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Comparison of Three Commercial Cellulases for Production of Glucose from Acid-Treated Hardwood

Scientific Note

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INTRODUCTION

Commercial cellulases are sold on a price per weight basis. This experiment was performed to compare the activities of three representative preparations on a weight basis. Three commercial cellulase preparations were compared, with and without added cellobiase, for their ability to hydrolyze sugars remaining in hardwood residue following pretreatment by means of dilute acid hydrolysis. Each cellulase preparation was tested at three enzyme loadings and two substrate concentration levels.

Hydrolysis results were evaluated by determination of potential sugars in the residue before and after hydrolysis, reducing sugar equivalents produced, and identification of specific monosaccharides resulting from hydrolysis of sugar polymers.

MATERIALS AND METHODS

Cellulase Preparations

Cellulase preparations included that provided by Mycotech (a division of Renewable Technologies, Inc., Butte, MT), Celluclast 1.5 L (Novo

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Laboratories, Inc., Danbury, CT), and Genencor 150 L (Genencor, San Francisco, CA). The cellobiase preparation was Novozym 188, supplied by Novo Laboratories, Inc.

Filter Paper Activity

Filter paper activity was determined by the method of the International Union of Pure and Applied Chemistry (*1*). Absorbances were read using a Gilford Response II spectrophotometer.

Substrate Preparation

The substrate was hardwood residue from the first stage of the Tennessee Valley Authority (TVA) dilute acid hydrolysis process. Uniformly sized hardwood chips, predominantly oak, were saturated with 0.75% sulfuric acid, exposed to 180 psig steam for 4 min, and washed to extract hemicellulosic sugars. Twenty-seven percent of the original dry weight of the wood was solubilized. Most of this weight loss was accounted for by the recovery of xylose. Analysis of the residue by a spectrophotometric method (*2*) showed the potential sugars to be 59 g glucose, 4 g xylose, and 0.7 g mannose/100 g dry residue.

The moist residue was pH 3.4. The residue was washed three times with volumes of water equivalent to three times the residue weight. The pH was adjusted to 4.8 in a final wash with the addition of sodium hydroxide. The final moisture content was 65.5%. The residue was distributed to sterile, disposable Erlenmeyer flasks in 5 and 10 g aliquots, based on dry weight.

Hydrolysis

The Mycotech preparation was a solid material, and the Celluclast 1.5 L, Genencor 150 L, and Novozym 188 preparations were liquids. The following procedure was followed to compare the solid and liquids. The appropriate amount of cellulase preparation was weighed into a volumetric flask and made to a 100-mL volume with 0.05*M* citrate buffer, pH 4.8. When cellulase preparations were supplemented with Novozym 188, it was weighed separately and added to the cellulase preparation before the combination was made to volume. All enzyme plus buffer solutions were filter sterilized. The 100-mL enzyme solutions were added to either 5 or 10 g of residue on a dry basis. The moisture contained in the residue added additional water to the combination, which was accounted for in potential sugar calculations.

Cellulase doses were 0.25, 0.50, and 0.75 g cellulase/5 g dry residue and 0.50, 1.00, and 1.50 g cellulase/10 g dry residue. When Novozym 188 was added, the corresponding amounts for the 0.25, 0.50, and 0.75 g cellulase preparations were 0.05, 0.10, and 0.15 g Novozym 188, respectively, and 0.10, 0.20, and 0.30 g Novozym 188, respectively, for the correspond-

ing 0.5, 1.00, and 1.50 g cellulase preparations (Table 1). Novo's recommendation of a ratio of 1:0.2 of Celluclast 1.5 L to Novozym 188 for initial industrial trials was the basis for the ratio of cellulase to cellobiase for all three cellulase preparations.

Controls consisted of residue to which 100 mL sterile buffer was added. The test flasks were incubated at 50°C and 100 rpm in a rotary shaker. Samples of 2-mL vol were taken at 6, 24, 48, and 96 h.

Analytical

Reducing sugar concentrations were determined by a modification of the dinitrosalicylate method of Miller (3). Glucose standards ranged to a concentration of 30 mg/mL. A Yellow Springs Instrument (YSI, Yellow Springs, OH) was used to determine glucose. Glucose, xylose, and mannose contents of wood residues before and after enzymatic hydrolysis were also determined by acid digestion, followed by a spectrophotometric determination (2). Glucose and xylose contents of hydrolysates were determined by high performance liquid chromatographic techniques (4).

RESULTS AND DISCUSSION

Filter Paper Activity

Mycotech reported filter paper unit activities (FPU) of 140 FPU for Mycotech, 144 FPU for Celluclast 1.5 L, and 218 FPU for Genencor 150 L as determined by the method of Montenecourt (5).

In this laboratory, filter paper activity per gram was determined to be 168 for Mycotech cellulase, 247 for Celluclast 1.5 L, and 285 for Genencor 150 L. The results are similar to those provided by Mycotech although different methods were used for the determinations.

Comparison of Cellulases for Recovery of Glucose from First-Stage Residue from the TVA Dilute Acid Hydrolysis Process

Typical time courses of reducing sugar recovery from the wood residue were observed (data not shown). Tables 2 and 3 contain results after 96 h of incubation.

The addition of Novozym 188 improved the recovery of reducing sugar for all three cellulase preparations. However, significant amounts of reducing sugar were recovered without the addition of cellobiase.

The percentage of the reducing sugar identified as glucose, using the YSI determination, at 96 h of hydrolysis was calculated for 10% residue hydrolysis tests (Table 3). The average percentages at the three dose levels of cellulase without added Novozym 188 were 49, 55, and 73% for Mycotech cellulase, Celluclast 1.5 L, and Genencor 150 L, respectively.

Table 1
Experimental Data

Test Number	Enzyme	g Cellulase: g residue	FPU: g residue
1	Mycotech	0.25:5	8.4:1
2	"	0.50:5	16.8:1
3	"	0.75:5	25.2:1
4	"	0.50:10	8.4:1
5	"	1.00:10	16.8:1
6	"	1.50:10	25.2:1

Test Number	Enzyme	g cellulase:g cellobiase:g residue
7	Mycotech/Novozym	0.25:0.05:5
8	"	0.50:0.10:5
9	"	0.75:0.15:5
10	"	0.50:0.10:10
11	"	1.00:0.20:10
12	"	1.50:0.30:10

Test Number	Enzyme	g Cellulase: g residue	FPU: g residue
13	Celluclast	0.25:5	12.4:1
14	"	0.50:5	24.7:1
15	"	0.75:5	37.1:1
16	"	0.50:10	12.4:1
17	"	1.00:10	24.7:1
18	"	1.50:10	37.1:1

Test Number	Enzyme	g cellulase:g cellobiase:g residue
19	Celluclast/Novozym	0.25:0.05:5
20	"	0.50:0.10:5
21	"	0.75:0.15:5
22	"	0.50:0.10:10
23	"	1.00:0.20:10
24	"	1.50:0.30:10

Test Number	Enzyme	g Cellulase: g residue	FPU: g residue
25	Genencor	0.25:5	14.3:1
26	"	0.50:5	28.5:1
27	"	0.75:5	42.8:1
28	"	0.50:10	14.3:1
29	"	1.00:10	28.5:1
30	"	1.50:10	42.8:1

Test Number	Enzyme	g cellulase:g cellobiase:g residue
31	Genencor/Novozym	0.25:0.05:5
32	"	0.50:0.10:5
33	"	0.75:0.15:5
34	"	0.50:0.10:10
35	"	1.00:0.20:10
36	"	1.50:0.30:10

Table 2
Yield Data, 96 h

Test Number	Reducing Sugar (g/L)	HPLC (g/L) Glucose	HPLC (g/L) Xylose	Glucose (g/L) by YSI
1	6.4	2.3	0.3	–
2	9.5	4.4	0.4	–
3	11.3	5.3	0.5	–
4	10.0	3.9	0.6	4.4
5	15.9	7.5	0.8	8.1
6	18.9	9.5	1.2	10.1
7	7.8	5.5	0.1	–
8	11.4	7.6	0.2	–
9	16.5	12.7	0.6	–
10	17.3	12.7	1.0	13.1
11	26.8	20.9	1.4	20.3
12	27.9	22.0	1.6	21.1
13	5.4	3.0	0.2	–
14	10.1	6.2	0.1	–
15	13.0	8.5	0.3	–
16	12.3	6.6	0.5	6.7
17	19.7	11.2	0.8	11.3
18	22.1	14.3	1.9	11.9
19	11.9	9.6	0.4	–
20	16.3	13.3	0.7	–
21	17.8	14.3	0.5	–
22	19.8	15.4	0.8	14.6
23	26.9	22.2	2.3	21.0
24	29.2	24.5	3.3	24.8
25	7.9	5.9	0.1	–
26	12.3	9.8	0.3	–
27	14.2	10.9	0.8	–
28	15.4	11.5	1.0	10.3
29	25.1	20.4	1.1	19.1
30	24.7	20.4	1.5	18.5
31	10.2	8.1	1.2	–
32	14.3	11.2	0.8	–
33	16.7	13.8	1.0	–
34	21.6	17.8	0.8	16.4
35	27.2	23.4	1.3	21.0
36	31.5	28.0	2.2	25.0

Table 3
Hydrolysis Results, 96 h

Test Number	g reducing sugar per g residue	g reducing sugar per g potential sugar	g Cellulase per g reducing sugar	FPU per g reducing sugar	% of reducing sugar identified as glucose by YSI	% of Reducing sugar identified as glucose by HPLC
1	.13	0.21	.36	60.5	-	36
2	.20	0.31	.48	80.6	-	46
3	.24	0.37	.61	102.5	-	47
4	.12	0.19	.42	70.6	44	39
5	.19	0.30	.53	89.0	51	47
6	.22	0.35	.66	110.9	53	50
7	.16	0.25	.36	60.5	-	71
8	.24	0.37	.49	82.3	-	67
9	.34	0.54	.51	85.7	-	77
10	.20	0.32	.29	48.7	76	73
11	.32	0.50	.38	63.8	76	78
12	.33	0.52	.54	90.7	76	79
13	.11	0.18	.42	103.7	-	56
14	.21	0.33	.46	113.6	-	61
15	.27	0.42	.53	130.9	-	65
16	.15	0.23	.34	84.0	54	54
17	.24	0.37	.43	106.2	57	57
18	.26	0.41	.57	140.8	54	65
19	.25	0.39	.23	56.8	-	81
20	.34	0.53	.34	84.0	-	82
21	.37	0.58	.46	113.6	-	80
22	.24	0.37	.25	61.8	74	78
23	.32	0.50	.31	76.6	78	83
24	.35	0.55	.43	106.2	85	84
25	.17	0.26	.29	82.7	-	75
26	.25	0.40	.38	108.3	-	80
27	.29	0.46	.49	139.7	-	77
28	.18	0.29	.27	77.0	67	75
29	.30	0.47	.34	96.9	76	81
30	.29	0.46	.51	145.4	75	83
31	.21	0.33	.27	77.0	-	79
32	.30	0.47	.39	111.2	-	78
33	.35	0.55	.50	142.5	-	83
34	.25	0.40	.24	67.4	76	82
35	.32	0.51	.37	105.5	77	86
36	.38	0.59	.48	136.8	79	89

The average percentages where each cellulase preparation was applied with added Novozym 188 were 75, 79, and 77% for Mycotech cellulase, Celluclast 1.5 L, and Genencor 150 L, respectively.

The percentages that glucose, as determined by HPLC, represents of the reducing sugar recovered at all three loadings with and without added Novozym 188 were calculated. When no additional cellobiase was added, 36–50% of Mycotech cellulase-derived reducing sugar was accounted for as glucose, 56–65% of Cellulast-derived reducing sugar was accounted for as glucose, and 75–83% of Genencor-derived reducing sugar was accounted for as glucose. The percentages that glucose comprises of the reducing sugar with added Novozym 188 are similar for the three cellulase preparations. Yield data (Table 2) show that the discrepancy is not owing to xylose. It is not known what reducing substance may account for the differences.

The recovery of reducing sugar improved with increased enzyme loading for all three cellulase preparations (Table 3). This was the case with and without added Novozym 188. The greatest ratio of reducing sugar recovered to potential sugar (0.59) was achieved with Genencor 150 L, using 10% residue supplemented with Novozym 188 at the highest enzyme loading. Similar results were achieved with 5% residue using Mycotech (0.54) and Celluclast 1.5 L (0.58) cellulase preparations supplemented with Novozym 188 and applied at the highest enzyme loading rates.

The ratio of cellulase, at 96 h, to reducing sugar recovered, with or without supplementation with Novozym 188, increased as the amount of cellulase loading increased. The smallest ratios of cellulase to sugar were achieved with 5% residue using Celluclast 1.5 L plus Novozym 188 (0.23), 5% residue using Genencor 150 L plus Novozym 188 (0.27), and 10% residue using Genencor 150 L plus Novozym 188 (0.24).

Reciprocal plots were made of reducing sugar recovered per unit of dry residue vs cellulase used per unit of dry residue (data not shown). This allowed the determination of the maximum reducing sugar from dry residue at infinite enzyme loadings.

The plots showed that the maximum reducing sugar that can be recovered at infinite loading ranged from 0.37 to 0.50 g/g dry residue. Without the addition of Novozym 188, the average value of the range was 0.41 g reducing sugar/g dry residue. This means that a recovery efficiency of 64% could be achieved at maximum cellulase loading, using the conditions of this test series. With added Novozym 188, the average recovery of reducing sugar per unit residue was 0.44 g/g or an efficiency of 69% at maximum cellulase loading.

Great differences exist in the actual amount of reducing sugar recovered at 96 h, using the highest cellulase loadings with and without Novozym 188 supplementation, and in the maximum potential for recovery of reducing sugar, as determined by plots for all three cellulase preparations (Table 3).

A number of explanations have been offered for the differences between actual recoveries from lignocellulosics and those that are possible.

Hydrolysis can be inhibited by factors, such as surface interferences (the size of lignocellulosic substrate was not reduced in this experiment, which made use of mild acid hydrolysis as the only pretreatment) and the presence of lignin and xylan, as well as product inhibition. Such factors may have contributed to the experimental results observed. Apparently, all three cellulase preparations were equally susceptible to these factors.

The experimental design did not include tests that would allow the study of the effects of substrate size on the activity of the cellulases. It is noteworthy that the experiment was originally designed for the testing of 15 and 7.5% residues. It was determined by trial that 15% residue in buffer could not be mixed using the rotary shaker in the experimental design. Thus, 5 and 10% residue concentrations were tested. If tests were to have included substrate concentrations much greater than 10% dry weight of residue, another means of agitation would have had to be found. Optimizing factors, such as particle size, ionic strength and composition, partial or total lignin removal, complete xylan removal, substrate concentration, and additional pretreatments, could benefit reducing sugar recovery from acid-treated wood residue.

The greatest sugar concentration achieved in this study was 31.5 g/L. This represents a yield of 0.59 g reducing sugar/g potential sugar in 96 h.

REFERENCES

1. Ghose, T. K. (1987), *Pure Appl. Chem.* **59,** 257.
2. Scott, R. W. (1976), *Anal. Chem.* **48,** 1919.
3. Miller, G. L. (1959), *Anal. Chem.* **31,** 426.
4. Beck, M. J. and Strickland, R. C. (1984), *Biomass* **6,** 101.
5. Montenecourt, B. S., Eveleigh, D. E., Elmund, G. K., and Parcells, J. (1978), *Biotechnol. Bioeng.* **20,** 97.