0273-2289/96/5758—0147$7.50

Fractionation of Herbaceous Biomass by Ammonia–Hydrogen Peroxide Percolation Treatment

SUNG BAE KIM† AND YOON Y. LEE*

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

ABSTRACT

Treatment with ammonia and hydrogen peroxide was investigated as a means of fractionating herbaceous biomass. The main feature of this process is that aqueous forms of these reagents are pumped simultaneously into a packed-bed flow-through-type reactor (percolation reactor) under a semibatch mode with ammonia being recycled. Experimental tests on corn cobs/stover mixture (CCSM) and switchgrass feedstocks have proven that a high degree of fractionation of biomass into three major components is attainable under this process scheme. The extent of delignification was 94–99% It was achieved at a representative condition of 170°C, 0.28 g loading of H_2O_2/g biomass, and 10 wt% ammonia concentration. At the same time, about 80% of total hemicellulose in the biomass was separated out into the effluent primarily in the form of xylose oligomers. Decomposition of sugar components was insignificant. The remaining solids had a composition of 80–93% glucan, 5–10% xylan, and 1–6% lignin. Selected solid samples. obtained under near-optimum conditions, exhibited a chemical composition close to that of commercial α-cellulose The enzymatic digestibilities of these solid samples were substantially higher than that of α-cellulose.

Index Entries: Biomass, herbaceous; ammonia; hydrogen peroxide; fractionation; pretreatment.

INTRODUCTION

Ammonia has a number of characteristics suitable for processing of lignocellulosic substrates. It is a proven delignification reagent *(1,2)*. It also carries other reactive properties, including hydrolysis of glucuronic acid ester crosslinks in biomass *(3)*, cleaving of the lignin–hemicellulose bonds *(4)*, and change of cellulose fiber structure *(5)*. High volatility of ammonia in comparison to water makes it easy to separate from aqueous mixture. To our knowledge, there is no harmful byproduct formation from ammonia–lignin–carbohydrate interaction at elevated temperatures. For these reasons, the use of aqueous ammonia in biomass pretreatment has been a subject of our laboratory investigation. Recently, we have reported on a novel biomass pretreatment process termed ammonia recycled percolation (ARP) process

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

(6). Its main technical features are: (1) aqueous ammonia ($NH_3 \cdot H_2O$) is used as the pretreatment reagent, and (2) a packed-bed flow-through-type (percolation) reactor is employed and operated under a recirculation mode The concept of percolation process applies well to chemical processing of lignocellulosic substrates. A distinctive feature of the percolation process in comparison to a straight batch process is that the process stream is continuously fed and withdrawn from the reactor. In connection with the biomass pretreatment, it offers a unique advantage that the lignin and other extraneous components are separated from the biomass structure, preventing recondensation of lignin within the biomass. In this study, the ARP process has indeed shown a great potential as a pretreatment method in that it provided the digestibility exceeding that of α-cellulose, high delignification, and negligible sugar decomposition. One result from the study was that a substantial amount of hemicellulose was also solubilized into the effluent along with lignin. In this article, we investigate the fractionation of herbaceous biomass by adding hydrogen peroxide to the ARP process to improve the performance of hemicellulose recovery and delignification. The process is henceforth referred to as ARP-H, H standing for hydrogen peroxide. There have been a number of pretreatment studies based on hydrogen peroxide reported in literature in which a high degree of delignification and enzymatic digestibility was claimed *(7–13)*. The observation by Gould and Freer *(10)* that 80% of the hemicellulose in agricultural residues was solubilized in the alkaline peroxide solution was particularly supportive of this approach. The scope of the work covered the technical factors pertaining to the overall assessment of ARP-H as a fractionation method.

MATERIAL AND METHODS

Materials

Milled corn cobs/stover mixture (CCSM), switchgrass, and hybrid poplar were supplied from National Renewable Energy Laboratory (NREL). They were screened to the nominal size of 2 mm ~ 60 mesh. The composition of untreated CCSM was 38.1 wt% glucan, 20 wt% xylan, 3.1 wt% arabinan, 1.2 wt% galactan, 0.6 wt% mannan, 16.0 wt% klason lignin, 4.7 wt% acid soluble lignin, 6 9 wt% ash, 6.7 wt% extractives, and 2.7 wt% unaccounted for. The cellulase enzyme, Cytolase CL (Lot No. 17–9226209), was supplied from Environmental Biotechnology, Inc. (Santa Rosa, CA). The average activities of the enzymes as determined by the supplier are: 95.9 IFPU/mL, β-glucosidase activity = 80.6 p-NPGU/mL, endoglucanase activity = 613 CMCU/mL.

Experimental Setup and Operation

A schematic diagram of the reactor setup is shown in Fig. 1. The system consists of a stock solution reservoir, pump, programmable drying oven, reactor, and liquid holding tank, which also served as a back-pressure vessel. Aqueous ammonia and hydrogen peroxide solutions were pumped simultaneously by a duplex metering pump (LDC minipump) to a packed-bed reactor through a preheating coil. The flow rate of ammonia solution was monitored by flow meter and hydrogen peroxide solution by a buret. The reactor was constructed out of SS 316 tubing to the dimension of 5/8 in. od × 6 in. L (33 cm^3 of internal volume). The reactor temperature was controlled in a temperature-programmable oven. An autoclave (600 mL, Parr Instrument, Moline, IL) was used as a liquid holding tank to which a nitrogen cylinder

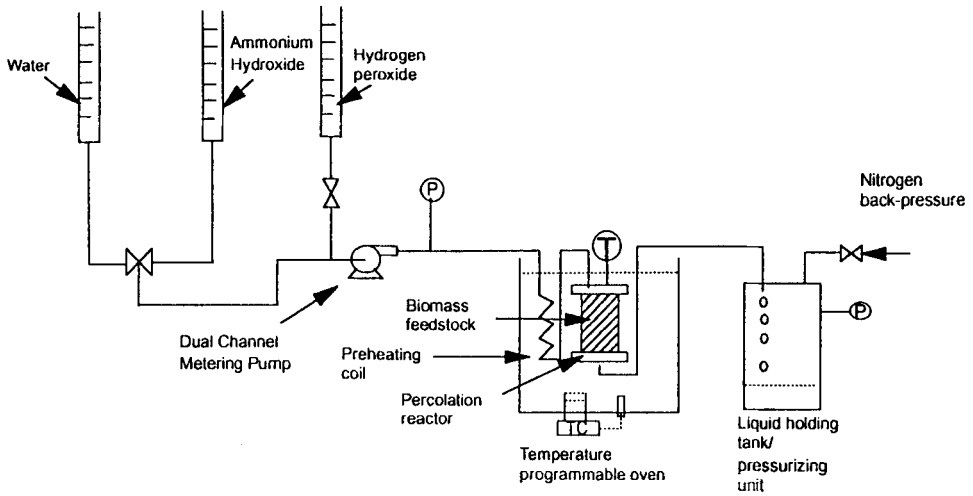

Fig. 1 . Schematics of ARP-H reactor system.

was connected to apply back pressure, preventing evaporation of reactant fluid. The biomass feed was presoaked with ammonia solution overnight. In an ARP-H experiment, 6 g of biomass sample were packed into the reactor. To carry out the reaction, oven temperature was initially set 10°C higher than the desired set point. When the reactor temperature reached within 6°C of the set point, it was reset to the original set point. This operation reduced the preheating time to 15 min. At the completion of a run, the reactor was pumped with water to remove the residual sugar and ammonia trapped in the treated biomass.

The effluent collected in the holding tank was filtered and analyzed for composition. The wet solids discharged from the reactor were separated into two portions. One was oven-dried at 105°C overnight for measurement of weight loss and further subjected to composition analysis. The other was used in the enzymatic digestibility test.

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 agitated at 150 rpm. The cellulase enzyme loading was 60 IFPU/g glucan. 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). Total glucose plus cellobiose content after 72 h of hydrolysis was taken for calculation of the enzymatic digestibility.

Analytical Methods

The solid biomass samples were analyzed for sugars, klason lignin, acid-soluble lignin, and ash, and extractives of solid samples were analyzed following the procedures described in NREL Chemical Analysis & Testing Standard Procedure (No 002 and 010). All experiments were done in duplicate. The hydrolysates from the ARP-H process were boiled until all free ammonia was evaporated. Since the hydrolysates contained oligomers, a secondary hydrolysis was carried out with 4 wt% sulfuric acid

at 121°C for 1 h. Sugars and decomposition products were measured by HPLC using Bio-Rad (Hercules, CA), Aminex HPX-87H column. Since Aminex HPX-87H column does not resolve xylose, mannose, and galactose, the combined value of xylan + mannan + galactan is used in part of this article, expressed as XMG.

RESULTS AND DISCUSSION

Effect of Hydrogen Peroxide on Delignification and Hemicellulose Recovery

The reaction of hydrogen peroxide with lignin goes through a rather complex mechanism *(14,15)*. Nonetheless, the net reaction can be expressed as the reaction between lignin and two lignin-oxidizing species, hydroxy radical (·OH) and perhydroxy anion (HO_2^-) formed during the decomposition of H_2O_2. The perhydroxyl anion is a mild oxidant that is not strong enough for prompting delignification, whereas the hydroxyl radical is a powerful lignin oxidant *(16)*. The latter also nonspecifically attacks carbohydrates resulting in depolymerization of carbohydrates *(16,17)*. The formation of both species can thus be expressed by the following two reactions:

$$H_2O_2 = H^+ + HOO^-, \qquad H_2O_2 + HOO^- = \cdot OH + O_2^{-}\cdot + H_2O$$

These reactions are strongly pH-dependent. Alkaline condition promotes formation of perhydroxyl anion by neutralizing the H^+ ions, whereas the perhydroxyl anion accelerates formation of hydroxyl radical through the second reaction. Table 1 shows various reaction schemes for ARP-H treatment of CCSM under a fixed level of H_2O_2 loading. The control reaction, No. 1, was conducted at the reaction condition of 170°C, 10 wt% ammonia without H_2O_2. In this run, about 60% hemicellulose removal and 85% delignification was achieved, an indication that the ARP treatment alone is quite effective in the delignification of CCSM. To improve delignification further, only ammonia solution was pumped initially, and then H_2O_2 was added into the ammonia stream to remove the residual lignin (run nos. 2–4). This scheme has improved the fractionation to achieve 77% hemicellulose removal, a phenomenal 98% delignification. From run 2–4, it seems clear that ammonia concentration is an important variable in hemicellulose recovery. In runs 5–7, H_2O_2 was added from the beginning of the experiment. This has increased hemicellulose recovery to 81% and delignification close to 100%. It is to be noted that the solubilization of cellulose portion increased slightly with increase in hemicellulose recovery. In view of the fact that commercial α-cellulose contains a considerable amount of xylan (about 6%), it seem to be extremely difficult to fractionate all of the xylan content selectively. We consider 80% of hemicellulose recovery as an upper limit of fractionation in ARP-H. The extent of fractionation achieved in this method is substantially higher than those reported for acid hydrolysis and two-stage organosol treatment of corn stover *(18)*, as well as those from similar studies on agricultural residues using H_2O_2. The degree of fractionation in these studies was reported to be 40–60% in delignification and 40–80% in hemicellulose removal *(7,11–13)*.

Effect of Reaction Parameters on Hemicellulose Recovery and Delignification

The temperature effect on ARP-H was investigated over the range of 165–180°C. The results are summarized in Table 2. The isolated effect of temperature was

Table 1
Effect of Ammonia Stream Concentration and Hydrogen Peroxide Feeding Mode on ARP-H Treatment of CCSM[a]

No.	reaction time, min	Ammonia stream conc., wt%	Duration of hydrogen peroxide input		Glucan, %	xmg,[b] %	Delignification, %	Solid remaining, %
1[d]	90	10	No addition	L[c]	2.6	13.1		
				S	34.9	8.6	85.1	49.2
2	90	10	60–90	L	3.0	13.8		
				S	33.5	7.6	96.2	40.6
3	90	15	60–90	L	3.4	15.3		
				S	32.3	6.3	95.3	39.9
4	90	20	60–90	L	3.6	16.7		
				S	31.8	5.1	98.2	38.6
5	60	15	0–90	L	3.5	15.7		
				S	32.2	5.6	97.9	37.5
6	60	20	0–90	L	3.9	16.9		
				S	32.1	5.0	98.6	38.5
7	90	20	0–90	L	4.0	17.6		
				S	30.7	4.3	98.3	37.1

[a] All sugar contents are based on oven-dried untreated biomass, and expressed as glucan, xylan, mannan, and galactan equivalents. Reaction condition: 170°C, hydrogen peroxide loading 0.84 g/g biomass, flow rate of ammonia and hydrogen peroxide stream 1 mL/min.
[b] xylan + mannan + galactan.
[c] L refers to hydrolysate after acid secondary hydrolysis, and S refers to the solid residue.
[d] flow rate of ammonia solution = 2 mL/min.

Table 2
Effect of Temperature on the Compositions of CCSM Hydrolysate and Solid Residue[a]

Temperature, °C		Glucan, %	XMG,[c] %	Delignification, %	Solid remaining, %
165	L[b]	4.0	17.1		
	S	32.4	4.8	98.6	38.9
170	L	4.0	17.6		
	S	30.7	4.3	98.3	37.1
175	L	4.0	17.4		
	S	30.8	4.2	98.7	35.4
180	L	4.1	17.3		
	S	30.5	4.4	98.6	34.8

[a] All sugar contents are based on oven-dried untreated biomass, and expressed as glucan, xylan, mannan, and arabinan equivalents. Reaction condition: 20 wt% ammonia, 90 min. (the hydrogen peroxide loading 0.84 9/9 dry biomass, the flow rate of ammonia and hydrogen peroxide 1 mL/min.

[b] L refers to hydrolysate after acid secondary hydrolysis, and S refers to the solid residue.

[c] xylan + mannan + galactan.

Table 3
Effect of Reaction Time on the Compositions of CCSM Hydrolysate and Solid Residue[a]

Reaction time, min		Glucan, %	XMG,[c] %	Delignification, %	Solid remaining, %
30	L[b]	3.5	15.2		
	S	33.7	6.6	96.8	42.2
60	L	3.8	16.8		
	S	31.8	4.8	98.0	38.5
90	L	4.0	17.6		
	S	30.7	4.3	98.3	37.1

[a] All sugar contents are based on oven-dried untreated biomass, and expressed as glucan, xylan, mannan, and arabinan equivalents. Reaction condition: 170°C, 20 wt% ammonia, 90 min, the hydrogen peroxide loading 0.84 9/9 dry biomass, the flow rate of ammonia and hydrogen peroxide 1 mL/min.

[b] L refers to hydrolysate afler acid secondary hydrolysis, and S refers to the solid residue.

[c] xylan + mannan + galactan.

such that the amount of solubilization of cellulose and delignification was insensitive to temperature over this range, remaining relatively constant at 4 and 98%, respectively. The hemicellulose recovery increased slightly as temperature was raised from 165 to 170°C. Further increase in temperature did not result in any improvement in hemicellulose recovery. For this reason, a reaction temperature of 170°C was chosen in most of the subsequent experiments.

In order to reduce total liquid input in the ARP-H, the reaction time was reduced from 90 min to 30 and 60 min. The results are shown in Table 3. The degree of delignification with lower reaction times was almost at the same level as that of 90 min. The hemicellulose recovery, however, has decreased from 81 to 70% (30 min) and 77%

Table 4
Effect of Hydrogen Peroxide Input on the Compositions of CCSM Hydrolysate and Solid Residue[a]

Hydrogen peroxide loading, g/g biomass		Glucan, %	XMG,[c] %	Delignification, %
1.12	L[b]	4.4	17.9	
	S	29.3	3.9	98.2
0.84	L	4.0	17.6	
	S	30.7	4.3	98.3
0.56	L	4.2	17.3	
	S	29.6	4.3	98.8
0.28	L	4.2	17.4	
	S	29.7	4.5	99.1
0.14	L	3.5	16.2	
	S	32.9	5.7	97.8
0.06	L	2.7	13.5	
	S	34.9	8.1	95.9

[a] All sugar contents are based on oven-dried untreated biomass, and expressed as glucan, xylan, mannan, and arabinan equivalents. Reaction condition: 170°C, 20 wt% ammonia, 90 min, the flow rate of ammonia, and hydrogen peroxide 1 mL/min.
[b] L refers to hydrolysate after acid secondary hydrolysis, and S refers to the solid residue.
[c] xylan + mannan + galactan.

(60 min). For a process in which delignification is the primary purpose, it seems logical to choose a reaction time of 30 min. However, if the fractionation aims at maximum recovery of each biomass component, the reaction time should be above 90 min.

Increase of H_2O_2 from 0.84 to 1.12 g/g caused a slight increase in solubilization of hemicellulose and cellulose, but no significant change in the delignification (Table 4). A decrease of H_2O_2 from 0.84 to 0.28 g/g again showed little effect on delignification and only a slight decrease in hemicellulose recovery. A further decrease of H_2O_2 down to 0.14 caused a slight decrease in delignification, but rather significant reduction in hemicellulose recovery. These results collectively indicate that 0.28 H_2O_2 g/g biomass is the minimum loading for maximum delignification and hemicellulose recovery within the conditions applied in this study.

Effect of Flow Pattern on Hemicellulose Recovery

The data in Table 4 confirms that a near-complete delignification is achievable by way of ARP-H treatment. Attaining hemicellulose recovery of above 80%, however, seems to be a difficult task, unless an extremely high loading H_2O_2 is applied. In our attempt to improve the ARP-H further, we have looked into the possibility of reducing the liquid throughput. It is a way of saving energy input and increasing the concentration of hemicellulose in the ARP-H effluent. The effect of flow rate and flow pattern on the ARP-H performance was therefore studied. As the flow rate, and thus the cumulative liquid throughput, was reduced to half, most of the performance indices remained unchanged, although the hemicellulose recovery decreased from 81 to 75% (Table 5). The gain here is the increase in the concentration level of hemicellulose sugars in the ARP-H effluent and, more importantly, the reduction in the cost in the downstream processing.

Table 5
Effect of Ammonia/Hydrogen Peroxide Delivery Flow Pattern on the Compositions of CCSM Hydrolysate and Solid Residue Under Fixed Total Hydrogen Peroxide Input[a]

Flow rate, mL/min						
Ammonia	Hydrogen peroxide		Glucan, %	XMG[c] %	Delignification, %	Lignin recovery, %
1	1	L[b]	4.0	17.6		
		S	30.7	4.3	98.3	59.8
1	0.5	L	3.8	16.9		
		S	30.5	4.8	97.5	—
0.5	0.5	L	3.8	16.4		
		S	31.4	4.8	98.2	61.8
1	1[d]	L	3.5	16.2		
		S	32.9	5.7	97.8	68

[a] All sugar contents are based on oven-dried untreated biomass, and expressed as glucan, xylan, mannan, and arabinan equivalents. Reaction condition: 170°C, 20 wt% ammonia, 90 min, the hydrogen peroxide loading 0.28 g/g biomass.
[b] L refers to hydrolysate after acid secondary hydrolysis, and S refers to the solid residue.
[c] Xylan + mannan + galactan.
[d] The hydrogen peroxide loading 0.14 g/g biomass.

Lignin Separation from Spent Liquid

Followup experiments were conducted to see how much of the lignin generated from the ARP-H could actually be separated from spent liquid. The hydrolysate collected from ARP-H reactor was evaporated until the pH dropped to 7.0 from the initial value of 11.5. Part of the lignin precipitates at this point. As the effluent was subjected to a secondary hydrolysis, lignin was further precipitated. The lignin precipitate was filtered, washed, and dried overnight at 105°C. The lignin content thus determined is summarized in Table 5. As H_2O_2 loading was doubled from 0.14 to 0.28 g/g, the lignin precipitated (based on the total lignin in the untreated biomass) decreased from 68 to 60%. The decrease in lignin recovery can be attributed to the fact that higher H_2O_2 loading increases the low-mol-wt fraction in the ARP-H effluent, which is not precipitated in acidic solution *(9,19,20)*. According to Gould and Freer *(10)*, these lignin degradation products are not toxic in either enzymatic hydrolysis or in subsequent fermentation.

Application of ARP-H Treatment to Switchgrass and Hybrid Poplar

The performance of the ARP-H was further tested using additional substrates of switchgrass and hybrid poplar. For these substratest the ARP-H runs were conducted at 170°C, for 90 min and 0.28 g H_2O_2/g biomass. The performance indices are depicted in Fig. 2. The hemicellulose recovery obtained from switchgrass was almost the same as that of CCSM. The degree of delignification, however, was lower. The ARP-H treatment was less effective on the woody substrate on both accounts, hybrid poplar yielding 50% hemicellulose removal and 80% delignification. Interestingly, the cellulose solubilized in the ARP-H effluent of hybrid poplar was only about one-fifth of the herbaceous substrates. This may be

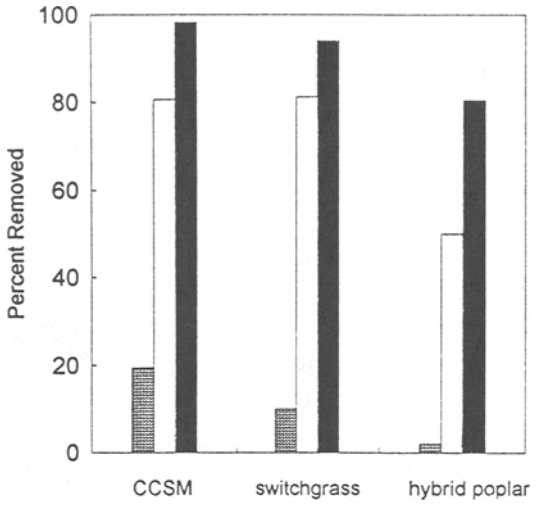

Fig. 2. Sugars and lignin removed from biomass after ARP-H treatment of CCSM, switchgrass, and hybrid poplar. Reaction conditions: 170°C, 20 wt% ammonia, 90 min, the H_2O_2 loading 0.28 g/g biomass, the flow rate of ammonia stream and H_2O_2 stream = 1 mL/min. ▩, glucan; □, xmg; ■, lignin.

explained by the fact that wood cellulose in general has a more rigid structure, higher crystallinity, and higher molecular weight than cellulose in herbaceous feedstock.

Enzymatic Digestibility

The enzymatic digestibilities of ARP-H-treated CCSM, switchgrass, hybrid poplar, untreated filter article (Whatman No. 1), α-cellulose and CCSM are shown in Fig. 3. The 72-h digestibilities of ARP-H-treated CCSM and switchgrass were 95 and 93%, respectively. These values were much higher than that of α-cellulose, which stands at 83% and is essentially in the same range of filter article (94%). The digestibility of hybrid poplar was 90%. The digestibility of the treated CCSM represents a 3- to 4-fold increase over the untreated one. The 24-h digestibility, 84%, of CCSM shown in Fig. 3 was much higher than that previously reported for prehydrolyzed and solvent extracted corn stover (20–37%) *(18)*.

Other Pertinent Comments

Although the process economics is beyond the scope of this work, we bring up a few relevant points. The use of hydrogen peroxide is undoubtedly a major cost factor in this process. From ammonia material balance based on Kjeldahl nitrogen analysis, it was found that the recovery factor of ammonia is 99.2%. The actual consumption of ammonia was 0.02 g NH_3/g dry biomass, a rather insignificant cost factor. The ammonia consumption was owing to reaction with the acetic acid/acetates formed during the ARP-H process. The lignin generated in this process is sulfur- and sodium-free, unlike the ones generated from pulping processes. It should receive due attention as such. A point of caution or limitation of this process is to be mentioned. It has been reported that the decomposition products of hydrogen

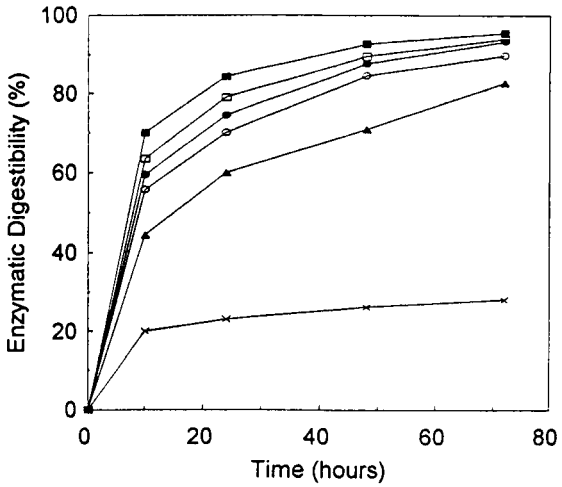

Fig. 3. Enzymatic digestibility of ARP-H treated and untreated CCSM, switchgrass, and hybrid poplar. Reaction conditions: 170°C, 20 wt% ammonia 90 min, the H_2O_2 loading 0.28 g/g biomass, the flow rate of ammonia stream and H_2O_2 stream = 1 mL/min. Enzymatic hydrolysis condition: 60 IFPU/g glucan, pH4.8, 50°C. ■, CCSM; □, switchgrass; ●, untreated filter paper; ○, hybrid poplar; ▲, alpha-cellulose; x, untreated CCSM.

peroxide in an alkaline solution not only act as lignin oxidants, but also attack carbohydrates, resulting in depolymerization and decomposition of carbohydrates *(16,17)*. We have paid particular attention to this matter and found that the significant decomposition of glucan occurs at temperatures above 170°C, a region that can be avoided for processing of herbaceous biomass.

REFERENCES

1. Jangalgi, N. R. (1983), *Chem. Abstr.* **98(14),** 109,117t.
2. Dale, B. E. and Moreira, M. J. (1982), *Biotechnol. Bioeng. Symp.* **12,** 31–43.
3. Han, Y. W. (1978), *Adv. Appl. Microbiol.* **23,** 119.
4. Wang, P. Y., Bolker, H. I., and Purves, C. B. (1967), *Tappi* **50,** 123,124.
5. Lewin, M. and Roldan, L. G. (1971), *J. Poly. Sci.* **36,** 213.
6. Yoon, H. H., Wu, Z. W., and Lee, Y. Y. (1994), *Appl. Biochem. Biotechnol.* **51/52,** 5–19.
7. Gould, J. M. (1984), *Biotechnol. Bioeng.* **26,** 46–52.
8. Holtzapple, M. T., Joseph, E. L., Sturgis, R., Lewis, J. E., and Dale, B. E. (1992), *Appl. Biochem. Biotechnol.* **34,** 5–21.
9. Abbott, T. and Peterson, R. (1985), *Biotechnol. Bioeng.* **27,** 1073–1076.
10. Gould, J. M. and Freer, S. N. (1984), *Biotechnol. Bioeng.* **26,** 628–631.
11. Gould, J. M. (1985), *Biotechnol. Bioeng.* **27,** 225–231.
12. Wei, C. and Cheng C. (1985), *Biotechnol. Bioeng.* **27,** 1418–1426.
13. Cunningham, R. L. and Carr, M. E. (1984), *Biotech. Bioeng. Symp.* **14,** 95.
14. Gierer, J. (1981), *Proc. Int. Symp. Wood and Pulping Chem.*, The Ekman-Days, Stockholm, vol. 2, p. 12.
15. Dencc, C. W. (1980), *Chemistry of Delignification with Oxygen, Ozone, and Peroxides*, Uni Publishers, Tokyo, Japan, p. 199.
16. Sjostrom, E. (1983), *Wood Chemistry—Fundamentals and Applications*, 2nd ed., Academic, San Diego, CA.
17. Samuelson, O. (1981), *Proc. Int. Symp. Wood and Pulping Chem.*, The Ekman-Days, Stockholm, vol. 2, p. 78.
18. Lee, Y., Robinson, C. W., and Moo-Young, M. (1987), *Biotechnol. Bioeng.* **29,** 572–581.
19. Lachenal, D., de Choudens, C., and Monzie, P. (1980), *Tappi* **63,** 119.
20. Bailey, C. W. and Dence, C. W. (1975), *Tappi* **58,** 104.