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Steam Explosion of Mixed Hardwood Chips, Rice Hulls, Corn Stalks, and Sugar Cane Bagasse

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Southern hardwood chips, rice hulls, corn stalks, and sugar cane bagasse were steam exploded. The hemicelluloses of all biomass materials were at least partially degraded. The hemicelluloses remaining were generally soluble in hot water. The cellulose content decreased only slightly. A portion of the lignin was soluble in hot aqueous alkali. However, up to half of the cellulose was also extracted by hot aqueous alkali. Acid hydrolysis of exploded hardwood chips, corn stalks, and sugar cane bagasse showed no rate enhancement. Exploded rice hulls showed a hydrolysis rate increase of approximately 2-fold. Enzymatic hydrolysis rates showed a 10-fold increase for exploded hardwoods, sugar cane bagasse, and rice hulls. No enzymatic rate increase was observed for exploded corn stalks; untreated corn stalks hydrolyzed at a rate similar to that of filter paper. The results suggest that the steam explosion pretreatment may not be as promising as suggested by researchers who have exploded aspen.

If efficient and economical processes can be developed for converting low-value biomass into commercially useful products, mankind would benefit in a substantial manner. Biomass conversion could provide an abundance of useful food, fuel, and chemical products from the cellulose in urban trash and the residues from forestry and agriculture (Cowling and Kirk, 1976). It would also help improve the management of forests by providing a market for the large amounts of low-quality, small-stem hardwoods that are currently growing on southern pine sites.

A variety of chemical and physical pretreatment methods have been developed for increasing the susceptibility of lignocellulose materials to enzymatic and acid hydrolysis. The ideal pretreatment not only should disrupt the plant cell structure but also should use inexpensive chemicals and require simple equipment. In addition, the pretreatment should fractionate the lignin and hemicelluloses and lower the crystallinity and molecular weight of the cellulose. Another important characteristic of a pretreatment, but one which has been largely ignored, is that it needs to be effective with a large range of biomass materials.

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One promising pretreatment appears to be steam explosion. This process was originally developed by Mason in 1925 and has been extensively used in the manufacture of hardboard (Spalt, 1977). In 1978, the Iotech Corp. Ltd., of Canada started using this process for the production of feed for ruminants. In view of the early results that showed the high digestibility of steam-exploded wood, Iotech decided to explore the use of this process as a method for pretreating aspen (Foody, 1980).

Since Iotech first reported their initial results, other investigators have also examined steam explosion as a biomass pretreatment for aspen (Marchessault et al., 1980, 1982; Marchessault, 1982; Foody, 1980; DeLong, 1981). These investigators have found that the following chemical changes occur in steam-exploded aspen: (1) The lignin is broken down into products with a molecular weight range of from 150 to 7000. Since the lignin is extensively depolymerized, it is soluble in alkaline solutions or certain organic solvents. (2) The hemicelluloses are partially broken down and are predominantly soluble in hot water. In addition, some degradation products are formed that apparently condense with lignin, thereby increasing the lignin content. (3) Steam explosion causes a large increase in the accessibility of the cellulose to enzymatic hydrolysis. Jurasek (1978) determined that the steam-explosion pretreatment resulted in approximately a 10-fold increase in the susceptibility of aspen wood to enzymatic hydrolysis.

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Marchessault et al. (1980) and Marchessault (1982) reported no noticeable change in the crystallinity of the cellulose, although changes in the size/perfection of the cellulose crystallites were observed.

In reviewing this past research, it should be remembered that aspen makes up only 3% of the woody biomass in the United States. More importantly, aspen has been shown to be a uniquely easy wood to pretreat. Millett et al. (1975) and Lipinsky (1979) both noted that aspen has unusual susceptibility to many pretreatments.

The enzymatic hydrolysis of other steam-exploded lignocellulosic materials (Foody, 1980) showed some variation, with oak giving lower glucose yields after enzymatic hydrolysis than birch, maple, or aspen. Examination of agricultural byproducts showed higher glucose yields from steam-exploded corn stover and bagasse than from sunflower stalks. Dekker and Wallis (1983a,b) found that sugar cane bagasse and sunflower seed hulls that had been steam exploded were highly susceptible to cellulose hydrolysis.

Acid hydrolysis of steam-exploded southern hardwoods showed no rate enhancement (Schultz et al., 1983). X-ray diffraction of exploded aspen chips showed little or no loss in the degree of crystallinity for the cellulose, although extensive morphological transformations occurred (Marchessault and St-Pierre, 1980; Marchessault et al., 1980; Marchessault, 1982). Dekker and Wallis (1983a) reported that the crystallinity of cellulose in sugar cane bagasse remained unchanged following an autohydrolysis–explosion pretreatment.

In view of these reported results, we felt that it would be worthwhile to determine the chemical changes and the acid and enzymatic hydrolysis rates of different biomass materials following a steam-explosion pretreatment. The materials examined included rice hulls, sugar cane bagasse, corn stalks, and mixed southern hardwood chips obtained from a commercial wood yard. Some results from the steam-explosion pretreatment of hardwoods have been previously reported (Schultz et al., 1983).

EXPERIMENTAL PROCEDURES

Unscreened, mixed southern hardwood chips, not sorted by size, were obtained from a commercial wood yard. The length of the chips varied from about 0.1 to 15 cm. Approximately 82% by weight of the mixed chips was actual wood chips. The remainder consisted of small twigs, leaves, bark, and other material. Examination of the wood chips showed a variety of woods present, the majority being oak and gum species. Pine chips accounted for about 1% of the wood present. Sugar cane bagasse was obtained from the USDA Sugar Research Station, located in Meridian, MS. The sugar cane bagasse was further ground in a Bauer mill. Ground rice hulls were obtained from Pirmi Delta, Inc., located in Greenville, MS. Corn stalks were obtained from a private farmer in central Mississippi. The leaves were removed, and the stalks then ground in a Bauer mill. The hardwood chips, sugar cane bagasse, and corn stalks were placed in 30-gal plastic bags with a small amount of formaldehyde added to inhibit decay and stored at 4 °C. The rice hulls had been previously dried, so no formaldehyde was added to the bags of rice hulls stored in the cold room.

Prior to steam exploding the biomass, bags containing about 3 L of green material were weighed. (Three liters of green hardwood chips weighed about 2.0 kg, 3 L of green sugar cane bagasse weighed approximately 0.4 kg, 3 L of green rice hulls weighed about 1.3 kg, and 3 L of green corn stalks weighed approximately 1.4 kg.) The following catalysts were added to some of the samples: NaOH powder (2% by weight, based on the green weight of the biomass material), H_2SO_4 (45 mL of 10% solution/kg of green weight), or $Fe_2(SO_4)_3$ (2% based on the green weight). The samples containing H_2SO_4 were neutralized with a sodium carbonate solution after the steam-explosion pretreatment.

A Masonite pilot reactor was used for this study. A description of the reactor and the procedure used is given in a previous paper (Schultz et al., 1983).

After steam explosion the biomass material, with the exception of the rice hulls, was air-dried and ground in a Wiley mill with a 2-mm screen. The rice hulls were air-dried but not ground, since they had been obtained in a ground form. The fiber samples were stored in a cold room prior to analysis. Duplicate analyses were performed, with the average reported. The analytical procedure used is the same as was used previously (Schultz et al., 1983).

Prior to acid and enzymatic hydrolysis of the exploded fiber, the samples were placed in boiling water for 1 h, then filtered, washed, and air-dried. The following tests were then run.

(*1*) *Carbohydrate Analysis by GC.* The water-insoluble fiber was hydrolyzed with sulfuric acid. The hydrolyzed sugars were then quantified by GC by use of the aldonitrile–acetate procedure (Chen and McGinnis, 1981).

(*2*) *Glucose Analysis of the Sulfuric Acid Hydrolysate.* A portion of the sulfuric acid hydrolysate was neutralized and analyzed for glucose by a glucose analyzer (YSI Model 23A) to verify the results by GC.

(*3*) *Acid Hydrolysis of the Water-Extracted Fiber.* Approximately 1.5 g of fiber was added to 100 mL of 20 vol % H_2SO_4. The samples were heated in an ethylene glycol bath set at 105 °C. After 6 h, a sample was removed, neutralized, and analyzed with a YSI glucose analyzer. Since the samples had different amounts of cellulose present in the starting fiber, the results were based on the total available glucose originally present in the water-extracted fiber.

(*4*) *Enzymatic Hydrolysis of the Water-Extracted Fiber.* Approximately 0.13 g of fiber was added along with 10 mL of enzyme solution to a test tube. The enzyme solution consisted of sodium acetate–acetic acid buffer (pH 5.0), 10 mg/mL cellulase enzyme (Meicelase CESB, from *Trichoderma viride*, purchased from Meiji Seika, Kaisha, Ltd., of Tokyo, Japan), and 0.0032 mg/mL sodium azide to inhibit microbial organisms. The Meicelase CESB enzyme had a cellulolytic activity measured by the Somogyi method of 1130 units/g. The test tubes were placed in a shaking water bath set at 40 °C. The glucose formed was measured at 6 and 24 h by using a YSI glucose analyzer. In addition to the fiber samples, each enzyme hydrolysis run included two control samples containing Whatman No. 1 filter paper. The cellulase enzyme was chosen because it could be stored for long periods; thus, the results from different runs could be directly compared.

RESULTS AND DISCUSSION

Wood. The effect of steam explosion on mixed hardwoods uncatalyzed or with NaOH has been published previously (Schultz et al., 1983). In this paper, the previous data were compared to runs made with H_2SO_4 as a catalyst.

Analysis of the cellulose content of the exploded wood fiber showed that the H_2SO_4 catalyst degraded the cellulose, as would be expected (Figure 1). Sulfuric acid was also found to increase the amount of material extracted by both water and aqueous alkali, which would be expected if the polysaccharides had been degraded by H_2SO_4.

A slight increase in the lignin content of the fiber steam exploded by using H_2SO_4, as compared to the other cata-

Table I. Carbohydrate, Lignin, and Ash Analyses of Selected Rice Hull Samples Steam Exploded for 1 min Using No Catalysts

		nonextracted samples[a]					
		carbohydrate analysis, wt %				ash +	
sample	reaction temp. °C	arabinose	xylose	mannose	glucose	lignin, wt %	ash, wt %
control		1.6	12.3	1.4	28.5	42.8	21.6
502	196	1.1	12.7	1.1	32.4	41.4	21.5
504	212	0.8	11.5	1.4	33.9	44.1	21.9
507	228	0.5	8.3	1.5	35.8	48.9	22.3
509	241	0.2	3.2	1.2	34.3	55.9	25.1
513	246	0.1	1.3	1.6	31.5	58.8	25.8
512	252	0.1	1.2	1.5	31.1	61.3	26.7

		water-extracted samples[b,c]					
		carbohydrate analysis wt %				ash +	
sample	% insoluble[b] in refluxing water	arabinose	xylose	mannose	glucose	lignin, wt %	ash, wt %
control-WE	88.5	1.6	13.3	1.9	30.8	38.1	19.6
502-WE	85.5	0.8	10.4	2.1	33.3	40.4	20.9
504-WE	78.9	0.4	5.5	1.7	30.6	40.3	20.7
507-WE	78.0	0.2	2.2	1.8	31.3	43.5	21.9
509-WE	82.6	0.1	1.1	1.8	32.9	47.4	23.3
513-WE	84.7	0.1	0.7	1.6	31.9	50.2	23.4
512-WE	86.0	0.1	0.7	1.5	31.9	52.1	25.9

		base-extracted sample[c,d]					
		carbohydrate analysis, wt %				ash +	
sample	% insoluble in refluxing 2% NaOH[d]	arabinose	xylose	mannose	glucose	lignin, wt %	ash, wt %
control-BE	51.7	1.2	10.0		28.0	11.3	1.6
502-BE	41.0	0.2	3.7		28.4	9.0	2.0
504-BE	36.2	0.1	1.8		29.1	8.2	1.6
507-BE	33.8		0.7		27.3	7.1	2.2
509-BE	31.0		0.5		27.1	5.6	2.0
513-BE	27.0		0.3	0.1	20.9	6.6	2.0
512-BE	28.6	0.1	0.6		16.3	11.0	2.0

[a] Each value is the average of two analyses. [b] Fiber samples were places in boiling water for 1 h, then filtered, and washed. [c] The values are based on the starting material and are the average of two analyses. [d] Fiber samples were placed in refluxing NaOH for 1 h, then filtered, and washed.

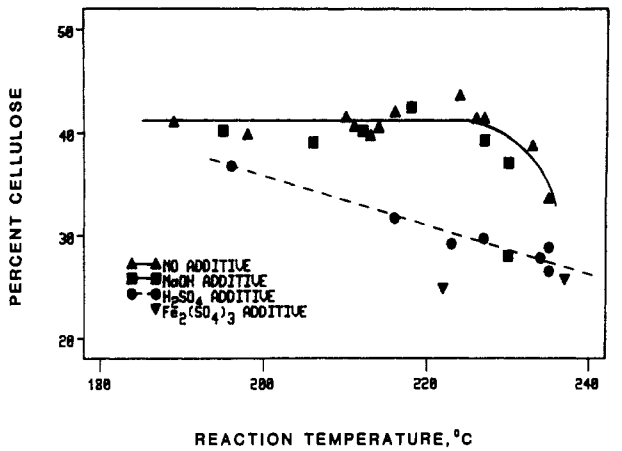

Figure 1. Cellulose content of wood fiber that had been steam exploded for 1 min.

lysts previously examined, was also observed.

Rice Hulls. Higher steam-explosion temperatures were reached by using rice hulls than with wood chips (252 vs. 235 °C). Another difference between the two materials was the high ash content of the rice hulls, i.e., 21.6%. Researchers have reported an ash content of about 20% for rice hulls and have shown that over 90% of the ash is amorphous silica (Hsu and Luh, 1980). The cellulose content was determined to be 39.4%, which is similar to that reported in the literature (Hsu and Luh, 1980; Garten et al., 1981).

Table I gives the carbohydrate, lignin, and ash contents of selected rice hull samples that had been steam exploded for 1 min with no catalyst. A significant portion of the hemicelluloses was degraded by the pretreatment, as were the hemicelluloses of the hardwood chips. The hemicelluloses that remained were soluble in hot water.

The cellulose content showed little change as the reaction temperature was increased for the uncatalyzed and NaOH-catalyzed runs. However, H_2SO_4 decreased the cellulose content, especially at the higher reaction temperatures. Although the uncatalyzed runs had no apparent effect on the cellulose content, carbohydrate analysis of the base-extracted samples showed that up to half of the glucose was extracted by hot aqueous alkali (Table I).

Lignin values were not corrected for ash content. Thus, the values are unrealistically large (Table I). When the lignin content of untreated rice hulls was corrected for ash content, a value of about 25% was obtained. This value is similar to the lignin value for rice hulls reported by other researchers (Hsu and Luh, 1980; Garten et al., 1981; Wilke, 1977). Extraction of the pretreated rice hulls with hot aqueous alkali removed both the lignin and the ash, as shown in Table I.

Sugar Cane Bagasse. The reaction temperature for the sugar cane bagasse ranged from 193 to 243 °C. The carbohydrate and lignin analyses of selected steam-exploded samples are given in Table II.

The hemicelluloses were partially degraded by the pretreatment. The hemicelluloses remaining were sufficiently depolymerized so that they were soluble in hot water. The cellulose content was not affected by the pretreatment, even when H_2SO_4 was added as a catalyst prior to steam explosion. As with all the biomass materials examined, extraction with hot aqueous alkali removed a significant fraction of the glucose, suggesting that the cellulose was affected by the pretreatment (Table II).

Table II. Carbohydrate and Lignin Analysis of Selected Sugar Cane Bagasse Samples Pretreated for 1 min

sample	reaction temp. °C	nonextracted samples[a] carbohydrate analysis, wt % arabinose	xylose	mannose	glucose	lignin, wt %
control		1.9	18.8	3.1	43.9	21.3
303	194	1.0	12.4	3.2	42.4	32.8
305	211	0.8	11.1	3.3	46.7	33.4
312	224	0.6	7.7	3.7	52.1	36.4

sample	% insoluble[b] in refluxing water	water-extracted samples[b,c] carbohydrate analysis, wt % arabinose	xylose	mannose	glucose	lignin, wt %
Control-WE	78.1	1.4	17.6	0.2	35.7	19.6
303-WE	68.2	0.3	6.3	0.2	35.7	25.1
305-WE	66.8	0.4	6.3	0.4	38.1	24.7
312-WE	69.1	0.2	1.5	0.7	43.3	26.0

sample	% insoluble in refluxing 2% NaOH[d]	base-extracted samples[c,d] carbohydrate analysis, wt % arabinose	xylose	mannose	glucose	lignin, wt %
control-BE	50.0	1.1	11.4	1.6	36.9	4.8
303-BE	35.8	0.1	1.8	1.6	30.3	10.1
305-BE	37.5		1.1	1.2	34.2	6.0
312-BE	33.7	0.1	0.8	1.5	32.1	5.6

[a] Each value is the average of two analyses. [b] Fiber samples were placed in boiling water for 1 h, then filtered, and washed. [c] The values are based on the starting material and are the average of two analyses. [d] Fiber samples were placed in refluxing 2% NaOH for 1 h, then filtered, and washed.

Table III. Carbohydrate and Lignin Analyses of Selected Corn Stalk Samples Steam Exploded for 1 min

sample	reaction temp. °C	nonextracted samples[a] carbohydrate analysis, wt % arabinose	xylose	mannose	glucose	lignin, wt %	ash, wt %
control		2.5	17.3	2.5	40.2	21.2	5.1
602	192	1.7	17.4	1.6	44.2	21.0	4.6
603	207	1.2	14.6	1.2	43.5	21.1	4.8
606	233	1.3	12.6	1.6	39.2	25.4	5.1
607	244	0.8	8.9	1.5	44.1	27.7	4.3

sample	% insoluble[b] in refluxing water	water-extracted samples[c] carbohydrate analysis, wt % arabinose	xylose	mannose	glucose	lignin, wt %	ash, wt %
control-WE	81.2	1.9	14.9	1.1	35.0	18.1	ND[e]
602-WE	83.8	1.2	15.1	0.8	38.0	22.8	ND
603-WE	77.9	0.5	9.7	0.1	36.0	25.2	ND
606-WE	64.9	0.5	7.2	0.6	32.0	26.8	ND
607-WE	73.7	0.4	6.2	0.4	37.9	28.2	ND

sample	% insoluble in refluxing 2% NaOH[d]	base-extracted samples[c] carbohydrate analysis, wt % arabinose	xylose	mannose	glucose	lignin, wt %	ash, wt %
control-BE	48.2	1.1	10.3	0.2	35.3	2.6	ND
602-BE	47.1	0.4	6.8	0.1	34.9	3.3	ND
603-BE	46.4	0.3	5.1	0.2	35.1	7.1	ND
606-BE	44.5	0.2	3.3	0.1	27.9	14.7	ND
607-BE	42.5	0.2	2.8		31.2	8.1	ND

[a] Each value is the average of two analyses. [b] Fiber samples were placed in boiling water for 1 h, then filtered, and washed. [c] The values are based on the starting material and are the average of two analyses. [d] Fiber samples were placed in refluxing 2% NaOH for 1 h, then filtered, and washed. [e] Not determined.

The apparent lignin content increased as the reaction temperature increased. We believe that the increase may have been partially caused by the hemicellulose degradation products. Hot aqueous alkali solubilized most of the lignin, including the lignin of the untreated bagasse.

Corn Stalks. The cellulose content of untreated corn stalks was determined to be 35% which agrees with that reported by Detroy (1981) of 29.3%. However, the lignin value (21.2%) is much higher than that reported for corn stover by Detroy (1981) (3.5%) and Harkin (1973) (4.2–8.8%). Analysis of the ash content of the lignin showed that ash accounted for only 3.1% of the lignin. A portion of the lignin value may have been due to the condensation of extractable material (proteins, polyphenols, etc.) with the lignin during the sulfuric acid hydrolysis procedure (Lai and Sarkanen, 1971). However, Wilke (1977) determined the lignin content after extraction with hot water and reported the lignin content to be 15.1% for corn stover and 18.5% for corn stalks. It is expected that hot water would have removed some of the extractable material, and Wilke's lignin values for corn stalks extracted with hot water (18.5%) is similar to our water-extracted value of 18.1%.

The corn stalks were steam exploded at temperatures

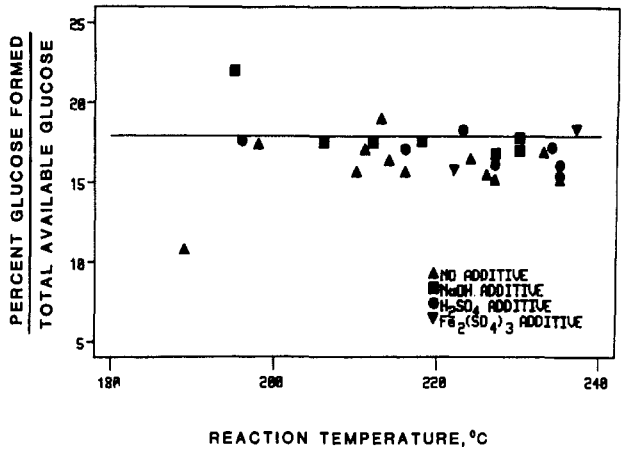

Figure 2. Acid hydrolysis rate of hardwood fiber samples that had been steam exploded for 1 min. Each value is the average of two analyses. The solid line shows the rate of acid hydrolysis of untreated mixed hardwoods.

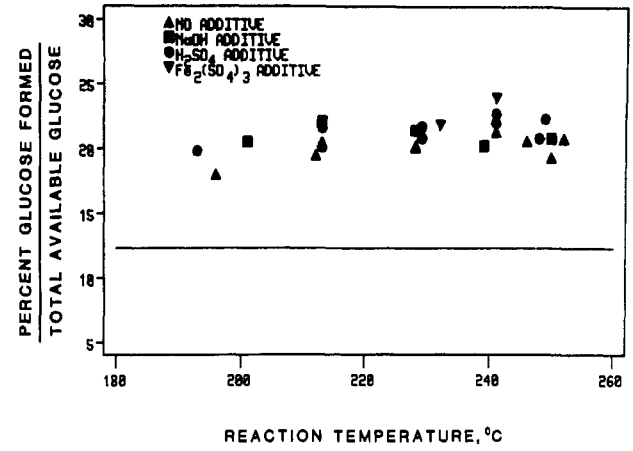

Figure 3. Acid hydrolysis of steam-exploded rice hulls. The solid line shows the average hydrolysis rate of three untreated samples.

ranging from 192 to 244 °C. Table III lists the carbohydrate, lignin, and ash analysis of selected samples steam exploded for 1 min.

As expected, the hemicelluloses were degraded by the pretreatment. However, extraction with hot water did not remove a significant portion of the remaining hemicelluloses.

The cellulose content was unaffected by the pretreatment except in the case of H_2SO_4-catalyzed runs, where the cellulose content decreased as the reaction temperature increased. Base extraction removed a portion of the glucose, as was also the case with the other three biomass materials examined (Table III). However, Table III shows that there is little difference in the amount of lignin extracted from untreated or exploded samples. It is interesting to note that about 90% of the lignin could be extracted from untreated corn stalks, while a lesser amount was extracted from the exploded samples.

Acid Hydrolysis. Acid hydrolysis of mixed hardwood chips that had been steam exploded for 1 min is shown in Figure 2. The solid horizontal line shows the acid hydrolysis rate of untreated hardwood chips. Figure 2 shows that steam explosion did not increase the hydrolysis rate. Indeed, the pretreatment appears to have caused a slight decrease in the hydrolysis rate. In addition, no important differences were noticed among the different catalysts. It was discussed earlier that sulfuric acid caused a reduction in the cellulose content. However, the cellulose may have been sufficiently altered by the acid catalyst, so that hot water extracted the degraded portion, leaving behind unaffected crystalline cellulose. Similar results were also obtained from the acid hydrolysis of steam-exploded corn stalks and sugar cane bagasse.

Marchessault and St-Pierre (1980) found that the crystallinity of steam-exploded aspen was not affected by the pretreatment. In addition, Dekker and Wallis (1983a) reported no change in the crystallinity of cellulose in sugar cane bagasse following an autohydrolysis–explosion pretreatment. Since the acid hydrolysis rate is highly correlated to the crystallinity of cellulose (Millett et al., 1979), the data are consistent with the cellulose crystallinity data reported by Marchessault and St-Pierre (1980) and Dekker and Wallis (1983a). However, it was reported earlier that hot aqueous alkali extracted approximately one-third of the cellulose of all four steam-exploded biomass materials. This suggests that the cellulose had been affected by the pretreatment in a manner that cannot be detected by crystallinity or acid hydrolysis studies.

The acid hydrolysis rates of exploded hardwoods, sugar cane bagasse, and corn stover suggested that rice hulls would also give negative results. However, acid hydrolysis of pretreated rice hulls showed a definite rate enhancement (Figure 3). The different catalysts did not appear to affect the results. In addition, higher reaction temperatures did not lead to an increased hydrolysis rate; 200 and 250 °C reaction temperatures gave similar results.

Chemical analysis of the rice hulls showed an ash content of 21%. Researchers have generally reported a high ash content of about 20% for rice hulls and have shown that over 90% of the ash is composed of SiO_2 (Hsu and Luh, 1980). The silica in rice hulls is amorphous and is highly concentrated in the outer layer. Since the only major difference in the chemical composition between rice hulls and the other three biomass materials examined appears to be the high ash content, this suggests that ash may be related to the increase in acid hydrolysis rates. A review of biomass literature showed only one reference in which a silicon compound was utilized as a catalyst. In this reference, a zeolite catalyst composed of SiO_2–Al_2O_3 was shown to give high yields of organic liquids when methyl β-glucoside was pyrolyzed in water (Frankiewicz, 1980). No references could be found in which silica was utilized as a catalyst for decrystallizing cellulose. We find it difficult to see how amorphous silica could act as a catalyst to decrystallize cellulose.

Another possibility could be that ash shields cellulose, reducing its accessibility. The steam-explosion pretreatment could have disrupted the ash shield, thus increasing the accessibility of cellulose to acids.

It is interesting to note that the cellulose of untreated wood (Figure 2), corn stalks, and sugar cane bagasse hydrolyzed at the same relative rate of about 18%. Untreated rice hulls hydrolyzed at a slower rate of approximately 12% (Figure 3). However, steam-exploded rice hulls hydrolyzed at a relative rate of 22%.

Additional investigations on rice hulls are being conducted at our laboratory.

Enzymatic Hydrolysis. Results from the enzymatic hydrolysis rates of exploded hardwood chips, rice hulls, and sugar cane bagasse (24-h hydrolysis is given in Figure 4) showed significant increases. In Figure 4, the solid horizontal line is the enzymatic hydrolysis rate for untreated material and the dashed line the hydrolysis rate of filter paper. The hydrolysis rate can be seen to increase as the reaction temperature increased. Jurasek (1978) reported a similar increase for steam-exploded aspen. Samples exploded at 240–250 °C hydrolyzed at about the same relative rate as filter paper. The increased enzymatic

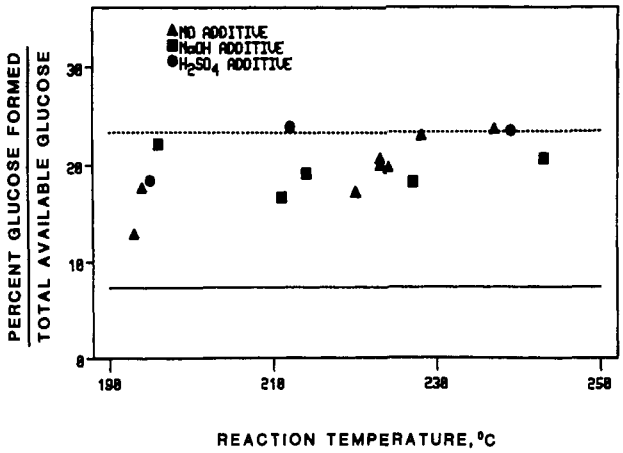

Figure 4. Enzymatic hydrolysis rates of steam-exploded sugar cane bagasse hydrolyzed for 24 h. The solid line shows the hydrolysis rate for untreated sugar cane bagasse and the dashed line the hydrolysis rate of filter paper.

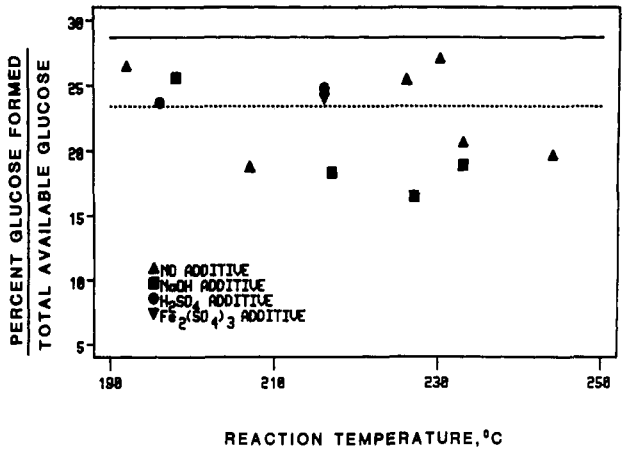

Figure 5. Enzymatic hydrolysis of steam-exploded corn stalks hydrolyzed for 24 h. The solid line is the hydrolysis rate of untreated corn stalks and the dashed line the hydrolysis rate of filter paper.

hydrolysis rates and the high solubility of the lignin in a basic solution indicate that the polymeric lignin network was disrupted by the steam-explosion pretreatment.

No differences in rates were noticed between mixed hardwood samples that had been stored 8 months prior to enzymatic hydrolysis and samples that had been recently steam exploded.

The only difference noticed between hardwood chips, sugar cane bagasse, and rice hulls was the hydrolysis rate of the *untreated* samples. The unexploded hardwood chips and rice hulls had a relative enzymatic hydrolysis rate of about 2% in 24 h, while untreated sugar cane bagasse had a 24-h rate of about 7%.

Different results were obtained from corn stalks, as compared to the other three materials examined (Figure 5). Untreated corn stalks had a relatively rapid hydrolysis rate of 28%. It is believed that the high value was due to the unique lignin in untreated corn stalks. Table III showed how lignin could be easily extracted from untreated corn stalks. Enzymatic hydrolysis rates are usually affected by lignin; however, the lignin in corn stalks may be of lower molecular size and thus may not sterically inhibit the enzymatic hydrolysis rate of the cellulose in corn stalks.

Foody (1980) and Perez et al. (1981) have both investigated the enzymatic hydrolysis of steam-exploded corn stover. However, we were unable to determine if either Foody or Perez et al. compared the hydrolysis rates of untreated and treated samples. Thus, it is difficult to say if corn stalks in general are relatively easy to hydrolyze with enzymes or if the high hydrolysis rate found by us is unique.

Conclusions. Weiss and Mednick (1983) have recently published a process and economic analysis of several ethanol production systems. One of the systems that they investigated was the steam-explosion pretreatment, using data furnished by Iotech (Foody, 1980). The proposed reaction conditions for exploding aspen chips were a temperature of 250 °C for 1.0 min. Several of the assumptions that Weiss and Mednick (1983) made from Iotech's data included (1) less than 50% of the hemicelluloses will form degradation products and (2) all the lignin will be soluble in a NaOH solution, while no cellulose will be soluble.

The results from this study, using mixed southern hardwood chips, ground rice hulls, sugar cane bagasse, and corn stalks, suggest that at 250 °C with a 1.0-min reaction time (1) over 90% of the hemicelluloses will be degraded and (2) a portion of the lignin will not be solubilized by a hot aqueous alkali, while up to 50% of the cellulose will be solubilized.

As expected, we found that the steam-explosion pretreatment dramatically increased the rate of enzymatic hydrolysis of mixed hardwood chips, sugar cane bagasse, and rice hulls. Corn stalks, however, were an exception. This study found that the enzymatic hydrolysis rate of untreated corn stalks was relatively rapid. No increase in the rate of acid hydrolysis was observed for exploded hardwood chips, sugar cane bagasse, and corn stalks. Exploded rice hulls, however, hydrolyzed faster in acid than untreated rice hulls.

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Determination of Glucosinolates in Canola Meal and Protein Products by Desulfation and Capillary Gas-Liquid Chromatography

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A rapid procedure for the quantitation of intact glucosinolates, including the indole glucosinolates, in rapeseed and canola meal is described. Me_3Si derivatives of the desulfoglucosinolates were separated on a glass capillary column by GLC within 10 min. Low-glucosinolate selections of *Brassica campestris* and *Brassica napus* were shown to contain relatively high concentrations of (1-methoxy-3-indolyl)methyl glucosinolate. Procedures designed to extract the glucosinolates from canola flours were not efficient, and plant breeding to reduce the levels of glucosinolates will continue to be necessary for seed improvement.

Glucosinolates are common constituents in species of Cruciferae, which are utilized for food and feed purposes. These sulfur-containing organic compounds contribute the characteristic pungent odor and sharp taste to many of the Cruciferae crops. However, the consumption of these compounds has been associated with goitrogenicity, toxicity, and antinutritional effects in experimental and farm animals. Recent reviews have dealt comprehensively with the nature of glucosinolates in food plants (Fenwick et al., 1983), the biological role of glucosinolates in plants (Kjaer, 1981), the effects of toxic products from glucosinolates on animals (Tookey et al., 1980), and analyses of glucosinolates (Olsen and Sørensen, 1981).

Glucosinolates occur as salts throughout the various plant parts of Cruciferae including the seeds but are readily hydrolyzed, under moist conditions, by an accompanying native enzyme, thioglucoside glucohydrolase (myrosinase). Usually the products of enzymatic decomposition are β-D-glucose and an organic aglucon moiety. Depending on the kind of glucosinolate and environmental conditions, the aglucon may undergo an intramolecular rearrangement and/or fragmentation to yield one or more products: isothiocyanates (Ettlinger and Lundeen, 1957), thiocyanates (Gmelin and Virtanen, 1960), nitriles (VanEtten et al., 1966), cyanides (Saarivirta, 1973), or oxazolidinethiones (Astwood et al., 1949; Daxenbichler and VanEtten, 1977).

Because of the instability of intact glucosinolates, enzyme-catalyzed hydrolysis under controlled conditions has been used in the determination of glucosinolate content. While total aglucon contents can be measured spectrophotometrically (Wetter and Youngs, 1976), most investigators have quantitated the individual degradation products by GLC (Youngs and Wetter, 1967; Daxenbichler and VanEtten, 1977) and, recently, HPLC (Mullin, 1978; Maheshwari et al., 1979). In these procedures, some glucosinolates escape detection (Olsen and Sørensen, 1980, 1981), and especially in the low glucosinolate cultivars, the proportion of undetected glucosinolates may be large.

Therefore, efforts have been made to elaborate procedures for analyses of intact glucosinolates. The GLC method of Underhill and Kirkland (1971) has been modified and greatly improved (Thies, 1980; Heaney and Fenwick, 1980; Olsen and Sørensen, 1980; Daun and McGregor, 1981). In the Heaney and Fenwick (1980) procedure, glucosinolates were extracted from the sample, purified, and desulfated with sulfatase on a DEAE-Sephadex A-25 column. The resultant desulfoglucosinolates were derivatized, separated, and quantified by GLC. Using a packed column, pertrimethylsilylated (Me_3Si) derivatives of 12 desulfoglucosinolates, including indole glucosinolates of low volatility, were satisfactorily separated in just over 30 min on a gas chromatograph.

The separation of Me_3Si derivatives of glucosinolates on glass capillary columns has been shown to reduce the elution time of the major glucosinolates quite markedly as compared to that on the packed column (Hiltunen et al., 1980). However, uneven base lines, numerous interfering peaks of sugars and other contaminants, and the absence of the indole glucosinolates had discouraged the adoption of this rapid technique.

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