

Simultaneous Saccharification and Fermentation of Lignocellulose: Process Evaluation

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ABSTRACT

Simultaneous saccharification and fermentation (SSF) processes for producing ethanol from lignocellulose are capable of improved hydrolysis rates, yields, and product concentrations compared to separate hydrolysis and fermentation (SHF) systems, because the continuous removal of the sugars by the yeasts reduces the end-product inhibition of the enzyme complex. Recent experiments using Genencor 150L cellulase and mixed yeast cultures have produced yields and concentrations of ethanol from cellulose of 80% and 4.5%, respectively. The mixed culture was employed because *B. clausenii* has the ability to ferment cellobiose (further reducing end-product inhibition), while the brewing yeast *S. cerevisiae* provides a robust ability to ferment the monomeric sugars. These experimental results are combined with a process model to evaluate the economics of the process and to investigate the effect of alternative processes, conditions, and organisms.

Index Entries: Saccharification; fermentation; lignocellulose.

INTRODUCTION

Lignocellulose (wood, grasses, and municipal solid waste) is an attractive feedstock for ethanol production because it is available at low cost and in large quantities. Although biological processes are inherently efficient, the price of this efficiency is the need to process each major

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component of lignocellulose separately. For example, the cellulose is difficult to hydrolyze to glucose, but it is simple to ferment the glucose to ethanol. Conversely, the hemicellulose (primarily xylose in hardwoods and grasses) is easily broken down to monomeric sugars at high yields, but the xylose is difficult to ferment to ethanol. Lignin is a phenolic polymer and once separated from the lignocellulosic matrix must be processed catalytically to yield useful products.

In the overall process for producing ethanol from wood (Fig. 1), the feedstock is pretreated and the xylan and possibly the lignin are removed for separate fermentation or chemical processing. The cellulose is hydrolyzed to glucose by either acid or enzyme, and the glucose is then fermented to ethanol. The ethanol from the glucose and xylose fermentations is then concentrated by distillation. Enzymatic hydrolysis processes are of interest because enzymes catalyze only specific reactions. Therefore, unlike acid hydrolysis, there are no side reactions or byproducts and the hydrolysis can potentially be run with yields approaching 100% of theoretical.

In this paper, we analyze the performance of simultaneous saccharification and fermentation (SSF) systems, compare their performance with that of separate hydrolysis and fermentation (SHF) systems that have been analyzed previously (1), and evaluate the sensitivity of the process to potential improvements. With the exception of the SSF, pretreatment, and enzyme production sections, the process uses proven technology. Therefore, the balance of the plant is based on a design prepared by Badger Engineers (2) for production of 25 million gal/yr of ethanol from mixed hardwoods. Capital cost estimates were produced with the ICARUS computer aided cost estimating program, and have an accuracy of $\pm 10\%$ for a completely defined process (3). Therefore, the overall accuracy of the cost estimate (130%) stems from the uncertainties in process design and performance, not the estimating technique. To rapidly

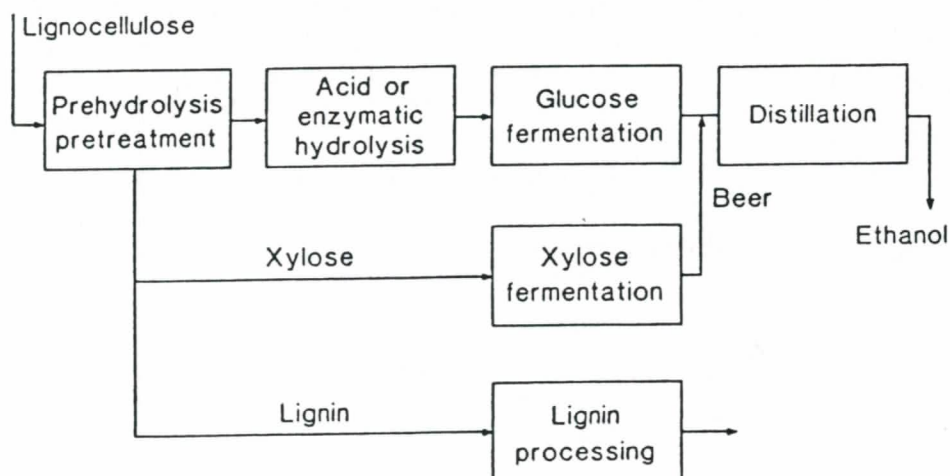


Fig. 1. Ethanol production from lignocellulose processing sequence.

assess the relationships among the various parts of the process, a mathematical model was developed to calculate material and energy balances, capital and operating costs, and the ethanol selling price using a Lotus 1-2-3 spreadsheet.

Because this paper is focused on the SSF process, we will not discuss the effects of utilization of the xylan and lignin fractions of the lignocellulose. However, it must be mentioned that utilization of these fractions greatly reduces the overall selling price of ethanol, and thus they are essential to the success of the process.

The design presented should not be viewed as that of a real operating plant, but as our best estimate of the current state of the technology, for use in investigating the effect of various improvements. Although the model accurately reflects the sensitivity of the process to the various process changes, uncertainty in the basic design means that the absolute ethanol selling price cannot be accurately estimated. Therefore, although great care was exercised in preparing the model and economics, caution must be used when comparing the results of this study to those of other authors who may have used different cost estimating or economic methodologies or other technologies.

Process Description and Overview of Enzymatic Hydrolysis Technology

All enzymatic hydrolysis processes consist of four major steps that may be combined in a variety of ways—pretreatment, enzyme production, hydrolysis, and fermentation. Although shielding of the cellulosic surface by lignin, crystallinity, and the inaccessibility of the cellulose to the enzymes have all been suggested as barriers to enzymatic attack (4), it now appears that the key to increasing the digestibility of lignocellulose lies in increasing the cellulose surface area that is accessible to the enzymes. Although the internal surface area of native wood is large, only 20% is accessible to large enzymes such as cellulase (MW of 30,000–60,000, major and minor dimensions of $300 \times 30 \text{ \AA}$) (5). By carrying out a prehydrolysis, the hemicellulose fraction can be removed, enlarging the pore size and thus opening the structure to attack by the enzymes (6). Further, the degree of digestibility is almost directly proportional to the fraction of the xylan removed (7). Thus, the effects of all the major pretreatment options—dilute acid, steam explosion (8), and organosolv processes (9)—are seen to consist of the acid-catalyzed removal of hemicellulose.

The pretreatment system used in the process model heats the solids in dilute (1.1%) sulfuric acid for 10 min at 160°C in a high solids reactor. The reactor has no free liquid phase and reduces sugar dilution and energy consumption by minimizing the amount of water that is processed. At these conditions, 93% of the xylan is hydrolyzed, resulting in a fully digestible cellulose pulp. Xylose yields are 60%, and furfural yields are 30%. This system is more fully described in (10).

Cellulase enzymes are efficiently produced by the filamentous fungus *T. reesei* (11). Traditional methods of production use solid cellulose as both the inducer and the carbon source for enzyme production and growth. Although productivities of up to 150 IU/L-h have been reported from such methods (12), the productivities are limited by the low rate of hydrolysis of the cellulose (and hence low rate of growth). This low production rate results in enzyme production costs being an important component of the overall processing cost. One promising alternative is to identify fungal mutants that produce enzyme while growing on soluble carbon sources (13).

The enzyme production system used in this study is based on experimental data (14). Rut C-30 is grown in a fed batch mode on pretreated substrate with a productivity of 100 IU/L-h, and a total batch time of 13 d. It is important to note that the original experiment used solka-floc as a carbon source, whereas this study assumes the use of dilute acid pretreated hardwood. Approximately 3% of the total feed is used to produce the enzymes.

The hydrolysis of cellulose is carried out by a complex of enzymes that have three different modes of action (Fig. 2) (15). First, the endoglucanase absorbs on the surface of the solid cellulose and attacks the interior of the polymer chain, splitting it and exposing two new chain ends. Next, exoglucanases remove cellobiose units from the nonreducing end of the cellulose chain. The cellobiose produced by this reaction can accumulate in solution and strongly inhibit the activity of the exoglucanases. Finally, in a liquid phase reaction, beta-glucosidase splits the cellobiose units into glucose. Similarly, the accumulation of glucose can inhibit the action of beta-glucosidase, causing a buildup of cellobiose,

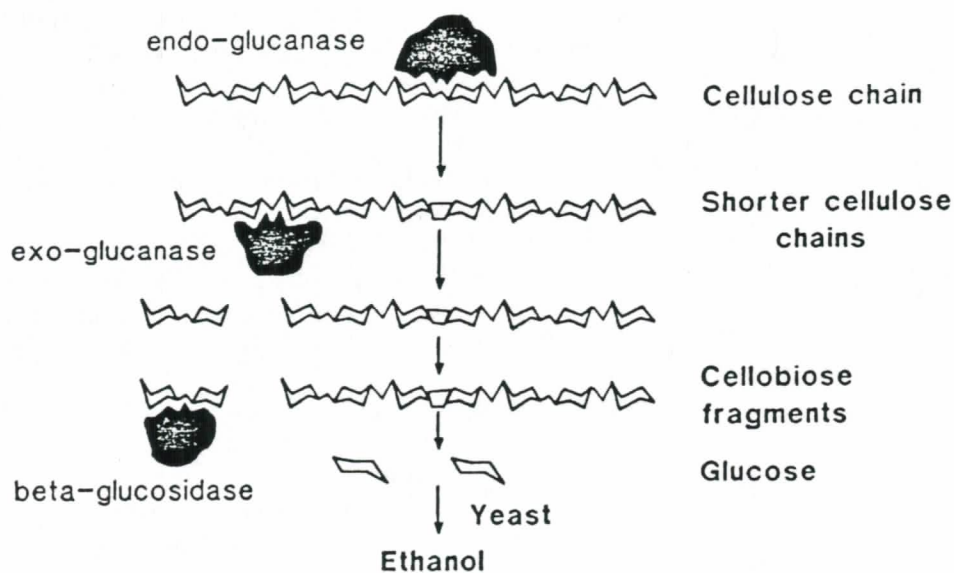


Fig. 2. Mechanism of enzymatic hydrolysis and SSF.

which again inhibits the exoglucanase activity. Thus, the successful production of glucose (the desired feedstock for ethanol production) can cause severe end-product inhibition, which can greatly limit the product concentration, yield, or reaction rate.

The SSF system used in the base case is a batch reactor, operated at 37°C, with an enzyme loading of 7 IU/g cellulose, using a mixed yeast culture of *B. clausenii* and *S. cerevisiae*, with a residence time of 7 d (16,17). The feed to the reactor is 16% solids, which translates to a cellulose input of 10% by weight. Of the total cellulose feed, 88% is hydrolyzed. Of this, 90% is fermented to ethanol and 10% is converted to cell mass and byproducts. The final ethanol concentration is 4.5%. The hydrolysis data used in this study were obtained using Genencor 150L cellulase, a preparation superior to Rut C-30. On the other hand, the enzyme production design was obtained using Rut C-30 productivity. Also, the hydrolysis data were obtained using Sigma-cell 50, a purified cellulase, whereas the study assumes the results will be the same for dilute acid pretreated hardwoods. These assumptions point out the need for integrated testing, where consistent methods and materials are used to develop all stages of the process.

The dilute beer from the SSF is purified by distillation. The stillage and waste solids streams are centrifuged to separate the solids and concentrated to produce a solution of 50% solids and mixed organics in water. These materials are then burned to produce steam and electricity to run the process. The energy production of the boiler and the process demand are almost exactly in balance.

Comparison of SHF and SSF Processes

To understand the rationale for SSF processes, it is useful to compare them with the simpler SHF process that was previously analyzed using the same design assumptions (1). A cost breakdown is shown in Fig. 3. The total cost of ethanol production is \$2.66/gal, with \$.65/gal (25%) of the cost contributed by enzyme production. Approximately \$.40/gal (15%) is contributed by the cellulose fraction of the feedstock that is converted to ethanol, and an additional \$.40/gal (15%) is attributable to the hydrolysis reactor section. The extremely high cost of enzyme production arises in part from the low productivity caused by the use of an insoluble cellulose carbon source. However, a much more important cause is the inhibition of cellulase caused by cellobiose and glucose. The higher the final glucose concentration, the higher the cellulase loading needed to achieve a given yield. Similarly, because the reaction is slowed or stopped by the presence of glucose and cellobiose, the hydrolysis is essentially halted before the reaction can proceed to completion. The optimal point for SHF is an enzyme loading of 20 IU/g substrate (33 IU/g cellulose) and a final glucose yield and concentration of 73% of theoretical and 4.5%, respectively. Thus, end-product inhibition is in large part responsible for the limitations in yield, product concentration, and reac-

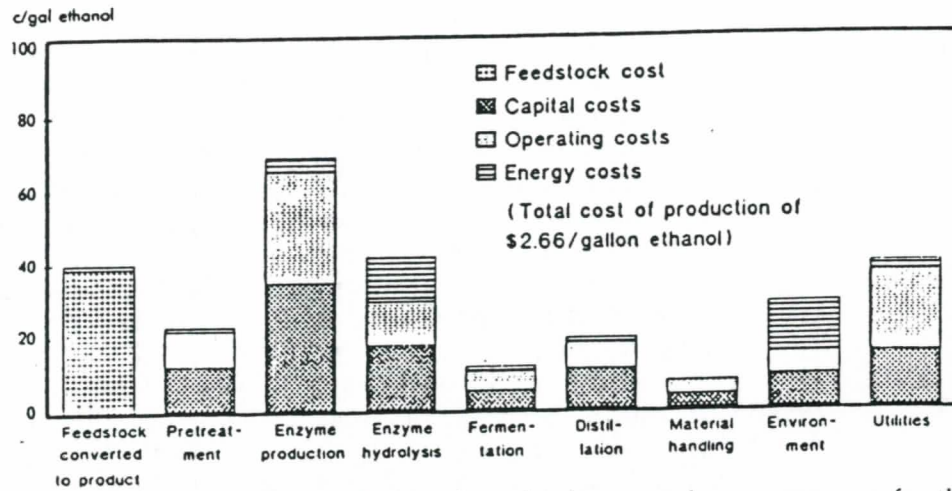


Fig. 3. Breakdown of ethanol production costs by process area for the separate hydrolysis and fermentation process.

tion rate, and the high enzyme loadings that result in SHF suffering such a high cost of production.

One means of alleviating this problem is to use cellulase preparations that have higher beta-glucosidase activities. These newer enzyme preparations (such as Genencor 150L) are less inhibited by glucose and also remove cellobiose more efficiently, allowing the reaction to proceed more swiftly to higher yields and glucose concentrations (16). The improvement from using such an enzyme preparation is shown in Fig. 4.

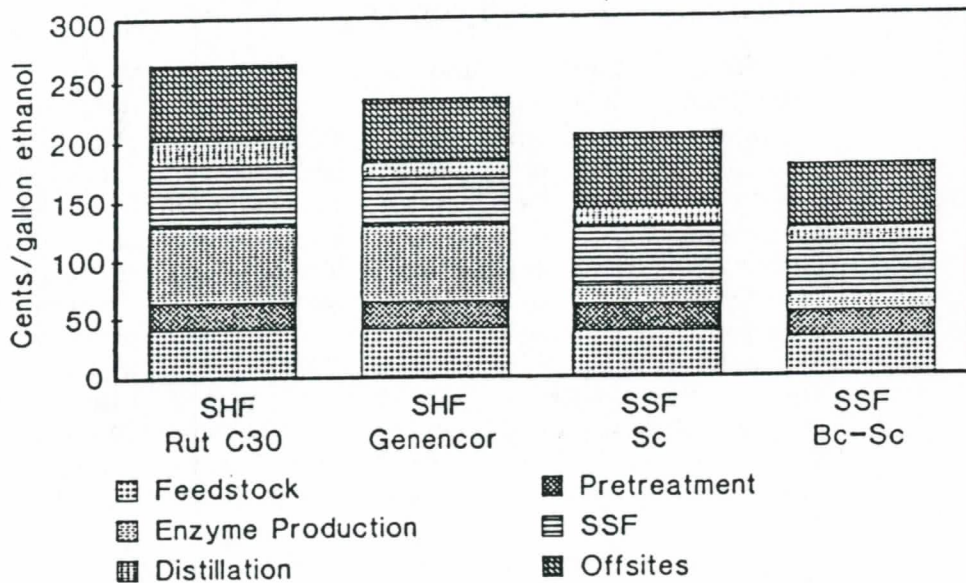


Fig. 4. Improvements in enzymatic hydrolysis by improved enzymes and SSF processes.

Even further improvement can be made by continuously removing the glucose through fermentation (SSF). Use of this process with Genencor 150L enzyme and *S. cerevisiae* (generally not considered one of the best single yeasts for SSF) further improves the performance. With the enzyme loading reduced by almost a factor of five to 7 IU/g cellulose, the hydrolysis yield remains at 73% and the ethanol concentration is increased to 3.7% (equivalent to a glucose concentration of 8.1%, roughly twice that of SHF). This further reduces the cost of ethanol production to \$2.06/gal (Fig. 4). *C. brassicae* (18), generally considered the organism of choice, gives a yield of 79% under similar conditions, resulting in an ethanol selling price of \$1.94/gal.

Since cellobiose is an even greater inhibitor than is glucose, a screening study was initiated to screen known cellobiose-fermenting yeasts for ethanol production (19,20). Of the yeasts studied, *C. lusitanae* fermented well at up to 41°C but had a relatively low ethanol tolerance, whereas *B. clausenii* fermented well up to 37°C and had a higher ethanol tolerance. Use of *B. clausenii* alone gave an improvement in yield to 83%. However, using a mixed culture of *S. cerevisiae* and *B. clausenii* together further increased the yield to 88%. The increased performance comes from the fact that the *B. clausenii* cell density increases quickly early in the SSF when glucose and cellobiose are being produced rapidly. Thus, the *B. clausenii* is active early and removes the cellobiose inhibition when it is most important, providing high initial rates. However, it is not a particularly robust yeast, and it loses viability later in the fermentation when the rate of glucose and cellobiose production drops. Here, the much more robust and ethanol-tolerant *S. cerevisiae* remains viable and allows the reaction to proceed essentially to completion without end-product inhibition (16). The mixed culture produces yields of 88% and ethanol concentrations of 4.5% from 10% cellulose, resulting in a predicted selling price of \$1.78/gal.

Optimization of SSF Processes Parameters

Taking the performance of the mixed culture of *S. cerevisiae* and *B. clausenii* as our new base case, we can again look at the breakdown of costs by process area (Fig. 5, Tables 1 and 2.). The largest difference is that the cost of enzyme production has dropped from \$.65 to \$.13/gal because of the large reduction in enzyme consumption brought about by the reduced loading and the increased yield. This is a major improvement in the design of such systems. The cost of feedstock is somewhat reduced because of the improved yield, and the increased ethanol concentration significantly reduces the cost of the distillation and environmental sections. Even the SSF section is slightly less expensive than the original combination of the hydrolysis and fermentation because the increased reaction time is offset by the increased concentration in the reactors and because the 7-d SSF is replacing a 3-d hydrolysis coupled to a separate fermentation system.

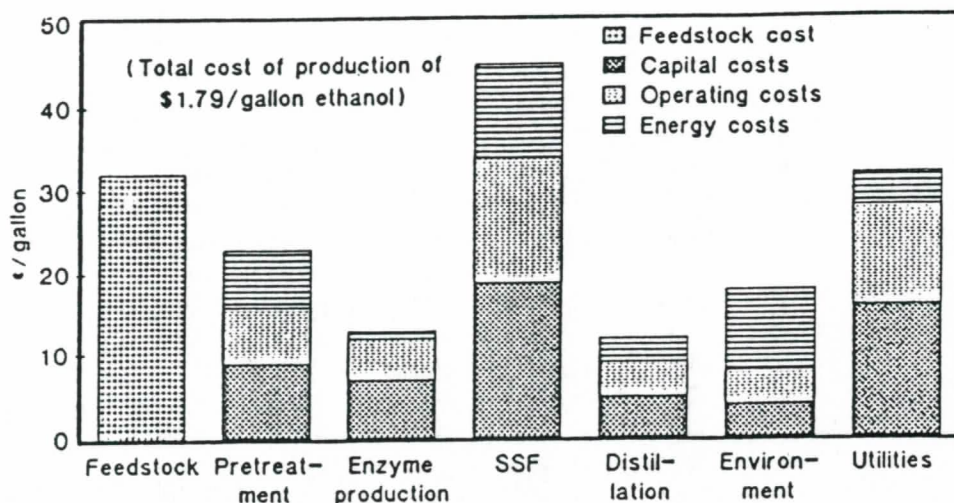


Fig. 5. Breakdown of ethanol production costs by process area for the simultaneous saccharification and fermentation process.

The most important parameters that determine the economic performance of any hydrolysis process are yield, product concentration, rate, and cost of catalyst. Using the model we can determine ethanol selling price as a function of the first three and graph the results three dimensionally (Fig. 6). The performance of the four previous yeast combinations are shown on the 7-d reaction time surface. Further, from our base-case design point (the mixed culture), we can take the partial derivative of price with respect to the major variables and determine the sensitivity of price to each. Figure 7 presents the sensitivity of price to a 1% incremental change in each of the major parameters. The most important parameter by far is yield, with product concentration approximately half as important, and rate half again as important. Enzyme cost and consumption, formerly the major factor, is now a relatively small variable, along with agitation power.

Optimization of Cellulose Concentration and Enzyme Loading

Realizing that there is a tradeoff between yield (favored by long residence times, low substrate and product concentrations, and high enzyme loadings), product concentration (favored by low yields, high enzyme loadings, and long residence times), and enzyme consumption, it is useful to determine the optimum combination of these (Fig. 8). Because yield is strongly dependent on reaction time, and because economics are proportionally four times more sensitive to yield than reaction time, this analysis was carried out for a constant reaction time of 7 d. Initial cellulose concentration ranged from 7.5% to 15%, and enzyme loading varied from 7 to 13 IU/g cellulose. As expected, yield was maximized at low concentrations and high enzyme loadings, but the shape is quite interesting. The yield is a strong function of the initial cellulose concen-

Table 1
 Cost of Production Summary for SSF Processing
 of Lignocellulose to Ethanol (25,000,000 gal/yr)

	Unit/gal	¢/unit	¢/gal
Raw materials			
Wood, lb	32.6	2.1	68.4
Sulfuric acid, lb	.7	3.3	2.2
Lime, lb	.7	1.8	1.2
Chemicals			1.7
Utilities			.6
Water, 1000 gal	.008	75	7.4
Labor, hr	.005	1540	35.3
Overhead and maintenance			116.2
Annual operating cost			
Capital charges, capital recovery factor = 0.13, 15% internal rate of return, 20 yr straight line depreciation			62.2
Ethanol selling price			178.4

Table 2
Capital Cost Breakdown, 1986\$, for SSF
Processing of Lignocellulose to Ethanol,
25,000,000 gal/yr

Process area	Capital cost million \$
Feedstock handling	7.1
Dilute acid pretreatment	10.4
Enzyme production	13.5
SSF	36.4
Distillation	10.2
Offsite tankage	4.1
Environmental control	8.5
Utilities	30.2
Total	120.5

tration, which suggests some type of end-product inhibition is occur. However, in a straight saccharification process, this inhibition is partly overcome by the addition of more enzyme. This essentially has the effect of increasing the beta-glucosidase activity and thereby decreasing glucose and cellobiose inhibition. In the SSF process, we see almost no effect of enzyme loading on yield, except at very high (15%) initial cellulose concentrations. This suggests that the extent of reaction is being controlled by the ethanol tolerance of the yeast. Further, the *B. clausenii* cellulose viability after the ethanol concentration passes 50 g/L, concentrations that are found in the runs with greater than 10% initial cellulose. Also, the insensitivity of the yield to enzyme loading implies that it could

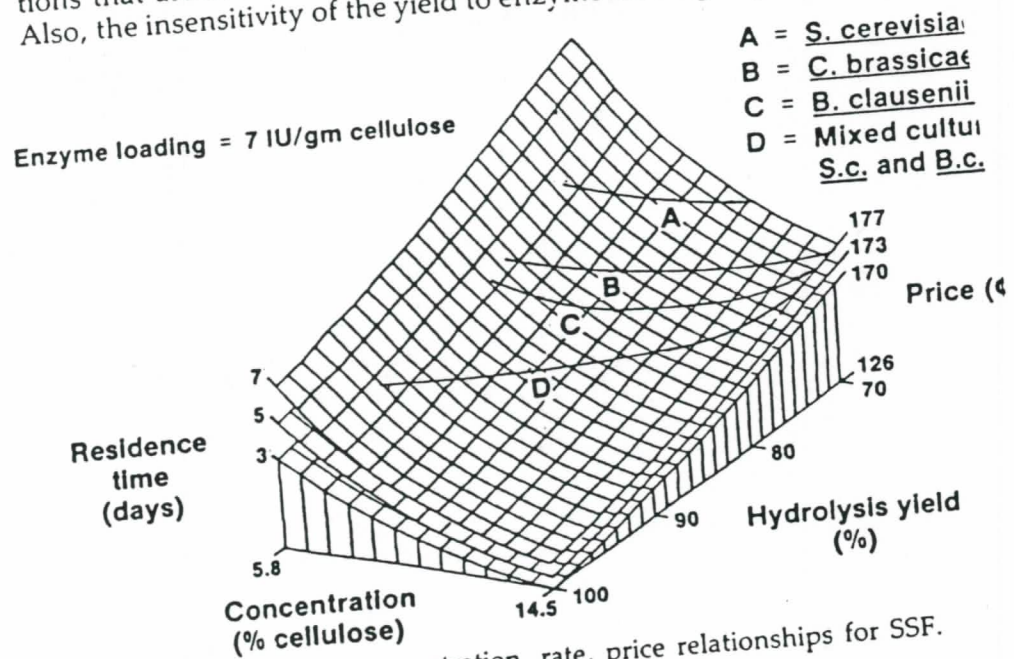


Fig. 6. Yield, concentration, rate, price relationships for SSF.

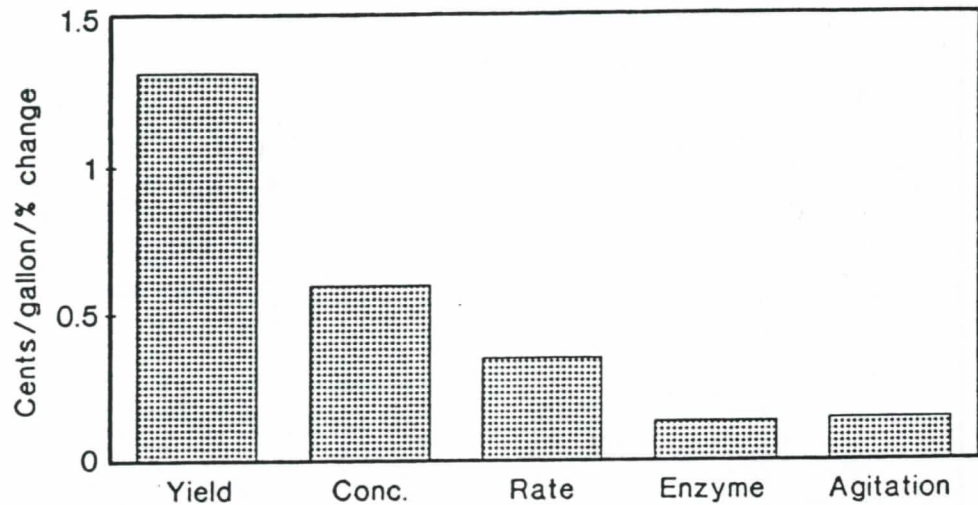


Fig. 7. Sensitivity of ethanol selling price to major process parameters.

be possible to further reduce the enzyme loading. Because of the shape of the yield curve, the price has an optimum at an intermediate value of cellulose concentration (10%), whereas the optimum experimental value of enzyme loading is the minimum tested (7 IU/g cellulose). Increases in enzyme loading only slightly increase the processing cost, but bring about no improvement in performance. This strongly suggests that enzyme loadings can be further reduced.

As the agitation power supplied to a fermenter is increased, the rate of hydrolysis increases at first and then decreases (21,22). This suggests that as agitation is increased, the power input disturbs the boundary layer around the solid particles, helping to reduce local buildups of concentration on or near the surface of the particle. At higher agitation rates, yield decreases, suggesting that enzyme is being inactivated by shear denaturation (23,24). Additionally, the rate and extent of denaturation appear to increase in the presence of an air-liquid interface (25). Studies of enzyme denaturation in stirred reactors suggested that enzyme denatur-

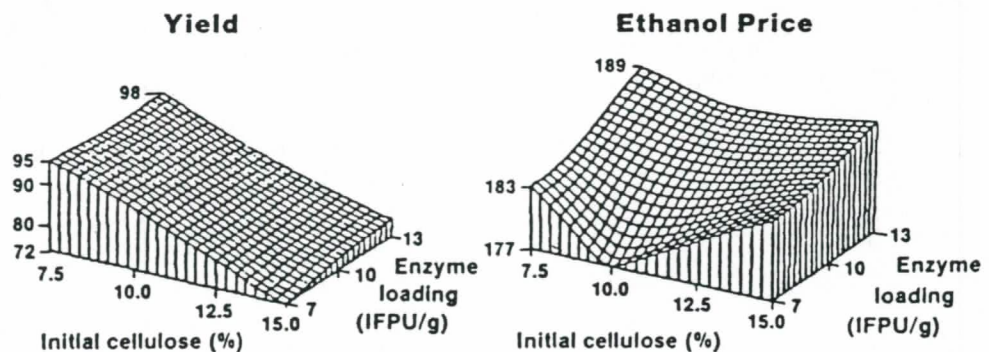


Fig. 8. Optimization of yield and ethanol price with respect to initial solids concentration and enzyme loading.

ation was caused either by high shear near the impeller tip or by thermal deactivation caused by local hot spots near the impeller (26). While these studies give us a qualitative understanding of the important phenomena, they offer no quantitative guide to the effect of power input on hydrolysis yield. This study uses a power input of 2 hp/1000 gal of reactor volume (27). This is a significant cost to the process (\$13.4/gal), but we have little knowledge of whether this is near the optimum or what the requirements for efficient hydrolysis are. Real optimization of this tradeoff awaits development of the data necessary for the analysis.

Process Improvements

The preceding analysis tells us how to optimize the process within the current parameters. However, there still remains the possibility of not merely optimizing the available performance but changing the process. Three examples are: increasing the temperature of the SSF process, taking steps to reduce the inhibition of the cellulose enzymes and yeast by ethanol, and recycling the unreacted solids to increase the hydrolysis efficiency and recover enzymes. Whereas the rate and extent of hydrolysis at 37°C are higher in SSF processes than in straight saccharification at either 37° or 50°C, the operating temperature remains below the long-term optimal operating temperature of the cellulase (45°C). Increasing the hydrolysis temperature should increase the reaction rate, assuming that yeasts can be found that are capable of carrying out the higher temperature process. Several yeasts (*C. lusitaniae*, *C. brassicae*, *S. uvarum*, and *C. acidothermophilum*) have been found that are capable of fermenting glucose at temperatures of 41°–43°C (17). In this temperature range, the activity of the cellulase enzyme complex increases approximately 8%/°C, suggesting that a total increase of up to 60% could be achieved by raising the hydrolysis temperature from 37° to 43°C. This would have the effect of decreasing the residence time from 7 to 4.5 d and decreasing the selling price from \$1.78 to \$1.67/gal. However, as was seen in the discussion of the tradeoff of substrate and enzyme loading, the limiting factor in SSF processes may no longer be the performance of the enzyme complex but the combined ethanol and temperature tolerance of the yeast. This tradeoff could become especially important because ethanol tolerance decreases as temperature is increased.

It is widely known that ethanol accumulation inhibits the activity of the yeast that carries out the fermentation. It is also reported that ethanol independently inhibits the activity of the cellulase enzyme complex. Although Blotkamp (28) reported that ethanol did not inhibit the action of cellulase, later investigations by the same group showed an inhibitory effect (29). More recent investigators have also reported significant effects. Gosh (30) and Takagi (31) reported that 50% inhibition of the cellulase complex occurs at a glucose or ethanol concentration of 2.5%. As glucose is converted to ethanol with a theoretical weight efficiency of 51%, this

means that a given amount of glucose is twice as inhibitory as sugar after it is converted to ethanol. Further, Ooshima (32) found that the inhibition is caused by the interference of ethanol with the absorption of the exoglucanase on the cellulose surface. This suggests that at the ethanol concentrations we are currently achieving, the ethanol may be strongly inhibiting not only the yeast but the enzyme as well.

Ghose (33) tested a system in which a flash unit was connected to the SSF reactor to periodically remove ethanol from the hydrolysis mixture when it built up to inhibitory levels. Using this technique, productivities were 44% higher than in a similar reactor without ethanol removal. Since the ethanol concentrations achieved in our experiments are higher than those of Ghose, it is possible that our degree of cellulase inhibition by ethanol may be greater. However, even a 44% increase in rate should reduce the selling price of ethanol by \$.08/gal over the base case. However, it should be kept in mind that there are other reasons for considering selective removal of the ethanol from the reactor. As discussed in the previous sections, ethanol tolerance of the yeasts may well be the factor limiting yield and concentration at high ethanol loadings and may also reduce the maximum temperature at which we can operate a SSF process at high yields.

Two major types of enzyme recycle schemes have been proposed: those in which enzymes are recovered from the liquid phase, and those in which enzymes are recycled by contact with unreacted solids. Systems of the first type have been proposed for separate hydrolysis and fermentation systems, which operate at temperatures of 50°C. These systems are favored at such high temperatures because increasing temperature increases the ratio of enzyme in solution to enzyme adsorbed on the solid (34) and at high cellulose conversions. Conversely, as the temperature is decreased, the amount of enzyme adsorbed on the solid increases and recycle of the enzyme by solids recycle becomes more attractive (35,36). At the lower temperatures encountered in SSF processes, it appears that solids recycle would be the most effective.

Recycle of the residual solids remaining after the 7-d SSF also offers the potential for increasing the overall process yield, and decreasing the enzyme requirements of the process. Yield would be increased because residence time of the recycled solids would be effectively doubled. In the simulations conducted, it was assumed that the hydrolysis behavior of the recycled unreacted cellulose was identical to that of the original feedstock. This is not strictly true. The cellulase remaining toward the end of the reaction is considerably more resistant to hydrolysis than the bulk of the original material. However, since the final days of the SSF reaction are operating primarily on such resistant cellulose, the error introduced by this assumption may not be great.

As the fraction of the residual solids recycled is increased from 0% to 60%, the yield of ethanol increases linearly from 88% to almost 95% (Fig. 9), decreasing the cost of feedstock to the process and slightly reducing

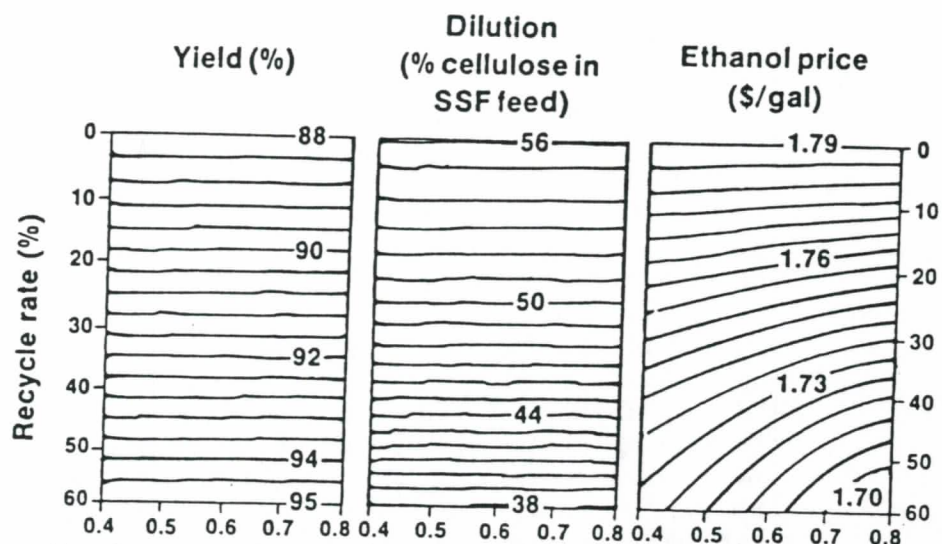


Fig. 9. Dependence of yield, cellulose dilution, and ethanol price on fraction of unreacted solids recycled, and percent of enzyme absorbed on solid substrate.

the costs of pretreatment and environmental control. Somewhat offsetting this is the fact that because the feedstock is not delignified before being sent to hydrolysis, recycle of unreacted solids actually implies a recycle of inerts (such as lignin), and a decreasing fraction of the solids in the reactor is composed of cellulose. Therefore, to maintain a constant 10% cellulose content in the reactor feed, it is necessary to process higher solids slurries at higher recycle rates. For example, with no recycle, a 15.6% solids slurry is charged to the reactor, whereas at 60% recycle, the reactor must process a 21% solid feed. Such a slurry would undoubtedly resemble a wet solid. However, it would not be as dry as a 21% solid slurry of fresh wood, because the water-absorbing capacity of lignin is not nearly as great as that of cellulose or hemicellulose. The reaction mixture would tend to liquify as the cellulose hydrolysis proceeded; however, even at the end of the reaction the reactor would contain approximately 10% lignin. Unfortunately, since we lack the data to accurately predict the tradeoff between agitation power, yield, and ethanol selling price in the basic design, we also lack the data to predict the effect of increasing solid content and viscosity. Thus although the model predicts a decrease in ethanol selling price of up to \$.08/gal for the high recycle case, the savings may be overstated because of the difficulty in processing and agitating the heavier suspension.

CONCLUSIONS

Simultaneous saccharification and fermentation systems offer large advantages over separate hydrolysis and fermentation systems for the production of ethanol from lignocellulosic materials because of their

great reduction in end-product inhibition of the cellulase enzyme complex. Because cellobiose is the most potent inhibitor of the complex, systems using a mixed culture of *S. cerevisiae* (a robust fermenter of glucose) and *B. clausenii* (a cellobiose-fermenting yeast) are the most promising so far identified. The SSF process has slightly increased yields (88% vs 73%) and greatly increased product concentrations (equivalent glucose concentrations of 10% vs 4.5%). However, the greatest improvement is that the enzyme loading can be reduced from 33 to 7 IU/g cellulose, which dramatically cuts what was formerly the largest single contribution to the cost of ethanol. SHF systems are predicted to produce ethanol for \$2.66/gal, whereas SSF processes with similar design bases are predicted to produce at \$1.78/gal.

The performance of SSF appears to be limited by the performance (combined temperature and ethanol tolerance) of the yeast rather than by the performance of the enzyme, and even enzyme loadings as low as 7 IU/g cellulose are saturated in enzyme. Power costs appear to be an important contributor to the overall cost, but they are not yet estimated accurately. Potential process improvements include the use of more thermotolerant yeasts (provided they can achieve high ethanol concentrations), selective removal of ethanol from the reaction mixture during processing to decrease inhibition of both the yeasts and enzyme, and recycle of unreacted solids to increase yields and decrease enzyme consumption.

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