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SO_2 Prehydrolysis for High Yield Ethanol Production from Biomass

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ABSTRACT

The advantages of the use of SO_2 in steam pretreatment are described. Two different large scale continuous reactors, the Stake and the Wenger, have been used for this purpose. Pine, aspen and corn stover were prehydrolysed by SO_2 in these reactors and hydrolysed by enzymes. The solution of hexoses and pentoses so obtained were fermented by *Pichia stipitis* R, yielding 372, 346 and 388 L ethanol/tonne for the 3 feedstocks, respectively. When a mixed culture of *P. stipitis* R, which is an excellent pentose fermenter, and *Brettanomyces clausenii* which is an excellent cellobiose fermenter, was used in a simultaneous saccharification-fermentation made with SO_2-prehydrolysed aspen, the yield rose to 384 L/tonne. These are higher yields than have been reported in the literature to date.

Index Entries: Prehydrolysis; sulphur dioxide; ethanol; cellulase; lignocellulosics; fermentation; cellobiose; pentose.

INTRODUCTION

Although the supply of petroleum-based liquid motor fuels has eased considerably since the "oil crisis" of the early 1970s, the US is far from being self-sufficient in this important commodity. This has given rise to a dramatic growth in the use of fermentation ethanol in gasoline. Furthermore, ethanol is an octane enhancer, and the phase-out of lead as an octane booster in gasoline has provided new markets for alcohol gasoline blends. Nearly all fermentation fuel ethanol in the US today is based on corn, a food or feed that is a relatively expensive feedstock. It is now widely accepted that the lignocellulosics such as wood, particularly saw-

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mill waste, or agriculture crops or residues hold the promise for a less expensive, long-term supply of fermentation substrates for ethanol production.

In the preparation of wood or other lignocellulosics for conversion to ethanol, it is highly desirable to hydrolyze hemicellulose components before hydrolysis of the cellulose, particularly if cellulose hydrolysis is to be acid-catalyzed and the hemicellulose sugars are to be fermented or otherwise utilized. Of the several proposals that we have studied for this "pretreatment" or "prehydrolysis," autohydrolysis, which is a steam pretreatment, and SO_2-catalyzed prehydrolysis, have been found most useful in laboratory-scale experiments (*1,2*). The purpose of this present paper is to describe the conversion of lignocellulosics to ethanol using SO_2 prehydrolysis on a large pilot-plant scale.

METHODS

SO_2 Prehydrolysis

Several forms of industrial equipment are available that are well established and are satisfactory for SO_2 pretreatment. We have used two kinds of continuous equipment, the Stake reactor and the Wenger reactor. Other candidate reactors may be the Defibrator as installed by TVA, and the Masonite gun. In the laboratory, batch reactors are most commonly used, ranging in size from 30 mL to 7 L.

Figure 1 is a general view of the Stake II Reactor as installed at the Université de Sherbrooke, which has a capacity of 1,500 kg/h. Figure 2 shows the SO_2 addition system of the Stake reactor, the gas being drawn from a cylinder and fed by a Milton Roy pump to the reactor along with the steam. Figure 3 shows a general view of the pilot Wenger reactor, with a capacity of 100–150 kg/h. In the runs reported below, the SO_2 was added in solution to the feedstock.

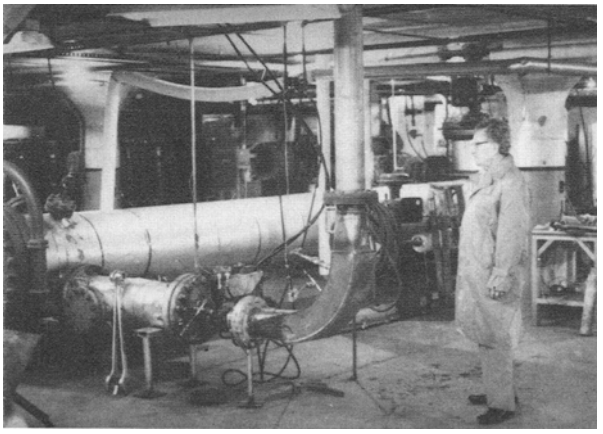

Fig. 1. The Stake II reactor: general view.

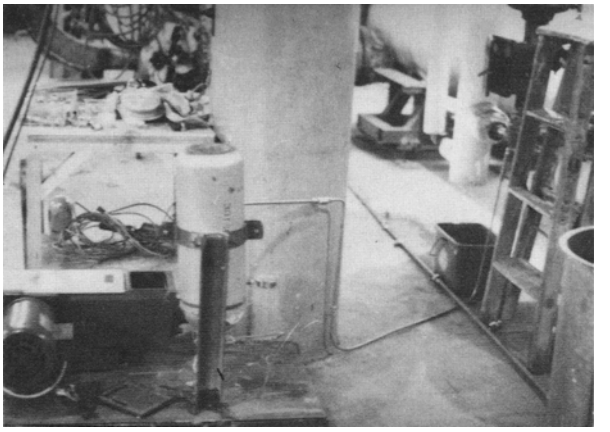

Fig. 2. The Stake II reactor: SO_2 addition system.

Until recently, we experienced a problem in using such equipment. The products of most of these reactors, the Stake, the Defibrator, and Masonite Gun, are coarse fiber bundles and chips that are only partially disintegrated. This is not surprising, since the Sunds Defibrator and the Masonite gun are used primarily to make fiberboard, and the products are normally put through a mechanical attrition process to separate the fiber bundles. If finely divided product is required such as would benefit further processing, relatively harsh pretreatment conditions are necessary. These produce lignin-hemicellulose condensation products and also fermentation inhibitors. Processing conditions necessary to obtain product most suitable for further processing in such equipment results in incompletely pretreated feedstock, requiring at a minimum screening out of large pieces, and probably subsequent grinding or attrition. This limitation simply does not apply to the Wenger reactor, the physical conditions being ideal for further processing without unwelcome chemical byproducts.

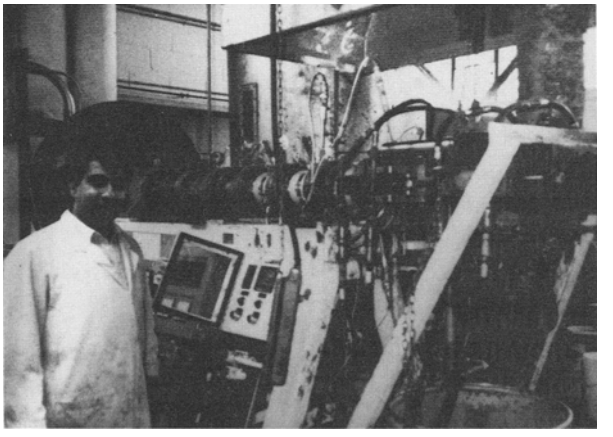

Fig. 3. The Wenger reactor.

When using the Wenger reactor, the product has a remarkably fine form, lacey, well-separated fibers ideal for further processing, whether by acid hydrolysis or by enzyme hydrolysis. The reason is that the internal helix in the Stake reactor is essentially a conveyor for moving the chips from inlet to outlet. In the Wenger reactor, the internal helix rotates many times more rapidly than is required for conveyance of the feedstock to the outlet, and it acts as an attrition device within the reactor. In consequence, the product is ready for the next stage of processing.

RESULTS

Table 1 presents the results of SO_2 pretreatment of pine, aspen, and corn stover in laboratory batch and in continuous equipment. The aim in these runs was to achieve about 30% solubilization of the feedstock, leaving a residue of about 70%. All laboratory batch SO_2-pretreatment reactions were with 2–3% SO_2 on fiber, at 150–160°C for 20–30 min. Under these conditions, a small amount of glucose is found with the wood feedstocks. Corn stover, which inevitably contains a significant amount of starch, gives more glucose in prehydrolysis. The hemicellulose of the two wood feedstocks dissolved completely under these conditions. The hemicellulose sugars were nearly all monomeric.

In the continuous equipment, the Stake reactor was operated on coniferous wood at a 2 min residence time with SO_2 and saturated steam at 208°C, with results very similar to those obtained in the batch reactor, the hemicellulose sugars dissolving completely. The results of processing the

Table 1
SO_2 Prehydrolysate Composition, Percent on Dry Feedstock

	Glucose	Hemisugars	Residue
Pine			
2.0% SO_2 on fiber, 150°C, 20 min, batch	5.3	19.6	69
2.6% SO_2 on fiber, 208°C, 2 min, Stake reactor	5.6	21.5	71
Aspen			
3.0% SO_2 on fiber, 160°C, 30 min, batch	3.5	25.2	70
2.5% SO_2 on fiber, 200°C, 45 s, Wenger reactor	4.1	21.9	69
Corn stover			
3.0% SO_2 on fiber, 160°C, 30 min, batch	8.8	13.0	71
2.5% SO_2 on fiber, 200°C, 45 s, Wenger reactor	4.4	17.1	73

aspen with SO_2 in the Wenger reactor at 200°C for 45 s were equally satisfactory.

For the purposes of this paper, the objective of our work is to make ethanol. Table 2 shows ethanol production from enzyme hydrolyzed SO_2-prehydrolyzed coniferous wood, aspen, and corn stover. The yields were 372, 346, and 388 L/t (dry wt) for the feedstocks respectively, being 78, 74, and 81% of theory based on the chemical composition of these woods. These yields on a three-stage process, that is, prehydrolysis, hydrolysis, and fermentation, represent more than 90% yields in each of the three stages.

Table 2 also compares ethanol yields from coniferous woods and from aspen, a deciduous wood, with SO_2 prehydrolysis with yields from steam only pretreatment (autohydrolysis). In both cases, the SO_2-pretreated wood gives higher ethanol yield than the autohydrolyzed wood. The difference is only 7.5% less for autohydrolyzed aspen than for SO_2-pretreated aspen. However, autohydrolysis of pine results in less than half, about 45%, of the amount of ethanol obtained with SO_2 pretreatment. Thus, one might consider autohydrolysis as a possibility for aspen, in spite of the lower yield, higher temperature requirement, and the oligomeric nature of the hemisugars, but this option is simply not available for coniferous woods. SO_2 catalyst must be used. This is an important matter, since most sawmill residues are from coniferous wood.

The process by which the results of Table 2 were obtained may be represented by the flowchart of Fig. 4. Wood chips or sawdust are fed to a reactor, either Wenger or Stake in our research, and SO_2 is fed in at the same time. It is desirable that the wood be at about 50% moisture, preferably reasonably fresh off the stump. If the wood has been allowed to dry up, it needs to be remoisturized, preferably by steam or hot water, to reswell the wood tissues. Agricultural crop residues—corn stover, straw, bagasse—are of low bulk density that much reduces the capacity of the equipment. Such feedstocks should be densified—pelleted, or in some other way—for use. SO_2 may be added as gas or as a strong solution, but the latter is a bit of a nuisance.

The product of the reactor is not washed, the hemisugars being left in place. A solution of enzymes is then added (*3*), solids content being in the range of 8–16%, and the pH raised to 4.8. This is shaken gently at 45–50°C for 24–72 h, depending upon feedstock and pretreatment conditions, and the residual lignin is filtered off. Yeast is then added to the filtrate.

The yeast we used to obtain the results of Table 2 was *Pichia stipitis* R. This is a remarkably efficient fermenter of hexoses and pentoses both together, which has been adapted to wood hydrolysate (*4,5*). Figure 5 is a scanning electron micrograph of the yeast before and after adaptation.

The ethanol is distilled from its solution. This makes for a straightforward flowchart.

Table 2
Comparison of SO_2 Prehydrolysis and Autohydrolysis, Followed by Enzyme Hydrolysis and *P. stipitis* R Fermentation

	Before fermentation		After fermentation				
Pretreatment conditions	sugars g/L	percent on feedstock	sugars g/L	ethanol g/L	g ethanol/ g sugar	L ethanol/ dry t feedstock	ethanol percent of theory
Coniferous wood							
SO_2 Prehydrolyzed	134	72	6	55	.43	372	77
Autohydrolyzed	36	32	3	15	.45	166	35
Aspen							
SO_2 Prehydrolyzed	123	68	6	52	.44	346	74
Autohydrolyzed	72	61	6	30	.45	320	67
Corn stover							
SO_2 Prehydrolyzed	120	65	6	52	.44	388	81

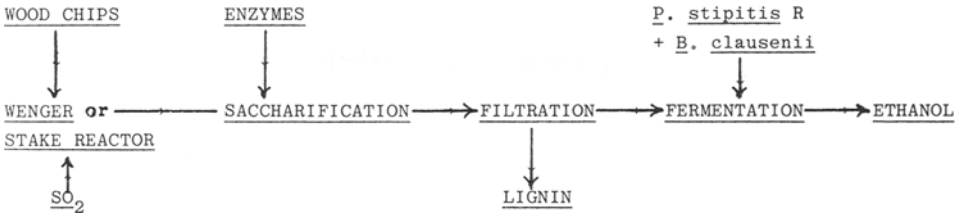

Fig. 4. Flowchart.

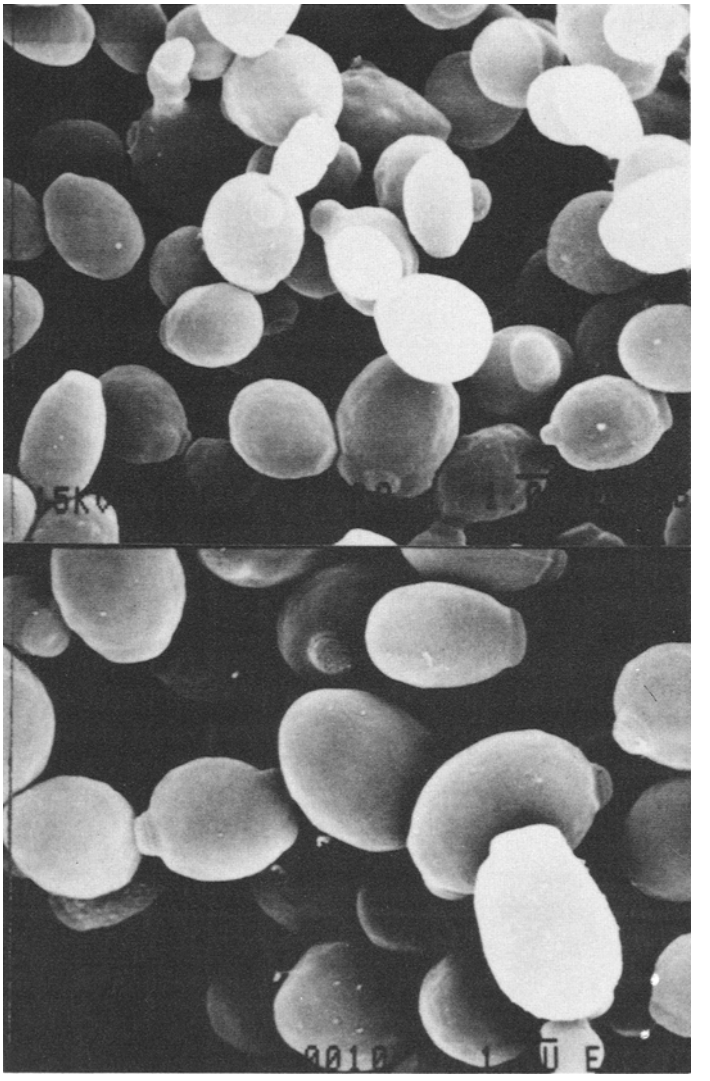

Fig. 5. Scanning electron micrographs of *Pichia stipitis* before (upper) and after (lower) adaptation to wood hydrolyzate.

Simultaneous Saccharification-Fermentation

The flowchart of Fig. 4 shows fermentation by a mixed culture. In addition to the *P. stipitis* R, the pentose fermenter, we also add *Brettanomyces clausenii*, which, in addition to fermenting hexoses, also ferments cellobiose. This is quite important when a simultaneous saccharification-fermentation (SSF) mode is to be employed. The flowchart of Fig. 4 may be modified by adding both enzyme and yeast to the product of prehydrolysis. In that case, saccharification by the enzyme and fermentation by the yeast takes place more or less simultaneously, because the optimum temperature for the enzyme system is 45–50°C, whereas that for the yeast is 30–35°C. The compromise temperature we used was 38°C. A thermophilic yeast, or even a mesophilic yeast, would probably be advantageous. Fortunately, pH 4.8 is suitable for both.

The theory behind the use of a mixed culture is shown in Fig. 6. SO_2 prehydrolysis is essentially a very good method of preparing a reactive cellulose free of entanglement with hemicellulose and lignin. Upon treatment with a cellulase enzyme system, cellulose hydrolyzes not to glucose but to cellobiose. A different enzyme, β-glucosidase, is required to convert the cellobiose to glucose. In most laboratories where enzyme hydrolysis is practiced, β-glucosidase is added. A difficulty with these enzyme reactions is that they are reversible, and are subject to powerful endproduct repression, which is how enzymes control themselves in biological systems. Cellobiose is a powerful inhibitor of cellulase activity, and glucose is a powerful inhibitor of the activity of β-glucosidase. As a result, these reactions are slow and incomplete.

In an SSF mode, the presence of a yeast such as *B. clausenii*, which ferments cellobiose rapidly and efficiently to ethanol (*6*), the cellobiose is

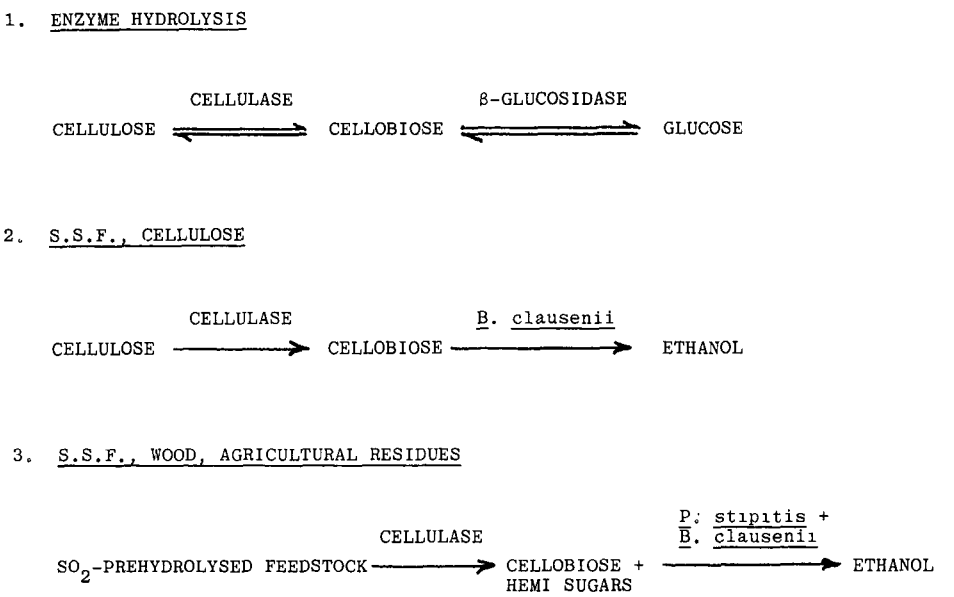

Fig. 6. Simultaneous saccharification-fermentation with mixed culture.

removed from solution as formed. No β-glucosidase is necessary. In the presence of cellulase, cellulose is fermented directly to ethanol.

Wood or agriculture residues contain in addition to cellulose also hemicellulose. These must be hydrolyzed to sugars and fermented. For this purpose, SO_2 prehydrolysis followed directly by the addition of cellulase and the two yeasts, the cellobiose fermenter and the pentose fermenter, leads directly to ethanol. Figure 6 shows this as though hydrolysis and fermentation were in two steps, and indeed we have operated them as two steps. But the simplest route is to combine hydrolysis and fermentation in one step. Then the flowchart becomes: SO_2 prehydrolysis → SSF with mixed culture → distillation. With this elegant procedure we have obtained 384 L ethanol/t aspen (dry wt), essentially 100 gal/t. This is the highest yield we have obtained so far from wood.

There are difficulties with this elegant procedure. The temperature we use, 38°C, is a compromise, which may be overcome by a brief hydrolysis with cellulase at 50°C ahead of SSF, a practice well-known in corn processing. Also, we have had slightly better results when we have added some commercial hemicellulase to our pure cellulase. Perhaps we have not optimized the SO_2 pretreatment for this particular purpose, to avoid the need for hemicellulase just as we have now avoided the need for β-glucosidase. Finally, cellulase is expensive.

This last point may be crucial. At this time we are not encouraged by enzyme producers to believe that the price of cellulase can come down to the point where it could be used to make anything as cheap as a gasoline substitute. We have approached this problem from several directions. One can use much less enzyme if the percent of solids is lowered to about 2% or less. But this produces a very dilute ethanol solution, and raises the cost of recovery substantially. One can recycle the enzyme as proposed by Hou-min Chang (*7*) or by Saddler (*8*). We can make our own enzymes, in-house or in-plant, and this seems to be an acceptable route according to IFP (*9*), which has built a pilot plant for this purpose. There are now several competing processes for in-house cellulase, including one of our own. Finally, we are now studying the immobilization of the enzyme. We did not believe immobilization of cellulase would be a useful process simply because of the solid nature of cellulose. However, when we made SO_2 prehydrolyzed aspen in the Wenger reactor, we saw a different product, which may well be suitable for hydrolysis by immobilized enzyme. Immobilization of these enzymes and of our mixed yeast culture together may provide a super-elegant process, especially when the immobilization takes place in our continuous dynamic immobilized biocatalyst bioreactor (*10*).

There is, however, a different approach which is to use SO_2 as the hydrolytic agent for SO_2 prehydrolyzed lignocellulosics. Table 3 shows the results of SO_2 hydrolysis of SO_2 prehydrolyzed pine, aspen, and corn stover. The sugars obtained by this process are quite respectable, ranging from 51–73% of the weight of the feedstocks. These, of course, include a great deal of pentose sugars, and the ethanol figures shown here are cal-

Table 3
Sugar Production by SO_2 Prehydrolysis and SO_2 Hydrolysis, Fermentation by *P. stipitis*

	Pine	Aspen	Bagasse	Corn cobs	Wheat straw
Sugars, actual, kg/t	670	590	512	735	685
Ethanol, calculated from sugars, L/t	413	356	312	434	405
Xylose contribution, percent	28	43	53	66	65

culated from the actual sugar figures based on fermentation by *P. stipitis* R. The yields of ethanol vary from a low of 312 L/t of bagasse to a high of 434 L/t of corn cobs, with pine near the high end and aspen in the middle. Fermentation of xylose makes an important contribution to these yields being greatest, one-half to two-thirds, with the agricultural crop residues, 28% with pine and 43% with aspen. These xylose contributions are based on the chemical analysis of the lignocellulosics.

SO_2 hydrolysis is not easy. Quite high temperatures are required, 190–200°C, and at these temperatures, the danger of rapid sugar destruction is great. As a result, it is necessary to carry out the hydrolysis in a series of short steps, recycling the unreacted residue. This appears to be true even when continuous equipment with short residence time is used (*11*). However, the results obtained when care is used and recycling is practiced are acceptable. With the present cost of enzymes this may be the most cost-effective process.

CONCLUSION

SO_2 prehydrolysis is an excellent process step for preparing lignocellulosics, including coniferous woods, for enzymatic hydrolysis or acid hydrolysis.

Two different large scale continuous reactors, the Stake and the Wenger, have proven to be effective for SO_2 prehydrolysis. The physical form of the Wenger product is more suitable for further processing.

SO_2 prehydrolyzed aspen processed in an SSF mode with enzymes and a mixed culture of *Pichia stipitis* R, an excellent pentose fermenter, and *Brettanomyces clausenii*, an excellent cellobiose fermenter, gave a yield of 384 L/t, which may be a new record.

Table 4 summarizes our best results to date, in the form of a scoreboard. The scoreboard emphasizes that the score is not cleverness at pretreatment, or elegant hydrolysis procedures, but yields of product. That is what pays off. Our yields are only about 80% of theoretical. There is thus plenty of scope for improvement.

Table 4
Scoreboard—Ethanol Yields Obtained from Lignocellulosics

	Pine	Aspen	Corn stover
SO_2 Prehydrolysis			
Sugars, kg/t	219	252	130
Enzyme hydrolysis			
Sugars, kg/t	717	680	650
Fermentation			
Ethanol, L/t	372	346	388
SSF, mixed culture			
Ethanol, L/t		384	
Theoretical yields, based on chemical composition			
Hemicellulose sugars, kg/t	245	275	176
Cellulose sugars, kg/t	510	470	576
Total sugars, kg/t	755	745	752
Ethanol, L/t	478	470	480
Ethanol yield, percent of theoretical	78	82	81
Practical yields that may be safely expected			
Ethanol: 350–400 L/t			

ACKNOWLEDGMENT

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REFERENCES

1. Wayman, M., A., Tallevi, and Winsborrow, B. (1984), *Biomass* **6,** 183–191.
2. Wayman, M. (1986), ch. 15 in *Cellulose: Structure, Modification and Hydrolysis,* Young, R. A. and Rowell, R. M., eds., Wiley, NY.
3. Wayman, M., Parekh, S., Chornet, E., and Overend, R. (1986), *Biotechn. Letts.* **8,** 749–753.
4. Parekh, S. R., Yu, S., and Wayman, M. (1986), *Appl. Microbiol. Biotechnol.* **25,** 300–304.
5. Jeng, R., Yu, S., and Wayman, M. (1987), *Can. J. Microbiol.,* in press.
6. Wyman, C. E., Spindler, D. D., Grohmann, K., and Lastick, S. M. (1986), Biotechnol. Bioeng. **17,** 227–238.
7. Cho, N. S., Matsumoto, Y., and Chang, H-m. (1986), *Proc. T.A.P.P.I. R. and D. Conf.,* Raleigh, NC, pp. 99–102.
8. Tan, Larry U. L., Yu, Ernest K. C., Campbell, N., and Saddler, J. N. (1986), *Appl. Microbiol. Biotechnol.* **25,** 250–255.
9. Pourquié, J., and Glikmans, G. (1986), *VII International Symposium on Alcohol Fuels,* Paris, France.
10. Parekh, S. R., and Wayman, M. (1987), *Enzyme Microbiol. Technol.,* **9,** 406–410.
11. Holysh, M. and Wayman, M. (1987), *Proc. Ninth Symposium on Biotechnology for Fuels and Chemicals,* Boulder, CO.