

## Effects of Inoculum Size on Solid-Phase Fermentation of Fodder Beets for Fuel Ethanol Production

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**This fuel ethanol study examined the effects of *Saccharomyces cerevisiae* inoculum size on solid-phase fermentation of fodder beet pulp. A 5% inoculum (wt/wt) resulted in rapid yeast and ethanol (9.1% [vol/vol]) production. Higher inocula showed no advantages. Lower inocula resulted in lowered final yeast populations and increased fermentation times.**

During conventional submerged fermentation of corn and other grains, fermentation is generally initiated by the addition of a quantity of yeast inoculum broth equal to 1 to 3% (wt/wt) of the saccharified mash (5, 12, 14, 15). This results in initial yeast concentrations ranging from  $2 \times 10^5$  to  $5 \times 10^6$  cells per g (3-5, 11, 15). During the typical 48 to 96 h of fermentation which follow, the yeast population increases in most cases to  $5 \times 10^7$  to  $5 \times 10^8$  cells per g, and ethanol concentrations rise to 8 to 12% (vol/vol) (2-5, 11, 15).

Solid-phase fermentation of pulpy feedstocks such as sweet sorghum and fodder beets may require a somewhat higher inoculum to ensure thorough yeast cell distribution on the pulp (7, 8). Kirby and Mardon evaluated concentrated yeast inocula of  $2 \times 10^7$  to  $9 \times 10^7$  cells per g of sugar beet pulp and noted that fermentation times decreased from 15 to 9 h over that range (K. D. Kirby and C. J. Mardon, Proc. 4th Int. Symp. Alcohol Fuels Technol. 1980, vol. 1, p. 13-19). Ethanol yields, however, were unaffected by the inoculum size and averaged 90% of theoretical. Kargi and co-workers also used concentrated yeast inocula, in this case ranging from  $10^6$  to  $7 \times 10^8$  cells per g of sweet sorghum pulp (9). They found that the optimum initial yeast concentration for sweet sorghum pulp was  $7 \times 10^8$  cells per g, based upon a compromise between ethanol yield (9% [vol/vol]) and fermentation time (17 to 20 h).

The relatively high inoculum levels used in the previously mentioned studies, however, may not be economically feasible for fuel ethanol production. Initial yeast levels above  $5 \times 10^7$  cells per g of pulp would likely require centrifugation of the inoculum broth to concentrate the yeast. This would add to both capital and operating costs. For that reason, we used various amounts of uncentrifuged inoculum broth (0.01 to 20.0% [wt/wt] of the beet pulp) to test inocula ranging from  $1.35 \times 10^4$  to  $1.2 \times 10^7$  cells per g of pulp.

All inocula of *Saccharomyces cerevisiae* NRRL Y-2034, the yeast used in this study, were prepared on 4% glucose-0.4% neopeptone (Difco Laboratories) (6). Fodder beets (*Beta vulgaris*, cv. Monorosa) were obtained from a Pennsylvania grower. After being hammer milled with an optimal 1.27-cm screen (6), the beet pulp was adjusted to pH 3.0 to inhibit bacterial contamination (W. R. Gibbons and C. A. Westby, Biotechnol. Lett., in press). This low pH did not inhibit the growth of the yeast.

Following pH adjustment, 1-kg amounts of beet pulp were thoroughly inoculated, by hand mixing, with 0.1, 0.5, 1.0,

5.0, 10.0, 50.0, 100.0, or 200.0 g of yeast inoculum broth. The yeast inocula contained  $0.5 \times 10^8$  to  $1.0 \times 10^8$  cells per g. Duplicate runs at each inoculum level were performed, and the results were averaged. Inoculated beet pulp was incubated at 30°C for 96 h with periodic agitation.

At 6-h intervals, fermenting fodder beet pulp was mixed, and samples were withdrawn for analysis. The reducing sugar concentration was determined by the dinitrosalicylic acid method (10) following incubation of 20 g of beet pulp in 80 ml of water with an excess of invertase (48 h at 50°C) (7). The yeast cell population was determined by a plate count method with potato dextrose agar (PDA) and tartaric acid (15). Bacterial contaminants were determined with PDA without tartaric acid. Ethanol was determined by specific gravity measurements of distilled samples by procedures recommended by the Association of Analytical Chemists (1).

When the inoculum was 0.5 to 20% (wt/wt) of the beet pulp, the maximum population of yeast cells that developed during fermentation remained relatively constant and averaged  $1.85 \times 10^8$  cells per g (Fig. 1). Below an inoculum of 0.5%, the maximum yeast population gradually decreased to  $9.4 \times 10^7$  cells per g at the 0.01% inoculum level. The average variability of yeast populations across replications for all trials was  $\pm 1.8 \times 10^7$  cells per g.

A high yeast population in the fermenting pulp is advantageous because it allows for shorter fermentation times and higher protein concentrations in the feed by-product (7, 8). The yeast population level immediately after inoculation was dependent upon the inoculum used and ranged from  $1.35 \times 10^4$  cells per g at the 0.01% inoculum level to  $1.2 \times 10^7$  cells per g at the 20% inoculum level.

Bacterial contaminants were not detected in any of the trials, even though the pulp was not pasteurized. This was presumably due to the low pH (3.0) of the pulp prior to inoculation (Gibbons and Westby, in press). In all trials, the pulp pH increased in a curvilinear fashion during fermentation but never exceeded 3.60.

The time to reach the maximum yeast population during fermentation showed a definite, and expected, dependency on inoculum size (Fig. 1). The shortest time to reach the maximum yeast population was 20 to 22 h at a 0.5% inoculum and above. The average variability between replications was  $\pm 1.69$  h. Below a 0.5% inoculum, the time to reach the maximum yeast population increased rapidly to 46 to 48 h.

The time to reach the maximum ethanol concentration also responded to inoculum size. In this case the shortest

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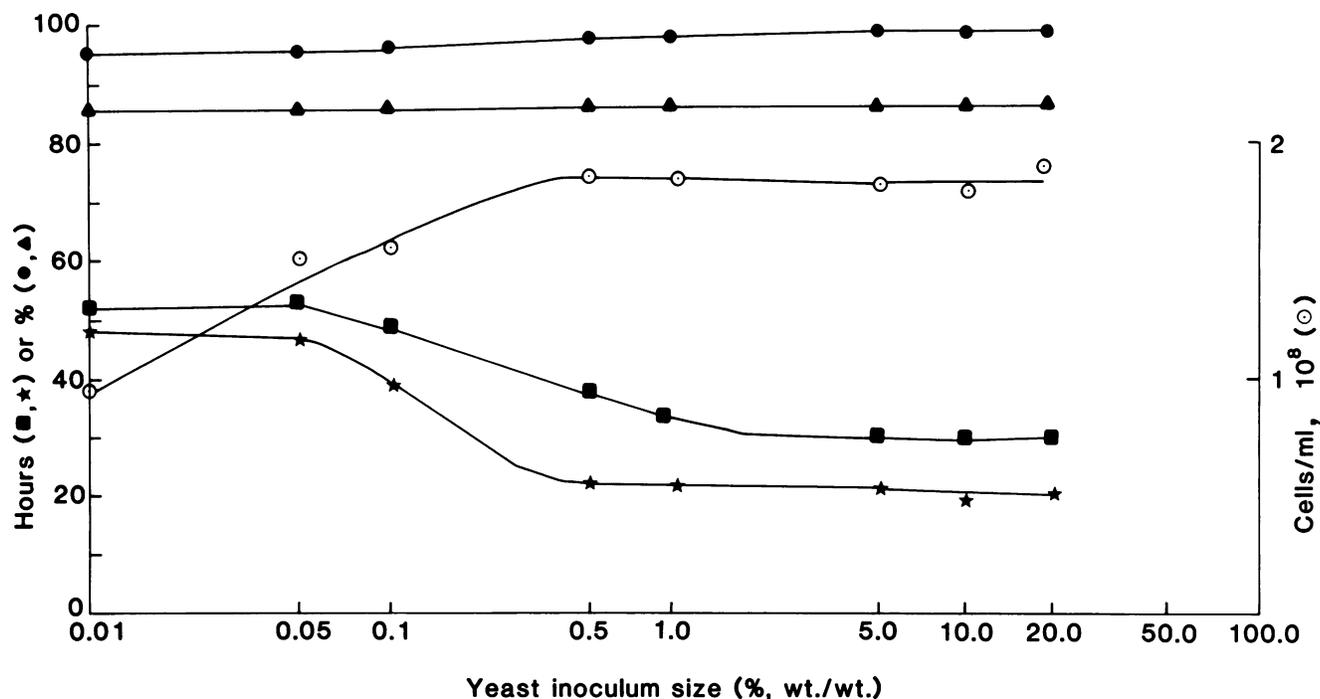


FIG. 1. Effect of *S. cerevisiae* NRRL Y-2034 inoculum size on ethanol and yeast cell production in the solid-phase fermentation of fodder beet pulp. Symbols: ■, time required for maximum ethanol concentration; ★, time required for maximum yeast population; ●, fermentation efficiency as a percentage; ▲, ethanol yield as a percentage of theoretical; ○, maximum yeast population.

fermentation time, 30 h, occurred at a 5% (wt/wt) inoculum and above. Higher inoculum levels did not reduce fermentation time further. At a 5% inoculum, the initial yeast cell population was  $3.8 \times 10^6$  to  $4.2 \times 10^6$  cells per g. The average variability between replications was  $\pm 1.75$  h. Below a 5% inoculum, fermentation time gradually increased to 53 h at a 0.05% inoculum.

The ethanol yield was independent of inoculum size and averaged 86% of theoretical (Fig. 1), with an average variability of  $\pm 0.75\%$ . Ethanol yields were calculated by assuming a conversion rate of 53.8 g of ethanol per 100 g of reducing sugar (13; W. L. Bryan, G. E. Monroe, R. L. Nichols, and G. J. Gascho. Winter Meet. Am. Soc. Agric. Eng. 1981, abstr. no. 81-3571). The maximum ethanol concentration that developed during solid-phase fermentation of fodder beet pulp inoculated with at least a 5% inoculum was 9.13% (vol/vol), and the average for all trials was 8.89% (vol/vol).

Fermentation efficiency was calculated by dividing the reducing sugar consumed during fermentation by the initial reducing sugar and multiplying the result by 100. Fermentation efficiency decreased slightly from 99% at a 5.0% inoculum and above to 96% at a 0.1% inoculum and below (Fig. 1). The average variability for all trials was  $\pm 0.57\%$ . The apparent explanation for this effect was that fewer yeast cells grew at lower inoculum levels (Fig. 1) and thus less sugar was converted to cell biomass, even though ethanol yields remained constant.

Overall, our results suggest that a 5% (wt/wt) inoculum ( $4 \times 10^6$  yeast cells per g [wet weight] of pulp) is the minimum amount necessary to ensure both maximum ethanol and yeast levels in a reasonably short fermentation time (30 h). Higher inocula (10 to 20%) provide no greater benefits and

would require higher investments in capital and operating costs to produce the greater quantity of inoculum.

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