Akreditasi LIPI Nomor : 452/D/2010
Tanggal 6 Mei 2010

ULTRASONIC PRETREATMENT OF WOODCHIPS FOR THE CONVERSION OF CELLULOSE TO GLUCOSE FOR BIOETHANOL PRODUCTION

Tutun Nugraha[1,2], Rettyana Ayuputri[1] and Mohammad Ihsan[2]

[1]*Faculty of Life Sciences, Swiss German University*
BSD City, Tangerang, Indonesia
[2]*Center for Technology of Nuclear Industry Materials, National Nuclear Energy Agency*
Kawasan Puspiptek Serpong 15314, Tangerang, Indonesia
e-mail : tutun.nugraha@sgu.ac.id

ABSTRACT

ULTRASONIC PRETREATMENT OF WOODCHIPS FOR THE CONVERSION OF CELLULOSE TO GLUCOSE FOR BIOETHANOL PRODUCTION. In this study, lignocellulosic biomass i.e. the woodchips of Albacia tree (Paraserianthes falcataria) were given different pretreatment methods, ie. chemical (acid) and physical (ultrasonic). The pretreatment was given in order to convert the cellulose to glucose for the production of bioethanol. 1% H_2SO_4 was applied for the acid pretreatment. Ultrasound pretreatment was carried out at varied time (10, 20 and 30 minutes) at 600 W, 20 kHz before or after the acid pretreatment. Enzymatic attack of the pretreated sample was also applied to enhance the saccharification process of cellulose. The objective of the research was to determine the most effective ultrasonic duration and the best combination of method for enzymatic hydrolysis of the woodchips. The data showed that the highest yield of glucose was achieved at 20 minutes ultrasonic time. It was also found that substantial amount of hydrolysis of cellulose to glucose occur during the ultrasonic stage even without the presence of acid or cellulose enzyme. It is likely that the highly energetic ultrasonic process alone could assist in enhancing rate of hydrolysis of lignocellulosic cellulose into glucose.

Key words : Ultrasonic, Sonochemistry, Bioethanol, Lignocellulose, Cellulose, Wood, Enzyme

ABSTRAK

PERLAKUAN AWAL ULTRASONIK TERHADAP SERBUK KAYU UNTUK KONVERSI SELULOSA MENJADI GLUKOSA PADA PRODUKSI BIOETANOL. Dalam percobaan ini, biomasa lignoselulosa berupa serbuk kayu dari pohon Albasia (*Paraserianthes Falcataria*) memperoleh perlakuan awal (*pretreatment*) yang berbeda yaitu perlakuan kimia (asam) dan perlakuan fisik (ultrasonik). Perlakuan awal ini diberikan untuk mengkonversi selulosa menjadi glukosa untuk produksi bioetanol. H_2SO_4 1 % dipergunakan sebagai bahan untuk perlakuan kimia. Perlakuan ultrasonik dilakukan pada 600 W, 20 kHz dengan variasi lama waktu 10 menit, 20 menit dan 30 menit. *Enzyme* selulase juga dipergunakan dalam tahap lanjutannya untuk membantu proses sakarifikasi selulosa ini. Tujuan dari percobaan ini adalah untuk menentukan kisaran waktu ultrasonik yang paling optimal, serta mencari kombinasi metode yang paling efisien untuk proses hidrolisis *enzymatic* dari serbuk kayu. Hasil menunjukkan bahwa produk glukosa optimal diperoleh dengan proses ultrasonik selama 20 menit. Selain itu, konversi selulosa menjadi glukosa juga telah terjadi selama proses ultrasonikasi, tanpa penambahan asam ataupun penambahan *enzyme* selulase. Kondisi energetik yang tinggi selama proses ultrasonik telah dapat memfasilitasi proses hidrolisis untuk mengubah kandungan selulosa dalam lignoselulosa menjadi glukosa.

Kata kunci : Ultrasonik, *Sonochemistry*, Bioetanol, Lignoselulosa, Selulosa, Kayu, *Enzyme*

INTRODUCTION

Awareness of climate change and the need to reduce human impacts on the environment has increased significantly over the past decade. Depletion of world's petroleum supply and over environmental pollution such as greenhouse gas resulted in a concern to find alternative fuels. To fulfill future energy needs, renewable energy such as bioethanol is identified as one of the sources of biofuel to replace the non renewable fossil fuels. The use of sugar or carbohydrates as raw materials for the production of

Special Edition on Materials for Energy and Device 2010, page : 5 - 9
ISSN : 1411-1098

bioethanol is in question due to competition with food requirements. Hence, from the point of view of food security, different sources should be explored. Being abundant in nature and placed outside of the human food chain make the cellulosic biomass suitable for bioethanol production.

Cellulose, which is a polymer chain of glucose, is considered as one of the best candidate for this purpose. In order to prepare the material for digestion into glucose, pretreatment step is needed. Wood in particular contains lignin which binds the cellulose that gives the wood its structural strength. It is for this reason that wood is also known as a ligno-cellulosic material. The lignin presents a problem when the cellulose is intended for chemical digestion into glucose. It hinders any attack on the cellulose to break it into glucose monomer. Concentrated H_2SO_4, for example, can be used as a substance to eliminate the lignin binding through chemical attack. Furthermore, other methods have also been developed for the pretreatment of cellulosic substance such as extrusion, steam explosion, extraction, and enzymatic conversion [1].

However, all these processes are considerably expensive, and also considered not so environmental friendly, due to the use of substantial amount of chemical substances at high concentration (eg. H_2SO_4, neutralizing agents, extraction agents), energy (eg. extrusion, steam explosion) and high investment costs [2,3]. Hence, an alternative method such as ultrasonic treatment of the cellulosic mixture is considered. The main objective of this research was to investigate the effects of ultrasonic pretreatment on hydrolysis process of lignocellulosic materials.

EXPERIMENTAL METHOD

Materials

Paraserianthes falcataria (Albacia) was obtained from Cileungsi, Bogor, Indonesia. The Albacia woods were collected in March 2010. They were sundried for 2 days, milled to the size of 0.25 mm, and were kept in airtight bottles at room temperature. Purified Cellulase powder from Aspergillus niger and concentrated sulfuric acid were obtained from Sigma Aldrich. All analysis were performed in triplicate or more, and the data were reported on a dry matter basis.

Methods

Acid Pretreatment

About 50 g of dried ground sample (mesh 60) was suspended in 1% H_2SO_4 solution in ratio of 1 : 10 (w/v). After that, the mixtures were autoclaved at 121°C for 15 minutes. Hydrolysate was pressed through cheese cloth and analyzed for reducing sugar level [4].

Ultrasonic Pretreatment

Acid pretreated sample was placed in a special glass vessel (Figure 1) and suspended in aquadest in ratio 1 : 20 (w/v). It was treated with ultrasonic at 600 W, 20 kHz (VCX 750, Sonics Vibra Cell, USA) for varied duration (10, 20 and 30 minutes). The temperature during ultrasonic treatment was kept at 30 °C. After that, the sample was centrifuged at 8,000 rpm, 4 °C for 15 minutes to clarify the solution. The supernatant were analyzed for reducing sugar concentration. The solid residue was finally subjected to enzyme treatment.

Enzymatic Hydrolysis

0.2 g of treated sample, 2.5 mL water, 1 mL citrate buffer pH 5, and 0.1 mL from 0.2 %cellulase enzyme solution were mixed, and incubated for 2 hours at 37 °C. The mixture was pressed through cheese cloth and then centrifuged at 8,000 rpm, 15 minutes, 4 °C to clarify the solution. 1 mL of the supernatant was taken into test tubes. Subsequently, 1 mL 3,5-dinitrosalisylic Acid (DNS) reagent was added, and the solution was boiled for 5 minutes, and then allowed to cool to room temperatures. The glucose released was measured by spectrophotometer at 540 nm.

RESULTS AND DISCUSSION

Ultrasonic energy was thought to affect both physical and chemical characteristics of the biomass. The process was carried out in a special glass vessel (Figure 1), so that the circulation of raw materials which was necessary to avoid agglomeration was easily observable. There were 4 external loops that continuously delivered the biomass under the vibrating tip of the probe. The vessel was suspended in a water bath to

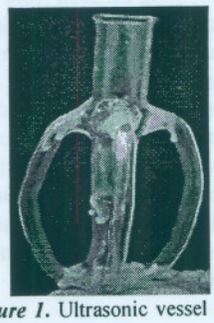

Figure 1. Ultrasonic vessel design

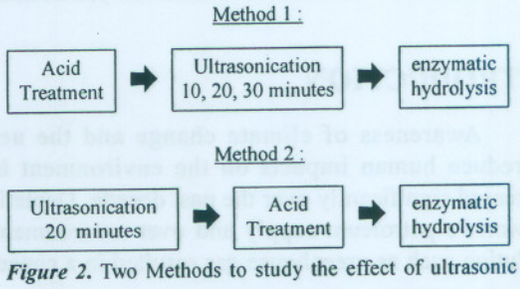

Figure 2. Two Methods to study the effect of ultrasonic

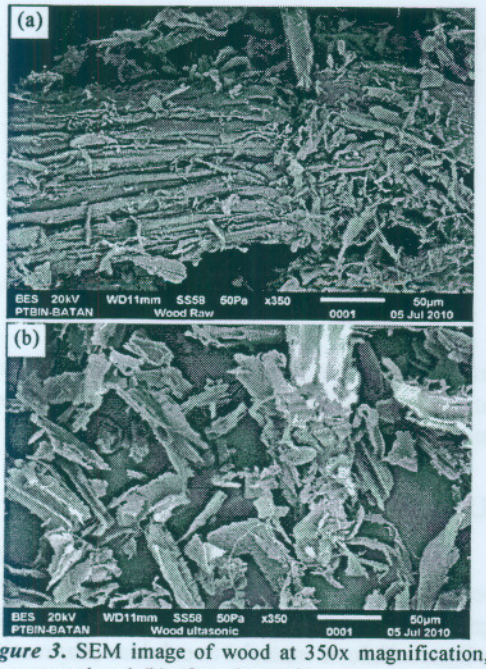

Figure 3. SEM image of wood at 350x magnification, (a) untreated and (b) after ultrasonic

keep the temperature at 30 °C. The experiments were carried out using 2 methods, in which the sequences of acid and ultrasonic pretreatment method were interchanged (Figure 2).

The Role of Ultrasonic as Physical Treatment of Raw Materials

Physical force exerted by ultrasonic wave caused a tremendous particle size reduction. This reduction in size yielded an increase in the ratio of surface area to volume ratio of the lignocellulosic materials. This would be beneficial since it would expose more cellulose moleculs that were available for chemical reactions. The heterogeneous reactions involving polymeric cellulose in the solid phase and the rest of the reactants in the aqueous phase required that the cellulose be physically exposed to the reactants in the aqueous phase.

Ultrasonic exerts its effects by means of generating bubbles inside a liquid medium, termed cavitation. The sound energy created by the ultrasonic probe acted as a source of vibrational wave energy that worked on the molecule within the liquid. This energy produced alternate compressions and stretches towards the liquid medium that produced bubbles. These bubbles are exposed to the same vibrational stresses within the liquid medium, and would grow and eventually collapse violently. During this collapse, theoretical temperature could reach over 5,000 K and pressure of up to 2,000 atmosphere. The uniform ultrasonic field creates millions of bubbles throughout the liquid, and destroyed thousands of times per second [5,6]. This local extreme of heat and pressure could cause the bonds within the biomass structure to break, resulting in torn and shattered structures as shown in Scanning Electron

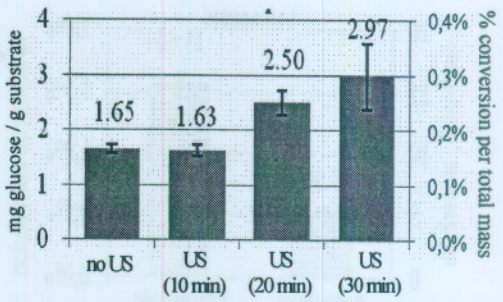

Figure 4. The role of ultrasonic on enzymatic hydrolysis of acid pretreated (first method) Woodchips

Microscope (SEM) results (Figure 3). The smaller the size of particles, the higher was the surface area. Due to this higher surface area, the enzyme or acid would have an easier access to hydrolyze the cellulose. Moreover, it is likely that the local high temperature would provide some energy for chemical reactions to occur, including the hydrolysis of cellulose into glucose. Figure 4 showed that ultrasonic treatment has significantly increased the enzyme hydrolysis of cellulose in woodchips. Based on statistical analysis, 20 minutes was the optimum duration for ultrasonic. The 30 minutes treatment did not give significant increase.

The amount of sugar released by the enzyme hydrolysis step in this study still required optimization in terms of the process conditions such as longer incubation period and higher enzyme cellulase concentration. Different cellulase sources should also be considered. Possibly the use of other sources of cellulase enzyme such as Trichoderma sp. could perform better than the results achieved in this research using cellulase enzyme from Aspergillus niger. Nevertheless, the impact of ultrasonic in the glucose production from lignocellulosic material has been shown here.

Ultrasonic Induced Hydrolysis

Other than its physical effects, ultrasonic appeared to be able to induce chemical effects in the form of hydrolysis of cellulose to glucose at low ambient temperature. It was found that substantial amount of glucose could be released during the ultrasonic stage. This phenomenon was illustrated by performing the lignocellulosic digestion using ultrasonic (30 °C) for 20 minutes, compared to digestion utilizing an autoclave device (121 °C, 15 minutes). In both cases, water was used as reaction medium. The results showed that substantial amount of glucose could be released during the ultrasonic process (Figure 5). This indicated that some cellulose molecules, which are polymers of glucose (Figure 6), were broken down into glucose during the ultrasonic stage, and that the amount was higher compared to the treatment at 120 °C in an autoclave system. Hydrolysis of cellulose that would typically happen at high temperature, could occur at relatively low temperature under ultrasonic irradiation. Some

Special Edition on Materials for Energy and Device 2010, page : 5 - 9
ISSN : 1411-1098

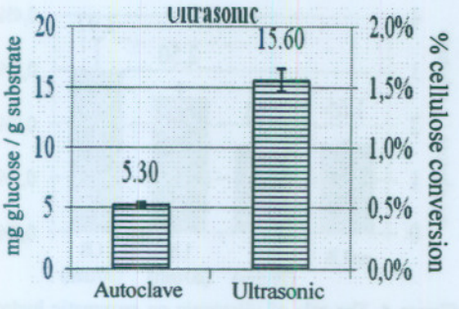

Figure 5. Comparison on the sugar released from woodchips treated with Autoclave and Ultrasonic

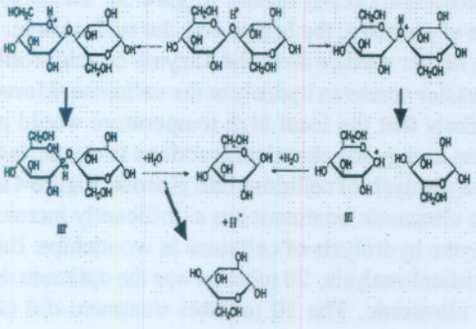

Figure 6. Mechanism of Hydrolysis [7]

authors who studied similar research did not report this phenomena, possibly due to the use of lower level of power during the ultrasonic treatment [4].

Ultrasonic As Initial Treatment Before Acid Treatment

In the method described above, the ultrasonic treatment was done after the acid pretreatment. In the second method (Figure 7), the sequence was reversed in which the ultrasonic was done prior to acid treatment. However, the results showed that the amount of sugar released was smaller using this second method. It appeared that acid digestion is best done during the initial stage, followed by the ultrasonic treatment.

This chemical treatment might have softened the lignocellulosic material by attacking the lignin bonds. This would make it easier for the ultrasonic wave to exert its effects on the material. Based on this observation, acid seemed to have attacked the lignin that binds the cellulose in a more efficient manner compared to ultrasonic. Once the lignin was damaged by the acid pretreatment, the ultrasonic could exert its impact without being hampered by the presence of lignin.

Based on this result, this 2nd method was not pursued further. It is interesting to note, however, that simultaneous ultrasonication and acid pretreatment could be performed as well. Under the circumstance, the acidity could attack the lignin while being assisted by the power of ultrasonic. This simultaneous process was not being

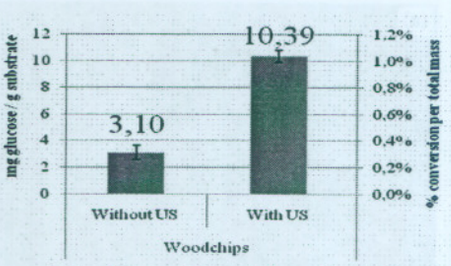

Figure 7. Total Sugar Released from Woodchips Using the 2nd method

done in this investigation since there was a possibility that the acid could damage the ultrasonic probe. This method, however, is noted for further study.

CONCLUSION

Physical treatment by ultrasonic treatment as attempted in this research (600 W, 20 kHz, 30 °C) has significantly increased the accessibility of cellulose in the lignocellulosic material to celulase enzymatic attack for its conversion to glucose. This effect was partially caused by the reduction in size of particle of the woodchips that increased the surface area as particle size was getting smaller. Hence, more cellulose was opened for chemical attack. Acid pretreatment prior to the ultrasonication yielded good result that was better than if the ultrasonication was done prior to the acid pretreatment. The chemical attack by the acid appeared to play important roles in damaging the lignin bond. Ultrasonic alone seemed to be unable to efficiently remove this lignin bond. Simultaneous atack by acid and ultrasonic energy was recommended to be attempted in further experiments. Moreover, ultrasonic was also found to induce the hydrolysis reaction of cellulose into glucose even at low temperature (30 °C). The highly energetic process that occured during the appearance and the colapse of the ultrasonic cavitation could have provided the energy for the conversion of cellulose into glucose, that otherwise could ony happen at high temperatures.

REFERENCES

[1]. H.L. CHUM, L.J. DOUGLAS, D.A. FEINBERG, H.A. SCHROEDER, *Evaluation of Pretreatments of Biomass for Enzymatic Hydrolysis of Cellulose*, Solar Energy Research Institute, Report Prepared for US Department of Energy, Golden, Colorado, (1985) 1-64

[2]. DANIEL SCHELL, QUANG NGUYEN, MELVIN TUCKER, BRIAN BOYNTON, *Biotechnology*, **70-72** (17) (1998)

[3] MELVIN P. TUCKER, KYOUNG H. KIM, MILDRED M. NEWMAN, QUANG A. NGUYEN, *Applied Biochemistry and Biotechnology*, **105** (1-3) (2003)

[4] N. YOSWATHANA, P. PHURIPHIHAT, P. TREYAWUTTHIWAT, M. N. ESHTIAGHI, *Energy Research Journal*, **1** (2010) 26-31

[5]. E.B. FLINT and K.S. SUSLICK, *Science*, **253** (1991) 248-249

[6] MING FEI LI, YONG MING FAN, RUN CHANG SUN, FENG XU, *Bioresources*, **5** (3) (2010) 1762-1778

[7]. Q. XIANG, Y.Y. LEE, P.O. PETTERSSON, R.W. TORGET, *Applied Biochemistry and Bioetchnology*, **107** (2003) 505-514