Bioconversion of Wheat Straw Cellulose/Hemicellulose to Ethanol by *Saccharomyces uvarum* and *Pachysolen tannophilus*

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**Summary**

The information presented in this publication represents current 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 30-60 min at a water-to-solids ratio of 7:1, the yield of cellulosic pulp was 70-82%. A sodium hydroxide extraction yielded a 60% cellulosic pulp and a hemicellulosic fraction available for fermentation to ethanol. The cellulosic pulps were subjected to cellulase hydrolysis at 55°C for production of sugars to support a 6-C fermentation. Hemicellulose was recovered from the liquor filtrates by acid/alcohol precipitation followed by acid hydrolysis to xylose for fermentation. Subsequent experiments have involved the fermentation ofcellulosic and hemicellulosic hydrolysates to ethanol. Apparently these fermentations were inhibited by substances introduced by thermal and alkali treatment of the straws, because ethanol efficiencies of only 40-60% were achieved. Xylose from hydrolysis of wheat straw pentosans supported an ethanol fermentation by *Pachysolen tannophilus* strain NRRL 2460. This unusual yeast is capable of producing ethanol from both glucose and xylose. Ethanol yields were not maximal due to deleterious substances in the WS hydrolysates.

**INTRODUCTION**

Abundant lignocelluloses in the United States could provide a reservoir of materials for conversion into liquid fuels. Because exploitation of these materials requires utilization of hemicellulose and lignin, techniques to remove hemicellulose from wheat straw (WS) were examined. Pentoses derived from hemicelluloses could be an economical source of carbohydrate for conversion to liquid fuels.¹ This easily hydrolyzable source of xylose was evaluated for support of an ethanol fermentation of *Pachysolen tannophilus*.

Until recently, no yeasts were reported to ferment pentoses, although some

¹The 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.
were capable of aerobic metabolism. Recent reports\textsuperscript{2-6} have identified a number of yeasts capable of producing alcohol from xylose, directly or indirectly. Several yeasts ferment \(\text{D-xylose, a catabolite of D-xylose, to alcohol. These indirect processes}\textsuperscript{2,3} involve a two-step process: first, D-xylose is obtained by xylose isomerase; then, D-xylose is fermented to ethanol. The direct fermentation of xylose to ethanol has been reported\textsuperscript{4-6} with \textit{Pachysolen tannophilus}. 

The demonstration of microbial systems for direct fermentation of glucose and xylose to ethanol provides a new dimension in the conversion of biomass to liquid fuels, especially ethanol. Processes for the conversion of cellulose and hemicellulose to ethanol could be integrated into existing cellulose-conversion plants where the hemicellulose-derived pentoses are underutilized. Future conversion industries would incorporate both the grain- and residue-derived sugars for optimum production of fermentation alcohol. Glucose liquors from acid- or enzyme-hydrolyzed cellulosics would provide a substrate for a \textit{Saccharomyces} fermentation that would be coupled to conversion of xylose and xylans to ethanol by other yeasts. A direct fermentation of xylose obtained from wheat straw hydrolysates to ethanol by \textit{P. tannophilus} is described herein.

\section*{MATERIALS AND METHODS}

\textbf{Substrates}

Two methods of removing hemicellulose from WS were employed.\textsuperscript{7} The raw material was straw of soft winter wheat (\textit{Triticum} sp., Arthur variety). One method was autohydrolysis or thermal pulping of WS chopped to 10-45 mm lengths. Wheat straw (908 g) was cooked in a digester equipped with steam jacket and rotated at 1 rpm. The WS was treated at 170°C for 30 or 60 min at a water-to-solids ratio of 7:1. Pulping liquors were frozen and freeze-dried.

The second method was an extraction procedure similar to that reported by Chen and Anderson.\textsuperscript{8} Milled WS was treated with 4\% NaOH overnight at room temperature. After passing through a 4-mm screen in a Wiley mill, 40 g of WS were mixed with 400 mL of 4\% NaOH (w/v). The sample was filtered, the residue was washed, and the combined filtrate was adjusted to pH 5 with HCL, and alcohol was added in a ratio of 1.5:1. The resulting precipitate was filtered and dewatered by exchange with 70\%, 95\%, and absolute alcohol. The precipitate was allowed to air dry before drying in a vacuum oven for 4 h at 50°C.

A cellulase treatment described by Detroy et al.\textsuperscript{9} was used to determine percent hydrolysis of the cellulosic pulps. All modified WS substrates were freeze-dried before chemical analyses. Cellulose contents were measured by the monoethanolamine method\textsuperscript{10} and were reported ash- and pentosan-free. Lignin contents were determined by the spectrophotometric method of Bagby,
Cunningham, and Maloney. Nitrogen was determined by the Kjeldahl method. Other analytical procedures were standards of the Technical Association of the Pulp and Paper Industry. Filtrates were analyzed for total glucose by high-performance liquid chromatography (HPLC). Stock cultures of Saccharomyces uvarum strain NRRL 1347 were used to ferment the glucose obtained from cellulase hydrolysis of modified WS pulps.

Stock cultures of Pachysolen tannophilus NRRL Y-2460 were maintained on agar slants at 25°C. The slant medium contained 3.0 g/L yeast extract, 3.0 g/L malt extract, 5.0 g/L peptone, 10.0 g/L dextrose, and 20.0 g/L agar.

The medium used for ethanol production was that described by Del Rosario. The carbohydrate and yeast extract plus salts base were autoclaved separately and combined before inoculation. Straw hydrolysates were not autoclaved but added directly to the production medium.

For ethanol production, vegetative cells were incubated in 17 mL of medium in 50-mL Erlenmeyer flasks at 25°C on a rotary shaker (200 rpm). After 40-h incubation, 0.5 mL of inoculum was transferred to 300-mL Erlenmeyer flasks containing 200 mL of medium. Flasks were incubated at 25°C in a rotary shaker, and aliquots were removed periodically for cell counts and ethanol assays. Ethanol was determined by gas-liquid chromatography.

RESULTS AND DISCUSSION

Wheat Straw Pretreatment

Various pretreatments were employed to disrupt the cellulose-hemicellulose-lignin complex to provide components rich in either cellulose or hemicellulose. Two of the methods of extraction to produce hemicellulose (xylan) and cellulosic pulps from WS with sodium hydroxide and with autohydrolysis are depicted in Figure 1. The resulting xylan is hydrolyzed to xylose for fermentation. Subsequent enzymatic hydrolysis of the residual cellulosic pulps provides glucose for a separate fermentation to ethanol.

Fig. 1. Protocol for wheat straw pretreatment and hydrolysis.
An 82\% yield of cellulosic pulp was realized from thermal pulping of WS with water for 30 min at 170°C (Table I). Pentosan content was reduced from 29\% to 18\% by the treatment. On the basis of original material, one-half of the pentosans in WS remained in cellulosic pulp. Little change was noted in lignin content. Free liquor was collected and compositional data of this freeze-dried hydrolysate indicated 35\% pentosan and a small amount of nitrogen.

The WS in water subjected to 170°C for 1 h yielded a cellulosic pulp in 70\% yield (Fig. 2). Although 91\% of the pentosans in hydrolysate were acid-hydrolyzed to xylose, pentosans in pulp represented 10\% of original solids. Improved technology should permit additional removal of pentosans from the WS.

A 60\% yield of cellulosic pulp was realized from the overnight extraction of WS with 4\% NaOH (Table II). This extraction method provided a cellulosic substrate slightly higher in pentosans but lower in lignin than substrates prepared by autohydrolysis. Forty-three percent of the original pentosans in the WS remained in the cellulosic pulp after significant delignification. The ethanol insoluble portion of the extraction was 18\% of the original WS. Hydrolysates of these aforementioned fractions were used to support a xylose fermentation of ethanol by *P. tannophilus* and will be described subsequently.

**Saccharification and Alcohol Fermentation of Cellulosic Pulps**

Preliminary findings\(^1\) in our laboratory have concerned primary chemical or thermal modifications of WS subsequent to enzymatic hydrolysis to glucose for ethanol fermentations.

A column reactor vessel as described by Detroy et al.\(^1\) was utilized for experiments with 300-500 g of straw. Various parameters were considered in

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<th>TABLE I</th>
<th>Characteristics of Thermally Pulped Wheat Straw(^a)</th>
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<tr>
<td><strong>Cellulosic pulp composition</strong></td>
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<tr>
<td>Residue yield (%)</td>
<td>Cellulose MEA (%)</td>
</tr>
<tr>
<td>82.5</td>
<td>46.3 (33)(^b)</td>
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<tr>
<td><strong>Hydrolysate composition</strong></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>Yield(^c) (%)</td>
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<tr>
<td>4.2</td>
<td>11.3</td>
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\(^a\)Pulped for 30 min at 170°C; MEA—monoethanolamine.

\(^b\)Values in parentheses represent analysis values for untreated WS.

\(^c\)Solids, basis original wheat straw. Free liquor collected through condenser.

\(^d\)Kjeldahl method.

\(^e\)After dilute acid hydrolysis.
BIOCONVERSION OF WHEAT STRAW TO ETHANOL

![Diagram of wheat straw bioconversion](image)

Fig. 2. Pentosans/xylose yields from wheat straw.

This scale-up procedure, with a view toward optimization of the chemical/thermal modification and enzymatic saccharification steps. Various chemical reagents were pumped continuously through the temperature-controlled column with aeration to optimize delignification as depicted in Figure 3. Saccharification of delignified WS was achieved by treatment with \textit{Trichoderma viride} cellulose (10 IU/g dry weight residue).

It should be noted that pretreatment of straw with NaOH, which appeared to be optimal for enzymatic hydrolysis, was accompanied by a considerable loss in dry weight, i.e., 18–25%. As a step towards practical processing, we evaluated the possibility that the alkali-treated straw might be neutralized with acid prior to enzymatic hydrolysis. Neutralization was accomplished by

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<th>CHARACTERISTICS OF ALKALI-EXTRACTED WHEAT STRAW</th>
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<tr>
<td>Residue(^a) yield (%)</td>
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<tr>
<td>--------------------------</td>
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<tr>
<td>60.3</td>
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<td>Hemicellulose ppt from WS</td>
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Yield (%) | Lignin (%) | Xylose\(^b\) (%)
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<tbody>
<tr>
<td>18.3</td>
<td>13.1</td>
<td>18.8</td>
</tr>
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\(^a\)Extracted overnight at room temperature with 4\% sodium hydroxide solution.

\(^b\)After dilute acid hydrolysis.
employing a water-wash procedure. Products formed during alkaline treatment and water washing of straw are not known, and their influence on enzymatic hydrolysis is not known. Recent experiments reported on the heat treatment of xylose with alkali indicate the formation of antimicrobial substances. Such substances could retard microbial fermentation of straw residues.

When 300-500 g of straw were reacted with 4.0% NaOH for 6 h, 15-18% of the biomass was lost. Conversion of the cellulose of WS to fermentable sugars for the various trials ranged between 30 and 50%. The crude fungal cellulase preparations used to treat the modified straw yielded also a substantial quantity of the fermentable 5C sugar, xylose. Three hundred grams of native WS yielded between 5 and 24 g of xylose from enzyme-treated, modified straws. This xylose fraction is exclusive of the hemicellulose fraction available in the aqueous extraction phase of the alkali-treated straws previously described (40 g xylose/500 g WS).

Ethanol production from the sugars in the column inoculated with S. uvarum at 10^8 cells/mL was 30-42% of theoretical yield. These lower fermentation values may be due partially to the presence of endogenous substances generated in the alkali modification processes, coupled to dilute sugar concentrations of 1-3% in the column reactor aqueous phase.

**Fermentation of Xylose and Wheat Straw Hemicellulose Hydrolysates**

*Pachysolen tannophilus* strain NRRL 2460 is capable of an ethanol fermentation of xylose under initial aeration conditions for generation of high cell populations. Detroy and co-workers have reported batch fermentations with 70 g/L D-xylose resulting in 0.3 g ethanol/g pentose metabolized in 6 days. Figure 4 depicts the replication cycle of *P. tannophilus* at 25°C on 7% xylose. Cell populations double every 24 h 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 hydrolysates. The yeast produced 7.2 g (0.72%) ethanol from a hydrolysate containing 43 g/L xylose (4.3%) as shown in Figure 5. All of the xylose was consumed within 4 days with only a slight effect upon cell replication. The suboptimal yield of ethanol is probably due to the presence of lignin by-products and degraded, sugar derivatives in the hydrolysate.
concentrated WS hydrolysates.\textsuperscript{16} Further optimization of both the processing of hydrolysates and the fermentation are underway in our laboratory.

Figure 6 depicts an overall schematic with an initial chemical pretreatment to yield a xylose/pentosan component plus the cellulosic residue for ethanol production. From 500 g of WS, one obtains 400 g of cellulosic pulp after chemical pretreatment with 4\% NaOH for 6 h.\textsuperscript{14} The liquor contains some 40 g of fermentable d-xylose that, although suboptimal, supports the \textit{P. tannophilus} 5C fermentation treatment of the cellulosic pulp with cellulase (10 IU/g) for 6 h, yielding 105 g of fermentable sugar, which is only 60\% of the available glucose in the pulp. Addition of \textit{S. uvarum} cells to the saccharified material yields 42 g of ethanol (80\% of theoretical amount) in 48 h.

Although the final yields of sugars from WS residues is not optimal, fermentation of the xylose and glucose produced in the aforementioned processes has been achieved with \textit{S. uvarum} and \textit{P. tannophilus}. With the advent of cultures such as the \textit{Pachysolen} yeast direct, total fermentations of residue polysaccharides to ethanol can be evaluated and worked toward optimization of such processes.

The authors thank C. E. Johnson, W. L. Orton, and D. M. Palmer for their technical assistance.

\textbf{References}


Accepted for Publication September 14, 1981