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Effect of Steam Explosion on the Physicochemical Properties and Enzymatic Saccharification of Rice Straw

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ABSTRACT

The effects of steam explosion pretreatment on the physical and chemical properties of rice straw and on the enzymatic saccharification of the straw were investigated. A wide range of pretreatment conditions were tested. Pretreatment effects were assessed by morphological changes, pore size distribution, pH, soluble sugars concentration, amounts of extracted components, and by enzymatic saccharification of pretreated samples. Rice straw was very finely defiberized by steam explosion. A great increase in pore volume accessible to cellulolytic enzymes was observed during pretreatment at 3.53 MPa for 2 min. On the other hand, pretreatment under very high steam pressure of 4.02 MPa reduced fiber porosity. The steam explosion products were separated into hemicellulose, cellulose, methanol-soluble lignin, and klason lignin. Increasing the steaming time at high steam pressures (temperatures) increased the amount of klason lignin and reduced the amount of hemicellulose, thus emphasizing the need to identify optimal pretreatment conditions for efficient use of biomass. A maximum 83% glucose yield was obtained by enzymatic saccharification of the sample exploded at 3.53 MPa for 2 min. These explosion conditions produce a material that is highly susceptible to attack by cellulolytic enzymes.

Index Entries: Rice straw; steam explosion; physicochemical properties; enzymatic saccharification.

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Abbreviations: Ai, accessible pore volume (mL/g); C_G, concentration of glucose (g/L); C_R, concentration of reducing sugar (g/L); C_X, concentration of xylose (g/L); di, solute molecule diameter (nm); f, frequency distribution of particle (%/μm);L, length of exploded rice straw fiber (μm); P, steam pressure (MPa); t, steaming time (min); W_C, weight of cellulose (g); W_H, weight of hemicellulose (g); W_K, weight of klason lignin (g); W_S, weight of methanol soluble lignin (g).

INTRODUCTION

Lignocellulose constitutes the world's most widely available low-cost renewable resource from which many useful substances, including fuel alcohols, can be derived (*1*). One way of utilizing lignocellulosic materials is to hydrolyze them enzymatically into fermentable sugars, which can then be converted to ethanol (*1,2*).

The variety of lignocellulosic materials available for bioconversion is truly enormous. Recently, low-cost agricultural residues have become attractive for the production of ethanol, because agricultural residues are available in large quantities and are rountinely cultivated (*3*).

The enzymatic degradation of heterogeneous lignocellulose is primarily governed by its physicochemical nature (*4*). Cellulose and the hemicellulose polysaccharides in native lignocellulose are largely protected from attack by cellulolytic enzymes. The inaccessibility to attack is mainly owing to the association of these polysaccharides with lignin and with each other, all of which act as a barrier shielding the polysaccharides. Some form of pretreatment is necessary for the polysaccharide components to become accessible to enzymes, providing high conversion yields to monosaccharides (*5*).

The steam explosion process has attracted attention recently as an effective pretreatment technique for efficient utilization of lignocellulosic biomass (*6–10*). This process has both physical and chemical actions that render the lignocellulose easily separable into its three main components: cellulose, hemicellulose, and lignin (*6,8,11*). This process involves steaming lignocellulosic substrates at high temperature and pressure followed by rapid reduction in pressure to expel the material from the reactor vessel. The treatment results in substantial breakdown of the lignocellulosic structure, hydrolysis of the hemicellulosic components, depolymerization of the lignin components, and defibration. Therefore, the accessibility of the cellulose components to degradation by enzymes is greatly increased (*6,12,13*).

In the steam explosion process, the chemical changes that take place in the three main components of lignocellulose during autohydrolysis are very much dependent on termperature, pressure, and steaming time (*14–16*). The initial reaction of all steaming processes involves a mild acidcatalyzed hydrolysis of glycosidic bonds of the hemicellulose and the α-ether linkages of lignin (*14,17,18*). Under severe steaming conditions, such as very high temperatures and pressures, and/or at longer steaming times, some of the pentoses are converted to furfural and hexoses are converted to 5-hydroxymethylfurfural, which may then react with lignin to form further condensation products (*14*). It is thus essential to evaluate the effects of steam explosion pretreatment conditions on all the constituents of the lignocellulosic material so as to maximize total product values.

Several investigations have been carried out to improve the enzymatic hydrolysis yields of sugars from lignocellulose by steam explosion (*7,9,19,20*). However, only a few have been concerned with the effects of pretreatment on the physical and chemical properties of the substrate. To utilize lignocellulosic biomass more efficiently by better understanding the process, it is important to know how the properties of the exploded products change under various conditions of pressure, temperature, and time of treatment.

In this article, the effects of explosion conditions on the physical and chemical properties of rice straw were investigated with respect to changes in morphology, pore size distribution, pH, amounts of extracted components, soluble sugar concentration, and subsequent enzymatic saccharification of pretreated samples.

MATERIALS AND METHODS

Substrate

Air-dried rice straw (obtained locally) was reduced to about 10 cm by chopping and then used as raw material.

Steam Explosion Apparatus

The steam explosion apparatus (shown in Fig. 1) consisted of a steam generator, a pressurized reactor, a receiver, and a condenser with a silencer. The reactor was insulated to maintain a constant temperature. The capacity of the reactor was 1.2 L, the maximum working pressure and temperature were 6 MPa and 275°C, respectively. Approximately 100 g of straw were introduced into the reactor, which was then steam-heated. After the desired steaming time, a ball valve at the bottom of the reactor was suddenly opened to bring the reactor rapidly to atmospheric pressure. The solid and liquid products were explosively released into a collection device. The gaseous products were passed from the top of the receiver into the condenser.

The operating variables examined in this study were the steaming time (0.5–10 min) and the steam pressure (2.55, 3.04, 3.53, and 4.02 MPa).

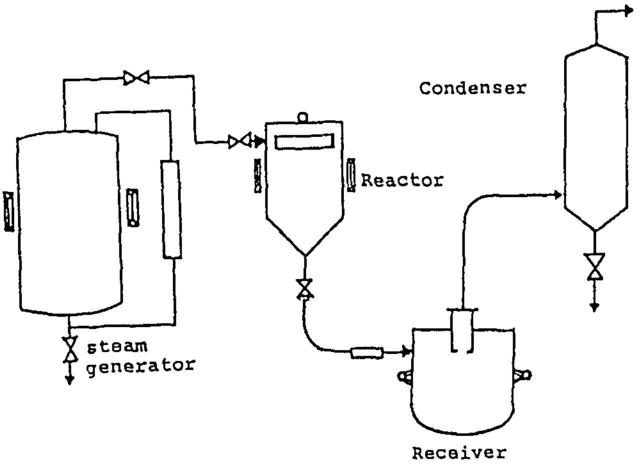

Fig. 1. Steam explosion apparatus.

The reaction temperatures for these pressures were 225, 235, 243, and 251°C respectively.

Measurement of Size Distribution

The explosion produced a mass of fibrous fragments, ranging in size from fairly large fibers to very small defiberized fibers, depending on the explosion conditions. The relationship between the shape of the fragments and the conditions of the explosion was studied by measuring the lengths of 400–500 fragments and determining their distributions.

Measurement of Pore Size Distribution

The pore size distribution in the untreated and exploded samples was measured by the solute exclusion technique of Stone and Scallen (*21*). In this technique, the solid substrate is contacted with a solution of molecules of known size. The pore volume available to molecules of a given size is then determined by measuring the dilution that occurs. Dextrans T-2000, T-500, T-70, T-40, T-10, and T-4, and analytical-grade raffinose and α-methyl-D-glucoside were used as pore volume probes. The molecular diameters of the above dextrans, raffinose, and α-methyl-D-glucoside are 56, 27, 11, 9, 5.1, 3.5, 1.2, and 0.8 nm, respectively. Water with a molecular diameter of 0.4 nm was the limiting solute.

The sample under investigation was first washed with distilled water until free of all water soluble material (i.e., until the refractive index of the washings matched that of the distilled water). Surplus water was then drained from the wet sample, and about 10 g of the sample (representing about 3 g of oven-dried fibers) were placed into tared plastic screw-cap bottles. The weight was recorded. Approximately 30 mL of the various 2% dextran solutions were poured into the bottles, and the weights recorded again. A distilled water blank was also processed for each sample.

The sample sugar solutions were periodically hand-shaken for about 1 min and allowed to stand overnight. The liquids were separated by suction filtration through preweighed 0.45 μm glass filters. The filtrate was collected in a clean, dry test tube. The solids were washed thoroughly with distilled water and set to dry overnight in the 103°C oven. The dry solid weights could then be calculated at a later time. Optical rotations of the filtered liquids, stock solutions, and blanks were measured on a spectropolarimeter at Hg-546-nm wavelength.

When a dextran solution is added to wet substrate, the concentration of dextran decreases as the dextran molecules penetrate macropores and micropores holding water. The inaccessible water is thus the water that is not available for diluting dextran solution. The inaccessible water in mL/g is calculated using the Stone and Scallan's (22) formula. For the largest solute probe, all the water is essentially inaccessible. The accessible pore volume for a given solute is calculated as the difference between the inaccessible water at 56 nm minus the inaccessible for the solute in question.

Measurement of pH

The pH of the exploded sample was measured by using a TOA pH meter, HM-26 S.

Determination of Soluble Sugars in Autohydrolyzate

One gram of freeze-dried exploded rice straw was extracted with 60 mL of distilled water for 12 h at ambient temperature. The solids and liquid were separated by filtration. The liquid was then analyzed for reducing sugars, glucose, and xylose content.

Extracted Components Analysis

The products of explosion were extracted by the method of Chua and Wayman (*14*). After first freeze-drying to sublimate the water, which constituted a large proportion of the product, 5 g of the dried product were added to 300 mL of distilled water and extracted for 12 h at ambient temperature. After filtration, the water-extracted hemicellulose fraction was recovered by concentrating the filtrate, then freeze-dried, and weighed. The solid residue was extracted for 12 h in a Soxhlet extractor with 10 mL methanol to dissolve the methanol-soluble lignin. After concentration and freeze-drying of the extract, the methanol-soluble lignin was weighed. The residue from the methanol extraction consisted of cellulose and methanol-insoluble lignin (klason lignin). One gram of this residue was added to 15 mL of 72% sulfuric acid and left at room temperature for 4 h. This was then placed in a 1-L conical flask, washed with 560 mL distilled water, and then boiled for 4 h with reflux cooling. After allowing the insoluble matter to settle, the liquid was filtered off. The solid material was then washed with hot water, dried to constant weight at 105 ± 3°C, and weighed. This substance was defined as klason lignin. The weight of cellulose was calculated by subtracting the weight of klason lignin from 1 g of the residue.

Enzyme

Meicelase, a commercial preparation of *T. ressei* cellulase (Meiji Seika Co. Ltd, Tokyo, Japan) was used. This cellulase contains the following enzyme activities (in U/mg) (*23*): FPU, 0.53; CM-cellulase, 7.90; β-glucosidase, 3.28; cellobiase, 2.27; xylanase, 0.31; and β-xylosidase, 0.02.

Enzymatic Saccharification

The exploded samples of rice straw were freeze-dried and then used in the saccharification experiment. The standard saccharification was carried out in a 300-mL conical flask in a thermostat incubator at 50°C for 72 h, agitated at 150 rpm. The pH of the suspension was adjusted to 5.0 with 0.5*M* phosphate buffer. The substrate and enzyme concentrations were 2% (w/v) and 0.2% (w/v), respectively, in a total volume of 100 mL. Samples (2 mL) were taken periodically, centrifuged, and the supernatants were analyzed for glucose concentration (the yield of glucose was corrected for those already present in the sample owing to autohydrolysis). The percent glucose yield was calculated using the following equation:

$$\%\ \text{Glucose yield} = [\text{glucose formed}\ (\times 0.9)\ /\ \text{cellulose in straw}] \times 100 \quad (1)$$

Analytical Methods

Reducing sugars were measured by the Somogyi-Nelson method (*24,25*). Glucose was measured specifically by the glucose oxidase method (*26*). Xylose concentration was determined by high-performance liquid chromatography (Shimadzu LC-9A) equipped with an Asahipak NH2P-50 column.

RESULTS AND DISCUSSION

Changes in Physical Properties of Steam-Exploded Rice Straw

Photograph

After the rice straw had been exploded under various conditions, the product consisted of a muddy brown liquid containing a relatively large amount of water and small fragments of fibers. The exploded rice straw had the characteristic smell of steam-scorched plants. Raising the steaming time had a considerable effect on the shape and size of the fibers. Figure 2 shows photomicrographs of the exploded rice straw for a steam pressure of 3.53 MPa and various steaming times. At the short time of 0.5 min, only parts of the surface of each fiber were shattered, and most of

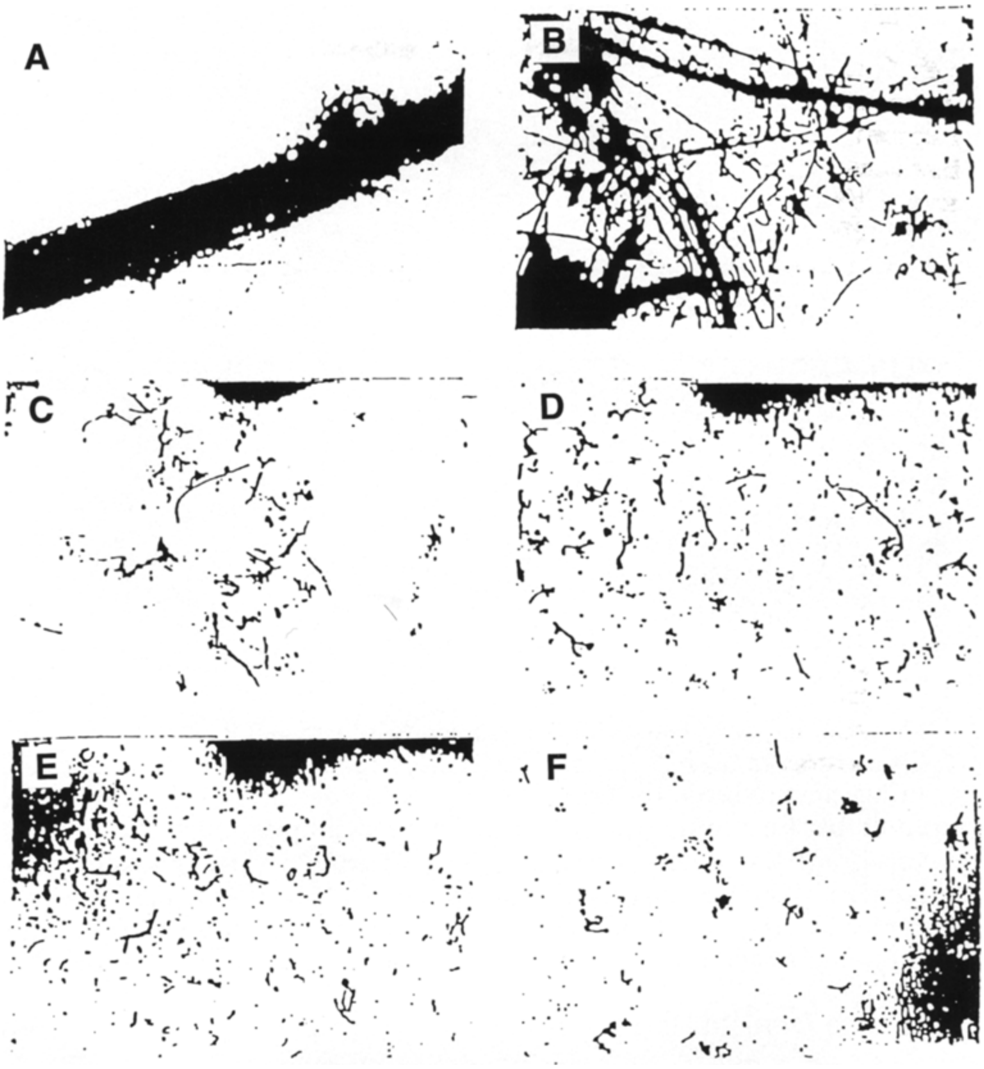

Fig. 2. Photograph of exploded rice straw at 3.53 MPa. **(A)** 0.5 min; **(B)** 1 min; **(C)** 2 min; **(D)** 3 min; **(E)** 5 min; **(F)** 10 min.

the fibers remained unchanged. At a steaming time of 1 min, the rice straw fibers began to shatter with some breaks occurring across the fibers. At a steaming time of 2 min and above, the fibers were very finely defiberized, giving a muddy appearance.

Size Distribution

Figure 3 (A–C) shows the frequency distributions obtained by measuring the lengths of 500 fiber fragments after explosion at 3.53 MPa for various steaming times. For the fibers of 10-mm length, an increase in the

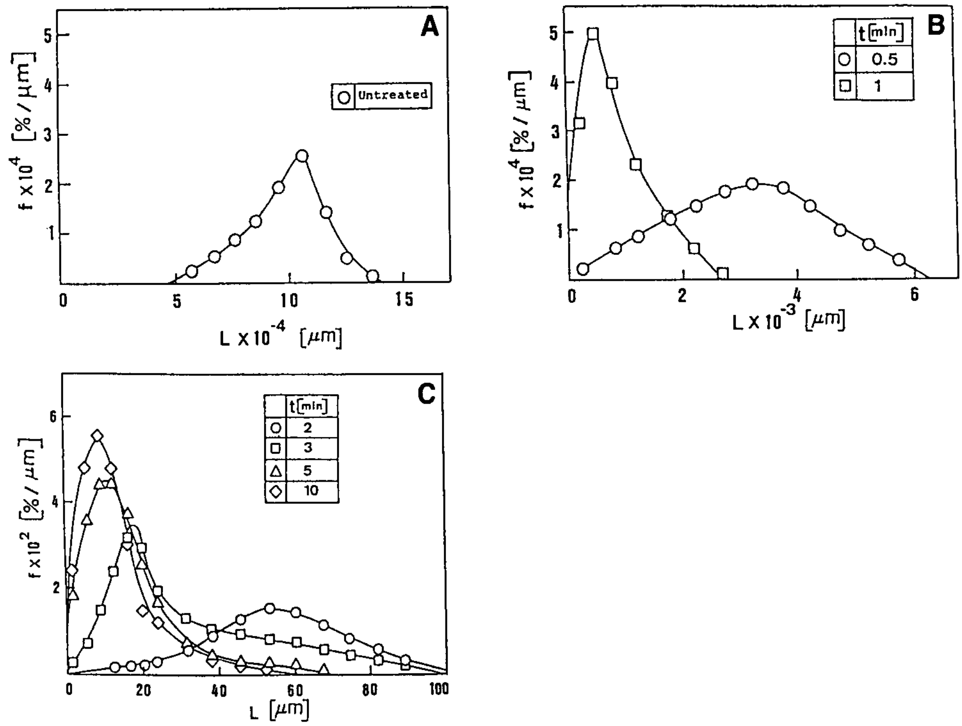

Fig. 3. Size distribution of exploded rice straw at 3.53 MPa.

steaming time reduced the size of the fibers considerably. This is probably not only because of hydrolysis during the reaction, but also the sudden expansion of the water in the rice straw fibers when the pressure was suddenly reduced. Such defibration was particularly noticeable at steaming times above 2 min, and is probably owing to the temperature of the steam being above the thermal softening temperature of the lignin.

Pore Size Distribution

During enzymatic hydrolysis of lignocellulosic materials, the enzymes must penetrate a network of capillaries and pores to reach all the available hydrolysis sites. The surface area available to cellulases is therefore expected to be the most important factor determining the enzymatic hydrolysis rate of cellulose (*27*).

The effect of pretreatment steaming time at 3.53 MPa on the change in the accessible pore volume is shown in Fig. 4. For comparison, pore volume accessible to solute probes in untreated substrate is also shown. Inspection of this figure indicates that more than 80% of the pore volume in the untreated substrate was in pores inaccessible to solutes of > 5.1-nm diameter. This highlights the inaccessibility of the cellulase in untreated substrate, since the major cellulase components are reported to have a tadepole-like shape consisting of a rigid, ellipsoidal head (6.7 × 4.4 nm) and an elongated, cylindrical, flexible tail (12.9 × 3.2 nm) (*28*). The low

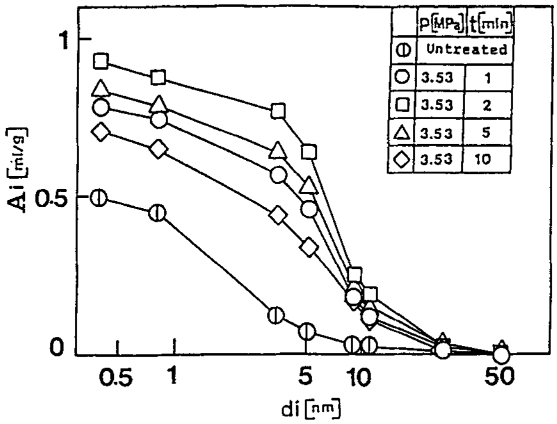

Fig. 4. Pore volume distribution of exploded rice straw at 3.53 MPa.

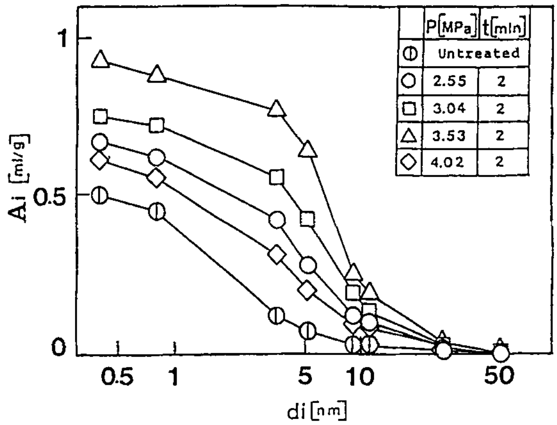

Fig. 5. Pore volume distribution of exploded rice straw at different steam pressures for 2 min.

accessibility in the natural cellulosic substrate is mainly owing to the highly ordered structure of cellulose itself and its association with hemicellulose and lignin in the native state. Steam explosion, on the other hand, dramatically increased the fiber porosity. It is apparent from Fig. 4 that with increased pretreatment steaming time, the accessible pore volume of the substrate was also increased and attained a maximum value at 2 min. The impact of the pretreatment can be seen as increasing the pore volume in the cell-wall capillaries (compared to the untreated substrate), especially in the important size range of 5.1 nm and larger. This remarkable change in fiber porosity is probably caused by extensive alterations in the lignocellulosic structure of rice straw during steam explosion.

To determine the effect of steam pressure on the change in fiber porosity, rice straw was further exploded at various steam pressures for a fixed steaming time, 2 min, and the results are presented in Fig. 5. It is evident from this figure that as the steam pressure (temperature) was raised, accessible pore volume in the substrate increased correspondingly. However, pretreatment under a very high steam pressure of 4.02 MPa significantly reduced fiber porosity. A very similar result was also observed at 3.53 MPa for longer steaming times (Fig. 4). The reason for this loss of accessibility under severe steam treatment conditions is probably the condensation of hemicellulose and lignin decomposition products, which are subsequently deposited on the micropores, possibly covering the surface of the cellulose microfibrils, and thus reducing the accessible pore volume.

Changes in Chemical Properties of Steam-Exploded Rice Straw

pH

At 3.53 MPa, the pH fell to 4.0 at a steaming time of 0.5 min. A very similar pH value was also achieved at 2.55 MPa for 2 min of steaming time (data not shown). This indicates that an acidic medium was easily created in the reactor vessel under the above mild explosion conditions of pretreatment. Further increase in pretreatment steaming time or steam pressure (temperature) appeared to have little effect on the pH value.

A reduced pH value achieved during explosion pretreatment is attributed to the formation of organic acids, such as acetic acid, generated from the thermally labile acetyl groups present in hemicellulose (*23*). Nevertheless, this weakly acidic condition in turn catalyzes the hydrolysis of hemicellulose itself during the pretreatment. This suggests that no additional substance, such as CO_2 gas (*29*), is required in the reactor to achieve autohydrolysis.

Soluble Sugars

Figure 6A depicts the effect of steam explosion on the concentration of reducing sugars (produced because of autohydrolysis during pretreatment) in autohydrolyzate. At 2.55, 3.04, and 3.53 MPa, the amounts of reducing sugars were produced during pretreatment to a maximum concentration (0.5 g/L) for exposure times of 3, 2, and 1 min, respectively. These results clearly indicate that when the steam pressure (temperature) was raised, the steam exposure time necessary to achieve maximum hydrolysis of carbohydrates decreased. However, at very high steam pressure, 4.02 MPa, the reducing sugar concentration dropped to 0.25 g/L even at a very short steaming time of 0.5 min. It is also evident from Fig. 6A that longer exposure times at any of the steam pressures (temperatures) resulted in a net decrease in the reducing sugar concentration. This is presumably owing to an increase in the severity of the explosion pretreatment, which destroyed more free sugars (*15*).

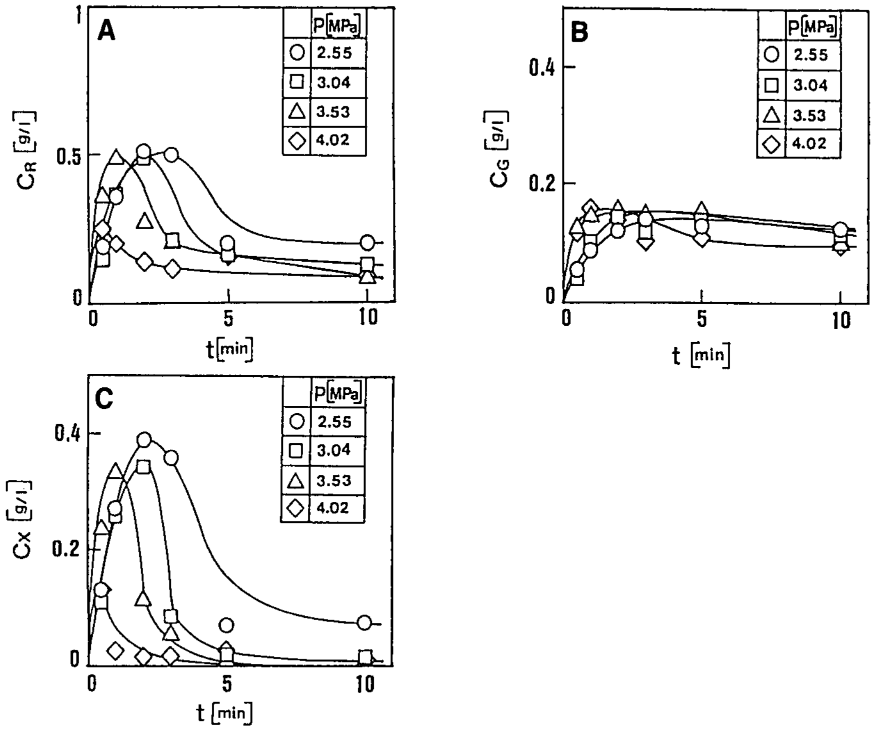

Fig. 6. Effect of steam explosion on the concentrations of reducing sugar **(A)**, glucose **(B)**, and xylose **(C)** in the autohydrolyzate.

The concentration of glucose in the autohydrolysate water extract is shown in Fig. 6B. The concentration of glucose reached its maximum value of about 0.15 g/L for all the steam pressures (temperatures) within 3 min of steaming time. Longer steaming times of pretreatment had no apparent effect on the concentration of glucose, except at 4.02 MPa, where the glucose concentration was slightly decreased with increasing steaming time. This result indicates that the destruction of glucose during steam explosion was minimal for most conditions studied.

Figure 6C shows the effect of steam explosion pretreatment on the concentration of xylose in autohydrolysate. Xylose was very quickly destroyed by this pretreatment, and xylose destruction increased with the increasing severity of the steaming conditions. The maximum of 0.4 g/L xylose concentration was measured at 2.55 MPa for 2 min of steaming time. At 4.02 MPa, extensive destruction of xylose was observed. As a result, its concentration dropped to 0.12 g/L, even at 0.5 min of steam exposure time. Furthermore, longer exposure times, at any of the steam pressures (temperatures), drastically reduced the concentration of xylose in autohydrolysate.

Xylose concentration decreases as the severity of the pretreatment conditions increases because of the relatively easy dehydration of xylose to furfural at elevated temperatures. Furfural, which is volatile, vaporizes through flashing during the steam explosion.

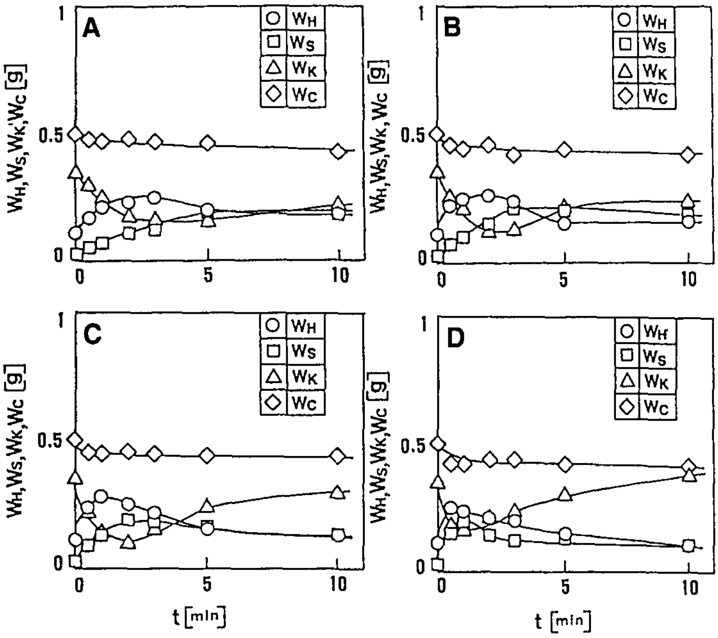

Fig. 7. Steam explosion pretreatment effects on the changes in extracted components at 2.55 (**A**), 3.04 (**B**), 3.53 (**C**), and 4.02 (**D**) MPa.

Extracted Components

Figure 7 (A–D) shows the effects of steam pressure (temperature) and steaming time on the extracted components. Inspection of this figure indicates that the cellulose component changed very little owing to changes in steaming time or steam pressure (temperature). However, significant variations occurred in the hemicellulose and lignin components.

At 2.55 MPa (Fig. 7A), the amount of hemicellulose increased with increased steaming time and attained a maximum value at 3 min, after which it decreased slightly. The amount of methanol-soluble lignin also increased as the steaming time increased. In contrast, the amount of klason lignin decreased with increasing methanol-soluble lignin. This result indicates that the methanol-soluble lignin was produced by the depolymerization of klason lignin by acidic hydrolysis. Depolymerization of lignin during steam explosion has been reported by some investigators (*16*) to occur mostly because of the cleavage of the β-*O*-4 ether bonds in the acidic medium.

At 3.53 MPa (Fig. 7C), the amounts of hemicellulose and also methanol-soluble lignin increased rapidly as the steaming time increased and attained their maxima at 1 and 2 min, respectively, but then gradually decreased as the heating continued. In contrast to methanol-soluble lignin, the amount of klason lignin initially decreased sharply and attained its lowest value at 2 min, but then started to increase progressively as the severity of the pretreatment was increased. These observations suggest that during steam explosion two types of reactions are ocurring: initially a faster reaction, depolymerization of the native lignin by acidic hydrolysis, and, as heating continues, condensation and repolymerization reactions take over, thus resulting in increased klason lignin. Such lignin degradation and modification during autohydrolysis of woody substrates has been reported by some investigators. A mechanism for depolymerization and condensation of the aspen wood lignin during autohydrolysis steam explosion has been proposed by Chua and Wayman (*14*). In general, cleavage of lignin bonds under acidic conditions involves the formation of resonance-stabilized carbonium ions. In addition, carbonium ions are strongly electrophilic and are able to participate in substitution-forming carbon–carbon bonds with aromatic C-1 or C-6 atoms.

Figure 7D shows the effect of steaming time at 4.02 MPa on the variation of the extracted components of exploded rice straw. It is evident from this figure that the initial hydrolysis reaction changes to a condensation reaction shortly after 1 min of steaming time, and as heating continued, the production of klason lignin increased. Increased klason lignin is attributed to the condensation reaction between the decomposition products of hemicellulose and lignin. This result is in agreement with Chua and Wayman (*14*), who also support Klemoda's postulate that under drastic conditions of pretreatment, the reactive hemicellulose degradation products, such as furfural and its precursors, can react with lignin during autohydrolysis. This also explains why the amount of hemicellulose decreased under severe pretreatment conditions.

The results, illustrated in Fig. 7 (A–D), are noteworthy in that the steaming time necessary to achieve maximum degradation of lignin became progressively shorter as the pressure (temperature) was raised. Nevertheless, a maximum degradation of lignin occurred at 3.53 MPa for 2 min of steaming time.

However, the above results conclusively show that pretreatment of lignocellulose by steam explosion must be carried out under conditions that will depolymerize the lignin (and thus open up the cellulose fiber to enzymatic attack) while causing minimal destruction of the valuable carbohydrate portion.

Enzymatic Saccharification of Exploded Products

Figure 8 shows the total glucose yield after 72 h of enzymatic saccharification of exploded samples for the different pretreatment cases. The yield increases with increasing steam pressure (temperature), except at 4.02 MPa, where the yield of glucose decreased.

Obviously, pretreatment at 3.53 MPa for 2 min of steaming time gives the highest yield. More than 80% yield of glucose was obtained based on the cellulose content in the pretreated sample. Thus, it appears that this explosion condition is adequate for producing a material that is highly susceptible to attack by cellulolytic enzymes.

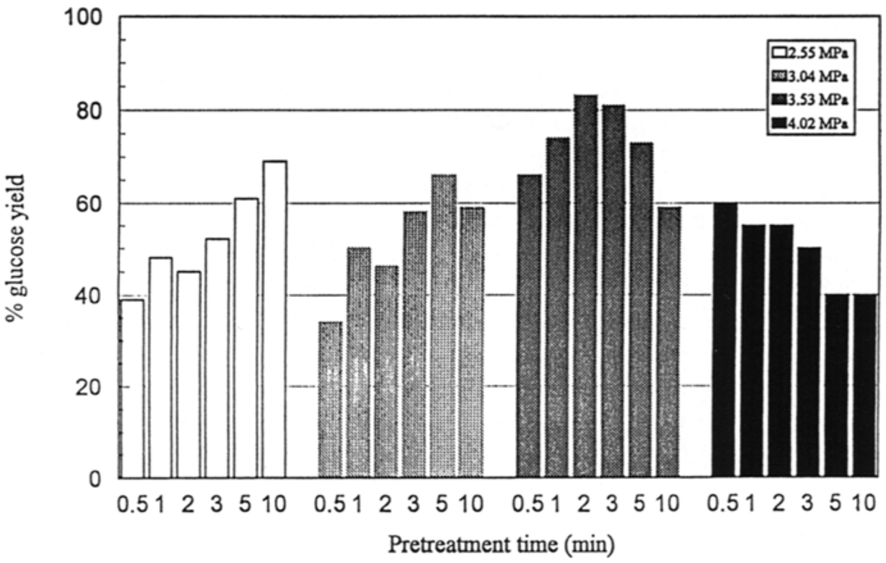

Fig. 8. Enzymatic saccharification of exploded rice straw.

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REFERENCES

1. Lutzen, N. W., Nielsen, M. H., Oxenboell, K. M., Schulein, M., and Stentebjerg-Olesen, B. (1983), *Phil. Trans. R. Soc. Lond.* **B 300,** 283.
2. Wright, J. D., Power, A. J., and Douglas, L. J. (1986), *Biotechnol. Bioeng.* **Symp. No. 17,** 285.
3. Wilke, C. R., Yang, R. D., Sciamanna, A. F., and Freitas, R. P. (1981), *Biotechnol. Bioeng.* **23,** 163.
4. Cowling, E. B., and Kirk, T. K. (1976), *Biotechnol. Bioeng.* **Symp. No. 6,** 95.
5. Weil, J., Westgate, P., Kohlmann, K., and Ladisch, M. R. (1994), *Enzyme Microb. Technol.* **16,** 1002.
6. Jurasek, L. (1979), *Dev. Ind. Microb.* **20,** 177.
7. Galbe, M., and Zacchi, G. (1986), *Biotechnol. Bioeng.* **Symp. No. 17,** 97.
8. Nakamura, Y., Moniruzzaman, M., Nagao, M., Sawada, T., and Motoi, M. (1991), *Kagaku Kogaku Ronbunshu* **17,** 504.
9. Ramos, L. P., Breuil, C., and Saddler, J. N. (1992), *Appl. Biochem. Biotechnol.* **34/35,** 37.
10. Oi, S., Matsuzaki, K., Tanaka, T., Iizuka, M., Taniguchi, M., and Prasertsan, P. (1994), *J. Ferment. Bioeng.* **77(3),** 326.

11. Beltrame, P. L., Carniti, P., Visciglio, A., Focher, B., and Marzetti, A. (1992), *Bioresource Technol.* **39,** 165.
12. Grous, W. R., Converse, A. O., and Grethlein, H. E. (1986), *Enzyme Microb. Technol.* **8,** 274.
13. Wong, K. K. Y., Deverell, K. F., Mackie, K. L., Clark, T. A., and Donaldson, L. A. (1988), *Biotechnol. Bioeng.* **31,** 447.
14. Chua, M. G. S. and Wayman, M. (1979), *Can. J. Chem.* **57,** 1141.
15. Brownell, H. H., Yu, E. K. C., and Saddler, J. N. (1986), *Biotech. Bioeng.* **28,** 792.
16. Hemmingson, J. A. and Newman, R. H. (1985), *J. Wood Chem. Technol.* **5(2),** 159.
17. Puls, J., Ayla, C., and Dietrichs, H. H. (1983), *J. Appl. Polym. Sci. Polym. Symp.* **37,** 685.
18. Hishiyama, S. and Sudo, K. (1992), *Mokuzai Gakkaishi* **38(10),** 944.
19. Mes-Hartree, M., Hogan, C. M., Hayes, R. D., and Saddler, J. N. (1983), *Biotechnol. Lett.* **5(2),** 101.
20. Mes-Hartree, M., Hogan, C. M., and Saddler, J. N. (1984), *Biotechnol. Bioeng.* **Symp. No. 14,** 397.
21. Stone, J. E. and Scallan, A. M. (1968), *Cellulose Chem. Technol.* **2,** 343.
22. Stone, J. E. and Scallan, A. M. (1968), *Pulp Paper Mag. Can.* **69,** 69.
23. Dekker, R. F. H. and Wallis, A. F. A. (1983), *Biotechnol. Bioeng.* **25,** 3027.
24. Nelson, N. (1944), *J. Biol. Chem.* **153,** 375.
25. Somogyi, M. (1945), *J. Biol. Chem.* **160,** 61.
26. Lloyd, J. B. and Whelan, W. J. (1969), *Anal. Biochem* **30,** 467.
27. Cowling, E. B. (1975), *Biotechnol. Bioeng.* **Symp. No. 5,**163.
28. Abuja, P., Schmuck, M., Pilz, I., Tomme, P., Claeyssens, M., and Esterbauer, H. (1988), *Eur. Biophys. J.* **15,** 339.
29. Puri, V. P. and Mamers, H. (1983), *Biotech. Bioeng.* **25,** 3149.