DISRUPTION OF BAKER'S YEAST BY A NEW BEAD MILL

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Summary

A continuous high-speed bead mill of novel design (Sulzer Annu Mill 01) was tested for cell disruption of baker's yeast as a model system. The efficiency of cell disruption was evaluated for the relative amount of released protein. The effects of rotation speed, cell concentration and flow rate of cell suspension on the cell disruption were investigated. The maximum yield of released protein was found to be 2.62 kg protein/L.h. This novel design appears to be more effective than existing commercially available mills.

INTRODUCTION

The disintegration of microorganisms for the recovery of various products is a common operation in biotechnology (Bailey and Ollis, 1986). Intracellular contents, such as chemicals, antibiotics and enzymes can be released from the cells by different methods. Among these methods, physical ones appear to be most attractive because they are relatively inexpensive for large-scale operations. Disintegration of microorganisms by different methods were reviewed recently by White and Marcus (1988) and Chisti and Moo-Young (1986). The basic principle of homogenization, the common method, relies on the use of high pressure, usually from 100 to 3,000 bar. The temperature increase in a homogenizer is about 2.6 °C per 103 bar, i.e., about 18°C to 25°C for each pass. Hedenskog and Enebo (1969) tested various methods for disintegration of algae and found that the best results were obtained by using a bead mill. The method of using high speed bead mill was first reported by Zetelaki (1969). A detailed discussion of high speed bead mills is given by Keshavarz and Dunnill (1987). High speed bead mills employ vertical or horizontal grinding chambers. In this work, cell disruption by a new type of bead mill (Sulzer Annu Mill 01) was studied for comparison purposes.

MATERIALS AND METHOD

The mill is manufactured by Sulzer Brothers Limited (Winterthur, Switzerland). It is based on a vertical cylindrical rotor and grinding chamber. The diameter and length of the rotor is 50 mm and 150 mm, respectively. Volume of the grinding chamber is 210 mL.

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Usually, 150 mL of glass beads (size ranging from 0.49 mm to 0.70 mm) are put into the grinding chamber. The rotor can be operated up to 6300 rpm. Schematics of the equipment and process operation are given in Figures 1 and 2. Rotation speed of the rotor can be controlled. The inlet suspension is introduced at the bottom of the grinding chamber. A filter screen at the outlet in the upper part of the chamber prevents the glass beads from flowing out.

Baker's yeast was chosen as the test material because it is one of the most difficult microorganisms to disrupt (Hetherington et al., 1971). Packages of the yeast were obtained from Lallemand Inc. (Ontario, Canada) and stored in a cold room (4°C) for no more than two weeks at a time. It was found that after storage for more than two weeks, the total amount of protein released decreased. Before each disruption experiment, yeast was suspended in a solution of 0.15M NaCl and 0.004M K$_2$HPO$_4$, pH 5.4 - 5.6. The suspension of fresh yeast cell was mixed until homogeneous. Water coolant at 2°C was used to provide cooling of test samples to 12°C before a disruption experiment started. Pressure in the grinding chamber depended on flow rate of the suspension by a metering pump and was usually less than 2 bar. Samples were collected and the temperatures of 17°C - 19°C reduced to 12°C before recycle to the mill.

Samples (10 mL) from the mill were centrifuged (Damon/IEC Division) for 30 min to remove cell debris and the supernatant was analyzed by the Lowry method for protein content. In this work, disruption of baker's yeast was conducted at different rotation speeds, from 1000 rpm to 4300 rpm (equipment maximum is 6300 rpm). Cell concentrations ranged from 225 to 600 g yeast/L.