BIOETHANOL PRODUCTION FROM NON-ACID PRETREATED WOODY BIOMASS BY USING ENZYMATIC REACTION

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ABSTRACT

Lignocellulose biomass, such as wood and agricultural residues, is considered a good candidate for bioethanol production. The present study was undertaken to investigate the effect of mechanochemical pretreatment and hydrothermal pretreatment, on chemical composition of sawdust samples from hardwood and softwood and subsequent bioethanol production applying pre-enzymatic hydrolysis and fermentation. The best condition for hydrothermal treatment gave the higher cellulose content from 52.5% to 84.9% and lesser lignin content from 26% to 3% than the other pretreatment conditions. Liberation of cellulose was confirmed by X-ray Diffraction (XRD).The pretreated sawdust was hydrolysed with inoculum containing crude enzymes from Trichoderma and Aspergillus niger. After enzymatic hydrolysis, the maximum glucose yield was 7.4% and 6% by inoculum containing crude enzymes from Trichoderma and Aspergillus niger. The total solid conversion were 30% for sawdust sample from softwood hydrolysed with inoculum containing crude enzyme from Trichoderma of 52 FPU/ml and 34% for sawdust sample from softwood hydrolyzed with crude enzyme from Aspergillus niger of 45 FPU/ml. From the fermentation studies, the sawdust sample from softwood gave 4% of crude ethanol by weight using inoculum containing crude enzyme from Trichoderma and 3% by using inoculum containing crude enzyme from Aspergillus niger. The crude ethanol was characterized and confirmed by FTIR spectra.

Keyword: - *Bioethanol, Lignocellulosic Biomass, Pretreatment, Enzymatic Hydrolysis, Fermentation*

1. INTRODUCTION

Ethanol has been a part of human culture since the dawn of time, but it was not until late ninetieth century that ethanol was first used as a source. Since the 20th century, our major energy demand has been supplied by fossil fuels such as: oil, coal, and natural gas. Fossil fuels originate from deceased organisms that lived several million years ago and by time have been embedded in the earth's crust [6].Myanmar forests consists of many species of wood which, after harvesting and processing, leave behind wood wastes in the forests and wood residues in the wood processing factories. Lignocellulose biomass, such as wood and agricultural residues, is attractive materials for the ethanol production since it is the most abundant reproducible resources on earth. Ethanol production from lignocelluloses biomass depends on the hydrolysis of cellulose and hemicellulose into simple reducing sugars that can be fermented into ethanol by microorganisms [2].Most of the developed countries have accepted the idea of obtaining sugars from hydrolysis of wood waste such as chips and sawdust from saw mills. Presently, enzymatic hydrolysis is considered the most promising technology for converting biomass into sugars and to be as a raw material for the production of other various biotechnical bulk chemical products. The hydrolysis of wood waste to sugars and molasses has developed into industrial production. After that, fermentation process is carried out to produce ethanol. This research work will benefit the commercial production of ethanol rather than using wood waste as fuel in general [2]. Environmental issues such as the threatening increase in temperature caused by the greenhouse effect and the fact that fossil fuels are non-renewable resources, has increased the interest in producing fuels from renewable resources, e.g. biomass. Ethanol as well as other biofuels produced from plant biomass, is an alternative to fossil fuels. Today the production cost of ethanol from lignocelluloses is still too high, which is the

major reason why ethanol has not made its breakthrough yet [1, 6].The technology for bioethanol production from lignocelluloses biomass is well defined; however, production from other feed stocks such as biomass still requires extensive research to develop a feasible production method. So, this work focused at improving its yield by using source of lignocelluloses namely: sawdust from hardwood and softwood. Thus assessing the effect of the sawdust on the yield of inoculum containing crude enzymes from *Trichoderma* and *Aspergillus niger*. This study focused on the technology to convert sawdust to ethanol [3].

2. MATERIALS AND METHODS

2.1 Raw Materials

The substrates used for this work are sawdust samples from hardwood and softwood; they are cheap and readily available sources of lignocelluloses from one of the twig in Phyu Township, Bago Division, in Myanmar for the pretreatment process. The substrates were individually screen analyzed in the British Standard, BSS 410 test sieve shaker and each sample was made to pass through 100 mesh number.

2.2 Mechanochemical Pretreatment

The substrates were soaked in 25 % (w/v) sodium hydroxide solution at a ratio of 1:10 (substrates: solution) for 90min at room temperature after washing free of the chemical. The treated substrates was then filtered and washed successively with distilled water until the wash water was neutral. The samples were dried at room temperature for two days. Then, the samples were ball milled at 1400rpm for 48hr. The treated sawdust samples were designated as HS-48 for hardwood and SS-48 for softwood. Grinding time 48 hr is an optimum condition from previous thesis by Dr. Mie Mie Kyaw, 2007.

2.3 Hydrothermal Pretreatment

The 50g of sawdust samples were added to 400ml water. The slurry was controlled to 80±5˚C by thermostat. Sodium hydroxide solution (5% wt of sawdust) was added to sawdust slurry. The mixture was heated to boil for various predetermined reaction time (60-240min) at 80±5˚C. After completing the boiling, solid and liquid portion of the mixture was separated by filtering. Then, the solid sample was washed several times with water to achieve neutral condition. The solid sample was dried at room temperature for 2days. At reaction time (180min), the best condition for hydrothermal treatment gave the higher cellulose content and lesser lignin content than the other pretreatment conditions. For optimum condition (180min), the treated sawdust samples were designated as HS-5 for hardwood and SS-5 for softwood.

2.4 Inoculum Preparation for Enzymatic Hydrolysis

The pure culture of Aspergillus niger and Trichoderma were provided by the Department of Biotechnology, Mandalay Technological University. The organisms were maintained as direct stock culture from which inocula were prepared.100ml of medium (Sabouraud broth) of sample with Aspergillus niger and 100ml of medium (Sabouraud broth) of sample with Trichoderma were used inoculum prepared in 250ml.The inoculum was shaken continuously on an environment-controlled at 25˚C before it was used for enzymatic hydrolysis and fermentation process.

2.5 Enzymatic Hydrolysis

The inoculum containing crude enzymes were used cellulases from Aspergillus niger and Trichoderma worked in Department of Biotechnology. The pretreated sawdust samples from hardwood and softwood were hydrolysed by cellulases from Aspergillus niger and Trichoderma at 50˚C, 85rpm in a water bath shaker with cellulose 5% (w/v).The cellulose powder was dissolved in 1ml of 0.05M citrate buffer (pH 4.8).
At each reaction time of 60min, 0.5ml of sample was taken and diluted for the glucose and the total reducing sugar analysis. In the enzymatic hydrolysis, filter paper, pretreated hardwood (HS-5) and softwood (SS-5) were used as the substrates.
At the end of the hydrolysis period, DNS reagent was added to stop the reaction. Then the process for colour development was continued. The undigested pulps were settled, separated and absorbance of liquid portion was

measured to find the amount of glucose produced. The untreated pulp was washed with water, dried at 100 ºC and weighed for determination of solid conversion.

2.6 Fermentation

Sawdust, 10X YP medium, baker yeast, crude enzymes from Aspergillus niger and Trichoderma and DI water were mixed in 250ml fermentation vessel .The fermentation period was allowed to 4 days or 96hr at pH 4.9-5.0 and temperature was maintained at 37˚C.The solution (100ml) obtained from fermentation process was taken and was added 100ml of water. This solution was put in a 1 L round-bottomed flask heated at 78°C. Crude ethanol was liberated and received in a trap by passing through a condenser.

2.7 Analytical Methods

The cellulose content ,hemicellulose content and lignin content of pretreatment sawdust were analyzed by heat-of-dilution dichromate method, extraction of alkali method,72%(v/v) sulphuric acid method respectively[2]. The degree of crystallinity and the crystal structure of sawdust were characterized by X-ray diffractometer (XRD). Total reducing sugars were determined by the DNS method using glucose as the standard [5]. Cellulase activity was assayed as filter paper units [4].The presence of glucose can be detected by absorbance measurement using the UV spectrophotometer. Crude ethanol percent was determined by using hydrometer. The crude ethanol was characterized and confirmed by FTIR spectra.

3. RESULTS AND DISCUSSION

3.1 Results from Mechanochemical Treatment

According to results from Mechanochemical pretreatment, these experimental data can be seen in Table 1.The compositions of untreated and pretreated sawdusts from hardwood and softwood are compared. According to Table 1, the percentage of cellulose content of untreated sawdust sample from softwood (SS-0) was more than that of hardwood (HS-0).But hardwood had lower lignin content than softwood. The hemicellulose could be produced significantly in hardwood. As softwood have higher lignin content which makes the hydrolysis step more difficult, they have generally produced less hemicellulose. For mechanochemical pretreatment, sample HS-48 and SS-48 of the lignin contents were decreased more than that of sample HS-0 and SS-0.

Table -1: Compositions of Hardwood and Softwood from Mechanochemical Pretreatment

Sample No.	Cellulose (%)	Hemicellulose (%)	Lignin (%)
HS-0[a]	43.5	23.5	24
HS-48[b]	62	17.5	11
SS-0[a]	52.5	9	26
SS-48[b]	73	8.25	7.5

[a]HS-0 andSS-0= untreated sawdust samples from hardwood and softwood

[b]HS-48 and SS-48= pretreated sawdust samples from hardwood and softwood

3.2 Results from Hydrothermal Pretreatment

According to results from hydrothermal treatment, the percentage of cellulose content is as shown in Fig. 1. The Figure shows time versus cellulose percent of the hardwood by using hydrothermal treatment process. At reaction time (180min), Sample No. (HS-5) gave the higher percentage of cellulose for optimum condition. The more reaction time, the higher percentage of cellulose. So, hardwood sample (HS-5) is the best conditions. Then, sawdusts from softwood treated by preheating to boil 80±5˚C followed by adding sodium hydroxide solution (5% by wt of sawdust) for 60min to 240min.So, sawdusts from softwood (SS-5) treated by preheating to boil 80±5˚C followed by adding sodium hydroxide solution (5% wt of sawdust) for 180min was best condition compared to that other conditions .

According to results from hydrothermal treatment, these experimental data can be seen in Table 2.The compositions of untreated and pretreated sawdusts from hardwood and softwood are compared. According to Table 2, the percentage of cellulose content of untreated sawdust sample from softwood (SS-0) was more than that of hardwood (HS-0).But hardwood had lower lignin content than softwood. The hemicellulose could be produced significantly in hardwood.

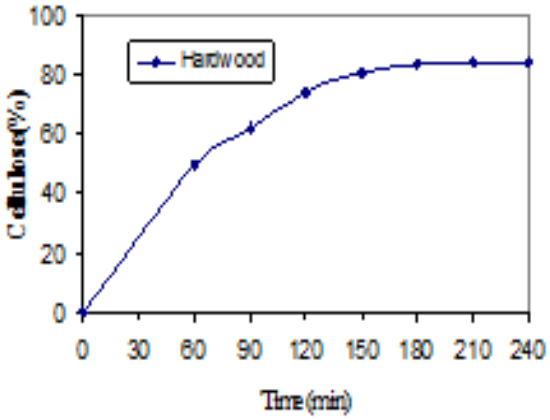

Fig -1: Percentage of cellulose from hardwood

Table -2: Compositions of Hardwood and Softwood from Hydrothermal Pretreatment

Sample No.	Cellulose (%)	Hemicellulose (%)	Lignin (%)
HS-0[a]	43.5	23.5	24
HS-5[b]	83.5	8	4
SS-0[a]	52.5	9	26
SS-5[b]	84.9	6.3	3

[a]HS-0 andSS-0= untreated sawdust samples from hardwood and softwood

[b]HS-5 and SS-5= pretreated sawdust samples from hardwood and softwood

As softwood have higher lignin content which makes the hydrolysis step more difficult, they have generally produced less hemicellulose. For hydrothermal treatment, sample HS-5and SS-5 of the lignin contents were decreased more than that of sample HS-0 and SS-0.

3.3 Results of XRD Patterns of Untreated and Pretreated Sawdust

The XRD patterns of untreated and pretreated sawdust samples from hardwood and softwood for mechanochemical treatment and hydrothermal treatment are shown in Fig. 2 and Fig. 3. According to Fig. 2, the strongest peak, $2\theta \approx 23^{\circ}$, originates from the cellulose crystalline plane. It could be seen that the longer reaction time heated more linkage with increase in the percentage of intensity reduction. Increasing the percentage of intensity reduction shows decreasing the degree of crystalline.

After the heating time 180min, this crystalline peak noticeably disappeared. So, sawdust sample from pretreated hardwood (HS-5) was better condition compared to that of (HS-48) pretreatment condition. Sample (HS-5) could reduce the linkages between lignin, hemicellulose, and cellulose in sawdust. According to untreated and pretreated sawdust samples from hardwood for mechanochemical treatment and hydrothermal treatment results, hydrothermal treatment on sawdust from hardwood was effective.

According to Fig. 3, the strongest peak, 2θ≈23˚, originates from the cellulose crystalline plane. It could be seen that the longer reaction time heated more linkage with increase in the percentage of intensity reduction. Increasing the percentage of intensity reduction shows decreasing the degree of crystallinity. After the heating time 180min, this crystalline peak noticeably disappeared. So, sawdust sample from pretreated hardwood (SS-5) was better condition compared to that of (SS-48) pretreatment condition. Sample (SS-5) could reduce the linkages between lignin, hemicellulose, and cellulose in sawdust.

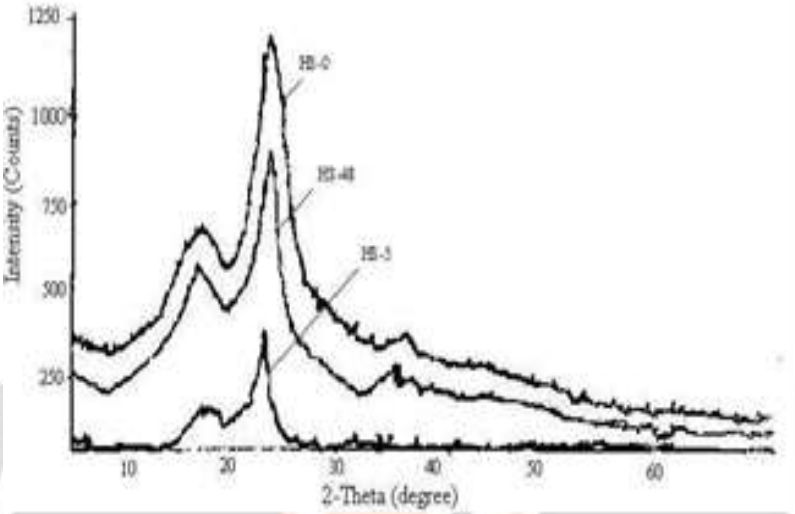

Fig -2: XRD patterns of untreated and pretreated sawdust samples from hardwood for mechanochemical treatment and hydrothermal treatment

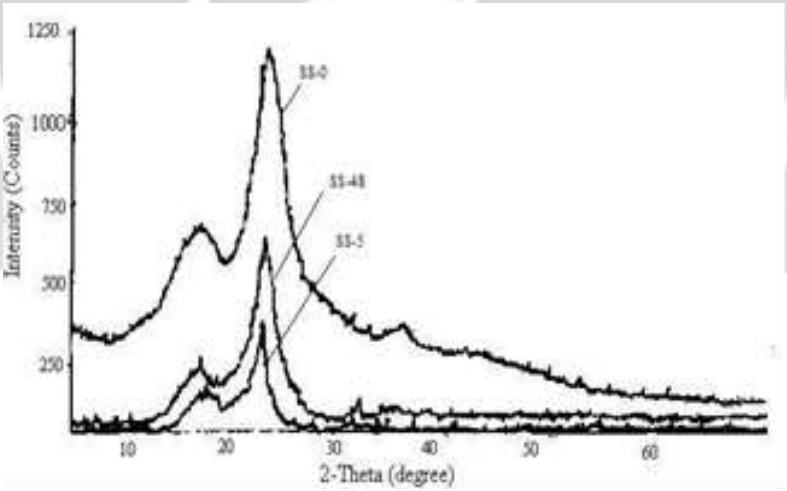

Fig -3: XRD patterns of untreated and pretreated sawdust samples from softwood for mechanochemical treatment and hydrothermal treatment

According to untreated and pretreated sawdust samples from softwood for mechanochemical treatment and hydrothermal treatment results, hydrothermal treatment was best condition observed in this study.It is sure that HS-5 and SS-5 were continued to treat for enzymatic hydrolysis and fermentation process.

3.4 Results of Glucose Concentration by using Enzymatic Hydrolysis

Inoculum containing crude enzymes concentrations (v/v) against glucose liberated from filter paper, pretreated sawdust samples from softwood (SS-5) and hardwood (HS-5) were plotted and shown in Fig. 4 and Fig. 5. According to Fig. 4, concentration of inoculum containing crude enzymes from *Trichoderma* that released 2 mg of glucose was 0.0068 for filter paper, 0.007 for pretreated softwood (SS-5) and 0.008 for pretreated hardwood (HS-5). According to Fig. 5, concentration of inoculum containing crude enzymes from Aspergillus niger that released 2 mg of glucose was 0.007 for filter paper, 0.0082 for pretreated softwood (SS-5) and 0.0084 for pretreated hardwood (HS-5). Comparing to Fig. 4 and Fig. 5, the cellulase activity of inoculum containing crude enzyme from Trichoderma was 54FPU/ml for filter paper, 52FPU/ml and 46FPU/ml for pretreated softwood (SS-5) and hardwood (HS-5). The cellulase activity of inoculum containing Crude enzyme from *Aspergillus niger* was 52FPU/ml for filter paper, 45FPU/ml and 46FPU/ml for pretreated softwood (SS-5) and hardwood (HS-5). Inoculum containing crude enzyme dilution from *Trichoderma* was less concentrated and more enzymatic cellulase activity.

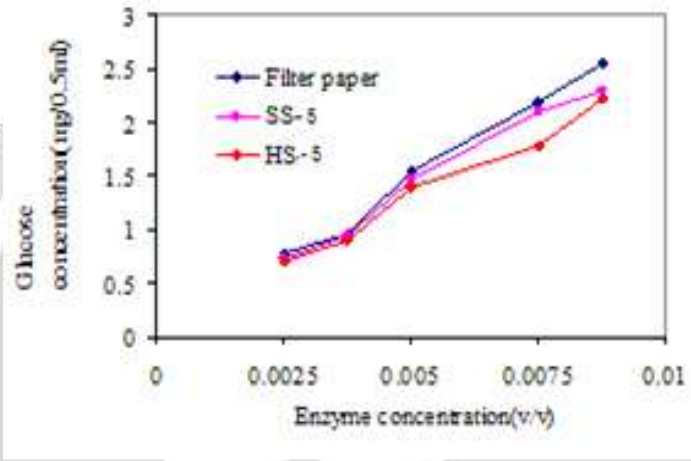

Fig -4: Amount of glucose liberated against Inoculum containing crude enzyme concentration from Trichoderma

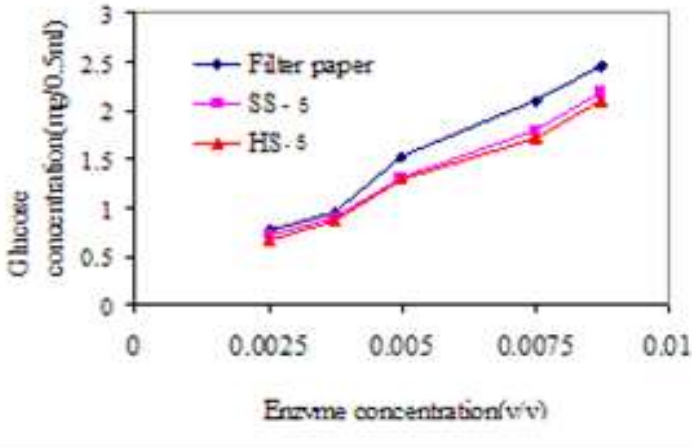

Fig -5: Amount of glucose liberated against inoculum containing crude enzymes concentration from Aspergillus niger

3.5 Results of Crude Ethanol Percent by using Hydrometer

Crude ethanol-water mixture from softwood distillate was measured by using hydrometer is as shown in Fig.3.6 It can be observed that the product ethanol concentration is 4% wt and 3%wt for *Trichoderma* and *Aspergillus niger* at the fermentation time 4days or 96hr. From the fermentation studies, the sawdust from softwood (SS-5) gave ethanol percent by weight of 4% and 3% by using inoculum containing crude enzymes from Trichoderma and Aspergillus niger.

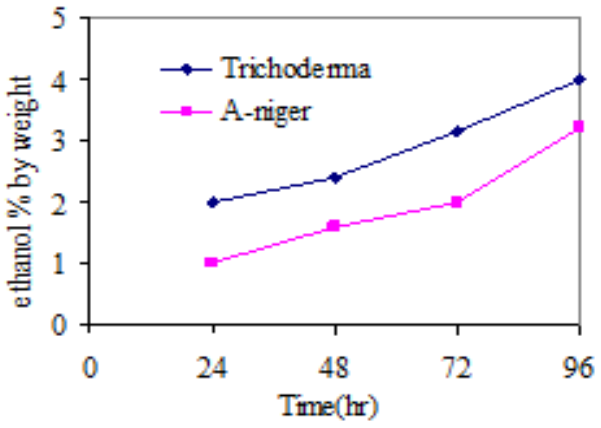

Fig -6: Ethanol % by weight from softwood distillate of the fermented Solution

3.6 Fourier Transformed Infrared (FTIR) Spectra of Ethanol

Local ethanol 93.43 v/v%, product crude ethanol samples by using inoculum containing crude enzymes from *Trichoderma* and *Aspergillus niger* were analysed with FTIR Spectrophotometer Genesis II, Maltson instruments. Inc 1001, Fourier Drive, Nadison, USA[7]. The FTIR spectrums are mentioned in Fig. 7. The spectra of ethanol (local) shows typical absorption bands at 3000-3700cm^{-1} correspond to O-H stretching in the region and the bands at 2700-3300 cm^{-1} correspond to C-H stretching in the region. Then, the bands at 1600-1700 cm^{-1} correspond to C=C stretching in the region. The bands at 1600-1900cm^{-1} correspond to rough calculation of primary alcoholic C=O stretching. The FTIR spectra of crude ethanol by using inoculum containing crude enzymes from *Trichoderma* and Aspergillus *niger* were similar as the ethanol (local). The FTIR spectra of crude ethanol are also described in Fig. 7.

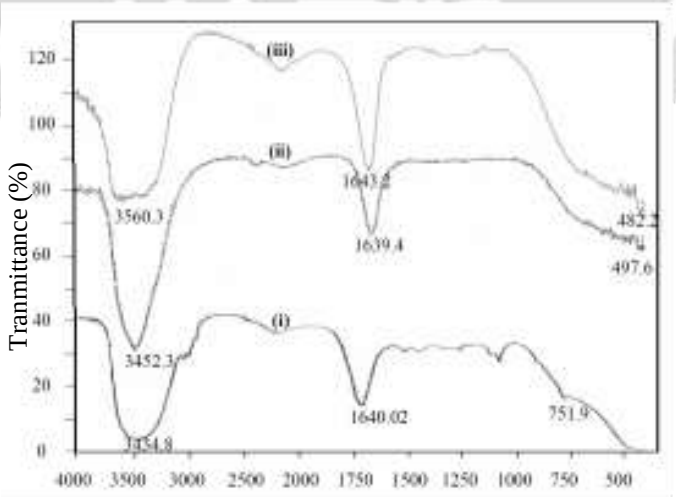

Fig -7: FTIR spectra of (i) ethanol(local)[7] (ii) crude ethanol by using Inoculum containing crude enzymes from Trichoderma and (iii) crude ethanol by using Inoculum containing crude enzymes from Aspergillus niger

4. CONCLUSIONS

The most prominent tested pretreatment condition was: hydrothermal treatment with a temperature of 80±5˚C during 180min. According to XRD results and sample composition analysis data, the lignin contents were decreased from 24% to 4% for hardwood (HS-5) and 26% to 3% for softwood (SS-5) for hydrothermal treatment. This condition gave the highest cellulose content and lesser lignin content than the other treatments in this investigation. Pretreated sawdusts from hardwood and softwood were then hydrolysed with two types of inoculum containing crude enzymes from *Trichoderma* and *Aspergillus niger* to produce glucose. The maximum glucose yield was 7.4% and 6% by crude enzymes from *Trichoderma* and *Aspergillus niger*. The total solid conversions were 30% for sawdust samples from softwood (SS-5) hydrolysed with inoculum containing crude enzyme from *Trichoderma* and 34% for sawdust samples from softwood (SS-5) hydrolysed with inoculum containing crude enzyme from *Aspergillus niger*. From the fermentation studies, the sawdust from softwood (SS-5) gave ethanol percent by weight of 4% by using inoculum containing crude enzyme from *Trichoderma* and 3% by using inoculum containing crude enzyme *Aspergillus niger*. The FTIR spectra of crude ethanol by using inoculum containing inoculum containing crude enzymes from *Trichoderma* and *Aspergillus niger* were similar as the ethanol (local).

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BIOGRAPHIES (Not Essential)

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