A METHOD FOR THE DETERMINATION OF THE THERMAL RESISTANCE OF BACTERIAL SPORES

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The heat resistance of bacterial spores at temperatures above 212 F was first studied systematically by Bigelow and Esty (1920), who introduced the procedure of heating suspensions in sealed tubes in an oil bath. Townsend, Esty, and Baselt (1938) have described a method using a specially designed can or sealed tubes heated in steam in a small retort. More complete details of the construction of the equipment are given by Pilcher (1947). An apparatus for determining spore destruction rates at temperatures higher than 212 F was described by Williams, Merrill, and Cameron (1937). Stumbo (1948) has recently described a technique for studying the resistance of spores in the higher temperature range (250 to 270 F).

In the course of investigations of high-temperature short-time processes a method was developed for the determination of the thermal resistance of bacterial spores or "thermal death time" at temperatures of 250 F or higher which possessed the following advantages: (1) more accurate measurement of temperature; (2) reduced lag correction with direct measurement of temperature lag in the product in each experimental run; and (3) the use of cotton-plugged tubes, permitting the addition of subculture media directly into the tube, thus eliminating the possibility of the loss of remaining viable spores during the transfer from sealed tubes.

The first two advantages were obtained by using a thermocouple system and recording potentiometer with the thermocouple introduced into a tube of the suspension during the run. The third advantage was obtained by the use of a pressure cooling system whereby the temperature of the tubes could be reduced from process temperature to 212 F under air pressure to prevent spattering of the liquid and blowing of the plugs.

The apparatus designed for this purpose has been called a miniature retort and has now been in satisfactory use for approximately two years, during which time some 1,500 retort runs have been made involving more than 15,000 tubes. It is the purpose of this paper to describe the construction of the miniature retort and the principles of its operation for the determination of the thermal resistance of bacterial spores.

Construction of miniature retort. The construction of the miniature retort will be described with reference to figure 1. The body of the retort is constructed of a pipe 6 inches in diameter and 12 inches high threaded and capped on the bottom end. To the upper end is attached a 2.5-inch flange with threaded bolts to pass through the 1-inch cover for use with wing nuts to hold the cover in place. In

addition to the bleeder valve, the cover is equipped with a standard safety valve shown in the diagram but not numbered.

Within the body of the retort is supported a pipe 4 inches in diameter with a capped bottom as shown in section *AA* of the diagram. The cooling water is introduced directly into this cylinder. The overflow outlet in the inner cylinder prevents the water level from covering and flooding the cotton-plugged tubes.

The retort is equipped with the following inlets and outlets: (1) incoming

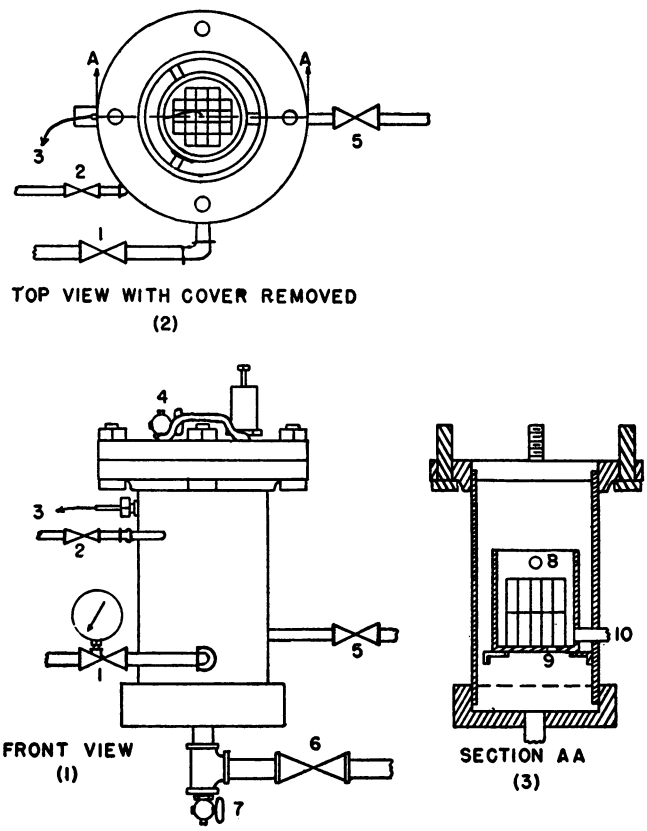

Figure 1. Diagrammatic representation of the miniature retort to show features of construction.

steam line and valve; (2) air-pressure line and valve; (3) thermocouple inlet; (4) bleeder valve; (5) incoming water line and valve; (6) water outlet and valve; and (7) bleeder valve.

Further reference will be made to these features of construction in the description of the operation of the retort. A 30-gallon galvanized tank serves as a ballast tank and is connected to the steam line through a type E. D. Spence valve. The ballast tank is connected to the miniature retort through a Mason-Neilan regulator and also through a by-pass around the regulator.

Temperature measurement. All temperature measurements are made by means

of copper-constantan thermocouples and a Brown recording potentiometer with a strip chart and pen. The temperature is measured by immersing the thermocouple in one tube of the inoculated liquid that is being run in the retort. Every retort run is recorded on the chart of the Brown recording potentiometer, using a chart speed of 1 linear inch per minute. The retort is operated and controlled manually upon the basis of the observation of the temperatures being traced by the pen line.

Thermocouples were calibrated against a Bureau of Standards thermometer using a type K potentiometer by immersing the thermocouple and the thermometer in the vapor of boiling toluene. When the latest temperature E.M.F. tables were used, the difference between the corrected Bureau of Standards thermometer reading and the thermocouple reading was of the order of 0.2 F. The constancy of response of the system was checked during several hours' continuous readings by the same system with the thermocouple attached to the Brown recording potentiometer. Later the boiling point of pure toluene corrected for atmospheric pressure was taken as a standard, and in some instances thermocouples were standardized by a procedure using the melting point of Bureau of Standards pure benzoic acid.

Operation of the miniature retort. By means of proper adjustment of the Spence valve, the ballast tank is set at a steam pressure corresponding to a temperature 4 F higher than is desired in the retort. The Mason-Neilan valve is adjusted to maintain the desired temperature in the retort. The top and bottom bleeder valves are left open, and with the by-pass open the entrance valve to the retort is opened. When the temperature reaches 212 F, the top bleeder valve is closed, the bottom bleeder valve being left open at all times. When the desired temperature is reached as indicated by the recorder, the by-pass valve is closed.

At the end of a run the steam valve is closed and simultaneously the air-pressure valve is opened to maintain a constant pressure. The cooling-water valve is opened and the air-pressure valve manipulated to maintain a constant pressure until a temperature of 212 F is reached. Cooling is very rapid, the temperature within the tube falling from 250 F or 260 F to 212 F within 0.1 minute. After a temperature of 212 F is reached, the air pressure is released and the bottom water outflow valve is opened.

Thermal death time determination with the miniature retort. All determinations are made in 13-by-100-mm pyrex tubes. The tubes are cotton-plugged and sterilized. Each tube receives 0.1 ml of a water or buffer suspension containing the desired number of spores, followed by 1 ml of the medium in which heating is to take place, such as phosphate buffer, brine, or puree. This procedure assures the presence of an equal number of spores in each tube and eliminates the difficulty of attempting to obtain a uniform dispersion of the spores through a viscous product. For dispensing the spore suspensions, 1-ml pipettes graduated in hundredths are used. The tubes are placed in the retort, and with the thermocouple in one of the tubes of the suspending medium the retort is operated in the manner previously described. A stop watch is started when the thermocouple indicates that the desired temperature is reached, and the retort is turned off

when the stop watch indicates the desired time of process. The actual process time is finally checked by measuring the recorded line on the chart with a rule graduated in tenths of an inch. The come-up time between 212 F and 250 to 260 F is short. With buffer or other thin liquids it is of the order of 0.6 to 0.8 minutes and with more viscous products such as puree 1.0 to 1.1 minutes. From the recorded temperatures on the chart a correction for the lethality of the come-up time may be calculated. Using the graphical method of Bigelow *et al.* (1920) and assuming $Z = 18$ F, the correction at 250 F was usually 0.2 to 0.4 minutes, and at 260 F, 0.1 to 0.2 minutes. These times are added to the time at maximum temperature in the retort to give the total process time. The proper correction value for each product and temperature can be calculated from the temperature records of each experiment.

Following thermal treatment in the retort the tubes are subcultured by the pouring of media directly into the tube. This operation is conducted in the plating room with proper aseptic precautions, and no significant contamination has been experienced. For thermophilic flat sour organisms 1 per cent tryptone, 0.5 per cent glucose broth is used with incubation at 130 F for 5 to 7 days. For putrefactive anaerobes a glucose tryptone yeast extract semisolid medium is used with incubation at 98 F for 21 to 30 days.

DISCUSSION

The method described has been used for the most part in the temperature range 240 to 260 F. An upper limit of the present equipment is established at 268 F by the characteristics of the Mason-Neilan valve in use and the slower come-up time at this temperature due to the use of one-half-inch pipe in the by-pass. If the control valve is changed and the diameter of the piping increased, satisfactory operation should be obtained at temperatures somewhat above 270 F. However, the upper limit of operation may be expected to occur somewhere in the temperature region between 270 F and 280 F at the point at which the calculated lethality of the come-up time will exceed the lethality of the process to be used.

The present procedure introduces two new principles into the technique of thermal death time determinations, the use of a thermocouple for recording the temperature of each run in such a manner as to provide accurate correction for the lethality of the come-up in each experiment, and the use of cotton-plugged tubes, which allow direct subculture by the addition of media to the tube. Although the method has been developed through the construction of a new piece of equipment, it would seem entirely possible to modify the equipment described by Pilcher (1947) to introduce thermocouple measurements and pressure cooling and provide a multiple retort unit based on these principles. Experiments are now being conducted in this direction.

This equipment and technique were designed in the hope that skips might be eliminated and end points considerably sharpened. To a certain extent the objective was accomplished by this procedure, except at time intervals very close to the end point, when results still were ambiguous at times. A combination

of the results obtained with the technique described and a new method of interpretation of the data has produced highly satisfactory results concerning the thermal resistance of bacterial spores, which will be described in subsequent communications.

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SUMMARY

A method for the determination of the thermal death time of bacterial spores has been described. This method introduces the new principles of the use of thermocouples to provide an accurate correction of lethality of the come-up time in each experiment and the use of cotton-plugged tubes cooled under pressure to allow subculture by direct addition of the medium to the heated spore suspension.

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