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EFFECTS OF ORGANISM TYPE AND GROWTH CONDITIONS ON CELL DISRUPTION BY IMPINGEMENT

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ABSTRACT

Data for disruption of C. utilis, S. cerevisiae and B. subtilis cells by impingement of a high velocity jet of suspended cells against a stationary surface are compared. Differences between organisms were observed, but there were no general differences found between yeast and bacteria. In addition, growth conditions were found to have an effect on disruption with cells grown at a high specific growth rate easier to disrupt than cells grown at a low rate.

KEYWORDS

Disruption, C. utilis, S. cerevisiae, B. subtilis.

INTRODUCTION

The need for efficient, large-scale processes for disrupting microorganisms is becoming increasingly important with the rapid development of new intracellular products from microorganisms. The advent of recombinant DNA technology may provide an almost unlimited range of microbial products, many of which will require disruption of the organism for recovery. While there have been numerous studies of equipment and processes suitable for large-scale disruption of microorganisms, much less consideration has been given to the effects of organism characteristics or growth conditions on disruption.

Large-scale disruption processes have been shown to have high energy requirements (Engler, 1979; Mogren and colleagues, 1974; Rehacek and Schaefer, 1977). Therefore, minimizing disruption costs can be quite important in the overall design of fermentation processes. When process design objectives allow freedom of choice in the organism selection or in the growth conditions to be used, it may be advantageous to make the selections on the basis of producing cells having the weakest cell walls thereby minimizing energy input requirements for disruption.

In this paper disruption characteristics of several different organisms are compared and effects of different growth conditions on disruption are evaluated.

MATERIALS AND METHODS

Organisms

Organisms used for this study were Candida utilis (ATCC 9226), Saccharomyces cerevisiae (NRRL Y2235) and Bacillus subtilis (ATCC 6051). Both yeasts were grown aerobically in continuous culture (D = 0.1/h) at 30°C and pH 4.5 using a synthetic medium reported elsewhere (Engler and Robinson, 1981). C. utilis also was grown in cyclic batch culture (μ_m = 0.5/h, 7% vol. retention, 6 h cycle time) using the same temperature, pH and medium. B. subtilis was grown aerobically in continuous culture (D = 0.2/h) at 37°C and pH 7.0 using a medium of the following composition (in g/liter): nutrient broth, 5; yeast extract, 5; glucose, 20. In addition to the above organisms, spent brewery yeast (S. cerevisiae) was obtained from Labatt's Brewery, Waterloo, Ontario.

Disruption

Cells were harvested and stored at 5°C for no more than 48 h prior to disruption. To prepare for disruption, cells were washed three times in 0.9% w/v aqueous NaCl and suspended in 0.9% w/v aqueous NaCl plus 0.1 M 2-mercaptoethanol. Disruption was accomplished by impingement of a high velocity jet of suspended cells against a stationary surface as described elsewhere (Engler and Robinson, 1981).

Analytical

Amounts of disruption were determined by Kjeldahl analysis of the supernatants from disrupted samples. Corrections were made for solids volumes in the samples using the calculation procedure reported previously (Engler and Robinson, 1979).

RESULTS

Results for the disruption of C. utilis grown both in cyclic batch and in continuous culture are shown in Fig. 1. Included are data for a single pass and for multiple passes through the impingement nozzle. The dashed line (1) represents the correlation for disruption of bakers' yeast in a Manton-Gaulin homogenizer reported by Hetherington and colleagues (1971). Solid lines were obtained by linear regression of the data and represent the equation

$$\ln[1/(1-R)] = K\ N\ P^a. \qquad (1)$$

Values of the parameters K and a are given in Table 1 along with values obtained from the data for the other organisms studied.

A detailed analysis of the C. utilis results has been reported elsewhere (Engler and Robinson, 1980) which indicates that cell wall strength is related to the specific growth rate of the cells. The data suggest that cells grown at a high specific growth rate are easier to disrupt than cells grown at a lower rate.

Disruption results for S. cerevisiae are shown in Fig. 2 along with the best-fit lines for the C. utilis data. These data also indicate that growth conditions affect cell wall strength with the spent brewery yeast being easiest to disrupt. However, these differences can not necessarily be attributed to different specific growth rates but may have been caused by differences in the growth medium, aeration, the presence of ethanol or other factors.