EVALUATION OF PAPER SLUDGES FOR AMENABILITY TO ENZYMATIC HYDROLYSIS AND CONVERSION TO ETHANOL

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ABSTRACT. Forty-four sludges from 39 paper mills were characterized with respect to composition and 36 of these sludges were examined with respect to enzymatic hydrolysis and conversion to ethanol in a simultaneous saccharification and fermentation system consisting of a commercial fungal cellulase preparation and fermentation by *Saccharomyces cerevisiae* For a significant fraction of mills paper-making is a highly effective fortuitous pretreatment for enzymatic hydrolysis although substantial variability is observed among sludges from the same process type.

APPLICATION STATEMENT. Biological conversion of waste sludges to ethanol offers potential benefits to paper mills by diverting a waste stream into a saleable product, and is also a point-of-entry and proving ground for emergent industrial processes featuring enzymatic hydrolysis of cellulosic biomass.

EXTENDED SUMMARY. Forty-four sludges from 39 paper mills were characterized with respect to composition and 36 sludges were examined with respect to enzymatic hydrolysis and conversion to ethanol in a batch simultaneous saccharification and fermentation system consisting of a commercial fungal cellulase preparation and fermentation by *Saccharomyces cerevisiae*.

For all 44 sludges tested, the mean solids content was 33 wt.% (on a dewatered sludge basis), the mean glucan content was 39.2% (dry sludge basis), and the mean ash content was 26.4% (dry sudge basis. More detailed compositional analysis on a subset of 15 sludges revealed that non-glucan carbohydrate (xylan and mannan) accounted for a mean value of 14.4% of glucan, with xylan present in higher amounts than mannan in 11 of the 15 sludges. Total carbohydrateaccounted for 42.3 $\pm$ 15% of the dry weight, with ash and acid insoluble volatile matter making up most of the balance. Most of the balance was made of acid-insoluble volatile

matter (17.4 ± 8.6) and ash (23.2 ± 18.7). Arabinose and galactose were not measurable at the limit of detection, corresponding to 1 wt.% dry sludge. Substantial variability was observed with respect to sludge composition, even for sludges produced via the same process type. Sludge composition was evaluated over time for samples taken at different times over a three-week period for 10 sludges and over five years for one sludge. Compositional variability over time was significant, but less than variability among sludges from different mills.

Reactivity screening tests were carried out in serum vials using an initial glucan concentration of 20 g/L. Supplemental β-glucosidase had a marked positive effect on ethanol yields, whereas cellulase loading (10 or 20 IU/g cellulose) and growth medium (lean or rich) did not have a significant effect. Of the 28 sludges tested with supplemental β-glucosidase, which increased the ethanol yield markedly, 19 achieved ethanol yields (as a % of theoretical based on glucan content) ≥ 80 %, 12 achieved ethanol yields ≥ 90%, and 8 achieved ethanol yields ≥ 95%. Ethanol production, indicative of yeast metabolism, was observed in all but two of the sludge samples tested. Mean ethanol yields were 100.3 ± 7.9% for three sulfite sludges, 94.2 % for one semichemical sludge, 87.6 ± 10.1% for eleven bleached Kraft sludges, 83.7 ± 20.6% for six deinking sludges, 81.7% for 3 sludges with no pulp mill, 69.7% ± 13.8% for unbleached Kraft sludges, and 36.1% for one thermochemical sludge. As with composition, SSF results exhibited great variability even for sudges of the same process type. Our results indicate that many paper sludges are highly susceptible to enzymatic hydrolysis in as-received form, and hence that paper-making is a highly effective fortuitous pretreatment process for a significant fraction of mills.

The mass of the post-SSF filter cake, obtained using a laboratory vacuum filter and measured for 9 sludges, was reduced by 28 to 85% as compared to the mass of the wet filter cake at the as-received solids concentration after dewatering at the mill. The amount of sulfuric acid required to adjust the pH of sludge slurries to 4.5 prior to SSF was < 10 g/Kg dry sludge for 80% of the sludges tested, and thus represents a relatively small cost.

Several factors make paper sludge an attractive raw material for emergent industrial processes featuring enzymatically-based processing of cellulosic biomass. These advantageous factors include the high reactivity of many paper sludges, the zero or negative cost of these materials, the potential for process simplification afforded by elimination of pretreatment as well as use of utilities and other infrastructure elements available at many mills, and the potential for enhanced mineral recovery as a result of enzymatic hydrolysis. The potential value of paper sludge processing is on the order of $220/dry ton, with contributing factors including ethanol production, avoided disposal cost, and mineral recovery. Substantial additional work is necessary in order to determine whether it is feasible to recover this value in an economically-viable manner.

INTRODUCTION

Paper sludges are solid residues arising in the course of pulping and paper-making operations. Sludges are typically removed from process wastewater in a clarifier, mechanically dewatered to between 20 and 35% solids, and may be available with or without biosolids associated with secondary wastewater treatment. As of 1995 (*1*), landfill was the disposal method used for approximately 51% of overall industry sludge production, with the remainder disposed of via incineration (e.g. in a bark boiler), land application, beneficial uses (e.g. aggregate, animal bedding, etc.), and recylcing. Because paper sludge has a high water and ash content and can lead to scaling problems, it is not a particularly desirable boiler fuel (*2-4*). However, the volume reduction accompanying incineration is sufficiently great that feeding sludge to a boiler can be

the least-cost disposal option. Alternatives to landfill disposal are being aggressively pursued by many paper companies (*5-9*), and are specified as a research priority in Agenda 2020 for America's Forest, Wood, and Paper Industry (*10*).

Based on values from the literature (*1*) and our own communication with paper mills, we estimate that sludge is produced at a rate of 4 to 10% of total paper product for processing of virgin fiber (dry basis), and 10 to 50% of total product for processing recycled fiber. Typical costs (capital and operating) for disposing sludge in industrial landfills are on the order of $20 to $30 per wet ton ($60 to $90/dry ton) in paper-making regions of the Northeast, Midwest, and Northwest, with lower costs common in Southern states. At a disposal cost of $25/wet ton, 30% moisture, and sludge output 6% of paper output (typical of paper production from primary fiber), the cost of sludge disposal is 5$/ton of paper produced; for sludge output 25% of paper output (typical of paper recycling), the cost of sludge disposal is $23/ton of paper produced. The cost of sludge disposal thus represents 1 to 5 % of the price of paper. While this cost is not large, it can be significant given the small profit margins characteristic of the paper industry. As well, mill operators have told us that sludge production is a significant factor impacting plant siting and public relations at many mills.

Paper sludges typically contain 25 to 75% carbohydrate with the balance lignin, inert clays and fillers. The three most prominent mineral components are typically kaolin and $CaCO_3$ (used for coating and/or as filler) and TiO_2 (used for whitening). The data in **Table 1** provide an indication of the potential value of paper sludge components. For a sludge with 50 wt.% carbohydrate (dry weight basis) and a 67% moisture content (wet filtercake basis), the potential value of ethanol production is $91/dry ton sludge. The potential value of sludge minerals, assuming the composition presented in Table 1, is about $54/dry ton sludge, with titanium dioxide the largest contributor. In addition, avoidance of sludge disposal has potential value of about $75/dry ton. Thus the potential value of paper sludge exclusive of the cost of value recovery is on the order of $220/dry ton for a typical sludges. This value is substantially higher for sludges that have higher than average carbohydrate content or tipping fees.

Biological conversion of paper sludge is potentially attractive relative to other options for sludge management because it can generate products of relatively high value, it is not hindered by the high water content typical of sludges, and because few if any additional waste materials of non-biological origin result from the process. Among biologically-derived compounds, ethanol has received the most attention as a product of paper sludge conversion. Duff and Murray (*12*) have comprehensively reviewed ethanol production technology in the context of forest industry waste materials. Prior studies have investigated hydrolysis of paper sludge via enzymes only (*13-16*), saccharification followed by subsequent fermentation (*15,16*), and simultaneous saccharification and fermentation (*14,17,18*). Duff and coworkers found that a thermomechanical sludge was less susceptable to enzymatic hydrolysis than a kraft and sulphite sludge (*13*), and that a deinking sludge was relatively recalcitrant to enzymatic hydrolysis. Moritz and Duff (*18*) demonstrated conversion of paper sludge to ethanol in a medium containing spent sulfite liquor as the liquid phase. Katzen et al. (*17*) report ethanol yields > 80% of theoretical for 13 chemical pulp/paper sludges in as-received form, but that groundwood sludge required acid-prehydrolysis to achieve high hydrolysis yields. Information was not given on the hydrolysis yields, origin, or composition of specific sludges. Jeffries and Schartman (*15*) examined several process configurations for enzymatic hydrolysis of a paper sludge, achieving saccharifications up to 65%.

Component	Typical Composition[a] (mass%)		Unit Value[b] ($/ton)	Value in sludge[c] ($/ton)	
	Wet Basis	Dry Basis		Wet Basis	Dry Basis
Disposal	-	-	-	25	75
Water	67	-	-	-	-
Carbohydrate	16.7	50	-	30.4	91.3
Mineral					
Kaolin	4.3	13	100	4.3	12.9
$CaCO_3$	3.6	11	100	3.6	10.8
TiO_2	0.5	1.5	100	10.0	30.0
Subtotal	8.4	25.5	-	17.9	53.7
GRAND TOTALS	**92.1**	**75.5**[d]		**73.3**	**220**

[a] Carbohydrate and total mineral composition based on results from Tables 2 and 3. Relative amounts of mineral components are for alkaline paper-making from (*11*). [b] Unit values for mineral components from (*11*). Values do not reflect the cost of mineral recovery, and will depend on attributes such as purity and particle size. Higher values are possible if minerals can be recovered in high quality form. [c] Minerals valued in terms of separated components; Carbohydrate valued as ethanol based on a 46% mass yield of ethanol per unit monomeric sugar, and an ethanol price of $1.20/gal. [d] In addition to carbohydrate and mineral components, sludge contains substantial amounts of volatile matter (see Table 3).

Table 1. Potential value of paper sludge components.

This paper reports screening studies to evaluate the amenability of paper sludges to enzymatic hydrolysis and subsequent conversion to ethanol. For this purpose we have used a simultaneous saccharification and fermentation (SSF) system comprised of a commercial cellulase preparation in combination with fermentation of soluble sugars to ethanol by the yeast *Saccharomyces cerevisiae.* Examined extensively in the context of converting cellulosic materials to ethanol (*e.g. 19*), SSF involves hydrolysis of cellulose fiber to cellobiose and other soluble products by exoglucanse and endoglucanase enzyme activities present in the cellulase preparation, conversion of cellobiose to glucose by β-glucosidase enzyme activity, and conversion of glucose to ethanol by yeast fermentation (**Figure 1**). By consuming soluble sugars as they are formed, the SSF process configuration lessens inhibition of the rates of glucan and cellobiose hydrolysis compared to sequential hydrolysis and fermentation. The objective of this work is to provide a

comparative evaluation of the susceptibility of different paper sludges to biological processing encompassing a greater number of sludges than has been examined previously in this context, and to determine the extent to which this susceptibility can be related to SSF conditions and sludge origin.

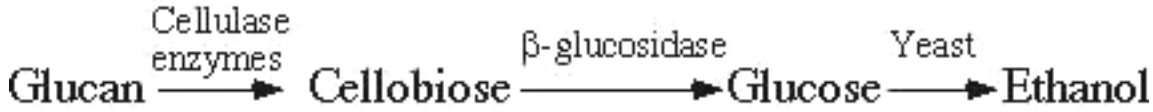

Figure 1. Schematic diagram of the SSF process.

MATERIALS AND METHODS.

Sources of sludges and other cellulosic substrates. Sludges were provided by 44 pulp and paper mills across the United States with the kind assistance of mill personnel, and were stored at 4°C in 5 gallon buckets for up to 6 months. Avicel, a microcrystalline cellulose, was provided by FMC Corporation (Newark, DE). Pretreated poplar, kindly provided by the National Renewable Energy Laboratory (NREL, Golden CO), was produced by batch reaction of 2 mm poplar chips at 160°C for 20 minutes in the presence of 0.45% H_2SO_4. Pretreated mixed hardwood was produced from wood flour (90 % birch, #60 mesh) obtained from the Louis O. Beede Co. (Lowell MA). Pretreatment was carried out in a flow reactor at 220°C for a 10 second residence time at 0.9% H2SO4 using equipment and procedures described previously (*20,21*).

Enzymes and microorganisms. *Saccharomyces cerevisae* strain D5A (kindly provided by NREL) was used for all SSF experiments. Cellulase (Genencor CL) kindly provided by Genencor International (San Fancisco, CA) was added in amounts sufficient to give the enzyme loading indicated in the text, reported in filter paper units (FPU) per g initial glucan. Where noted in the text, cellulase activity was supplemented with ß-glucosidase (Novozyme 188, Novo Nordisk, Wilton, CT) at a ratio of 4 IU ß-glucosidase/FPU. Cellulase activity determined as described by Ghose et al. (*22*) was 90 FPU/ml the Genencor cellulase preparation.

Simultaneous saccharification and fermentation. Simultaneous saccharification and fermentation (SSF) was performed in 150 mL sealed serum vials (Bellco, Vineland NJ) with a working volume of 100mL and an N_2 gas phase. Two growth media were used as noted in the text: "rich" medium contained 10 g/L yeast extract (Difco, Detroit, MI) and 20 g/L peptone (Difco); "lean medium" contained 0.15% by volume corn steep liquor (CPC International, Summit-Argo, IL) and 2.5 mM Mg SO_4 as described by Newman et al. (*23*). An appropriate amount of never-dried sludge was added to medium-containing serum vials to give an initial glucan content of 20 g/L medium. The pH was adjusted with sulfuric acid to an initial value of 4.5, and sludge-containing serum vials were autoclaved at 121°C for 20 minutes. Appropriate volumes of cellulase and β-glucosidase were added to give the enzyme loading indicated in the text, reported in filter paper units per g initial dry glucan. SSF tests were initiated by adding a 0.2 vol% inoculum and incubating on a rotary shaker at 37°C.

Analytical methods. Total solids (gravimetric) and solids composition including % ash (muffle furnace), % glucan, % xylan and % mannan (quantitative saccharification and HPLC), % acid-soluble lignin (absorbance of the quantitative saccharification hydrolyzate at 205 nm), and % volatile matter (muffle furnace) were determined for each sludge according to procedures described elsewhere (*24*). Samples were analyzed for ethanol and soluble sugars using an

Aminex HPX-87H column (Biorad, Foster City, CA). Higher resolution analysis of soluble sugars in quantitative saccharification hydrolyzates was carried out using an Aminex HPX-87P column. Refractive index detection was used for both columns. External validation of carbohydrate analysis was provided by Mark Davis of the USDA Forest Products Laboratory (Madison WI) for two sludges. Concentration measurements made in the two laboratories differed by < 1% for glucan and mannan and < 25% for xylan. Carbohydrate is reported on an anhydrous sugar basis (e.g. glucan and xylan rather than glucose and xylose).

Definition of parameters. Conversion and ethanol yield were calculated based on the glucan content:

$$\text{Conversion (\%)} = 100 \times \left(1 - \frac{\text{Mass final glucan}}{\text{Mass initial glucan}}\right)$$

$$\text{Ethanol yield (\%)} = 100 \times \frac{\text{Ethanol produced}}{(\text{Glucan initial} - \text{glucan final}) \times (92/162)}$$

The filter cake mass reduction was calculated in order to provide a measure of dewaterability:

$$\text{Filter cake mass reduction} = 1 - \frac{\text{Final mass wet filter cake}}{\text{Initial mass wet filter cake}}$$

Final filter cake mass was measured by vacuum filtration using a 0.4 μm pore size membrane (Nucleopore, Pleasanton, CA) with filtration terminated 20 seconds after the filtercake nolonger appeared wet. Initial mass wet filter cake was the mass of wet sludge added at the moisture content received from the mill in order to give an initial glucan concentration of 20 g/L.

$$\text{Glucan reacted (mass fraction)} = \text{Conversion} \times \frac{\text{Mass initial glucan}}{\text{Mass initial solids}}$$

Error analysis. All values reported are averages for at least two replicate tests, with three tests used for most samples in which xylan and mannan were analyzed. The root mean squared errors for the reported parameters were:

Parameter	Root mean square error[a]
Glucan	1.6 mass %
Xylan	0.58 mass %
Mannan	0.46 mass %
Solids	0.72 mass %
Ash	0.62 mass %
Ethanol yield	3.1 %
Conversion	1.4 %

$$^{a} = \sqrt{\frac{\sum (Yij - \bar{Y}i.)^2}{b(n-1)}}$$

b = number of different samples analyzed
n = number of replicates/sample
Yij = individual measurement of the indicated parameter
$\bar{Y}i.$ = average of replicate measurements for one sample

It may be noted that explanations other than measurement error may account for some apparent discrepancies in the ethanol yield and conversion values. In particular, calculated ethanol yields > 100 % may occur because of the possibility of ethanol production from mannose and small amounts (corresponding to ≤ 5.1% of paper sludge glucan) of soluble sugars introduced with the cellulase preparation. Although similar values are generally observed for conversion and ethanol yield as expected, conversion in excess of ethanol yield (which was observed for several tests without β–glucosidase) could be explained by accumulation of unfermented soluble sugars. Values preceded by ± in the text reflect standard deviation including potential sources of variability in addition to measurement error.

RESULTS

Sludge origin, composition, and temporal variation Sludges were obtained from mills in the Northeast, Midwest, and Northwest areas of the United States. In choosing sludges for testing we gave preference to relatively large mills, mills using chemical (as opposed to purely physical) processes, and obtaining sludges from mills with a variety of process types. Features of the sludges tested are presented in **Table 2**. Each sludge is referred to by an identification number, used throughout this paper. The origin of the sludges is characterized with respect to the furnish (virgin and/or recycled, hardwood and/or softwood where known) and process used by the mill producing the sludge are specified. A total of 18 sludges were tested from Kraft mills, 7 from deinking mills, 7 from facilities with no pulp mill (for which little information on the pulping process was available in most cases), 4 sulfite mills, 1 semichemical mill and 1 thermochemical mill. The presence or absence of bleaching is indicated in cases for which this is known. Finally, compositional information is provided with respect to wt. % solids (100 x mass solids/mass wet sludge as received from the mill), wt. % glucan (100 x mass glucan/mass dry matter), and wt.% ash (100 x mass ash/mass dry matter), for which respective median values of were 33.0, 39.2, and 26.4 were obtained.

A subgroup of 15 sludges was selected for more detailed compositional analysis, including glucan, xylan, mannan, ash, acid soluble lignin, and acid-insoluble organics (**Table 3**). Sludge composition varied considerably from mill-to-mill and for sludges from different locations from the same mill. Variability in composition over the 3 week sampling period examined for sludges from a particular location in a particular mill was less important than mill-to-mill variation. As an indication of this, the standard deviations of the mean values for different sludges (bottom row in Table 3) are greater than the standard deviations for particular sludges based on measurements taken over a 3-week period (other rows in Table 3) for 58 of the 60 sludge ID/parameter combinations reported.

Xylan was present in higher amounts than mannan in 11 of the 15 sludges. Non-glucan carbohydrate (xylan and mannan) together represented from 7.4% to 25.6% of the glucan content, with a mean value of 14.4%. Total carbohydrate (glucan + non-glucan carbohydrate) represented from 13.8% to 73.7% of sludge dry mass, with a mean value of 48.4%. Acid-insoluble volatile matter is assumed to represent a combination of acid-insoluble lignin as well as other volatile compounds. Mass recovery averaged 90%. Arabinose and galactose were not measurable at the limit of detection (corresponding to 1.0 wt.% dry sludge).

Sludge	Origin		Composition		
I.D. #	Furnish[1]	Process[2]	Solids[3]	Glucan	Ash
1	V (HW, SW)	K, B	15.30%	37.38%	42.73%
2	R	D, B	30.11%	28.83%	28.33%
3	V	NPM, Paper	24.68%	38.05%	38.52%
4	V/R	D, U	42.13%	20.53%	65.17%
5	V	K, B, Paper	19.26%	57.14%	24.08%
6	V (HW)	K, B	40.81%	20.57%	51.62%
7	V (HW)	K, B	27.36%	42.82%	32.86%
8	R	NPM, Paper	31.88%	40.30%	4.96%
9	V	S, U	49.26%	40.85%	20.68%
10	R	D, B, Paper	32.94%	34.99%	21.83%
11	R	NPM, Paper	29.71%	53.10%	2.81%
12	V	NPM, Paper	30.48%	33.49%	15.98%
13	V/R	D, B	37.89%	26.80%	42.24%
14	V (HW, SW)	K, B	40.91%	47.40%	33.65%
15	R	NPM, Paper	36.09%	42.14%	12.39%
16	V	NPM, Paper	95.65%	19.47%	54.05%
17	R	SC, U,Paper	34.86%	42.29%	3.48%
18	R	D, B, Paper	29.82%	24.98%	34.41%
19	V	NPM, Paper	42.76%	27.87%	50.36%
20	V (HW)	S, B	36.00%	35.28%	29.60%
21	R	D, B, Paper	30.03%	22.86%	48.50%
22	R	K, B, Paper	24.84%	54.90%	26.40%
23	V	K, B	37.72%	41.69%	30.11%
24	R	D, B, Paper	32.60%	67.25%	7.20%
25	R	D, U, Paper	40.98%	47.63%	25.32%
26a	V	K, B, Paper	18.00%	60.66%	12.00%
b	V	K, B, Pulp	38.63%	17.99%	-
27	V (SW)	K, B, Pulp	37.53%	36.27%	14.85%
28	V (SW)	K, U, Pulp	19.92%	43.52%	29.43%
29a	V (SW)	S, B, Pulp	31.52%	68.59%	5.55%
b	V (SW)	S, B, Pulp	25.52%	53.97%	1.80%
30a	V	K, B, Pulp	16.57%	47.27%	19.90%
b	V	K, B, Paper	25.15%	56.53%	5.03%
31	R	D, U, Paper	36.33%	42.72%	34.88%
32	R	D, U, Paper	40.39%	33.08%	31.53%
33	V (SW)	K, B, Pulp	34.45%	32.10%	47.82%
34a	V (SW)	K, U, Pulp	37.16%	50.32%	13.59%
b	V (SW)	K, U, Pulp	7.85%	56.28%	8.00%
c	V (SW)	K, U, Pulp	13.20%	11.66%	53.98%
35	V	K, B, Pulp	40.34%	23.33%	57.37%
36	V (SW)	K, U, Pulp	16.91%	51.44%	2.35%
37	V	TC, B, Pulp	40.91%	27.20%	22.38%
38	R	D, B, Paper	48.00%	59.00%	-
39	R	D, B, Paper	33.00%	30.00%	-

[1]V=virgin, R = recycled, HW = hardwood, SW = softwood. [2]D = deinking, K = kraft, S = sulfite, TC = thermochemical, B = bleached, U = unbleached, NPM = no pulp mill, Pulp = pulp sludge, Paper = paper sludge. [3]As received dewatered from the mill.

Table 2. Origin and composition of sludges tested.

Sludge I. D. #	n[b]	Total Carbo-hydrate	Glucan	Xylan	Mannan	Ash	Acid Soluble Lignin	Acid Insoluble Volatile Matter	Mass Balance[c]
		%	%	%	%	%	%	%	%
27	3	41.59	36.27 ± 4.26	3.07 ± 0.03	2.25 ± 1.76	14.85 ± 7.04	1.74 ± 0.39	25.09 ± 3.04	83.27 ± 2.53
28	3	48.76	43.52 ± 4.67	2.97 ± 0.81	2.28 ± 0.61	29.43 ± 4.58	0.64 ± 0.06	17.79 ± 4.54	96.63 ± 3.51
29 a b	 2 1	 73.67 57.99	 68.60 ± 5.86 53.97	 2.43 ± 0.08 1.45	 2.65 ± 0.20 2.57	 5.56 ± 0.06 1.80	 0.87 ± 0.01 1.06	 13.53 ± 4.16 32.50	 93.63 ± 3.50 93.35 ± 3.47
30 a b	 2 1	 53.38 65.79	 47.27 ± 0.46 56.54	 3.33 ± 0.25 5.61	 2.79 ± 0.15 3.64	 19.90 ± 0.42 5.03	 1.17 ± 0.09 0.99	 19.26 ± 0.47 18.95	 93.71 ± 3.24 90.75 ± 3.93
31	3	48.72	42.72 ± 8.74	4.66 ± 1.62	1.33 ± 0.17	34.88 ± 5.96	0.84 ± 0.11	8.64 ± 0.50	93.08 ± 2.83
32	3	39.48	33.08 ± 6.26	5.70 ± 0.67	0.69 ± 0.53	31.53 ± 11.02	0.89 ± 0.14	9.51 ± 0.61	81.41 ± 4.08
33	3	36.21	32.10 ± 5.96	1.96 ± 0.38	2.14 ± 0.91	47.81 ± 3.02	0.49 ± 0.05	6.34 ± 1.45	90.85 ± 4.03
34 a b c	 1 1 1	 58.60 66.28 13.76	 50.13 56.28 11.66	 4.98 6.17 1.29	 3.30 3.84 0.80	 13.59 8.00 53.98	 0.89 0.90 1.57	 22.99 19.35 8.61	 96.07 ± 3.34 94.54 ± 3.68 77.92 ± 2.92
35	3	26.80	23.33 ± 14.47	1.89 ± 1.08	1.58 ± 1.04	57.37 ± 29.11	0.37 ± 0.16	4.97 ± 1.43	89.51 ± 5.71
36	3	60.05	51.44 ± 3.71	5.09 ± 0.54	3.53 ± 0.46	2.35 ± 0.36	1.65 ± 0.02	23.56 ± 2.43	87.61 ± 4.72
37	3	34.16	27.20 ± 2.35	3.02 ± 0.80	3.94 ± 1.12	22.38 ± 1.88	1.70 ± 0.09	30.36 ± 2.11	88.60 ± 3.86
Mean[d]		48.35	42.29 ± 15.00	3.58 ± 1.65	2.49 ± 1.06	23.23 ± 18.72	1.05 ± 0.43	17.43 ± 8.63	90.06 ± 3.69

[a] Standard deviations for each sludge reflect variability between mean values for different samples taken at 1-week intervals. Each sample was analyzed in triplicate. [b] n = # samples from a given mill (and different locations within the mill for sludges 29, 30, and 34) taken at 1-week intervals. [c] Mass balance standard deviations are calculated based on the difference between individual mass balance measurements relative to the mean mass balance for replicate analyses of a given sample. [d] Standard deviations for mean values at the bottom of each column except the mass balance column reflect variability among the time-averaged values for the different sludges tested.

Table 3. Detailed compositional analysis of selected sludges[a].

Data taken over a five-year period is shown for sludge 5 in **Figure 2**. Although the glucan, ash, and solids concentrations varied somewhat, the SSF conversion remained above 90% for all samples.

SSF results

Batch SSF was carried out in serum vials containing never-dried paper sludge at a concentration corresponding to 20 g glucan/L. **Figure 3** presents ethanol production data over time at a cellulase loading of 10 FPU/g glucan for sludge 5 in relation to other cellulosic substrates: dilute-acid pretreated poplar (2 mm particle size prior to pretreatment) prepared at the National Renewable Energy Laboratory, dilute-acid pretreated mixed hardwood flour prepared at Dartmouth, Avicel (a purified microcrystalline cellulose), and mixed hardwood flour that has not been pretreated. Dilute acid-pretreated materials are included to compare paper to cellulosic materials intentionally prepared to be accessible to enzymatic hydrolysis. Avicel is one of the more common laboratory substrates used to characterize systems featuring the enzymatic hydrolysis of cellulose. As may be seen from the data in the figure, sludge 5 is as reactive as the more reactive of the two pretreated substrates (dilute acid- pretreated hardwood flour) and is substantially more reactive than either dilute acid-pretreated poplar) or Avicel. The effect of paper making is seen to be a marked increase in the rate and extent of hydrolysis for sludge 5 as

well as most other sludges (described subsequently), as even the least reactive sludges are far more reactive than non-pretreated mixed hardwood flour. A 7-day reaction period was found sufficient to achieve complete reaction in batch SSF tests.

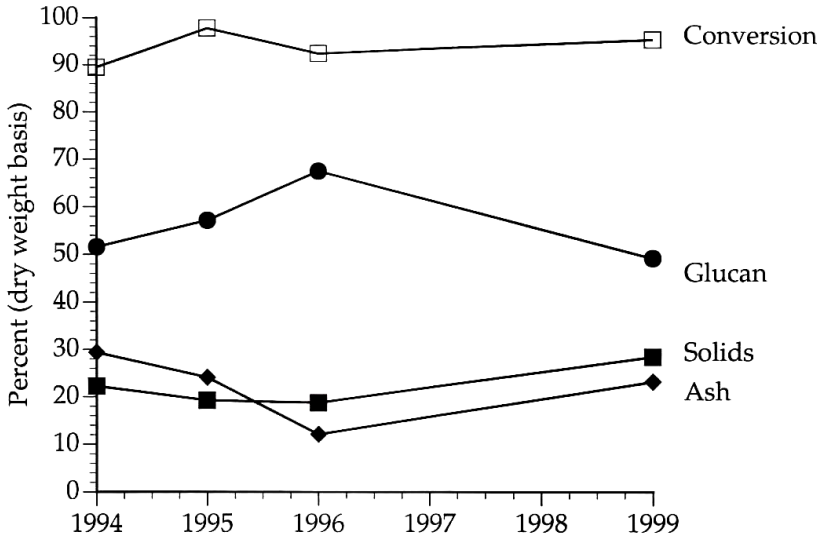

Figure 2. Characteristics of sludge 5 over a 5-year period.
□, conversion; ●, cellulose; ■, solids; ◆, ash. Cellulase loading: 10 FPU/g glucan.

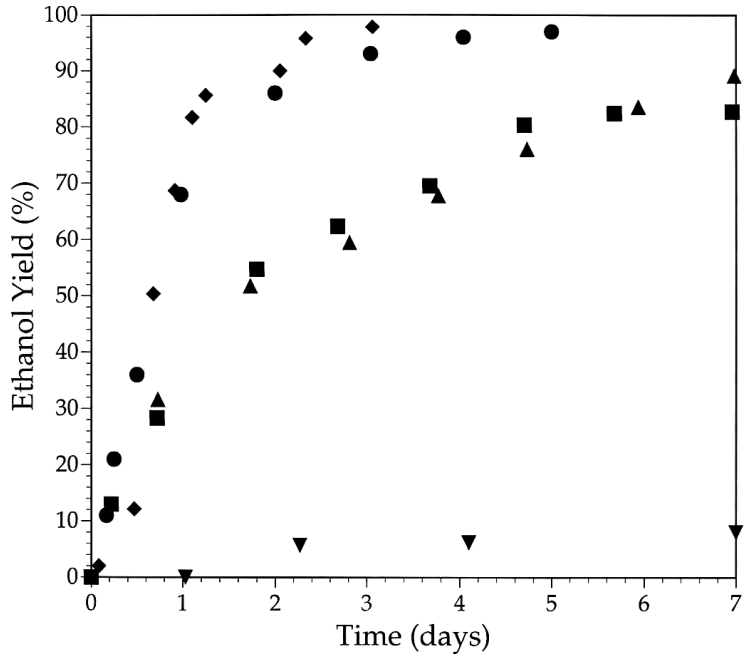

Figure 3. SSF timecourse for sludge 5 in comparison to other substrates.
◆, sludge 5; ●, pretreated hardwood; ■, Avicel; ▲, pretreated poplar P, unpretreated mixed hardwood. Cellulase loading: 10 FPU/g glucan.

To be implemented in the field, paper sludge conversion to ethanol must utilize a growth medium for the yeast that is low-cost and does not contain excessive quantities of complex growth factors. **Figure 4** compares SSF performance on a "rich" medium (containing yeast extract and peptone) to that on a "lean" medium (containing corn steep liquor and $MgSO_4$) for sludge 10 and Avicel. Results from this and other controlled experiments (Table 5) indicate that SSF performance was essentially equivalent for both media.

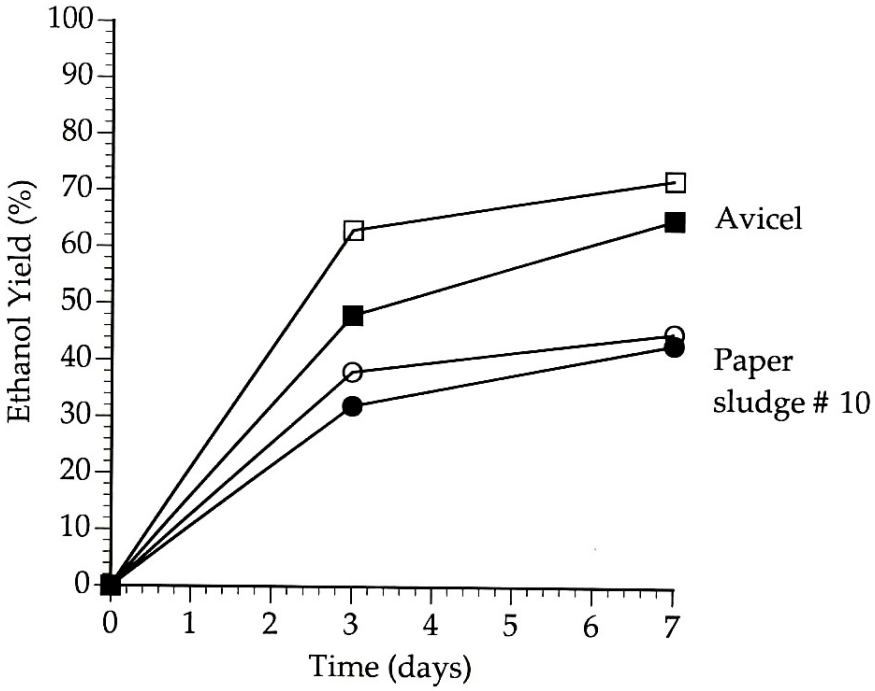

Figure 4. Comparison of SSF results for rich and lean media. Squares represent Avicel, circles represent sludge 10, open symbols represent lean medium, closed symbols represent rich medium. Cellulase loading: 10 FPU/g glucan.

Although the yeast used in these studies was only capable of fermenting six-carbon sugars, the fate of xylan is also of interest. **Figure 5** shows that xylan hydrolysis accompanies glucan hydrolysis with essentially equal fractional conversion of xylan and glucan.

Table 4 presents SSF results for all sludges tested, representing 48 SSF trials carried out in duplicate on 36 distinct sludges from 35 different mills. Results are reported in relation to SSF conditions (β-glucosidase addition, cellulase loading, growth medium) in terms of both glucan conversion and theoretical ethanol yield, with glucan conversion (mean value = 79.9%) somewhat higher than ethanol yield (mean value = 75.0%) for most sludges tested. The discrepency between conversion and ethanol yield is probably due at least in part to the consumption of sugars originating from the paper sludge for production of yeast cells in addition to ethanol. Ethanol production, indicative of yeast growth, was observed in all but two of the sludges tested. In ethanol-producing samples with added β–glucosidase, soluble sugars were not observed to accumulate, indicating that hydrolysis rather than fermentation was rate-limiting.

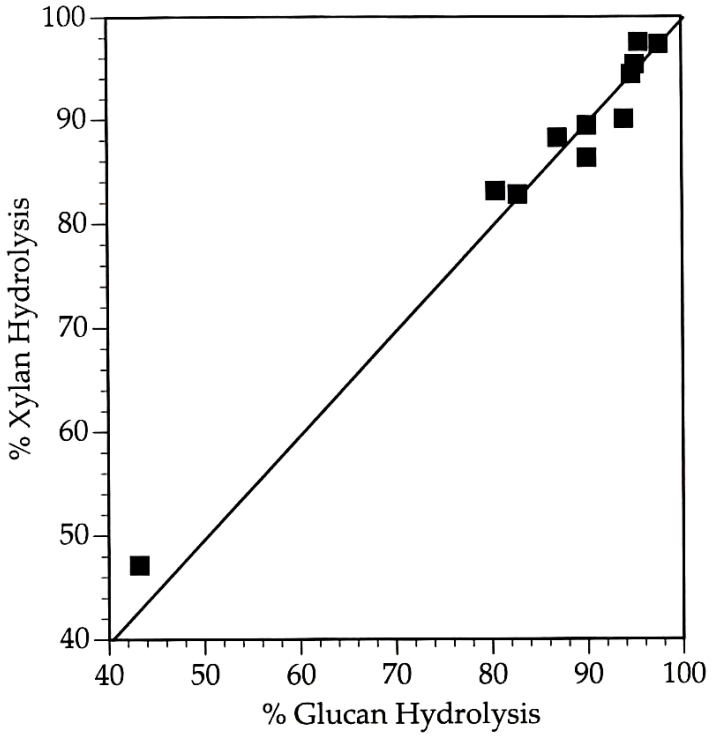

Figure 5. Hemicellulose hydrolysis in relation to glucan hydrolysis during SSF for 11 different sludges. Cellulose loading: 20 FPU/g glucan.

Data for the impact of SSF conditions on performance, as measured by the ethanol yield, is presented in **Table 5**. Addition of β-glucosidase had a very strong impact on the results. Analysis of variance indicated that β-glucosidase addition had a significant effect even at a 0.001 confidence level. The beneficial effect of β-glucosidase is further indicated by the observation that sludges tested with and without β-glucosidase gave higher ethanol yields with β-glucosidase in 8 of 10 cases. The effect of β-glucosidase addition varies from slight (sludges 10, 11, 26a) to dramatic (sludges 15, 17, 20) with ethanol yields increased by more than 2-fold in some cases. Given the strong effect of β-glucosidase addition, the impact of other factors is reported in Tables 5 and 6 only for samples run in the presence of supplemental β-glucosidase. Cellulase loading (10 or 20 FPU/g glucan) and growth medium (rich or lean) resulted in similar mean ethanol yields, and did not have a significant effect on the SSF ethanol yield even at P = 0.25.

The impact of sludge origin and composition on observed SSF performance as measured by the ethanol yield was also examined (**Table 6**). The pulping process had a significant effect at P = 0.25, but not P = 0.1. Mean yields in relation to the process from which the sludge originated were: sulfite, 100.3; bleached Kraft, 87.6; deinking, 83.7; unbleached Kraft, 69.7%; thermochemical and semichemical, 94.2 (one sample); and thermochemical, 36.1 (one sample). The very high yields obtained with the three sulfite sludges tested are notable, and would be significant at higher P values if the sample size were larger. Sludges from mills with no pulp mill had a mean ethanol yield (81.7%) falling in the middle of this range. This result suggests that pulping sludge and paper-making sludge do not differ substantially in their amenability to SSF at least when considering average results.

Sludge I.D.#	Conditions			Conversion	Theoretical Ethanol Yield
	Cellulase (FPU/g Glucan)	Beta Glucosidase Addition?	Medium	%	%
1	10	no	rich	89.0	55.1
2	10	no	rich	64.2	55.8
3	10	no	rich	91.0	77.8
3	10	yes	lean	97.8	116.7
4	10	no	rich	65.7	61.4
5	10	no	rich	89.5	103.2
5	10	yes	lean	97.7	85.7
6	10	no	rich	79.2	63.1
9	10	yes	lean	97.0	109.1
10	10	no	rich	38.3	53.2
10	10	yes	lean	37.7	46.0
11	10	no	rich	45.1	52.7
11	10	yes	lean	49.3	57.8
12	10	no	rich	DNG	DNG
13	10	no	rich	45.6	67.3
14	10	no	rich	80.0	76.3
14	10	yes	lean	97.3	93.7
15	10	no	rich	35.1	46.4
15	10	yes	lean	71.2	71.1
17	10	no	rich	44.9	30.0
17	10	yes	lean	84.9	94.2
19	10	yes	lean	DNG	DNG
20	10	no	rich	90.8	46.8
20	15	yes	rich	94.3	98.0
21	10	no	rich	81.5	68.9
22	10	no	rich	92.4	62.6
23	10	no	rich	83.3	66.6
23	10	yes	rich	82.8	85.3
23	20	yes	rich	88.4	87.1
24	10	no	rich	87.7	91.8
25	10	no	rich	72.8	39.0
26a	20	no	rich	92.2	73.1
26a	20	yes	rich	95.2	82.4
26b	10	yes	rich	98.1	98.5
26b	20	yes	rich	92.6	98.3
27	20	yes	rich	90.1	82.1
28	20	yes	rich	95.5	73.2
29a	20	yes	rich	97.6	93.9
30a	20	yes	rich	94.0	70.4
31	20	yes	rich	95.1	81.4
32	20	yes	rich	94.7	104.5
33	20	yes	rich	90.1	78.8
34a	20	yes	rich	80.5	54.4
35	20	yes	rich	82.8	104.3
36	20	yes	rich	87.0	81.4
37	20	yes	rich	43.2	36.1
38	20	yes	rich	91.4	91.9
39	20	yes	rich	80.9	81.2

Table 4. Summary of SSF results.

Factor	n	Mean ethanol yield (% of theoretical)
SSF conditions		
β-glucosidase[a]		
with	28	84.0 ± 18.8
without	18	63.1 ± 17.9
cellulase loading[b]		
10 IU/g glucan	11	86.9 ± 21.2
20 IU/g glucan	16	81.2 ± 17.5
growth medium[a,b]		
rich	19	83.2 ± 16.9
lean	9	85.7 ± 23.3

[a] Cellulase loading 10 to 20 IU/g glucan, as specified for individual sludges in Table 4. It may be noted that we did not observe a significant difference between SSF results over this range of cellulase loadings.
[b] Includes only tests with β-glucosidase in which ethanol was produced.

Table 5. Analysis of SSF results in relation to SSF conditions.

Factor	n	Mean ethanol yield (% of theoretical)
Furnish		
Virgin	18	84.9 ± 19.2
Recycled	8	80.6± 19.3
Process		
Bleached Kraft	11	87.6 ± 10.1
Unbleached Kraft	3	69.7 ± 13.8
Deinking	6	83.7 ± 20.6
Sulfite	3	100.3 ± 7.9
Thermochemical	1	36.1
Semichemical	1	94.2
No pulp mill	3	81.7 ± 30.9
Bleached (all processes)	17	86.6 ± 13.8
Unbleached (all processes)	8	79.3 ± 24.7
Composition		
High glucan	9	78.8 ± 14.9
Medium glucan	9	88.9 ± 15.4
Low glucan	10	84.3 ± 24.5

[a] Footnotes a and b from Table 5 are applicable.

Table 6. Analysis of SSF results in relation to sludge origin and composition[a].

With the exception of β-glucosidase addition and the paper-making process, all other factors tested were not significant as indicated by a single factor analysis of variance even at P = 0.25. Sludges produced from virgin fiber processing resulted in a mean yield (84.9%) somewhat higher than sludges produced from recycled fiber processing (80.6%). Taking all sludges into consideration, the mean yield with bleaching (86.6%) was somewhat higher than without bleaching (79.3%). The nine SSF tests carried out with sludges having the highest glucan content (47.3 to 68.6 wt.% glucan) had a mean ethanol yield of 78.8%, whereas tests carried out on the nine sludges having an intermediate glucan content (38.1 to 43.5% glucan) had a mean ethanol yield of 88.6%, and the tests on the ten sludges with the lowest glucan content (17 to 39.3 wt.% glucan) had a mean ethanol yield of 84.3 %.

Paper sludges are typically difficult to dewater, as indicated by the fact that many sludges are disposed of as filtercakes with solids content ≤ 35 wt.% (Table 2). **Table 7** examines the effect of SSF on sludge dewaterability for 9 sludges in terms of the filter cake mass reduction (FCMR). FCMR represents the fractional decrease in the weight of the wet filter cake after SSF (determined using a laboratory vacuum filter) as compared to the initial weight of the wet filter cake (at the as-received solids concentration after dewatering at the mill). For the sludges examined, FCMR values varied from 0.28 to 0.85. The ratio of FCMR:glucan reacted provides an indication of the change in the ease of dewatering accompanying SSF. FCMR:glucan reacted ratios were ≥ 1 for 7 of the 9 sludges tested indicating that a fractional reduction of the mass of the wet filter cake in excess of the fractional reaction of glucose occurred.

Sludge	As-received solids (wt.%)	Filter cake mass reduction[a]	Glucan Reacted[b]	FCMR[c] Reacted fiber
1	29.8	0.28	0.16	1.70
3	24.7	0.74	0.37	1.99
5	19.3	0.85	0.56	1.52
9	49.3	0.38	0.40	0.96
10	32.9	0.36	0.13	2.73
11	29.7	0.45	0.26	1.72
14	40.9	0.63	0.46	1.37
15	36.1	0.29	0.30	0.97
17	34.9	0.43	0.36	1.18
Ave				1.41

[a] Expressed as a fraction of the initial wet filter cake mass, see materials and methods.
[b] Expressed as a fraction of the initial sludge dry mass, see materials and methods.
[c] FCMR = filter cake mass reduction

Table 7. Solids reduction and dewaterability of selected sludges.

For almost all sludges tested, acid addition was required to bring the pH to 4.5 prior to SSF. As presented in **Figure 6**, the acid demand was < 120 g H_2SO_4/kg dry sludge (corresponding to about \$10/ton dry sludge for H_2SO_4 purchased at \$85/ton) for 25 of the 30 sludges tested, and < 60 g H_2SO_4/kg dry sludge for 19 of the 30 sludges tested. Sludges with high SSF ethanol yields were well-represented among those with low acid demands.

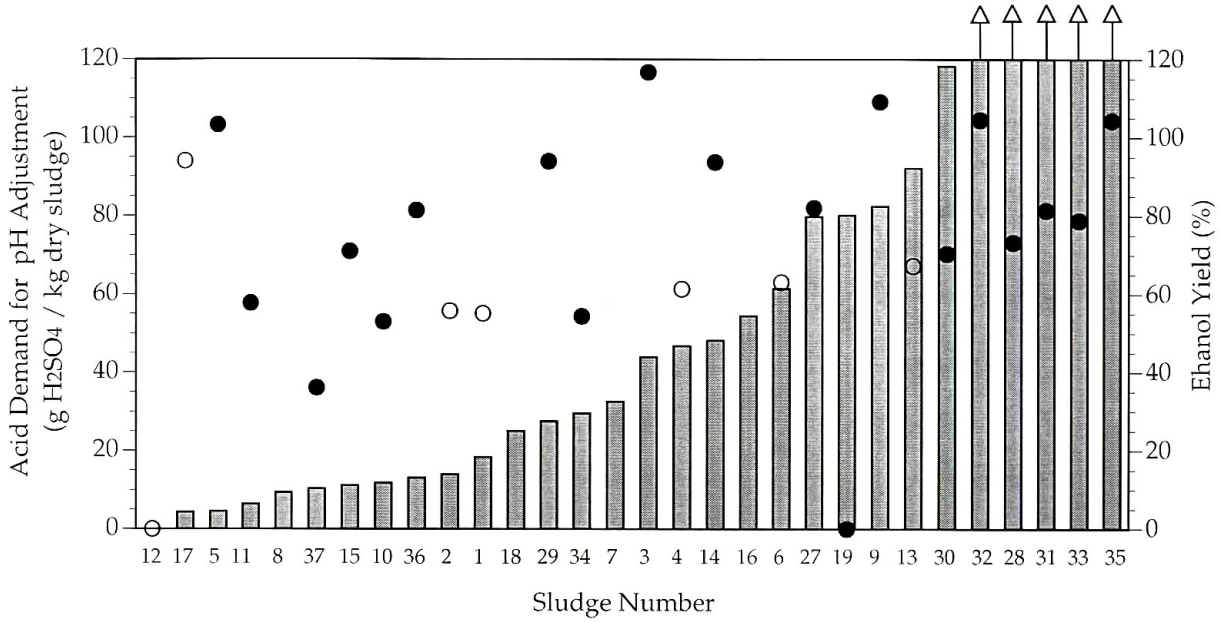

Figure 6. Acid demand for pH adjustment in relation to SSF ethanol yields. Bar height represents acid demand. Circles represent ethanol yields. ●, + β-glucosidase; O, - β-glucosidase.

Discussion

The results reported here indicate that for a significant fraction of paper mills, paper-making is a highly effective, albeit fortuitous, pretreatment for enzymatic hydrolysis. Amenability of as-received sludges to enzymatic hydrolysis does however vary substantially among sludges. Moreover, SSF results show wide variability with respect to process type and furnish, suggesting that it is difficult to predict SSF reactivity based on these variables in the absence of test data.

Of the 28 sludges tested with β-glucosidase, 19 achieved ethanol yields in excess of 80%, 12 achieved ethanol yields in excess of 90%, and 8 achieved yields in excess of 95%. These values support widespread potential applicability of enzymatic hydrolysis and ethanol production from paper sludge, as do data on the dewaterability of solids remaining after SSF and acid demand for pH adjustment. Dewaterability is improved by SSF for most sludges in spite of the fact that growth of yeast both adds mass to the filter cake and impedes filtration. Visual inspection of SSF residues in settling columns (data not shown) suggests that essentially colloidal yeast can likely be separated from readily sedimented mineral components, which may well improve dewaterability and could also facilitate mineral recovery. For 80% of the sludges tested, the cost of acid required for pH adjustment is ≤ \$10/dry ton sludge. This value is small compared to potential avoided tipping fees (Table 1). The cost of sludge neutralization is thus unlikely to impede practical application for all but the most alkaline of paper sludges.

SSF results were strongly impacted by the presence or absence of supplemental β-glucosidase. Growth medium (rich or lean) and cellulase loading (10 FPU/g glucan or 20 FPU/g) had no significant effect on SSF results. The best way to provide the needed β-glucosidase in practice may well be to use a microorganism that synthesizes this enzyme such as engineered strains of *Klebsiella oxytoca* (*25*) or *Saccharomyces cerevisiae* (*26*). Recombinant xylose-utilizing microorganisms (*27,28*) are also attractive since xylan as well as glucan is hydrolyzed during SSF (Figure 6). The similar SSF results observed at 10 and 20 FPU/g suggest that yet lower amounts of cellulase might also be effective. This is further supported by the paper sludge SSF data of Duff et al. (*14*) featuring similar results at 5 FPU/g glucan as compared to 10 FPU/g. Operation at low cellulase loadings is important in terms of process economics because cellulase is typically a dominant cost factor (*29*).

Yeast growth was observed in all but 2 samples, indicating that inhibition of fermentation was not important under the conditions tested. It should be noted that the glucan concentration used (20 g/L) was chosen based on convenience for rapid screening in simple batch culture vessels, and that substantially higher concentrations would almost assuredly be used in practical application. Inhibition of fermentation, or perhaps cellulase, could potentially be manifested at these higher concentrations that was not observed at the lower concentrations employed herein. We have observed no evidence of inhibition in work with one sludge at commercially viable concentrations (Lynd et al., in preparation).

Biological conversion of cellulosic biomass to commodity products has been hailed in prominent fora during 1999 (*30-33*) as offering potentially large benefits in terms of sustainable resource supply and environmental quality. Several factors make paper sludge an attractive raw material for emergent industrial processes featuring enzymatically-based processing of cellulosic biomass. These advantageous factors include the high reactivity of many paper sludges, the zero or negative cost of these materials, the potential for process simplification afforded by elimination of pretreatment as well as use of utilities and other infrastructure elements available at many mills, and the potential for enhanced mineral recovery as a result of enzymatic hydrolysis. In addition to these distinctive advantages, paper sludge also presents distinctive challenges as a feedstock for biological processing. One such challenge is the difficulty of mixing unreacted paper sludge at the concentrations necessary in order to produce recoverable product concentrations. A second challenge is the small amount of sludge available at particular locations as compared to the scale typical of most current and contemplated ethanol plants, and associated diseconomies of small scale and/or costs for transporting sludge with a high water content. Finally, the matter of the cost and feasibility of mineral recovery from spent SSF residues has not been addressed in any detail to our knowledge. Further experimentation and analysis relative to these advantages and challenges is an important area for future work.

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