PRO EXPERIMENTIS

Continuous Disintegration of Microorganisms in a New Laboratory Apparatus

Laboratory mechanical disintegration of micro-organisms has been carried out so far mainly in single-run disintegrators with designs based either on forced passage of the frozen suspension through a hole[1], intensive mixing[2–4], or vibration[5–7] of the microbial suspension with abrasive elements present. The earliest continuous method of micro-organisms disintegration was elaborated by FREEDMAN and ROSS[8] and is based on the last-mentioned mode of operation. The principle of this equipment has, however, not yet been applied for purposes other than those within the field of laboratory work. Research in the fields of microbiology, biochemistry and pharmacology necessitates, to a growing extent, equipment to achieve continuous disintegration, a principle which can also be applied at production-scale.

In the present paper, the main characteristics of micro-organisms disintegration in a continuously operated, horizontal, laboratory-scale disintegrator developed by the Willy A. Bachofen Company of Basel, Switzerland, in accordance with a Czechoslovak patent[9], and the results obtained with the single-run disintegrator[10], will be given. The parameters of these disintegrations are applicable for modeling disintegration processes ranging from pilot-plant[11] up to factory-scale in the large units now built (for the flow rate of 150 l/h and more suspension) operating on the same principle.

Materials and methods. In order to establish the main parameters of disintegration, 2 micro-organism patterns were used – yeast (commercial baker's yeast), the suspension with a dry-matter content of 14 or 16% (weight/volume), and bacterial suspension (*Bacillus subtilis*) with a final dry-matter content of 5.5%.

The equipment is the prototype of the continuous horizontal mill (Figure 1) with exchangeable disintegrator containers (with a cooling mantle) with a total internal capacity of 0.6 l (A), 0.3 l (B) and an additional container (C) of 0.3 l capacity, especially designed for batch disintegration. The stirrer (D) in the 0.6 l container is formed by 4 grooved disks, 6.4 cm in diameter, fitted on a common shaft with an adjusting plate (I) to achieve separation of the glass beads from the disintegrated material during disintegration; the stirrer (E) for the content of the 0.3 l container has 2 grooved disks and a separator plate (I) equal in diameter to the aforementioned one. The stirrer for batch disintegration is formed by a single grooved disk, 9.5 cm in diameter, bearing flat (F) or deep (G) tangential slots. The stirrers move with a variable peripheral speed of 10, 15 or 20 m/sec. As disintegrating elements, glass beads having a specific gravity of 2.6 g/cm³ and a diameter of 0.25 to 0.5 mm for the destruction of yeasts were employed; beads 0.1 mm in diameter were used for the bacterial suspension. The ratio of the dry bed volume of beads to that of the microbial suspension in the disintegrator con-

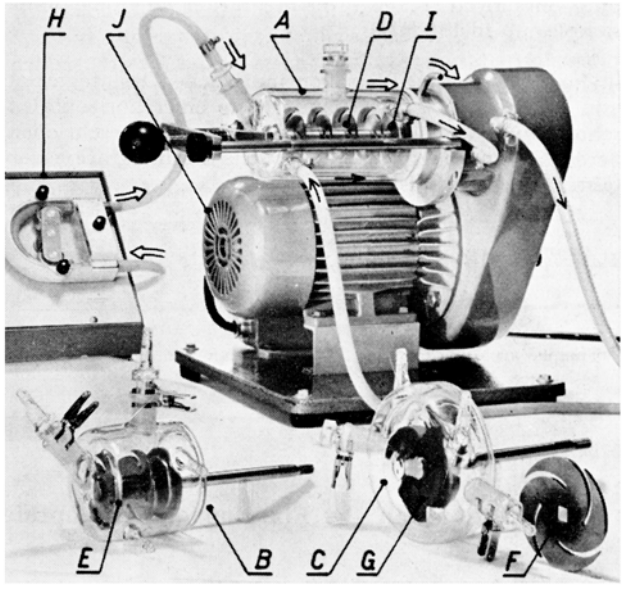

Fig. 1. Continuously operated disintegrating unit. Disintegrator vessel: A) volume 0.6 l; B) volume 0.3 l; C) volume 0.3 l for batch disintegration. Agitator: D) for the 0.6 l vessel; E) for the 0.3 l vessel. Agitator for batch disintegration: F) with flat grooves; G) with deep grooves; H) pump; I) separator disk; J) driving engine.

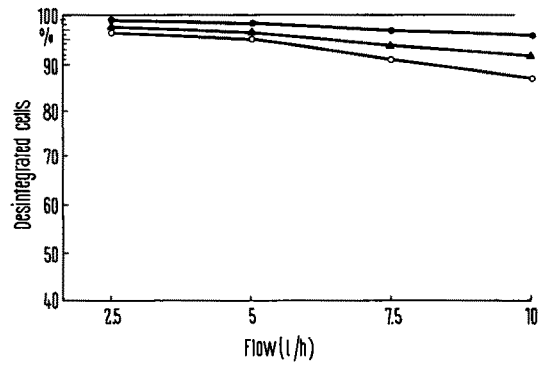

Fig. 2. Functional relationship between the degree of disintegration of yeasts (14% suspension) and the flow rate as well as the peripheral speed of the stirrer. ●, peripheral speed of the stirrer 20 m/sec. ▲, peripheral speed of the stirrer 15 m/sec. ○, peripheral speed of the stirrer 10 m/sec.

Degree of disintegration (%) for a 16% yeast suspension	Viscosity (cP) at 25 °C
Prior to disintegration	14.0
25	16.5
50	19.0
75	22.0
100	26.0

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tainer at rest was 1.7:1 for the yeasts and 1.6:1 for the bacteria. The rate of the yeast disintegration was determined by direct counting of cells and cell walls stained with methylene blue after heating and then transferred onto the graduated screen of a microscope. With bacteria, the disintegration speed was determined by measuring the extinction of their supernatant layer at 280 nm. An orienting control of the disintegration course was carried out by measuring the viscosity of the disintegrated suspensions.

Results and discussion. Continuous disintegration of micro-organisms in the apparatus presented is based on the principle of intensively mixing the suspension passing through the cylindrical container of the disintegrator filled with glass beads. The suspension is conveyed (the direction of flow being indicated by a bold arrow in Figure 1) by a pump (H) to the container of the disintegrator (A). The grooved disks of the horizontally disposed agitator (D) impart a preferential rotatory movement to the individual layers of the mixture; the beads move along the streamlines in the suspension, involving, in addition, colliding and rolling of the beads. The suspension is separated from the beads prior to leaving the disintegrator. The container surface and the bearing of the stirrer are cooled by flowing water or salt water (the direction of flow being indicated by an ordinary arrow in Figure 1). The disintegration temperature of the suspension can be influenced when different cooling liquids are used. Salt water (−4 °C) with a flow rate of 190 l/h for example allows to disintegrate with a temperature of until less than 10 °C (peripheral speed of stirrer 10 m/sec).

The disintegrating effect of the 0.6 l unit is evident from Figure 2 for yeast suspensions with a dry-matter content of 14% and at flow rates of 2.5 to 10 l/h. Figure 3 shows the appearance of the yeast material after different degrees of disintegration and separation of protoplasm released by a single washing-out process with a physiological solution and by centrifuging. The different degrees of disintegration can also be judged by the number of residual unopened cells (Figure 3B), but chiefly by the damage of cell walls (Figure 3D) and the quantity of protoplasm adherring to them.

Protoplasm released from the cells into the solution causes an increase of the whole suspension's viscosity. This circumstance was utilized to perform an orienting determination of the degree of disintegration for yeasts, as is shown by the example in the Table.

Continuous disintegration of bacterial suspensions in a 0.6 l container of the equipment is documented by the results obtained by milling a suspension of *Bacillus subtilis* cells with glass beads at a peripheral speed of the stirrer of 15 m/sec. In this case, 80–85% of the cells is disintegrated at a flow rate of the suspension equal to 1 l/h, 75–80% at 3 l/h, 70–75% at 5 l/h and 60–65% at 7 l/h.

The unit equipped with a 0.3 l container for continuous disintegration was sufficiently effective to achieve the destruction of the fibrillary micro-organisms tested (*Aspergillus niger*) resulting in a 100% disintegration. The 0.3 l container for batch use with stirrer disks bearing tangential grooves of different depths was successfully employed in the disintegration of low-volume samples up to 150 ml[12].

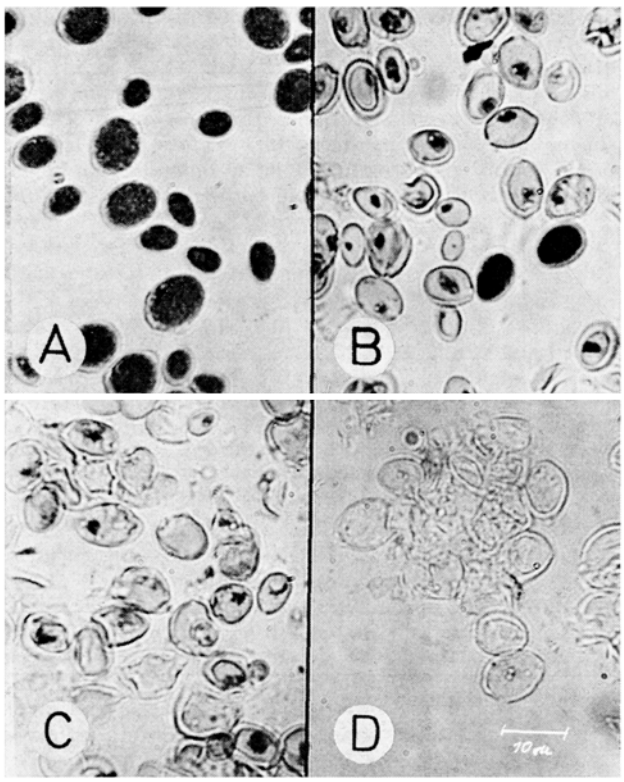

Fig. 3. A) Yeasts prior to disintegration. Cells and cell walls after removal of protoplasm released following continuous disintegration. B) Flow rate 7.5 l/h, and peripheral speed of the stirrer 10 m/sec. C) Flow rate 5.0 l/h, and peripheral speed of the stirrer 15 m/sec. D) Flow rate 2.5 l/h, and peripheral speed of the stirrer 20 m/sec.

Zusammenfassung. Die kontinuierliche Desintegration von Hefen und Bakterien mit Hilfe einer horizontalen, schnellaufenden Labor-Kugelmühle wird beschrieben, deren Arbeitsprinzip auf industrielle Desintegrationsverfahren anwendbar ist.

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[12] Thanks are due to Mrs. I. TRAUTMANN for skilled technical assistance.

Carbodiimide Fixation for Immunohistochemistry: Observations on the Fixation of Polypeptide Hormones

Of the fixatives normally used in immunofluorescence[1a], those which act chemically rely heavily on attack of primary amino groups to form cross-links between neighbouring structures. The stimulus to seek alternative fixatives arose recently in connection with our immunohistochemical studies of polypeptide hormones. The use of water-soluble carbodiimides (CDI) suggested itself because these reagents effect cross-linking by initial attack of carboxyl groups, and because native antigenic determinants survive in immunogen conjugates prepared with CDI.