An Efficient, Easily Constructed Cell Homogenizing Press

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ABSTRACT

SAGERS, RICHARD D. (Brigham Young University, Provo, Utah). An efficient, easily constructed cell homogenizing press. Appl. Microbiol. **10**:37–39. 1962.—An easily constructed, highly efficient cell homogenizing press is described which should be available to laboratories at moderate cost. The press allows the preparation of homogenates from up to 20 g of packed cell paste in a single batch without danger of damage to the press. Cell breakage and recovery of cell homogenates is accomplished at temperatures which maintain the material in the frozen state. The press may be assembled, disassembled, and cleaned with minimal effort.

Among several available methods of disrupting bacterial cells for preparing subcellular components and soluble enzymes, a homogenizing press of the type designed by Hughes (1951) has proved useful in our experience. Cells may be disrupted and homogenates recovered in the frozen state, thus aiding in the preservation of enzymes and other materials which are particularly unstable. Other useful hydraulically operated tissue homogenizing presses have been designed by French and Milner (1955) and by Emanuel and Chaikoff (1957). The last two instruments are somewhat less efficient, however, than the Hughes press for rupturing bacteria and yeasts.

After considerable experience with several Hughes presses, it seemed desirable to construct an instrument which eliminated a number of encountered difficulties. The small bolts used with this press stretch rather readily under the high pressures applied, allowing the halves of the press to separate during cell breakage and reducing the efficiency of the operation. After repeated usage the threads on the bolts may become stripped, making the opening of the press difficult or awkward. A most serious difficulty is the shortness of the cylinder and its relatively low capacity. When more than 3 or 4 g of frozen cell paste are to be crushed there is a possibility of inserting the piston into the cylinder at a slight angle. When the piston is driven into the cylinder under high pressure, a slight misalignment may result in severe scoring and damage to the cylinder wall, requiring major repair or replacement of the instrument.

The cell press described in this communication has been designed to overcome the above difficulties and to provide an easily constructed, larger capacity instrument, which is less susceptible to damage, and which is readily assembled and disassembled with minimal effort.

COMPONENTS AND CONSTRUCTION OF THE PRESS

The components of the press shown in Fig. 1, 2, and 3 with specifications given in Fig. 1 are as follows:

A, a 5-in. diameter base, hollowed out to provide a polished surface A_1 and threaded to accomodate

B, a 3½-in. diameter threaded inner component provided with a ⅞-in. diameter cylinder with a circular collection chamber. The latter is separated from the cylinder by polished surface B_1. The cylinder accomodates

C, a ⅞-in. piston (tolerance 0.001 in.)

To be positioned atop the piston during the crushing operation is

D, a ⅝-in. steel ball and

E, a steel cap. The ball is accomodated in the recessed areas in the cap and the top of the piston, and serves to compensate for possible slight misalignments in the pressure plates of the hydraulic press (Carver Laboratory Press[1]) employed to drive the piston.

Two additional components (Fig. 2) used for opening the press as described later are as follows:

F, a metal plate fitted with two bosses which fit into end mill slots on oposite sides of component *A*. This plate may be bolted to a table or to the floor and is used to hold the press while opening by means of

G, a spanner wrench. The spanner fits into a ½-in. diameter hole drilled ⅜ in. deep in component *B*.

Parts *A* and *B* of the press were constructed from circular molybdenum tool steel (Rockwell hardness scale, 20 (Rc 20)), and heat-hardened after construction to Rc 50. The piston (*C*) was made from Graphmo[2] tool steel (Rc 55). A good grade of stainless steel offers advantages with respect to corrosion and rust resistance, but soft stainless steel is definitely not acceptable. If the press described herein is cleaned and dried immediately after use, coated with a thin layer of light machine oil, and stored in a dry place, the steel retains its bright finish and is not readily subject

[1] Manufactured by Fred S. Carver, Inc., Summit, N. J.

[2] Manufactured by Steel and Tube Division, Timken Roller Bearing Company, Canton, 6, Ohio.

to rusting. Prior to use the oil is easily removed from the parts coming in contact with cells.

The design of the press is such that all major operations during construction of components A, B, and C can be made on a lathe, and can be accomplished without removing the individual components from the lathe chuck until each unit is completed. The large threaded surfaces insure against binding or stripping under the high pressures used. Consequently, components A and B are easily assembled and disassembled after repeated usage.

When A and B are assembled, only surface B_1 comes in contact with A_1. The most critical requirement of the press is that these surfaces should be carefully polished to insure that all of B_1 is seated on A_1, since maximal cell breakage occurs under these conditions. Surface B_2 is machined to give 0.01-in. clearance between A_1 and B_2, assuring a close fit between A_1 and B_1 and also reducing friction between the contacting surfaces when opening the press. B_2 serves mainly as a revetment to prevent homogenized cellular material from becoming mixed in the threads.

The no. 9 threads used are coarse enough to allow rapid assembly, but fine enough to allow precision adjustment of the distance between surfaces A_1 and B_1 if desired. Since a no. 9 thread gives a rise of 0.111 in. per turn (360°), each 3.6°-turn gives approximately 0.001-in. rise. Thus, the clearance between A_1 and B_1 may be adjusted by engraving marks 3.6° apart on the circumference of A, to be aligned with an index marker on B.

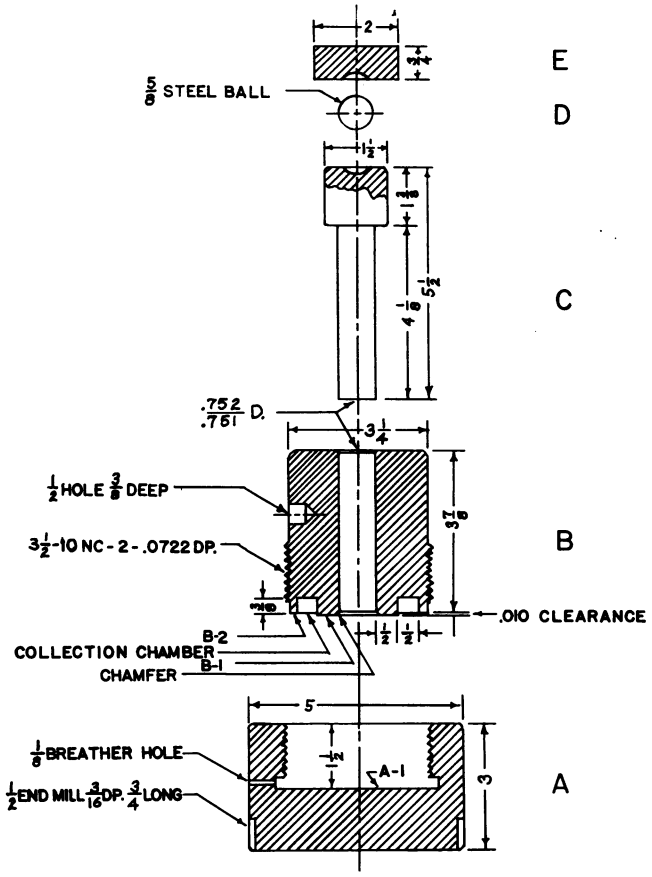

FIG. 1. *Detailed specifications for cell homogenizer*

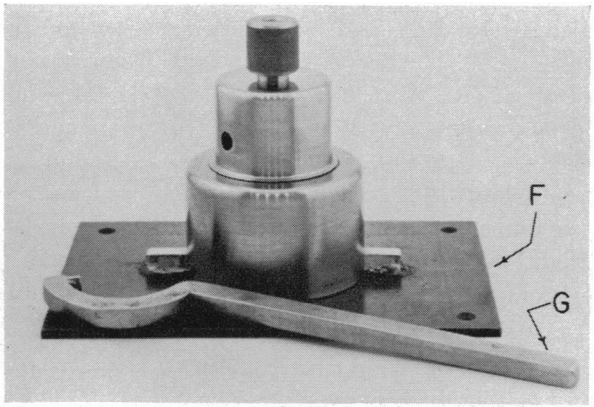

FIG. 2. *Assembled view of cell homogenizer with base plate (F) and spanner wrench (G).*

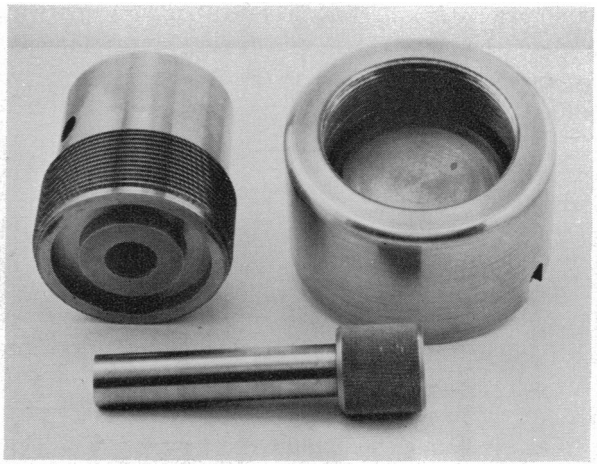

FIG. 3. *Exploded view of homogenizer, showing interior details*

TABLE 1. *Efficiency of the cell press in rendering cellular material nonsedimentable after 15 min at 25,000 × g*

Organism	Packed wet wt prior to breakage	Sedimentable residue	Solubilized material	
	g	g	g	%
Bacillus subtilis	8.9	5.0	3.9	44
Clostridium acidiurici	17.2	8.6	8.6	50
Diplococcus glycinophilus	7.9	4.5	3.4	43
Escherichia coli	7.0	3.9	3.1	44
Leuconostoc mesenteroides	8.5	4.6	3.9	46
Sarcina lutea	12.3	6.0	6.3	51
Serratia marcescens	6.6	3.5	3.1	47
Streptococcus faecalis	9.9	4.8	5.1	52
Hansenula anomala	15.0	10.4	4.6	31
Saccharomyces cerevisiae	19.4	11.8	7.8	39

The organisms were grown in appropriate media and harvested by Sharples centrifugation. The collected cells were washed in 0.05 M potassium phosphate buffer, pH 7.0 and centrifuged at 25,000 × g for 15 min in a Servall SS-3 superspeed centrifuge using an SS-34 rotor and 50-ml plastic cups. The packed cells were then frozen at −20 C, crushed, resuspended, and the viscosity of the homogenates reduced by brief sonic oscillation as described in the text.

The dimensions and mass (20 lb) of the press are such that when cooled, the low temperature desired for keeping cells and homogenates in the frozen state is easily maintained throughout the homogenizing operation described below until the material is removed from the press.

Up to 20 g of packed wet weight of cells are easily accommodated in half the length of the 7/8-in. diameter cylinder. Even with this amount of material, the piston can be inserted to a distance of 5 cm (approximately half the total distance) before high pressure is applied, thus insuring proper positioning of the piston and reducing the danger of bending or of scoring the cylinder wall.

Operation of the Press

In preparing the press for use, *B* is turned into *A* by means of the threaded surfaces which are lightly oiled. It is not necessary to use force to close the press, since the components turn freely into a closed position by hand pressure. The assembled press with the piston inserted, is cooled to about −20 C. (If the temperature is lowered much below this value, excessive pressure, generally beyond the safe capacity of the hydraulic press, is required for crushing cells.) After cooling, the piston is withdrawn and a pellet of frozen cells inserted into the cylinder. The piston is replaced and pushed in by hand pressure as far as possible. The cold press is then quickly centered on a hydraulic press with the ball and cap assembly positioned atop one piston. Light pressure is applied at first to drive the piston further into the cylinder by taking up any remaining free space and compacting the cells. Then high pressure is applied and the piston driven all the way in. The entire operation should take less than a minute. Homogenization is effected by extrusion under high pressure (approximately 14,000 psi) of frozen cells between surfaces A_1 and B_1. The crushed cells are collected in the circular chamber which has a capacity of approximately 23 cm^3.

After cellular material has been extruded between surfaces A_1 and B_1, these surfaces become "frozen" together and force required to separate them. To facilitate opening, the press is positioned on the metal plate (*F*), the spanner inserted into component *B*, and a sharp blow applied to the spanner with a wooden or lead mallet to break the frozen seal. If difficulty is experienced in breaking the seal immediately after crushing cells, the press is probably still too cold. If allowed to stand at room temperature for a few minutes, or if tap water is run over the bottom of the press for a few seconds to raise the temperature to about −10 C, a single sharp below to the spanner is generally sufficient to break the seal, and the parts are then easily separated without further need of the spanner. A small breather hole from the outside to the inner chamber allows pressure equilibration during opening of the press and prevents additional friction on the threads. The cellular material should remain solidly frozen throughout the operation and can be easily removed from the collection chamber with a small spatula.

Efficiency of the Press

The efficiency of cell breakage is very high and comparable to that of the Hughes press (Hughes, 1951). We have noted that when packed wet cells of a number of bacterial species (centrifuged at 25,000 × *g* for 15 min) are frozen at −20 C and crushed, about 50% of the total weight of the cellular material is rendered nonsedimentable upon recentrifugation at the above force and time. Representative data are shown in Table 1. With cells of the yeasts *Saccharomyces cerevisiae* and *Hansenula anomala*, efficiency of cell breakage is somewhat lower than observed with bacteria but similar to the efficiency observed with the Hughes press.

Upon suspension of the homogenized cellular material in suitable buffers to prepare cell-free extracts it is commonly observed, especially with bacterial homogenates, that the suspension is highly viscous. This is apparently due to the release from the cells of deoxyribonucleic acid, since the enzyme deoxyribonuclease readily reduces the viscosity of such preparations. Another convenient method of reducing the viscosity is to subject the homogenized material (in a 1:10 suspension, w/v) to sonic oscillation for 0.5 to 2 min. Cellular debris is removed by centrifugation after employing either of the above methods of rendering the maximal amount of material nonsedimentable at the centrifugal forces applied.

We have experienced good success in making enzyme preparations from the bacteria mentioned as well as from the yeasts. We have not fully determined the usefulness of this press for making preparations of cell walls, cell membranes, mitochondria, ribonucleic and deoxyribonucleic acids, or other subcellular fractions, but its potential seems great.

Acknowledgment

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LITERATURE CITED

Emanuel, C. F., and Chaikoff. I. L. 1957. An hydraulic homogenizer for the controlled release of cellular components from various tissues. Biochim. et Biophys. Acta **24:** 254–261.

French, C. S., and H. W. Milner. 1955. Disintegration of bacteria and small particles by high-pressure extrusion, p. 64–67. *In* S. P. Colowick and N. O. Kaplan, [ed.], Methods in enzymology, v. I. Academic Press, Inc., New York.

Hughes, D. E. 1951. A press for disrupting bacteria and other microorganisms. Brit. J. Exptl. Pathol. **32:**97-109.