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Hydrothermal Pretreatment Enhanced Enzymatic Hydrolysis and Glucose Production from Lignocellulose Biomass

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Abstract - *The present works investigate hydrothermal pretreatment of Lignocellulose biomass, such as wood and agricultural residues, on chemical composition of sawdust samples from hardwood and softwood and subsequent bioethanol production applying pre-enzymatic hydrolysis and fermentation. Sawdust samples from hardwood and softwood were treated by preheating to boil 80±5˚C followed by adding sodium hydroxide solution (5% wt of sawdust) for 60min to 240min in the hydrothermal treatment. At reaction time (180min), 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 by absorbance measurement using the UV spectrophotometer. 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.*

Key Words: **Hydrothermal Pretreatment, Enzymatic Hydrolysis, Lignocellulosic Biomass, Sawdust, Glucose Production**

1. INTRODUCTION

Recent economic developments in many countries all around the globe have heightened the need for alternative energy resources due to the well-documented drawbacks of fossil fuels: (1) their finite supply (2) greenhouse gasses emission and global warming and (3) increasing price and unexpected fluctuation. All these weaknesses have strengthened the interest in alternatives, renewable, sustainable, and economically viable fuel such as bioethanol [1]. In the first generation bioethanol production, expensive starch and sugar derived from sugar cane and maize are employed as feedstock but in the second generation process, lignocellulosic materials, which are cheap, abundant and renewable, are used [2]. 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 [3]. However, the natural recalcitrance of lignocellulosic biomass has hindered its potential applications such as biochemical and biofuel production if no pretreatment is performed. Pretreatment is necessary to remove barriers such as hemicellulose and lignin that limit the penetration of enzyme to cellulose. Hydrothermal pretreatment has advantages over other pretreatments since the system only use water and the hydronium ion from water ionization act as catalyst in the reaction medium [4]. Under hydrothermal pretreatment process, several soluble inhibitors were produced, which hampered the efficiency of enzymatic hydrolysis and fermentation [5]. 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 [6]. The aim of the current study was to investigate the chemical changes in the process of hydrothermal pretreatment and the effect of the sawdust on the yield of inoculum containing crude enzymes from Trichoderma and Aspergillus niger. Factors affecting maximal conversion of cellulose to glucose was highlighted and discussed.

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 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.

2.3 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.

2.4 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 °C and weighed for determination of solid conversion.

2.6 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[3]. 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 [7].The presence of glucose can be detected by absorbance measurement using the UV spectrophotometer.

3. RESULTS AND DISCUSSION

3.1 Chemical Compositions of sawdust samples 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 .

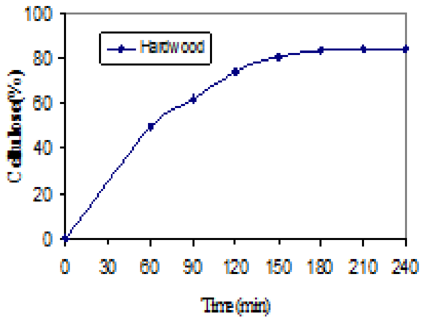

Fig -1: Percentage of cellulose from hardwood

According to results from hydrothermal treatment, these experimental data can be seen in Table 1.

Table -1: 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

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 hydrothermal treatment, sample HS-5and SS-5 of the lignin contents were decreased more than that of sample HS-0 and SS-0.

3.2 XRD Patterns of Untreated and Pretreated Sawdust Samples

The XRD patterns of untreated and pretreated sawdust samples from hardwood and softwood for hydrothermal treatment are shown in Fig. 2 and Fig. 3. According to Fig. 2, 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 crystalline.

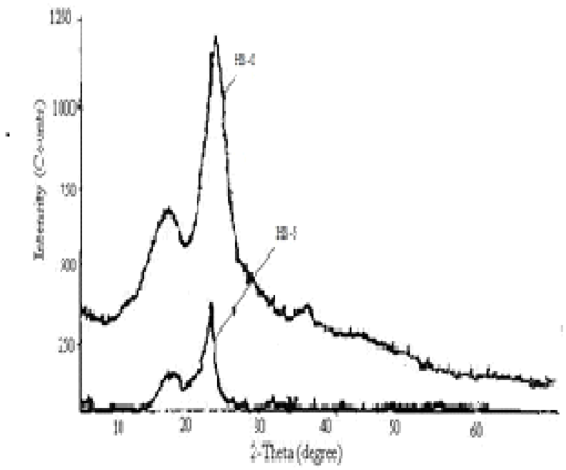

Fig -2: XRD patterns of untreated and pretreated sawdust samples from hardwood for hydrothermal treatment

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-0) 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 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-0) pretreatment condition.

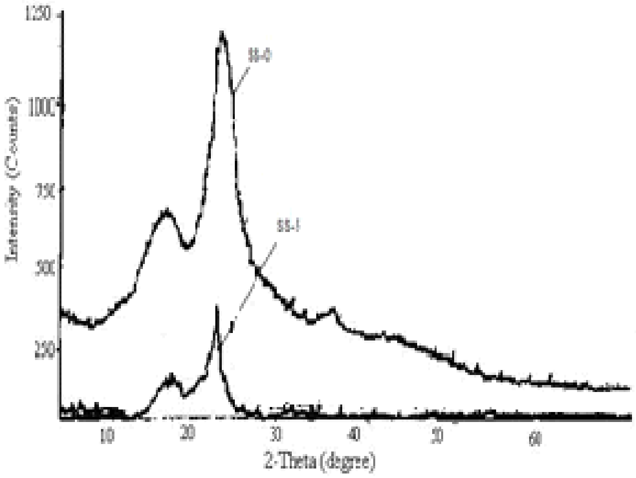

Fig -3: XRD patterns of untreated and pretreated sawdust samples from softwood for hydrothermal treatment

Sample (SS-5) could reduce the linkages between lignin, hemicellulose, and cellulose in sawdust. According to untreated and pretreated sawdust samples from softwood for 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.

3.3 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.

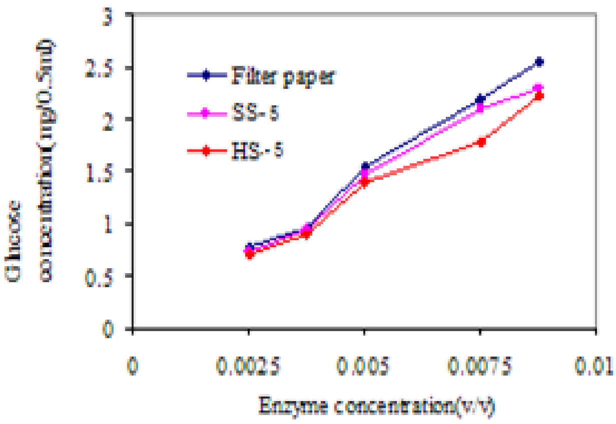

Fig -4: Amount of glucose liberated against Inoculum containing crude enzyme concentration from Trichoderma

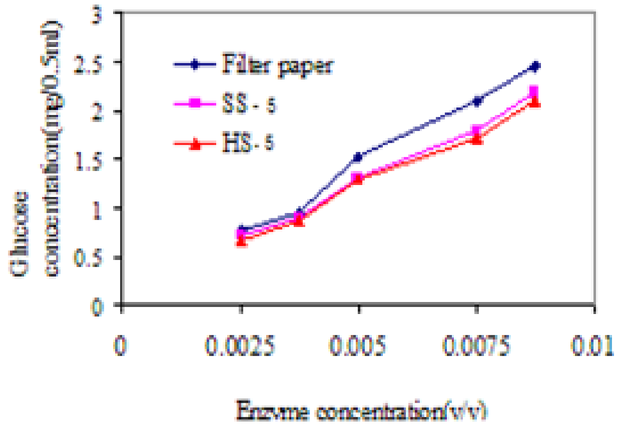

Fig -5: Amount of glucose liberated against Inoculum containing crude enzymes concentration from Aspergillus niger

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.

4. CONCLUSION

Hydrothermal pretreatment and enzymatic hydrolysis of Lignocellulosic biomass demonstrated high conversion of cellulose to glucose. 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.*

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BIOGRAPHIES

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