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Ethanol Production from Spent Sulfite Liquor Fortified by Hydrolysis of Pulp Mill Primary Clarifier Sludge

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ABSTRACT

Some low-yield sulfite pulping operations ferment spent sulfite liquor (SSL) to remove biochemical oxygen demand associated with dissolved sugars while at the same time generating ethanol as a salable product. Simultaneous saccharification and fermentation (SSF) of primary clarifier sludge in a medium of SSL was proposed as a means of reducing the amount of sludge to be disposed of while at the same time increasing ethanol productivity. In this article, the option of fortifying existing SSL fermenting processes with the sugars produced via *in situ* enzymatic hydrolysis of sulfite primary clarifier sludge (PCS) has been explored. In 100% SSL PCS hydrolysis rates as high as 3.4 g/(L·h) were observed at an initial enzyme loading of 10 filter paper units (FPU)/g PCS. To reduce the deleterious effects of glucose inhibition, single-stage SSF was carried out using cellulase enzymes and *Saccharomyces cerevisiae*. The production rate of ethanol in SSL was increased by as much as 25% through the SSF process.

Index Entries: SSF; primary clarifier sludge; ethanol production.

INTRODUCTION

The environmental advantages of biomass-derived fuels has continued to drive advancements in technologies for the production of ethanol from lignocellulosic materials. However, several factors have hindered economic exploitation, including: low prices for conventional energy resources, high costs of dedicated biomass crops, and costly, multiple-step biomass processing schemes that result in significant capital and operating expenditures *(1–4)*. As such, in order for technology to move from laboratory to pilot or commercial scale, it will be necessary to exploit niche opportunities to minimize feedstock and processing costs. One such opportunity exists in the pulp and paper sector.

The pulp and paper industry is a natural choice for integration of ethanol production with fiber processing. Particularly in light of a tightening supply of fiber in North America, there is little economic incentive to divert fiber from pulp produc-

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tion to lower value ethanol. However, the pulp and paper industry produces large quantities of predominantly cellulosic solid waste in the form of primary clarifier sludges (PCS). Use of such materials as feedstocks has a number of advantages over dedicated woody feedstocks. First, the material is a waste and therefore represents a disposal cost to mills. Second, in chemical pulping processes, waste cellulosics like PCS have been pretreated to remove lignin and hemicellulose. Third, bioconversion technologies can use aqueous slurries of PCS as opposed to conventional disposal options, which require that the material be dewatered. Finally, a range of facilities are present at pulp mills, which would benefit an integrated bioconversion facility. These facilities can include low-grade heat, materials handling equipment, skilled personnel, and in some cases, existing full-scale fermentation and distillation operations. In previous work, we have shown that PCS from chemical pulping operations is readily amenable to enzymatic hydrolysis to produce fermentable sugars *(5)*. In this article, we describe the development of a technology for increasing the ethanol productivity at a sulfite pulp mill by the *in situ* simultaneous saccharification and fermentation (SSF) of PCS in spent sulfite pulping liquor (SSL).

BACKGROUND

Enzymatic Hydrolysis of PCS

The enzymatic hydrolysis of cellulose can be modeled as a two-step heterogeneous catalytic reaction requiring the synergistic activity of two groups of enzymes, namely (endo- and exo-) glucanases and β-glucosidase. Endo- and exoglucanases work in synergy to degrade the cellulose strands to cellobiose, a glucose dimer. Endoglucanases randomly attack the β-1,4 glycosidic bonds of the water-insoluble cellulose, yielding water-soluble oligomers of varying molecular weights. Exoglucanases attack the nonreducing ends of these intermediates, in the process yielding cellobiose. β-glucosidases convert the cellobiose to glucose.

The rates at which the series of reactions progress are limited in part by the inhibition of enzyme activities as reaction products (sugars) accumulate in the bulk liquid. Exo- and endoglucanases are inhibited by cellobiose. The activity of β-glucosidase is inhibited to a lesser extent by glucose, which causes an accumulation of cellobiose and glucanase inhibition *(6)*. A low (2%) sugar concentration is sufficient to reduce the rate of hydrolysis significantly. Accordingly, considerable effort has been directed toward developing a technology whereby sugars could be removed as they are produced. One of the most successful of these technologies is the SSF process.

Simultaneous Fermentation of Hydrolysis Sugars to Ethanol

During SSF, fermentative organisms are used to convert sugars to alcohol as they are produced. Kinetic advantages are achieved through reduced end-product inhibition, whereas single-vessel processing further reduces production costs. Chief among the disadvantages of SSF technology is the kinetic penalty in hydrolytic efficiency resulting from operating at temperatures that are below the optimum for cellulases. The following equation summarizes the conversion of cellulose to ethanol:

$$(CH_2O)_n \xrightarrow{\text{cellulases}} C_6H_{12}O_6 \xrightarrow{\text{yeast}} 2CH_3CH_2OH + 2CO_2\uparrow + \text{yeast cells} \qquad (1a)$$

Simultaneous hydrolysis and fermentation has been the subject of intense research and development, with recent work focusing on conversion of agricul-

tural residues *(6)*, modeling and reactor development *(7,8)*, and metabolism of oligomeric and five-carbon sugars *(9)*.

Integration at Tembec's Sulfite Pulping Operation

Canadian low-yield sulfite operations do not recover pulping chemicals, choosing rather to produce alternative products from SSL, such as ethanol and lignosulfonates. Tembec operates a low-yield, ammonia-based sulfite-pulping operation in Temiscaming, Quebec, Canada. Tembec currently produces 8–10 million L/year of ethanol by fermenting the hexose sugars in SSL in a continuous process (3 continuous stirred tank reactors in series). The ethanol is upgraded on site through azeotropic distillation. Since the fermentation and distillation plants are operating well below design capacity of 18–20 million L/year, there is a desire to fortify the SSL with sugars to increase ethanol production. It would be advantageous to carry out PCS hydrolysis simultaneously in the fermentation vessels to reduce capital (fewer reaction vessels) and operating (reduced enzyme inhibition) costs.

MODEL DEVELOPMENT

Modeling of sugar uptake, ethanol production, and yeast cell yield in Tembec's continuous process will be carried out using the method of Aiba et al. *(10)*. The model is of the form:

1. Yeast cells:

$$dX_n/dt = D(X_{n-1} - X_n) + \mu_n X_n \tag{1b}$$

2. Reducing sugars:

$$dG_n/dt = D(G_{n-1} - G_n) - (dG_n/dt)_{consumption} + (dG_n/dt)_{production} \tag{2}$$

3. Ethanol:

$$dP_n/dt = D(P_{n-1} - P_n) + Y_{P/X}\mu_n X_n \tag{3}$$

4. PCS:

$$dS_n/dt = D(S_{n-1} - S_n) - (dS_n/dt)_{consumption} \tag{4}$$

In these equations, X_n = the yeast cell concentration in reactor n (g/L), G_n = the reducing sugar concentration in reactor n (g/L), P_n = the ethanol concentration in reactor n (g/L), S_n = the PCS concentration in reactor n (g/L), D = the dilution rate (h^{-1}), μ_n = the specific cell growth rate (h^{-1}), and the term $Y_{P/X}$ is the ethanol yield coefficient for cell growth (ethanol produced [g]/yeast cells produced [g]).

The sign convention in the above equations is that the rates subscripted *production* and *consumption* are positive. Also,

$$\mu_n = \mu_{max}\,(G_n/K_s + G_n)\,[1 - (P_n/P_{max})]^n \tag{5}$$

which is a modification of the Monod equation to account for ethanol inhibition. P_{max} is the ethanol concentration (in 100% SSL) at which further ethanol production ceases.

The term

$$(dG_n/dt)_{consumption} = (1/Y_{X/G})\mu_n X_n \tag{6}$$

Also

$$\upsilon = (dS_n/dt)_{consumption} = a\,(dG_n/dt)_{production} = [V_{max}S_n/K_m + (K_m/K_{ig})G_n + S_n] \quad (7)$$

where V_{max}, K_m, and K_{ig} are kinetic constants; $a = 0.909$.

Ideal mixing is assumed (D = constant = F/V, where V= tank volume and F= volumetric flow rate). This model is presently under development, and some preliminary results are presented in this article.

MATERIALS AND METHODS

Substrate and Enzymes

PCS (30.3% solids) and softwood SSL were obtained from Tembec. The fraction of PCS that was cellulose-accessible to enzymes was 66.4%. Cellulase enzymes were obtained from Novo-Nordisk (Bagsvaerd, Denmark) as Celluclast CCN #1.5L (78 FPU/mL glucanase activity) and Novozym TN #188 (780 IU/mL β-glucosidase activity). Unless otherwise specified, enzyme loading was 10 FPU of glucanase and 100 IU of β-glucosidase activity/g of PCS solids in all hydrolyses and SSF runs. PCS, SSL, and enzymes were stored at 4°C until use.

Hydrolyses

Reactions were performed in 250-mL Erlenmeyer flasks in media containing SSL of various concentrations up to full-strength. To mimic the continuous fermentation system at Tembec in which steady-state sugar concentrations are low, the SSL was prefermented to remove hexoses (and the resulting ethanol was removed by flash vaporization). The flasks were incubated in a rotary shaker (New Brunswick Scientific Co., Edison, NJ) at 33 and 37°C and 150 rpm. All experiments were run in duplicate.

Fermentation and SSF Experiments

The conditions for fermentation and SSF experiments were nearly identical to those under which hydrolyses were performed. Fermentation and SSF runs were performed at 33 and 37°C, which correspond to the temperature range of Tembec's fermentation process.

Saccharomyces cerevisiae (Baker's yeast) inoculum for fermentation and SSF experiments was grown for 24 h prior to use in an enriched medium consisting of 5% glucose, 2.5% peptone, and 0.3% yeast extract, in addition to trace elements and vitamins *(5)*. To initiate a fermentation, 37 vol% inoculum solution was added to flasks containing unfermented SSL in various concentrations, including full-strength. For SSF reactions, the unfermented SSL was fortified with various concentrations of PCS, and cellulase enzymes were added at the same time as the yeast inoculum. All runs were carried out at pH 5.3.

Analyses

Representative samples were withdrawn and centrifuged (13,000*g*, 5 min) using a Fisher (Toronto, ON) microcentrifuge (Model 235C). The concentration of total reducing sugars in each sample was determined colorimetrically using a dinitrosalicylic acid (DNS) reagent *(11)* as previously described *(5,12)*.

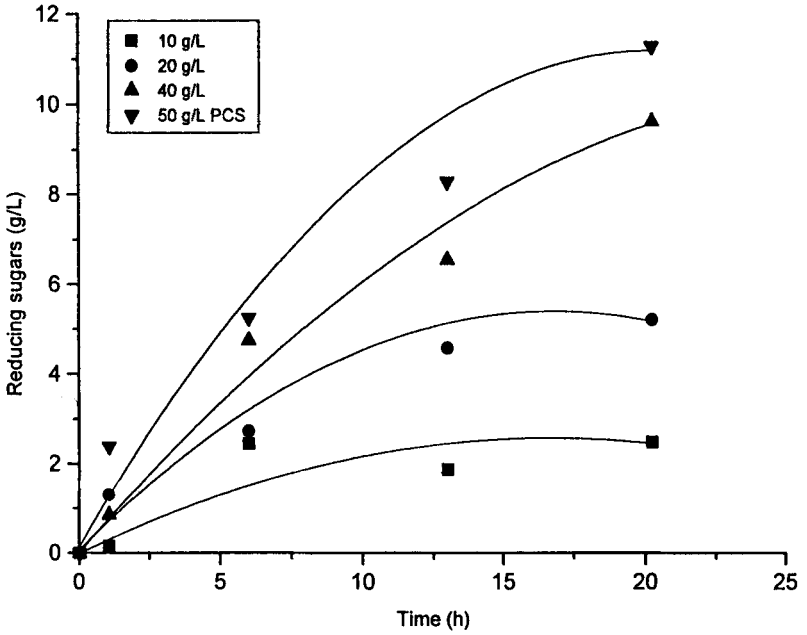

Fig. 1. Enzymatic hydrolysis of PCS in 100% SSL at different initial PCS concentrations. Temperature: 33°C; enzyme loading: 10 FPU/g PCS. SSL concentration equivalent to the liquid feed to Tembec's continuous fermentation system. The resulting data were applied to Eq. (7) to obtain Eq. (8).

A Hewlett-Packard Gas Chromatograph (5880A Series) equipped with a flame ionization detector (FID) and a Carbowax 20M packed column (Supelco, Bellefonte, PA) was used to determine the ethanol concentrations of the samples. External standards ranging from 0.1 to 1.0 g/L ethanol and containing 1.5 g/L isopropanol as an internal standard were used. The oven temperature was 70°C.

RESULTS

Hydrolyses of PCS in SSL

Experiments focused on obtaining kinetic parameters for use in a mathematical model to predict the ethanol production rate of Tembec's fermentation system when modified to run in SSF mode. All data to be applied toward the simulation were taken from experiments running at a temperature of 33°C (the normal operating temperature of Tembec's system). Typical results from a set of hydrolyses of PCS in full-strength (100%) SSL (the feed concentration to Tembec's fermentation system) are given in Fig. 1.

An analysis of the initial rate data using Lineweaver-Burk coordinates ($1/\upsilon$ vs $1/S_o$, where υ and S_o are the sugar production rate and initial substrate [PCS] concentration, respectively) produced a linear relationship between $1/\upsilon$ and $1/S_o$ ($R^2 = 0.996$). The average rates of hydrolysis over the first hour were used to determine the rate law in the absence of competitive inhibition by glucose:

$$\upsilon = dG/dt = (7.9S/113 + S)\ [g/(L{\cdot}h)] \quad (8)$$

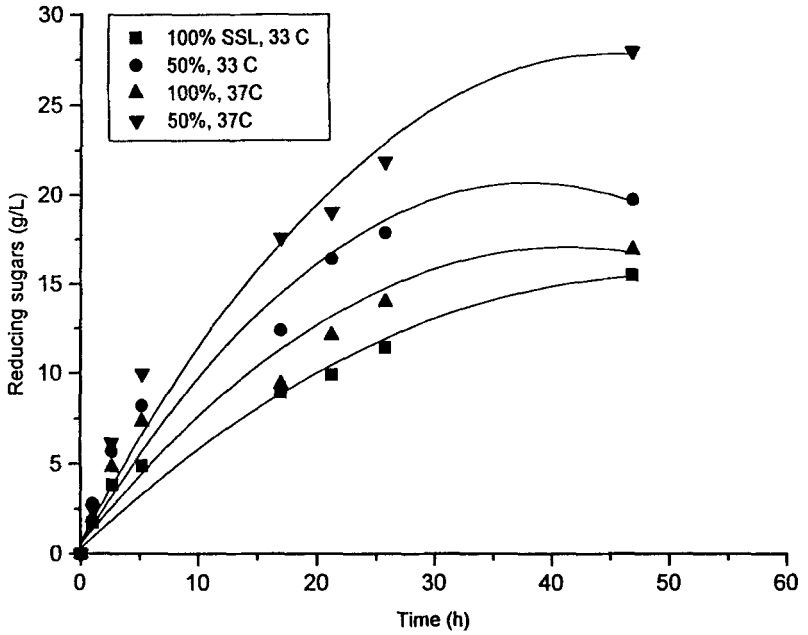

Fig. 2. Effects of temperature and SSL concentration on hydrolysis of PCS. The temperatures (33 and 37°C) are representative of the temperature range of Tembec's process. Enzyme loading: 10 FPU/g PCS.

where G and S are the reducing sugar and PCS concentrations (g/L), respectively. Further analysis using Foster-Niemann coordinates revealed no detectable effect of competitive inhibition by glucose on the hydrolysis rate, υ. This was the result of SSL inhibition of enzyme activity, which masked the effect of glucose inhibition over the range of sugar concentrations (0–11.2 g/L) encountered in the experiment. Equation (8) is therefore valid over this range of sugar concentrations.

To determine the interactive effect of SSL concentration and temperature on hydrolysis rate, hydrolyses were carried out at two SSL concentrations (100 and 50%) and two temperatures (33 and 37°C) (Fig. 2). Higher hydrolysis rates were observed at the higher reaction temperature (37°C), although this effect was more pronounced for the runs performed in the more dilute (50%) SSL. For example, the hydrolysis rate in 50% SSL was 43% higher at 37°C (0.60 g/[L·h]) than at 33°C (0.36 g/[L·h]). By contrast, the hydrolysis rate in 100% SSL was only 9% higher at 37°C (0.36 g/[L·h]) than at 33°C (0.33 g/[L·h]).

Fermentation of SSL

Batch experiments were performed to quantify the kinetic parameters and yield coefficients of cell growth and ethanol production. In one such experiment, 33% SSL fortified with glucose to achieve initial total reducing sugar concentrations of 23, 38, 66, and 85 g/L was fermented (Fig. 3).

Ethanol accumulation in the bulk liquid (or more significantly within the yeast cells) is known to inhibit cell activity and, hence, lower the ethanol production rate. A set of experiments performed in 100% SSL at 33°C were designed to observe this effect (Table 1).

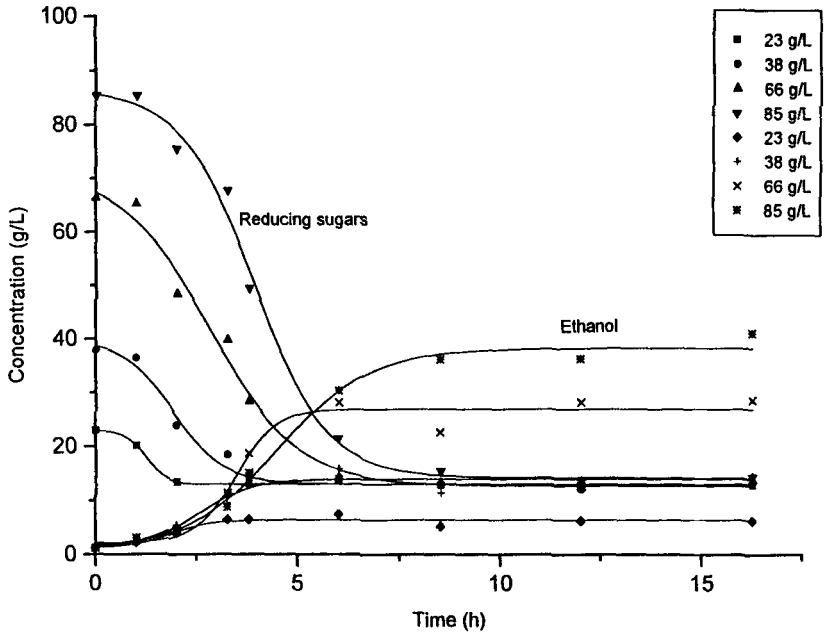

Fig. 3. Effect of initial sugar concentration on fermentation of glucose-fortified 33% SSL. Temperature: 33°C.

Table 1
Overall 21-h Ethanol Production Rates in 100% SSL

Initial ethanol concentration, g/L	Final ethanol concentration, g/L	Ethanol production rate, g/(L·h)
12.5	32.4	0.95
17.9	37.0	0.91
22.9	39.9	0.81

The interactive effects of SSL and ethanol concentration on yeast cell production were determined at one initial ethanol concentration (23 g/L) in both 33 and 100% SSL (Fig. 4). At an initial ethanol concentration of 23 g/L, the maximum specific cell growth rates were 0.07 and 0.38/h in 100 and 33% SSL, respectively. In runs performed in 100% SSL, the maximum specific cell growth rates at initial ethanol concentrations of 12.5 and 23 g/L were 0.12 and 0.07/h, respectively. These observations are qualified by the fact that different sizes of yeast inoculums were used.

SSF of PCS in 100% SSL

The SSF of PCS was conducted in unfermented, 100% SSL as a means of comparing the results predicted by the model to be developed. The ethanol concentrations after 24 h corresponding to initial PCS concentrations of 20, 35, 50, and 65 g/L as well as a control (0 g PCS/L) are presented in Table 2.

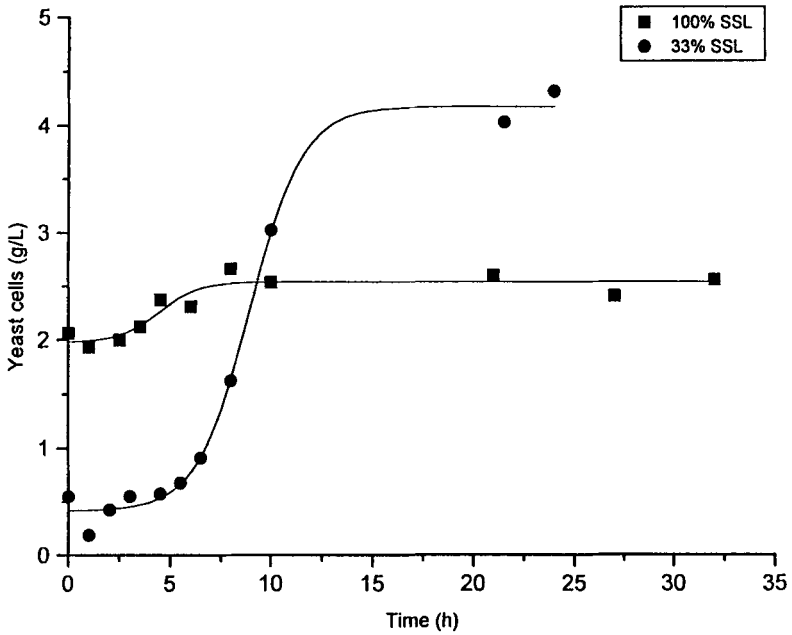

Fig. 4. Yeast cell production in 50 and 100% SSL at an initial ethanol concentration of 23 g/L. Temperature: 33°C.

Table 2
SSF of PCS in 100% SSL: Ethanol Concentrations After 24 h

PCS concentration, g/L	Control	20	35	50	65
Final ethanol concentration	18.7	21.4	21.6	22.5	23.4
Increase over control, %	—	14.4	15.3	20.0	25.0

DISCUSSION

Effect of Temperature and SSL Concentration on PCS Hydrolysis Rate

Hydrolysis of PCS is inhibited by SSL (Fig. 2), an observation that contrasts our earlier findings with more dilute SSL concentrations at a higher temperature (50°C); however, the fact that inhibition was less severe at the higher temperature also supports these findings (*5*). It is possible that the difference in degree of inhibition is partly owing to batch-to-batch variation in the SSL. This contention is supported by observed wide variations in fermentability of the SSL as delivered from the mill. If SSL inhibition of hydrolysis continues to be a concern, it is possible that sugar production could be increased by hydrolyzing PCS in SSL prior to its evaporation (from 50–100% SSL). However, this advantage might be offset by a hydrolysis rate reduction owing to the competitive inhibition of the enzymes by the higher levels of reducing sugars that would result from choosing this method over an SSF process (where the sugars produced via hydrolysis would be continuously removed via fermentation).

Effect of SSL and Ethanol Concentration of the Fermentation of Wood Sugars

Over a range of ethanol concentrations typical of those found in Tembec's full-scale system, the presence of SSL (Fig. 4) and ethanol in the bulk medium was found to inhibit yeast growth (results regarding ethanol inhibition not shown), although SSL was found to inhibit yeast to a much greater extent as compared to a very mild effect of ethanol inhibition. Despite the low cell concentrations in the batch experiments (a maximum of about 3 g/L in 100% SSL vs approx 5 g/L for Tembec's continuous, cell recycle process), *S. cerevisiae* is capable of consuming SSL sugars at rates comparable to those achieved in Tembec's process and remains active when ethanol levels in 100% SSL are as high as 39 g/L. For example, in batch experiments, the overall ethanol production rate over a 21-h period in 100% SSL with an initial ethanol concentration of 12.5 g/L was 0.95 g/(L·h) (Table 1), yielding a final ethanol concentration of 32.4 g/L. When one compares this rate with the overall ethanol production rate of Tembec's continuous system (0.8 g/[L·h] with a final ethanol concentration of 20 g/L), it is evident that an increase in sugar loading on their system resulting from the *in situ* enzymatic hydrolysis of their PCS would be readily handled in their existing process.

SSF of PCS in SSL

Preliminary SSF results indicate that Tembec could significantly increase its ethanol production rate while at the same time reduce the amount of PCS of which it must dispose. For example, over a period of 24 h (comparable to the residence time of Tembec's process), a 25% increase in the ethanol production rate over the control (no PCS addition) was forthcoming when 65 g PCS/L were simultaneously hydrolyzed in the fermentation medium. The fact that there was marginal benefit to increasing PCS loading into the vessels indicated a limited degree of hydrolysis occurring in the SSF reactions. It is likely that this inhibition was mainly the result of the presence of hexoses in the unfermented SSL, and that significant hydrolysis may not have occurred until after these sugars were consumed. Consumption of approx 65 g/L of sugar initially present in 100% SSL requires nearly 24 h (result not shown). As such, by using unfermented SSL we effectively minimized the benefit to PCS fortification over the 24-h period of observation.

As previously noted, our batch reactions have two major differences from the full-scale system at Tembec. First, the concentration of hexoses in our system (initially 65 g/L) is significantly higher than the steady-state concentration in Tembec's full-scale system. Second, in our batch reactors, yeast concentrations range from 1 to 3 g/L, increasing over the time-course of the SSF reaction. These values are initially much lower and rise to a yeast concentration only 60% of that observed in the full-scale system. Since both of these factors have exerted strongly negative impacts on the extent of cellulase inhibition observed in the batch experimental system, it is likely that much greater improvements in ethanol production can be achieved through SSF of PCS than those already observed.

CONCLUSION

1. Sulfite primary clarifier sludge is a suitable substrate for hydrolysis in SSL. The rate equation for the hydrolysis of PCS in 100% SSL was found to be

$\upsilon = dG/dt = (7.9S/113 + S)$ (g/[L·h]). Glucose inhibition of enzyme activity was not evident at the reducing sugar levels encountered. The rate law is valid as long as the enzyme loading is maintained at a level of 10/100 IU/g PCS at a temperature of 33°C. Although it was derived from data produced in batch trials, it is also valid for a continuous system like the one proposed for the Tembec mill as long as perfect mixing is assumed.

2. SSL was found to inhibit enzyme activity. At 37°C, the rates of hydrolysis were found to be 0.60 and 0.36 g/(L·h) in 50 and 100% SSL, respectively. Temperature also affected the hydrolysis rate. The rate of hydrolysis of PCS in 100% SSL was 9% greater at 37°C than at 33°C. The temperature chosen for the model was 33°C. Hence, the model would underestimate the rate of hydrolysis (and the ethanol production rate) at times when Tembec's full-scale system operated at higher temperatures.
3. The rate of ethanol production in 100% SSL was comparable to that currently achieved by Tembec's existing SSL fermentation process, but over a higher range of ethanol levels. Therefore, Tembec's fermentation system should be able to handle the increase in sugar loading following its modification to an SSF process utilizing PCS.
4. Preliminary tests have shown that the rate of ethanol production in SSL can be increased by as much as 25% through the simultaneous enzymatic hydrolysis of PCS. Work is continuing to improve on this result.

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