A high speed shaker for the disruption of cells at low temperatures*

The apparatus to be described is comparatively simple, inexpensive and easily available. Temperatures near 0° C can be maintained for prolonged periods and about 6 g of dry bacteria can be disrupted in a single short operation, by using a shaker head in a refrigerated centrifuge.

A suspension of bacterial cells is shaken with 0.2 mm glass beads at high speed (1900–2000 r.p.m.) in a stainless steel capsule on an International Equipment Co. No. 6007** shaker head mounted in an International refrigerated centrifuge. Capsules and shaker head are shown in Fig. 1. In order to hold the capsules securely at high speeds, the spring is removed from the clamp supplied with the shaker, the entire lengths of the vertical shafts are threaded, and lock nuts are used above and below the side arms. The overall shaking effect is a vertical reciprocation with a slight horizontal movement, producing circulation as well as concussion of the sample. The slight horizontal movement eliminates the problem of vortex formation. The length of the stroke at the capsule is $1^3/_8''$. No mechanical difficulties have been encountered in well over 200 h of operation. However, the centrifuge should be level and all sources of vibration should be eliminated.

A variety of glass and metal shaking vessels have been used, but for adequate cooling narrow diameter stainless steel capsules, of the type illustrated in Fig. 1, have proven to be the most satisfactory. The tight fitting screw caps are fitted with polyethylene liners. To reduce fouling by the glass beads the screw threads are placed on the outside of the capsules. The capsules can be cleaned by shaking with glass beads and water for consecutive periods until metallic abrasion is reduced to a minimum. The abrasive effect can be nullified by chromium electroplating*** the inner surface of the capsule.

In our experiments equal weights of an aqueous cell suspension (or water) and 0.2 mm glass beads (thoroughly washed with acid and water) were used and the capsules were never more than two-thirds filled. In order to maintain the internal temperature at below 5° C and above freezing, the centrifuge temperature must be adjusted to the conditions of the run. For instance, when the smaller capsule was shaken for 30 min at 1900 r.p.m. with a 20 g charge (10 ml water plus 10 g beads) freezing of the contents occurred if the centrifuge temperature was lower than —10° C; with one-half the charge, centrifuge temperatures lower than —5° C caused freezing. The proper centrifuge temperature for a 60 g charge in the larger capsule shaken at

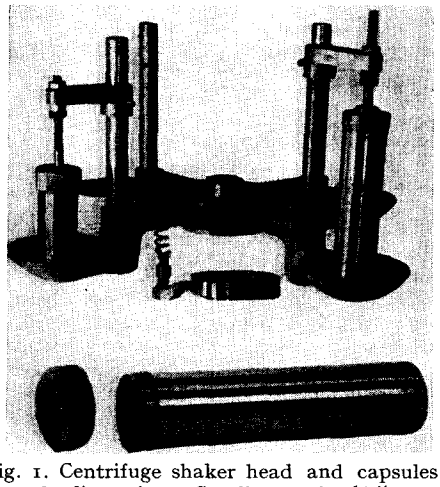

Fig. 1. Centrifuge shaker head and capsules. Capsule dimensions: Small: length $3^1/_8''$; outside diameter $1^1/_{16}''$; wall thickness $^3/_{32}''$; capacity 27 ml. Large: length 5″; outside diameter $1^3/_{16}''$; wall thickness $^1/_{32}''$; capacity 80 ml.

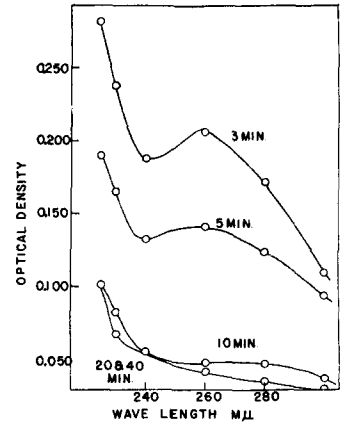

Fig. 2. Absorption spectra of insoluble fractions of exponential cells after different shaking periods. Values plotted are the differences between those of the original suspension (76 μg dry bacteria/ml) and the corresponding supernatants.

* Work done under a research contract with the Office of Naval Research, Washington, D.C., U.S.A.

** No. 6007 is the part number given by the International Equipment Co., Boston, Mass., U.S.A., to the shaker head that will fit the tapered shaft of the refrigerated centrifuge. Otherwise it is identical to their No. 6061 shaker head for which they will also supply the proper tapered bearing.

*** Chromium, when electroplated, has the hardness of 9 on the scale where diamond is 10.

TABLE I

DISRUPTION OF *S. faecalis* BY SHAKING

Individual 5 ml portions of a 1% suspension of lyophilized cells were shaken with 5 g beads at —5° C and 1900 r.p.m. for the stated time intervals. The capsule contents were filtered through a coarse sintered glass funnel; capsule, beads and funnel were thoroughly washed and the combined suspension was centrifuged at 20,000 *g* in the cold for 1 h. Nitrogen was determined by a semi-micromodification of the method of MILLER AND MILLER[1] on the total suspensions, precipitates and supernatants. Ultra-violet absorption spectra were obtained on the total suspensions and the supernatants. The soluble nitrogen represents the average of values obtained from the analysis of the supernatants and the corresponding precipitates. Their average difference is less than 2%. The nature of the *S. faecalis* cells used will be described elsewhere[2].

Shaking time (min)	*% nitrogen soluble*		*% of U.V. at 260 mμ soluble*	
	Exponential cells	*Stationary cells*	*Exponential cells*	*Stationary cells*
0	20	15	20	20
3	67	65	69	77
5	75	71	82	83
10	89	77	98	91
20	89	78	97	94
40	89	79	96	94

1900 r.p.m. for 30 min was —20° C. The thinner wall of the larger capsule favors better heat transfer. Four of the large capsules, shaken simultaneously, each with 30 ml of a 5% cell suspension, would give a working capacity of 6 g dry bacteria.

The data of Table I demonstrate the speed and completeness of the solubilization of nitrogen and 260 mμ absorption peak (nucleic acids and derivatives) of two *Streptococcus faecalis* (ATCC 9790) preparations. Both sets of results are particularly striking since they were obtained without washing the insoluble material. The high speed (20,000 *g*) supernatants contained only 4% of the total rhamnose in the cell as determined by the method of DISCHE AND SHETTLES[3]. When the absorption spectra of the insoluble residues were calculated (Fig. 2), a progressive decrease in the 260 mμ peak was observed with increasing time of disruption until it was no longer present after 20 min of shaking. Since rhamnose seems to be specifically located in the cell walls of *Streptococci*[4,5,6], and the nucleic acid absorption is specific for cytoplasm[4,5], an apparently clean separation can be obtained by this method. The overall recovery of lyophilized, white, highly soluble supernatant material is 70 to 80% of the initial weight of cells. The difference in the soluble nitrogen fraction of exponentially grown and stationary *S. faecalis* cells, as well as further data supporting the method will be discussed elsewhere[2].

The cells were well washed prior to lyophilizing but a significant soluble fraction was found at zero time (Table I). Back extrapolation of the data suggests that the additional time required to disrupt fresh cells would not exceed one min. Even if the difference in fragility of lyophilized and fresh cells were greater, the method would be highly effective, especially since *S. faecalis* seems to be more resistant to mechanical disruption than some other organisms[7].

The primary advantage of this procedure, as compared to previous ones[7,8,9], is the ease of maintaining low temperatures within the shaken capsule without interrupting the run. The process is rapid and reproducible, and the equipment is easily available. When only small quantities of cells are to be disrupted or low temperature is not essential, ordinary screw capped bottles may be used. The shaker can be used for other purposes and has been employed in this laboratory for the preparation of cell free nuclei from animal tissues[10].

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Received January 7th, 1957