Alcohol from cellulose

T. A. Hsu, M. R. Ladisch, and G. T. Tsao

Biomass is a term which encompasses cellulosic residues, including agricultural residues, municipal wastes, livestock wastes, paper wastes, and forestry residues. Conversion of biomass into fermentable sugars could help to stretch petroleum resources. Sugar fermentation gives ethanol, which when used in gasoline (a 10% ethanol/90% gasoline mixture) extends gasoline supplies. Other sugar fermentations yield chemical intermediates such as butanol, acetic acid, acetone, and butanediol (1).

Presently, fuel alcohol production is associated with grain as the sugar source. While grain may become a significant source of alcohol, its use as a food and an export commodity imposes limits. Thus, we must also look to biomass for additional fermentable sugars.

While the total grain production in the U.S. is 355 million tons, the annual output of cellulosic residues exceeds 1000 million tons. This figure is comparable to the 400 million tons of oil annually imported by the U.S. (2).

Both starch and cellulose could be thought of as polysaccharide. Starch has a structure which is readily hydrolyzed. In comparison, cellulose (Fig. 11) forms a crystalline structure (Fig. 1(b) which protects the internal bonds from hydrolysis (3, 4). Also, cellulose in plants is protected by lignin. This polyphenolic material forms a seal around the cellulose (Fig. 1c) for further protection against hydrolysis (8).

Before we can use cellulosic residues as a source of chemical intermediates, they must first be broken down to sugars. This problem has been worked on for over 50 years with limited success (6). Here, we report on an approach to disrupt the cellulose structure with chemical solvents (5, 7). Our approach, when followed by hydrolysis, completely converts cellulose to sugars as compared to 50-70% conversion obtained with other methods.

Biomass materials in general consist of cellulose, hemicellulose, and lignin in the ratio of approximately 4:4:3 (9). Based on laboratory data and prior literature, the concept of obtaining two separate sugar streams by sequential hydrolysis seems well suited for biomass processing (Fig. 2).

Hemicellulose hydrolysis is done either with 0.1-1% aqueous sulfuric acid at 95-120 °C for short reaction times, or with 2-4% acid at 90 °C for about 4 h (10), and yields primarily pentoses. Since pentose fermentation to alcohols is done at different conditions than glucose fermentation, it is advantageous to obtain two sugar streams, one consisting primarily of pentoses and the other primarily of glucose.

The remaining material is known as ligno-cellulose and must be exposed to a pretreatment before cellulose hydrolysis is done to obtain glucose (Fig. 3). One

![Figure 1. Structure of cellulose in biomass materials: (a) cellulose, (b) cellulose crystallite, (c) cellulose crystalline in microfibril](image)

![Figure 2. A sequential hydrolysis/pretreatment hydrolysis scheme for biomass conversion](image)
approach to breaking the physical structure and the lignin seal is shown in Figure 3. In this case, cellulose material is exposed to cadexen, a cellulose solvent which consists of 5-7% cadmium oxide in 28% aqueous ethylenediamine (11, 12).

During the dissolution step, ligno-cellulose is allowed to stand with the solvent at room conditions from 15 min to 12 h. Conditions will vary with the material. When cadexen is the solvent, methanol is used to reprecipitate the cellulose into a flocculent material.

The reprecipitated cellulose material is quickly subjected to enzyme cellulase hydrolysis. Enzymes, usually derived from the microorganism Trichoderma reesi, catalyze the breakdown of cellulose to soluble sugars (13-15). While cellulose is converted to sugars, the lignin remains intact and is later filtered off (Fig. 2). This lignin has a heating value of about 11,000 Btu/lb (wet) and could be used to supply energy for part of the process.

The effect of pretreatment is to greatly increase the enzyme-catalyzed hydrolysis of cellulose by disrupting its lignin seal and crystalline structure. A schematic representation of this process is shown in Figure 4 (3, 4).

The solvent-imposed change of crystalline structure is characterized by the reduction in fold length, so that the number of folds or regions accessible to hydrolysis by catalyst is greatly increased (4). Hence, hydrolysis proceeds more rapidly and completely than is the case for untreated cellulose. The solvent approach to pretreatment is energy efficient since it is carried out at ambient conditions with little or no mechanical energy input. For complete reprecipitation of cellulose, approximately one volume of methanol per volume of cadexen solution is required (Fig. 5). Another important aspect of this process is solvent recovery. Figure 6 shows that three to four volumes of wash water per volume of cadexen will completely wash out both the cadmium and ethylenediamine from Avicel® (a microcrystalline cellulose).

Cadmium precipitates out as cadmium hydroxide, which can be regenerated in a kiln at 270-360 °C with a net heat input of approximately 300 Btu/lb (16). Ethylenediamine can be recovered by distilling off the water to recondensate the ethylenediamine to 20%.

Once cellulose has been reprecipitated, it can stand, under certain conditions, without recrystallizing at a pH > 11 or pH < 5. The rate of recrystallization, which is measured as a function of extent of hydrolysis, varies significantly with conditions, as is shown by data obtained with Avicel® (Fig. 7). Apparently storage of reprecipitated cellulose at high pH is more effective in preventing recrystallization than at low pH.

Reprecipitated cellulose, washed free of solvent, is more susceptible to hydrolysis than untreated cellulose (Fig. 8). The reaction was carried out at pH 4.8 and 50 °C using cellulase enzymes. Note that pretreated cellulose (Avicel) gives 90% conversion to glucose in a short time, while maximum conversion for untreated cellulose is 40%. Interestingly, cellulose pretreated with pure ethylenediamine alone (a strong organic base) also gave much higher conversion than the control, although the rate was slower.

Why did we not observe 100% conversions to glucose? Examine the LPLC (17) data shown in Figures 9 and 10. With low enzyme concentration, the formation of glucose is significant. However, smaller quantities of higher oligosaccharides (in this case, cellobiose and cellootiose)

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**Figure 3. Solvent pretreatment procedure**

**Figure 4. Effects of solvent pretreatment**
Figure 5. Methanol requirement for reprecipitation

Figure 6. Wash water requirement (Avicel®)

Figure 7. Effect of precipitate storage conditions on relative susceptibility to hydrolysis.

Figure 8. Timecourse hydrolysis of pretreated and untreated recrystallization (Avicel®)
Figure 9. LPLC (low-pressure liquid chromatography) chromatogram of cellulose hydrolysate obtained at low enzyme level.

Figure 10. LPLC chromatogram of cellulose hydrolysate obtained at high enzyme level.

Teh An Hsu recently received his Ph.D. in chemical engineering with George T. Tsao. He is presently employed by Hawaiian Sugar Cane Growers Association, Hawaii.

Michael R. Ladow is assistant professor of food and agricultural engineering and assistant professor of chemical engineering at Purdue. His research areas deal with the conversion of cellulose residues to fermentable sugars, sugar separations, enzymic kinetics, and alcohol recovery. He holds a Ph.D. in chemical engineering from Purdue and is a group leader in the Laboratory of Renewable Resources Engineering (LORRE).

George T. Tsao is professor of chemical engineering and agricultural engineering at Purdue. He is also the director of the Laboratory of Renewable Resources Engineering (LORRE). Dr. Tsao received his Ph.D. from the University of Michigan. He directs research on cellulose conversion, fermentation, food engineering, starch hydrolysis, and other biochemical engineering topics.
are also formed. Thus, if the conversion is based on glucose analysis only, the conversion will appear to be less than 100%. The cause for this incomplete conversion is inhibition of cellulases by the products formed (celllobiose and glucose) (17, 18). If higher enzyme concentrations are used—in other words, if there is sufficient catalyst present to partially overcome the inhibition effect—complete conversion to glucose is obtained.

After we developed conditions for the conversion of crystalline cellulose (which has no lignin associated with it) to soluble sugars and glucose, we applied our process to some “real” substrates. We used corn residue, which is prevalent in Indiana, and bagasse, which can be found at sugar cane plants in the southern part of the U.S. As seen in Figure 11, pretreated corn residue and bagasse (where hemicellulose had not been previously removed) showed much improved conversions over untreated corn residue and bagasse. Soluble sugars from bagasse and corn residue (Fig. 12) again include some celllobiose and other oligosaccharides. In addition, a small amount of xylitol and arabinoxylose is present due to hemicellulose activity of the enzyme. Similar results are obtained when the hemicellulose is first hydrolyzed by acid and the remaining ligno-cellulose is solvent pretreated.

Conclusion

The process described in this brief overview is one of: 1) hemicellulose hydrolysis, 2) solvent pretreatment, 3) solvent recovery, and 4) cellulose hydrolysis. Solvent caclon, while not necessarily amenable to large-scale use due to its toxic properties, is useful as a model from which applications of other, easier to handle solvents are being developed. Solvent pretreatment appears to be a key factor in utilizing biomass. Solvent pretreatment enhances the conversion of cellulosic materials to fermentable sugars. These raw sugars have great potential as fermentation substrates to produce fuels and chemicals which can supplant part of the U.S. oil imports.

References

(2) G. Tien. Testimony before the 9th Congress, Special Hearing on Alcohol Fuel. 2nd Session (Oct. 21, 1978).

Authors' address: Laboratory of Renewable Resources Engineering, A. A. Putter Engineering Center, Rm. 216, Purdue University, West Lafayette, Ind. 47907.

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