0273-2289/92/3435—0651$02.00

Preliminary Studies on the Processing Sequence for Southern Red Oak and Municipal Solid Waste Using a Hybrid Dilute Acid/Enzymatic Hydrolysis Process for Ethanol Production

ROBERT D. JOHNSON[1] AND MICHAEL H. ELEY*[,2]

[1]Tennessee Valley Authority, Muscle Shoals, AL 36550; and [2]K. E. Johnson Research Center, The University of Alabama in Huntsville, Huntsville, AL 35899

ABSTRACT

Based on this preliminary study, a metric ton of dry southern red oak chips subjected to a first-stage dilute sulfuric acid hydrolysis would yield 132 kg of xylose and 40 kg of glucose and mannose. A second-stage dilute sulfuric acid hydrolysis on the first-stage residue would yield only 128 kg of additional glucose, but a second-stage cellulytic enzyme hydrolysis on the first-stage residue would yield an additional 265 kg of glucose. Fermentation of these hydrolyzates would show that the hybrid process would yield over 50% more ethanol. Results on other biomass are also included.

Index Entries: Woody biomass; cellulose hydrolysis; acid hydrolysis; enzymatic hydrolysis; ethanol.

INTRODUCTION

Woody biomass, such as southern red oak (*Quercus falcata*), is usually a difficult substrate for cellulytic enzymes to degrade because of the relative inaccessibility of the enzyme substrate in the lignocellulosic complex (*1–3*). A variety of pretreatments have been devised to disrupt the lignocellulosic complex of woody biomass (*2–6*). Once the cellulose polymer is

*Author to whom all correspondence and reprint requests should be addressed.

exposed, the cellulytic enzymes can readily hydrolyze most of the polymer to glucose.

Strong mineral acids have often been used both to disrupt the lignocellulosic complex and to hydrolyze the carbohydrate polymers to single sugars (*3,6,7*). The rate of acid hydrolysis is dependent on acid concentration, reaction temperature, and the sugar composition of the polymers (*7*). Unfortunately, under the hot acidic conditions that are optimum for hydrolysis, secondary reactions occur leading to the production of sugar degradation products, such as furfural, hydroxymethylfurfural, and levulinic acid (*8*). Such secondary products both reduce the overall yield of fermentable sugars and in sufficient concentrations act as inhibitors of microbial fermentations (*9*). To avoid or at least minimize secondary acid catalyzed reactions and to increase total fermentable sugar yields, a two-stage dilute acid hydrolysis has been used (*10*). In this dilute acid two-stage process, the first-stage hydrolyzate contains predominantly xylose, and the second-stage hydrolyzate contains mostly glucose. These two hydrolyzates are fermented separately. The first-stage hydrolyzate would be fermented with an organism capable of fermenting both pentoses and hexoses. The second-stage hydrolyzate would undergo classical yeast fermentation (*10*).

Under optimum conditions, dilute sulfuric acid disrupts the lignocellulosic complex of woody biomass, partially solubilizing the lignin component and hydrolyzing most of the hemicellulose, while leaving the cellulose mainly intact. The cellulose is partially hydrolyzed with a second dilute sulfuric acid hydrolysis under somewhat harsher reaction conditions. Attempts to hydrolyze the cellulose in this manner have met varying degrees of success (*11*).

Direct cellulytic enzyme hydrolysis of woody biomass is virtually impossible, and both the glucose yields and the fermentability of the second-stage dilute acid hydrolyzates are potential problems for commercialization of a lignocellulose-to-ethanol process. Previous studies have shown that dilute acid pretreatment of lignocellulosic biomass followed by enzymatic hydrolysis of the solid residue can achieve high yields of glucose for fermentation into ethanol (*3*). The objective of this study is to obtain data on this processing sequence for southern red oak and municipal solid waste, and compare it to results for dilute acid hydrolysis alone.

METHODS

The primary feedstock for this study was southern red oak chips. The chips were approx 2.5 cm long × 2.5 cm wide and 0.5 cm thick. The chips were acid-impregnated by drawing a 1% by weight sulfuric acid solution into an evacuated chamber containing the chips (*9*). Excess acid solution was drained off, and the chips were processed in a masonite gun at 180

psig with saturated steam for 4 min and explosively released. The processed solids were washed with water, and the first-stage hydrolyzate and solid residue were recovered by vacuum filtration. In other experiments, refuse-derived fuel pellets (RDF) replaced the wood chips as the feedstock. For the RDF hydrolysis, a rotating digester was used with 2% by weight sulfuric acid in a 5:1 liquid:solid ratio at 160°C under saturated steam pressure for 15–20 min.

For the second-stage acid hydrolysis, the first-stage residue was soaked in a 2–4% sulfuric acid solution and drained of excess acid. The acid-soaked residue was processed in a masonite gun at 240–320 psig with saturated steam for 2–6 min and explosively released. The processed material was washed with water, and the second-stage dilute acid hydrolyzate and solids were recovered by vacuum filtration. No second-stage acid hydrolysis was performed on first-stage residues of RDF.

For the cellulytic enzyme hydrolysis experiments, three different strains of *Trichoderma reesei* were used. The cultures were obtained from the American Type Culture Collection, and designated as QM 9414, RUT C30, and MCG 77. The cultures were maintained on both slants and plates. For sporulation and subsequent transfers to liquid media, plate cultures were preferred. Spore suspensions were made in sterile distilled water for transfer to liquid media. Liquid cultures were grown in a mineral salts medium of Mandels (*12*), except the urea was omitted from the RUT C30 cultures (*13*). The spore suspensions were initially transferred to 200 mL of liquid medium in 500-mL Erlenmeyer flasks. The flasks were incubated at 28°C for 24 h in an environmental shaker. Four hundred milliliters of liquid shake flask cultures were used as the inoculum for 10 L of liquid medium in 16-L New Brunswick Microgen SF-116 Fermenters. Twenty-five grams per liter of α-cellulose (Sigma Chemical Co.) were added to each fermenter for enzyme induction. Aeration was 1–2 L/min, the agitation speed was 300 rpm, and the temperature was maintained at 28°C. The pH fluctuated during the incubation period, but was generally maintained above pH 3.0 with 2*N* NH_4OH. Foaming was controlled by the addition of Antifoam 1520 (Dow Chemical Co.). The cultures were incubated 9–12 d and then stored at 4°C. The crude culture broth was filter sterilized prior to use in hydrolysis tests.

The first-stage residue was washed with water, and filtered two additional times and with a third wash with the pH adjusted to 4.8 with NaOH. This third filtered residue was autoclaved prior to use in hydrolysis tests. Moisture analyses were performed, and sufficient residue was added to the hydrolysis tests to give the desired solids content.

Sterile wood residues were weighed into 250-mL Erlenmeyer flasks. Twenty-five milliliters of sterile 1*M* citrate buffer (pH 4.8) and 75 mL of the filter-sterilized crude culture broth were added to each flask. The flasks were incubated at 50°C in an environmental shaker for 72 h. Samples were removed at various times during the hydrolysis and analyzed for glucose using a YSI Glucose Analyzer.

For the second-stage acid hydrolysis, the first-stage residue was soaked dues, the residues were dried and weighed to give the desired solids content. The residues were suspended in 0.05*M* citrate buffer (pH 4.8) with 100-g total sample, in 250-mL Erlenmeyer flasks, and autoclaved. Celluclast (0.5 mL) and Novozyme (0.2 mL) (Novo Laboratories) were filter sterilized and added to each flask. Flasks were incubated at 50°C in an environmental shaker for 24 h.

Analytical procedures included moisture analyses using a Dietert Moisture Teller. The cellulose content and potential sugar composition of wood chips and residues were determined spectrophotometrically (*14*). Glucose was also measured using a YSI Model 27 Industrial Glucose Analyzer (Yellow Springs Instrument Co., Inc.). Cellulase activity was measured with filter paper (Whatman No. 1) as described by Mandels (*15*).

RESULTS AND DISCUSSION

Southern red oak chips were subjected to first-stage dilute sulfuric acid hydrolysis. The processed material was washed with water equivalent to four times the dry chip weight and vacuum filtered. The material was washed and filtered three times. The quantities of xylose, glucose, and mannose in the three hydrolyzates were determined. The combined totals for each of the three sugars based on a metric ton of chips were 131 ± 1.2 kg of xylose, 27.6 ± 0.4 kg of glucose, and 13 ± 0.4 kg of mannose. About 33% of the dry chip weight was solubilized by the first-stage dilute acid hydrolysis.

The solid residue from the first-stage dilute acid hydrolysis was analyzed for potential sugars, and found to be composed of 58% glucose, 10% xylose, and 3% mannose. This would correspond to 52.2% cellulose. This residue was used in both the second-stage dilute sulfuric acid hydrolysis and the second-stage cellulytic enzyme hydrolysis. The residue on a dry-weight basis represents 67% of the dry chip weight.

A known quantity of residue based on dry weight was subjected to a second-stage dilute sulfuric acid hydrolysis. The processed material was vacuum filtered to recover the hydrolyzate, and the solids were washed with water equivalent to four times the dry weight of the first-stage residue and vacuum filtered. The solids were washed and filtered twice. The quantity of glucose was measured in each of the three hydrolyzates. Total glucose based on a metric ton of the original chips was 120 ± 26 kg. About 16% of the residue or about 11% of the original dry chips is solubilized in the second-stage dilute acid hydrolysis. The solid residue from the second-stage acid hydrolysis was not analyzed further for carbohydrates, but on a dry-weight basis, the residue represents about 56% of the chip dry weight.

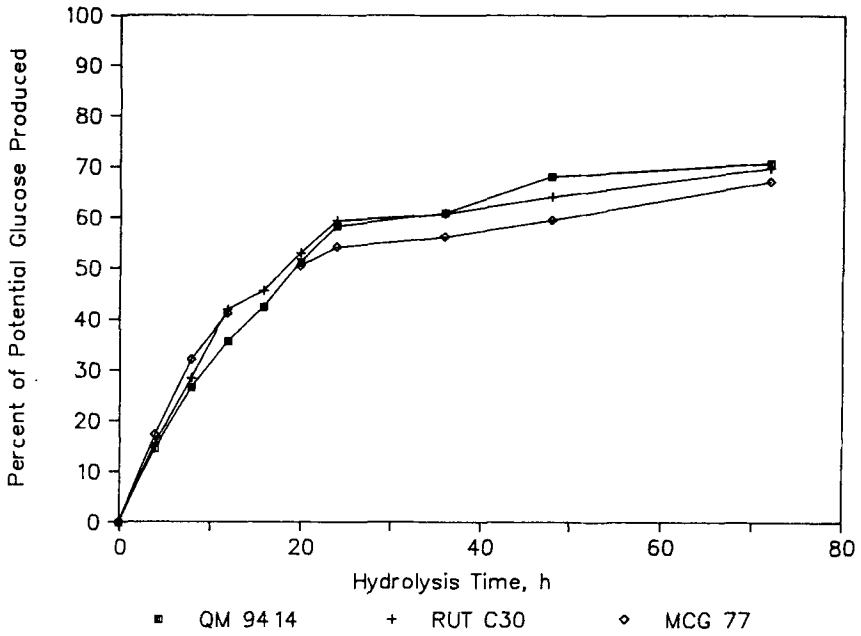

Fig. 1.

A known quantity of the first-stage dilute acid residue was washed, neutralized, and autoclaved. Crude filter-sterilized culture broth and buffer were added, and the mixture was incubated for 72 h with aliquots taken at various times for glucose analyses. Figure 1 shows the results of the enzymatic hydrolysis of first-stage residue by the crude culture broths from all three fungal strains. The results for all three crude enzyme preparations are quite similar at almost all time points and show less than 10% differences after 72 h. This is somewhat surprising, since the filter paper assays of these enzyme preparations indicated that the RUT C30 strain exhibited the lowest activity with 0.33 IEU/mL, the QM 9414 strain exhibited the intermediate activity with 0.44 IEU/mL, and the MCG 77 strain had the highest activity with 0.62 IEU/mL. It must be noted that the values are based on glucose produced and not reducing sugars. With such differences in enzyme activity, one might expect to see a 33% and an 88% faster rate of hydrolysis for the QM 9414 and MCG 77 enzymes, respectively, over the RUT C30 enzyme. Apparently, the type of substrate material has an effect on the hydrolysis rates.

After 72 h of enzymatic hydrolysis, about 68% of the potential glucose was produced, which on the basis of a metric ton of dry chips would yield an additional 270 kg of glucose. The enzymatic hydrolysis solubilizes about 35% of the first-stage residue or about 24% of the original dry chip weight. The solid residue from the second-stage enzymatic hydrolysis was not analyzed for carbohydrate content, but on a dry-weight basis, the residue represents 43% of the chip dry weight.

Table 1
Calculated Average Ethanol Yields from a Dilute Sulfuric Acid Hydrolysis Process and a Hybrid Dilute Sulfuric Acid/Enzymatic Hydrolysis Process*

	First stage acid hydrolysis	Second stage acid hydrolysis	Enzymatic hydrolysis	Total process yield
Dilute acid process	71.7	65.5	—	137.2
Hybrid process	71.7	—	134.9	206.6

*Values are in Kg of ethanol per metric ton of dry chip input.

Based on possible fermentation of the first- and second-stage hydrolyzates to ethanol, the results are summarized in Table 1. The results show that the hybrid acid/enzyme process would yield more than 50% more ethanol than the two-stage dilute acid process. We have also noted that more biomass is solubilized by the hybrid process, leaving less solid residue. It is difficult to make far-reaching conclusions about the merits of such a hybrid process for commercial application to woody biomass based on such limited results, but it does appear that a hybrid process is technically feasible. Although we have also shown that the hybrid process is superior to the two-stage acid process, it should again be noted that the range of conditions for acid hydrolysis used in these test may not be optimal.

For enzymatic hydrolysis to be most effective, the lignocellulosic complex of woody biomass must be disrupted. The pulp and paper industry has demonstrated its ability to accomplish this goal. Paper products, therefore should be much more susceptible to cellulytic enzyme hydrolysis than the wood from which the paper is made. We have been interested for some time in the hydrolysis of paper products, but most particularly in the hydrolysis of paper products in municipal solid wastes (MSW). Separation of these waste paper products from MSW is accomplished commercially, and the end product of such separation is called refuse-derived fuel (RDF). We have obtained samples of pelletized RDF and subjected these pellets to the hybrid acid/enzyme process. In the first-stage dilute sulfuric acid hydrolysis in the rotating digester, we obtained hydrolyzates that on a metric ton basis yielded about 120 kg of glucose under the particular conditions used. The residue was washed, dried, suspended in buffer, and sterilized. The second-stage enzymatic hydrolysis was initiated in this experiment by the addition of commercially available cellulytic enzyme concentrates. After 24 h at 50°C, the hydrolyzate from a 2% suspended solids mixture yielded about 161 kg based on a dry metric ton of residue. Fermentation of the hydrolyzates would yield about 61 kg ethanol/metric ton (18.5 gal/t) from the first-stage dilute acid hydrolyzate and an additional 55 kg ethanol/metric ton of RDF (16.8 gal/t). The combined ethanol yield from the hybrid dilute acid/enzyme process would be about 116 kg/metric t (35.3 gal/t). As was the case with the woody biomass, the

results are based on a limited amount of test data under very specific conditions, and additional research to optimize both the acid and enzymatic steps of the hybrid process on RDF are necessary before arriving at any far-reaching conclusions.

ACKNOWLEDGMENTS

We wish to thank Dr. Richard Strickland, Dr. Mary J. Beck, Chiquita Baker, Dawn Hardy, Larry Softley, and Deborah Tuten for their support and assistance in this study.

REFERENCES

1. Cowling, E. G. (1975), *Biotechnol. & Bioeng. Symposium* **5,** 163–181.
2. Walker, L. P. and Wilson, D. B. (1991), *Bioresource Technology* **36,** 3–14.
3. Grethlein, H. E. and Converse, A. O. (1991), *Bioresource Technology* **36,** 77–82.
4. Millett, M. A., Baker, A. J., and Satter, L. D. (1975), *Biotechnol. & Bioeng. Symp.* **5,** 193–219.
5. Torget, R., Himmel, M., Wright, J. D., and Grohmann, K. (1988), *Applied Biochemistry and Biotechnology* **15,** 89.
6. Torget, R., Werdene, P., Himmel, M., and Grohmann, K. (1990), *Applied Biochemistry and Biotechnology* **24/25,** 115.
7. Goldstein, I. S. (1983), *Wood and Agricultural Residues, Research on Use for Feeds, Fuels and Chemicals,* Saltec, J., ed., Academic, New York, pp. 315–328.
8. Harris, J. F., Baker, A. J., and Conner, A. H. (1985), *Two-Stage Dilute Sulfuric Acid Hydrolysis of Wood, USDA Forest Service Gen. Tech. Report FPL-45,* Forest Products Laboratory, Madison, WI.
9. Beck, M. J. and Strickland, R. C. (1984), *Biomass* **6,** 101–110.
10. Strickland, R. C., Griffith, R. L., Beck, M. J., and Watson, J. R. (1987), *Symposium Papers: Energy from Biomass and Wastes XI.*
11. Wright, J. D. and Power, A. J. (1986), *Comparative Technical Evaluation of Acid Hydrolysis Processes for Conversion of Cellulose to Alcohol.* Presented at the Energy from Biomass and Wastes X Conference, Washington, D.C.
12. Andreotti, R. E., Mandels, M., and Roche, C. (1977), *Proceedings of Bioconversion Symp. I.I.T.,* Delhi, p. 249.
13. Wilke, C. R., Maiorella, B., Sciamanna, A., Tangnu, K., Wiley, D., and Wong, H. (1983), *Enzymatic Hydrolysis of Cellulose: Theory and Applications,* Noyes Data Corp., N.J., pp. 16–20.
14. Scott, R. W. (1976), *Analytical Chemistry* **48,** 13.
15. Mandels, M., Andreotti, R., and Roche, C. (1976), *Biotechnol. & Bioeng. Symposium* **6,** 21–34.