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Cooling Device for Use with a Sonic Oscillator

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ABSTRACT

ROSETT, THEODORE (Duke University Medical Center, Durham, N.C.). Cooling device for use with a sonic oscillator. Appl. Microbiol. **13**:254–256. 1965.—A cooling cell is described that facilitates maintenance of biological materials at low temperatures during prolonged sonic treatment. Using this cell and a Branson S-75 Sonifier, I examined temperatures and the release of protein and enzyme activity from suspensions of *Saccharomyces cerevisiae*. With the Sonifier at full power, it was possible to maintain cell temperature within 9 C of the cooling-bath temperature, and to disrupt 10% (w/v) suspensions of *S. cerevisiae* in 10 min.

The disruption of microorganisms in large quantities by exposure to ultrasonic vibrations has become a standard adjunct to biochemical studies in many laboratories. As larger amounts of protein or enzyme have been required, instruments of increasing power have come into use. Even though such ultrasonic generators are coupled to extremely efficient transducers, the very high intensity of the vibrations results in the production of large amounts of heat in the solution being treated. Maintaining a low temperature in such preparations has become a problem of considerable importance. This report describes the design of a chamber capable of maintaining a low temperature during prolonged sonic treatment of solutions or suspensions. The efficiency of this chamber has been tested for maintenance of low temperatures and disruption of *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

When an ultrasonic probe is immersed in water and the power turned on, there is considerable radiation pressure away from the bottom of the probe tip. This is particularly noticeable if the vessel is a beaker where the patterns of cavitation bubbles streaming away from probe tip go down to the bottom, out to the sides, and then back up to the top. Unfortunately, beakers and test tubes possess relatively small exterior surfaces, so that heat loss from that surface does not equal the heat generated by the ultrasound until the internal temperature is very high. Moreover, if microorganisms are disrupted by forcible contact with cavitation streamers or bubbles (Hughes and Nyborg, 1962), allowing such streamers to emanate outward in the shape of an expanding cone is less efficient than confining them in a vessel shaped like a converging cone wherein most of the material in suspension would be in contact with relatively high energy cavitation.

Accordingly, I designed a chamber (Rosett cooling cell; Branson Sonic Power, Danbury, Conn.) which utilizes the radiation pressure of an ultrasonic probe as a pump to circulate fluid through a conical chamber and then through a series of coils having large surface-to-volume ratios (Fig. 1).

These cells are designed to operate immersed in a stirred cooling bath, as illustrated in Fig. 2. With the Sonifier S-75 (Branson Sonic Power, Danbury, Conn.) at its highest power setting, the temperature inside the cooling cell at equilibrium is about 9 C above that of the cooling bath. Because the fluid inside the cell is in rapid motion during high-intensity oscillation, it is possible to immerse the cell in cooling baths as low as −8 C; if the bath temperature is lower, the fluid in the side arms of the cell may freeze. The temperature range of 0 to 5 C has been shown to be satisfactory for most enzyme preparations.

Salt-ice water mixtures with temperatures of 0, −5, and −7 C were prepared in a 3-liter glass beaker fitted with a magnetic stirrer.

For each test, 50 ml of distilled water were measured into a cooling cell. The ultrasonic probe was clamped into place so that the probe tip was centered in the cooling cell, 3.8 cm below the surface of the liquid and 3.8 cm from the bottom of the cell. The Sonifier was connected to an electrical timer which was set for an appropriate interval. The power setting on the generator was no. 8, drawing 8 to 9 amp. Temperature was measured with a mercury thermometer and a thermistor probe immersed in the fluid in the cooling cell. Temperature changes were recorded until the temperature inside the cell was approximately 42 C. Then the cooling bath was rapidly positioned until the cell was immersed as shown in Fig. 2. The temperature change was then recorded until it reached equilibrium.

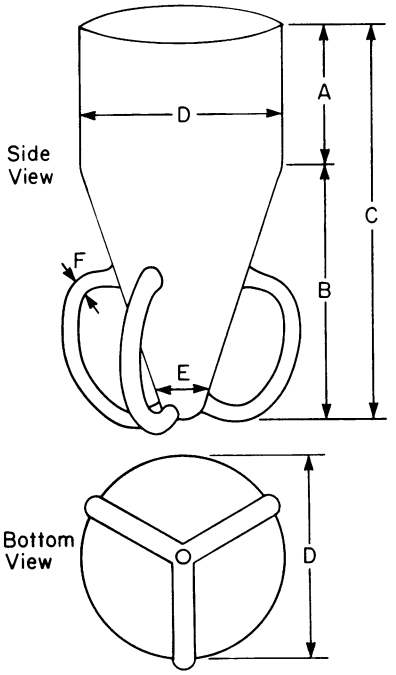

FIG. 1. *Cooling cell. Outside dimensions (in mm): A, 40; B, 140; C, 180; D, 70; E, 15; F, 8.*

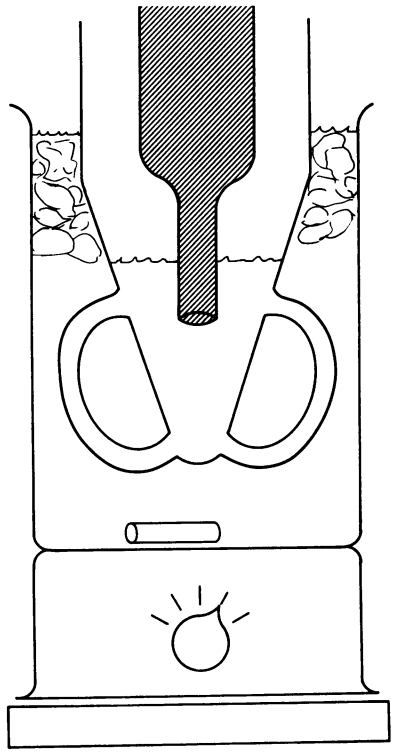

FIG. 2. *Position of cooling cell in use.*

A suspension of 10% (w/v) fresh yeast (Fleischmans Yeast, Standard Brands, Inc., New York, N.Y.) in distilled water was prepared, and 50-ml portions were sonically treated for 5, 10, 15, 20, and 25 min with an outside bath temperature of 0 C. The yeast suspension was prechilled; otherwise, the conditions were the same as previously noted. Suspensions were centrifuged at 25,000 × *g* in a refrigerated centrifuge. The material floating on top was aspirated off, and the supernatant fluids were decanted and centrifuged at 31,000 × *g* for 20 min.

Soluble protein was measured by the method of Warburg and Christian (1941). Alcohol dehydrogenase activity in the supernatant fluids was measured by a modification of the method of Racker (1950).

RESULTS

The temperature inside the cooling cell was within 10 C of that of the cooling bath within 2 min (Fig. 3). In 3 min, the internal temperature had reached equilibrium and remained within 9 C of that of the cooling bath. When the treated

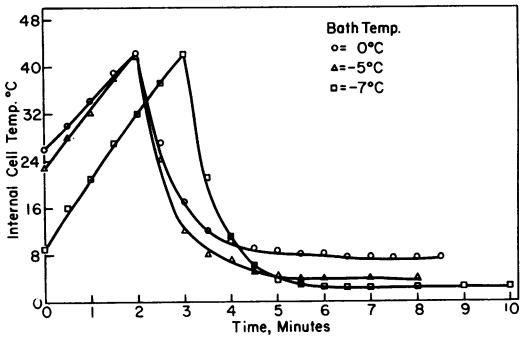

FIG. 3. *Internal cooling-cell temperature as a function of bath temperature.*

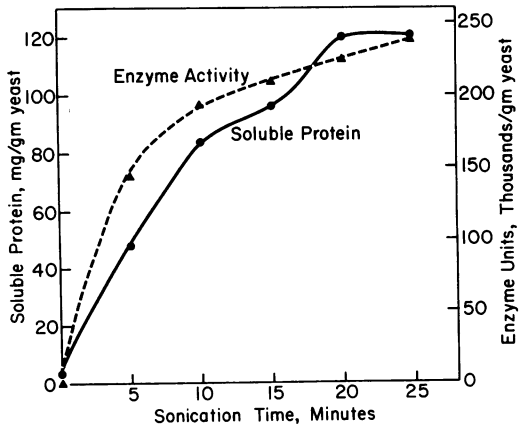

FIG. 4. *Soluble protein and alcohol dehydrogenase release from Saccharomyces cerevesiae.*

sample was precooled, the temperature never rose more than 9 C above the temperature of the cooling bath.

The yields of soluble protein and alcohol dehydrogenase from sonic disruption of *S. cerevisiae* are summarized in Fig. 4. One unit is defined as the amount of enzyme catalyzing an optical density change of 0.001 per min under the conditions of assay. Racker (1950) reported a yield of 275,000 units of enzyme per g of dried yeast, with a specific activity of 3,700. This corresponds to 75 mg of soluble protein per g of yeast (dry weight). Long (1961) reported that *S. cerevisiae* contains about 0.140 g of protein per g of yeast. In the present experiments, ultrasonic treatment of *S. cerevisiae* yielded more than four times as much enzyme and almost seven times the soluble protein obtained with other procedures.

Discussion

Use of the cooling cell in conjunction with high-intensity ultrasound results in adequate temperature control. Good yields of enzyme and soluble protein from relatively resistant organisms such as *S. cerevisiae* can be obtained in a relatively short period.

A discussion of possible mechanisms of cell disruption by ultrasound is available elsewhere (Hughes and Nyborg, 1962). In the present experiments, the yeast cells were all ruptured at the end of 10 min. Microscopic examination under phase contrast revealed many "ghosts" with holes in the cell wall, containing no visible cytoplasmic granules. The data suggest that, within the first 10 min, virtually all of the cytoplasm leaked out of the cells into the medium; however, some of the enzyme must still be bound to insoluble, sedimentable particles, as shown by the slow rise in the latter part of the enzyme-release curve. The double break in the curve for release of soluble protein has repeatedly been observed. It seems likely that the second phase reflects further fragmentation of the cell wall after the initial release of cytoplasm.

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