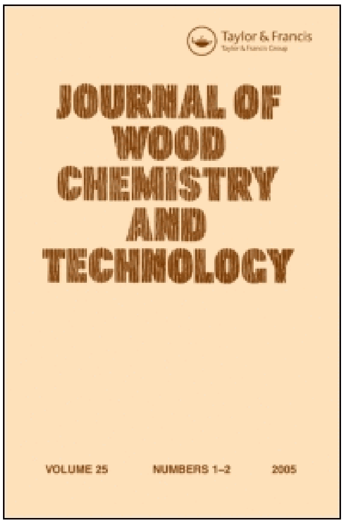

Journal of Wood Chemistry and Technology

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597282

Enhancement of Enzymatic Hydrolysis of Lignocellulosic Wastes by Microwave Pretreatment Under Atmospheric Pressure

P. Kitchaiya[a]; P. Intanakul[a]; M. Krairiksh[b]

[a] Department of Chemical Engineering, Faculty of Engineering, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand [b] Department of Telecommunication Engineering, Faculty of Engineering, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand

Online publication date: 06 March 2003

To cite this Article Kitchaiya, P. , Intanakul, P. and Krairiksh, M.(2003) 'Enhancement of Enzymatic Hydrolysis of Lignocellulosic Wastes by Microwave Pretreatment Under Atmospheric Pressure', Journal of Wood Chemistry and Technology, 23: 2, 217 — 225

To link to this Article: DOI: 10.1081/WCT-120021926

URL: http://dx.doi.org/10.1081/WCT-120021926

JOURNAL OF WOOD CHEMISTRY AND TECHNOLOGY
Vol. 23, No. 2, pp. 217–225, 2003

Enhancement of Enzymatic Hydrolysis of Lignocellulosic Wastes by Microwave Pretreatment Under Atmospheric Pressure

P. Kitchaiya,[1,*] P. Intanakul,[1] and M. Krairiksh[2]

[1]Department of Chemical Engineering and [2]Department of Telecommunication Engineering, Faculty of Engineering, King Mongkut's Institute of Technology Ladkrabang, Ladkrabang, Bangkok, Thailand

ABSTRACT

A technique to improve enzymatic hydrolysis of lignocellulosic wastes by microwave pretreatment under atmospheric pressure was developed. Ground rice straw or sugar cane bagasse immersed in a glycerine medium with small amounts of water, was treated with 240 W of microwave irradiation for 10 min at atmospheric pressure. A temperature of about 200°C could be reached in this medium without high pressure build up. More than twice the amount of reducing sugars was produced from enzyme saccharification utilizing the microwave pretreatment, compared with no pretreatment. The microwave pretreatment which achieves high temperatures at

*Correspondence: Dr. P. Kitchaiya, Department of Chemical Engineering, Faculty of Engineering, King Mongkut's Institute of Technology Ladkrabang, Ladkrabang, Bangkok 10520, Thailand; E-mail: kkprakob@kmitl.ac.th.

DOI: 10.1081/WCT-120021926
0277-3813 (Print); 1532-2319 (Online)

atmospheric pressure provides some advantages over a steam explosion process which requires high pressure and a subsequent sudden pressure drop.

Key Words: Microwave pretreatment; Lignocellulosic wastes; Enzyme hydrolysis.

INTRODUCTION

Lignocellulosic wastes have been proposed as large renewable resources for chemicals and sugars. Effective conversion of lignocellulosic materials to fermentable sugars would significantly reduce the production cost of subsequent fermentation products. There are three major components in lignocellulosic materials: hemicellulose, lignin, and cellulose. The susceptibility of lignocellulosic materials to enzymatic saccharification of the cellulosic is limited due to the presence of the complex structure of lignin and hemicellulose with the cellulose. Therefore, various pretreatment techniques of lignocellulosic materials to enhance saccharification by cellulase enzyme have been developed.[1] An efficient pretreatment of lignocellulosic wastes by steam explosion has been reported.[2,3] In this pretreatment, the lignocellulosic wastes are subjected to saturated steam at high temperature (200–270°C) and high pressure (14–60 atm) for a short time (20 s–10 min). The pressure is then dropped quickly as the material exits the pressure vessel. Steam at temperature above 170°C can soften hemicellulose and lignin. The sudden release of the high pressures (above 14 atm) to atmospheric pressure in the process can partially fibrize the softened material. This pretreatment increases the accessibility of enzymes to the cellulose in a later saccharification step.[4] Further, steam explosion at this high temperature also causes hydrolysis of hemicellulose and some delignification such that smaller molecules that can be removed from the cellulose with water and methanol, respectively. Thus, the physical treatment coupled with hydrolysis removes some hemicellulose and lignin from the cellulose.

Brownell et al.[5] varied the pressure drop for the steam explosion pretreatment of wood at 32.4, 6.9, and 3.4 atm and concluded that the explosion part of the steam explosion process contributes essentially nothing to the enzymatic accessibility in saccharification. Azuma et al.[6] enhanced the enzymatic susceptibility of cellulose in lignocellulosic wastes by microwave irradiation. They soaked rice straw (60–80 mesh, 2 g) in water (14 mL) in a *tightly* closed glass vessel and applied microwave irradiation of 2.4 kW at 2450 MHz for 8 min. Hemicellulose and lignin were separated, hydrolyzed, and dissolved in water and methanol, respectively. The cellulose residue without a notable change in degree of crystallinity was saccharified by cellulase producing large amounts of reducing sugars. Thus, experimental results similar to steam explosion were obtained with the microwave irradiation procedure. The glass vessels with stainless-steel stoppers that they used in their experiments could withstand the pressure that developed at 230°C in the treatment. Ooshima et al.[7] performed similar microwave experiments in sealed glass vessels and obtained similar results.

The studies of Brownell et al.,[5] Azuma et al.,[6] and Ooshima et al.[7] all involved elevated pressures. This investigation was directed toward whether the reducing sugar yield could be enhanced without the use of the higher pressure but still using the microwave irradiation. The vapor pressure of water at 200°C is about 15 atm.[8] However, a compound could be added to the water to depress the vapor pressure of the system and absorb the microwave irradiation. According to the vapor–liquid equilibrium data,[8] a mixture of water and glycerine with 10.3 mol% water has a total pressure of 1 atm at 202°C. Compounds with high dielectric constant can absorb microwave irradiation well. The dielectric constant of glycerine at 20°C is 41.1, compared with 80.4 for water.[9] Thus, a mixture of water and glycerine was selected as the immersion medium for the lignocellulosic waste during the microwave treatment in this work.

MATERIAL AND EXPERIMENTAL METHODS

Microwave Pretreatment

Ground rice straw (0.2 g, 60 mesh, from a paddy field in the central region of Thailand) or sugar cane bagasse (from a local cane sugar factory) was immersed in glycerine solution in 25-mL screw cap vials (Kimble). The vial caps were loosely closed to release any built up pressure. Glycerine solutions containing different amount of water were prepared by adding water to 7 and 10 g glycerine (Merck). Four vials, containing the ground lignocellulosic wastes immersed in glycerine solutions, were placed in a 250-mL empty beaker. This beaker was positioned at the center of a rotating circular glass plate in a domestic microwave oven (Turbora Model TRX-1963, 2450 MHz) for the microwave treatment. The applied microwave irradiation was 240 W, for 10 min, for all experiments.

Extraction of Hemicellulose and Lignin

Water (40 mL) was added to the mixture of microwave pretreated lignocellulosic waste and glycerine, and the mixture was stirred and centrifuged. The liquid was filtered through a nylon-6,6 membrane (0.45 μm, 13 mm diameter, Alltech), washed five to six times with water, and the filtrate was analyzed for the amount of reducing sugars hydrolyzed from hemicellulose by DNSA method.[10] Next, the hydrolyzed lignin was removed by immersing the pretreated material in methanol overnight. The liquid was separated from the solid material by centrifuge, and the solids were washed three times with water.

Enzyme Saccharification of Cellulose

The rice straw or bagasse residue (with the hemicellulose and lignin removed) was hydrolyzed by a cellulase. Celluclast 1.5 L (2 mL, a cellulase from *Trichoderma reesei*, 1,500 NCU/gm, from Novo Nordisk) in 50 mL of 0.1 M acetate buffer (pH 4.8) with 12 drops of toluene added as an antiseptic, was used to saccharify residual cellulose wastes. The incubation temperature was 40°C and samples were shaken at 150 cycles/-min in a shaker for two days. The mixtures were centrifuged and the supernatants were filtered through the 0.45 μm Nylon-6,6 membrane. The filtrates were analyzed for the amount of reducing sugars saccharified from cellulose by the DNSA method.[10] Each experiment was repeated four times and the average value was reported. It was observed that the microwave irradiation pretreatment in glycerine solution resulted in only a small amount of paste at the bottom of the centrifuge tube after the saccharification, compared with untreated samples in which large amounts material remained after saccharification.

RESULTS AND DISCUSSION

The temperatures of ground rice straw immersed in glycerine solution as functions of microwave irradiation time are shown in Fig. 1. The temperatures were quickly measured using a thermometer after the specified irradiation time. The temperature above 180°C, which is necessary for softening of hemicellulose and lignin, could be reached within 4 min of treatment.

The release of reducing sugars from the hemicellulose of the rice straw pretreated by microwave irradiation was in the range of 2–4%

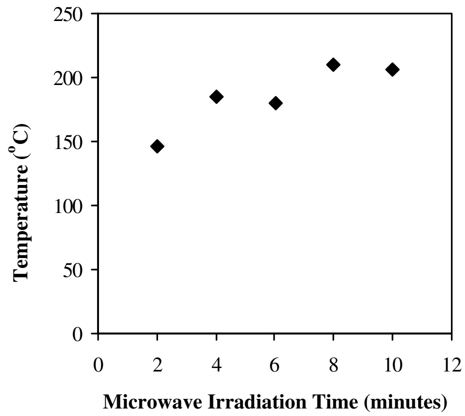

Figure 1. Temperature as a function of time for ground rice straw in a glycerine solution (7 g glycerine containing 0.7% water) subjected to 240 W of microwave irradiation.

(Fig. 2, empty symbols). A significant increase in the yield of reducing sugars (43–55%, Fig. 2, filled symbols) was obtained by further extraction of the rice straw with methanol to remove lignin followed by saccharification with cellulase. The amounts of reducing sugars obtained in our experiment are comparable to those reported by Azuma et al.[6] They reported 33–55% reducing sugars produced from saccharification of cellulose in rice straw immersed in water and pretreated with microwave irradiation in a *tightly sealed* high pressure glass tube. However, the amount of reducing sugars produced from hemicellulose from rice straw after only microwave pretreatment reported by Azuma et al.[6] was somewhat higher (1–10%) than that found in our work.

In control experiments where the rice straw immersed in glycerine solutions was untreated by microwave irradiation, the reducing sugars produced from hemicellulose hydrolysis and cellulose saccharification were found to be 0.6 and 22%, respectively. Further experiments were carried out by replacing glycerine with *pure water* following by microwave irradiation. In such experiments, the amounts of reducing sugars obtained were close to those found in previous control experiments. However, the temperatures of the mixture after microwave irradiation were around 100°C as pure water was used instead of glycerine. Thus, the results from these control experiments suggested that the yield of reducing sugars could be improved by microwave irradiation using water in conjunction with glycerine as the immersion medium.

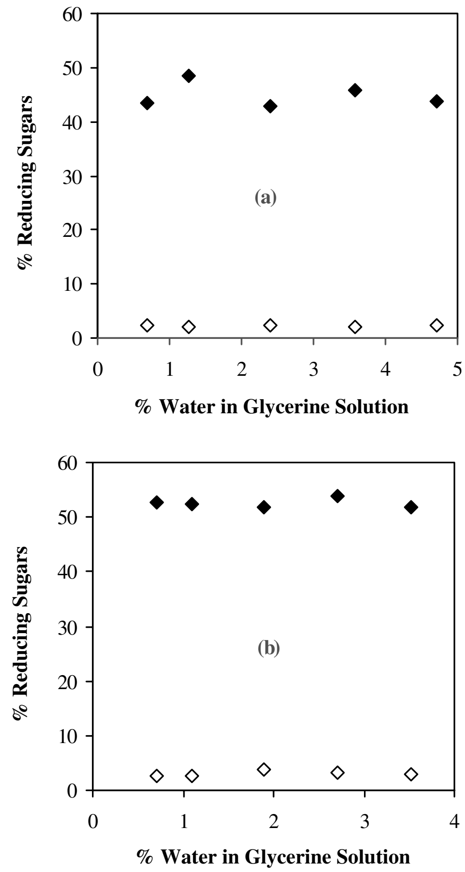

Figure 2. Reducing sugars produced from microwave treatment of rice straw immersed in glycerine solutions at different amounts of water filled in *loosely closed* vials. Empty and filled symbols represent reducing sugars produced from hydrolysis of hemicellulose and subsequent saccharification of cellulose, respectively. Figures (a) and (b) are 7 and 10 g glycerine in the glycerine solution, respectively.

Figures 3 and 4 show the amount of reducing sugars obtained from hemicellulose hydrolysis and cellulose saccharification for the case in which the mixture of rice straw or bagasse immersed in glycerine solutions was fully exposed to the atmospheric pressure during the microwave irradiation. Although the mixture vials were uncapped, there was no spillover observed. The amount of reducing sugars obtained from the loosely capped vials (Fig. 2) and uncapped vials (Figs. 3 and 4) show no

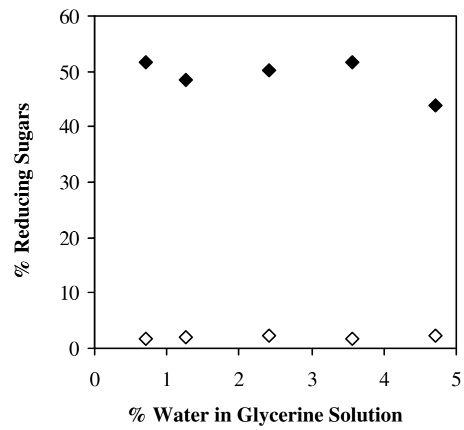

Figure 3. Reducing sugars produced from microwave treated rice straw immersed in glycerine solutions (7 g glycerine) in *open* vials. Empty and filled symbols represent the reducing sugars produced from the hydrolysis of hemicellulose and subsequent saccharification of cellulose, respectively.

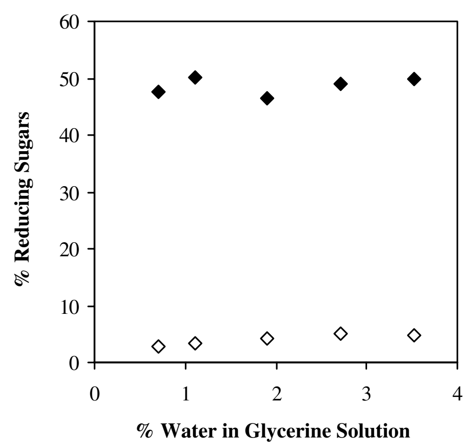

Figure 4. Reducing sugars produced from microwave treated bagasse immersed in glycerine solutions (10 g glycerine) in *open* vials. Empty and filled symbols represent the reducing sugars produced from hydrolysis of hemicellulose and subsequent saccharification of cellulose, respectively.

significant difference, suggesting that high pressure is not necessary for high yield of reducing sugars.

From the experimental results, the microwave pretreatment enhanced the removal of hemicellulose and lignin from cellulose in rice straw and bagasse. The key factor that affects the separation is the high temperature, i.e., 175–210°C. In this high temperature range, acetic acid in the presence of water is hydrolyzed from the hemicellulose of the lignocellulosic materials and catalyzes the hydrolysis of hemicellulose and lignin. Autohydrolysis of hemicellulose and lignin by the release of acetic acid from hemicellulose when the aspen wood pretreated by water under high pressure at temperatures of 175–220°C has been reported.[11] Azuma et al.[6] also observed that the release of acetic acid from hemicellulose at high temperature obtained during microwave irradiation caused hydrolysis of hemicellulose and lignin.

Further, the fact that the microwave pretreatment of lignocellulosic wastes at atmospheric pressure provided large amount of reducing sugar from hemicellulose hydrolysis and cellulose saccharification indicates that the high pressure and sudden pressure drop of steam explosion are not required for effective pretreatment. The small amounts of water in glycerine solutions, within the range studied, had no significant effect on the amount of reducing sugar produced. As previously stated, the small amounts of water added to glycerine contribute to the high temperature of the mixture (ca. 200°C), and only a small amount of water is required for the hydrolysis of hemicellulose and lignin in rice straw and bagasse.

CONCLUSIONS

Enhancement of enzyme saccharification of rice straw by the microwave irradiation at atmospheric pressure could be accomplished by immersing rice straw or sugar cane bagasse in the glycerine solution. High pressure and a sudden pressure drop were not necessary for the hydrolysis of hemicellulose and lignin in the pretreatment step. High temperature played a significant role in the acid-catalyzed hydrolysis of hemicellulose and lignin. This result could be applied to design a new lignocellulosic waste pretreatment process for chemical separation operating at atmospheric pressure and high temperatures.

ACKNOWLEDGMENTS

This work was fully supported by The Thailand Research Fund. The authors thank East Asiatic (Thailand) Company for supplying Celluclast 1.5 L and Dr. Apinan Namkanisorn for some helpful discussions.

REFERENCES

1. Hon, D.N.S.; Shirashi, N. Chemical degradation In: *Wood and Cellulosic Chemistry*; Marcel Dekker, Inc.: New York, 1991; 478–483.
2. Saddler, J.N.; Ramos, L.P.; Breuil, C. Steam pretreatment of lignocellulosic residues. In *Bioconversion of Forest and Lignocellulosic Plant Residues*; Saddler, J.N., Ed.; CAB International: UK, 1993; 73–91.
3. Higuchi, Y. Steam explosion of wood. In *Biomass Handbook*; Kitani, O., Hall, C.W., Eds.; Gordon & Breach Science Publishers: USA, 1989; 470–474.
4. Puri, V.P.; Mamers, H. Explosive pretreatment of lignocellulosic residues with high-pressure carbon dioxide for the production of fermentation substrates. Biotechnol. Bioeng. **1983**, *25* (12), 3149–3161.
5. Brownell, H.H.; Yu, E.K.C.; Saddler, J.N. Steam-explosion pretreatment of wood: effect of chip size, acid, moisture content and pressure drop. Biotechnol. Bioeng. **1986**, *28* (6), 792–801.
6. Azuma, J.; Tanaka, F.; Koshijima, T. Enhancement of enzymatic susceptibility of lignocellulosic wastes by microwave irradiation. J. Ferment. Technol. **1984**, *62* (4), 377–384.
7. Ooshima, H.; Aso, K.; Harano, Y. Microwave treatment of cellulosic materials for their enzymatic hydrolysis. Biotechnol. Lett. **1984**, *6* (5), 289–294.
8. Perry, R.H.; Green, D.W. *Perry's Chemical Engineers' Handbook*, 6th Ed.; McGraw-Hill: USA, 1984; 13–14 and 3–237.
9. Akhadov, Y.Y. *Dielectric Properties of Binary Solutions*; Pergamon Press: UK, 1980; 284.
10. Miller, L.G. Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal. Chem. **1959**, *31*, 426–428.
11. Lora, J.H.; Wayman, M. Delignification of hardwoods by autohydrolysis and extraction. Tappi **1978**, *61* (6), 47–50.

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016