Saccharification versus simultaneous saccharification and fermentation of kraft pulp

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Abstract: Enzymes are a significant cost in cellulosic ethanol production, minimizing their use would be desirable as long as ethanol yields and productivities are not reduced. The aim was to evaluate the effects of enzyme dosage on conversion of cellulose to ethanol. Kraft pulp, an intermediate in paper production, was used to represent a fractionated cellulose feedstock. Trials were conducted in a 5 L BioFlow bioreactor (2-3 L working volume) with agitation rate varied (80-900 r/m) to provide acceptable mixing. Based on survey of the literature, an average dosage for cellulase (34 FPU/g glucan) and β-glucosidase (135 CBU/g glucan) was calculated, and these were set as the 100% dosages. Dosages of 1%, 7%, 13%, 33%, 67%, 100%, 133% were tested, using Novozyme Celluclast 1.5 L (cellulase) and Novozyme 188 (β-glucosidase) in a 4.8% (dry mass) kraft pulp slurry. Novozymes recommended dosages are at the low end of this spectrum, at 12 g/g glucan for Celluclast 1.5 L (35% dosage) and 1.2 g/g glucan for Novozyme 188 (0.9% dosage). Saccharification trials (50°C) showed a typical dosage response, with the 133% dosage achieving the highest sugar concentration (~59 g/L glucose) and saccharification rate (2.45 g/L/h), with a specific rate of 2.2×10^{-4} g glucose/unit enzyme/h. However the 13% enzyme dosage resulted in the highest specific saccharification rate (2.9×10^{-4} g glucose/unit enzyme/h). Simultaneous saccharification and fermentation (SSF) trials (35°C) were conducted using *Saccharomyces cerevisiae* or *Candida molischiana* to compare enzyme dosages of 33%, 67%, 100%, and 133%. Ethanol titers and productivities were similar for trials with 67% or more of the literature average enzyme dosage, however were lower at the 33% enzyme dosage. Thus enzyme dosage can be substantially reduced from levels typically cited in the literature, but cannot be reduced to levels recommended by the manufacturer, without reduction in ethanol yield and productivity.

Keywords: cellulosic ethanol, fermentation, saccharification, renewable energy

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1 Introduction

Interest in producing renewable liquid fuels has increased vastly as petroleum prices have risen to over $50/ barrel. Corn ethanol production in the United States was estimated at 10.6 billion gallons in 2009[1]. Research to develop processes to convert cellulosic biomass into ethanol has also increased over the past two decades. This is being intensely evaluated because lignocellulose offers a large un- or under-utilized renewable resource[2]. The United States Department of Energy estimated that 1.3 billion tons of biomass would be available annually in the US alone for conversion to biofuels[3].

Research to improve the biomass ethanol process is needed, since biomass-derived ethanol is not presently economically competitive with petroleum or corn ethanol[2]. Various pretreatments strategies are being researched to enhance cellulose and hemicellulose accessibility for enzymatic conversion into fermentable sugars. One approach is solvent-based fractionation of

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biomass into cellulose, hemicellulose, and lignin streams[4]. The advantage of this process is that the resulting fractions of cellulose pulp and hemicellulose aqueous phase could be separately converted by enzymes and microbes specific for those carbohydrates.

Cellulose deconstructing enzymes are another critical cost element. In 2011, K. Creamer of Novozymes mentioned that cellulase enzyme cost $13.50/kg and β-glucosidase enzyme cost $42/kg (personal communication). The literature contains a broad range of recommended enzyme dosages, and enzyme manufacturer recommendations can also be quite variable. Table 1 provides a range of enzyme dosages that have been used in biomass ethanol research. The average dosage for cellulase enzyme is 33.1 FPU/g glucan β-glucosidase dosage is 124.36 CBU/g glucan, thus yielding 1:4 ratios. The 100% level was set to 34 FPU for the cellulase enzyme and 135 CBU for the β-glucosidase.

Table 1 Typical lignocellulose deconstructing enzyme dosages and conditions

Cellulase (FPU/g)	β-glucosidase (CBU/g)	Ratio (wt/wt)	Hydrolysis time/h	Biomass	Sugar recovery /%	Pretreatment method	Reference
7.5/g glucan [a]	85/ g glucan [a]	1:6	48	Corn fiber, Switchgrass, Rye straw	54-71	Ammonia fiber expansion	(5)
35/ g glucan [a] 60/g glucan [a]	111/ g glucan [a]	1:4	72	Corn stover	92.5-99	Aqueous ammonia	(6)
75/ g glucan [a]	225/ g glucan [a]	1:3	48	Rye straw Bermuda grass	30-52 46-81	Dilute acid	(7)
45/ g glucan [a]	85/ g glucan [a]	1:6	72	Corn stover	53-92	Lime	(8)
15/ g glucan [a]	250/ g glucan [a]	1:16	72	Corn stover	93.2	Lime	(9)
15/ g glucan [a]	60/ g glucan [a]	1:4	72	Switchgrass, Corn stover Hybrid poplar, Douglas fir	97	Fractionation	(10)
16.5 /g glucan	56 /g glucan [a]	1:3	72	DDGS	83	Ammonia fiber expansion	(11)
65 /g glucan [a]	376 /g glucan [a]	1:6	96	Barley husks	88	Catalytic steam pretreatment	(12)
15 /g glucan [a]	39 /g glucan [a]	1:3	72	Barley and wheat straw	35-50 35-40	Acid/water impregnation and steam explosion	(13)
15 /g glucan [a]	40 /g glucan [a]	1:3	24	DDGS	89.4	Soaking in aqueous ammonia	(14)
45 /g glucan [a]	180 /g glucan [a]	1:4	72	Corn stover	75	Extrusion	(15)
60 /g glucan [a]	120 /g glucan [a]	1:2	60	Corn stover	90	pH controlled liquid hot water	(16)
15 /g glucan [a]	64 /g glucan	1:4	72	Corn stover	70	Ammonia fiber expansion	(17)
12 /g glucan [a]	50 /g glucan [a]	1:4	24	Sugarcane bagasse	92.8*	Organosolv	(18)

Note: [a] converted to a per gram of glucan unit * theoretical ethanol yield.

The purpose of this project was to evaluate the effectiveness of various dosages of a commonly used enzyme cocktail, Novozyme Celluclast 1.5 L and Novozyme 188. Initial saccharification trials identified an optimal range of dosages that I then evaluated in simultaneous saccharification and fermentation (SSF) trials with two yeast strains.

2 Materials and methods

2.1 Enzymes, yeast, substrate, and other materials

The enzymes used in this experiment were obtained as a gift from Novozyme. Celluclast 1.5 L is a cellulase enzyme, and has an activity of 4 460.6 FPU/mL. Novozyme 188 is a β- glucosidase, and has an activity of 18 150 CBU/mL. Filter paper units (FPU) are identified as the amount of enzyme needed to release 1 μmol of glucose (under standard conditions) from a known substrate under specific conditions[19] and cellobiase units (CBU) are identified as the amount of enzyme needed to release 2 μmol of glucose (under standard conditions) using cellobiose as the substrate[19]. Enzymes were stored at 4℃ prior to use. *Saccharomyces cerevisiae* NRRL Y-2034 and *Candida molischiana* ATCC 2516 were obtained from the respective culture collections. Short term maintenance cultures were stored on Potato Dextrose Agar (PDA) plates and slants stored at 4℃. Lyophilization was used for long term storage.

The inoculum for all experiments was prepared by

transferring colonies into a 5% glucose, 0.5% yeast extract medium (100 mL in a 250 mL Erlenmeyer flasks), then incubating for 24 h at 35℃ in a rotary shaker (250 r/m).

Kraft pulp was used as the substrate in this experiment. It was obtained as a gift from the Paper Science and Engineering Department at University of Wisconsin – Stevens Point, and consisted of: 76.7% glucan, 0.5% arabinan, 7.7% xylan, 0.3% galactan, 6.7% mannan, and 3.2% lignin. The buffer solution consisted of 1 951 mL of distilled water and 14 g sodium citrate. The pH was adjusted to 4.8 using 14 M HCl. A stock solution of tetracycline (10 μm/mL in 70% ethanol) was prepared and stored in the freezer. To control contamination, 2.7 mL was added to each 1 L of buffer solution prior to addition of kraft pulp.

2.2 Effect of enzyme dosage on saccharification of kraft pulp

Trials in triplicate were conducted in a 5 L, New Brunswick BioFlow III Bioreactor, to which 2,070.3 mL of buffer/enzyme/tetracycline and 100 g of kraft pulp were added (4.8% solid loading). The temperature was set to 50℃ and agitation was initially set to 900 r/m. As the viscosity dropped during the first 30-120 min, the agitation rate was reduced to 75-100 r/m. Saccharification trials were performed for 72 h. Table 2 lists the dosages which were used during the saccharification.

Table 2 Amounts added for different saccharification dosages

Enzyme Dosage/%	Cellulase (mL) / g kraft pulp	Cellulase (mL) /g glucan	β-glucosidase (mL) /g kraft pulp	B-glucosidase (mL) /g glucan	Buffer/ Antibiotic (mL)
133	54	70.40	60	78.23	1 958.3
100	40.5	52.80	45	58.67	1 984.8
67	27	35.20	30	39.11	2 013.3
33	13.5	17.60	15	19.56	2 041.8
13	5.4	7.04	6	7.82	2 058.9
7	2.7	3.52	3	3.91	2 064.6
1	0.54	0.70	0.6	0.78	2 069.16

2.3 Effect of enzyme dosage on simultaneous saccharification and fermentation of kraft pulp

Trials in triplicate were again conducted in the New Brunswick BioFlow III bioreactor, using the same amounts of kraft pulp, buffer, and an antibiotic as the saccharification trials. The only difference was that 20 mL of buffer was replaced by 20 mL of yeast inoculum and 10 mL of buffer was replaced by 10 g of condensed corn solubles (CCS), which provided yeast nutrients. CCS was used over a yeast extract to decrease cost in fermentation. Table 3 provides an analysis of the CCS used, which was obtained as a gift from a dry mill ethanol plant. Enzyme dosages of 133%, 100%, 67%, and 33% of the literature recommended levels were tested. At 0 h, 20 mL of a 24 h yeast culture was added. Both *Saccharomyces cerevisiae* and *Candida molischiana* were tested, as the latter is also capable of xylose fermentation. The temperature was set to 35℃ and agitation was initially set to 900 r/m, until the solution achieved adequate mixing (30-120 min). The agitation rate was then lowered to 75-100 r/m. Simultaneous saccharification and fermentation trials were performed for 96 h.

2.4 Analytical method

Samples (5 mL) were aseptically removed using wide mouth 10mL pipet throughout both saccharification (0, 3, 6, 9, 12, 24, 36, 48 and 72 h) and simultaneous saccharification and fermentation trials (0, 3, 6, 9, 12, 24, 36, 48, 72 and 96 h). After measuring pH, samples were placed in sealed centrifuge tubes and boiled for five min. to denature enzymes. Samples were then filtered through 0.2 μm filters into high performance liquid chromatography (HPLC) vials, which were frozen until analysis. Carbohydrates, organic acids, and ethanol were measured in a Waters HPLC (Milford, MA), with an Aminex HPX-87H column operated 65℃, and Waters 2 410 refractive index detector. The mobile phase was 0.01 N H_2SO_4 at a flow rate of 0.6 mL/min.

Table 3 Analysis of condensed corn solubles (CCS)

Component	As received basis	100% dry matter basis
Total moisture/%	72.3	0
Total dry matter/%	27.7	100
Crude protein, combustion/%	5.25	18.9
Crude fat (diethyl ether extract)/%	5.71	20.6
Ash/%	3.28	11.9
Fat: roese gottieb/%	5.71	20.6
Crude fiber, crucible method/%	0.48	1.74
Nitrogen free extract/%	13.0	47.0
Calcium/%	0.03	0.10
Copper/ug · g^{-1} (ppm)	1.20	4.34
Magnesium/%	0.22	0.80
Phosphorus/%	0.46	1.66
Potassium/%	0.77	2.70
Sodium, %	0.20	0.74
Zinc/ug · g^{-1} (ppm)	29.9	108

3 Results and discussion

3.1 Effect of enzyme dosage on saccharification of kraft pulp

Based on the glucan content of kraft pulp, one can calculate the theoretical maximum glucose level from the cellulosic component as follows for a 48 g/L solution (4.8% solids loading)

48 g/L×76.7% glucan = 36.82 g/L glucan

36.82 g/L glucan×1.11 (conv. factor for cellulose to glucose) = 40.87 g/L glucose

Based on the xylan content of the kraft pulp, one can also calculate the total amount of xylose that should be present after saccharification:

48 g/L×7.7% xylan= 3.7 g/L xylose

3.7 g/L xylan×1.12 (conv. Factor) =4.14 g/L xylose.

This experiment was conducted to determine the effect of enzyme dosage on saccharification efficiency of kraft pulp. Minimizing enzyme use would enhance ethanol production economics, as along as saccharification yield and rate were not significantly reduced. Figure 1 shows the effect of enzyme dosage on glucose concentration during saccharification of kraft pulp. As expected, glucose concentrations and saccharification rates were higher at the higher enzyme dosage levels (33%-133% of the literature recommended levels). There was a significant drop at 33% enzyme dosages and lower. These data were consistent with a normal dosage response, although the large difference between the 67% and the 33% dosage levels was unexpected.

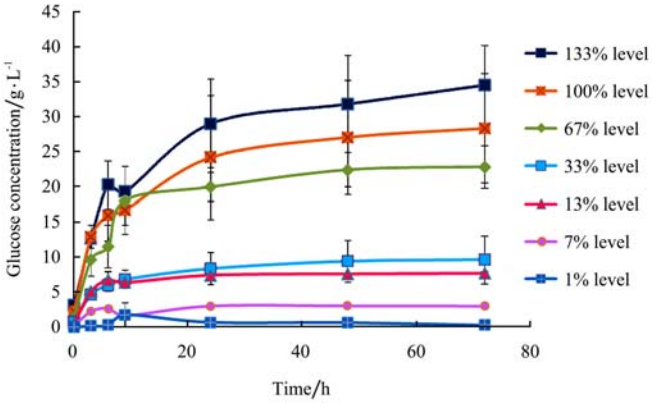

Figure 1 Glucose concentrations during saccharification of 48 g/L kraft pulp with various enzyme dosages
(100% dose: cellulase 34 FPU and β-glucosidase 135 CBU)

Figure 2 shows the effect of enzyme dosage on xylose concentration during saccharification of kraft pulp. Once again, both xylose concentration and saccharification rate were higher at the 133%, 100% and 67% enzyme dosage level. A significant drop was again observed at the 33% enzyme dosage and below.

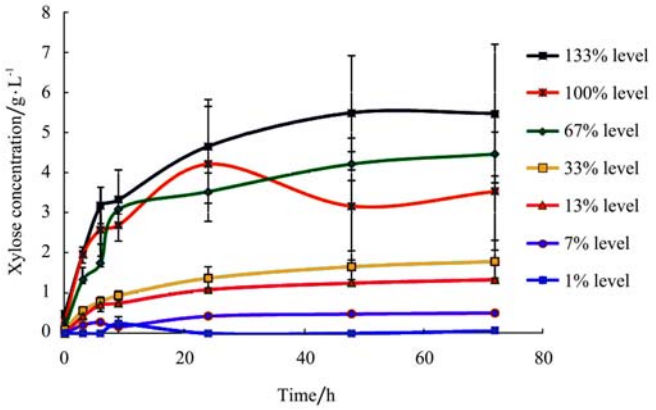

Figure 2 Xylose concentrations during saccharification of 48 g/L kraft pulp with various enzyme dosages
(100% dose: cellulase 34 FPU and β-glucosidase 135 CBU)

Figure 3 shows the effect of enzyme dosage on dextrin (4 glucose unit) concentration during saccharification of kraft pulp. Dextrin concentrations were lowest at the highest enzyme dosage levels (133% and 100%), since there were sufficient enzymes to degrade dextrins into glucose.

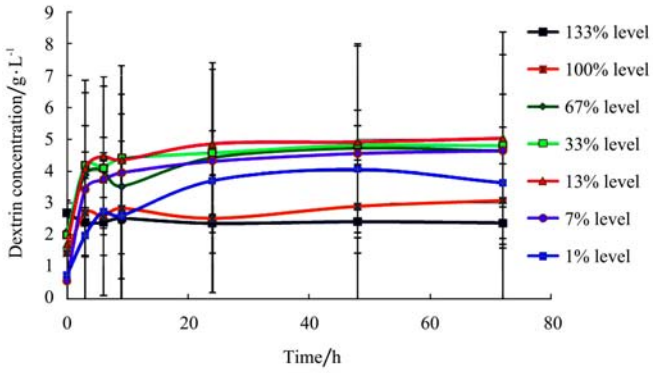

Figure 3 Dextrin concentrations during saccharification of 48 g/L kraft pulp with various enzyme dosages
(100% dose: cellulase 34 FPU and β-glucosidase 135 CBU)

Table 4 provides a summary of carbohydrate concentrations, yields, saccharification rates, and specific saccharification rates at 24 h. Glucose and xylose concentrations and saccharification rates were highest at the 67%-133% enzyme dosages, and fell significantly at enzyme levels of 33% and less. As expected, specific saccharification rates were relatively constant, as these were normalized based on enzymes present.

As expected, glucose yields fell as enzyme dosages were reduced. This was likely exacerbated by feedback inhibition of enzymes by the released glucose, at least in the middle and upper dosage trials[20,21]. The reduction in sugar yields was more pronounced at enzyme dosages of less than 67%. In some cases xylose yields were over 100% and this may have been due to discrepancies in the methods by which xylan was measured by the source. Also there may have been batch to batch variability in the xylan content of the kraft pulp. It was also observed that mannose eluted at the same time as xylose in the HPLC, thus increasing apparent xylose concentrations[15].

Table 4 Carbohydrate concentrations, yields, saccharification rates, and specific saccharification rates at 24 h saccharification of kraft pulp at different enzyme dosages

Enzyme dosage/%[1]	Saccharification rate at 24 h (g glucose/L/h)	Specific saccharification rate at 24 h (g glu/total unit enz/h)	Glucose concentration at 72 h (g/L)[2]	Glucose yield (% theoretical)[2]	Xylose concentration (g/L)[2]	Xylose yield (% theor)[2]
133	1.21	5.36E-3	34.47 (±5.67)	84% (±14%)	6.69 (±1.73)	162% (±42%)
100	0.648	5.9E-3	28.29 (±7.80)	69% (±23%)	3.53 (±1.45)	85% (±41)
67	0.791	7.34E-3	22.75 (±3.02)	56% (±1.8%)	4.45 (±0.98)	107% (±2%)
33	0.360	6.15E-3	9.57(±3.39)	23% (±8.3%)	1.78 (±0.53)	43%(± 12%)
13	0.321	1.36E-2	7.62 (±0.22)	19% (±0.5%)	1.34 (±0.03)	32% (±0.7%)
7	0.129	1.09E-2	2.91 (±0.02)	7% (±0%)	0.51 (±0.0)	12% (±0.1%)
1	0.0132	5.57E-3	0.25 (±0.01)	0.1%(±0%)	0(±0.0)	0% (±0%)

Note: [1]100% enzyme dosage equals 34 FPU/g glucan for Celluclast 1.5 L and 135 CBU/g glucan for Novozyme 188.
[2] ± values represent one standard deviation

Karunanithy and Muthukumarappan[15] also used a cellulase to β-glucosidase ratio of 1:4, with an enzyme dosage that was equivalent to 133% of the literature average. They reported 58.4%-74.6% glucose recovery on extrusion pretreated corn stover, which was less than observed (84% yield) on kraft pulp in this study. Mesa et al.[18] also used a 1:4 enzyme ratio, but an enzyme dosage of only 33% of the literature average to saccharify organosolv pretreated bagasse. Mesa et al.[18] observed a 33%-52% glucose recovery, which was higher than the 23% recovery found here. These studies, as well as the other noted in Table 1, indicate that the enzyme dosage needed to achieve an acceptable glucose yield are predicated on the feedstock and pretreatment method. Hence, an enzyme dosage curve must be developed for each feedstock and pretreatment.

3.2 Effect of enzyme dosage simultaneous saccharification and fermentation of kraft pulp

Figures 4 and 5 show the effects of enzyme dosage on ethanol titer of kraft pulp using *Saccharomyces cerevisiae* and *Candida molischiana*, respectively. In both sets of trials, the yeast performed similarly at enzymes dosages of 67%-133% of the recommended level, with final ethanol titers in the range of 16-18 g/L. Final ethanol titers with *C. molischiana* were slightly higher at each enzyme dosage compared to *S. cerevisiae*. These titers were 77%-86% of theoretical maximum of 20.84 g/L based on just glucose concentrations. However the initial rates of ethanol production were higher with *S. cerevisiae*. In these cases, ethanol production was

largely completed by 48 h.

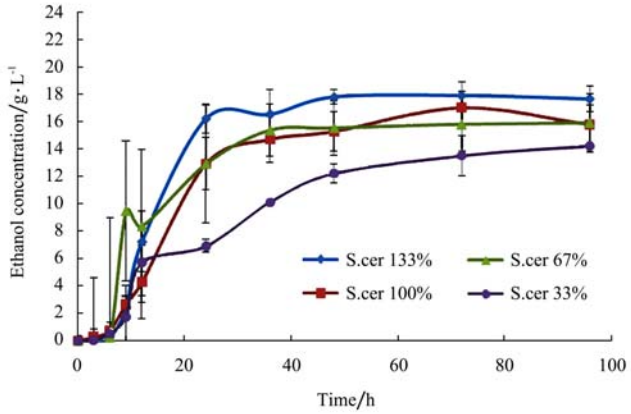

Figure 4 Ethanol concentrations during SSF of 48 g/L kraft pulp with various enzyme dosages using *S. cerevisiae* (100% dose: cellulase 34 FPU and β-glucosidase 135 CBU)

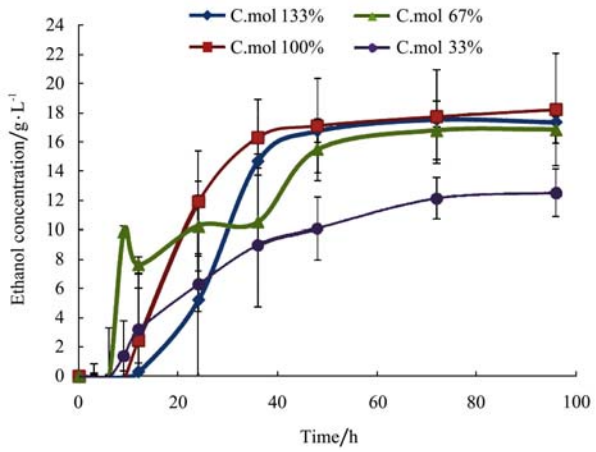

Figure 5 Ethanol concentrations during SSF of 48 g/L kraft pulp with various enzyme dosages using *C. molischiana* (100% dose: cellulase 34 FPU and β-glucosidase 135 CBU)

At the 33% enzyme dosage, ethanol production rate and final titer (13-14 g/L) were both reduced, resulting in ethanol yields that were only 62%-67% of theoretical. It appears that 67% of the normal enzyme dosage was the minimal acceptable level, at least for a solids loading level of 4.8%. Moreover, *C. molischiana* matched the ethanol productivity of *S. cerevisiae* at this solids loading.

Figures 6 and 7 show the glucose concentration during SSF with *S. cerevisiae* and *C. molischiana*, respectively. The initial spike in glucose concentration at 6-18 h was expected, since enzymatic saccharification rates initially exceed glucose consumption rates during the growth phase of the yeast. As expected, the maximum glucose peak level correlated well with enzyme dosage (i.e., higher glucose peaks at higher enzyme dosage levels). After yeast populations peaked, glucose was rapidly consumed and fermented to ethanol. Thus by 24-36 h little free glucose was present, as fermentation rates exceeded saccharification rates. Glucose levels in the *S. cerevisiae* trials were consistently below levels in the *C. molischiana* trials, indicating a more rapid glucose metabolism. This is consistent with the ethanol production data shown in Figures 4 and 5. Based on residual glucose levels after 48 h, there appears to be little difference between 33%-133% enzyme dosages.

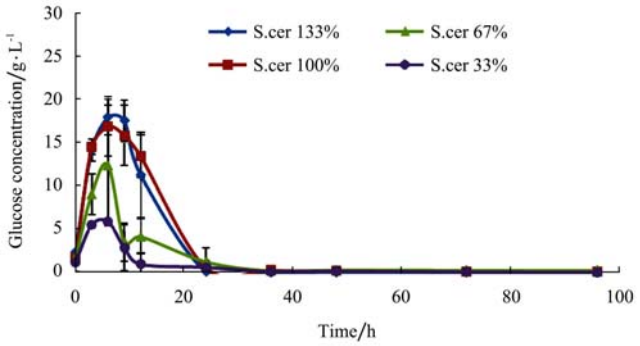

Figure 6 Glucose concentrations during SSF of 48 g/L kraft pulp with various enzyme dosages using *S. cerevisiae* (100% dose: cellulase 34 FPU and β-glucosidase 135 CBU)

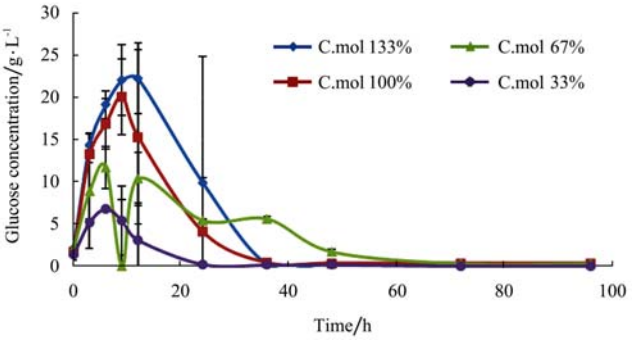

Figure 7 Glucose concentration during SSF of 48 g/L kraft pulp with various enzyme dosages using *C. molischiana* (100% dose: cellulase 34 FPU and β-glucosidase 135 CBU)

Table 5 provides an overall comparison of yeast performance at the different enzyme dosages for SSF of a 4.8% solids loading of kraft pulp. Based on the parameters shown, there appears to be no significant difference between the 67%, 100%, and 133% enzyme dosages during SSF for either yeast. There was, however, a significant reduction in ethanol titer, yield, and productivity when only 33% of the recommended enzyme dosage was used. This means that enzyme use

can be cut in half from the average levels listed in the literature, without sacrificing ethanol production.

Table 5 Comparison of yeast performance at different enzyme dosages during SSF

Yeast strain	Enzyme dosage (% of recomm)[1]	Net max ethanol titer (g/L)[2]	Ethanol yield (% of theoret)[2]	Ethanol productivity (g/L/h)[2,3]	Residual Glucose (g/L)[2,4]	Residual Xylose (g/L)[2,4]
Saccharomyces cerevisiae NRRL Y-2034	133	17.90(±0.99)	85.90%(± 5.3%)	0.25(±0.015)	0	2.20(±0.28)
Saccharomyces cerevisiae NRRL Y-2034	100	17.02(±1.17)	81.70%(±5.7%)	0.24(±0.017)	0.10(±0.18)	2.32(±0.23)
Saccharomyces cerevisiae NRRL Y-2034	67	15.89(±2.11)	76.25(± 10.2%)	0.17(±0.022)	0.21(±0.28)	2.11(±0.36)
Saccharomyces cerevisiae NRRL Y-2034	33	14.24(±0.18)	68.33%(± 0.5%)	0.15(±0.001)	0	1.81(±0.16)
Candida molischiana ATCC 2516	133	17.54 (±0.51)	84.17%(±3%)	0.24(±0.010)	0.24(±0.22)	2.09(± 0.69)
Candida molischiana ATCC 2516	100	18.21(±3.85)	87.38%(±20%)	0.19(±0.043)	0.31(±0.34)	2.13(± 0.29)
Candida molischiana ATCC 2516	67	16.84(±0.91)	81.0%(±8.9%)	0.17(±0.019)	0.24(±0.26)	2.19(±0.22)
Candida molischiana ATCC 2516	33	12.51(±1.62)	60.0%(± 7.9%)	0.13(±0.017)	0	2.04(± 0.36)

Note: [1]100% enzyme dosage equals 34 FPU/g glucan for Celluclast 1.5 L and 135 CBU/g glucan for Novozyme 188; [2] ± values represent one standard deviation;
[3] At maximum ethanol concentration; [4] At 96 h.

A broad range of enzyme dosages have been reported for lignocellulose ethanol production. At the high end are Palmarola-Adrados et al.[12], who obtained 81% of the theoretical ethanol yield from steam/acid catalyst treated barley husk, using 192% of the literature average dosage of cellulase and 278% for β-glucosidase. Similar ethanol yields with kraft pulp were obtained using 100% of the average enzyme dosages. Zhang et al.[22] reduced the cellulase dosage to 65% of the literature average, and still obtained an ethanol yield of 81.2% of theoretical from corncobs pretreated with formic acid and aqueous ammonia. Our results at the 67% enzyme dosage were only slightly lower, at 76% of theoretical ethanol yield. Erdei et al.[23] used much lower enzyme dosages of 44% cellulase and 13% β-glucosidase on steam pretreated wheat straw, but ethanol yields dropped to 68% of theoretical. 68% ethanol yields at 33% enzyme dosage were obtained. Thus it appears that enzyme levels can be reduced to 67% of the literature average without a significant difference in ethanol production. The manufacturer's recommended dosage was much lower, at 12 g/g glucan for the cellulase enzyme (35% dosage) and 1.2 g/g glucan for the β-glucosidase enzyme (0.9% dosage)[24,25]. At these low levels ineffective saccharification was observed (Table 4), and therefore would have expected even lower ethanol yields in SSF.

4 Conclusions

Using kraft pulp as a model substrate to represent cellulosic material recovered from a biomass fractionation process, Cellulose degrading enzyme dosages of 67%-133% of the levels typically reported in the literature were found to perform similarly in saccharification trials. When only 33% of the dosage was used, a significant reduction in glucose and xylose release was observed. This may have been partially due to repression of the enzymes by the glucose, which would have been more pronounced under the limited enzyme levels. The 133% enzyme dosage had the highest saccharification rate and the 13% enzyme dosage had the highest specific enzyme activity rate at 24 h. In simultaneous saccharification and fermentation trials, ethanol yields were similar (76%-81% of theoretical for the 67%-100% enzyme levels). At 33% of the literature average dosage, ethanol yields fell to 68% of theoretical. The manufacturer's recommended dosage for cellulase was 35% of the literature average, but less than 1% for the β- glucosidase, and these levels were not high enough to create significant levels of sugars or ethanol. These studies, as well as the other noted in Table 1, point out that the enzyme dosage needed to achieve an acceptable glucose and ethanol yields are predicated on the feedstock and pretreatment method. Hence, an enzyme dosage curve must be developed for each feedstock and pretreatment.

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