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SIMULTANEOUS SACCHARIFICATION AND FERMENTATION OF SEVERAL LIGNOCELLULOSIC FEEDSTOCKS TO FUEL ETHANOL

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Abstract—When ethanol fuel is produced from lignocellulosic materials such as wood, herbaceous plants, and agricultural and forestry wastes, its use as a transportation fuel reduces dependence on imported petroleum, decreases the balance of trade deficit, improves urban air quality, contributes no net carbon dioxide to the atmosphere, and provides new markets for depressed farm economies. The simultaneous saccharification and fermentation (SSF) process is a favored option for conversion of the lignocellulosic biomass into ethanol because it provides enhanced rates, yields, and concentrations of ethanol with less capital investment compared to competing processes. In this study, the performance of four woody crops (aspen, two hybrids of populus, and a native strain of sweetgum), three herbaceous crops (switchgrass, weeping love grass, and Sericea *lespedeza*), and three agricultural residues (corn cobs, corn stover, and wheat straw) is reported for the SSF process. For the pretreatment conditions employed for these feedstocks, excellent results were obtained for corn cobs followed by corn stover, wheat straw, weeping love grass, the woody crops, and switchgrass. Only the legume *S. lespedeza* did not give good ethanol yields for the pretreatment conditions chosen.

Keywords—Simultaneous saccharification and fermentation, lignocellolosic biomass, ethanol, woody crops, herbaceous crops, agricultural residues.

1. INTRODUCTION

Although ethanol is now produced from sugar and starch crops, it can also be made from lignocellulosic biomass such as trees, herbaceous plants, municipal solid waste, and agricultural and forestry residues; enough ethanol could be made from these abundant domestic feedstocks to replace all gasoline used in the United States. When made from such materials, ethanol is a clean-burning fuel that can address increasing concerns about urban air pollution, global climate change, energy security, and international trade while creating new agricultural markets. Because these feedstocks have no competing uses for food and can be produced inexpensively, they are low in cost. Therefore, the price of ethanol produced from lignocellulosic materials has the potential to be competitive with petroleum.

Three enzyme-catalyzed processes have been emphasized for the conversion of lignocellulosic biomass into ethanol: separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), and direct microbial conversion (DMC). Through process evaluations, the SSF process, in which lignocellulosic biomass, hydrolysis enzymes, and fermentative microorganism are added to one vessel, has been selected for emphasis.[1–3] The SSF process is depicted in Fig. 1.

The SSF process was originally developed for lignocellulosic biomass by researchers at Gulf Oil Company in 1974.[4–6] The key to the SSF process is its ability to rapidly convert sugars into ethanol, reducing their buildup in the fermentation broth. Because sugars are much more inhibitory to the conversion process than ethanol is,[7,8] the SSF process can achieve greater rates, yields, and concentrations than competing processes now known. The SSF process also eliminates expensive equipment and reduces the probability of contamination by unwanted organisms that are less ethanol tolerant than the microbes selected for fermentation. Over the years, various groups have worked on the SSF process to improve the choice of enzymes, fermentative microbes, biomass pretreatment, and process conditions.[9–20]

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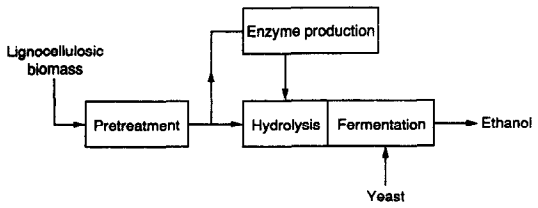

Fig. 1. The simultaneous saccharification and fermentation (SSF) process combines cellulose hydrolysis to glucose and fermentation of glucose to ethanol in one vessel.

In this paper, a summary will be provided of the evaluations of several potential feedstocks for ethanol production via the SSF process. Because only selected data can be presented here, particular emphasis will be placed on the variation in SSF performance with substrate, fermentative microbe, and enzyme augmentation. It will be shown that a wide range of feedstocks is suitable for ethanol production. Recommendations will then be made for future research to achieve economic goals.

2. MATERIALS AND METHODS

2.1. *Materials*

Four woody crops were employed in this study: *Populus maximowiczii* × *nigra* (Hybrid NE388), *Populus trichocarpa* × *deltoides* (Hybrid N11), *tremuloides* (aspen), and a native strain of sweetgum (*Liquidambar styraciflua*). The herbaceous crops for this study were weeping love grass (*Eragrotis curvula*), Sericea lespedeza (*Lespedeza cuneata*), and switchgrass (*Panicum virgatum*). *P. maximowiczii*, *P. trichocarpa*, *L. styraciflua*, and the herbaceous crops were supplied to us through the coordination of the Biomass Production Program managed by Oak Ridge National Laboratory (ORNL), Oak Ridge, Tennessee for the U.S. Department of Energy (DOE). The corn residues used in this study were harvested from the eastern Colorado slope in the fall of 1984 and selected for absence of rot. Wheat straw was also obtained locally. The fermentation yeast used were *Sacchromyces cerevisiae* (D_5A), a National Renewable Energy Laboratory (NREL) strain genetically derived from Red Star brewers yeast, and *Brettanomyces clausenii* Y-1414 obtained from the Northern Regional Research Laboratory (NRRL), U.S. Department of Agriculture, Peoria, Illinois. Chemicals were purchased from the Sigma Chemical Company, St Louis, Missouri, and yeast extract and peptone growth media were ordered from Difco, Detroit, Michigan. Cellulase enzyme came from Genencor Inc., San Francisco, California, and β-glucosidase (Novozyme-188) from NOVO Laboratories, Inc., Wilton, Connecticut. Shaker flask, 250 ml Pyrex graduated vessels, and Braun Biostat V fermentation vessels were used for the fermentations.

2.2. *Methods*

Shaker flask SSFs were carried out in 250 ml flasks outfitted with stoppers constructed to vent CO_2 through a water trap. These flasks contained 100 ml of fermentation broth and were agitated at 150 rpm in a shaker incubator at 37°C. A 1% yeast extract and 2% peptone (w/v) media (YP) were used with a substrate loading of 7.5% (w/v) lignocellulose. A lipid mixture of ergosterol (5 mg l^{-1}) and oleic acid (30 mg l^{-1}) was added to the media for improved ethanol yield.[21] Also, penicillin and streptomycin at 10 mg l^{-1} were used to minimize bacterial contamination. The inocula were grown in a shaker flask with YP media and 2% (w/v) glucose at 37°C, and 1/10 (v/v) yeast culture to total volume of media was added to the fermentation. The substrate was autoclaved in fermentation flasks, and sterile media, lipids, antibiotics, and enzyme were added before the inoculum.

Ethanol concentrations in the supernatant were measured by gas chromatography using a Porapak Q80/100 column. The internal standard was 4% isopropanol. Larger scale 3 l SSFs were also run at selected conditions. For these experiments, residual sugars (glucose and cellobiose) were determined as glucose by incubation of the sample with 2 mg ml^{-1} almond extract β-glucosidase from Sigma for 1 h at 37°C, and total sugars were measured on the model 27 glucose analyzer from Yellow Springs Instruments, Yellow Springs, Ohio. Viable cell densities were measured as colony forming units (CFU) by plating serial dilutions on YP medium supplemented with either glucose (YPD) or cellobiose (YPC).

Cellulase enzyme loadings of 7, 13, 19, and 26 IU g^{-1} lignocellulose were used in the shaker flask screening experiments to span the range of activity previously shown to be important for SSFs. In this paper, IU stands for international units of filter paper activity in μmol glucose min^{-1}.[22] β-glucosidase enzyme was reported in this study at ratios of 1 and 8 parts to 1 part of cellulase as measured by IU of β-glucosidase per IU of cellulase. The β-glucosidase activity

was determined by *p*-nitrophenyl-β-glucoside (*p*Npg) assays at a temperature of 37°C because this is the temperature for the SSFs. The activity of cellulase increases as temperatures are increased to an optimum at 45°C,[17,18] the temperature selected for saccharification without fermentation studies. The IUPAC revisions of measured cellulase activities indicate that the level of β-glucosidase in an enzyme preparation may affect the results of the cellulase assay in filter paper units.[22]

Woody crops were completely debarked. Two 500 mg batches of Wiley-milled (2 mm screen) wood, herbaceous plants, or crop residues were pretreated with dilute sulfuric acid (0.45–0.50% v/v) in a 2-gal Parr Reactor. All materials were pretreated at about 140°C for 1 h with stirring at 185 rpm. After reaction, the slurries were washed several times with hot water in a large Buchner funnel lined with a linen sheet to bring the pH of 1.3 up to 4.5. These batches were combined, immediately placed in freezer storage bags, and stored at −20°C. Approximately 70% of the pretreated woods' dry weight was cellulose, 29% was lignin and acid-insoluble ash, and 1% was xylan. Approximately 58% of the pretreated grasses' dry weight was cellulose, 40% was lignin and acid-insoluble ash, and 2% was xylan. The pretreated legume gave a lower dry weight of cellulose at 45%, with 51% lignin and acid-insoluble ash, and 2.5% xylan.

Shaker flask results are reported as a percentage of maximum theoretical ethanol yields and do not account for the substrate used in cell growth. Thus, the maximum expected ethanol yield is about 95%, assuming that about 5% of the substrate is needed for cell growth. These calculations are based on the measured ethanol concentrations and a 56.7% theoretical ethanol yield for conversion of cellulose to ethanol only. However, the saccharifications of cellulose are reported on the basis of the percentage of the maximum amount of sugars possible. Thus, comparison of the SSF and straight saccharification results must consider that subsequent fermentation of the sugars produced in the latter will also result in about a 5% loss to cell growth and maintenance.

A method was developed to estimate the residual cellulose by poisoning the yeast cells with NaF and allowing the excess enzyme to complete the saccharification from a given time of SSF. Background measurements of glucose were subtracted, which stem from the β-glucosidase enzyme itself and from some residual glucose not taken up by the cells at the time of the sample. At lower enzyme levels, additional cellulase and β-glucosidase were added to complete the saccharification.

The straight saccharification yields are calculated as the amount of glucose produced compared to the potential glucose in the cellulose feed. The substrate level was limited to 7.5% cellulose for a small- and large-scale SSFs because mixing problems were encountered at higher cellulose levels during SSF evaluations performed with *S. cerevisiae* and pretreated wheat straw at higher substrate levels.[18]

3. RESULTS

Supplementation of the Genencor 150L enzyme (batch II) used in this study with β-glucosidase was used to increase ethanol yields and saccharification rates to values observed with a previous batch of this enzyme. Previous work indicated an approximately 35% reduction in pNpg activity g^{-1} of protein for batch II when compared with batch I.[17,18] Without β-glucosidase supplementation, a decrease of approximately 12% in ethanol production was observed.

Table 1 illustrates the final saccharification yields at 45°C for all woody and herbaceous crops studied plus corn cobs, corn stover, and wheat straw at selected cellulase loadings and supplementation with β-glucosidase. For woody crops, the best overall rates of hydrolysis and final conversions to simple sugars were observed for *P. maximowiczii* and *L. styraciflua*. The highest enzyme loadings of 26 IU cellulase with an 8:1 ratio of β-glucosidase achieved a maximum cellulose conversion of 82% with *P. maximowiczii*. The saccharifications took up to 8 days to achieve the highest yields.

For herbaceous crops, the best overall rates of hydrolysis and final conversions to simple sugars were observed for weeping love grass and switchgrass. The highest enzyme loading of 26 IU cellulase g^{-1} cellulose, with an 8:1 ratio of β-glucosidase, was necessary to achieve 70–73% conversion for these grasses, and the saccharifications took 5 days to achieve these yields. Only 39% of the cellulose remaining in the pretreated legume, *S. lespedeza*, was saccharified under these conditions. It has been reported[23] that the legume requires harsher conditions of pretreatment (180°C for 20 min using 0.5% H_2SO_4 v/v) to achieve the high conversion yields

Table 1. Summary of final (8-day) saccharification yields for acid pretreated woody and herbaceous crops with selected cellulase and β-glucosidase loadings at 45°C

IU β-glucosidase:IU	0:1				8:1			
IU Cellulose/g lignocellulose	7	13	19	26	7	13	19	26
Woody crops								
Populus *maximowiczii* × *nigra**	24	48	66	72	23	50	82	82
Populus *trichocarpa* × *deltoides*	14	28	40	47	14	31	35	65
Populus *tremuloides*	17	30	48	65	16	30	43	64
Sweetgum *Liquidambar styraciflua**	32	46	53	66	34	48	68	74
Herbaceous crops								
Weeping love grass*	30	47	60	69	47	60	68	73
Switchgrass*	32	45	53	61	47	56	64	70
Sericea lespedeza	8	12	15	19	9	17	24	39
Corn cob*	55	64	78	86	69	83	90	90
Corn stover	48	64	77	84	64	80	86	89
Wheat straw*	34	48	—	64	60	73	—	77

*Saccharification yields are expressed in percentage of theoretical conversion.

(approximately 70%) observed with the other two grasses.

Table 2 compares the final (7–8 day) ethanol yields for SSF with different substrates at selected enzyme loadings and yeast combinations run in 100 ml shaker flasks. The rates and yields are consistently higher for *P. maximowiczii* compared to the other woody crops with *S. cerevisiae* as the fermenting yeast at all enzyme loadings. However, the mixed culture generally achieves the best overall rates and highest yields for *L. styraciflua* at the lower enzyme loadings without β-glucosidase supplementation. With β-glucosidase supplementation, *P. tremuloides* gives the best results with the mixed culture at lower cellulase loadings while *P. maximowiczii* performs best with greater cellulase use. Lower rates and yields result with *P. trichocarpa* than with other substrates.

Table 2 also compares the final ethanol yields for SSF with herbaceous substrates at selected enzyme loadings and yeast combinations run in 250 ml flasks. Just as for the straight saccharification, the rates and yields are consistently higher for weeping love grass and switchgrass

Table 2. Summary of final yields in SSFs for pretreated ligocellulosic biomass run at 37°C for selected cellulase and β-glucosidase loadings

IU β-glucosidase:IU	0:1				8:1			
IU Cellulase/g lignocellulose	7	13	19	26	7	13	19	26
Yeast	*S. cerevisiae*							
Woody crops								
Populus maximowiczii × *nigra**	42	61	75	80	79	86	88	90
Populus *trichocarpa* × *deltoides*	39	47	55	62	56	61	77	82
Populus *tremuloides* (aspen)	34	51	66	76	69	81	83	84
Sweetgum *Liquidambar styraciflua*	41	61	70	77	77	83	86	86
Herbaceous crops								
Weeping love grass	47	57	64	75	63	71	85	89
Switchgrass	41	52	59	61	56	70	81	84
Sericea lespedeza	18	22	27	30	28	33	44	52
Corn cob	58	63	80	87	87	91	94	94
Corn stover	54	59	77	84	82	86	90	92
Wheat straw	45	57	—	76	67	77	—	90
Yeast	Mixed Culture*							
Woody crops								
Populus *maximowiczii* × *nigra*	53	67	74	77	65	84	86	86
Populus *trichocarpa* × *deltoides*	39	43	47	52	52	66	73	80
Populus *tremuloides* (aspen)	63	66	74	76	73	78	79	79
Sweetgum *Liquidambar styraciflua*	68	72	77	78	67	79	82	84
Herbaceous crops								
Weeping love grass	62	65	70	77	71	85	88	86
Switchgrass	49	57	63	67	62	73	83	87
Sericea lespedeza	25	28	33	33	29	41	49	50
Corn cob	76	85	89	92	92	93	96	96
Corn Stover	75	84	87	89	86	89	92	92
Wheat Straw	66	73	—	76	79	83	—	85

*Mixed culture of *Brettanomyces clausenii* and *Saccharomyces cerevisiae*.

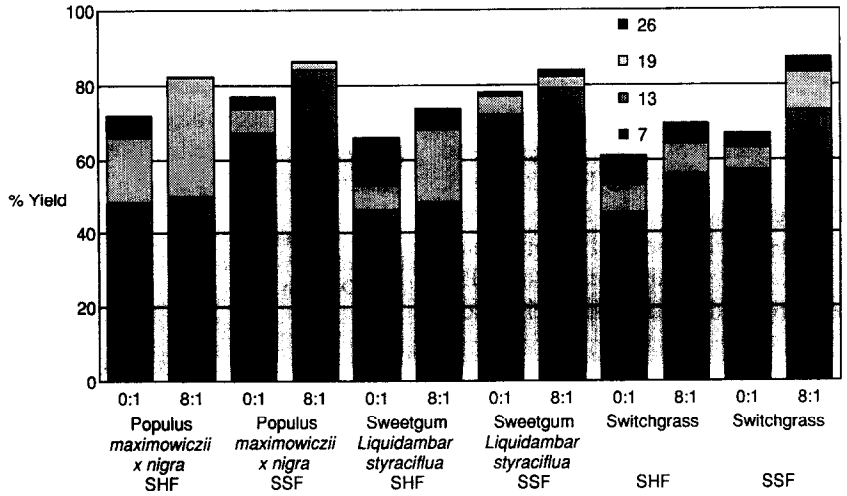

Fig. 2. Comparisons of final yields as a percentage of the theoretical maximum product potential for saccharification only (SHF) and SSF of selected hardwoods and herbaceous crops. Cellulase loadings of 7, 13, 19, and 26 IU g^{-1} cellulose are employed without (0:1) and with (8:1) β-glucosidase supplementation.

than for the legume, *S. lespedeza*, for all enzyme levels. It is also apparent that higher yields are observed for weeping love grass and generally for switchgrass than for any of the woody crops at lower cellulase loadings without β-glucosidase supplementation. The fermentation with the mixed culture particularly excels at low cellulase usage without β-glucosidase additions.

Similar trends are observed for corn cobs, corn stover, and wheat straw. However, it is evident that the yields for corn cobs are the best of all of the substrates evaluated, followed closely by corn stover. With high cellulase loadings and β-glucosidase supplementation, yields of up to 96% are realized with corn cobs and stover. However, even at low cellulase levels of 7 IU g^{-1} without additional β-glucosidase, yields of about 75% are achieved with the mixed culture while values of 54–58% are attained with *S. cerevisiae* alone. The performance for wheat straw was generally slightly better than or equivalent to the herbaceous crops but not as good as for corn cobs or stover throughout.

All of the substrates tested reached the highest conversions of cellulose to ethanol with *S. cerevisiae* at higher cellulase enzyme loadings with β-glucosidase added. The mixed culture of *S. cerevisiae* and *B. clausenii* was slightly less sensitive to β-glucosidase supplementation. This observation can be explained by production of β-glucosidase during SSFs by the cellobiose-fermenting yeast *B. clausenii* while

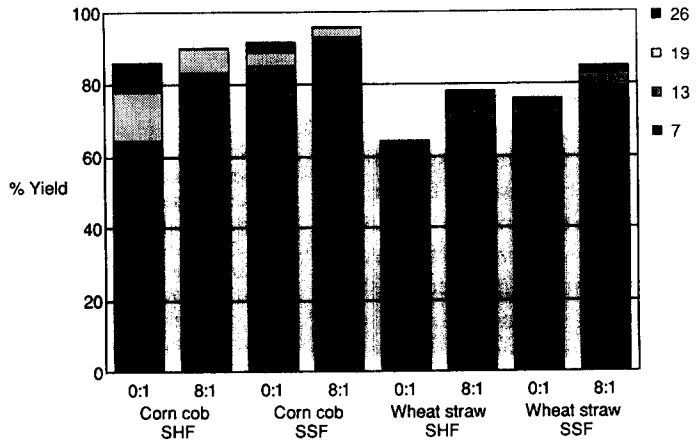

Fig. 3. Comparison of final yields as a percentage of the theoretical maximum product potential for saccharification only (SHF) and SSF of corn cobs and wheat straw. Cellulose loadings of 7, 13, 19, and 26 IU g^{-1} cellulose are used without (0:1) and with (8:1) β-glucosidase supplementation.

S. cerevisiae alone can achieve similar results with β-glucosidase supplementation.

It is apparent that the SSF process gives higher yields than straight saccharification if we compare the final yields of both Tables 1 and 2. This comparison is shown in Figs 2 and 3. The increased yield is because of the low residual sugars present in the SSF from yeast fermentation that relieve inhibition of the cellulase enzyme experienced in straight saccharification.

4. CONCLUSIONS

From our data, we can conclude that corn cobs, corn stover, wheat straw, and weeping love grass respond well, in descending order, to dilute acid pretreatment; achieve fast enzymatic hydrolysis rates; and give high ethanol yields by the SSF process. Switchgrass performed somewhat more poorly. All the pretreated woody crops reached similar final conversions of cellulose to ethanol in the SSF process, but the final yields and rates of hydrolysis for *P. maximowiczii* were the highest for *S. cerevisiae* with or without β-glucosidase supplementation. However, the mixed culture of *S. cerevisiae* and *B. clausenii* generally achieved higher final yields at lower cellulase loadings without β-glucosidase supplementation. Only the legume *S. lespedeza* did not respond as well to the dilute acid pretreatment conditions used for the other two grasses, and the conversion of cellulose to sugars or ethanol was much less for this substrate. However, it may be possible to increase the yields by alteration of the pretreatment conditions. On the other hand, corn cobs and stover exhibited the best performance in the SSF process of any substrate we have evaluated.

Without β-glucosidase supplementation at low cellulase loadings, the overall rates and yields were generally better for the mixed culture than for *S. cerevisiae* because of the additional β-glucosidase activity produced by *B. clausenii*. However, *S. cerevisiae* performed about the same with substantial β-glucosidase addition and/or high cellulase loadings. These results point out the benefit of providing proper β-glucosidase levels to prevent accumulation of the powerful inhibitor cellobiose. Thus, the use of a mixed culture of a cellobiose-fermenting organism with an ethanol-tolerant strain or supplementation of the enzyme broth with β-glucosidase will continue to be desirable to obtain high yields until a cellulase enzyme that has higher β-glucosidase activity or is less inhibited by cellobiose is developed or a more ethanol-tolerant cellobiose-fermenting microorganism is found.

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REFERENCES

1. J. D. Wright, Ethanol from lignocellulose: an overview. *Energy Prog.* **8**(2), 71 (1988).
2. J. D. Wright, Ethanol from biomass of enzymatic hydrolysis. *Chem. Eng. Prog.* **62,** August (1988).
3. J. D. Wright, C. E. Wyman and K. Grohmann, Simultaneous saccharification and fermentation of lignocellulose: process evaluation. *App. Biochem. Biotechnol.* **18,** 75 (1988).
4. W. F. Gauss, S. Suzuki and M. Takagi, Manufacture of alcohol from cellulosic materials using plural ferments. *U.S. Pat. No.* 3,990,944, November 9, 1976.
5. M. Takagi, S. Abe, S. Suzuki, G. H. Emert and N. Yata, A method for production of alcohol directly from cellulose using cellulase and yeast. *Proc. Bioconversion Symp.* ITT, Delhi, India, 551 (1977).
6. P. J. Blotkamp, M. Takagi, M. S. Pemberton and G. H. Enert, Enzymatic hydrolysis of cellulose and simultaneous saccharification to alcohol. *AIChE Symp. Ser.* **74,** 85 (1978).
7. M. Holtzapple, Inhibition of *Trichoderma reesei* cellulase by sugars and solvents, American Institute of Chemical Engineers 1989, Annual Meeting, San Francisco, CA, November 8, 1989.
8. M. Takagi, Inhibition of cellulase by fermentation products. *Biotechnol. Bioeng.* **24,** 1506 (1984).
9. S. M. Lastick, D. D. Spindler and K. Grohmann, Fermentation of soluble cellooligosaccharides by yeasts. *Wood Agric. Residues* (E. J. Soltes, Ed.), 239 pp. (1983).
10. S. M. Lastick, D. D. Spindler, S. Terrel and K. Grohmann, Simultaneous saccharification and fermentation of cellulose. *Biotechnology* **84,** 277 (1984).
11. J. Szczodrak, The use of cellulases from a β-glucosidase hyperproducing mutant of *Trichoderma reesei* in simultaneous saccharification and fermentation of wheat straw. *Biotechnol Bioeng.* **9,** 1112 (1989).
12. J. Szczorak and Z. Targonski, Selection of thermotolerant yeast strains for simultaneous saccharification and fermentations of cellulose. *Biotechnol. Bioeng.* **31,** 300 (1988).
13. J. N. Saddler, M. Mes-Hartree, E. K. C. Yu and H. H. Brownell, Enzymatic hydrolysis of various pretreated lignocellulosic substrates and the fermentation of the liberated sugars to ethanol and butanediol. *Biotechnol. Bioeng. Symp.* **13,** 225 (1983).
14. J. N. Saddler, *Conversion of Cellulose to Ethanol Using a Two-Stage Process.* Forintek Canada Corp., Ottawa, Ontario, Canada, 45, May (1982).
15. J. N. Saddler, C. Hoagen, M. K. Chan and G. Louis-Seize, Ethanol fermentations of enzymatically hydrolyzed pretreated wood fractions using *Trichoderma* cellulase, *Zymomonas mobilis* and *Saccharomycea cerevisiae. Can. J. Microbiol.* **28,** 1311 (1982).
16. D. B. Rivers, G. M. Zanin and G. H. Emert, Evaluation of sugar cane bagasse and rice straw as process substrates for production of ethyl alcohol. *Proc. Arkansas Acad. Sci.* **38,** 95 (1984).

17. D. D. Spindler, C. E. Wyman, A. Mohagheghi and K. Grohmann, Thermotolerant yeast for simultaneous saccharification and fermentation of cellulose to ethanol. *Appl. Biochem. Biotechnol.* **17,** 279 (1988).
18. D. D. Spindler, C. E. Wyman, K. Grohmann and A. Mohagheghi, Simultaneous saccharification and fermentation of pretreated wheat straw to ethanol with selected yeast strains and β-glucosidase supplementation. *Appl. Biochem. Biotechnol.* **20/21,** 529 (1989).
19. D. D. Spindler, C. E. Wyman and K. Grohmann, Evaluation of thermotolerant yeasts in controlled simultaneous saccharifications and fermentation of cellulose to ethanol. *Biotechnol. Bioeng.* **34**(2), 189 (1989).
20. C. E. Wyman, D. D. Spindler, K. Grohmann and S. M. Lastik, Simultaneous saccharification and fermentation of cellulose with the yeast *Brettanomyces clausenii*. *Biotechnol. Bioeng.* **17,** 221 (1986).
21. J. H. Janssen, N. Burris, A. Woodward and R. B. Bailey, Lipid enhanced ethanol production by *Kluyveromyces fragilis*. *Appl. Environ. Microbiol.* **45,** 598 (1983).
22. T. K. Ghose, Measurements of cellulase activities. *Pure Appl. Chem.* **59,** 257 (1987).
23. R. Torget, P. Walter, M. Himmel and K. Grohmann, Dilute acid pretreatment of short rotation woody and herbaceous crops. *Appl. Biochem. Biotechnol.* **24/25,** 115 (1990).