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Improvements in Titer, Productivity, and Yield Using Solka-Floc for Cellulase Production

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Abstract

Researchers studying cellulase enzymes for the economical production of fuel ethanol envision cellulose as the carbon source. However, submerged *Trichoderma reesei* cultures grown on cellulose exhibit high run-to-run variability. Thus, an investigation of 30 batch cellulase production experiments was instrumental in determining fermentation conditions that improved enzyme titers, yields, and productivities. Eighteen of the 30 batch experiments experienced minimal process upsets and were classified into eight groups based on agitation rate, gas sparge rate, and the use of oxygen supplementation. Comparing corn steep liquor with yeast extract/peptone also tested the effect of different sources of nitrogen in the media. Average 7-d enzyme titers were doubled from 4 to 8 FPU/mL primarily by increasing aeration.

Index Entries: Cellulase; *Trichoderma reesei* L27; aeration; agitation; oxygen supplementation.

Introduction

Cellulase production using lignocellulosic biomass as the primary carbon source offers significant opportunities for reducing the cost of fuel ethanol. The total production cost of ethanol from biomass is targeted at below $1.50/gal. Many consider commercially available cellulase enzymes too costly for economical ethanol production. To achieve the goal of cheap ethanol from biomass, cellulase production is envisioned as a unit operation within an ethanol production facility *(1)*. This unit operation would use inexpensive lignocellulosic feedstocks to produce the enzyme. Whole broth cellulase including residual fungal cell mass would be continuously transferred to the simultaneous saccharification and cofermentation (SSCF) unit operation to reduce the need for purification, concentration, and

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stabilization of the protein slurry. Economic models of the proposed biomass-to-ethanol processes have been created at the National Renewable Energy Laboratory (NREL). These models demonstrate the sensitivity of parameters to the overall cost of ethanol and provide goals and guidelines for research initiatives. Volumetric productivity of the cellulase enzyme was identified as the single most important technical target to focus on because economic analyses show that enzyme production costs are much more sensitive to changes in volumetric productivity than changes in yield. In fact, economic projections of the operation of the cellulase unit show that with a lignocellulosic feedstock cost of $25/dry t, increasing enzyme volumetric productivity from 30 to 55 FPU/(L·h) reduces the production cost of fuel ethanol by nearly $0.40/gal (Ruth, M., personal communication). These projections are shown in Fig. 1.

One of NREL's current research projects is designed to test and improve the cellulase production process to meet the economic goals. This demonstration involves the bench scale growth of *Trichoderma reesei* in submerged cultures for enzyme production using cellulose as the carbon source. These submerged fungal cultures can exhibit severe foaming (especially with pretreated substrates) and high run-to-run variability. In an earlier study, the results of cellulase production runs using pretreated yellow poplar hydrolysate were presented *(2)*. Acetic acid, furfural, and lignin present in the pretreated biomass inhibited enzyme production by *T. reesei*.

Although research on enzyme production on pretreated biomass continues, it is important to develop a baseline experimental data set under optimal conditions using pure cellulose (Solka-floc). Three primary goals of this research will aid the development of this baseline data set:

1. To demonstrate the ability of *T. reesei* L27 to achieve the economic goals of high titer, productivity, and yield using cellulose as the sole carbon source
2. To gain a better understanding of the biochemistry of the production process and determine what operational settings are needed to increase titer, productivity, and yield
3. To conduct replicate experiments to discern effects of important variables from run-to-run variation.

Over the course of almost 2 yr, we conducted more than 30 batch cellulase production runs using Solka-floc (obtained from Fiber Sales and Development Corp., St. Louis, MO). A subset of this data set of Solka-floc-based enzyme production runs was used to determine the effects of nutrient media (yeast extract/peptone [YE/P] vs corn steep liquor [CSL]) and aeration (agitation rate, gas sparge rate, and oxygen supplementation). Improving aeration resulted in substantial and statistically significant increases in enzyme titers, yields, and productivities. Although early runs obtained average 7-d titers of 4 FPU/mL, later runs achieved average 7-d titers of 8 FPU/mL. In essence, a doubling of the enzyme titer was demonstrated. Corresponding process yields increased from 72 to 144 FPU/g of

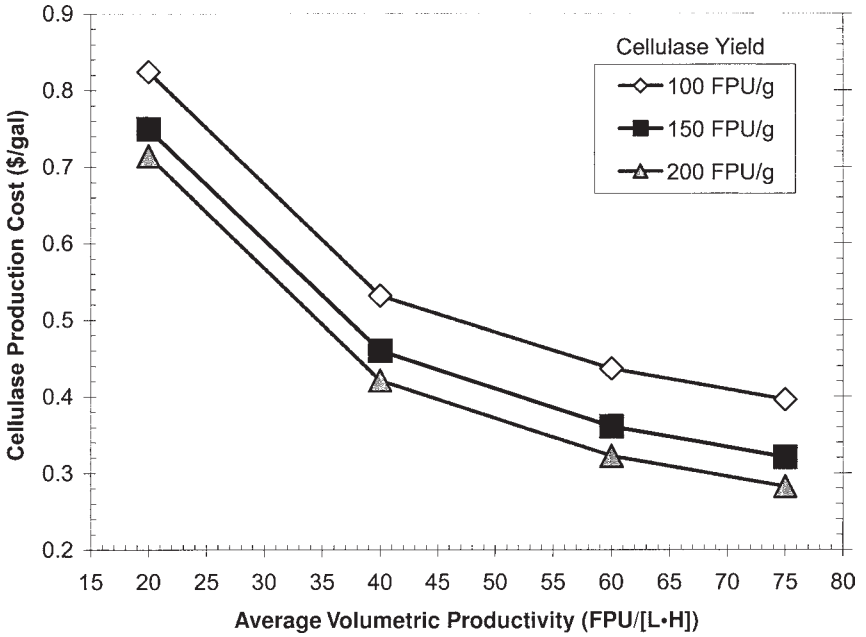

Fig. 1. Cellulase production cost as a function of volumetric productivity.

carbohydrate, and average volumetric productivities higher than 55 FPU/(L·h) were achieved.

Materials and Methods

Microorganism

The microorganism used for this research was *T. reesei* strain L27. After testing four strains of *T. reesei* (L27, Rut C-30, RL-P37, and QM9414) on Solka-floc and pretreated yellow poplar sawdust, *T. reesei* strain L27 was determined to be the best producer of enzymes on cellulose. In addition, *T. reesei* L27 produced enzyme in the presence of wood hydrolysate *(2)*. This strain is a hyperproducing mutant created at Cetus Corporation, Berkeley, CA through classic strain improvement techniques *(3,4)*. As a classically developed mutant, this strain does not need to be grown under the potentially more stringent containment requirements dictated for many recombinant microorganisms. Taken together, these attributes make *T. reesei* strain L27 an attractive candidate for demonstrating low-cost enzyme production on cellulosic feedstocks.

Preparation of Inoculum

Frozen stock cultures of *T. reesei* L27 spores were prepared as suggested by Schell et al. *(5)* and stored at –70°C. The seed culture was typically grown in two stages. In the first stage, a single cryovial of frozen stock culture was used to inoculate a baffled 250-mL shake flask containing a

50-mL working volume of 2% (w/v) glucose nutrient medium. The flask was incubated in a temperature-controlled orbital shaker operating at an agitation rate of 200 rpm at 28°C. After 36–48 h, the first-stage culture was transferred at a 5% (v/v) inoculum level to an appropriate number of baffled 1-L shake flasks containing a 300-mL working volume of a 1% w/v Solka-floc nutrient medium. The second-stage flasks were incubated for 36–48 h prior to inoculating the production vessel(s) at 5% (v/v).

Standard Cellulase Production Methodology

Enzyme production vessels were prepared by adding 5% (w/v) Solka-floc as the sole carbon source, a complex nitrogen source, water, and corn oil, and autoclaving at 121°C for 30 min. Because a suspension of 5% (w/v) Solka-floc is too thick to achieve effective mixing or aeration in shake flasks, all the production experiments were carried out in Bioflo 3000 fermentors (New Brunswick Scientific, New Brunswick, NJ). Seven-liter vessels with a working volume of 2.5 L were used. The cultures were grown for 7 d at 28°C. The pH was maintained at 4.8 using automatic base and acid additions of 4 *M* ammonium hydroxide and 2 *M* phosphoric acid, respectively. The fermentors were also continuously sparged with sterile filtered air (vvm was varied based on the experiment). Vogel's media including basal salts, trace minerals, and Tween-80 were used in all cellulase production vessels, as in our previous research *(4)*. The fermentors were connected to a data acquisition system (Biocommand software, New Brunswick Scientific), which recorded data points for temperature, pH, gas sparge rate, dissolved oxygen, and agitation every 30 min.

Tested Run Conditions

Experiments were conducted to study the effect of the complex nitrogen source on enzyme production. YE/P media at a concentration of 10 g/L of yeast extract and 20 g/L of peptone was tested as a laboratory grade source of amino acids. By contrast, 1% (v/v) CSL (obtained from Grain Processing Corporation, Muscatine, IA) was tested as an industrial source of complex nitrogen and vitamins. The CSL was tested as either a 1% (v/v) clarified filter-sterilized solution or a 1% (v/v) whole autoclaved stock containing the corn solids. Because scale-up dictated the use of tap water instead of deionized water, this variable was also tested.

Three aeration factors were tested: agitation rate, gas sparge rate, and oxygen supplementation. Runs without molecular oxygen supplementation simply received air at a given sparge rate; vessels with oxygen supplementation used auxiliary oxygen tanks and the Bioflo 3000 dissolved oxygen control system. For the experiments involving oxygen supplementation, the dissolved oxygen concentration was maintained at or above 20% of air saturation. The second aeration parameter tested was the agitation rate. Agitation was tested at three levels: 300, 450, and 600 rpm. Agitation remained constant over the course of the 7-d runs. The third aeration parameter tested was the average achieved gas sparge rate in the range of

0.2–5.0 vvm. Although a needle valve on the Bioflo 3000 fermentors was used to set the gas flow, fluctuations in the delivered flow rate were observed. The Biocommand data acquisition system was used to take measurements every 30 min on the actual sparge gas rate achieved (liters per minute) in each vessel. These data were averaged and used to categorize a run as having a low, medium, or high gas sparge rate.

Analytical Methods

Samples were drawn daily from the fermentors, and centrifuged, and the supernatant was analyzed for cellulase activity and amounts of protein. The cellulase enzyme activity was measured using the procedure recommended by the International Union of Pure and Applied Chemistry *(6)*. The BCA protein assay *(7)* was used to determine the total protein amount in the supernatant.

Adjusting FPU Titers to Account for Reduction in Culture Volume

Despite the use of a chilled condenser, which is part of the Bioflo 3000 fermentation unit, significant reduction in culture volumes occurred in many of the week-long highly aerobic fermentation runs. These reductions in volume presumably resulted from evaporation or stripping of the water in the culture media. In cases in which volume losses are significant, large errors will result in reported performance parameters (such as volumetric productivity and yield) if corrections are not made to account for the reduced working volume. In a bioethanol production process, a specified total amount of cellulase enzyme (as filter per units or mass of enzyme protein) must be fed to the SSCF unit operation. The total amount of enzyme produced in the enzyme production unit operation is the product of the final enzyme titer and the final cellulase production volume (assuming batch operation). The assumption that the cellulase production volume has remained constant at its initial volume during enzyme production can lead to erroneous estimates of the amount of installed enzyme production capacity required for the process.

We observed that the amount of reduction in volume that occurred over the course of a cellulase production run depended strongly on the gas sparge rate. To compare the productivity of the different enzyme production runs on an equivalent basis, measurements of the culture volume in the fermentor were routinely taken at each sampling point. The volume of base or acid added for pH control approximately equaled the volume withdrawn during sampling, so a simple correction for volume loss was employed. Measured enzyme activity titers were corrected for volume reduction by multiplying by the ratio of the culture volume at the time of the sample to the initial culture volume, according to Eqs. 1 and 2:

$$E_c = \phi E_t \tag{1}$$

in which

$$\phi = (V_t / V_o) \tag{2}$$

and in which E_c is the corrected enzyme titer (FPU/mL or FPU/L); E_t is the enzyme activity at time t (FPU/mL or FPU/L); ϕ is the volume reduction factor (dimensionless); V_t is the volume at time t (L); and V_0 is the initial volume (L).

This correction became significant in the high sparge rate experiments in which vessels lost almost 50% of their original volumes by the seventh day of operation. Titers corrected for the reduced working volume, and productivities and yields calculated using these corrected titers, are reported here.

Calculation of Average Volumetric Productivity

Cellulase enzyme average volumetric productivity (Q_P) was calculated by dividing the corrected enzyme titer at a given time (filter paper units/liter) by the elapsed time of cultivation (hours) according to Eq. 3.

$$Q_P = (\phi E_t / t) \tag{3}$$

in which E_t is the enzyme activity at time t (FPU/L); and t is the time (h). As such, Q_P is an estimate of process performance over the duration of the fermentation rather than a measure of the maximum rate of enzyme production. Instantaneous or interval volumetric productivities that are considerably higher than Q_P can be obtained during specific periods over the course of a typical batch cellulase production run.

Calculation of Process Yield

The yield of cellulase enzyme based on total carbohydrate substrate fed (Y_P) was also calculated using corrected enzyme titers. These values were calculated by dividing the corrected enzyme titer at the final time (filter paper units/liter) by the concentration of the carbohydrate substrate added to the fermentor at the beginning of the batch run, according to Eq. 4.

$$Y_P = (\phi E_t / C_0) \tag{4}$$

in which C_0 is the initial glucose or glucose equivalent concentration (g/L).

The concentration of substrate initially charged to the system was expressed in terms of glucose equivalents. In the case of Solka-floc substrate, the initial amount of glucose equivalents was calculated assuming 1.11 grams of glucose/g of cellulose based on hydration (55 g/L of glucose equivalents for the 50 g/L [5% w/v] of Solka-floc cellulose in the system).

Results and Discussion

Effect of Media Formulations

Figure 2 shows that, on the average, process-relevant nutrient medium (whole, autoclaved 1% [v/v] CSL and tap water) produced similar (if not superior) enzyme titers compared with expensive laboratory grade media (YE/P and deionized water). This is a particularly advantageous result

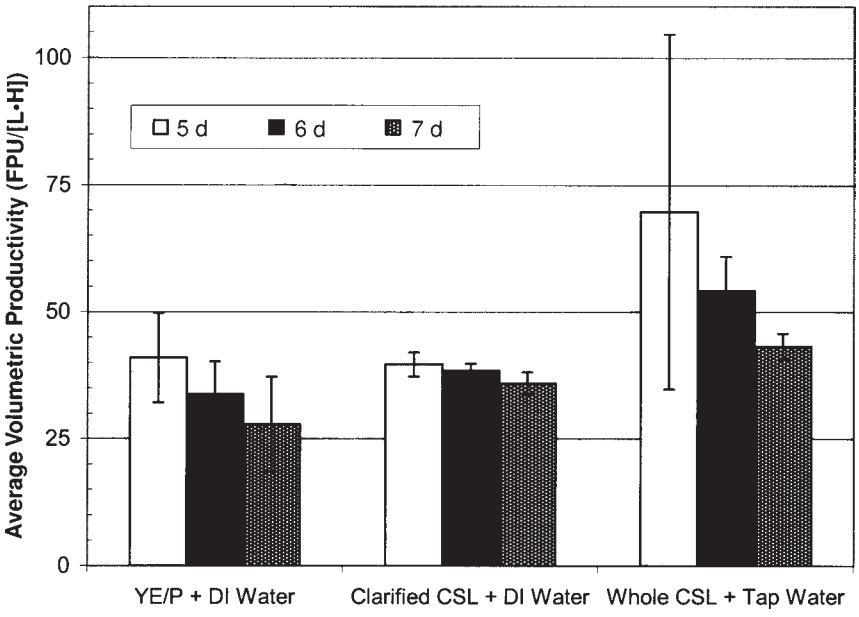

Fig. 2. Effect of nutrient media on volumetric productivity. DI, deionized.

because it is much easier to use tap water and whole CSL at larger scales than it is to use filter-sterilized CSL and deionized water.

Effect of Aeration Parameters on Titer, Productivity, and Yield

Based on our understanding of aerobic protein production processes in general, and of the cellulase enzyme production process in particular, we decided to target oxygen transfer to the culture as a key strategy for improving enzyme titers. Three different methods of achieving increased maximum oxygen transfer rates were examined. First, the benefit afforded by using molecular oxygen supplementation of the sparge gas stream was examined. However, oxygen supplementation is not expected to be an economical approach for achieving high oxygen transfer rates at large scales. Therefore, protocols that would not require the use of oxygen supplementation were also sought. Second, increasing the gas sparge rate during fermentation was tested as a more economical method of improving oxygen transfer capabilities. Unfortunately, it proved difficult to precisely control sparging rates in terms of volumes of gas introduced per volume of culture broth per minute. An averaged delivered gas sparge rate was used to classify each run into low (0.9–1.5 vvm), medium (1.5–3 vvm), or high (3–5 vvm) categories. Third, either alone or in combination with the other two approaches, higher agitation rates were examined as a means to improve oxygen transfer. Agitation rates of 300, 450, and 600 rpm were considered.

Because of operational difficulties that occurred during many of the 30 batch cellulase production runs, several runs were excluded from subsequent analysis. Table 1 presents the refined data set consisting of 18 runs,

Table 1
Summary of Experimental Parameters for Each Run

Experiment ID		Experimental parameters			
Run no.	Experiment no.	Media type[a]	Agitation rate (rpm)	Gas sparge rate (vvm)	Oxygen supplied
1	22	YE/P	300	0.94	Yes
2	22	YE/P	300	1.03	Yes
3	22	YE/P	300	1.01	Yes
4	22	YE/P	300	0.99	Yes
5	29	cCSL	450	1.38	No
6	30	wCSL	450	1.84	Yes
7	35	wCSL	450	0.96	Yes
8	35	wCSL	450	0.95	Yes
9	36	wCSL	450	0.97	Yes
10	36	wCSL	450	2.16	No
11	36	wCSL	450	2.99	No
12	36	wCSL	450	0.97	No
13	38 I	wCSL	450	1.83	Yes
14	38 II	wCSL	450	1.98	Yes
15	39	wCSL	450	1.73	No
16	39	wCSL	450	1.39	Yes
17	39	wCSL	600	1.79	No
18	39	wCSL	300	1.91	No

[a]cCSL, clarified CSL; wCSL, whole CSL.

Table 2
Grouping of Individual Batch Cellulase Runs into Eight Categories (*see* Table 3)

	Without oxygen supplementation		With oxygen supplementation	
Condition no.	Gas sparge rate (vvm)	Agitation rate (rpm)	Gas sparge rate (vvm)	Agitation rate (rpm)
1	0.9–1.4	450		
2	1.7–2.2	300		
3	1.7–2.2	450		
4			0.9–1.4	300
5			0.9–1.4	450
6			1.7–2.2	450
7	>2.5	450		
8	1.7–2.2	600		

which provided enough replicates to assess the effects of actual process improvements from run-to-run variations.

Table 2 provides a means of grouping these experiments into eight categories depending on the levels of agitation (low, medium, or high), gas sparge rate (low, medium, or high), and type of gas sparging (air alone or oxygen-supplemented air). The bold face numbers in the first column of

Table 2 represents the fractional factorial design that was tested for significance of oxygen supplementation. A coding scheme was employed to identify the combination of these factors. For example, condition no. 1 corresponds to a run at 450 rpm, without oxygen supplementation, and with an average achieved gas sparge rate of 0.9–1.4 vvm. Condition no. 4 corresponds to a run at 300 rpm, with oxygen supplementation, and an average achieved gas sparge rate of 0.9–1.4 vvm. Table 3 summarizes the raw data of averaged corrected enzyme titers, average productivity, and average yield for each of the eight different tested conditions at d 5, 6, and 7 for each fermentation. Standard deviations (SDs) of performance levels are also provided in Table 3 for those sets of conditions where multiple runs were made. This information is also presented in Fig. 3 (titers), Fig. 4 (productivities), and Fig. 5 (yields). The error bars depicted in these and in subsequent figures represent 1 SD limit. The text labels for the different bars shown for each of the eight tested conditions presented in Table 2 match the "Condition Description" column provided in Table 3.

As the error bars in Figs. 3–5 demonstrate, there were cases in which large differences in performance were achieved between runs carried out at ostensibly similar operating conditions. Nevertheless, grouping the data as done in Tables 2 and 3 enabled us to make several important observations. For example, the lowest enzyme titers achieved at d 5–7 were approx 1 to 2 FPU/mL and were produced using a combination of low agitation rate (300 rpm), moderate gas sparge rate (1.7–2.2 vvm), and no supplementation of the air sparge stream with molecular oxygen. Modestly better, but still low, corrected enzyme titers of approx 3 FPU/mL were achieved using a combination of low agitation rate, low gas sparge rate, and oxygen supplementation. By contrast, the highest corrected titers of more than 8 FPU/mL were obtained when enzyme production was carried out using a moderate agitation rate (450 rpm) and either a low or moderate gas sparge rate in the presence of oxygen supplementation, or at a high gas sparge rate without oxygen supplementation. In terms of the more economically relevant performance measures of volumetric productivity and yield on cellulosic substrate, comparison of Figs. 3–5 shows that the conditions producing the highest and lowest titers are the same ones that produce the highest and lowest average volumetric productivities and enzyme yields. This is to be expected because productivity and yield are simply calculations based on titer, and the substrate level was the same for all the runs.

Effect of Gas Sparge Rate

Figure 6 offers a closer look at the effect of gas sparge rate on enzyme production performance. The data in Fig. 6, which are a subset of the data presented in Fig. 4, are for the runs carried out without oxygen supplementation using an agitation rate of 450 rpm. Clearly, volumetric productivity increased with increasing gas sparge rates even when the titers were corrected for volume loss. In fact, the titer increased with time at the highest gas sparge rate, further suggesting that oxygen transfer is an important

Table 3
Performance Data of Averaged Conditions for d 5–7

Day	No. of replicates	Coded condition no.	Condition description	Corrected titer (FPU/mL)	Productivity (FPU/[L·h])	Yield (FPU/g)
5	2	1	Med rpm, low vvm, no O_2	4.3 ± 0.1	36.2 ± 1.2	78.1 ± 2.6
	1	2	Low rpm, med vvm, no O_2	1.3	10.6	22.9
	2	3	Med rpm, med vvm, no O_2	5.9 ± 0.3	49.0 ± 2.2	105.9 ± 4.7
	4	4	Low rpm, low vvm, with O_2	3.3 ± 0.4	27.5 ± 3.0	58.6 ± 6.3
	4	5	Med rpm, low vvm, with O_2	7.3 ± 1.7	61.0 ± 14.2	131.1 ± 30.0
	3	6	Med rpm, med vvm, with O_2	8.5 ± 2.3	70.8 ± 19.0	152.7 ± 40.8
	1	7	Med rpm, high vvm, no O_2	7.8	65.3	141.0
	1	8	High rpm, med vvm, no O_2	6.8	56.4	121.8
6	2	1	Med rpm, low vvm, no O_2	4.4 ± 0.2	30.3 ± 1.6	78.3 ± 3.8
	1	2	Low rpm, med vvm, no O_2	2.2	15.5	39.7
	2	3	Med rpm, med vvm, no O_2	6.7 ± 1.3	46.5 ± 8.6	119.8 ± 23.0
	4	4	Low rpm, low vvm, with O_2	3.2 ± 0.4	22.6 ± 2.9	57.9 ± 7.4
	4	5	Med rpm, low vvm, with O_2	7.7 ± 1.3	53.7 ± 9.1	138.6 ± 23.8
	3	6	Med rpm, med vvm, with O_2	8.5 ± 0.8	58.8 ± 5.8	152.4 ± 14.8
	1	7	Med rpm, high vvm, no O_2	8.3	57.9	149.6
	1	8	High rpm, med vvm, no O_2	6.6	46.1	118.3
7	2	1	Med rpm, low vvm, no O_2	4.6 ± 0.4	27.5 ± 2.5	83.2 ± 7.6
	1	2	Low rpm, med vvm, no O_2	2.4	14.3	43.4
	2	3	Med rpm, med vvm, no O_2	7.5 ± 3.5	44.7 ± 21.0	135.1 ± 63.5
	4	4	Low rpm, low vvm, with O_2	3.4 ± 0.4	20.5 ± 2.3	61.4 ± 6.9
	4	5	Med rpm, low vvm, with O_2	8.2 ± 1.1	48.7 ± 6.6	146.9 ± 19.4
	3	6	Med rpm, med vvm, with O_2	10.0 ± 2.2	59.5 ± 13.1	179.5 ± 39.6
	1	7	Med rpm, high vvm, no O_2	13.6	80.8	244.4
	1	8	High rpm, med vvm, no O_2	6.2	36.6	110.7

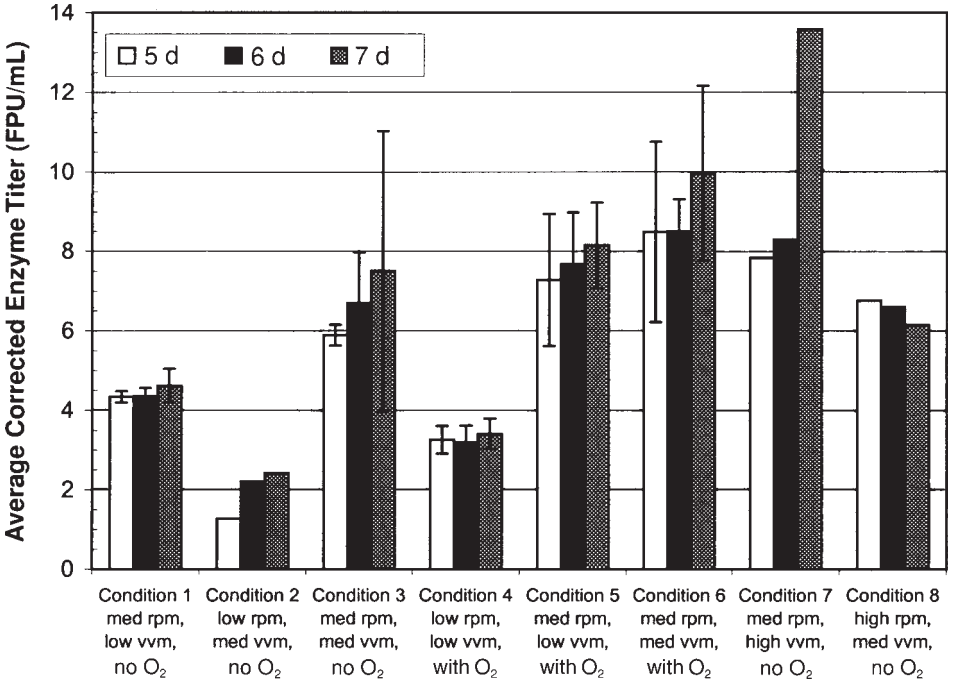

Fig. 3. Comparison of averaged titers for the eight batch conditions.

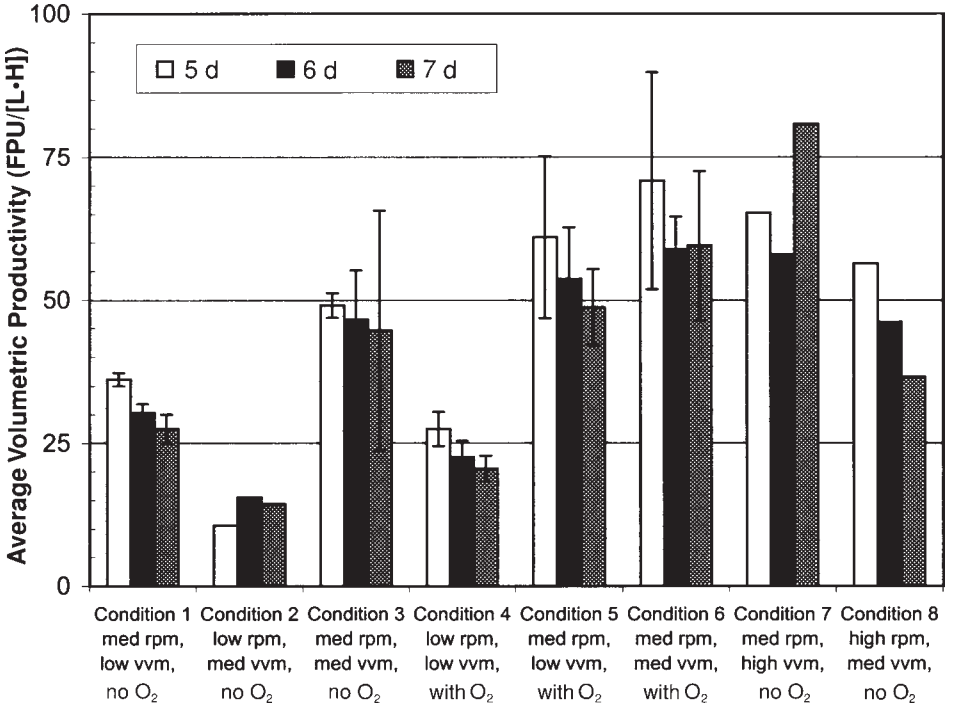

Fig. 4. Comparison of averaged productivities for the eight batch conditions.

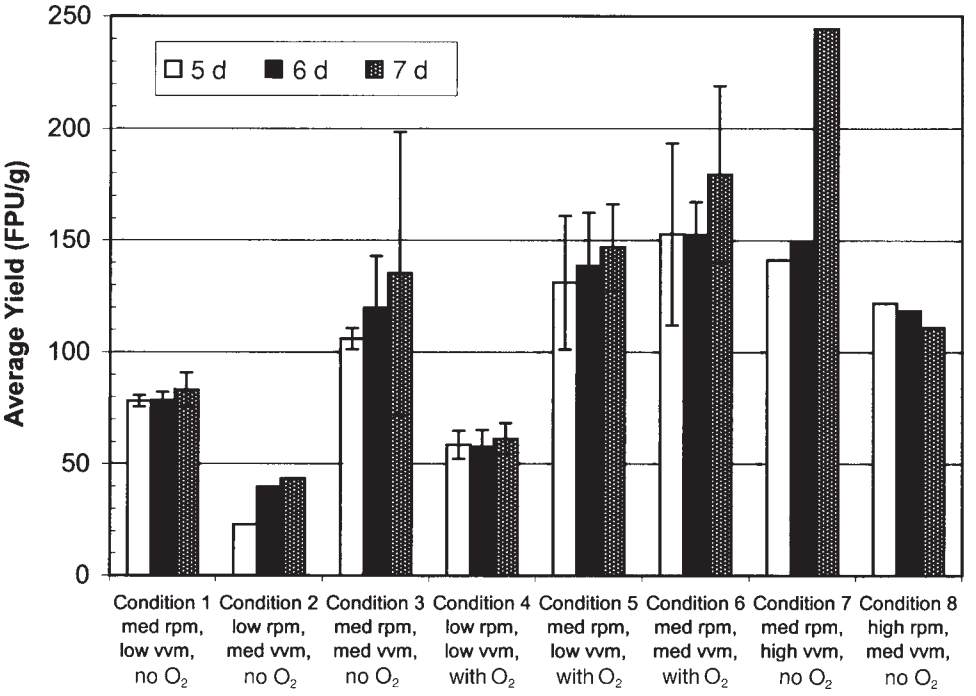

Fig. 5. Comparison of averaged yields for the eight batch conditions.

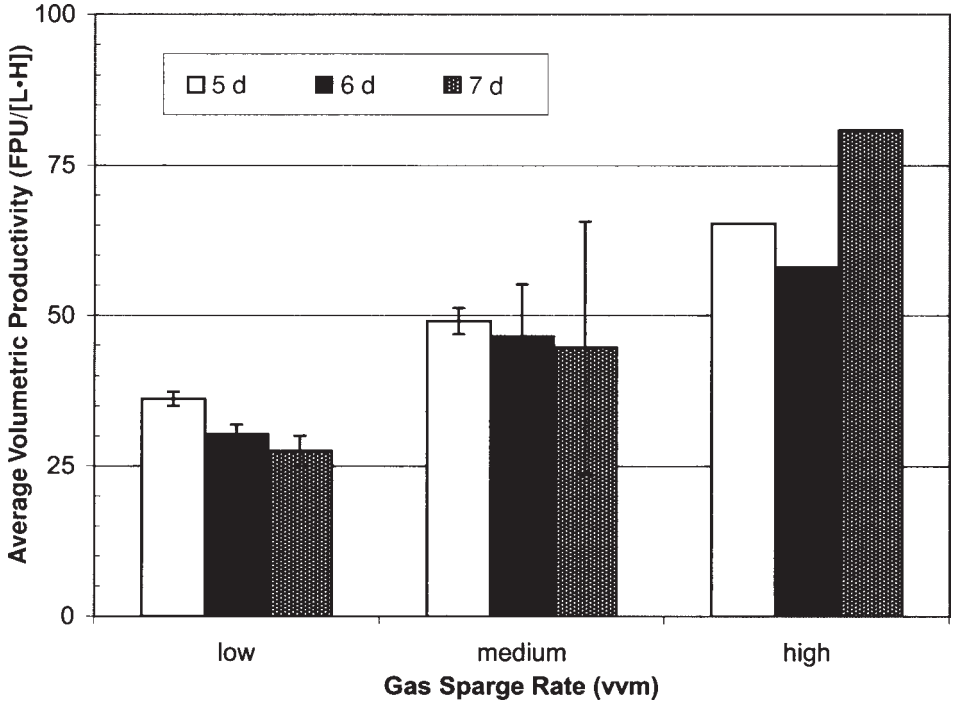

Fig. 6. Effect of gas sparge rate on volumetric productivity.

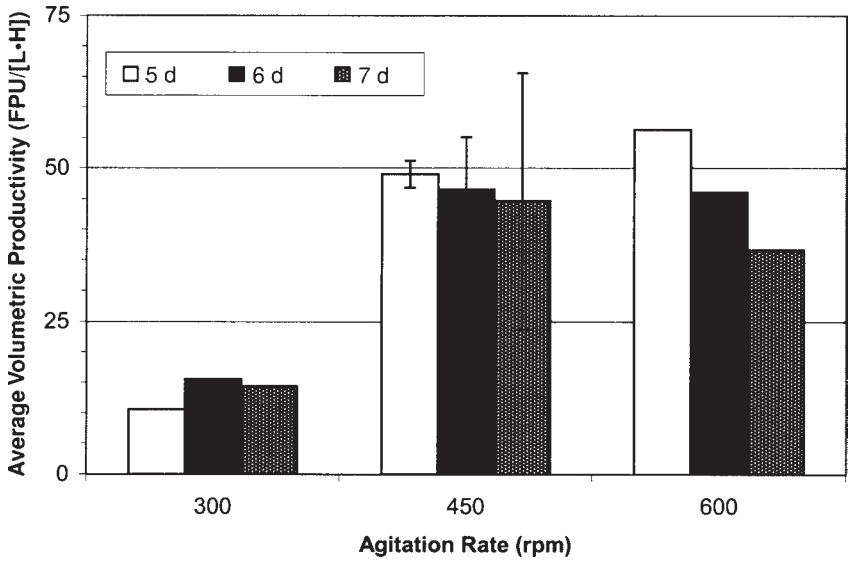

Fig. 7. Effect of agitation rate on volumetric productivity.

variable. Because gas sparge rate was found to significantly improve titers, it naturally increased productivity and yield as well.

Effect of Agitation Rate

Figure 7 illustrates the effect of agitation rate on enzyme production performance for runs carried out using a moderate gas sparge rate without oxygen supplementation. There was a pronounced increase in enzyme titer, volumetric productivity, and yield when the agitation rate was increased from 300 to 450 rpm, but the effect of agitation rate then diminished. The results suggest that increasing the agitation rate from 450 to 600 rpm may be beneficial in terms of enabling superior performance at 5 d, but no benefit is evident at the 6- and 7-d performance levels. Figure 8 shows the effect of agitation rate on total protein production for a single experiment in which the effect of agitation was studied. Once again, the measured protein in the solution was corrected for measured volume losses during the fermentation. As Fig. 8 clearly illustrates, protein production was much lower in the low agitation experiment in which the culture was presumably oxygen limited. The protein production curves for the 450 and 600 rpm conditions are quite similar, suggesting that oxygen transfer is extremely important for this system. Given the large run-to-run variation in the system, the single run at 600 rpm does not provide enough evidence to claim that the protein or the fungus was significantly affected by shear at the higher agitation rate of 600 rpm.

Analysis of Variance for Oxygen Supplementation, Agitation Rate, and Gas Sparge Rate

Figure 9 displays the results of a retrospective analysis of variance (ANOVA) performed on the data from the aeration study. The results for

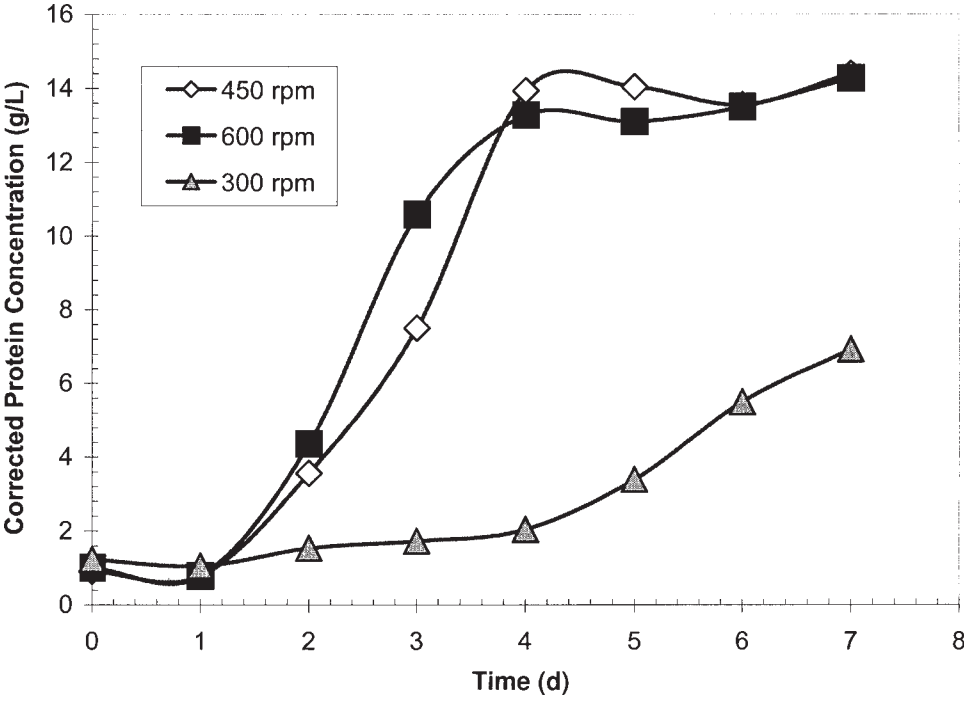

Fig. 8. Effect of agitation rate on protein production.

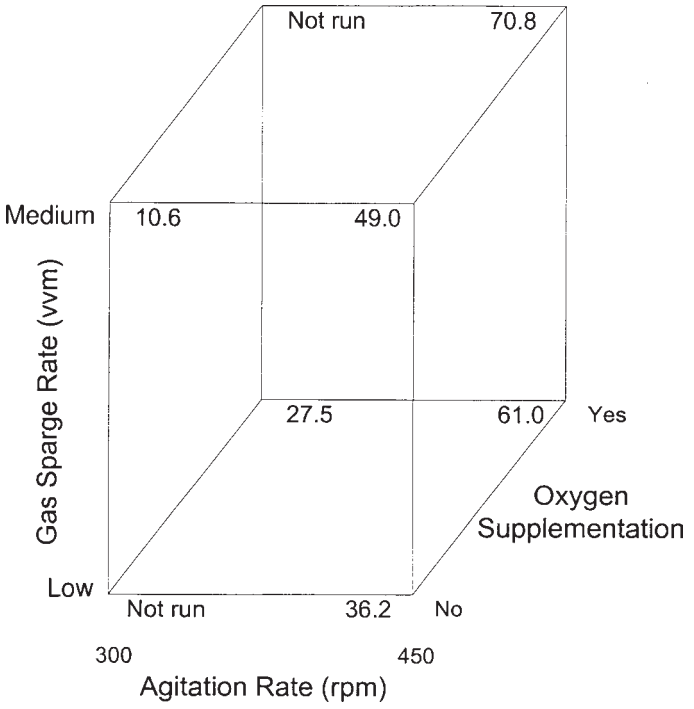

Fig. 9. Highlights of ANOVA analysis: 5-d Qp results (FPU/[L·h]).

volumetric productivity were analyzed for an incomplete 3-factor 2-level (2^3) subset of the data made up of conditions 1–6, which comprise the low and medium levels of agitation rate and gas sparge rate, either with or without oxygen supplementation. ANOVA of this data set shows that all three aeration factors—agitation rate, gas sparge rate, and oxygen supplementation—have a pronounced influence on enzyme production performance, with agitation rate and oxygen supplementation having the largest single effects. Using an inverse transformation of the response data, we found the effects of all three factors on 5-d average volumetric productivities to be statistically significant at above the 95% confidence level. A large two-way interaction was observed between the agitation rate and the gas sparge rate, primarily as a consequence of not having a full factorial set of experiments. In other words, the oxygen supplementation effect confounds the observation of the gas sparge effect.

Conclusion

The results presented herein indicate that all three aeration variables considered in this study are important. In addition, we have demonstrated that a combination of a higher agitation rate and moderate to high gas sparge rates (i.e., conditions 3, 7, and perhaps 8) can be used to achieve high productivity enzyme production. Thus, the need to supplement the sparge gas supply with molecular oxygen can be eliminated if effective aeration can be otherwise achieved by increasing the agitation and gas sparge rates to sufficient levels. It has also been shown that the *T. reesei* strain L27 is physiologically capable of achieving the economic production target of above 55 FPU/(L·h) average volumetric productivity using pure cellulose as the sole carbon source.

As the results shown in Figs. 4–6 also demonstrate, we also were able to achieve significant increases in cellulase enzyme production performance in the batch production runs carried out using 5% Solka-floc substrate. Equally important, we have developed an improved enzyme production protocol that incorporates industrially relevant media and eliminates the requirement for sparging with molecular oxygen. This improved production protocol shows potential for production at higher scales, both in terms of technical feasibility and in economics.

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