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Ammonia-Recycled Percolation Process for Pretreatment of Biomass Feedstock

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ABSTRACT

An ammonia-based biomass pretreatment method named ammonia recycled percolation (ARP) process was investigated. The process characteristics are:

1. Aqueous ammonia is used as a pretreatment reagent;
2. A packed bed flowthrough-type reactor (percolation reactor) is used in recirculation mode;
3. ARP-Ammonia is continuously recycled.

The experimental data on hybrid poplar indicate that the ARP is a highly effective pretreatment method. The digestibility of the best-case ARP sample approached that of filter paper. The extent of delignification in the ARP process was in the range of 23-63%. The ARP process solubilized significant amounts of xylan into the pretreatment effluent, yet left most of the glucan fraction intact. It was demonstrated that pretreatment with pure water at elevated temperatures (autohydrolysis) prior to the ARP selectively removed most of the hemicellulose. A scanning electron microscope (SEM) examination of the solid samples revealed that ARP induces considerable morphological changes reflecting an increase in pore size and porosity. The overall ammonia material balance has shown that in excess of 99% can be recovered.

Index Entries: Delignification; ammonia; cellulose/hemicellulose; enzymatic hydrolysis; autohydrolysis.

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INTRODUCTION

Pretreatment is a necessary element in biomass-to-ethanol conversion processes. The primary purpose of pretreatment is to make the cellulosic biomass amenable to the action of cellulase. To date, a number of pretreatment methods have been proposed and investigated, including steam explosion (*1–3*), alkaline treatment (*4,5*), treatment with sulfur dioxide (*6–8*), organosolv pretreatment (*9*), treatment with hydrogen peroxide (*10–12*), treatment with supercritical ammonia (*13,14*), dilute acid pretreatment (*15–19*), and ammonia freeze explosion process (AFEX) (*20*). Interesting claims have been made with regard to the effectiveness of these pretreatment methods. The economic and environmental constraints, however, limit the applicability of these known methods.

In this article, we introduce an alternative pretreatment based on aqueous ammonia (ammonium hydroxide). Ammonia has a number of characteristics suitable for pretreatment of lignocellulosic substrates. It is a proven delignification reagent (*3a,5*). It causes significant changes in the biomass other than delignification. For example, ammonia increases the accessibility to carbohydrates by hydrolyzing glucuronic acid ester crosslinks (*21*). Ammonia is known to cleave the bonds between lignin and hemicellulose (*22*) and C—C and C—O bonds of lignin macromolecule (*23*), and causes ammonolysis of uronic acid ester groups in hemicellulose (*24*). Ammonia also changes the cellulose fiber structure from cellulose I to cellulose III (*25*). Use of ammonia causes not only pretreatment effects, but also fractionates of biomass, especially separating lignin from biomass. Furthermore, the lignin generated in this process is sulfur- and sodium-free, unlike the lignin generated in conventional pulping processes. It is therefore of high quality and may command high by-product credits. Ammonia is one of the most heavily used industrial commodity chemicals. On a molar basis, ammonia costs only one-fourth the price of sulfuric acid. It is easily recoverable from aqueous mixture because of high volatility. Ammonia/ammonium hydroxide is far less corrosive than sulfuric acid at high temperatures.

With this understanding, we have investigated a novel pretreatment method based on aqueous ammonia. It is named the ammonia recycled percolation (ARP) process because of the following technical features:

1. Aqueous ammonia (ammonium hydroxide) is used as the pretreatment reagent;
2. A packed bed, flowthrough-type (percolation) reactor is employed and operated under a recirculation mode; and
3. Ammonia is continuously regenerated and recycled during the process.

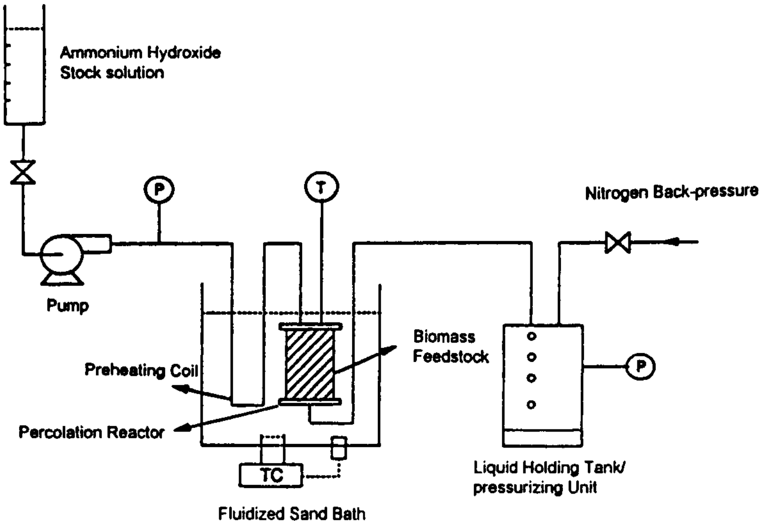

Fig. 1. Schematic diagram of the APR system.

The overall objective of this investigation was to evaluate the ARP as a pretreatment process. The scope of this work covered the technical factors concerning the operating conditions, effectiveness as a pretreatment, nature of the pretreatment reactions, and other process aspects.

MATERIALS/METHODS

Materials

Hybrid poplar milled from whole tree (including bark) to the nominal size of 1/16 in. to 60 mesh was supplied from NREL and used throughout this study. The cellulase enzyme, Cytolase CL, Lot No. 17-92262-09, was supplied from Environmental Biotechnology, Inc. The specific activity of the enzyme as determined by the supplier is as follows: Filter paper activity = 103 FPU/mL, β-G activity = 88.9 pNPGU/mL, Endo-G activity = 269 CMCU/mL.

Experimental Setup and Operation

The batch experimental apparatus for the ARP process is shown in Fig. 1. The process involves treating biomass with an ammonium hydroxide solution at temperatures above 150°C and pressure around 325 psi. The system is comprised of an aqueous ammonia reservoir, pump, sand bath, packed-bed reactor, and liquid holding tank, which also serves as a back-pressure vessel. The reactor was constructed of SS-316 tubing, 5/8 in. OD × 6 in. L (33-cm^3 internal reactor volume). It is flanged, and a screen seals both ends. An autoclave (600 mL, Parr Instrument) was used as a liquid holding tank. It was connected to a nitrogen cylinder to apply back pressure to the system. A positive displacement pump (Metering Minipump, LDC) was used to deliver aqueous ammonia against the reactor pressure. Aqueous ammonia was pumped through a preheating coil and then to the reactor prepacked with biomass. The preheating coil and the reactor were submerged and heated in a sand bath. The aqueous ammonia that passed through the reactor was collected at the liquid holding tank. The flow rate of aqueous ammonia was controlled by the metering pump speed and monitored by the flow gage. The biomass samples taken from the reactor were washed with water and filtered. A portion of the wet solid residues was oven-dried at 105°C overnight to measure weight loss and further subjected to chemical composition analysis. The remaining solid residues were stored in a refrigerator for the digestibility test. The ARP effluent collected in a holding tank was transferred into an air-tight sample bottle and stored in the refrigerator for further composition analysis.

Digestibility Test

Enzyme hydrolysis of pretreated substrate was performed at 50°C, pH 5.0, on a shaker bath agitated at 150 rpm. For 1 g of dry biomass, 50 mL buffer (phosphate) and 0.3 mL of cellulase enzyme (Cytolase CL), equivalent to 30 IFPU, were added. Samples were taken periodically and analyzed for glucose content using HPLC. The total glucose content after 72 h of hydrolysis was taken to calculate the enzymatic digestibility.

Analytical Methods

The biomass samples were analyzed for sugar and Klason lignin content following the procedure described in NREL-CAT Standard Procedure-#002. Sugars (glucose, xylose, and mannose) in the solids were analyzed by HPLC using Bio-Rad, Aminex HPX-87C column and HPX-87P column where applicable. Liquid samples from the pretreatment process were boiled until all the free ammonia evaporated and most of the dissolved lignin precipitated, and then put through HPLC (HPX-87H column) for analysis of sugar, acids, and decomposition products. The same HPLC analysis was repeated for the liquid samples after they were subjected to a secondary hydrolysis. The secondary hydrolysis was performed to convert the oligomer content (mostly xylose oligomer) to monomers. The conditions of secondary hydrolysis were: 4% sulfuric acid, 121°C, 1-h reaction time. The dissolved lignin in the liquid samples was determined by UV spectrophotometric method at 280 nm. Indulin (Westvaco) solution in aqueous ammonia was used as a standard. The surface area of treated and untreated biomass samples was measured by BET apparatus (Quantachrome). The surface structure of the treated and untreated bio-

Table 1
Effect of Ammonia Concentration on the Composition of Solid Residues in ARP[a]

Pretreatment condition						
Ammonia, wt%	Temp., °C	%Wt remaining	%Lignin content	%Glucan content	%Xylan content	%Digestibility, 72 h
Untreated biomass		100.0	26.0	42.9	14.8	7.8
2.5	160	77.5	22.7	40.9	13.7	62.7
5.0	160	75.5	21.1	41.0	13.4	73.3
10	160	72.5	15.4	40.5	12.8	78.2
20	160	70.7	14.6	39.5	12.5	83.8
2.5	175	75.1	21.1	39.6	11.6	78.9
5.0	175	73.0	18.2	40.9	9.7	78.5
10	175	69.2	15.3	41.3	9.5	87.0
20	175	68.5	15.2	41.8	10.6	81.4

[a] All sugars and lignin content in the table based on oven-dried untreated biomass. Pretreatment condition: 1 h, 325 psi, reagent flow rate = 1.0 mL/min. Enzymatic hydrolysis condition: 30 IFPU/g treated biomass, 50°C, pH 5.0.

mass samples was also observed by an SEM. The undissociated ammonia content in liquid and solid samples was determined by total Kjeldahl nitrogen analysis following the EPA method (600/4-79-020). It was done at Mid-South Testing, Inc., Decatur, AL.

RESULTS AND DISCUSSION

ARP Process

The effect of ammonia concentration, reaction temperature, and reaction time on the ARP pretreatment performance was studied in the first series of ARP experiments. Ammonia concentrations were varied over 2.5–20.0 wt%, and two different reaction temperatures of 160 and 175°C were employed. The pressures ranged from 175–325 psi. Table 1 summarizes the composition data for pretreated biomass. Shown in Fig. 2 are the enzymatic digestibility data for biomass samples pretreated at various ammonia concentrations. Figure 2 clearly shows that the ARP samples indeed exhibit enhanced enzymatic digestibility of cellulose. At 160°C, the enzymatic digestibility increased with the ammonia concentration. At 175°C, however, the cellulose digestibility increased with the ammonia concentration only up to 10.0 wt% level. Within the experimental conditions covered here, the digestibility of ARP samples was in the range of 63–87%. On ARP pretreatment, the biomass samples lost 20–35% of its weight. Lignin and hemicellulose accounted for the major part of the weight loss. As shown in Table 1, the amount of lignin removed increased as the ammonia concentration was varied from 2.5–20 wt%. The maximum

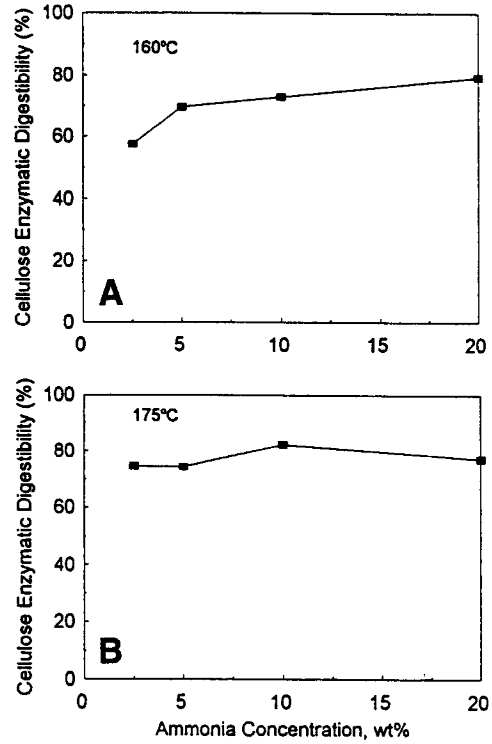

Fig. 2. The effect of ammonia concentration on cellulose enzymatic digestibility. Pretreatment condition: 1 h, 325 psi, reagent flow rate = 1.0 mL/min. Enzymatic hydrolysis condition: 30 IFPU/g treated biomass, pH 5.0, 50°C.

lignin loss in these runs was 44%. As much as 36% of hemicellulose was extracted into the ARP effluent and recovered as xylose oligomer. However, <8% of the total glucose content in the original biomass was extracted during the pretreatment.

In the next series of runs, the pretreatment reaction temperature was varied between 150 and 190°C, while keeping ammonia (10 wt%), reaction time (1 h), and pressure (325 psi) constant. Table 2 summarizes the composition data for solid residues after pretreatment. Figure 3 shows the enzymatic digestibility and biomass composition data as a function of pretreatment reaction temperature. The results collectively indicate that the reaction temperature has indeed a significant effect on the pretreatment. As reaction temperature increases, the enzymatic digestibility generally increased. The maximum observed enzymatic digestibility was 95%. However, at 180°C, the actual glucose yield decreased owing to significant glucan loss during pretreatment. This was more evident at 190°C in which as much as 14% of glucan was extracted from the biomass during pretreatment. At 180 and 170°C, the glucan loss was somewhat lower, 6.8 and 3.7%, respectively. The results in Fig. 3 also indicate that the extent of delignification and xylan extraction are the key factors controlling the pretreatment effectiveness. The trend was that biomass

Table 2
Effect of Pretreatment Temperature
on the Composition of Solid Residues and Enzymatic Digestibility[a]

Pretreatment temperature, °C	%Wt remaining	%Lignin content	%Glucan content	%Xylan content	%Digestibility at 72 h
Untreated biomass	100.0	26.0	42.9	14.8	7.8
150	84.7	18.7	42.0	12.4	70.6
160	76.5	15.4	41.0	11.2	77.2
170	70.9	15.6	41.3	10.4	81.9
180	68.9	13.2	40.0	8.8	95.2
190	69.8	13.1	37.0	7.7	95.2

[a] All sugars and lignin content in the table based on oven-dried untreated biomass. Pretreatment condition: 10 wt% ammonia, 1 h, 325 psi, reagent flow rate = 1.0 mL/min. Enzymatic hydrolysis condition: 30 IFPU/g treated biomass, 50°C, pH 5.0.

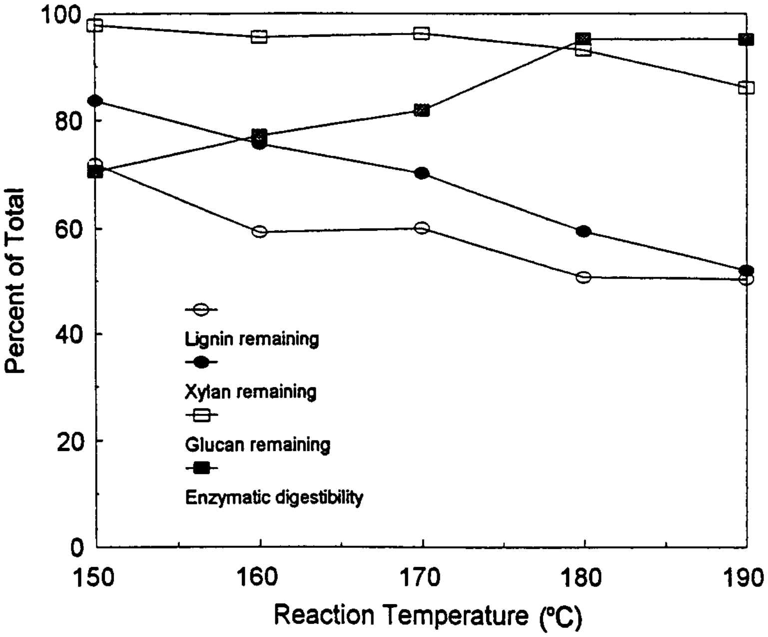

Fig. 3. Compositional changes and cellulose enzymatic digestibility in ARP. Pretreatment condition: 10 wt% ammonia, 1 h, 325 psi, reagent flow rate = 10 mL/min. Enzymatic hydrolysis condition: 30 IFPU/g treated biomass, pH 5.0, 50°C.

samples with lower lignin and xylan content exhibited higher exzymatic digestibility. To reaffirm this, digestibility data obtained from various ARP experiments were plotted against the amount of lignin and xylan removed from each sample (Fig. 4). Despite the scatter of the data, this plot convincingly shows a correlation between lignin and xylan removal and digestibility. The figure also shows that the enzymatic digestibility generally increases with the lignin and xylan removal, and then levels off at about 15% xylan and 20% lignin removal.

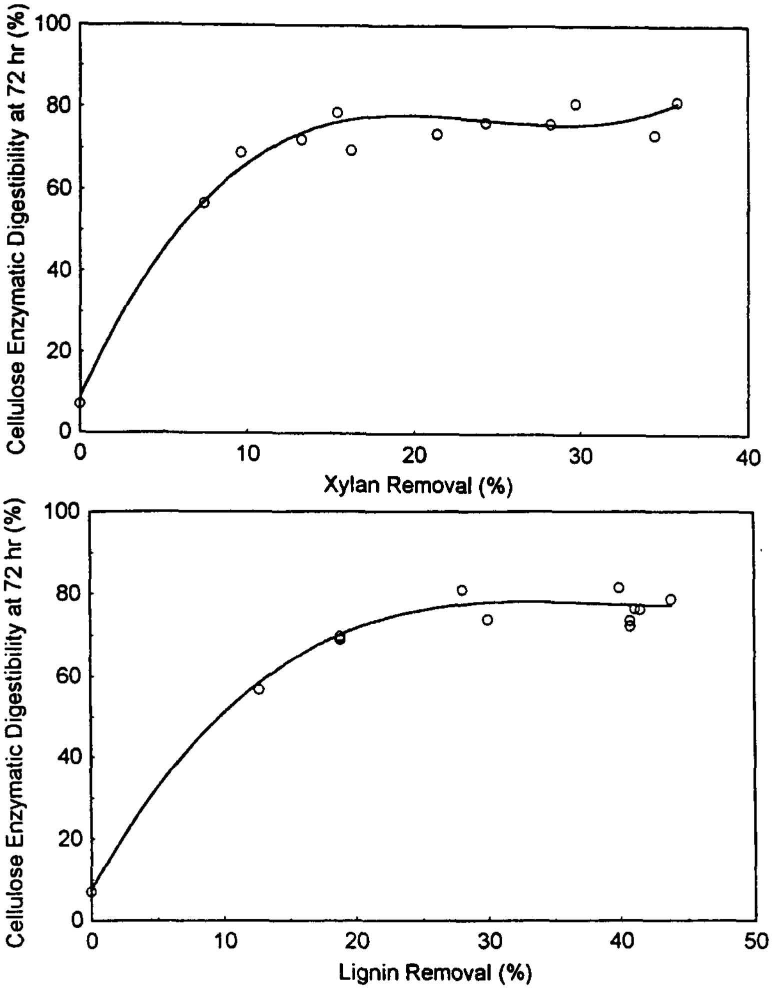

Fig. 4. Digestibility vs degree of delignification and percent xylan removal in ARP.

In most of the ARP experiments, a 1-h reaction was applied. A slight increase in digestibility was noticed at reaction times longer than 1 h. This increase, however, was offset by greater loss of cellulose at longer reaction times (Fig. 5). The experimental results gathered to this point suggest that at 180°C, 1 h of reaction time, 10 wt% ammonia, and 325 psi of pressure represent a near optimal set of pretreatment conditions for the ARP process. The enzymatic digestibility of the biomass pretreated at this condition (measured with enzyme loading of 30 IFPU/g dry biomass) was consistently above 90%, and the overall glucose yield (on the basis of glucose content in the original biomass) was also high at 85–90%.

Physical Characteristics of ARP Solid Samples

The solid samples were observed by SEM. The morphological changes reflecting the increase in pore size and porosity were quite evident for the

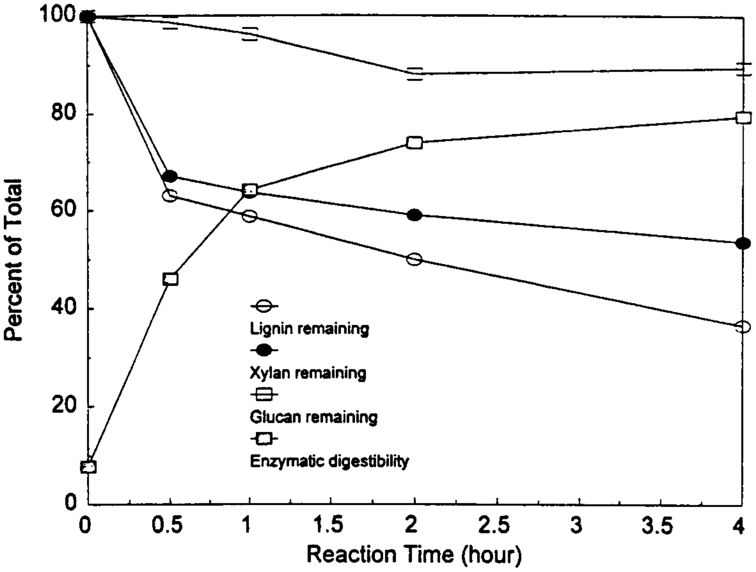

Fig. 5. Compositional changes and cellulose enzymatic digestibility in ARP. Pretreatment condition: 175°C, 10 wt% ammonia, 325 psi, reagent flow rate = 1.0 mL/min. Enzymatic hydrolysis condition: 18 IFPU/g treated biomass, pH 5.0, 50°C.

pretreated samples (Fig. 6). Especially in the cross-sectional pictures, the fibers are seen to be collapsed owing to the pressing action of the knife blade during cutting. Reduction of mechanical strength seems to be an effect of ARP.

In order to examine the changes of the surface area on ARP pretreatment, the BET surface area was measured for the treated and untreated biomass samples. The BET surface area of the biomass sample pretreated at a typical condition (175°C, 1 h, 10 wt% ammonia) was 1.2 m^2/g, whereas the untreated sample was 0.8 m^2g. This increase, however, did not provide sufficient grounds to explain the 7–10-fold increase in digestibility.

ARP Effluent

The effluent of the ARP process was analyzed by HPLC (using Bio-Rad HPX-87H column and 0.005*N* H_2SO_4 as the mobile phase). The effluent as recovered from the ARP process contained high amounts of lignin (0.07–0.1 g lignin/g untreated biomass). On evaporation of NH_3, most of the lignin precipitated. Among identifiable components in the HPLC chromatograms were oligomeric sugars and acetic acid (acetate and undissociated acid). The average total acetic acid in the effluent was 0.026 g acetate/g untreated biomass. Secondary hydrolysis of this effluent by dilute sulfuric acid sharply increased xylose monomers and slightly increased of acetic acid. The glucose level, however, was < 10% of xylose. This indicates that dissolution of cellulose, although it may have been affected in overall

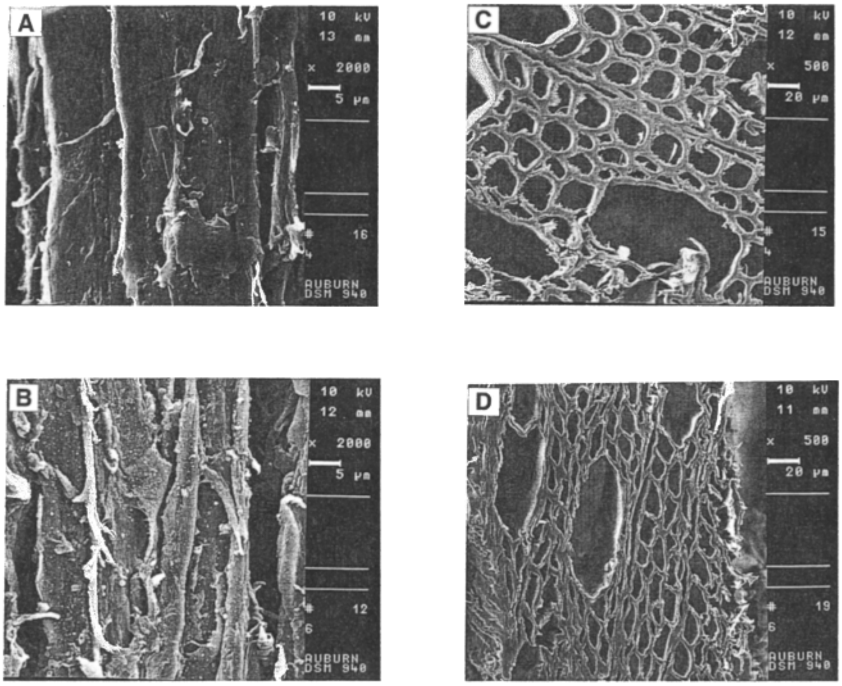

Fig. 6. Pretreatment conditions: 165°C, 5 wt% ammonia, 30 min. (**A**) Untreated biomass (side view), (**B**) pretreated biomass (side view), (**C**) untreated biomass (cross-sectional view), (**D**) pretreated biomass (cross-sectional view).

molecular structure, is insignificant during the ARP process. The decomposition products of glucose and xylose (HMF and furfural) were undetectable in any of the ARP effluents.

Additional Factors Influencing Digestibility

The effect of drying pretreated biomass was examined. One of the samples from the pretreated runs was divided into three portions. The first one was oven-dried at 105°C overnight. A second sample was soaked into distilled water, and the third sample was washed with NaOH solution before enzymatic hydrolysis. The enzymatic digestibility data for these samples are presented in Fig. 7. It was found that drying the pretreated biomass decreased the digestibility, reducing it by 30%, perhaps because of recrystallization of cellulose in the biomass.

The effect of enzyme loading on the glucose yield in the enzymatic hydrolysis of the ARP samples was also investigated. The enzymatic hydrolysis was performed at 50°C and pH 5.0 with two different enzyme loadings of 15 IFPU and 30 IFPU/g treated biomass. In order to examine the enzyme deactivation, an additional enzyme of 15 IFPU was added at 72 h of enzymatic hydrolysis. The effect of β-glucosidase supplementation was also studied. The biomass tested in this study was a sample

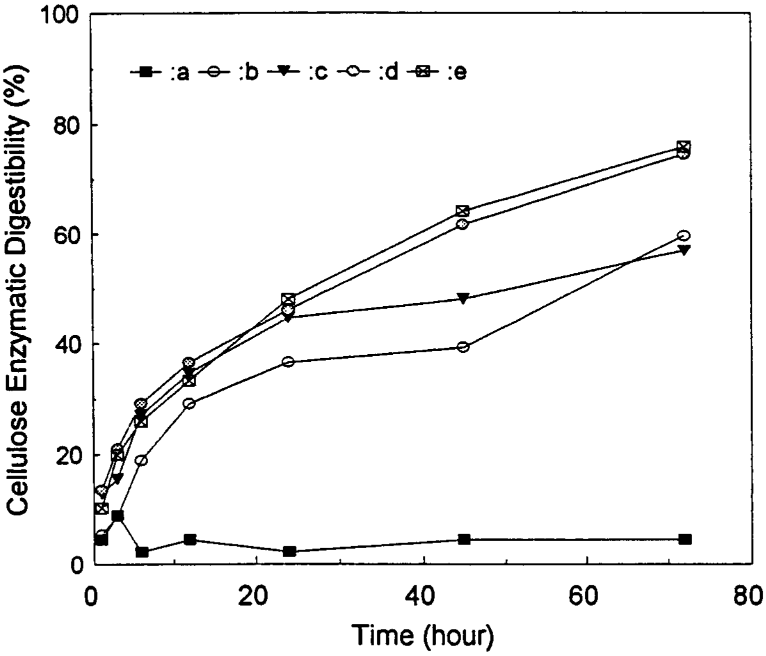

Fig. 7. Enzymatic hydrolysis of various biomass samples: untreated, pretreated, and filter paper. Pretreatment condition: 175°C, 10 wt% ammonia, 4 h, 325 psi, reagent flow rate = 2.0 mL/min. Enzymatic hydrolysis condition: 12.5 IFPU/g treated biomass, pH 5.0, 50°C. (A) Untreated, (B) pretreated and oven-dried, (C) pretreated wet sample washed with NaOH, (D) pretreated wet sample soaked in H_2O, and (E) untreated filter paper.

pretreated at 175°C with 10 wt% ammonia for 1 h. As seen in Fig. 8, high enzyme loading (30 IFPU/g dry biomass) brought about a 20% increase in the glucose yield at 72 h. However, at 120 h, there was only a 10% increase over that of low loading (15 IFPU/g dry biomass). It was also found that the sequential addition of enzyme (15 IFPU at time = 0, than an additional 15 IFPU at 72 h) produced the same glucose yield as that of the high loading. Supplementation of β-glucosidase, however, showed no discernible effect on the glucose yield, probably because of sufficient β-glucosidase activity in Cytolase CL.

Lignin Separation from Spent Liquid

Tests were conducted to see how effectively the lignin, as well as ammonia, can be separated from the spent liquid. The liquid residue collected from a pretreatment run was evaporated from 80 to 40 mL. During the process, the pH changed from the initial value of 11.5 to 7.0, proof that most of the ammonia had been evaporated. The resulting liquid (thin slurry) was then centrifuged at 3600 rpm for 10 min. The supernatant was poured out, and the precipitate was dried at 105°C overnight and weighed (0.421 g). We believe most of the precipitate is lignin. Lignin is perhaps

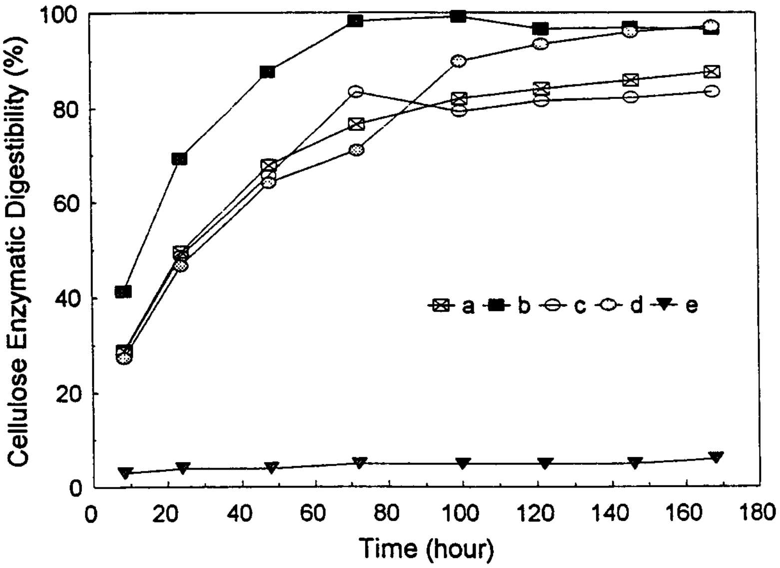

Fig. 8. Enzymatic hydrolysis of pretreated biomass at different enzyme loadings. Pretreatment condition: 175°C, 10 wt% ammonia, 2 h, 325 psi, reagent flow rate = 1.0 mL/min. Enzymatic hydrolysis condition: 50°C, pH 5.0, (phosphate buffer), 1.0 g biomass / 50 mL buffer. a: Treated sample with low loading (15 IFPU/g biomass), b: treated sample with high loading (30 IFPU/g biomass), c: treated sample with low loading + β-glucosidase (15 U), d: treated sample with low loading and add enzyme (15 IFPU) at 72 h, and e: untreated sample with low loading.

the only component in the ARP effluent that has pH-dependent solubility. During the pretreatment (175°C, 1 h, 5 wt% NH_3), 0.480 g of lignin (Klason), equivalent to 40% of lignin content in the original untreated biomass, was extracted from 4.6 g of biomass feed (dry basis) into the liquid effluent. It is then calculated that 0.421/0.480* 100 = 88% of the dissolved lignin is separated out from the liquid effluent simply by ammonia evaporation and centrifugation. The lignin generated in this process, although subject to further evaluation, is considered to be of high quality because it is free of sulfur or sodium. The delignified liquid effluent may be recycled after ammonia supplementation.

Ammonia Consumption

The consumption of ammonia, if any, is a critical issue for the ARP process from an economic standpoint. The overall material balance of ammonia for the process as determined from one run is as follows. For 1 g of dry biomass, 26 mL of 10 wt% ammonia solution were introduced into the percolation reactor in a semicontinuous manner, the solid being stationary and liquid effluent passing through it. The total ammonia throughput is therefore 2.6 g/g dry biomass. Of this quantity, 0.016 g of ammonia is left in the liquid stream unreacovered after boiling. It is likely most of am-

Table 3
Effect of Water and Ammonia Pretreatment
on the Composition of Solid Residues and Enzymatic Digestibility[a]

Pretreatment condition	%Wt remaining	%Lignin content	%Glucan content	%Xylan content	%Digestibility at 72 h
Untreated biomass	100.0	26.0	42.9	14.8	7.8
ARP (10 wt% NH_3, 180°C, 1 h)	68.9	13.1	40.0	7.7	95.2
AHP (pure water, 180°C, 1 h)	66.1	18.3	41.7	3.4	69.8
AHP + ARP (180°C, 0.5 h each, 10 wt% NH_3)	62.8	15.7	37.9	5.7	95.0
ARP + AHP (180°C, 0.5 h each, 10 wt% NH_3)	67.0	14.6	40.6	8.0	84.3
AHB (pure water, 180°C, 1 h)	71.1	20.4	40.7	2.2	71.7

[a] All sugars and lignin content in the table based on oven-dried untreated biomass. ARP: ammonia-recycled percolation. AHP: autohydrolysis run in percolation mode. AHB: autohydrolysis run in batch mode. Pretreatment condition: 1 h, 325 psi, reagent flow rate = 1.0 mL/min. Enzymatic hydrolysis condition: 30 IFPU/g treated biomass, 50°C, pH 5.0.

monia exists as ammonium acetate. An additional 0.001 g of ammonia was affixed to the treated solid residue as the result of pretreatment. The net consumption of ammonia is therefore 0.017 g/g dry biomass. The recovery factor of ammonia by simple boiling of the treated liquid effluent is then calculated to be 1 − (0.016 + 0.001)/2.6 = 99.3%. One of the key claims of the ARP process is hereby proven, e.g., that ammonia consumption is extremely low, although the throughput is high. A possibility also exists that additional steam-stripping of the liquid may further increase ammonia recovery.

Autohydrolysis

A primary result of the ARP process is delignification. However, it seems inevitable that a substantial portion of hemicellulose (as much as 40% of total initial xylan) is also solubilized during the process. We were interested to see if it is feasible to extract all of the xylan during pretreatment. Since the ARP process alone is not satisfactory for that purpose, a two-stage pretreatment process—a combination of autohydrolysis and ARP—was experimentally studied as a method to remove and recover all the xylan from the biomass. The biomass feed was pretreated with pure water alone (autohydrolysis), with 10 wt% ammonia solution alone, pure water followed by 10 wt% ammonia solution, and finally 10 wt% ammonia solution followed by pure water. The pretreated samples were enzymatically hydrolyzed. Table 3 summarizes the composition and digestibility data after these pretreatments. The results indicate that pure water treatment increases xylan extraction from the biomass. The major role of ARP, on the other hand, appears to be delignification and subsequent increase of digestibility. It is particularly noteworthy that when the biomass was pretreated with pure water at 180°C for 1 h in a straight batch mode (without percolation), about 85% xylan, but only 5% of glucan, was extracted, demonstrating a good selectivity. Autohydrolysis was more effective in the batch reactor operation mode than in the percolation mode. Without the ARP treatment, however, the autohydrolysis samples have an enzymatic digestibility of only about 70%. From these results, we feel that autohydrolysis followed by the ARP treatment would eventually prove to be an ideal pretreatment method, one that will ensure selective separation of xylan from the biomass as well as sufficient delignification to give acceptable enzymatic digestibility.

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REFERENCES

1. Brownell, H. H., Mes-Hartree, M., and Saddler, J. N. (1986), *Biotechnology and Renewable Energy,* Murray, M. Y., ed., p. 36.
2. Galbe, M., and Zacchi, G. (1986), *Biotechnol. Bioeng. Symp.* **17,** 97.
3. Jurasek, L., (1986), *Dev. Ind. Microbiol.* **20,** 177.

3a. Waiss, A. C. (1972), *J. Anim. Sci.* **35(1),** 109.

4. Detroy, R. W. (1980), *Biotech. Bioeng. Symp.* **10,** 135.
5. Jangalgi, N. R. (1983), *Chem. Abstr.* **98(14),** 109117t.
6. Millet, M. A., Baker, A. J., and Satter, L. D. (1975), *Biotechnol. Bioeng. Symp.* **6,** 193.
7. Mackie, K. L., Brownell, H. H., West K. L., and Saddler, J. N. (1985), *J. Wood Chem. Technol.* **5(3),** 405.
8. Wayman, M., Parekh, S., Chornet, E., and Overend, R. P. (1986), *Biotech. Lett.* **8(10),** 749.
9. Park, J. K. and Phillips, J. A. (1988), *Chem. Eng. Commun* **65,** 187.
10. Cunningham, R. L. and Carr, M. E. (1984), *Biotechnol. Bioeng. Symp.* **14,** 95.
11. Gould, J. M. (1984), *Biotechnol. Bioeng* **26,** 46.
12. Helmling, O., et al. (1989), *Biotechnol. Bioeng.* **33,** 237.
13. Chou, Y. C. T. (1986), *Biotechnol. Bioeng. Symp.* **17,** 19.
14. Chen, S. L. and Sunol, A. K. (1988), *AIChE For. Prod. Div. Sess.* **33.**
15. Cahela, D. R., Lee, Y. Y. and Chambers R. P. (1983), *Biotechnol. Bioeng.* **25,** 3–17.

16. Lee, Y. Y., Lin, C. M., Johnson, T., and Chambers, R. P. (1979), *Biotechnol. Bioeng. Symp.* **8,** 75–88.
17. Lee, Y. Y. and McCaskey, T. A. (1983), *Proc. Tappi R & D,* 317–326.
18. Kim, S. B. and Lee, Y. Y., (1986), *Biotechnol. Bioeng. Symp.* **17,** 71–84.
19. Grohmann, K., Torget, R., and Himmel, M. (1985), *Biotechnol. Bioeng. Symp.* **15,** 59.
20. Dale, B. E. and Moreira M. J. (1982), *Biotechnol. Bioeng. Symp.* **12,** 31.
21. Han, Y. W. (1978), *Adv. Appl. Microbiol.* **23,** 119.
22. Wang, et al. (1967), *TAPPI* **50(3),** 123.
23. Segal, et al. (1954), *J. Poly. Sci.* **13,** 193.
24. Tarkow, H. and Feist, W. C. (1969), *Adv. Chem. Ser.* **95,** 197.
25. Lewin, M. and Roldan, L. G. (1971), *J. Poly. Sci.* **36,** 213.