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Simultaneous Saccharification and Fermentation of Pretreated Wheat Straw to Ethanol with Selected Yeast Strains and β-Glucosidase Supplementation

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ABSTRACT

Previous shake flask and stirred tank evaluations of temperature tolerant (37–43°C) yeasts in simultaneous saccharification and fermentation (SSF) on Sigmacell-50 cellulose substrates to ethanol have identified several good microorganisms for further SSF studies (*17*). Of these, the glucose fermenting yeast *Candida acidothermophilum, C. brassicae, Saccharomyces cerevisiae, S. uvarum,* and a mixed culture of the cellobiose fermenting yeast *Brettanomyces clausenii* with *S. cerevisiae* as a control were chosen for shake flask SSF screening experiments with pretreated wheat straw. This study indicates that the *Saccharomyces* strains *cerevisiae* and *uvarum,* give very good performance at high cellulase loadings or when supplemented with Novo-188 β-glucosidase. In fact, with the higher enzyme loadings these yeast will give complete conversion of cellulose to ethanol. Yet at the lower, more economical enzyme loadings, the mixed culture of *Brettanomyces clausenii* and *S. cerevisiae* performs better than any single yeast.

Index Entries: Simultaneous saccharification and fermentation; β-glucosidase supplementation; cellulase activity.

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INTRODUCTION

The simultaneous saccharification and fermentation (SSF) process has been investigated as a method of converting lignocellulosics to ethanol since the 1977 publication by Takagi et al. (*19*). Lignocellulosic biomass provides a low cost substrate for ethanol production, but the conversion costs are currently high. To make ethanol from the SSF process competitive with the price of petroleum derived fuels, factors such as yeast viability, enzyme cost, rate of hydrolysis, and ethanol yield need to be improved.

From preliminary glucose and simultaneous saccharification and fermentation (SSF) screening tests of 10 promising microbial strains at temperatures ranging from 37 to 47°C, five yeast, *Saccharomyces cerevisiae, S. uvarum, Candida lusitaniae, C. brassicae,* and *Brettanomyces clausenii,* were selected as good fermenters in pure or mixed culture for more detailed evaluations on Sigmacell-50 cellulose substrate (*18*). The parameters measured included ethanol concentration, yeast viability, yeast cell density, residual sugars, and cellulose concentration. Mixed cultures of *S. cerevisiae* with *B. clausenii* and *S. uvarum* with *C. lusitaniae* fermented best on Sigmacell-50 cellulose substrate, apparently because of rapid cellobiose removal. Enzyme supplementation with β-glucosidase enzyme was also investigated. β-glucosidase activity compensated for changes in the cellulase enzyme batches from commercial sources and also alleviated possible problems of mixed culture maintenance in continuous fermentations.

The evaluation of pretreated wheat straw was introduced to determine the performance of the best combinations of yeast and cellulase identified in previous studies with a substrate of potential commercial interest. Mixed cultures of cellobiose-fermenting yeast with ethanol-tolerant strains gave better results than either yeast alone (*17,18,22*) since reduction in cellobiose, a strong enzyme inhibitor, appeared to be beneficial. Therefore, supplementation with β-glucosidase was evaluated as an alternative to use of cellobiose-fermenters. However, since a recent economic evaluation of SSF performed by Hinman (*8*) determined that high β-glucosidase supplementation is uneconomical, addition of β-glucosidase does not appear to be desirable. Nonetheless, the measurements of the effects of β-glucosidase supplementation provide valuable knowledge about the desirable characteristics of the enzyme/yeast system.

Yeasts selected for this work have been reported in many publications concerning cellulose, glucose, and cellobiose fermentations at various temperatures. SSF studies were done by Blotkamp et al. (*3*) with *Saccharomyces cerevisiae* (*14,16*) and *S. uvarum* (*4,17*) with the cellulase enzyme from *T. reesei* to observe the effects of different yeasts, cellulase loadings, and substrate concentrations at 40 and 45°C. *Candida acidothermophilum* (*6*) and *C. brassicae* (*1–3,12,13,15,19*) have been reported for the production of ethanol. The use of a mixed culture of *Brettanomyces clausenii* and a *S. cerevisiae* mutant strain (D_5A) have been reported in recent publications on SSF (*10,11,17,18,22*).

MATERIALS AND METHODS

Materials

Yeast strains obtained from the American Type Culture Collection (ATCC), Rockville, Maryland, are as follows: *Candida acidothermophilum* 20831, *Candida brassicae* 32196, and *Saccharomyces uvarum* 26602. Strains obtained from the Northern Regional Research Laboratories (NRRL), USDA, Peoria, Illinois, were *Candida lusitaniae* 5394 and *Brettanomyces clausenii* Y 1414. *Saccharomyces cerevisiae* (D_5A) is a SERI strain genetically derived from Red Star baker's yeast. Yeast extract and peptone media were obtained from Difco, Detroit, Michigan, whereas sugars and other chemicals were obtained from Sigma Chemical Company, St. Louis, MO, and other national laboratory supply houses. Sigmacell-50 cellulose substrate was from Sigma. The cellulase used was Genencor 150L from Genencor Inc., San Francisco, CA. β-glucosidase (Novozyme-188) came from NOVO Laboratories, Inc., Wilton, CT. Fermentation vessels for more comprehensive stirred reactor studies are 6-L Braun Biostat V fermenters from B. Braun Instruments, Burlingame, CA, and 250 mL Pyrex erlenmeyer flasks were used for the shake flask screening tests.

Methods

Small-scale SSFs (100 mL) were run in 250 mL culture flasks, modified to vent CO_2 into water traps. All fermentations used 1% yeast extract and 2% peptone as media and were agitated at 37°C and 150 rpm, either in a shaker incubator or the stirred reactors. A lipid mixture of 5 mg/L ergosterol and 30 mg/L oleic acid plus penicillin and streptomycin at 10 mg/L were added to the SSF media to improve ethanol tolerance (*9*) and to minimize bacterial contamination, respectively. The substrate for the SSFs was dilute acid pretreated wheat straw at 7.5% (w/v) glucan concentration. Cellulase enzyme (batch II) was employed at selected loadings between 7 and 26 IU/g cellulose in the substrate, in which IU represents an international unit of filter paper activity in micromoles of glucose/min (*5*). Preliminary experiments revealed the best method for achieving good mixing was to autoclave the straw separately and then to add all of the liquid fraction mixed together including enzyme, media components, water, inoculum, and antibiotics. The moisture content of the straw (65%) did not change after autoclaving. Ten percent (v/v) inocula were used in all SSFs. Several shake flask SSFs were run on pretreated wheat straw with 5, 20, and 40% inocula to determine whether the level of inocula affected performance, but no effect was noted.

The wheat straw (12.5 kg) was pretreated with 137 L of dilute sulfuric acid (0.5% (v/v)t) in a Pfaudler glass lined and jacketed reactor. The pH of the dilute sulfuric acid was 1.54, and temperature was held at 140°C for 1 h with impeller stirring. The heating up and cooling down times were approximately 1 h each. The pretreated straw was then centrifuged, washed

several times with distilled water, and the pH was brought up to between 4.0 and 4.5 with 70 wt% NaOH followed by a final rinsing. About 63% of the pretreated wheat straw dry wt was determined to be cellulose, 32% lignin and acid insoluble ash, and 2.5% xylan.

A substrate level of 7.5% cellulose was chosen for the shake flask and stirred reactor SSFs of wheat straw owing to mixing problems revealed in preliminary SSF evaluations performed with *S. cerevisiae* and pretreated wheat straw at higher substrate levels. Regardless of the cellulase level, cellulose levels of greater than 10% (w/v), which represent at least 15% (w/v) of dry straw solids, took 3 or more d to liquefy. These were difficult to mix, and, therefore, were not included in this study. Since wet straw was shown to provide higher saccharification yields than oven dried straw, wet straw (65% moisture) was chosen for this study. The pretreated wet straw was frozen for storage to prevent microbial degradation.

Ethanol concentrations in the supernatant were measured by gas chromatography using a Porapak Q80/100 column. The internal standard was 4% (w/v) isopropanol. Glucose and cellobiose were determined as glucose by incubation of the sample with 2 mg/mL almond extract β-glucosidase from Sigma for 1 h at 37°C, and total sugars were measured on the glucose analyzer from Yellow Springs Instruments, Yellow Springs, OH. Viable cell density was measured as colony forming units (CFU) by plating serial dilutions on YPD or YPC plates.

Results reported in percent equivalent conversion of cellulose represent the percent of the feed cellulose that is required to provide the measured ethanol concentration assuming a 90% yeast conversion efficiency; the remaining 10% of the sugars produced from the cellulose are assumed to be used for cell growth and maintenance. This value indicates the amount of substrate required to produce the ethanol concentrations measured in the screening experiments, and puts the fermentations on the same basis for comparison with the straight saccharification of cellulose to sugars. Although we were able to measure residual cellulose in previous work with Sigmacell-50, a method for measuring residual cellulose in the pretreated wheat straw has not yet been developed because of lignin interference, and the percent equivalent conversion provides an estimate of cellulose utilization that has proven to be reasonably accurate in previous studies (*17,18,22*). The straight saccharification yields are calculated as the amount of glucose produced compared to the potential glucose in the cellulose present.

The β-glucosidase activity of the Novo-188 cellobiase enzyme used in supplementation for SSFs was determined by running *p*-nitrophenyl-β-glucoside assays at selected temperatures of 37, 40, and 50°C. It was found that at 37°C the activity is 125 IU/mL, at 40°C the activity is 200 IU/mL, and at 50°C it is 500 IU/mL. Since the wheat straw SSFs were run at 37°C, the ratios of β-glucosidase reflect this temperature whereas the IUs for the cellulase activity are measured at 50°C, according to the IUPAC procedures (*5*).

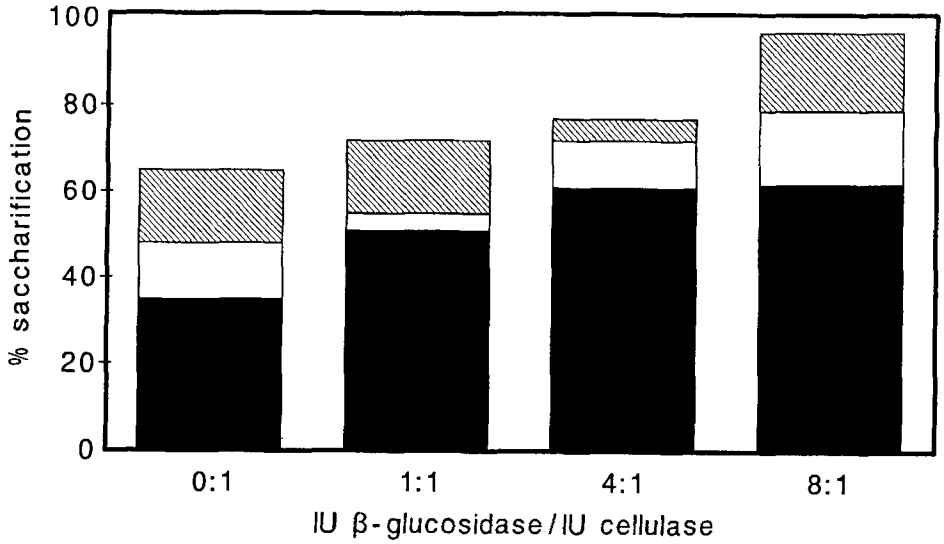

Fig. 1. Saccharifications of pretreated wheat straw (45°C) with Genencor (batch II) cellulase at 7 (■), 13 (□), and 26 (▨) IU/g of cellulose at selected ratios of cellulase to β-glucosidase (IU/IU).

RESULTS

Genecor 150L cellulase enzyme was used in current experiments since it decreases enzyme variability when results for pretreated wheat straw are compared to other previously obtained data with Sigmacell-50 cellulose (*17*). Since our original supply of cellulase (batch I) was exhausted on preliminary small-scale screenings with Sigmacell-50, we used a new cellulase (batch II) for saccharifying the substrate in all the following work. Although about a 20% decrease in ethanol production was observed for SSFs conducted with batch II enzyme, it was still valuable to compare the yeast characteristics on a consistent basis for the pretreated wheat straw SSFs. When batch II of Genencor enzyme was supplemented with β-glucosidase, the specific cellulase activity increased substantially (Fig. 1).

The most recent IUPAC revision (1987) "Measurements of Cellulose Activities" states that the level of β-glucosidase in an enzyme preparation may affect the results of the cellulase assay, particularly for filter paper units (*5*). Results in Table 1 verify this statement. Changes in apparent specific cellulase activity of the cellulase enzyme are given as a function of increasing β-glucosidase supplementation.

Shake flask wheat straw SSFs were performed with *Saccharomyces cerevisiae* (D_5A), *S. uvarum, Candida brassicae,* and *C. acidothermophilum,* with and without β-glucosidase (Novo-188) supplementation. The levels of cellulase activity were 7, 13, and 26 IU of cellulase/g of (7.5%) cellulose in the straw substrate. The β-glucosidase was added as a supplement at ratios of 1:1, 4:1, and 8:1 IUs of β-glucosidase to IU cellulase. SSFs were run at 37°C, so the activity of the cellobiase supplementation reflects this

Table 1
Apparent Specific Cellulase Activity of Genencor 150L Cellulase Enzyme Stock as a Function of β-Glucosidase Supplementation

β-glucosidase:cellulase	IFPU/mL
0:1	85
1:1	127
2:1	160
4:1	180
8:1	217

temperature. However, *S. uvarum* was also run at 41°C to examine if the higher temperature would improve *S. uvarum's* performance over the 37°C SSF run. In addition, SSF using a mixed culture of *B. Clausenii* and *S. cerevisiae* was run on pretreated wheat straw with and without β-glucosidase supplementation as a control.

A separate series of nine SSFs were run with mixed culture I and 19 IU of cellulase/g of cellulose to check the repeatability of these shake flask SSFs. From these fermentations, a standard deviation of 2.6% of the mean of the final ethanol concentration of 34.9 g/L was calculated. Thus, results appear reliable and repeatable.

Table 2 summarizes the results as measured in percent equivalent cellulose conversions at the end of each SSF run, e.g., after approx 4–7 d. The data for the β-glucosidase ratios with 7 IUs cellulase/g substrate reveal that mixed culture I of *B. clausenii* and *S. cerevisiae* gives the best overall performance because of the β-glucosidase activity of *Brettanomyces clausenii*. For the 13 IU loadings, mixed culture I outperforms the other yeast at the 0:1 ratio of β-glucosidase to cellulase only to be surpassed by *S. uvarum* for the β-glucosidase supplemented SSFs. At the 26 IU level of cellulase, *Saccharomyces* strains *uvarum* and *cerevisiae* reach 100% conversion efficiencies with a 4:1 ratio of β-glucosidase to cellulase, and *C. brassicae* reaches 100% conversion at a 8:1 ratio. In actuality, the *Saccharomyces* strains probably achieve virtually complete estimated equivalent cellulose conversions at the highest β-glucosidase loading, because they ferment with better than the 90% efficiency assumed. The results of these two strains are too close to distinguish their performance. At this high level of cellulase (26 IU), mixed culture I and *C. acidothermophilum* do not improve over the 4:1 ratio of enzymes and give a 94% final estimated cellulose conversion.

The results show that the two *Saccharomyces* strains give the best final conversions at high enzyme loadings and high β-glucosidase supplementations. At the lower, more economical enzyme loadings, mixed culture I performs better than any single yeast, but then cellulose conversions are incomplete. *S. uvarum* did not substantially improve in performance at the higher temperature of 41°C without β-glucosidase supplementation. The conversions seem to be limited at this point by fermentation efficiencies of individual yeast strains and not by the cellulase activity.

Table 2
Summary of Equivalent Cellulose Conversions in Small Scale Batch SSFs of Pretreated Wheat Straw at Various Cellulase and β-Glucosidase Loadings[a]

IU β-glucosidase:IU cellulase		0:1			1:1			4:1			8:1		
IU Cellulase/GM cellulose Yeast	Temperature	7	13	26	7	13	26	7	13	26	7	13	26
Saccharomyces uvarum	41°C	73[1]	81	83	76	84	87	82	91	94	85	95	99
Saccharomyces uvarum	37°C	67	73	86	81	83	94	85	96	100	87	100	100
Saccharomyces cerevisiae	37°C	50	63	85	70	81	88	77	84	100	74	86	100
Candida brassicae	37°C	47	72	76	70	81	84	72	82	87	77	89	100
Candida acidothermophilum	37°C	70	77	85	83	86	90	85	87	94	81	90	94
Mixed culture I[b]	37°C	73	81	84	76	86	89	78	88	94	88	92	94

[a]All cellulose conversions are estimated as percent equivalent conversion. Initial cellulose concentration was 7.5 wt%.
[b]Mixed culture I = *Brettanomyces clausenii* and *Saccharomyces cerevisiae*.

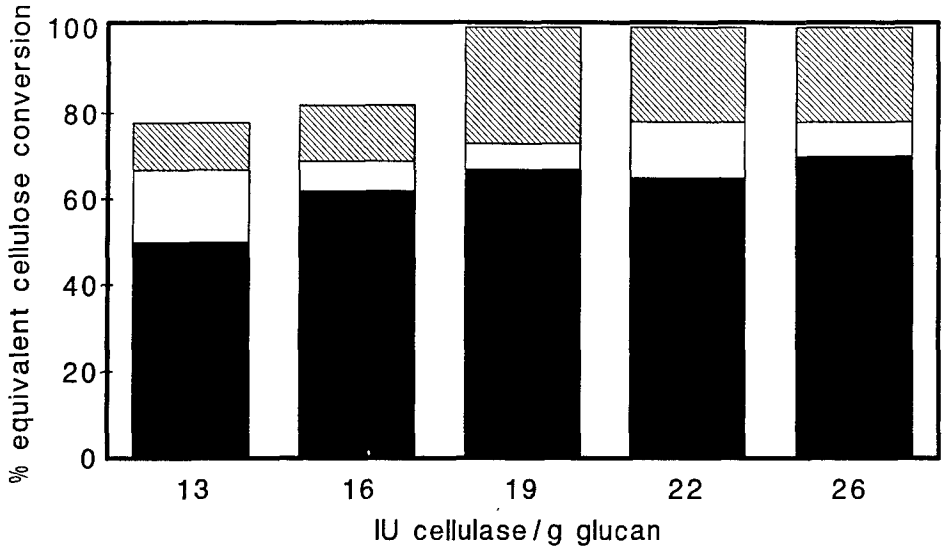

Fig. 2. Percent equivalent cellulose conversions for *S. uvarum* at 3d with Genencor cellulase (batch II) loadings ranging from 13 to 26 IU/g of cellulose at selected ratios (0:1 (■), 1:1 (□), and 8:1 (▨) (IU/IU)) of β-glucosidase supplementation to cellulase for shake flask SSFs at 37°C with pretreated wheat straw.

For our next experiment, *S. uvarum* was studied in a series of shake flask SSFs with cellulase enzyme loadings of 13, 16, 19, 22, and 26 IU/g of cellulose with and without β-glucosidase supplementation. In previous work with Sigmacell-50 substrate, we found 13 IU/g cellulose to be the optimal enzyme loadings (*17*). The runs reported here were made to better define the effect of β-glucosidase and cellulase loadings on performance with the pretreated straw substrate as compared to the previous experiments. As Fig. 2 reveals, 19 IU/g of cellulose gave virtually complete cellulose conversion in 3 d with a 8:1 ratio of β-glucosidase to cellulase. This level of cellulase enzyme was, therefore, chosen for the stirred fermenter SSFs. Recent results indicate that there is an adsorptive loss of the cellulase enzyme component β-glucosidase that binds to lignin in dilute acid pretreated aspen wood. This may account for increased enzyme loadings with the pretreated straw substrate versus Sigmacell-50 cellulose (*20*).

Three-L batch SSFs were run with *Saccharomyces cerevisiae, S. uvarum,* and mixed culture I at 0:1, 1:1, and 8:1 ratios of β-glucosidase to cellulase at 37°C and 7.5% initial cellulose concentrations. A mixing problem was encountered with the stirred reactor SSFs on pretreated wheat straw. In the 6-L Braun Biostat fermenters, the stirring shaft with three turbine impellers was initially unable to mix the 12% substrate slurry, whereas the shaker flask SSFs mixed well with a rotating shaker. Consequently, the larger-scale stirred reactor SSFs took at least 24 h longer to liquefy than did the shaker flask SSFs. Some of the stirred reactor SSFs had to be repeated because the delay in the onset of the fermentation impaired yeast

Table 3
Summary of Equivalent Cellulose Conversions for Controlled SSFs at 37°C on Pretreated Wheat Straw with 19 IUs Cellulase/g of Cellulose and Varying Ratios of β-Glucosidase Supplementation

	2 d			4 d			Final		
	0:1	1:1	8:1	0:1	1:1	8:1	0:1	1:1	8:1
Saccharomyces uvarum	60	73	79	75	78	99	81	84	100
Saccharomyces cerevisiae	42	68	73	63	78	99	75	84	100
Mixed Culture I[a]	47	52	65	63	67	89	78	81	94

[a]Mixed Culture I = *Brettanomyces clausenii* and *Saccharomyces cerevisiae.*

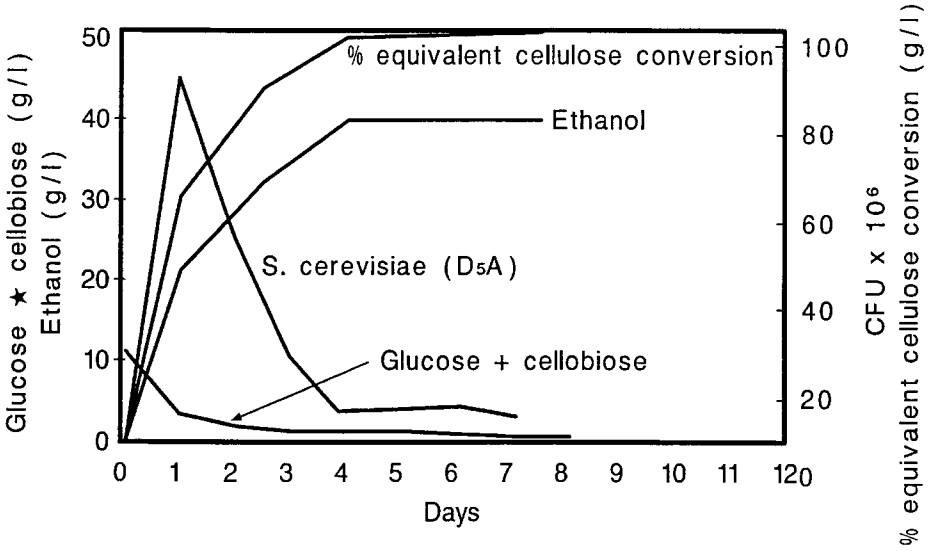

Fig. 3. Controlled stirred reactor SSF with *S. cerevisiae* (D_5A) at 37°C with Genencor cellulase loading of 19 IU/g pretreated wheat straw cellulose substrate (7.5% (w/v)) and 8:1 β-glucosidase supplementation to the cellulase enzyme (IU/IU).

growth and the fermentation would either cease or proceed slowly. Nevertheless, the *Saccharomyces uvarum, S. cerevisiae,* and mixed culture stirred reactor studies were completed. Table 3 reports the percent cellulose conversions at 2, 4, and final day intervals.

From this data, *S. uvarum* is the fastest fermenter (4–6 d) followed closely by *S. cerevisiae* (4–7 d) and then the mixed culture (7–8 d). Both the *Saccharomyces* strains attained virtually complete conversions with the higher level of β-glucosidase supplementation. These results verify the shake flask SSF results, although approximately one extra day was required. Figure 3 shows an example of results from a controlled stirred reactor SSF with *S. cerevisiae* at a 8:1 ratio of β-glucosidase to cellulase.

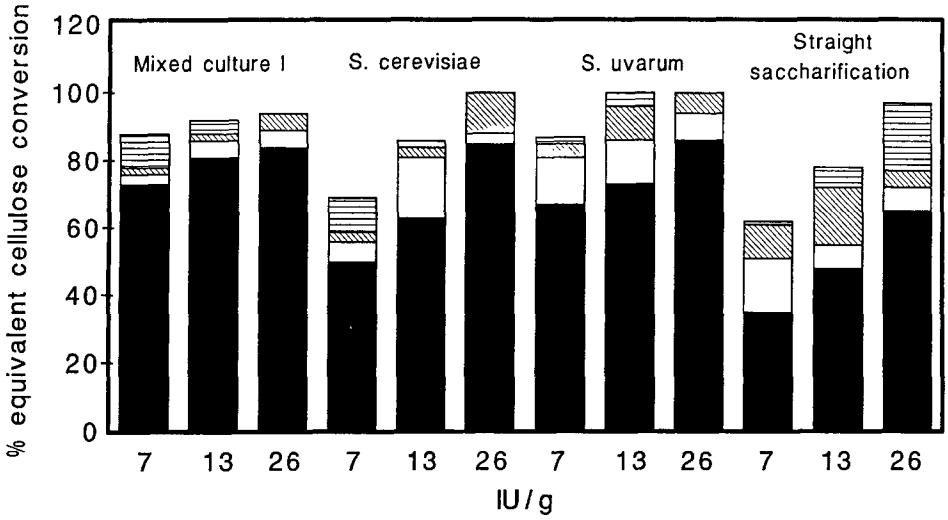

Fig. 4. Comparison of equivalent cellulose conversions for shake flask pretreated straw SSFs at 37°C and straight saccharification at 45°C for various cellulase enzyme loadings with varying ratios (0:1 (■), 1:1 (□), 4:1 (▨), and 8:1 (⊟) (IU/IU)) of β-glucosidase supplementation.

The soluble sugar concentrations and colony forming units (CFUs) were monitored in all the stirred reactor studies. A sugar concentration of about 1 g/L was measured at the start of the fermentation and dropped to less than 0.5 g/L at the finish for the 0:1 and 1:1 β-glucosidase to cellulase ratios, and *Saccharomyces* yeasts. At 8:1 β-glucosidase:cellulase ratios the soluble sugar level for these yeast started out at about 10 g/L and dropped to 1 g/L or less. For the mixed culture, a similar trend was seen, except at an 8:1 ratio of enzyme, the sugar concentration starts at 5.0 g/L, because the two yeast keep the starting sugars down. All yeast showed about the same viable cell density as measured in colony forming units (CFUs): values started at around 2×10^7, reached 8×10^7 in 24 h, and then gradually dropped to around 4×10^7 cells in 6–7 d. *B. clausenii* showed lower overall viability.

Unfortunately, substrate (cellulose) disappearance could not be measured directly as had been done for other stirred reactor studies using Sigmacell-50 as a substrate (*10,11,18*). Thus, the percent equivalent cellulose conversion was used to estimate the cellulose conversion. A comparison of equivalent cellulose conversions for *Saccharomyces uvarum, S. cerevisiae,* and mixed culture I in the shake flask SSFs to saccharification without fermentation can be seen in Fig. 4. The latter was conducted at 45°C on wheat straw with and without β-glucosidase supplementation at ratios (based on 37°C assay) of 1:1, 4:1, and 8:1 β-glucosidase to cellulase. All of these experiments were completed using 7, 13, and 26 IU Genencor cellulase/g of cellulose in the wheat straw. Ninety-four percent saccharification was achieved in 5 d with 8:1 β-glucosidase supplementation at 26 IU cellu-

lase loading. In the two *Saccharomyces* wheat straw SSFs, complete equivalent cellulose conversions at 4:1 and 8:1 were seen in 5 d, showing faster saccharification of substrate in SSF was obtained at 37°C than with saccharification without fermentation at 45°C. The mixed culture again outperformed all of the other yeast at the lower cellulase levels of 7 and 13 IUs without β-glucosidase supplementation.

CONCLUSIONS

SSFs were performed on pretreated wheat straw to evaluate some previously selected yeast for fermentations on a substrate representative of those potentially useful in commercial ethanol production. Results show an improvement in SSF for the pretreated straw substrate with increasing enzyme loadings and β-glucosidase supplementation. In addition, saccharification rates improved using the pretreated wheat straw substrate vs Sigmacell-50 SSFs (*16,17*).

Clearly, a mixed culture of a cellobiose fermenting yeast and a strong glucose-fermenter reduces the sugar concentrations (cellobiose, in particular) throughout the fermentation. The rate of hydrolysis and product yield at low cellulase enzyme loadings is increased. Also, β-glucosidase supplementation greatly improves the fermentation rates when used with a strong glucose-fermenting yeast by minimizing cellobiose accumulation. Both saccharification and SSF results show that addition of β-glucosidase increases the specific activity of cellulase from *T. reesei*. The saccharification rate and SSF performance increase at lower, more economical, cellulose enzyme loadings if β-glucosidase is added.

Pretreated wheat straw performs well in the SSF process, and a single culture of a strong glucose fermenter like *S. uvarum* appears to be a good choice when supplemented with β-glucosidase enzyme at a high level. However, β-glucosidase supplementation has been shown to be uneconomical. Thus, an alternate approach of improving the balance of cellulase properties, developing an ethanol tolerant yeast that ferments glucose and cellobiose to high ethanol concentrations, or a combination of these approaches is desirable.

REFERENCES

1. Becker, D. K., Blotkamp, P. J., and Emert, G. H. (1980), "Pilot Scale Conversion of Cellulose to Ethanol," American Chemical Society, Div. Fuel Chem. Prepr. **25**(4), 279-308.
2. Becker, D. K., Blotkamp, P. J., and Emert, G. H. (1981), *Fuels from Biomass and Wastes*, Klass, D. L. and Emert, G. H., eds., Ann Arbor Science Publications, Ann Arbor, MI, chapter 20, pp. 375-391.

3. Blotkamp, P. J., Takagi, M., Pemberton, M. S., and Emert, G. H. (1978), *AICHE Symp. Ser.* **74**(181), 85–90.
4. Deshpande, V., Sivaraman, H., and Rao, M. (1983), *Biotechnol. Bioeng.* **25**(6), 1679–1682.
5. Ghose, T. K. Measurements of cellulase Activities, Pure and Applied Chemistry, Biochemical Engineering Centre, Indian Institute of Technology, New Delhi-110016, India, IUPAC, (1987).
6. Ghosh, T. K., Roychoudhury, P. K., and Ghosh, P. (1984), *Biotechnol. Bioeng.* **26**(6), 377–381.
7. Gonde, P., Blondin, B., Leclerc, M., Ratomahenina, R., Arnaud, A., and Galzy, P. (1984), *Appl. Environ. Microb.* **48**(2), 265–269.
8. Hinman, N. (1988), Solar Energy Research Institute, personal communication. (1988).
9. Janssen, J. H., Burris, N., Woodward, A., and Bailey, R. B. (1983), *Appl. Environ. Microb.* 598–602.
10. Lastick, S. M., Spindler, D. D., and Grohmann, K. (1983), *Wood Agric. Residues* 239–251.
11. Lastick, S. M., Spindler, D., Terrell, S., and Grohmann, K. (1984), *Biotech 84*, 277–289.
12. Pemberton, M. S., Brown, R. D., Jr., and Emert, G. H. (1980), *Can. J. Chem. Eng.* **58,** 723–729.
13. Rivers, D. B., Zanin, G. M., and Emert, G. H. (1984), *Proc. Ark. Acad. Sci.* **38,** 95–96.
14. Saddler, J. N., Hoagan, C., Chan, M. K.-H., and Louis-Seize, G. (1982), *Can. J. Microbiol.* **28,** 1311.
15. Shoemaker, S. P. (1984), *Biotech 84* 593–600.
16. Spangler, D. J. and Emert, G. H. (1986), *Biotechnol. Bioeng.* **28,** 115–188.
17. Spindler, D. D., Wyman, C. E., Mohagheghi, A., and Grohmann, K. (1988), *Appl. Biochem Biotech.* **17,** 279.
18. Spindler, D. D., Wyman, C. E., and Grohmann, K. (1989), *Biotech & Bioengineering* 5424.
19. Takagi, M., Abe, S., Suzuki, S., Emert, G. H., and Yata, N. (1977), *Proc. Biocon. Symp.* IIT, Delhi, 551–571.
20. Takagi, M. (1986), *Biotechnol. Bioeng.* **28,** 115–118.
21. Tatsumoto, K., Baker, J. O., Tucker, M. P., Oh, K. K., Mohagheghi, A., Grohmann, K., and Himmel, M. E. *Biotechnol. Bioeng. Symp.* **18,** in press.
22. Wyman, C. E., Spindler, D. D., Grohmann, K., and Lastick, S. M. (1986), *Biotechnol. Bioeng.* **17,** 221–238.