Grinding Microorganisms with a Peristaltic Pump

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Abstract

PHILLIPS, JAMES W. (Pennsylvania State University, University Park), CARL LAMANNA, AND M. F. MALLETTE. Grinding microorganisms with a peristaltic pump. Appl. Microbiol. 13:460–463. 1965.—The Randolph Co. model 610 peristaltic, ½-hp pump was effective for preparative purposes in disrupting baker's yeast and spores of Bacillus globigii when suspended with glass beads. Best results were obtained with use of a slurry just fluid enough to flow through tubing while stirred. Beads of 0.2 and 0.1 mm were used to best advantage for the yeast cells and spores, respectively. Yeast cells were disrupted completely within 15 min, and the spores in 10 to 30 min. Temperature and surface denaturation are readily controlled, and the system is easily modified for use with large quantities of microorganisms.

None of the methods of cell rupture is suitable in all applications, and many are not useful for work with microorganisms on a large, preparative scale. Rapid mixing of cells with a suspension of small glass beads (Lamanna and Mallette, 1954) has proved to be one of the most effective techniques and has been widely modified, e.g., by Hamilton and Knight (1962). A recent, commercial high-speed shaker (Nossal Cell Disintegrator, McDonald Engineering Co., Cleveland, Ohio) is water-cooled, but its capacity is limited.

In one adaptation, Lamanna, Chatigny, and Colledge (1959) passed a closed loop of flexible tubing containing the cell-bead suspension between rollers at high speed. Although effective in disrupting cells, their procedure required care in sealing the tubing and did not permit effective control of the temperature or wide flexibility in the quantities processed. The present modification avoids these problems by employing a peristaltic pump in which the tubing remains stationary, while the slurry of microorganisms and beads is forced through the tubing.

Materials and Methods

Two organisms were used in testing the procedure. Commercial baker's yeast was employed both because of its availability and because it is widely regarded as one of the most difficult of organisms to rupture. To test application of the method to an equally resistant but smaller form, lyophilized spores of Bacillus globigii (B. subtilis) at a count of 6 × 10^11 per gram were supplied by R. D. Housewright, Fort Detrick, Frederick, Md.

Glass beads were obtained from Minnesota Mining and Manufacturing Co. in two sizes. By use of the Howard mold-counting chamber, as described by Triebold and Aurand (1963), the two sizes of beads were estimated to average about 0.2 and 0.1 mm in diameter.

A Randolph Co. (Houston, Tex.) model 610 peristaltic pump (½ hp) with Zero-Max speed control was used. This pump is rated at 185 gal (700 liters) per hr at 575 rev/min, the maximal recommended speed, with the size of tubing used here. Other peristaltic pumps of which we have knowledge are not rapidly effective in disrupting cells, probably because of a lower energy input into the movement of fluid in tubes. Rubber tubing of ½ inch (1.6 cm) outside diameter, ⅜ inch (0.95 cm) inside diameter, and 60 cm long was closed into a loop with a U-shaped 30-cm piece of 12-mm glass tubing. The segment of rubber tubing acted on by the rollers of the pump was lubricated with Silicone lubricant supplied with the pump.

The filled tubing was supported externally in an ice bath. Other temperature-control devices might be used instead of this simple but effective expedient. The tubing was filled with a cell-bead suspension except for a small air space acting as a cushion against pressure changes. Large air spaces were avoided to reduce foaming and the accompanying denaturation. However, in larger-scale work than that actually described, a closed circuit was found to be unnecessary, thus permitting flexibility in the volume of suspension treated. In such cases, the suspension was pumped into a large glass bulb, and then fed out the bottom of the bulb, thus insuring continuous repassage of the beads in large quantities through the pump. Foaming was kept negligible in this arrangement by placing the outflow tube below the surface of the liquid in the reservoir bulb.

For grinding yeast cells, 50 g of commercial cake were suspended in 100 ml of 0.5 M phosphate buffer
GRINDING WITH A PERISTALTIC PUMP

Fig. 1. Mechanical disruption of baker's yeast and spores of Bacillus globigii ground with glass beads in the peristaltic pump. A, B, and C are photomicrographs of yeast suspensions after grinding for 0, 5, and 15 min, respectively, and Gram staining; magnification factor, ×612. D, E, and F represent spore suspensions after grinding for 0, 10, and 46 min and malachite green staining; magnification factor, ×960.

(pH 7), and 28 ml of this suspension were mixed with 65 ml (99 g) of glass beads (0.2 mm in diameter). This material, just fluid enough to flow through the system, was poured and scraped through a powder funnel into the tubing, and then the ends of the tubing were joined. Samples were ground at maximal rated speed for various lengths of time, the resulting mixture was diluted with 7 ml of buffer, and the beads were allowed to settle for a few minutes. Filtration and washing permit complete separation of beads from the mixture. Release of soluble cell components was followed by allowing the beads to settle, diluting 1.2-ml samples of the supernatant material to 5 ml with 0.5 M phosphate buffer (pH 7), centrifuging for 0.5 hr at 12,000 × g and 2°C, decanting the faintly hazy liquid and diluting it 1:100 with water, and reading the absorbancy with a Beckman DU spectrophotometer. Light scattered by the remaining suspended material was corrected for by means of

\[ A_{\text{corrected}} = A_{\text{observed}} - \tau \left( \frac{\lambda_r}{\lambda_{\text{observed}}} \right)^4 \]
Table 1. Changes in oxygen uptake and absorbancy on grinding yeast

<table>
<thead>
<tr>
<th>Grinding time (min)</th>
<th>O2 uptake (µl/min)</th>
<th>Wavelength (µm)</th>
<th>Absorbancy</th>
<th>Absorbancy (Corrected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>11.7</td>
<td>300</td>
<td>0.021</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>280</td>
<td>0.036</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>260</td>
<td>0.058</td>
<td>0.021</td>
</tr>
<tr>
<td>5</td>
<td>10.1</td>
<td>300</td>
<td>0.045</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>280</td>
<td>0.195</td>
<td>0.136</td>
</tr>
<tr>
<td></td>
<td></td>
<td>260</td>
<td>0.329</td>
<td>0.249</td>
</tr>
<tr>
<td>15</td>
<td>6.2</td>
<td>300</td>
<td>0.064</td>
<td>0</td>
</tr>
<tr>
<td></td>
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<td>280</td>
<td>0.278</td>
<td>0.194</td>
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<tr>
<td></td>
<td></td>
<td>260</td>
<td>0.462</td>
<td>0.349</td>
</tr>
</tbody>
</table>

* Oxygen uptake was measured at pH 7 by the standard method of Umbreit, Burris, and Stauffer (1964) with KOH to absorb the CO₂ evolved; 2 ml of the suspension were added directly to 50 µmoles of glucose without centrifuging, and included intact cells, debris, and particulates.

† See Materials and Methods for the correction procedure.

where absorbancies are represented by \( A \) and wavelengths by \( \lambda \). The symbols \( \tau \) and \( \lambda \) are, respectively, turbidity \( (\tau = \log I_0/I) \) and absorbancy \( (\lambda \) at which \( \tau \) was measured. The wavelengths for \( \tau \) were chosen to provide values of useful magnitude but yet to lie outside the range of light absorption by the usual cell components and solvents; the wavelengths used were 300 µm for yeast and 330 µm for spores.

The spores were ground in the same way except that 12 g of lyophilized material were suspended in 80 ml of buffer, and 0.1-mm beads were used, being more effective than the larger size. After grinding, the mixture was diluted with 10 ml of buffer, the supernatant material was decanted and centrifuged as above, and a sample of the centrifuged solution was diluted 1:100 and examined.

Gross changes in the cells were observed with a Leitz Ortholux microscope and xenon light source. The photographic records were obtained with Kodak Panatomic-X sheet film (9 by 12 cm). The prints reproduced in Fig. 1 represent approximately the center 35% of the actual fields, which were not flat and, therefore, were out of focus at the edges. The yeast systems were stained by the Hucker modification of the Gram method and the spores, with malachite green, both according to Farrell (1966).

**RESULTS AND DISCUSSION**

Effectiveness of the peristaltic pump in rupturing baker’s yeast is evident in Fig. 1. Since damaged cells do not retain the Gram stain, the typical samples shown in the figure reveal extensive disruption in 5 min and nearly complete absence of cells staining normally after grinding for 15 min. Owing to limited depth of field, yeast cells retaining the Gram stain appear out of focus in the photomicrographs. The apparently normal but unstained cells represent only cell envelopes, having lost their contents. Thus, loss of cytoplasmic contents without extensive trituration of the cell wall is possible.

Table 1 summarizes the physiological effects of the treatment. Oxygen uptake data (not reported) revealed a pronounced lag period for intact yeast cells, a slight one after grinding for 5 min, and a barely perceptible one after 15 min. The tabulated data were calculated after attainment of a steady state maintained for at least the duration of measurement (1 hr). A decline of 50% in O₂ uptake after grinding for 15 min shows a major loss in respiratory capacity even though debris, active particles, and any remaining intact cells were included in the measurement.

Absorbancy data corrected for the scattered light in centrifuged and diluted suspensions of supernatant fluid reveal no significant light absorption prior to grinding when measured at wavelengths characteristic of proteins and nucleates. After grinding for 5 min, a marked absorption indicates extensive solubilization of protein and nucleate. This process had continued in the 15-min sample. No further increase was noted on prolonged grinding. Hence, disruption of yeast cells and solubilization of cellular contents was essentially complete in 15 min. Isolation of a specific component might exhibit some other time optimum. Comparisons of various ratios of absorbancy data from the table for
ground microorganisms reveal consistency in the procedures for centrifuging and correcting for scattered light.

In a similar way, destruction of the spores is demonstrated. Figure 1 shows a decline with time in the number of stainable spores and an increase in the amount of debris in typical suspensions. The absorbancy data (Table 2) show little or no absorbing material prior to grinding, with a marked increase in 10 min. There was no significant change in 20 min more, and disruption was complete by this measure within about 10 min. Again, comparison of absorbancy ratios for the ground spores reveals consistency of the technique.

Other variations in time and temperature were compatible with the foregoing discussion. Use of fewer beads or relatively more liquid reduced grinding effectiveness somewhat for these two organisms. Less resistant cells may be ground readily in a more fluid system, although the ratio of beads to water must become important at some level for any organism and must be determined empirically.

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LITERATURE CITED


