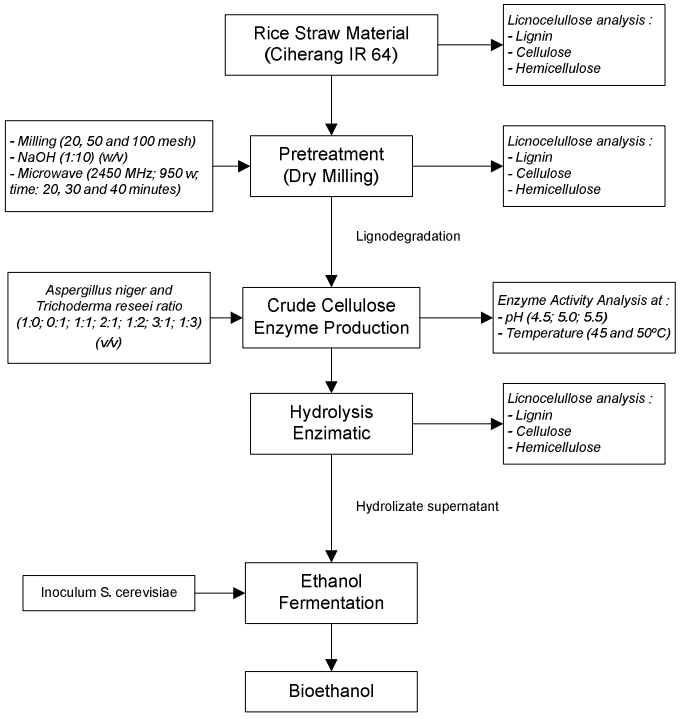


Fig. 1. Schematic Diagram of Rice Straw Bioethanol Production

A. Rice Straw Pretreatment

Rice straw which is cleaned from impurities and leaves residual then dried under the sun for 12 hours and cut into pieces (± 2 cm) then dried in an oven (T = 100°C, for 4 hours). After that, dried straw was milled using a disc mill and screened with 20, 50 and 100 mesh. The straw pretreatment procedure is 10 g of powdered straw which is passes each sieve (20, 50 and 100 mesh) was placed in a 250 ml Erlenmeyer and then added with 0.5 M NaOH solution at a ratio of 1: 10 (w/v).

After the flask was closed using aluminum foil and then put in a microwave oven with a frequency of 2450 MHz, 950 watts for a certain time (20, 30 and 40 minutes). Straw treated was neutralized using distilled water, and then dried in an oven at 50°C for 12 hours. Dry straw after pretreatment levels were observed for lignin, cellulose and hemicellulose. The microstructure was observed to find the best results using Scanning Electron Microscopy (SEM). Data obtained were analyzed by ANOVA and further tested by Duncan test. The best results of the pretreatment process will be used to process rice straw hydrolysis.

B. Rice Straw Hydrolysis

The straw hydrolysis process was carried out using crude enzymes of *T. reesei* derived from *A. niger* and previously undertaken activity test (exoglukanase, and β-glucosidase endoglukanase) at different pH and temperature. Crude enzyme activity was tested at 4.5; 5.0; 5.5 pH and temperatures of 45 and 50°C. Data obtained from the test results of the activity will be tested using ANOVA and further testing using a Least Significant Different (LSD). The treatment of crude cellulase enzymes from both fungi (*T. reesei* and *A. niger*) was using different ratio, i.e: 1:0; 0:1; 1:1; 2:1; 1:2; 3:1; 1:3 (v/v).

Furthermore, 5 g of straw from the best results in pretreatment process was weighed and put into a glass beaker and added to a solution of pH 5 citrate buffer with a volume of 50 ml of the appropriate enzyme treatment. After that, put in a water bath shaker for 72 hours at a temperature of 50°C and a stirring speed of 75 rpm. The observations of glucose was conducted every 8 hours. Hydrolyzates were taken periodically every 8 hours. Hydrolyzates were centrifuged at a speed of 10000 rpm for 20 minutes. Supernatant was used for analysis of the levels of reducing sugars using the Dinitro Salicylic Acid (DNS) method. The analysis of the data obtained was using ANOVA and further testing using Duncan test.

C. Production of crude cellulose enzyme from Trichoderma reesei and Aspergillus niger

Isolates of *T. reesei* and *A. niger* maintained on Potatoes Dextroce Agar (PDA) and performed subculture every month. Preparation of the inoculum used for the production of enzymes made according to Mahamud and Gomes [19]. Enzyme production was using Solid State Fermentation (SSF). Fermentation medium made by: 5 g of powder straw 100 mesh sieve was added 25 ml of nutrient solution predetermined pH 5.5 (using HCl 0.1N). Composition of nutrient solution per liter was performed according to Ahamed and Vermette [20].

Harvesting the enzyme was using a solution of 1% Tween 80. The harvesting was conducted by adding a solution of 1% Tween 80 for 100 ml into the fermentation medium, then shaking it at 120 rpm for 1 hour, then performed using a centrifuge speed of 4000 rpm for 30 min at 4°C. The supernatant was a crude enzyme to be used for hydrolysis of rice straw. The crude enzyme then tested by endoglukanase, exoglukanase and β-glucosidase activities.

D. Preparation of inoculum Saccharomyces cerevisiae for Ethanol Fermentation

Saccharomyces cerevisiae isolates were sub cultured every month using media yeast extract peptone dextrose (YEPD) agar, this medium contains yeast extract (1%), peptone (2%), dextrose (2%) and agar (2%). Preparation of inoculum by growing *Saccharomyces cerevisiae* on sterile medium containing dextrose (6%), peptone (0.5%), and yeast extract (0.5%). Inoculum was incubated in a water bath shaker (100 rpm), at 30°C for 18 hours. After the incubation is complete, *Saccharomyces cerevisiae* cells were separated from the growth medium by means of centrifuge speed of 8000 rpm for 15 minutes. Pellet obtained will be inoculated in the ethanol fermentation process.

E. Ethanol Fermentation

A total of 50 ml of rice straw cellulose hydrolysis (hydrolyzate) which has the highest glucose levels specified pH to pH 4 by adding HCl or NaOH 0,1N 0,1N and then sterilized by autoclave at 121°C for 15 minutes and cool to room temperature. Hydrolyzate sterile inoculated with 0.5% (w/v) of *Saccharomyces cerevisiae*, then incubated in a water bath at 30°C for 48 hours. *Saccharomyces cerevisiae* from the hydrolyzate was separated by centrifugation of 10000 rpm at 4°C for 10 minutes. Hydrolyzate fermented ethanol content was tested using gas chromatography.

F. Analytical Methods

Moulds spores calculation (haemocytometer) based on [18], the assay of cellulose, hemicellulose and lignin was measured by Chesson method (1978) *in* [21], endoglukanase activity test (CMC-ase) method based on Mandels and Weber (1969) *in* [22], exoglukanase activity test (FP ase) method based on Mandels *et al*. (1976) *in* [22], β-glucosidase activity assay method based on Berghem (1974) *in* [17]. Total Reducing sugar for detecting glucose based on dinitrosalicylic acid (DNS) method [23].

III. Result and Discussions

A. Rice Straw Physical Properties during Microwave-NaOH Pretreatment

Rice straw used for bioethanol production process was measured on moisture content and pH values before and after the microwave-NaOH pretreatment. It was done to determine the effect of microwave treatment temperature and the concentration of NaOH solution. The yield of rice straw after pretreatment process was also calculated to determine the effectiveness of pretreatment. The results of water content and pH value of rice straw before and after pretreatment and straw yield of rice based on the size of the mesh are shown in Table 1.

TABLE I
THE PHYSICAL PROPERTIES AND YIELD OF STRAW PRETREATMENT PROCESS BASED ON THE MESH SIZE

Mesh	Before Petreatment		After Pretreatment Microwave-NaOH		Yields (%)
	Moisture content (%)	pH	Moisture content (%)	pH	
20	10.07	13.09	4.85	12.16	30.5
50	8.88	13.07	3.97	12.13	30.1
100	9.53	13.00	4.05	12.06	21.5

Based on Table 1, the moisture content obtained before and after the pretreatment process shows that there is a downward trend. This is presumably due to the influence of temperature on the microwave when pretreatment causes, evaporation of water at the surface of the material faster than the evaporation of water in the material, resulting in the decreement of water content is greater. Increasing pretreatment temperature resulted in a decrease in the water content of the input material is substituted with NaOH during the pretreatment process.

In addition, the pH value before and after the process pretreatment have a significant difference. This difference is due to the ionization of H + ions from water treatment due to compression, resulting in the decreement of pH value before and after pretreatment. Therefore, at high temperatures the water can be applicable as a result of acid decomposition of H_2O into H + and OH-ions 0.5 M NaOH immersion treatment alkaline solution causes the pH is high, but with the support of an alkaline compound, one of them by using an alkaline chemical such as NaOH compounds was able to degrade lignin from the cellulose structure.

Based on the mesh size of the straw, the value obtained yield tends to decrease, in which the smaller the size of the yield of rice straw powder obtained the less. This is because the process of filtration or separation of solids wasted a lot of rice straw because the size is so small that it is carried by the water. In addition, during the process of filtering and neutralizing the pH were probably left in the filter paper when the oven drying process takes place. The loss of material certainly affects the resulting yield.

B. Rice Straw Lignocellulose during Microwave-NaOH Pretreatment

The results of the analysis of rice straw varieties IR 64 Ciherang before pretreatment process contains cellulose hemicellulose and lignin with Chesson method can be seen in Table 2. These results are not much different from the study of [1]. Differences in the composition of lignocellulose are influenced by the variety, age and condition of plant growth.

TABLE II
THE LIGNOCELLULOSIC CONTENT OF RICE STRAW BEFORE PRETREATMENT

References	Lignocellulosic Content		
	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Present study	30.38 $\pm$ 1.40	18.49 $\pm$ 0.50	7.93 $\pm$ 0.25
Sun & Cheng [1]	24-45	20-30	10-15
Ibrahim *et al.* [24]	38.83	68.09	14.55

The main purpose of the pretreatment process is to obtain high value cellulose to be utilized as a substrate in the production of cellulase enzymes on the enzymatic hydrolysis process. Based on the testing method Chesson rice straw after pretreatment process, it was found that the content of cellulose and hemicellulose after microwave-NaOH pretreatment process increased, whereas the lignin content decreased. The result of measurements of the content of cellulose, hemicellulose and lignin of rice straw before pretreatment process is shown in Table 3.

Based on Table 3, the lignocellulose contend tends to increase in line with increasing of microwave time and mesh size powder of straw. This is because the size reduction using mechanical pretreatment disc mill is capable of damaging the cellulose crystal, lowering the degree of crystallization and increasing the surface area [25]. The smaller the surface area of straw has increased so that the exposed surface of the material is also more extensive microwave so that the cellulose content is increased. In addition, the process of the interaction of microwaves with this material resulted in binding cellulose content of

hemicellulose can be separated, and the content of lignin in the cell walls of cellulose that blocks began to fall.

TABLE III
THE LIGNOCELLULOSIC CONTENT OF RICE STRAW AFTER MICROWAVE-NAOH PRETREATMENT

Mesh	times (min)	Cellulose (%)	Hemicellulose (%)	Lignin (%)
20	20	61.78 ± 1.15 a	22.18 ± 1.99 b	5.32 ± 0.16 a
	30	64.71 ± 3.31 ab	19.62 ± 0.14 ab	4.78 ± 1.10 a
	40	69.25 ± 0.49 bc	18.61 ± 0.08 ab	3.29 ± 0.05 a
50	20	66.68 ± 0.37 b	30.11 ± 4.36 c	3.67 ± 0.05 a
	30	69.26 ± 0.13 bc	18.51 ± 2.25 ab	3.76 ± 0.56 a
	40	68.99 ± 1.88 b	20.93 ± 1.01 b	3.61 ± 0.25 a
100	20	69.34 ± 0.82 bc	18.29 ± 0.02 ab	5.25 ± 0.23 a
	30	70.00 ± 0.71 bc	17.35 ± 0.66 a	4.45 ± 0.02 a
	40	72.70 ± 1.10 c	16.93 ± 1.29 a	3.66 ± 0.19 a

Notes: The numbers followed the same letter in the same column indicates of not significantly different according to Duncan test (α = 5%)

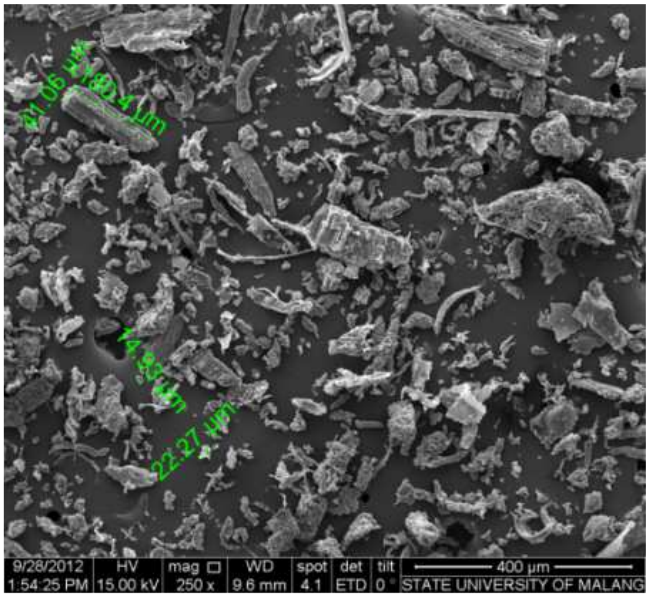
Increased levels of hemicellulose allegedly caused damage to the bonds that connect with cellulose and hemicellulose with lignin, namely hydrogen bonding, ester or ether. The destruction of the bonds in lignocellulosic components in alkaline solution (NaOH) and the termination of the bond due to the heat generated by the microwave radiation that unleashed hemicellulose with lignin and cellulose. While the reduction in particle straw hemicellulose occurred at 100 mesh, hemicellulose content decreases with the longer time of microwave exposure.

The longer exposure to microwaves will result in longer heat effect also on the material, so that the process of breaking chemical bonds between molecules and hemicellulose degradation occurs. This is consistent with the statement of Zheng *et al.* [25] that alkaline pretreatment can lead to decomposition of the polymer in the cell wall by way of breaking the hydrogen bonding and covalent. According to Sun and Cheng [1], mechanism of hydrolysis of lignocellulose by NaOH saponification of the ester bond is the intermolecular cross-linking between xylan, hemicellulose and other components, such as lignin and hemicellulose.

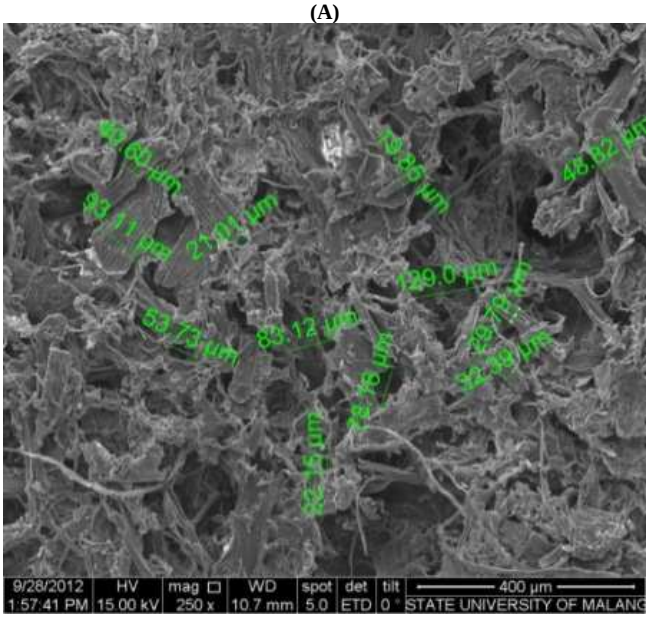
Components that can inhibit performance cellulase enzymes hydrolyze cellulose in addition to hemicellulose is lignin. The decrement in lignin as shown in Table 3, occurs along with the longer time of microwave exposure. The heat effect also increased so as to degrade lignin more ties in well so that the lignin content decreases. In addition, the smaller size of the surface area of the material will be widened so that the absorption of microwave energy to break the bonds of lignin is also getting bigger. The results of Hu and Wen study [26], a decline in lignin along with the size reduction of switch grass which is pretreated by using a combination of alkali and radio wave heating.

C. Microstructure of Rice Straw during Microwave-NaOH Pretreatment

Analysis of the rice straw microstructure due to a pretreatment process was conducted by using SEM which is shown in Fig. 2.



(A)



(B)

Fig. 2. rice straw microstructure at 250x magnification. (A) before pretreatment, (B) after microwave-NaOH pretreatment

Figure 2A shows that straw looks as fractions harder material shiny and irregular shape with particle sizes ranging between 14.93-180.4µm. Figure 2B shows that straw into a shape changing materials look more stringy and loose with diameters ranging between 53.73-129.0µm cavity and the diameter of the fractures ranged between 19.85-93.11µm. Alkaline solution (NaOH 0.5N) gave the ester bond and saponification reaction between the molecules of cellulose ether with lignin with hemicellulose and lignin molecules. The saponification reaction will result in breaking the bonds of the ester or ether that macromolecular lignin, cellulose and hemicellulose which originally linked to each other will be separated and dissolved lignin in alkaline solution.

This reaction will be enhanced by the heat generated by microwave exposure. Microwave exposure will be absorbed by the molecules present in straw that has been treated with a solution of alkali. Microwave energy will be easily absorbed by the polar compounds (water), energy that has

been absorbed will use these molecules to move (vibrate), movement (vibration) will cause thermal effects that can accelerate the termination of intermolecular bonding. The movement of these molecules will also lead to collisions between molecules so that will give the effect of a decrease in the crystalline structure of cellulose and creating amorphous structure [8].

D. The Exoglucanase Activity

The exoglucanase activity of crude enzymes separately or mix produces different activity at different pH and temperature which is shown in Fig. 3.

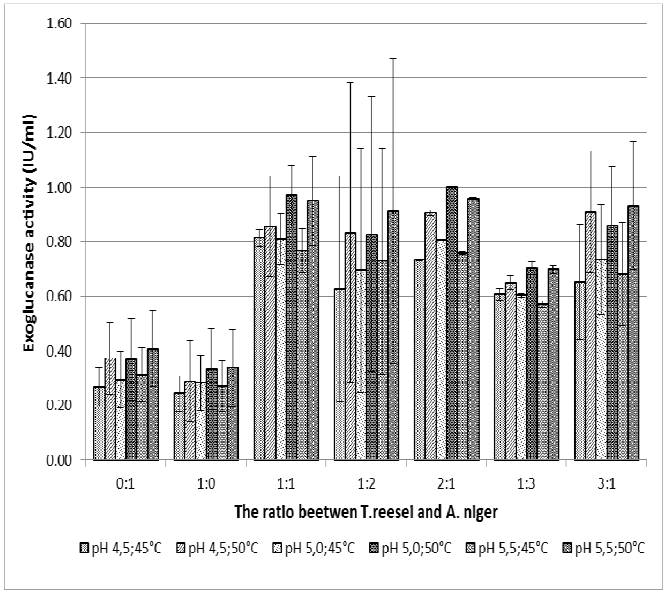


Fig. 3. The Exoglukanase crude enzyme activity of *T. reesei* and *A.niger* as well as a mixture both pH and temperature

Based on Fig. 3, it can be seen that the activity of the crude enzyme exoglucanase *T. reesei* and *A. niger* are not much different, but after two enzymes combined with a variety of comparisons, results in a significant increase in activity. Increased activity of crude enzyme was also strongly influenced by temperature, where the enzyme has a high activity when incubated at 50°C than when incubated at a temperature of 45°C. The increase in temperature causes the atoms in the enzyme molecule has a great energy and a great tendency to move. Eventually they gained enough energy to form a bound globular protein structure and subsequent deactivation of the enzyme occurs. The sensitivity of each protein to denature varies depending on the pH. Cellulase enzymes from both fungi have increased activity at 50°C, allegedly ions on the protein molecule is not formed globular structures and denaturation have not experienced.

E. The Endoglucanase Activity

The testing of endoglucanase activity in crude enzyme cellulose using Na-CMC as substrate. Endoglucanase activity of *T. reesei* and *A. niger* and crude enzyme mixture generally has a high activity at pH 5.0. Crude enzyme activity was also greatly influenced the temperature. Endoglucanase activity of both mold increases when the temperature is raised 5°C as shown in Fig. 4.

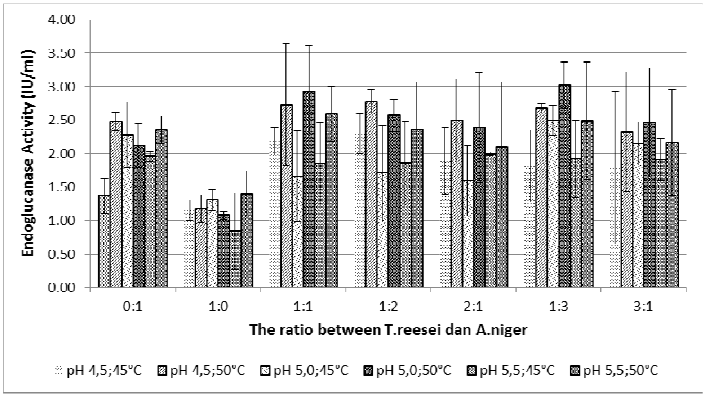


Fig. 4. The endoglukanase crude enzyme activity of *T. reesei* and *A.niger* as well as a mixture of both pH and temperature

Based on Fig. 4, it can be seen that an increase in activity of the crude enzyme of *T.reesei* and *A. niger* separately or a mixture of both. High crude enzyme activity at 50°C was compared to the temperature of 45°C. Increasing of 5°C in temperature may increase the activity of crude enzyme significantly. According to Perez *et al.* [27] cellulase enzyme system of *T.reesei* belong to the mesophilic group, which has the optimum temperature for the activity of 50°C.

F. The β-glucosidase Activity

Activity of β-glucosidase from *T. reesei* crude enzyme was lower than *A. niger* and declining crude enzyme activity when the second of the two molds are merged. β-glucosidase activity was optimum at pH 4.5 and continued to decline with increasing pH. β-glucosidase activity was also increased when the incubation temperature is raised 5°C. The low activity at temperatures of 45°C and 50°C increase in temperature as shown in Fig. 5.

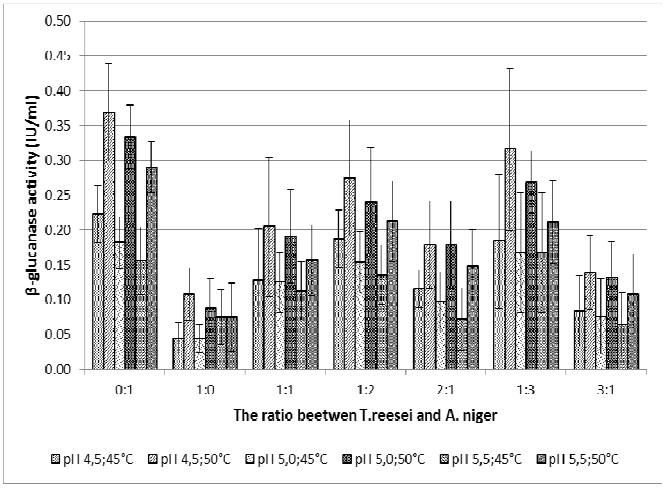


Fig. 5. The β-glucosidase crude enzyme activity of *T. reesei* and *A.niger* as well as a mixture of both pH and temperature

The ANOVA results of each crude enzyme mixture is known that there is no interaction between pH and temperature. However temperature treatment effect on the exoglucanase activity of the crude enzyme mixture.

Furthermore the pH treatment only affects the activity of β-glucosidase crude enzyme mixture of *T. reesei* and *A. niger* (1: 2), (2: 1) and (1: 3).

G. Reducing Sugar

Hydrolysis of rice straw microwave-NaOH pretreatment results using the enzyme from *T. reesei* and *A. niger* showed a significant difference. The results of hydrolysis using crude enzyme from *A. niger* higher than the crude enzyme from *T. reesei* and isolation increased when the two are combined in a crude enzyme hydrolysis as shown in Table 4.

TABLE IV
LEVELS OF GLUCOSE DUE TO HYDROLYSIS OF RICE STRAW TREATMENT WITH A MIXTURE OF CRUDE ENZYME FROM *T. RESEEI* AND *A. NIGER* ISOLATION

Comparison of crude enzymes mixing from *T. reesei* and *A. Niger*	Glucose content (mg/ml)
0 : 1	8.97 b
1 : 0	0.99 a
1 : 1	8.69 b
1 : 2	7.28 b
2 : 1	12.89 c
1 : 3	8.81 b
3 : 1	9.99 bc

Notes: The numbers followed the same letter in the same column indicates of not significantly different according to Duncan test (α = 5%)

From Table 4 it can be seen that the results of the hydrolysis of a mixture of both enzymes resulted in higher sugar hydrolysis compared with the results of the two enzymes with work separately. The *T.reesei* crude enzymes of glucose produces the lowest (0.99 mg/ml) with exoglukanase activity, endoglukanase activity and β-glukosidase activity respectively of 0.33 IU/ml, 1.09 IU/ml and 0.09 IU/ml. This proves that the ability of crude cellulase enzyme to hydrolyze cellulose was not optimum because the content of β-glucosidase which is low. This is consistent with the statement of Taherzadeh and Karimi [28] that the loss of the use of *T. reesei* cellulase for hydrolysis is still at a suboptimal level and not optimal due to low β-glucosidase content. The increased hydrolysis results in the combined use of both crude enzyme indicating that the addition of the crude enzyme from *T. reseei* and *A.niger* can improve endoglukanase composition, and β-glucosidase exoglukanase be better so as to degrade cellulose in straw to glucose compared with the composition of single crude enzyme.

H. Levels of Rice Straw Lignocellulose Using enzyme hydrolyzed cellulase after the crude Isolation of T.reesei and A.niger

Residual straw after dry hydrolyzed back to content analyzed it lignocelullose. The results of measurements of cellulose, hemicellulose and lignin are shown in Table 5.

From Table 5 it can be seen that after the enzymatic hydrolysis of hemicellulose decreased levels, initially fell 16.93% to reach 6.67% as well occur in lignin content tend to have increased from 3.67% to 4.33% increase during the hydrolysis process enzymatically. The straw cellulose content after pretreatment increased the majority of straw hydrolyzed using enzymes rough, but at a certain treatment has decreased, but the decrease was not significantly happens. Levels decreased in the treated cellulose hydrolysis using an enzyme mixture ratio *T reesei: A. niger* of 1: 0, 1: 3, 3: 1. This shows that there is still a lot of cellulose in the straw which is still not perfectly hydrolyzed into simple sugars, so the results are also very low sugar level.

TABLE V
MEAN LEVELS OF CELLULOSE, HEMICELLULOSE AND LIGNIN HYDROLYZED RICE STRAW AFTER USING CRUDE CELLULASE ENZYME AND ISOLATION OF *T. REESEI* AND *A. NIGER*

Comparison of crude enzyme *T.reesei : A. niger*	Hemicellulose (%)	Cellulose (%)	Lignin (%)
0 : 1	6.83 a	77.19 b	3.85 a
1 : 0	15.24 c	69.80 a	4.33 a
1 : 1	7.97 ab	74.75 ab	4.29 a
1 : 2	6.67 a	76.17 b	4.08 a
2 : 1	8.68 ab	74.10 ab	3.88 a
1 : 3	9.48 ab	72.36 a	4.14 a
3 : 1	10.21 b	71.44 a	3.70 a
Control	16.93 c	72.70 a	3.67 a
Duncan test (α=5%)	2.57-2.80	3.10-3.38	-

I. Ethanol Fermentation

The results of hydrolysis with the highest sugar levels continued to ethanol fermentation process. *Saccharomyces cerevisiae* with a cell density of $5.5x10^7$ (0.5% w/v) was added to the hydrolyzate results with glucose levels of 12.89 mg/ml. Fermentation is carried out at 30^oC for 48 hours to produce ethanol at 0.15%, equivalent to 1.5 g/l. Ethanol yield which calculated based on which hydrolysis of straw by 3% (0.03 g/g). The results of ethanol fermentation resulting from the very small amount of sugar in a straw hydrolysis are also small. It is contrary to the results of straw after experiencing pretreatment microwave able to produce alkali cellulose was 70.68%. The resulting sugar levels only reached 12.89 mg/ml or 25.47% based on the weight of straw (yield).

Low sugar is caused by a rough performance in the enzyme hydrolysis process is not maximized in converting cellulose to glucose. It can be associated with the low enzyme activity of crude enzyme which is added exoglucanase activity 1.002 IU/ml, endoglucanase 2.23 IU/ml and β-glucosidase activity of 0.17 IU/ml. According to Zheng *et al*. [25] the granting of the activity of 15 FPU enzyme capable of converting glucan (cellulose) rice straw into glucose by 68.9% so as to produce ethanol at 37g/l or about 3.7% on the straw which has undergone pretreatment ammonia fiber expansion and fermented (AFEX) using *Saccharomyces cerevisiae* 424A (NHL-ST). The study results by Yoswathana and Phuriphipat [29] also shows the yield of ethanol from straw no more than 15 g/l by using *Saccharomyces cerevisiae* cells at a density of 2-8 $x10^8$ and fermented for 6 days to get a yield of 0.42 g/g.

IV. CONCLUSIONS

The best of microwave pretreatment of rice straw with alkali is obtained at straw particle size of 100 mesh and the long exposure of microwave for 40 minutes (72.70±1.10% cellulose; 16.93±1.29% hemicellulose; and 3.66±0.19%

lignin). Crude cellulase enzyme activity was optimum at the temperature of 50^{o}C. The crude enzyme of cellulase enzymes *A.niger* - *T.reesei* on a ratio of 1: 2 (v/v) was able to increase the yield which produces sugar at 12.89 mg/ml (1.29% w/v) with the conversion into ethanol of 0.15% (w/v). Glucose yield from rice straw by 25.47% and 3% ethanol.

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