Hydrolysis of Wheat Straw Hemicellulose with Trifluoroacetic Acid. Fermentation of Xylose with Pachysolen tannophilus

G. F. Fanta, T. P. Abbott, A. I. Herman, R. C. Burr, and W. M. Doane
Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture,* Peoria, Illinois 61604

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Treatment of wheat straw with 1N trifluoroacetic acid (TFA) for 7 h at reflux temperature yielded 23% xylose based upon initial straw weight. This corresponds to about an 80% xylose yield based on the xylan content of the hemicellulose. The cellulose component of wheat straw was largely unaffected, as evidenced by low glucose yields. Decomposition of xylose by prolonged refluxing (23 h) was minimal in 1N TFA compared to 1N HCl. Treatment of wheat straw with refluxing 1N TFA converts about 10% of the lignin initially present in straw into water-soluble lignin fragments. Fermentation of the xylose-rich wheat straw hydrolyzate to ethanol with Pachysolen tannophilus was comparable to the fermentation of reagent grade xylose, indicating that furfural and toxic lignin by-products were not produced by 1N TFA in sufficient amounts to impair cell growth and ethanol production. Cellulase treatment of the wheat straw residue after TFA hydrolysis resulted in a 70-75% conversion of the cellulose into glucose.

INTRODUCTION

Efficient utilization of lignocellulose both as an energy source and as a source of useful monomers and polymers depends to a large extent on our ability to separate these materials into their hemicellulose, cellulose, and lignin components. Bioconversion to ethanol is a good example of the necessity for such separations, since different organisms are required to ferment glucose (derived from cellulose) and xylose (the major component of hemicellulose). Because hemicellulose is more susceptible to acid hydrolysis than cellulose, selective hydrolysis of hemicellulose to xylose has been used to achieve this separation.1 Also, an autohydrolysis or thermal pulping technique for separation of hemicellulose from wheat straw has been described by Detroy et al.2,3 A serious drawback to these methods, however, is the subsequent inhibition of xylose fermentation with the yeast Pachysolen tannophilus by toxic by-products of the hydrolysis (e.g. furfural and soluble lignin fragments).

*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.
by filtration, washed with water, and dried. Yields of xylose, arabinose, and glucose in the clear, yellow filtrate were determined by HPLC (Waters ALC 201 with R.I. detector) without neutralizing or removing acid. A Bio-Rad HPX-42 column was used with water as the mobile phase at a flow rate of 0.6 mL/min. Xyloextrins were separated on an amino column (Regis Chemical Co.) with 70:30 acetonitrile-water as the mobile phase at a flow rate of 2 mL/min.

**Bioconversion of Hydrolysis Products**

A stirred slurry of 50.0 g of wheat straw in 500 mL 1N TFA was heated under reflux for 7 h, and cooled to 25°C, and then the solid was removed by filtration and washed with four 100-mL portions of water. The filtrate and washings were concentrated to a syrup on a rotary evaporator (30°C). To remove volatile TFA, three successive additions of 100 mL water were made to the syrup, followed by evaporation to almost dryness. The residue was freeze-dried from a water solution and redissolved in water, and the pH was adjusted to 4 with sodium hydroxide. The final volume was 200 mL. This solution was fermented with *P. tannophilus* NRRL Y-2460 as described by Detroy et al. 3

A portion of the wet, TFA-insoluble straw residue (0.2 g, dry basis) was digested with 20 mL of a 6.25 g/L cellulase solution according to the method of Detroy et al. 11

**RESULTS AND DISCUSSIONS**

**Acid Hydrolyses**

Stirred suspensions of 5 g of ground wheat straw in 50 mL of either 1N TFA (Fig. 1), 0.1N TFA (Fig. 2), or 1N HCl (Fig. 3) were heated under reflux for varying periods of time, and yields of xylose, arabinose, and glucose based on the starting weight of straw were calculated from HPLC analyses of the acidic solutions. Sugar yields at zero time in Figures 1-3 were obtained by cooling and analyzing the reaction mixtures as soon as the temperature reached 99°C.

Xylose yields shown in Figure 1 indicate rapid hydrolysis of the hemicellulose by 1N TFA. The 23% yield of xylose observed after 7 h reflux corresponds to about 80% of theoretical, assuming that straw contains 29% hemicellulose, 12 80-90% of which is xylan. The decrease in xylose yield observed after a reflux time of 23 h can probably be attributed to the formation of furfural; as we will discuss later, furfural was indeed isolated from the hydrolyzate. Arabinose yields remained fairly constant throughout the range of reflux times. It is noteworthy that the yield of glucose remained low and was only 1.6% after a reflux time of 23 h. Refluxing 1N TFA is thus an effective medium for hydrolyzing hemicellulose without significantly attacking cellulose.

As expected, hydrolysis occurs more slowly in 0.1N TFA (Fig. 2), a reflux time on the order of 23 h being required to give the same xylose yield as 1.5 h reflux in 1N TFA. Arabinose formation was roughly comparable to that observed with 1N TFA.

Hydrolysis in 1N HCl (Fig. 3) was carried out to provide a comparison with 1N TFA. Although the xylose yield increased rapidly, it decreased sharply on prolonged refluxing and reached a value of only about 6% after 23 h. Xylose decomposition thus takes place more readily in HCl than in TFA. Also, the glucose yield reached about 6% after
23 h, indicating that wheat straw cellulose is more susceptible to hydrolysis in HCl than in TFA.

Acid hydrolyzates were also examined by HPLC for xylooligomers, and di- and trisaccharides were indeed observed with short reflux times (0-1 h with 1N acid and 0-3 h with 0.1N TFA). Yields of xylooligomers, however, were only ca. 2-3% based on the weight of starting straw, indicating that further hydrolysis to form xylose occurs rapidly.

We also carried out a limited number of experiments to determine whether the use of 5N TFA would permit us to carry out the hydrolysis at a lower temperature. A 24-h hydrolysis at 50°C gave only 2% yields of xylose and arabinose, whereas increasing the temperature to 70°C produced xylose, arabinose, and glucose in yields of 16, 2, and 0.6%, respectively. No xylooligomers were detected in either experiment.

Since refluxing 1N TFA hydrolyzed hemicellulose without significantly hydrolyzing cellulose, we wished to determine whether the lignin component of straw was similarly inert. Perhaps the most straightforward method for acquiring this information was to conduct lignin analyses both on the initial straw and on the TFA-insoluble residue remaining after 7-h hydrolysis. The difference between the calculated weight of lignin in the initial straw and that in the residue was taken as the weight of lignin solubilized during TFA hydrolysis.

Two published methods were used to analyze for lignin. In the first method,5 straw or residue was reacted with hot acetyl bromide-acetic acid. Lignin in the resulting solution was then determined by measuring ultraviolet absorbance at 280 nm, a region of the UV in which lignin fragments commonly absorb. Although straw itself readily dissolved in the acetyl bromide-acetic acid mixture to give a reproducible lignin value of 14.6% by weight, the residue isolated after 7 h reflux with 1N TFA gave an appreciable quantity of insoluble material after acetyl bromide-acetic acid treatment. A low lignin value could thus be obtained, leading to an erroneously high value for percent lignin solubilized by 1N TFA.

To avoid the solubility problems inherent in the spectroscopic determination of lignin, we used a gravimetric method developed by Bagby et al.,10 in which straw or residue is digested at 5°C with 80% sulfuric acid to remove polysaccharides. Wheat straw and its hydrolysis residue gave lignin values of 18.3 and 26.2% by weight, respectively, by this method; calculations based on these values showed that 7% of the lignin originally present in straw had been solubilized by 7-h reflux in 1N TFA.

To further verify the amount of lignin solubilized during TFA hydrolysis, we examined the 7-h TFA hydrolyzate for soluble lignin fragments. The volatile fraction obtained by freeze-drying the hydrolyzate, absorbed strongly in the 280-nm region. The compound responsible for this absorption was isolated from the volatile fraction by chloroform extraction and was identified as furfural by infrared and mass spectra. Furfural was the only major UV-absorbing material in this volatile fraction, and the absence of volatile lignin-derived fragments was confirmed by the weak UV absorption remaining after chloroform extraction.

When the nonvolatile residue from freeze-drying was dissolved again in water, the UV spectrum showed a maximum at 277 nm, presumably due to lignin fragments. Using an absorptivity value of 18 given for lignin at 280 nm13 and the experimentally determined absorbance at 277 nm, we calculated that 7% of the starting lignin was solubilized during TFA hydrolysis.

These water-soluble lignin fragments could be partially removed from the TFA hydrolyzate through successive extractions with 95:5 (v/v) chloroform-methanol and their weight determined by evaporation of the solvent. Furfural was completely removed by the first few extractions and was easily evaporated from lignin-derived products. Subsequent extractions removed increasing amounts of lignin products, and their removal could be followed by the decrease in UV absorbance. By accurately measuring these absorbance differences and then correlating them with the grams of product isolated, we could calculate the total weight of lignin products in our aqueous solution. The water-soluble lignin products amounted to 10% of the lignin initially present in wheat straw, in good agreement with previous findings.

Infrared spectra of products removed by extraction with 95:5 chloroform–methanol were consistent with published spectra for lignin. Thin-layer chromatography on silica gel, eluting first with 70:30:1 (v/v/v) hexane-ethyl acetate-methanol and then with 90:10 (v/v) ethyl acetate–methanol showed that at least eight components were present. Spots on the chromatogram were clearly visible under short-wavelength UV irradiation.

Chloroform–methanol extraction of the 1N TFA hydro-
lyzate from the zero-time reaction gave about the same yield of soluble lignin fragments as that obtained from the 7-h hydrolyzate. Apparently, reaction of TFA with labile portions of the lignin structure occurs largely in the early stages of hydrolysis.

The UV spectra showed that lignin products formed initially are unstable and are not the same as those formed later in the hydrolysis. The TFA hydrolyzate at zero time (after removal of any furfural by extraction) showed the major band at 317 nm and a less intense band at 285 nm. A similarly extracted 7-h hydrolyzate showed the major band at 275 nm with a shoulder in the 320-nm region. Isolation of lignin fragments by a series of 95:5 chloroform-methanol extractions, followed by evaporation of solvent at near room temperature, caused the 317–285 nm spectrum to revert to a spectrum more similar to that observed after the 7-h hydrolysis. Absorbance in the 320-nm region for lignin fragments is consistent with Gould’s photoacoustic UV spectra of native lignin.14

Bioconversions of Hydrolysis Products

A xylose-rich hydrolyzate resulting from the 7-h hydrolysis of wheat straw with 1N TFA was first subjected to vacuum distillation to remove volatile TFA and was then fermented to ethanol by *P. tannophilus* NRRL Y-2460 (Fig. 4). The rate of xylose utilization was close to that observed by Detroy and co-workers for fermentation of pure xylose under similar conditions. Also, maximum cell counts and maximum yields of ethanol were similar for the two fermentations, although Figure 4 shows that ethanol production from wheat straw hydrolyzate did not begin until after 48 h. The diauxie observed between the third and fifth day is probably caused by preferential fermentation of the small amounts of six-carbon sugars (glucose, mannose, and galactose) normally present in straw hydrolyzates.

The similarity between fermentation of our straw hydrolyzate (with no purification other than removal of TFA) and that of pure xylose is noteworthy. Published reports on attempted fermentations of xylose resulting from either autohydrolysis or sulfuric acid hydrolysis of wheat straw state that fermentations are strongly inhibited by the presence of furfural and water-soluble lignin derivatives formed as by-products of the hydrolyses.1,2 Although we have indeed found furfural and lignin fragments in our TFA hydrolyzate, their concentrations are apparently too low to affect xylose fermentation.

Detroy and co-workers1,2,12,15 investigated a number of chemical pretreatments for wheat straw in an attempt to increase the susceptibility of the cellulose to attack by cellulase. Glucose produced in this enzymatic saccharification was then fermented to ethanol by *Saccharomyces uvarum*.15 With no pretreatment, 10–46% of the cellulose was converted to glucose, depending on particle size12; however, this conversion could be increased significantly by chemical pretreatment of the straw, for example with ammonium hydroxide, ethylenediamine, acid, or alkali.11 When the insoluble residue remaining after the 7-h hydrolysis of wheat straw with 1N TFA was similarly digested with cellulase, we observed a 70–75% conversion of cellulose to glucose. Glucose yields of this magnitude are comparable to those reported earlier by Detroy et al. for other chemical pretreatments.

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References