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MICROBIAL PROCESSES FOR THE CONVERSION OF LIGNOCELLULOSE/ HEMICELLULOSE RESIDUES TO ENERGY AND FEEDSTUFFS*

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LITERATURE REVIEW

Lignocellulosic Residues and Composition

Residues are the excesses and wastes from the growing and processing of raw agricultural products. An accurate measure of the present and potential supply of agricultural byproducts from domestic crops is not known in the United States with certainty; however, the annual total certainly exceeds 700 million tons (dry basis). The feasibility of utilizing these residues for producing energy or chemicals depends on the following characteristics: composition, availability, current usage, value of the residue for other uses and the economics of collection and storage.

Cellulose, our most abundant renewable resource is available as a renewable plant material from sources such as wood pulp, newsprint, urban waste, agri-residues, and manure in an almost unlimited supply. An estimated 22 X 10^9 tons of cellulose is generated by photosynthesis annually worldwide (Loehr, 1977). Only about 4 X 10^9 tons/yr (20%) is readily available for conversion to energy or feedstuffs. Cellulose derived from wood and grasses is the most available.

The ever-rising cost of petroleum causes rapidly changing economic conditions and makes it difficult to fix a price on various residues. Wood cellulose is currently the

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most economical. Cellulose derived from cane bagasse, corn refuse, and certain tree crops may be the cellulose produced most efficiently by photosynthetic processes (Humphrey, 1975). With the price of petroleum crude between $33-46 per barrel and cellulosic wastes at less than one-third of that price (Miller, 1975), residue now is viable as a source of energy and feedstuff.

Dry cellulosic materials (in average) have a heat value of about one-third that of hydrocarbon of equal weight. Therefore, cellulosic materials can be looked at seriosly in terms of renewable, industrial resource. One alternative to the conversion of cellulose is the production of alcohol. Alcohol can be burned in internal combustion engines and can be used in solvents, in beverages, and in hydrocarbon synthesis (via ethylene). The basic steps in processing cellulose to alcohol include conversion of cellulose to glucose, followed by microbial fermentation to alcohol and recovery of alcohol by distillation.

Several chemical and biological processes have been used to modify the abundant lignocellulosic byproducts of agricultural and wood-processing industries to increase their digestibility for animals. Sodium hydroxide, in particular, has been used to increase the nutritional value of roughages (Van Soest, 1973; Rexen and Thomsen, 1976).

Cellulose/Hemicellulose Hydrolysis

Most plant fibers contain cellulose, hemicellulose, and lignin in approximate ratios of 4:3:3 (Tsao, 1978; Rydholm, 1965; Otto et. al., 1954; Bikales and Segal, 1971). Cellulose is a homogeneous polymer of glucose, whereas hemicellulose molecules are often polymers of pentoses (xylose and arabinose), hexoses (mannose), and a number of sugar acids. Lignin, a polyphenolic macromolecule (Brauns and Brauns, 1960; Sarkanen and Ludwig, 1971) is relatively higher in C and H and lower in O content than are cellulose and hemicellulose, and it has the highest heat value of the three (Falkehag, 1954). Hydrolysis of hemicellulose to mono- and oligosaccharides can be accomplished with either acids or enzymes under moderate conditions (Browning, 1967; Lee et al., 1979). Unlike hemicellulose, cellulose is resistant to hydrolysis. Cellulose fibers generally consist of a highly ordered crystalline structure of cellulose surrounded by a lignin seal, which becomes a physical barrier to easy hydrolysis. The difficulty in obtaining fast and complete hydrolysis of the secondary hydroxyl-linked polysaccharides is the inherently more resistant β-1,4-glucan materials. The easily hydrolysable portion of cellulose (amorphous region) is about 15% and the remainder,

the resistant residue, is crystalline cellulose. Crystalline cellulose may be hydrolyzed by strong acid, but this also causes degradation of the glucose monomer.

The strong crystalline structure and lignin barrier limits cellulose hydrolysis by either acids or enzymes. Acids are non-specific catalysts; they attack cellulose as well as lignin. On the other hand, enzymes (cellulases) are specific catalysts that convert cellulose into glucose with little byproduct. However, cellulase has no effect on lignin and, therefore, the cellulose is not accessible to the enzyme. It should be understood that the configuration of cellulose is still undefined. Models of cellulose fibrils with folded chains have been proposed, as well as models based on extended cellulose molecules. A review of the cellulose configuration as related to hemicellulose and lignin has been presented recently by Tsao (1978).

Since polysaccharides of the plant cell wall are water insoluble, they are presumably polymerized and crystallized simultaneously in biosynthesis. This fact indicates a nascent morphology, and it is this morphological characteristic rather than crystallinity, as such, which yields the problems relative to biomass conversion. The relationship between the degree of crystallinity and susceptibility to acid hydrolysis has been well-documented in cellulose fine structure studies (Battista, 1950). The relationship between fine structure and enzymatic susceptibility is less understood. In general, the greater the amount of decrystallization the greater the cellulose hydrolysis to glucose. Mechanical pretreatments of cotton (roll-milled) decreases the degree of crystallinity with a concommitant five- to tenfold increase in enzyme susceptibility (Marchessault and St.-Pierre, 1978). These mechanical pretreatments destructure the dense packing of cellulose microfibrils in cell walls and result in a high surface-to-volume ratio.

Hemicellulose is one of the major components of renewable resources, comprising upwards of 35% of the plant material (Dunning and Lathrop, 1945). Hydrolysis of hemicellulose yields a mixture of sugars, primarily D-xylose as the major component. For complete utilization of biomass-derived sugars in the production of fermentation alcohol, the conversion of both the cellulosic and hemicellulosic components is warranted. Until recently, no yeasts were reported to ferment pentoses, although some were capable of aerobic metabolism (Barnett, 1976). Hemicellulose-derived pentoses can be obtained easily in good yield from residues using a relatively simple process (Lee et al., 1979). Xylan can be degraded by yeast of the genera Aureobasidium, Cryptococcus, and Trichosporon and Candida utilis and Candida albicans utilize D-xylose (Chakravorty et al.,

1962). Clostridium thermosaccharolyticum ferments xylose to a mixture of ethanol, lactic acid, and acetic acids (Wang et al., 1979). Aeromonas hydrophilia and Aerobacter sp. produce ethanol and 2,3-butanediol from xylose, both chemicals being suitable as fuel. Fusarium oxysporam and other Fusarium species degrade xylose and have been used in combination with the yeast Saccharomyces cerevisiae (Batter and Wilke, 1977; Loughran et al., 1945). Several of these processes use a yeast to convert glucose to ethanol and later inoculation with Fusarium to produce additional alcohol from pentoses. Recent reports have identified a number of yeasts capable of alcohol production from xylose, directly (Schneider et al., 1981; Slininger et al., accepted for publication) and indirectly (Wang et al., 1980; Gong et al., 1981). Several yeasts have been found capable of fermenting D-xylulose, a catabolite of D-xylose, to alcohol. These indirect processes include a two-step process. First, D-xylose is converted to D-xylulose by xylose isomerase. D-xylulose is subsequently fermented to ethanol by yeasts. The direct fermentation of xylose to ethanol has been reported with Pachysolen tannophilus.

The demonstration of microbial systems for direct fermentation of D-xylose to ethanol provides a new dimension in the conversion of biomass to liquid fuels, especially ethanol. Processes of hemicellulose to ethanol could be adopted into existing cellulosic conversion plants where the hemicellulosic-derived pentoses are not being utilized. Future conversion industries would incorporate both the grain and residue-derived sugars for optimum production of fermentation alcohol.

Microorganisms: Lignocellulosic Conversions

Degradation of lignin, cellulose, and hemicellulose of wood by microorganisms has been reviewed by Wilcox (1973). According to Ishikawa et al., 1967, phenol oxidase-rich white-rot fungi degrade more lignin than phenol oxidase-poor white-rot fungi.

A procedure for extracting hemicellulose from ryegrass straw for the production of glucose isomerase and use of the extracted straw residue for animal feed has been reported recently by Chen and Anderson (1980). The hemicellulose fraction of ryegrass straw was extracted with NaOH and used as a substrate for production of glucose isomerase by Streptomyces flavogriseus. About 15% of the total available hemicellulose is obtained by treating ryegrass straw with 4% NaOH for either 3 hours at 90°C or 24 hours at room temperature. S. flavogriseus grown at 30°C for two days on 2% straw hemicellulose produced intracellular glucose isomerase

at a level of 3.04 units/ml of culture. The alkali-treated residue (after separation of hemicellulose) had approximately 75% higher digestibility and 20% higher feed efficiency for weanling meadow voles than did untreated straw.

Various cellulase productions on bagasse and rice straw, wheat straw, woody waste, and other plant materials by the fungi Trichoderma reesei, T. viride, and Aspergillus niger have been reported (Mandels, et al., 1974). Trichoderma viride may be the most active enzyme producer. Numerous workers have used T. viride to produce several enzymes, including "cellulase onozuka," xylanase, cellulase, B-1,3 glucanase, and chitinase, plus several others at lesser potency levels. Cellulase onozuka or T. viride grown on wheat bran or rice straw at various substrate concentrations yielded a 5 to 10% sugar solution after incubation at pH 5.0, 45°C for 48 hours. Mitra and Wilke (1975) have described the production of T. viride QM 9414 enzyme in a multistage continuous fermentation system. Another cellulase preparation, Meicelase, is being produced by a culture of T. viride on a solid substrate of wheat bran at Yodogawa Factory of Meije Seika, Osaka, Japan. Studies by Han and Anderson (1975) and by Han (1975) utilized Candida utilis and Trichoderma viride to ferment ryegrass pretreated with alkali to upgrade it for animal feed. These workers reported a 40% increase in digestibility, accompanied by a 20- to 200-fold increase in substrate weight. Numerous white-rot fungi are capable of lignocellulose degradation (Kirk, 1971). The production of upgraded animal feeds through biological conversion of plant residues centers on preferential modification of the lignin moiety.

Direct Bioconversion To Liquid Fuels

Usually cellulase is isolated and used to hydrolyze cellulose in a separate reactor. An alternative approach, in which cellulase production, cellulose hydrolysis, and a fermentation are carried out simultaneously in a single operation, has been presented by Cooney et al. (1978). In this scheme, the anaerobic, thermophilic bacterium Clostridium thermocellum is utilized to produce hexose sugar, ethanol, and acetic acid from corn residue, solka floc, and cellobiose. C. thermocellum is unable to metabolize pentoses to ethanol; however, Clostridium thermosaccharolyticum ZC can. Therefore, a combination of these two cultures can directly convert substantial cellulose and hemicellulose (hexoses and pentoses) to ethanol (Wang et al., 1979; Brooks et al., 1979). This mixed-culture system directly converts solka floc and ground corn stover to a mixture of ethanol and acetic and lactic acids (Wang et al.,

1979). Selected isolates of the two Clostridia species produce more ethanol and less acetic acid than the parents. These isolates grown in mixed cultures yield 0.57 gram total of ethanol and acetic and lactic acids per gm of cellulose. Mixed-culture systems of Clostridia also are being applied to conversion of hardwoods to ethanol (Brooks et al., 1979). Another direct conversion fermentation combines Trichoderma with another yeast (Emert, 1979; Hoge, 1977). The fungus-yeast fermentation was tested on municipal solid wastes, pulp mill primary sludge and digester rejects, and agricultural and forest residues. In such experiments, continuous removal of the glucose formed during hydrolysis substantially reduced product inhibition.

RESULTS

Current Research: NRRC

The Northern Agricultural Energy Center at the Northern Regional Research Center studies new or improved fermentation and chemical methods of converting biomass into alcohol, recovery of plant hydrocarbons to be used as petrochemical replacements, use of low-proof alcohol as fuel, and conversion of alcohol and methane gas byproducts into food and feed products. As a small part of this work, we have concentrated on differential modification of the lignin, cellulose, and hemicellulose in wheat straw by chemical, enzymatic, and fermentation processes (Lindenfelser et al., 1979).

Fungal Bio-Modification: Feedstuffs from Lignocellulosic Residues

The oyster mushroom, P. ostreatus, NRRL 2366, increases digestibility and nutritional content of wheat straw (WS) for ruminants by modification of its lignin and cellulose as depicted in Fig. 1. The fungus was grown on native WS with amended ammonium sulfate, urea, or oatmeal (Fig. 2). P. ostreatus degrades the lignocellulosics, as evidenced by 10 to 45% and 15 to 55% decreases in lignin and cellulose, respectively, after 36 days (Detroy et al., 1980). Lignocellulose degradation and CO_2 production reflected substrate weight losses of 15 to 30%.

Soluble reducing sugars during fermentation of WS remained constant during the first 15 days of incubation; however, free reducing sugars increased twofold to threefold the remainder of the fermentation period regardless of nitrogen amendment. The in vitro digestibility of WS as

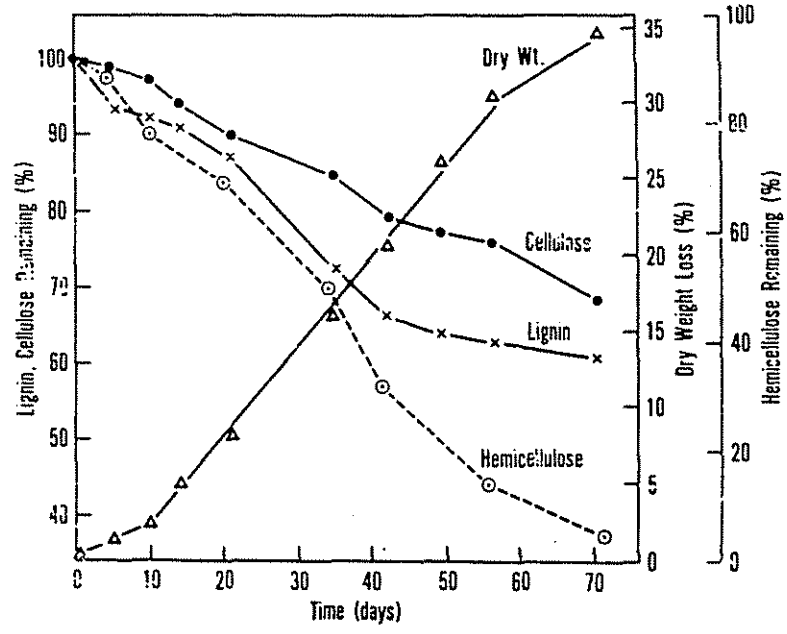

Fig. 1. Hemicellulose, cellulose, lignin, and weight losses in wheat straw (WS) during 70-day fermentation with P. ostreatus NRRL 2366. Water was added to straw at 2X weight of substrate (v/w), incubation at 25°C.

measured by release of reducing sugar after cellulase treatment increased twofold to threefold after a 90 day fermentation period.

The WS fermented by P. ostreatus also was subjected to cellulase treatment. During the first 20 days, the mushroom organism utilized soluble nutrients, lignin, cellulose, and a sizable hemicellulose fraction and yielded a residue having no more susceptibility to cellulase action than control WS (17 and 15% conversion, respectively). After 30 days incubation, however, the available cellulose doubled in the fermented WS, and after 40 to 50 days' fermentation, the conversion of cellulose to glucose increased four- and fivefold, respectively (Table 1). Biological modification of WS, coupled with cellulase hydrolysis, resulted in a 72% conversion of the cellulose component to glucose, without milling or other physical preparation.

Degradation of ^{14}C Labeled-Lignocelluloses By Fungi

Differentially ^{14}C-labeled, naturally complexed lignocelluloses (LC) of maple were employed to evaluate specific

Fig. 2. P. ostreatus NRRL 2366 growing in WS moistened with water added at 2X weight of substrate (v/w), showing growth amounts after incubation at 25°C, left-to-right: 5 and 45 days.

fungi for degradation and solubilization of lignin and for cellulose hydrolysis (Wicklow et al., 1980). Fungi were grown on ^{14}C-lignin (L*C) or ^{14}C-glucan (LC*) substrates for 48 days at 26°C. Lignin biodegradation was assayed by the conversion of L*C residue to $^{14}CO_2$ and other ^{14}C-lignin water-soluble components, whereas cellulose was measured by conversion of the (LC*) residue to $^{14}CO_2$ (Table 2). Because solubilization of labeled materials, microbial incorporation of ^{14}C into cellular mass, or other bioconversions such as humification were not accounted for in this system, these percent recovery values must be considered minimal decomposition values. The ^{14}C recovered as CO_2 after 48 day incubation in fermentations with labeled ^{14}C cellulose ranged between 26-51%. Late-appearing colonists of pasture dung generally were more efficient in hydrolyzing cellulose. Sordaria fimicola, Ascobolus furfuraceus, Podospora decipiens, and Poronia punctata exhibited little hydrolysis of cellulose after 28 days of growth. In contrast, the unidentified basidiomycete and Hypocopra merdaria effected significant evolution of labeled $^{14}CO_2$ in later stages of the fermentation. These results suggest that cellulose is hydrolyzed at different rates during different phases of growth. Rates of degradation of

Table 1

Enzymatic Hydrolysis of Cellulose from Biologically Treated Wheat Straw

Fermentation[a] (days)	Cellulose content of 200 mg treated straw,[b] (mg)	Glucose from 200 mg sample by enzymatic hydrolysis,[c] (mg)	Conversion of cellulose to glucose,[d] (%)
0	90	15	15
10	89	15	15
20	81	15	17
30	78	30	34
40	72	45	56
50	69	55	72

[a] Pleurotus ostreatus NRRL 2366 fermentation of wheat straw substrate.

[b] Analyses for cellulose content were made using standard analytical methods.

[c] Hydrolysis of 200 mg fermented straw sample with 10 I.U. cellulase per gram of residue. On I.U. = 1 μmole produced as glucose from filter paper/minute.

[d] Conversion data were adjusted for weight gain due to water addition to glycosyl moiety on hydrolysis. Formula:

$$\frac{(\text{gram glucose produced})\ (162/180)\ (100)}{\text{dry wt cellulose, gram}} = \%\ \text{conversion}$$

labeled cellulosic components of natural lignocelluloses from maple were fairly uniform through the 6 day fermentation (28-38%). At 48 days, however, the three late-appearing species on dung (e.g., Hypocopra merdaria, Poronia punctata, and the unidentified basidiomycete) accounted for cumulative losses of $^{14}CO_2$ from 64.5 to 72.6%, whereas the earlier appearing forms resulted in smaller losses (39-53%).

Cumulative lignin degradation by coprophilous Ascomycetes was minimal during the 48-day fermentation (3.8-6.5%), but the basidiomycete produced losses up to 42% of the labeled material. These results support those obtained earlier in direct chemical measures of the lignin remaining in fermented WS, where this basidiomycete was the only fungal isolate that produced significant losses of lignin.

Table 2

Evolution of $^{14}CO_2$ from Specifically Labeled [^{14}C-(Lignin)- and ^{14}C-(Cellulose)-]Lignocellulosic of Young Maple Stems Selected by Isolates of Coprophilous Fungi

	Specifically labeled lignin[a,b]								
	C*			LC*			L*C		
Taxa	6 Day	28 Day	48 Day	6 Day	28 Day	48 Day	6 Day	28 Day	48 Day
Sordaria fimicola	22[c]	35	39	33	45	48	2	5	7
Ascobolus furfuraceus	19	32	32	31	49	53	1	3	4
Podospora decipiens	11	26	26	28	42	48	1	3	4
Hypocopra merdaria	33	40	51	37	60	64	2	3	5
Poronia punctata	30	37	41	36	53	65	1	4	5
Unidentified basidiomycete	7	21	35	38	64	73	8	30	42

[a] C* = ^{14}C-labeled cellulose, 780 cpm/mg; LC* = ^{14}C-labeled cellulose in lignocellulosics, 1500 cpm/mg; L*C = ^{14}C-labeled lignin in lignocellulosic, 2100 cpm/mg. Labeled lignocellulosic prepared by method of Crawford and Crawford (1976).

[b] 10 mg of labeled material was blended with 100 mg Wheat Straw and pelletized.

[c] Percent ^{14}C recovered as CO_2 at 6, 28, and 48 days incubation.

Conversion of Wheat Straw To Ethanol: Chemical Pretreatment/Enzymatic Hydrolysis

Preliminary investigations (Detroy et al., 1980) in our laboratory concerned primarily chemical/biological modification of straw subsequent to enzymatic hydrolysis to sugar. Experiments described herein involve primary and secondary chemical modification steps prior to enzymatic saccharification and alcohol production as depicted in Fig. 3.

Table 3 depicts the enzymatic saccharification of wheat straw that has been pretreated with various chemical reagents. Pretreatment of WS with acid (5% H_2SO_4) or alkali (1% NaOH) resulted in little loss in the cellulosic component. Acid pretreatment at 90°C for 4 hr resulted in only a 3% cellulose loss compared to 13% with alkali treatment. Acid pretreatment at room temperature (4 hr) resulted in a 15% decrease in the hemicellulose fraction; at 90°C for 4 hr, 93% was lost. Delignification was 11% or less in the acid pretreatments compared to 17 to 35% for alkali-treated samples.

At 25°C, the acid-pretreated WS treated with a fungal cellulase yielded a 30% conversion of the cellulosics to glucose, whereas the alkali-treated straw upon incubation with cellulase yielded 51% conversion.

Primary treatments of WS with H_2SO_4 or NaOH at 90°C for 4 hr yielded cellulose conversions of 25 and 75%, respectively.

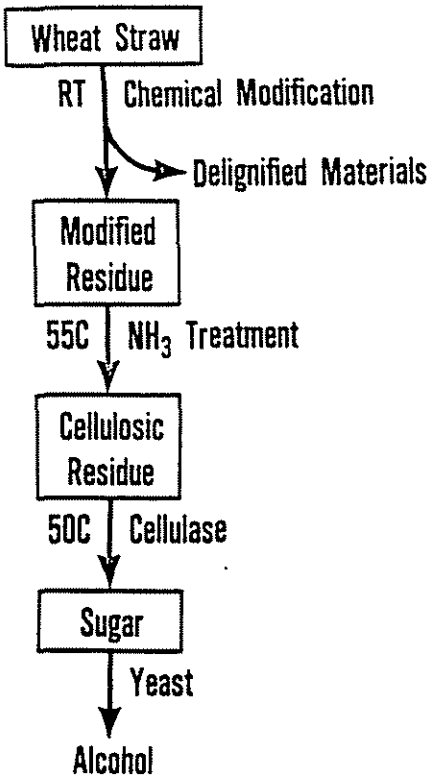

Fig. 3. Protocol for wheat straw pretreatment and hydrolysis.

Primary/Secondary Treatment-Enzymatic Hydrolysis

Secondary treatment of room-temperature acid-pretreated straw with EDA yielded a 51 and 69% loss in hemicellulose and lignin, respectively. The highly corrosive EDA yielded an amorphous-appearing WS residue that, when treated with enzyme, yielded a 98% conversion as documented in Table 3. A similar result was obtained with the 90°C acid-treated straw and EDA; however, the glucose conversions were considerably reduced (59%). The production of furfural derivatives in the initial heat-step may inhibit the enzymatic hydrolysis; this will be discussed later.

Secondary treatment with EDA of straw initially heated with alkali at room-temperature yielded significant hemicellulose and lignin losses, 51 and 61%, respectively. Enzymatic conversion was 61%.

Secondary treatment with NH_4OH gave best results with the alkali-pretreated straw. Delignification was 40%, with

Table 3

Primary and Secondary Pretreatments of Straw Followed by Enzymatic Saccharification

Chemical[a] pretreatment		Modified residual straw (%) Loss[b]			Conversion[c] of cellulose to glucose (%)
Primary	Secondary	Hemicellulose	Cellulose	Lignin	
5% H_2SO_4, 25°C for 4 hr	None	15	2	6	30
	EDA	51	5	69	98
	NH_4OH	40	1	25	55
5% H_2SO_4 90°C for 4 hr	None	93	3	11	26
	EDA	93	10	75	59
	NH_4OH	92	7	19	30
1% NaOH, 25°C for 1 hr	None	32	13	17	51
	EDA	51	11	61	61
	NH_4OH	50	14	40	70
1%, NaOH, 90°C for 1 hr	None	60	9	35	75
	EDA	60	17	47	72
	NH_4OH	60	9	29	83
Control (H_2O)		0	0	0	11

[a] As described in Materials and Methods.

[b] As determined by standard procedures for acid-detergent fiber analysis, cellulose, permanganate lignin, and neutral detergent for hemicellulose.

[c] Hydrolysis of 200 mg treated straw sample with 10 I.U. cellulase per g of residue. One I.U. = 1 μmole produced as glucose from filter paper/min. Conversion data were adjusted for weight gain from water addition to glycosyl moiety on hydrolysis.

Formula: $$\frac{(\text{g glucose produced})\ (162/180)\ (100)}{\text{dry wt of cellulose, g}} = \%\ \text{conversion}$$

a 50% loss in hemicellulose with alkali straw pretreated at room-temperature. Conversion rates to glucose were 70%. Straw pretreated with hot alkali and then treated further with NH_4OH yielded cellulose that was 83% convertible.

Primary/Secondary Treatment: Time Course

Since conversion percentages to glucose were highest with the alkali-pretreated straws, further evaluations were done by varying the alkali/acid concentrations and reaction time (1-4 hr) in the pretreatments prior to secondary NH_4OH treatment. Figure 4 depicts the percent conversion of

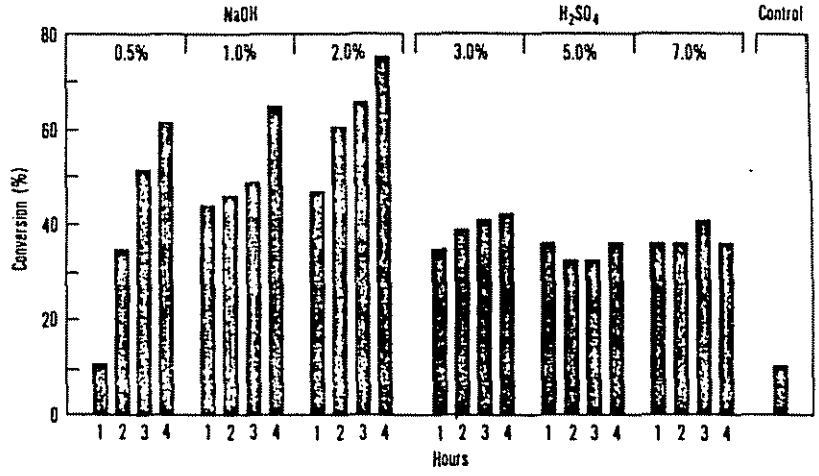

Fig. 4. Primary/secondary chemical pretreatment of straw prior to enzymatic saccharification. NaOH pretreatment: 0.5, 1.0, and 2.0% for 1 to 4 hr prior to secondary treatment with NH_4 at 55°C for 72 hr. H_2SO_4 pretreatment: 3, 5, and 7% for 1-4 hr prior to secondary treatment with NH_4OH at 55°C for 72 hr.

cellulose to sugar after 1-4 hr primary and 72 hr secondary chemical treatments prior to enzyme hydrolysis.

Maximal saccharification was obtained with the alkali-treated straws. Pretreatment with 0.5 NaOH for increasing periods of time yielded progressively higher percent saccharification, reaching 62% after 4 hr. Pretreatment with 1% NaOH yielded conversion rates of 44 to 65% over 1 to 4 hr. The highest percent conversion, 75%, was achieved with 2% NaOH for 4 hr.

Acid pretreatments for 1 to 4 hr with varying H_2SO_4 concentration (3-7%) yielded approximately equal conversion rates, 32 to 41%. Control wheat straw treated with enzyme without prior chemical modification resulted in 11% conversion of cellulose component to glucose.

As evidenced in Table 3, extensive treatment with alkali resulted in exhaustive solubilization of hemicelluloses coupled to delignification. Cellulose component losses at room temperature were 14% or less with alkali treatment. Since cellulosic residues contain from 10-35% hemicellulose, pretreatment or modification with chemicals may yield a carbon loss or solubilization and, therefore, must be calculated in terms of efficiency of total sugar conversion.

Batch Column Reactor Vessel for Saccharification and Alcohol Fermentation

Figure 5 depicts a scale-up unit used for hydrolysis for wheat straw similar to the small-scale experiments. The column reactor was used for experiments with 300-500 g of straw. Various treatments were considered in the scale-up studies, with a look toward optimization of the chemical modification and enzymatic saccharification steps. Chemical reagents were pumped continuously through the temperature-controlled column with aeration. The modified WS was H_2O-washed with 3-4 volumes before addition of buffered enzyme at 50°C.

Table 4 contains data from several experiments on sugar formation and alcohol production from chemically modified straw in the reactor column. Pretreatment of straw with NaOH, which appeared to be optimum for enzymatic hydrolysis, was accompanied by a considerable loss in straw dry weight, i.e., 18 to 25%. As a step towards practical

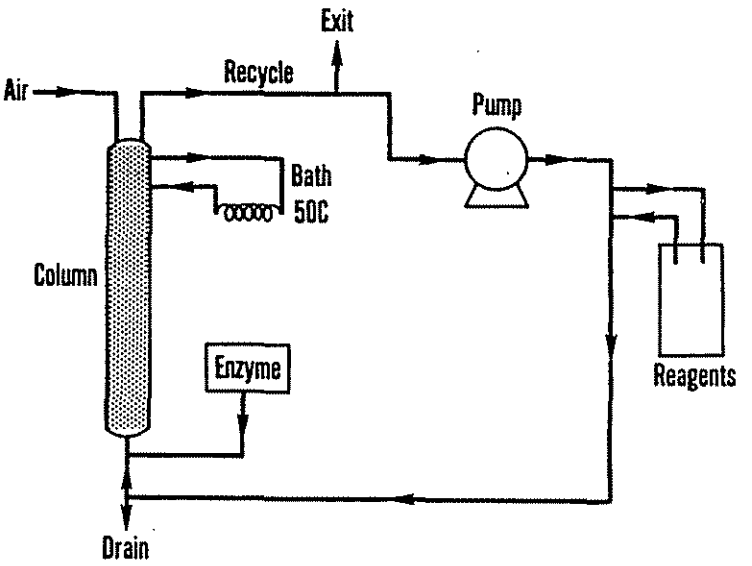

Fig. 5. Reactor column for pretreatment, saccharification, and fermentation.

Table 4

Glucose and Alcohol Production from Chemically Modified and Enzymatically Treated Straw in Column Reactor

Experiments	Total sugar produced[b]		Conversions[c,d]	
Chemical pretreatments[a]	Glucose (gram)	Xylose (gram)	Cellulose to glucose (%)	Glucose to ethanol (%)
NaOH (1%) 24 hour	35	13.5	26	42
NaOH (0.5%) 6 hour	39	23.9	30	41
NaOH (0.5%) 24 hour	40	16.4	18	30
NH_4OH (10%) 24 hour	20	5.2	15	31
Anhydrous NH_3 (6 gram) 6 hour	20	7.0	15	33

[a] Chemical modification experiments conducted at room temperature.

[b] Enzymatic saccharification conducted on the resultant modified residue with 10 I.U. per mg of residue at 45°C for 6 hours.

[c] Hydrolysis of 200 mg treated straw sample with 10 I.U. cellulase per gram of residue. One I.U. = 1 μmole produced as glucose from filter paper/minute. Conversion data were adjusted for weight gain from water addition to glycosyl moiety on hydrolysis. Formula:

$$\frac{(\text{gram glucose produced})\ (162/180)\ (100)}{\text{dry wt of cellulose, gram}} = \%\ \text{conversion}$$

[d] Represents alcohol produced from the saccharified glucose available compared to theoretical yield of 2 moles ethanol from 1 mole glucose.

processing, the alkali-treated straw was neutralized with acid or water prior to enzymatic hydrolysis. Neutralization was quite satisfactory by washing with H_2O wash. The nature of the products formed during alkaline treatment of straw and H_2O wash is unknown. Recent experiments reported the formation of antimicrobial substances during heat treatment of xylose with alkali (Kato and Shibasaki, 1974). Such materials could retard fermentation, and the separation of alkali-treatment liquor would be indispensable.

A large percent of the dry weight loss in alkali treatment is the hemicellulose component, which is 25% of the wheat straw. In the column reactor, hemicellulose losses of 50 to 60% were observed in the alkali-treated straws.

In experiments conducted with 300-500 g or more of straw, reacted with 0.5% $NaOH/NH_4OH$ for 6 hr, biomass losses were 15 to 20%.

This hemicellulose fraction obtained by alkali treatment could be neutralized and precipitated with ethanol for use as carbon source for xylose-fermenting organisms.

In the various trials, conversion of the cellulosic components of wheat straw to fermentable sugar ranged between 15 to 30%. The crude fungal cellulase-hemicellulase preparations used to treat the modified straw yielded also a substantial quantity of the fermentable C-5 sugar, xylose. From 300 g of native WS, the yield of xylose from enzyme-treated, modified straws was 5 to 24 g. This xylose fraction is exclusive of the hemicellulose fraction lost in the aqueous extraction phase of the alkali-treated straws.

Ethanol yields from the saccharified sugars in the column inoculated with a S. cereviseae strain NRRL 2034 at 10^8 cells/ml were 30 to 42% of theoretical. Possibly, yields were low because inhibition substances were generated during the chemical treatments or enzymatic saccharification, and because the sugar concentration was only 1 to 3% in the column reactor.

Fermentation of Xylose and Wheat Straw Hemicellulose Hydrolyzates

The idea of using a fermentation process to convert renewable plant biomass to ethanol is increasingly more attractive as non-renewable fossil fuel reserves dwindle, but many technological pitfalls still block its realization. An efficient process for deriving ethanol from lignocellulosic carbohydrates has been particularly slow to evolve. Flickinger (1980), Wang et al. (1980), Gong et al. (1981), and Cooney et al. (1980) describe the current state of the lignocellulose conversion process.

According to Sciammanna et al. (1977), pentoses comprise nearly one-third and hexoses nearly two-thirds of the reducing sugars obtained from lignocellulose hydrolysis. Yeasts familiar to the brewing industry efficiently produce ethanol from the hexose fraction, composed of D-glucose, but fail to do so from the pentose fraction, composed largely of the aldopentose D-xylose. Several researchers have reported that yeasts are able to assimilate aldopentoses oxidatively but are not able to ferment them to ethanol except when exogenous isomerases are added to the growth medium. Nevertheless, through systematic screening of yeasts from the AR Culture Collection at the Northern Regional Research Center, Pachysolen tannophilus has been found to produce ethanol rapidly and in good yield from D-xylose. This discovery not only promises a solution to the problem of utilizing the pentoses obtained from lignocellulose hydrolysis but also disproves the belief that yeasts cannot convert aldopentoses to ethanol.

While this account was in preparation, Schneider et al. (1981) published a report on the ability of P. tannophilus

NRRL Y-2460 to ferment D-xylose in the presence of oxygen. Our work confirms and adds significantly to their findings. Batch experiments were conducted under various conditions to identify those conditions necessary for optimum ethanol production.

The yeast Pachysolen tannophilus was found to be capable of converting D-xylose to ethanol. Batch cultures initially containing 50 g/liter D-xylose yielded 0.34 g of ethanol per gram of pentose consumed. Aerobic conditions were required for cell growth but not for ethanol production. Both alcohol formation and growth were optimum when incubation temperature was 32°C, when pH was near 2.5, and when D-xylose and ethanol concentrations did not exceed 50 g/liter and 20 g/liter, respectively.

The most recent information from our laboratory represents research findings on the production of glucose and xylose from straw and subsequent direct fermentation of both sugars to ethanol.

Agricultural straw was subjected to thermal or alkali pulping prior to enzymatic saccharification. When wheat straw (WS) was treated at 170°C for 30 to 60 min at water-to-solids ratio of 7:1, the yield of cellulosic pulp was 70 to 82% (Fig. 6). A sodium hydroxide extraction yielded a 60% cellulosic pulp and a hemicellulose fraction available for fermentation to ethanol. The cellulosic pulps were subjected to cellulase hydrolysis at 55°C for production of sugars to support a C-6 fermentation. Hemicellulose was recovered from the liquor filtrates by acid/alcohol precipitation followed by acid hydrolysis to xylose for fermentation.

Pachysolen tannophilus strain NRRL 2460 is capable of an ethanol fermentation of xylose under initial aeration conditions for generation of high cell populations (Slininger et al., 1981). Detroy et al. (1981) have reported batch fermentations with 70 g/liter D-xylose resulting in 0.3 g ethanol/g of pentose metabolized in 6 days. Figure 7 depicts the replication cycle of P. tannophilus at 25°C on 7% xylose. Cell populations double every 24 hr for approximately 3 days. Ethanol concentrations of 2.0% were achieved by 6 days with complete utilization of the xylose available.

Our most recent experiments with P. tannophilus involve fermentation of xylose from crude WS hydrolyzates. The yeast produced 7.2 g (0.72%) ethanol from a hydrolyzate containing 43 g/liter xylose (4.3%) as shown in Figure 8. All of the xylose was consumed within 4 days with only a slight effect upon cell replication. The sub-optimal yield for ethanol is probably due to the presence of lignin byproducts and degraded sugar derivatives in the concentrated WS

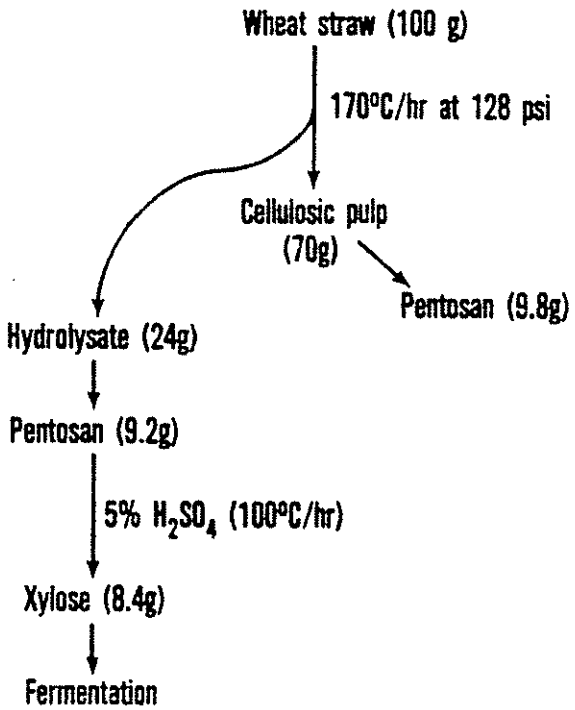

Fig. 6. Pentosans/xylose yields from wheat straw.

hydrolyzates. Further optimization of both the processing of hydrolyzates and the fermentation are underway in our laboratory.

Although the final yields of sugars from WS residues are not optimal, fermentation of the xylose and glucose produced in the aforementioned processes havebeen achieved with S. uvarum and P. tannophilus. With the advent of cultures such as the Pachysolen yeast, direct total fermentations of residue polysaccharides to ethanol can be evaluated and worked together for optimization of such processes.

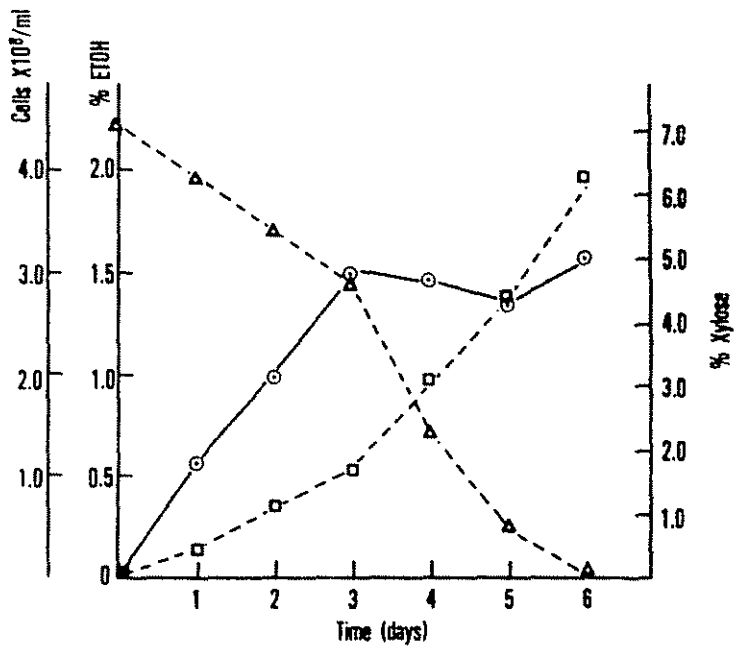

Fig. 7. Ethanol production by Pachysolen tannophilus 2460 on 7% xylose.

cells X 10^8/ml
% xylose
% ethanol

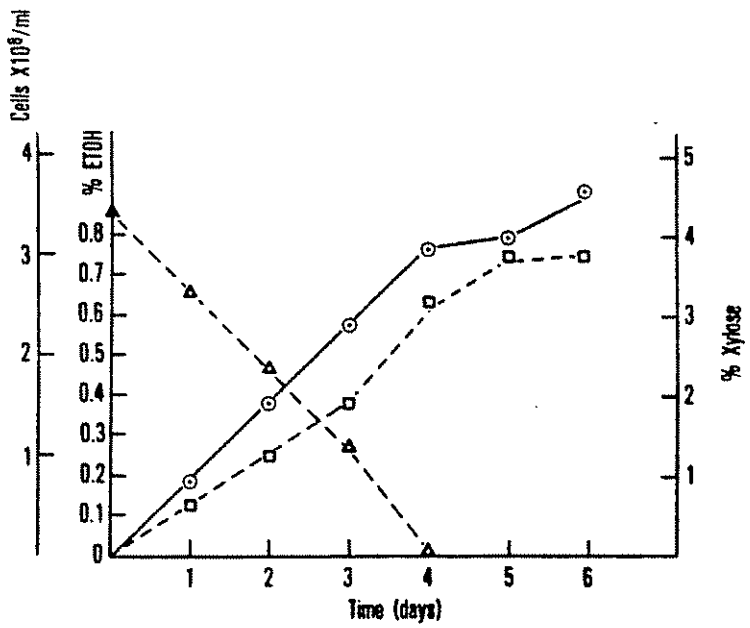

Fig. 8. Ethanol production by Pachysolen tannophilus 2460 on wheat straw xylose hydrolyzates (4.3% xylose).

cells X 10^8/ml
% xylose
% ethanol

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