Method for Rupturing Large Quantities of Microorganisms[1]

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Many procedures have been described (Umbreit *et al.*, 1957) for the mechanical rupturing of yeast and bacterial cells. These techniques vary from simple hand grinding with abrasives to the use of extrusion or high speed shaking devices. These techniques, although generally satisfactory when dealing with small quantities of cells, are unsuitable for breaking larger quantities of cells required in many investigations.

To provide a more suitable method for grinding larger quantities of cells, an attempt was undertaken to rupture cells in a cell-glass bead slurry under conditions where high shearing forces could be obtained. The Eppenbach[3] laboratory colloid mill Model QV-6 was chosen for this purpose. This colloid mill has a rotor 2 in. in diameter and operates at 8000 rpm. The clearance between the rotor and stator can be varied from 0.0005 to 0.125 in. The homogenizing zone and the lower portion of the feed reservoir are jacketed for cooling purposes and a bypass line provided for recirculating the discharged slurry back to the feed reservoir.

Runs were made with 300 to 600 ml batches of heavy cell suspensions to which were added Superbrite[4] glass beads. The beads used were 120 to 130 μ in diameter except for a single run with Bakers' yeast where 1 to 20 μ beads were tried. Permanent antifreeze at -25 C was circulated through the jacket to maintain the slurry temperature at 15 to 20 C. Lower temperatures could not be maintained without material freezing on the interior surfaces of the mill; however, this problem could readily be solved by increasing the heat transfer area and raising the coolant temperature.

Two representative organisms were chosen to survey the conditions for optimal cell breakage: one was *Escherichia coli* and the other a semiconstitutive β-glucosidase hybrid yeast strain (*Saccharomyces fragilis* 610 $\times$ *Saccharomyces dobzhanskii* 1974) furnished by Dr. Wickerham. The influence of bead concentration, mill setting, and time on cell breakage is summarized in table 1. Protein and enzyme assays showed that the optimal breakage was obtained with 60 ml of beads per 100 ml of suspension and with a mill setting of 0.030 in. Microscopic examination following such treatment of yeast (20 min) and *E. coli* (15 min) showed 99 per cent breakage.

The rate of breakage of a suspension (170 mg dry wt per ml) of commercial bakers' yeast cake,[5] as indicated by supernatant fluid protein determinations, is shown in figure 1. Under condition A (60 ml of 120 μ beads per 100 ml suspension) maximum protein liberation was observed after 10 min. The dry weight of the supernatant after 30 min was 110 mg per ml. The decrease in supernatant protein after 10 min is thought to result from protein loss during centrifugation. Condition B (40 ml of 1 to 20 μ beads per 100 ml suspension), however, showed only slight breakage and 16.2 mg dry wt per ml of supernatant after 30 min.

Preliminary experiments suggest that this method can be used equally well for breaking mold mycelium. In an experiment analogous to that with *E. coli* (table 1), over 95 per cent breakage of *Aspergillus niger* mycelium was obtained in 3 min.

The success of this method is thought to result from the very high shearing forces to which the cells are subjected. The shearing forces obtained with cell bead slurries in devices such as the Waring Blendor are much less than can be achieved with a colloid mill. Presum-

[5] Red Star Yeast Co., Milwaukee, Wisconsin.

TABLE 1

Effect of bead concentration and mill setting on cell breakage

Organism	Bead Concentration*	Mill Setting	Volume	Minutes				
				2	5	10	15	20
			ml	*enzyme/ml*†				
Yeast‡	60	0.030	300		75			120
	60	0.060	300		55	70		85
	60	0.020	300		66	72	79	
	30	0.030	300		35	50		60
				mg protein/ml				
Escherichia coli§	60	0.030	565	7.1	8.1		39.4	

* Ml 120 μ beads per 100 ml cell suspension.

† Units β-glucosidase per ml $\times$ 10^{-3} (Duerksen and Halvorson, 1958).

‡ *Saccharomyces fragilis* strain 610 $\times$ *Saccharomyces dobzhanskii* strain 1974; 100 g Sharples paste per 50 ml buffer.

§ 300 g Sharples paste plus 300 ml buffer.

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[3] Gifford-Wood Co., Hudson, New York.

[4] Minnesota Mining and Manufacturing Co., St. Paul, Minnesota.

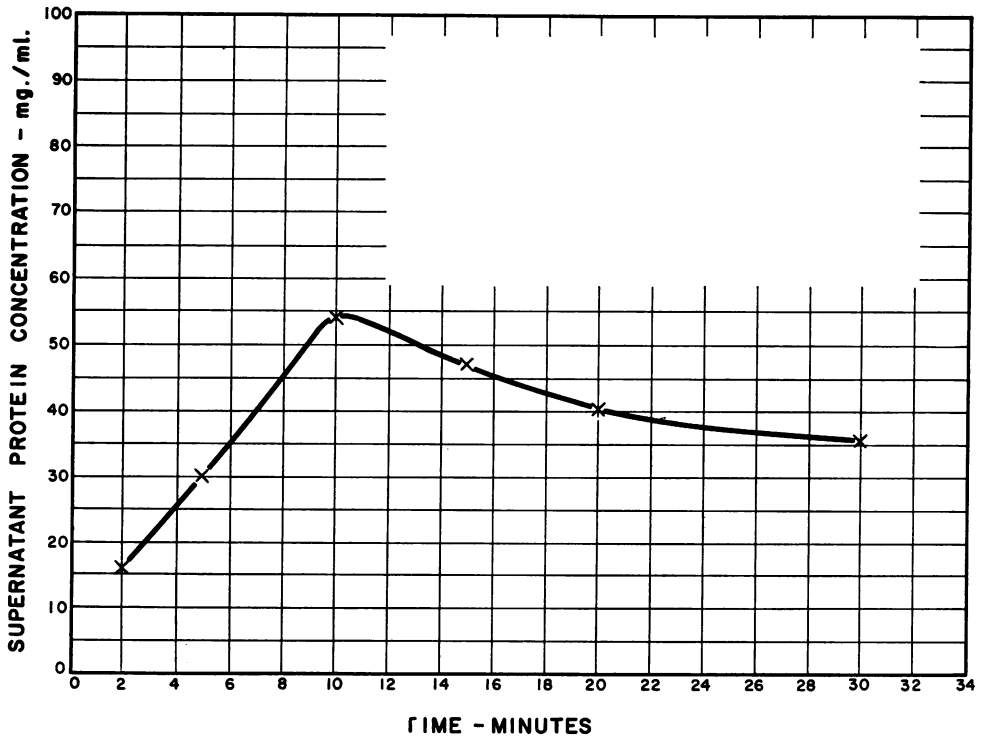

Figure 1. Rupture of bakers' yeast. *Assay:* Supernatant protein determined spectrophotometrically from ratio of optical densities at 260 and 280 mμ.

ably other abrasive agents could be used instead of glass beads; however, the influence of size distribution should be investigated. Many other types of colloid mills and homogenizers, which are available commercially in a wide range of sizes, could be expected to function as well as the Eppenbach mill.

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