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

C.R. Engler* and C.W. Robinson**

*Bioengineering Program, Texas A&M University, College Station, Texas 77843
** Department of Chemical Engineering, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

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.

Comparison of the disruption results for S. cerevisiae grown aerobically in continuous culture with those for C. utilis grown under identical conditions shows C. utilis to be more difficult to disrupt, particularly at the low end of the pressure range studied. Previous investigators also have reported that C. utilis was more difficult to disrupt than bakers' or brewers' yeasts (Mogren and colleagues 1974; Rehacek and Schaefer, 1977). However, in those studies growth and post-

TABLE 1 Disruption Parameters

Organism	Culture	Growth Rate (h^{-1})	a (-)	K (Pa^{-a})
C. utilis	Cyclic Batch	0.5	1.17	$8.78(10^{-10})$
C. utilis	Continuous	0.1	1.77	$8.53(10^{-15})$
S. cerevisiae	Continuous	0.1	0.86	$2.24(10^{-7})$
S. cerevisiae	Brewery	-	1.87	$4.76(10^{-15})$
B. subtilis	Continuous	0.2	1.07	$4.93(10^{-9})$

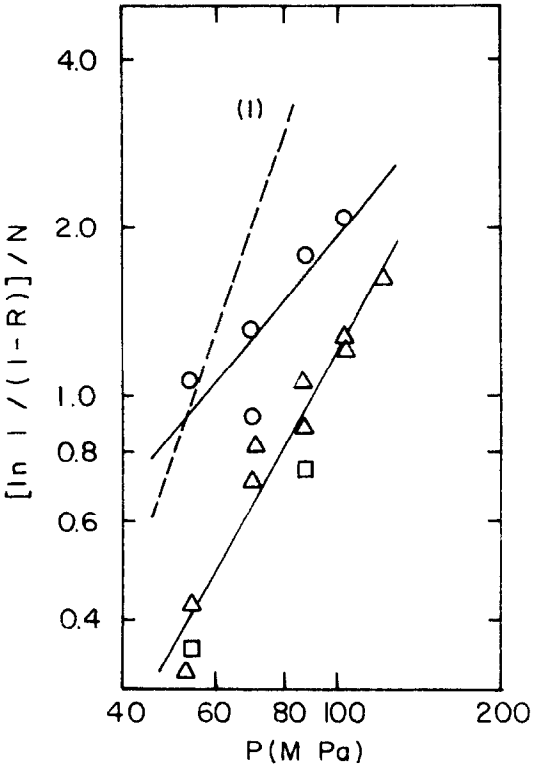

Fig. 1. Impingement disruption of C. utilis. (Δ) continuous, D = 0.1/h, single pass; (O) cyclic batch, μ_m = 0.5/h, single pass; (□) continuous, D = 0.1/h, multiple pass; (1) correlation of Hetherington and colleagues (1971).

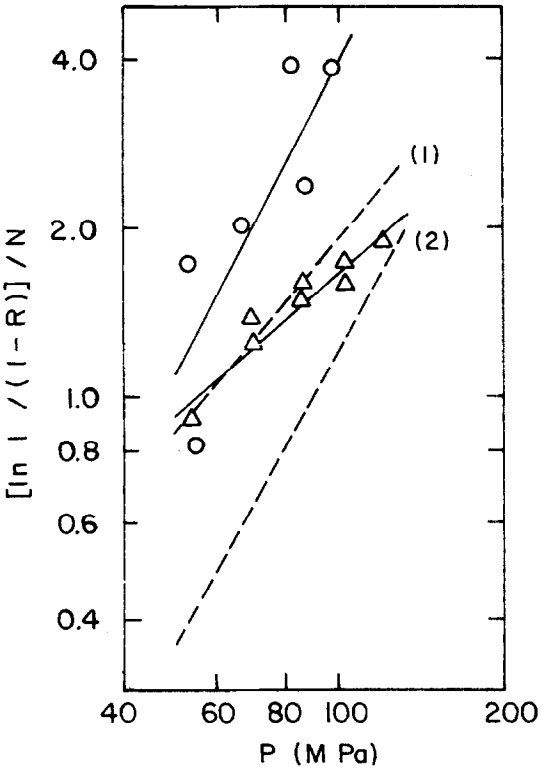

Fig. 2. Impingement disruption of S. cerevisiae. (Δ) continuous, D = 0.1/h, single pass; (O) spent brewery yeast; (---) best-fit lines for C. utilis data.

harvest handling conditions for the different organisms were not identical so the disruption differences could have been caused by any of several factors.

Disruption results for B. subtilis are shown in Fig. 3. Also shown are the best-fit lines for the C. utilis data. The disruption characteristics for B. subtilis are similar to those of batch-grown C. utilis and also aerobically grown S. cerevisiae. A comparison of the percentage of cells disrupted at two levels of operating pressure for the microorganisms tested is given in Table 2.

DISCUSSION

These results (Table 2) show there can be significant differences in the disruption characteristics of different organisms. For example, the C. utilis cells grown in continuous culture were more difficult to disrupt than either S. cerevisiae or B. subtilis cells. However, the results do not suggest any general trend between type of organism (bacteria or yeast) and susceptibility to disruption since disruption characteristics of B. subtilis, S. cerevisiae grown in continuous culture and C. utilis grown in batch culture were all similar.

The results also show that disruption characteristics of a given organism can be altered significantly by changing growth conditions. For yeasts, it appears that a high growth rate produces cells having weaker cell walls as shown by the C. utilis data. Although the effect of growth rate on disruption of bacteria was not studied, similar results may be expected since fast-growing cells would not have time to produce material for reinforcing the cell wall structure. Also, a greater percentage of cells in a fast-growing culture are in the separation stage and therefore may be more susceptible to disruption.

The effects of other growth conditions such as medium composition and aeration were not studied in this work. Gray and colleagues (1972) reported that E. coli cells

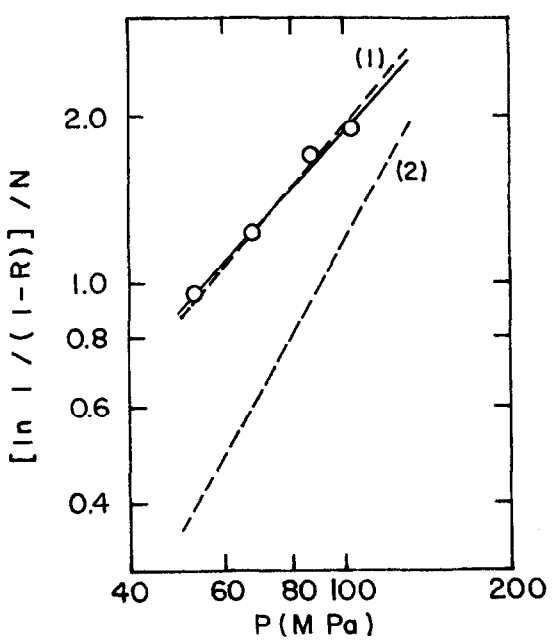

Fig. 3. Impingement disruption of B. subtilis.
(O) continuous, D = 0.2/h, single pass;
(---) best-fit lines for C. utilis data.

grown on a complex medium were more difficult to disrupt with a Manton-Gaulin homogenizer than cells grown on a simple medium. In addition, the S. cerevisiae results in this work suggest that such growth conditions may be important in determining cell wall strength. Therefore, growth conditions in addition to specific growth rate could be expected to affect cell wall strength.

Because of the large number of factors which may affect the susceptibility of microorganisms to disruption, a general correlation for disruption does not appear possible. Although it is reasonable to expect the growth rate effect observed for C. utilis to be generally applicable for other organisms, effects of changing medium composition or other growth parameters likely would not follow general trends. Therefore, such effects would have to be evaluated for individual organisms as necessary.

TABLE 2 Comparison of Disruption Data

Organism	Culture	Growth Rate(h^{-1})	N	Percentage Disrupted 54 MPa	88 MPa
C. utilis	Cyclic Batch	0.5	1	69	87
C. utilis	Continuous	0.1	1	30	53
			2	47	77
			3	65	87
S. cerevisiae	Continuous	0.1	1	60	79
S. cerevisiae	Brewery	-	1	56,82	91,98
B. subtilis	Continuous	0.2	1	61	82

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NOMENCLATURE

- a exponent of pressure (dimensionless)
- D dilution rate (h^{-1})
- K dimensional rate constant (Pa^{-a})
- N number of passes (dimensionless)
- P operating pressure (Pa)
- R fraction of cells disrupted (dimensionless)
- μ_m maximum specific growth rate (h^{-1})

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