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Morphological Behaviour of Saccharomyces cerevisiae During Continuous Fermentation

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SUMMARY

Saccharomyces cerevisiae were observed to undergo drastic morphological changes when grown in continuous culture. Peak elongations and minimum cell volumes were found at intermediate dilution rates when it was believed the in-situ glucose concentration was at its lowest. The shapes and sizes were reproducible and have been quantified at two different glucose feed concentrations.

INTRODUCTION

Saccharomyces cerevisiae has the largest commercial production of any single-celled microorganism. It is produced aerobically for the purpose of Bakers' yeast and anaerobically for the purpose of brewing. It has also found renewed scientific use in genetic engineering studies (Davies, 1988). The production of ethanol from glucose by Saccharomyces cerevisiae is now being used for fuel alcohol purposes in various parts of the world. Lovitt et al. (1988) recently reviewed the literature on this latter topic.

Several investigators have reported anomalous morphological behaviour of Saccharomyces cerevisiae. Usually this has involved subjecting the cells to a severe chemical perturbation in the fermentation environment (McMurrough and Rose, 1967; Lipke et al., 1976; Tyson et al., 1979; Maiorella et al., 1983; Morris et al., 1986; Lumsden et al., 1987). On the other hand, cells grown using "normal" fermentation media are reported to undergo little or no morphology changes even if grown at different rates in a chemostat (Mor and Fiechter, 1968; Adams, 1977; Tyson et al., 1979; Lafforgue et al., 1987). Franco et al. (1984) have shown that the levels of metabolites inside Saccharomyces cerevisiae can shift not only due to a change in the fermentation medium but also due to a shift in the dilution rate in a chemostat. In this paper, we report on a major change in the morphology of Saccharomyces cerevisiae due to a shift in the dilution rate (growth rate) of a chemostat. This behaviour was observed to occur at different dilution rates depending on the level of glucose in the feed medium.

MATERIALS AND METHODS

Microorganism: A 'high ethanol' tolerant strain of Saccharomyces cerevisiae was used. It was obtained from the United States Dept. of Agriculture, NRRL Y-132. This is a normal diploid strain and has been used by others for growth on bagasse (Tyagi and Ghose, 1977) and inside an immobilized fermentor (Robinson et al., 1980).

Fermentation Media: The ingredients of the fermentation broth were similar to those used by previous investigators (Bazua and Wilke, 1977; Robinson et al., 1980). It contained (per litre of de-ionized water): NH_4Cl, 2.5g; Na_2HPO_4, 2.91g; KH_2PO_4, 3.00g; $MgSO_4$, 0.25 g; $CaCl_2$, 0.08g; citric acid, 5.3g; sodium citrate, 2.5g; yeast extract (technical), 3.0g; and glucose, 5.0 or 20.0g. The

glucose was sterilized separately and added aseptically to the sterilized nutrients. The pH of the mixture was continually tested and found to lie between 4.0 and 4.1.

Culture Conditions: The chemostat used was a New Brunswick Bioflo C30. It had a working volume of 483 cc. No aeration was used so the only oxygen source was that in the dissolved feed. The peristaltic pump was pre-calibrated and found to be stable and reproducible. The feed was aseptically mixed and stored in 16 litre glass bottles while the effluent discharged into a sterile container. Samples were taken directly from the fermentor using the supplied sampling system. The temperature was controlled at 30°C using a heating tape wrapped around the outside of the fermentor. No cooling was necessary other than natural convection. The pH was found not to vary between the feed and effluent (not detectable) so pH control was also unnecessary.

Measurements: The cells were photographed by placing them on a gridded surface (Petrof-Hauser apparatus) and taking pictures using a photomicroscope at a total magnification of 400. The cells were 'manually' sized using a Bausch & Lomb measuring magnifier. The total number of cells sized for each steady state condition varied depending on the results of the photography but generally was around 80. Dry weights were performed by centrifuging, washing, centrifuging again and drying. Optical densities were performed at 600 nm. Glucose analysis was done by an enzymatic assay (Sigma 115) while ethanol was analyzed by GC.

RESULTS AND DISCUSSION

Initial batch growth experiments in culture flasks resulted in little or no morphological changes as observed by others. This trend was observed even for batch cultures which were severely inhibited due to the presence of external ethanol addition. Table 1 shows that cells grown with low initial ethanol reached a mean volume of 62.0 $(\mu m)^3$ with an axis ratio (long axis length divided by short axis length) of 1.15. Although cells grown with a high ethanol concentration grew much slower in the batch mode, Table 1 shows that they achieved a mean volume of 54.6 $(\mu m)^3$ and an axis ratio of 1.08, quite similar to those grown at much lower concentrations of external ethanol. These experiments showed that the growth rate of Saccharomyces cerevisiae does not cause a large alteration in morphology.

Growth in continuous culture was a completely different story. It was discovered that, in spite of the fact that no external chemical perturbations were applied, the microorganism could take on a continuous range of shapes from the elliptical form seen in batch, (see Figure 1a) to a rod shaped form not previously reported under these conditions (see Figure 1b). This morphological behaviour was completely adjustable and reproducible simply by varying the flowrate (dilution rate) of feed into the chemostat. The morphological data are listed in Table 1 and shown in Figures 2, 3 and 4.

It can be seen that at very low dilution rates and at high dilution rates, the Saccharomyces cerevisiae cells grew in their typical elliptical shape. However, at intermediate rates, they elongated in a continuous fashion reaching a peak length and a minimum volume. These peaks and minimums were a function of the concentration of glucose in the feed medium. At 5 g/l the greatest length (and minimum volume) occurred at about $D=0.15\ hr^{-1}$ while at 20 g/l this peak (and minimum) were shifted to about $D=0.25\ hr^{-1}$. The lines on these graphs represent best fit parabolic curves to the data and it can be seen they are a reasonable approximation to the data at intermediate flows. The elongation (expressed here

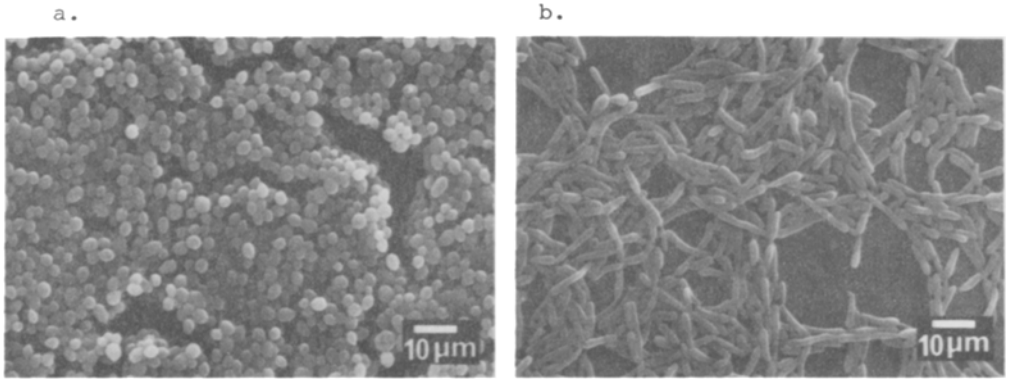

Fig. 1: Cell morphology as seen under a scanning electron microscope: a) Normal elliptical shape from batch or high dilution rate, b) Rod shape from intermediate dilution rates.

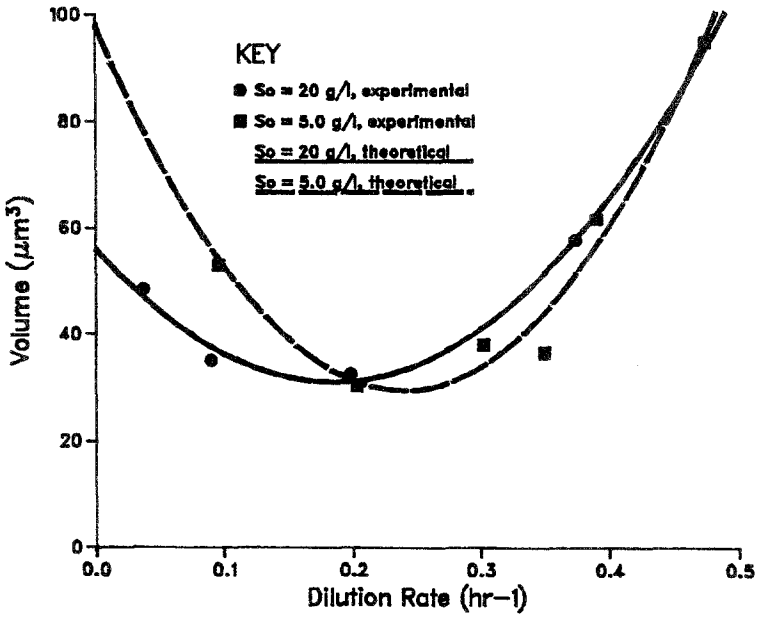

Fig. 2: Mean cell volumes at two different feed concentrations.

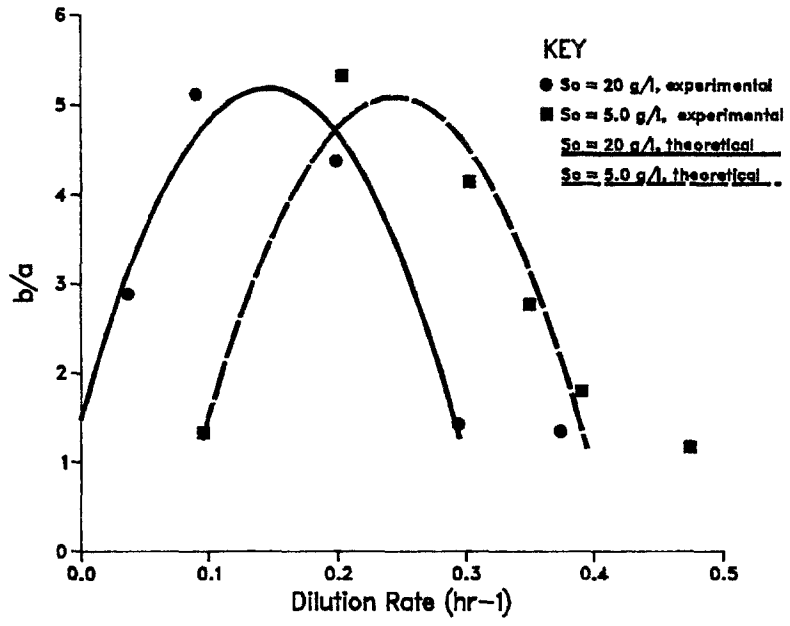

Fig. 3: Mean cell elongation at two different feed concentrations.

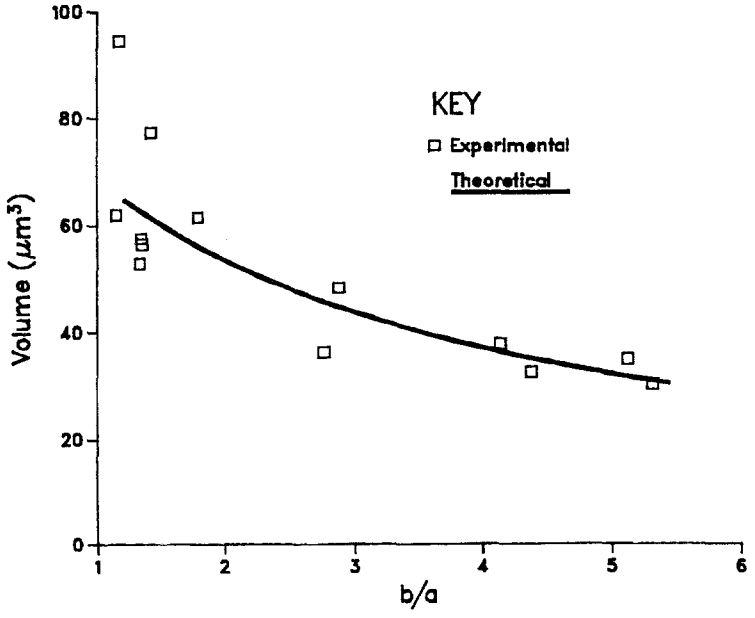

Fig. 4: Relationship between cell morphology (elongation) and mean cell volume.

Table 1: Summary of Morphologic Behaviour of *Saccharomyces cerevisiae*

So (g/l)	(C)ontinuous or (B)atch	D or μ (hr^{-1})	No. of Cells Sized	Mean Volume $\pm$ Std Dev $(\mu m)^3$	b/a $\pm$ Std Dev
20	B	.31	98	62.0 $\pm$ 16.5	1.15 $\pm$ 0.11
20	B	<.01	22	54.6 $\pm$ 14.8	1.08 $\pm$ 0 09
20	C	0.037	73	48.4 $\pm$ 19.8	2.88 $\pm$ 1.15
20	C	0.090	84	35.0 $\pm$ 10.1	5.12 $\pm$ 1.63
20	C	0.198	82	32.5 $\pm$ 9.8	4.37 $\pm$ 1.45
20	C	0.294	74	77.3 $\pm$ 16.8	1.42 $\pm$ 0.26
20	C	0.374	84	57.5 $\pm$ 15.6	1.34 $\pm$ 0.28
20	C	0.090*	83	56.5 $\pm$ 13.8	1.35 $\pm$ 0.29
5	C	0.302	67	37.9 $\pm$ 13.0	4.14 $\pm$ 1.19
5	C	0.474	18	94.6 $\pm$ 18.0	1.17 $\pm$ 0.09
5	C	0.350	46	36.4 $\pm$ 13.5	2.76 $\pm$ 0.82
5	C	0.203	79	30.3 $\pm$ 11.3	5.32 $\pm$ 2.00
5	C	0.390	20	61.5 $\pm$ 11.0	1.79 $\pm$ 0.33
5	C	0.096	13	52.9 $\pm$ 12.6	1.33 $\pm$ 0.17

*Impulse of Ethanol, Morphology Change

as the ratio of axis lengths = b/a) appears to be directly related to the average cell volume as shown in Figure 4. The volume varies from the largest size seen in batch runs of 65 μm^3 to a minimum size of 35 μm^3. The elongation appears to reach a maximum ratio of about 5.0. In one run, when the microbe was highly elongated at an intermediate dilution rate, an impulse of ethanol was injected (about 100 g/l). Although the dilution rate was not changed the size of the microorganisms shifted back to an elliptical form. This shift did not occur in old microorganisms, however, only newly budded cells were in an elliptical shape. This indicated that the cells were not directly responding to flowrate but to some sort of environmental condition in the medium and that once formed the cell was resistant to further morphological change. There was concern that the morphological behaviour might have a direct affect on cell mass determinations by the optical density technique. It was found, however, that even the most elongated condition resulted in the same linear OD vs dry weight curve up to an OD of 0.45.

In spite of this experimental phenomenon, no chemical analysis was found to explain the shift in morphology. Both the HPLC analysis of carbohydrates and the GC analysis (capillary column, FID) for ethanol failed to detect any other spurious chemical in the medium. One possible explanation was that there may be some low critical glucose concentration at which the cells 'decide' to form elongated buds. This level was below the minimum concentration detectable by HPLC or enzyme assay (about 0.1 g/l). This would make sense because by elongating, the cells are greatly increasing their surface area to volume ratio, thus making it easier for them to 'find' the scarce glucose molecules. This would explain the constant elliptical shape in batch runs and the return to elliptical shape in the impulse test where the glucose concentrations were high. Further, at very low dilution rates, there may be a slight increase in glucose level due to cell death, thus once again resulting in elliptical shapes. This would also explain the shift in the location of peak elongation and minimum volumes at different feed glucose concentrations because at lower feed concentrations there will be a different S vs D curve when the substrate in the

feed approaches the value of the Michaelis-Menten constant.

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