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Ammonia Recycled Percolation Process for Pretreatment of Herbaceous Biomass

PRASHANT V. IYER, ZHANG-WEN WU, SUNG BAE KIM,† AND YOON Y. LEE*

Department of Chemical Engineering, Auburn University, Auburn, AL 36849

ABSTRACT

An ammonia-based pretreatment method termed ammonia recycled percolation (ARP) was developed for pretreating herbaceous biomass like corn cobs/stover mixture (CCSM) and switchgrass. The process involves treatment of biomass with aqueous ammonia through a percolation reactor (packed-bed, flow-through type). The effects temperature, reaction time, and ammonia concentration were studied. The extent of delignification in the ARP process was in the range of 60–80% for CCSM and 65–85% for switchgrass. The ARP process solubilized significant amounts of the hemicellulose fraction into the pretreatment effluent, yet left most of the glucan fraction intact. The experimental data on CCSM and switchgrass indicate that the ARP is a highly effective pretreatment method. Near-complete conversion of cellulose to glucose was obtained by enzymatic hydrolysis of ARP-treated solid samples of CCSM, whereas conversion was slightly lower for switchgrass. The rate of enzymatic hydrolysis of ARP-treated samples was substantially higher than that of α-cellulose. The ARP effluents were evaluated for fermentability/toxicity by the xylose-fermenting yeast *Pichia stipitis* (NRRL Y-11545). The adaptability of ARP-treated solid samples to simultaneous saccharification and fermentation (SSF) was tested for ethanol production using cellulase enzyme and the yeast, *Saccharomyces cerevisiae* (NREL, D5A).

Index Entries: Pretreatment; ammonia; delignification; switchgrass; corn cobs/stover.

INTRODUCTION

Pretreatment is a necessary element in bioconversion of lignocellulosics to fuels and chemicals. The primary purpose of pretreatment is to make the cellulosic biomass amenable to the action of cellulase enzymes. A number of pretreatment methods have been proposed and investigated *(1–7)*. Although most of these pretreatments do increase the yields of sugars by enzymatic hydrolysis, the reported yields are usually below the theoretical maximum *(1–5)*. There are some exceptional cases where quantitative conversion of cellulose into glucose has been reported *(6,7)*. These pretreatment methods, however, require a high-energy input or utilize

*Author to whom all correspondence and reprint requests should be addressed.
†On leave from Gyongsong National University, Chinju, Korea.

Table 1
Initial Composition of CCSM and Switchgrass

Components identified[a]	Percentage	
	CCSM	Switchgrass
Glucan	38.10	35.20
Xylan	20.00	17.76
Arabinan	3.10	3.72
Galactan	1.20	1.92
Mannan	0.60	3.40
Klason lignin[b]	15.95	19.88
Acid-soluble lignin	3.10	3.30
Ash	4.29	5.25
Extractives	6.70	6.09
Others	6.96	3.48

[a]Based on oven-dry untreated biomass.
[b]For Klason lignin determination, biomass was treated with 95% ethanol to remove extractives.

expensive and/or toxic reagents. There are other problems associated with pretreatments, such as loss of sugars through decomposition and generation of toxic products that inhibit microbial action in the subsequent fermentation *(8,9)*. The economic and environmental constraints further limit the applicability of these known methods. Recently, a novel pretreatment method based on aqueous ammonia has been developed in our laboratory *(10)*. This process was termed the ammonia recycled percolation (ARP) process. The ARP when applied to woody biomass brought about not only pretreatment effects, but also a partial fractionation of biomass into cellulose, hemicellulose, and lignin. Lignin generated from the ARP process is sulfur- and sodium-free, unlike the lignin generated from conventional pulping processes. It is quite conceivable that the uncontaminated lignin can be a marketable byproduct, enhancing the overall economics of the bioconversion process.

In this study, we extended our investigation on the ARP as a pretreatment method to cover herbaceous biomass feedstocks, including corn cobs/stover mixture and switchgrass. Switchgrass has a number of desirable characteristics as an energy crop *(11)*. The scope of this work included the technical factors, primarily the reaction conditions, effectiveness as a pretreatment, and fermentability/toxicity of the effluents to simultaneous saccharification and fermentation (SSF).

MATERIALS AND METHODS

Materials

Corn cobs/stover mixture (CCSM) and switchgrass feedstocks were supplied from National Renewable Energy Laboratory (NREL, Golden, CO). The CCSM was milled and screened to the nominal size of 0.25–2.00 mm. Switchgrass was used as supplied (the size being very fine—5 mm). The initial compositions of CCSM and switchgrass are shown in Table 1. The cellulase enzyme, Cytolase CL, Lot No. 17-92262-09, was obtained from Environmental Biotechnology (Santa Rosa, CA). The average specific activity of the enzyme as determined by the supplier is as

follows: Filter paper activity = 95.9 FPU/mL, β-glucosidase activity = 80.6 p-NPGU/mL, endoglucanase activity = 613 CMCU/mL. A culture of xylose-fermenting yeast *Pichia stipitis* (NRRL Y-11545) was obtained from USDA and was used for fermentability and toxicity studies on ARP effluents. An NREL culture of *Saccharomyces cerevisiae*, D_5A strain, was used for SSF studies. The Sigma (St. Louis, MO) α-cellulose was supplied from NREL.

Experimental Setup and Operation

The details of the experimental apparatus are described elsewhere *(10)*. A slight modification was made in that a temperature-programmable oven replaced a sand bath. In operation of ARP, 5–7 g of air-dried biomass sample were packed into the reactor, soaked with ammonia solution, and left overnight. The ratio of liquid to solid inside the reactor was kept in the range of 7–10. The preheating time to reach the desired reaction temperature was about 15 min. Nitrogen back pressure was applied to the reaction system at 325 psi to prevent the ammonia vaporization. After the reaction, water was pumped to remove residual sugars and ammonia trapped in biomass. The reactor was then flushed with nitrogen to remove the excess water. The biomass samples discharged from the reactor were divided into two portions. One portion of the wet solid residues was oven-dried at 105°C overnight to measure moisture content and then subsequently the weight loss on pretreatment. It was further subjected to chemical composition analysis. The other remaining portion of solid residues was stored in a walk-in cool room (4°C) under wet condition for carrying out the digestibility test. The ARP effluent collected in a holding tank was transferred into an air-tight sample bottle and stored in the cool room for further composition analysis.

Digestibility Test

Enzymatic hydrolysis of pretreated substrate was performed at 50°C and pH 4.8 (0.05*M* sodium citrate buffer) on a shaker bath (New Brunswick Scientific, Edison, NJ) agitated at 150 rpm. The enzyme loading was 60 IFPU/g glucan, and the initial glucan concentration was 1% (w/v). Samples were taken periodically and analyzed for glucose and cellobiose content using high-performance liquid chromatography (HPLC). The total glucose content after 72 h of hydrolysis was taken to calculate the enzymatic digestibility. Untreated switchgrass, CCSM, and α-cellulose were also subjected to the same digestibility test as a control and as a reference.

Analytical Methods

The solid samples were analyzed for sugars, Klason lignin, acid-soluble lignin, and ash following the procedures of NREL-CAT Standard Procedures *(12)*. Prior to Klason lignin determination, the feedstocks were put through ethanol reflux to remove the extractives. The effluents from the ARP process were boiled until all free ammonia was evaporated. Since most of the sugars contained in the effluents were oligomers, a secondary hydrolysis was carried out at 121°C with 4% (w/w) sulfuric acid for 1 h of reaction time to convert oligomers into monomers. Sugars and decomposition products were measured by HPLC using Bio-Rad (Hercules, CA) Aminex HPX-87H column. The total amount of xylan, mannan, arabinan, and galactan was used to represent the hemicellulose content in this study. The fermentation samples were analyzed using the H-column and/or YSI 2300 Stat Plus sugar analyzer.

Fermentation of ARP Effluents and ARP Solid Samples

Fermentability/toxicity tests of ARP effluents containing xylose were done following the procedure obtained from the USDA *(13)*. The fermentation was performed at 30°C, pH 6.0 with 3% (w/v) xylose. The adaptability of ARP-treated solid samples for ethanol production by SSF was determined by following NREL-CAT Standard Procedure No. 00 8 *(14)*. The SSF conditions were: 38°C, pH 5.0, 3% (w/v) glucan, and 25 IFPU/g glucan enzyme loading.

Determination of Fermentation Parameters

For the ARP effluent fermentability/toxicity tests, the yield was calculated as the ratio of maximum ethanol concentration detected minus the concentration of ethanol at zero time to the initial concentration of sugars (glucose + xylose) present. Volumetric productivity (g/L/h) was calculated as the maximum ethanol concentration detected minus the concentration of initial ethanol divided by the fermentation time (time at which the ethanol concentration reaches maximum). For the SSF of ARP-treated solid substrates, the ethanol yield was defined as the ratio of the amount of net ethanol produced to the amount of the initial glucose content in the pretreated substrate divided by 0.51.

RESULTS AND DISCUSSION

Effect of Ammonia Concentration

Ammonia concentrations were varied over 2.5–20% (w/w) keeping other conditions at 170°C, reaction time of 1 h, flow rate = 1 mL/min. Figure 1 shows the weight remaining, amounts of glucan, hemicellulose, lignin remaining, and cellulose enzymatic digestibility on the ARP pretreatment. During the ARP, CCSM lost 43–47% of its weight. The weight loss for switchgrass was slightly higher at 49–56%. Lignin and hemicellulose accounted for the major part of the weight loss. In the case of CCSM, as high as 56% of the original amount of hemicellulose of CCSM was extracted into ARP effluent, whereas <8% of the total glucose content was extracted. These numbers were slightly higher for switchgrass, 64 and 14%, respectively. The amount of glucan and hemicellulose solubilized remained essentially constant showing little effect of ammonia concentration over this range. The lignin remaining, however, decreased by 48 to 19% for CCSM (32 to 16% for switchgrass) as the concentration of ammonia was increased from 2.5 to 20%. The increase in ammonia concentration beyond 10% had negligible effect on the degree of delignification. Figure 1 also shows that the enzymatic digestibility of ARP samples exceeded 90% for CCSM and 87% for switchgrass in all cases. The digestibility generally increased as the ammonia concentration was increased. However, above 5% for CCSM, the increase of digestibility was insignificant. Results were similar for switchgrass, but the leveling off occurred at 10%. An ammonia concentration of 10% is therefore regarded as a near-optimum level for pretreatment of switchgrass. For CCSM, ammonia concentration of 5% was sufficient to achieve high digestibility. Considering that an ammonia concentration of 10% gives a higher degree of delignification (79%) and that almost all of it is recycled, it was taken as the optimum level for CCSM as well.

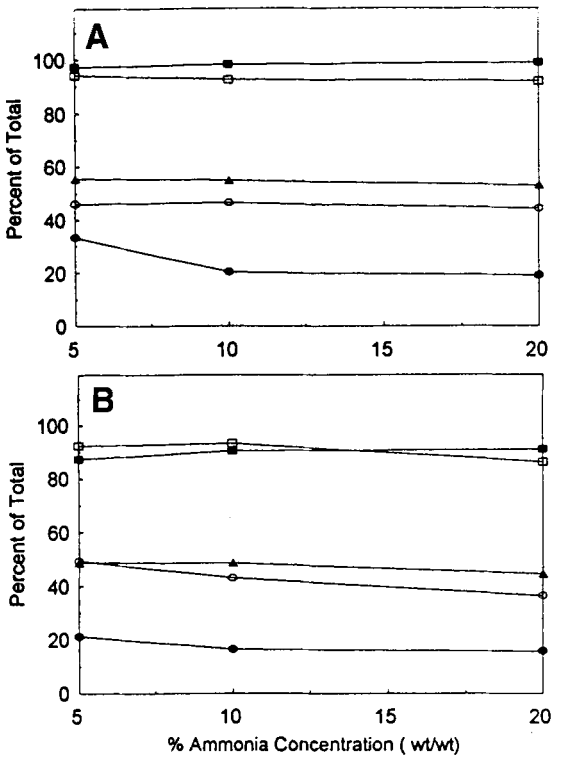

Fig. 1. Compositional changes and enzymatic digestibility in ARP at various ammonia concentrations. **(A)** CCSM; **(B)** switchgrass. Pretreatment conditions: 170°C, flow rate = 1 mL/min. Enzymatic hydrolysis conditions: 60 IFPU/g glucan, pH 4.8, 50°C. ▲, solid remaining; □, glucan remaining; ○, HC remaining; ●, lignin remaining; ■, enzymatic digestibility.

Effect of Temperature

ARP on CCSM retained 44% of hemicellulose (43% for switchgrass) at 170°C, 10% ammonia, treatment time of 60 min, flow rate = 1 mL/min (Fig. 1). Complete retention or complete removal of the hemicellulose (depending on the subsequent fermentation strategy) is desirable for efficient utilization of hemicellulose sugars. Increased solubilization of the hemicellulose is expected with increase in the reaction temperature. Ensuing runs were made over 160–200°C for CCSM, maintaining other conditions at 10% ammonia, 1-h reaction time, flow rate = 1 mL/min. Table 2 summarizes the composition data for the hydrolysate and the remaining solid residue after pretreatment. From the data on CCSM, it is seen that the amounts of glucan and hemicellulose solubilized increased from 8 to 15% and 46 to 80%, respectively, as the pretreatment temperature was increased from 160 to 200°C. However, the maximum amount of hemicellulose recovered as oligomers in the ARP effluent was only about 72% of the original amount and occurred at 200°C. The ARP treatment of CCSM gave a near-complete sugar balance at temperatures below 200°C, indicating sugar decomposition is insignificant. With regard to delignification, temperature effect gave a complicated picture. As the temperature was increased from 160 to 180°C, lignin remaining in the solid decreased to 19% of the original. As the temperature was increased from 180 to 200°C, however, lignin remaining increased to 35%.

Table 2
Effect of Reaction Temperature on Composition of Hydrolysates and Solid Residues in ARP[a]

Temperature, °C	% Solid remaining	% Lignin, Klason	% Glucan			% Hemicellulose		
			Solid	Liquid	Total	Solid	Liquid	Total
CCSM								
Untreated	100.00	15.95	38.10	—	38.10	24.90	—	24.90
160	55.56	5.03	35.10	1.89	36.99	12.53	11.04	23.57
170	54.90	3.26	35.30	2.04	37.34	11.01	12.65	23.66
180	51.26	3.10	34.78	2.11	36.89	9.51	14.53	24.04
190	49.70	5.32	33.41	2.36	35.77	7.96	16.51	24.47
200	43.24	5.63	32.18	2.39	34.57	5.09	18.07	23.16
Switchgrass								
Untreated	100.00	19.88	35.20	—	35.20	26.80	—	26.80
160	52.15	4.84	33.34	1.58	34.92	13.24	12.26	25.50
165	50.22	4.26	32.02	1.28	33.30	11.60	13.30	24.90
170	48.57	3.26	32.88	1.27	34.15	10.94	14.34	25.28
175	45.12	3.53	31.94	1.11	33.05	8.51	15.40	23.91

[a]All sugar and lignin contents based on the oven-dry untreated biomass. Hemicellulose: the total amount of xylan, mannan, arabinan, and galactan. Pretreatment conditions: 10% (w/w) ammonia, 60 min, flow rate = 1 mL/min. Secondary hydrolysis conditions: 121°C, 45 min, 4% sulfuric acid.

We do not have a clear explanation for this unusual behavior at this time. The data in Tables 2 and 3 show that the material balance of the treated solids do not close out because ash content and extractable and gaseous degradation components (if any) were not measured, and were thus unaccounted for. The enzymatic digestibility was investigated for all samples of CCSM treated at various temperatures (Fig. 2). The maximum cellulose enzymatic digestibility after 72 h was 98% of theoretical maximum for CCSM, and it occurred with the ARP conducted at 170°C. With one exception of ARP-treated sample (CCSM at 160°C), all treated samples are seen to exhibit high digestibility, most of them showing above 90% in 36 h. At the 36-h point, the digestibility of α-cellulose was only 56%. Although the reaction temperatures of both 170 and 180°C offer good pretreatment performance for CCSM, reaction temperature of 170°C would be preferred to 180°C from process viewpoint.

A 10°C increment was used in the study of temperature effect on CCSM. After a finding that the optimum temperature for CCSM is near 170°C, a 5°C increment was then used for switchgrass, applying four levels over 160–175°C. The composition data of treated switchgrass are also included in Table 2. The enzymatic digestibilities for ARP-treated switchgrass samples are shown in Fig. 2. As the temperature was increased from 160 to 175°C, there was a substantial increase in hemicellulose removal. However, the lignin content in the solid remained essentially constant. The glucan removal was below 10% for all the temperatures. Again the initial rate of hydrolysis of ARP-treated samples was much higher than that of α-cellulose and the digestibility at 72 h of the best-case ARP-treated switchgrass was above 90% as opposed to 77% for α-cellulose. From the data in Table 2 and Fig. 2, 170–175°C is judged to be the optimum region for treatment of switchgrass in ARP.

Table 3
Effect of Reaction Time on Composition of Hydrolyzates, Solid Residues, and Cellulose Enzymatic Digestibility in ARP

Reaction time, min	% Solid remaining	% Lignin, Klason	% Glucan			% Hemicellulose			% Digestibility 72 h
			Solid	Liquid	Total	Solid	Liquid	Total	
CCSM									
Untreated	100.00	15.95	38.10	—	38.10	24.90	—	24.90	22.90
15	56.07	4.16	35.48	1.82	37.30	11.95	12.61	24.56	94.10
30	55.68	3.82	34.43	1.88	36.31	11.15	13.01	24.16	95.28
45	55.28	3.44	34.98	2.16	37.14	11.39	13.35	24.74	96.45
60	54.90	3.26	35.30	2.04	37.34	11.01	12.65	23.66	98.30
Switchgrass									
Untreated	100.00	19.88	35.20	—	35.20	26.80	—	26.80	17.30
30	50.10	5.76	31.07	1.88	32.95	12.88	12.72	25.60	87.30
60	48.57	3.26	32.88	1.27	34.15	10.94	14.34	25.28	90.46

Notes are the same as for Table 2.

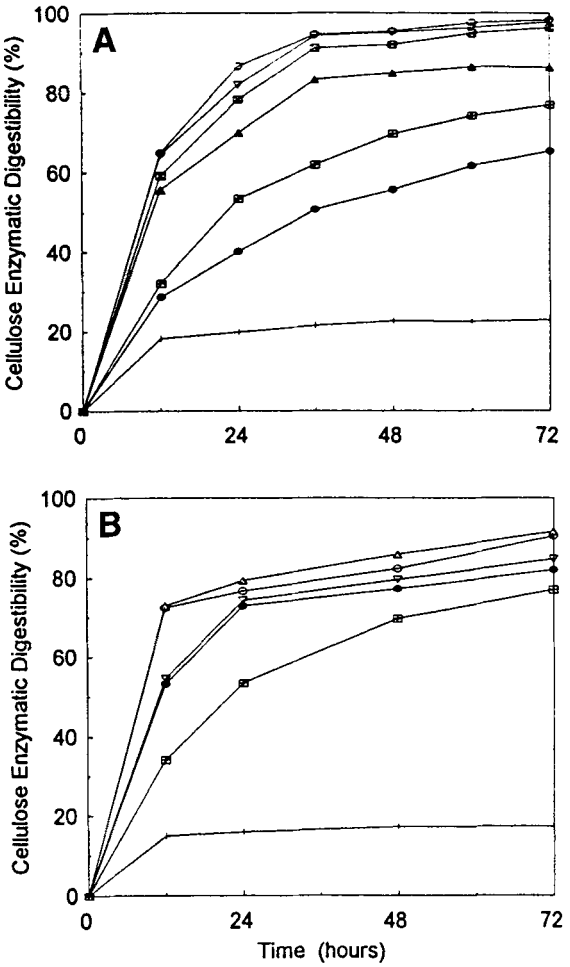

Fig. 2. Enzymatic digestibility of substrates treated in ARP at various temperatures. **(A)** CCSM; **(B)** switchgrass. Pretreatment conditions: 10% ammonia, 60 min, flow rate = 1 mL/min. Enzymatic hydrolysis conditions: 60 IFPU/g glucan, pH 4.8, 50°C. (A) ●, 160°C; ○, 170°C; ▽, 180°C; ⊠, 190°C; ▲, 200°C; +, untreated; ⊞, alpha-cellulose. (B) ●, 160°C; ▽, 165°C; ○, 170°C; △, 175°C; +, untreated; ⊞, alpha-cellulose.

Effect of Reaction Time

The time effect on the ARP process was first studied by taking on-line samples of the ARP effluent of switchgrass. The conditions were: 10% ammonia, 170°C, reaction time of 1 h, flow rate = 1 mL/min. These samples were analyzed for sugars and lignin. Lignin contents were determined by the UV at 280 nm using 10% ammonia solution as a reference. The data indicate that most of the hemicellulose removal and delignification occur within first 30 min of the ARP operation (Fig. 3). In the ensuing runs, reaction time was varied over 15–60 min for CCSM, keeping other conditions at 10% ammonia, 170°C, flow rate = 1 mL/min. The composition data for the hydrolysate and the remaining solid residues after the ARP pretreatment and the enzymatic digestibility for both switchgrass and CCSM are presented in Table 3. From the data, it is quite evident that the reaction time of 15 min is sufficient to increase the 72-h digestibility from the control level of 23–94% for CCSM. For switch-

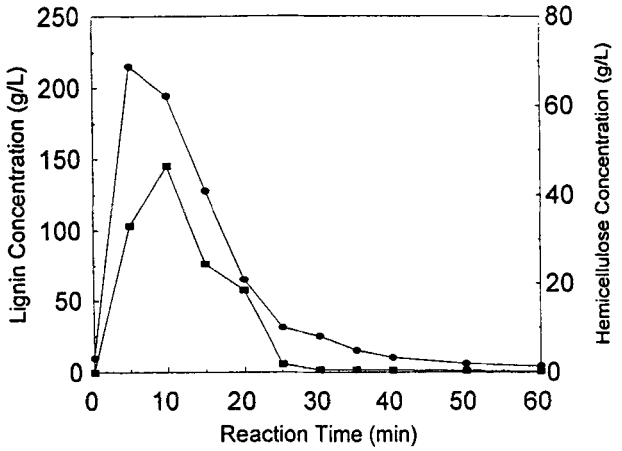

Fig. 3. Time-course of lignin and hemicellulose in ARP effluent of switchgrass. Pretreatment conditions: 170°C, 60 min, flow rate = 1 mL/min. Secondary hydrolysis conditions: 121°C, 4% sulfuric acid, 1 h; HC, hemicellulose. ●, lignin; ■, hemicellulose.

grass, a reaction time of 30 min was required. The digestibilities were somewhat lower at 17.3% for control and 87.3% for treated samples. Further increases in reaction time showed only a marginal increase in digestibility for both CCSM and switchgrass. As the reaction time was increased from 15 to 60 min for CCSM (30–60 min for switchgrass), the remaining lignin decreased from 26 to 20% of original for CCSM (29 to 16% for switchgrass). However, the hemicellulose and glucan remained relatively constant (Table 3). Data in Table 3 collectively indicate that the acceptable range of reaction time under the previously described reaction conditions is 15–60 min.

ARP Effluent and Lignin Separation

ARP treatment of CCSM and switchgrass solubilized about 80% of original lignin into the ARP effluent at the pretreatment condition of 170°C, 1 h, 10% ammonia, 1 mL/min. At the same time, about 55% of the original hemicellulose was extracted into the ARP effluent, mostly in the form of oligomers. The ARP effluents were boiled until all the free ammonia evaporated (or until pH reached 7.0 from 11.5) and then subjected to a secondary acid hydrolysis. The acidic condition of secondary hydrolysis induced precipitation of lignin from the ARP effluent. The effluent was cooled to room temperature and filtered. The precipitated lignin was further washed with deionized (DI) water, and then dried at 105°C overnight and weighed. During the pretreatment, 80% of the original Klason lignin was extracted into the liquid effluent for CCSM. It was 84% for switchgrass. However, only 50–60% of the original Klason lignin was precipitated and recovered. The balance of the lignin is believed to be broken down into low-mol-wt lignin fragments and remained soluble even under acidic conditions.

Ammonia Consumption

From an economic standpoint, the consumption of ammonia is an important issue in the ARP process. Ammonia material balance was calculated using data on Kjeldahl nitrogen analysis on both solids and liquids. The results have shown that the recovery of ammonia is in excess of 99%, essentially the same as that for hybrid

poplar *(10)*. The ammonia consumption was 0.02 g/g dry biomass, not a significant cost factor. Most of the unrecovered ammonia is believed to be in the form of ammonium acetate in the ARP effluent. Only a small fraction is affixed to the treated solid residue as a result of pretreatment. This fraction may even be utilized as a source of nitrogen in the subsequent fermentation *(15)*.

Fermentability/Toxicity Tests on ARP Effluents

The ARP treatment of CCSM and switchgrass under near-optimum conditions solubilized 50–56% of total hemicellulose into the ARP effluent. Extraneous components, such as extractives, acetic acid, furfural, and dissolved lignin components, present in the hydrolysates are potential inhibitors of microorganism fermenting the xylose. A toxicity test was therefore conducted to determine the maximum effluent loading for efficient fermentation of xylose to ethanol. The effluent loading is defined as the volume percentage of the ARP hydrolysate in the total fermentation liquid.

Multiple runs of ARP were made at 170°C, 10% ammonia, flow rate = 1 mL/min, at the reaction time of 15 min for CCSM (30 min for switchgrass) to generate a sufficient amount of liquor to be tested. Effluent was boiled to remove ammonia, and its final volume was readjusted to initial volume adding DI water. Since ARP effluent contained a large amount of oligomeric sugars (mainly xylan), a secondary hydrolysis was conducted to convert them into monomers. In this process, lignin was precipitated and filtered out before subjecting the effluent to fermentability/toxicity tests.

Figure 4 shows the yield (g/g) and volumetric productivity (g/L/h) at various effluent loadings for CCSM and switchgrass. The yield at 40 and 60% effluent loading for CCSM was essentially the same as that of the control (0% loading). The volumetric productivity, however, has decreased significantly with effluent loading of 60%. Fermentation with 80% effluent loading was not completed within 72 h, leading to lower concentration of ethanol than other runs. For switchgrass, the results were quite similar to those of CCSM in that 60% effluent loading showed little effect on the product yield, whereas the volumetric productivity was adversely affected.

SSF

ARP-treated solid samples used in the SSF test were prepared under the conditions of 170°C, 10% ammonia, flow rate = 1 mL/min, 15-min reaction time for CCSM (30 min for switchgrass). The glucan content of pretreated biomass was 65% (dry basis) for CCSM and 66% for switchgrass. The glucan content of α-cellulose, which was used as a control, was 94%.

The ethanol yield data for both ARP-treated CCSM and switchgrass are presented in Fig. 5. The ethanol yield for pretreated CCSM reached 83% of theoretical maximum at 168 h. It was essentially same as that of α-cellulose. The pretreated switchgrass gave about the same level of ethanol yield, 84% at 168 h. At the 24-h point, the ethanol yield of the pretreated CCSM reached 60% and switchgrass, 52%. These values were substantially higher than the 35% yield observed for α-cellulose (Fig. 5). The SSF results on the ARP-treated CCSM and switchgrass are deemed quite satisfactory in view of the fact that a certain fraction of the substrate is channeled into cell growth.

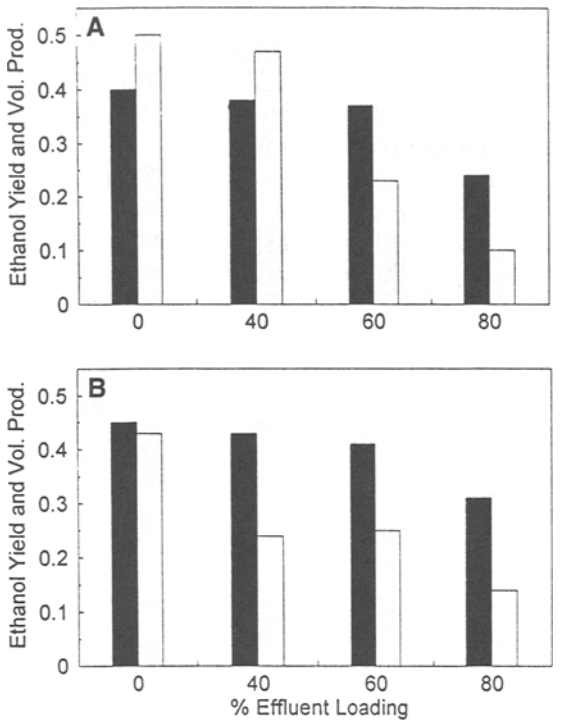

Fig. 4. Ethanol yield (■, g/g) and volumetric productivity (□, g/L/h) at various effluent loadings. **(A)** CCSM, 15 min in ARP; **(B)** switchgrass, 30 min in ARP. Pretreatment conditions: 170°C, 10% ammonia, flow rate = 1 mL/min; 15 min for CCSM, 30 min for switchgrass. Fermentation conditions: 30°C, pH 6.0, 72 h, 3% (w/v) xylose.

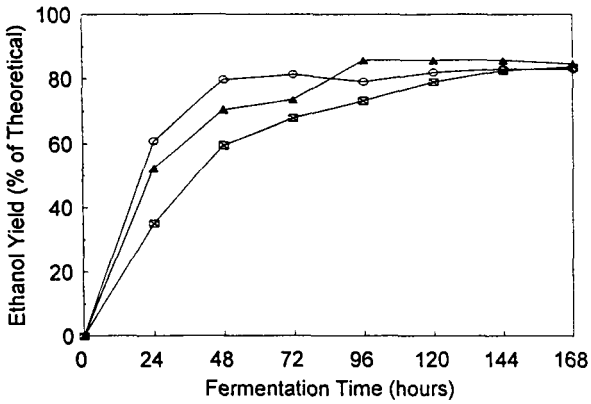

Fig. 5. Ethanol yield substrates pretreated in ARP at different fermentation times. Pretreatment conditions: 170°C, 10% ammonia, flow rate = 1 mL/min, 15 min for CCSM, 30 min for switchgrass. SSF condition: 38°C, pH 5.0, 3% (w/v) glucan, enzyme loading 25 IFPU/g glucan. ○, pretreated CCSM; ▲, pretreated switchgrass; ⊠, alpha-cellulose.

CONCLUSIONS

ARP as tested against CCSM and switchgrass is a highly effective pretreatment method. The enzymatic digestibility of the pretreated substrates was found

to be in the vicinity of 90%. Hemicellulose and lignin removal and modification of physical structure are attributed to the increase in the enzymatic hydrolysis. The optimum condition identified for ARP on CCSM is: 170°C, 10 wt% ammonia concentration, flow rate = 1 mL/min, and reaction time 15–60 min. For switchgrass, it is: 170°C, 10 wt% ammonia concentration, flow rate = 1 mL/min, and reaction time 30–60 min. The extent of delignification was 74–80% for CCSM and 71–84% for switchgrass. Under the optimum conditions, 50–56% of the total hemicellulose in CCSM and switchgrass, but <8% of the total glucan in CCSM (10% for switchgrass), was solubilized. The toxicity of the ARP effluent is such that its loading up to 60% could be employed without adversely affecting the ethanol yield. ARP-treated solid samples of CCSM and switchgrass are easily adaptable to SSF for ethanol production.

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