

# MODIFICATIONS OF WHEAT STRAW TO ENHANCE CELLULOSE SACCHARIFICATION BY ENZYMATIC HYDROLYSIS

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## ABSTRACT

Successful utilization of lignocelluloses for fermentation alcohol or other chemicals requires development of economic conversion techniques. Chemical, physical, and thermal processes were applied to wheat straw (WS), and the modified products were assayed by enzymatic hydrolysis. Treatments that remove pentosans or lignin make the cellulose more readily available to saccharifying enzymes. For example, autohydrolysis at 170°C for 30 min decreased pentosan content and increased fourfold the conversion of cellulose to glucose. "Cold soda" pulping removed lignin and pentosans to effect a sixfold improvement. Good results were obtained by more complete delignification without an appreciable loss in pentosans.

## INTRODUCTION

The abundant, renewable, cellulosic materials are attractive as potential sources for alcohol fuels and other chemicals. Emert and Katzen (1980) indicated that although 144 million tons of lignocellulose wastes is collected annually in the United States, 465 million tons of available lignocellulose is uncollected. Detroy and Hesseltine (1978) reviewed some of the most promising and successful technological processes available for the production of chemicals from agricultural raw materials. Many comprehensive studies have been undertaken to improve the efficiency of hydrolyzing cellulose (Brown and Jurasek, 1979). Economical means to hydrolyze cellulose would provide fermentable sugars for the production of chemicals and fuels. At our Center we have studied the accessibility of cellulose to enzymatic hydrolysis as an effect of biological and chemical pretreatments (Detroy *et al.*, 1980, 1981). Using straw from wheat grown in central Illinois, we have investigated feasible techniques to prepare

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\*The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

substrates that could be readily hydrolyzed by enzymes. Chemical composition data and SEM photomicrographs of these substrates permitted us to evaluate the effect of chemical and physical modifications on wheat straw (WS) as they relate to cellulose conversion as assessed by enzymatic hydrolysis.

## MATERIALS AND METHODS

The raw material for pretreatment studies was straw of soft winter wheat (*Triticum* sp. Arthur variety). The WS was chopped into 15-45 mm lengths with a Taylor, Stiles, and Company cutter. Autohydrolysis or thermal pulping was performed on chopped WS. A 10-gallon, stainless-steel jacketed digester tumbling at 1 rpm was used for pulping. With a water-to-solids ratio of 7:1, the WS was pulped 30 min at 170°C. The internal pressure was reduced to 75 psi by venting, and contents were discharged into blow pit. Pulp was washed and yield was determined.

The chopped WS was ground in an Abbe, hammer, or Wiley mill and, subsequently, was modified by the following procedures: (1) WS ground to pass 4 mm screen in an Abbe mill was extracted 48 hr in a Soxhlet apparatus consecutively with acetone and cyclohexane. (2) WS (-0.7 mm, hammer mill) was steeped 24 hr at room temperature with sodium hydroxide (4% solution) in half-gallon jars. (3) A sodium chlorite procedure in preparation of holocellulose similar to that described by Adams (1965) was performed on unextracted WS ground to pass 1 mm screen in a Wiley mill. (4) Two levels (10 and 15%, based on WS solids) of sodium hypochlorite (expressed as available Cl<sub>2</sub>) were applied to WS (-1 mm, Wiley mill) maintaining a 3% consistency to permit adequate mixing. Treatments were continued for 3 hr at 50°C. (5) In addition, hypochlorite treatments were combined with sodium hydroxide (4% solution) extractions. WS treated with sodium hydroxide and/or sodium hypochlorite was water washed.

All modified substrates were freeze dried before chemical analyses or cellulase saccharifications. Crude and alpha cellulose contents were measured by the monoethanolamine method of Nelson and Leming (1957). Lignin contents were determined by the spectrophotometric method of Bagby *et al.* (1973). Other analytical procedures were standards of the Technical Association of the Pulp and Paper Industry.

A cellulase method was used to determine hydrolysis efficiencies of cellulose to glucose on modified WS substrates as described by Detroy *et al.* (1980). WS specimens were mounted on double-sided Scotch tape placed on aluminum stubs for observation with the stereoscan SEM. Mounted samples were coated with gold-palladium (60:40) to a thickness of about 15 nm while rotating at 10 rpm in a Denton DV-502 high-vacuum evaporator. The coated samples were observed in a SEM (Mark 2A, Cambridge Scientific Instruments Co., Ltd., London England) at a beam specimen angle of 45°C. Accelerating voltage was 20 KV, and final aperture was 200 um.

## RESULTS AND DISCUSSION

Conversion yields of glucose from cellulose by enzymatic hydrolysis were much improved when the lignin contents of the substrates were reduced to 10% or less (Table 1). Acetone and cyclohexane extraction removed minute quantities of oil and hydrocarbon and had a somewhat adverse effect upon the susceptibility of

the WS to enzymatic attack. The alcohol-benzene extractives of modified WS were less than for the control. The quantity of minerals removed during pretreatments did not greatly affect cellulase activity.

Extraction of WS with sodium hydroxide (4% solution) removed lignin and pentosans, which resulted in substrates higher in M.E.A. cellulose (ash and pentosan free) contents. Pentosans in the alkali solutions can be recovered. Flickinger (1980) indicated that pentoses could be used for conversion to liquid fuels also. Even though some minerals and alcohol-benzene solubles were removed during treatments with sodium hypochlorite, the substrates did not respond well to enzymatic hydrolysis. However, when sodium chlorite was added to WS in sufficient quantity under proper conditions to reduce lignin content to a low level (5%), enzymatic hydrolysis was good. The residual pentosans remaining after extractions would require removal for their inherent value before cellulase treatment or could be utilized as residue after hydrolysis. A two-step extraction of ground WS with sodium hydroxide and sodium hypochlorite provided substrates in which 82-90% of the cellulose could be converted to glucose by cellulase.

The effect of particle size of WS before subsection to enzyme treatment is demonstrated in Table 2. A fourfold increase in conversion is noted for WS passing a 0.7 mm screen during hammer milling. Similar results were observed for the Abbe, hammer, and Wiley milling of WS with particle size ranging from 1 to 6 mm. Millett *et al.* (1979) commented on the enhancing effect of fine grinding and reported a dramatic response of enzymatic hydrolysis to the effect of vibratory ball milling. A photomicrograph (Fig. 1, a) shows the magnitude of surface area available for enzymatic attack after hammer milling. When this material is treated with sodium hydroxide, the substrate has a 30% lower lignin content and 23% lower pentosan content than the control (Table 1). The removal of these constituents provides a more open structure for cellulase hydrolysis (Fig. 1, b) and the conversion is 67%. The physical effect of removing the cementing agent, lignin, to a low level (5%) in a WS substrate is demonstrated in Figure 2.

When WS is subjected to thermal pulping or autohydrolysis (Table 3), a net decrease is observed in the pentosan, lignin, mineral, and alcohol-benzene soluble contents of the pulp calculated on basis of components in original WS. Additional study should permit an improvement over this 65% (29.1 to 10.2%, basis original) reduction in pentosan content. The liquor could be a valuable byproduct, because it would be pentosan rich. This pretreatment permitted a fourfold increase in enzymatic hydrolysis. The defibering action associated with depressurization at 75 psi of chopped WS pulped in water is exhibited in Figure 3. Material balance determinations for modified WS (Tables 1 and 3) indicate that the cellulose had not been degraded by pretreatment techniques.

Applicability of experimental results from wheat straw to other lignocellulosic materials should be possible with modifications necessitated by inherent differences in raw materials. Increasing the surface area of lignocellulose and some purification of the cellulosic component appear necessary to improve the efficiency of enzymatic hydrolysis. Economical pretreatments that provide usable byproducts (hemicellulose and lignin) are required to attain good yields of glucose from cellulose and to ensure total biomass utilization.

## ACKNOWLEDGEMENT

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Table 1. Enzymatic Saccharification of Wheat Straw after Extraction

Extraction	Particle size, <sup>2</sup> mm	Yield, <sup>3</sup> %	Chemical composition <sup>1</sup>				Enzymatic hydrolysis	
			Solubility in alcohol-benzene, %	Cellulose, M.E.A., %	Pentosans, %	Lignin, Ash, %	Conversion of cellulose to glucose, %	
Acetone, cyclohexane	4	97.1	4.20	32.9	28.9	15.4	11.4	14
Sodium hypochlorite, 10% <sup>4</sup>	1	88.6	1.98	37.1	31.9	14.4	6.05	27
Sodium hypochlorite, 15% <sup>4</sup>	1	88.2	2.06	37.6	32.7	16.2	5.92	31
Sodium hydroxide, 4% <sup>5</sup>	0.7	58.1	2.24	56.2	22.4	10.1	4.82	67
Sodium hydroxide, 4% <sup>5</sup>								
Sodium hypochlorite, 10% <sup>4</sup>	1	53.8	1.29	61.2	24.0	6.72	2.64	82
Sodium hypochlorite, 15% <sup>4</sup>								
Sodium hydroxide, 4% <sup>5</sup>	1	54.8	1.93	59.1	24.3	8.68	2.71	90
Sodium chlorite <sup>6</sup>	1	75.0	1.62	44.6	34.3	5.10	6.34	62
None	0.7, 1, 4	—	6.46	32.9	29.1	14.5	9.46	46, 35, 31

<sup>1</sup>Duplicate analyses are  $\pm 2\%$  of the mean value reported.

<sup>2</sup>Material passed screen openings of that size.

<sup>3</sup>Recovered solids after pretreatment.

<sup>4</sup>Percent based on dry material expressed as available chlorine.

<sup>5</sup>Percent solution.

<sup>6</sup>According to Adams (1965) with modifications.

Table 2. Influence of Milling Wheat Straw on Cellulose Conversion

Milling process	Particle size <sup>1</sup> mm	Enzymatic hydrolysis, conversion of cellulose to glucose, %
(Chopped only)	25	10
Abbe	4	31
Hammer	0.7	46
	6.4	33
Wiley	1	35
	2	30

<sup>1</sup>Material passed screen openings of that size.

Table 3. Characteristics of Thermally Pulped Wheat Straw 170°C, 30 min, 7:1 (Water to Solids) 15-45 mm Sections

	<u>Pulp properties</u>	
Yield, %	64.9	
Liquor pH	4.2	
	<u>Chemical composition<sup>1</sup></u>	
	<u>Pulp</u>	<u>Control</u>
Solubility in alcohol-benzene, %	7.59	6.46
Cellulose,		
M.E.A., %	50.3	32.9
Alpha, %	46.1	30.2
Pentosans, %	15.7	29.1
Lignin, %	14.4	14.5
Ash, %	7.46	9.46
	<u>Enzymatic hydrolysis</u>	
	<u>Pulp</u>	<u>Control</u>
Conversion of cellulose to glucose, %	39	10

<sup>1</sup>Duplicate analyses are  $\pm 2\%$  of the mean values reported.

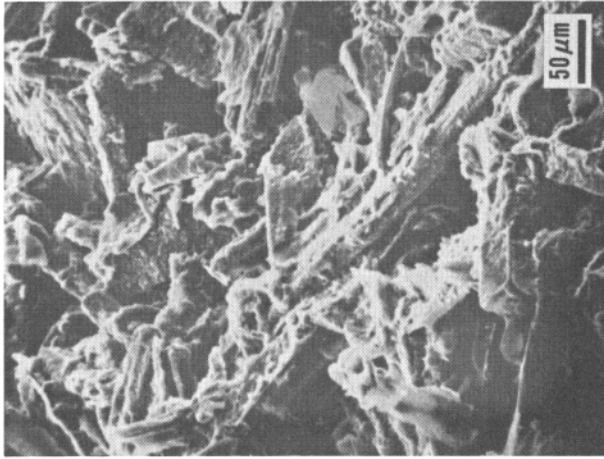
## FIGURE LEGENDS

Figure 1. Wheat straw milled in hammer mill (-0.7 mm). (a) Untreated (b) Treated with 4% sodium hydroxide.

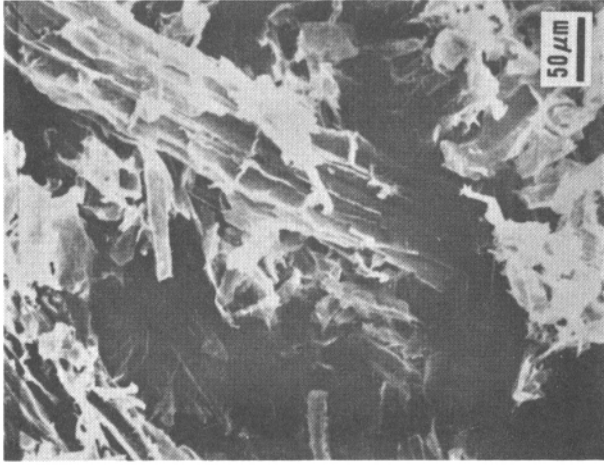
Figure 2. Wheat straw milled in Wiley mill (-1 mm). (a) Untreated (b) Treated with sodium chlorite.

Figure 3. Autohydrolyzed wheat straw after depressurization at 75 psi.

# SEM Photomicrographs



(a) Untreated

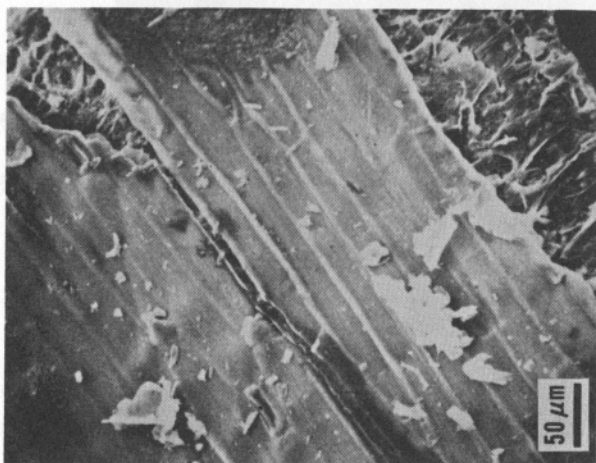


(b) Treated with 4% Sodium Hydroxide

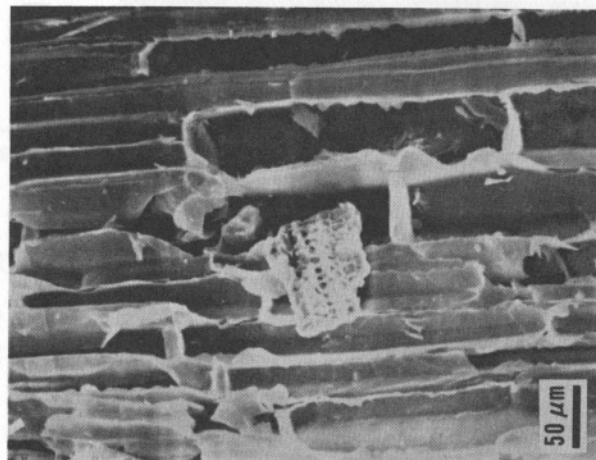
Wheat Straw Milled ( $-0.7$  mm) in Hammer Mill

Figure 1

## SEM Photomicrographs



(a) Untreated



(b) Treated with Sodium Chlorite

Wheat Straw Milled (-1 mm) in Wiley Mill

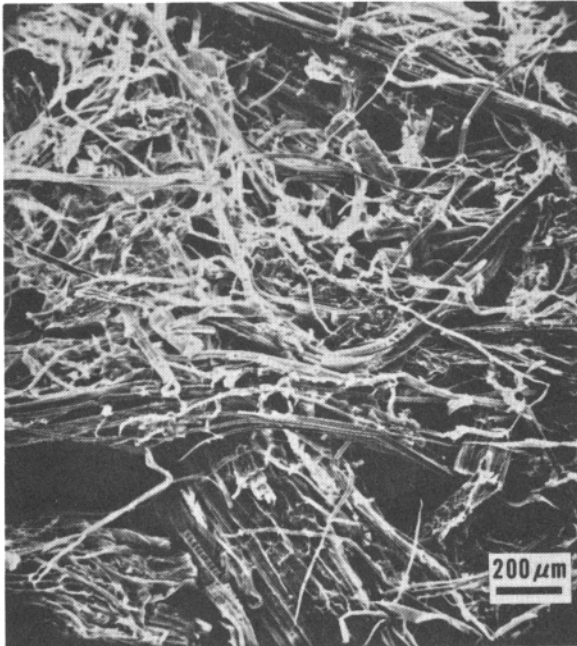
Figure 2

## FIGURE LEGENDS

Figure 1. Wheat straw milled in a Wiley mill (-1 mm) and untreated. (a) Treated with 1% sodium chlorite.

Figure 2. Wheat straw milled in a Wiley mill (-1 mm) and untreated. (a) Treated with sodium chlorite.

Figure 3. A cellulolytic wheat straw after approximately a 24 hr.



**Autohydrolyzed Wheat Straw After  
Depressurization at 75 psi.**

Figure 3