Time Temperature Studies of Spore Penetration Through an Electric Air Sterilizer

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Experiments which include the liberation of highly infectious aerosols in an exposure cabinet require equipment for sterilization of air from the exposure cabinet prior to discharge to the outside. For such sterilization, some institutions use a gas-fired incinerator or a centrally located oil-fired incinerator. Frequently neither is applicable because of the limited capacity of the former and the prohibitive installation and maintenance expense of the latter.

A review of biological literature revealed little data on the rate of sterilization of air as a function of temperature. Bourdillon, Lidwell, and Raymond (1948) tested two types of furnaces: 1) a furnace with internal heating, and 2) an externally heated furnace. The temperature needed for complete sterilization was found to be between 210 and 240°C when the exposure times were 6 to 8 seconds for the internally heated furnace and 0.4 to 0.6 seconds for the externally heated furnace. Although the exposure time in the internally heated furnace was nearly 10 times that in the externally heated furnace, the temperature required for sterilization was the same. No explanation was given for this variation. The authors concluded that it was desirable to aim at a possible air exit temperature of 300°C (572°F) when complete freedom from spore-bearing organisms was essential, except that in a well-designed furnace 250°C (482°F) might be fully adequate. In view of the absence of reliable experimental data, tests were undertaken to determine the time temperature rate required for sterilization of Bacillus globigii spores suspended in an air stream.

MATERIALS AND METHODS

The electric grid air sterilizer is a cylindrical unit approximately 3 feet long and 2 feet in diameter. It will sterilize air at a rated capacity not to exceed 75 cfm. The sectional view of the sterilizer in figure 1 shows the position of the heating element, the baffle, and the glass wool insulation.

The ductwork and additional apparatus for testing the sterilizer is illustrated in figure 2. The test organism was atomized into the air-mixing chamber by means of an all-glass direct spray, peripheral air jet atomizer (Chicago type). Relative humidity data for the air passing through the mixing chamber were obtained by a Brown recording psychrometer. The air passed from the mixing chamber into a 6-inch diameter duct, 6 feet in length. The flow rate of air passing through the system was measured 42 inches from the Trent with a Hastings Precision Air Meter. The air containing the test organisms then passed through the Trent sterilizer into a 9-inch well-insulated retention duct, 9 feet in length. Brown thermocouples, inserted at 12, 31, 50, 69, 89, and 108 inches from the Trent outlet, recorded automatically the temperature gradient along the duct by means of a Brown recording potentiometer. The air then was drawn through an additional 100 inches of insulated duct to a blower and thence to the outside air. The rate of air flow was controlled by a sliding damper located in the nebulization chamber.

The temperature control system was installed by a commercial electrical service organization experienced with this type of sensitive instrumentation; proper


installation is extremely important. The primary functions of this instrument were to: 1) place the exhaust fan in operation automatically once the temperature of the air reached the minimum safe limit, 2) maintain a determined temperature within safe and economical limits by controlling power input to the Trent and 3) provide a continuous exhaust.

Air samples were collected in three cross-sectional sampling planes (fig. 3). The sampling tubes installed in the system are shown in figure 4. The tubes were so designed that they could be traversed across the horizontal diameter of the duct. The purpose of selecting various sampling points across the horizontal diameter was to assure satisfactory sampling as well as to determine whether the system was properly baffled to provide kill of organisms across the entire duct diameter. Five positions were chosen, which were etched on the periphery of the sampling tube. Initial tests demonstrated uniform distribution of the test organisms across the duct diameter.

Air samples were collected with liquid impingers to determine cloud concentrations before the Trent sterilizer. The liquid impingers were constructed with a critical orifice through which air could be drawn into the collecting medium at approximately 0.5 cfm. The medium consisted of 20 ml of nutrient broth, and 6 to 8 drops of olive oil. For sample evaluation, one-tenth ml of exposed fluid was pipetted on a nutrient agar plate which then was streaked. One-tenth-ml samples of 1-ml serial dilutions of the impinger fluid also were streaked on agar plates.

Air to be sampled after the Trent sterilizer was collected by means of an inverted funnel and sieve sampler mounted over dry ice as illustrated in figure 5. The bacteria in the air were impinged on the agar Petri plate which was placed in the sieve sampler.
Two related factors were investigated: 1) the rate of air flow through the sterilizer and retention duct and 2) the effect of varying temperature. Four rates of flow were used: 10, 25, 50 and 75 cfm, and five temperatures were studied averaging 425, 475, 525, 575 and 625 F.

A test pattern was adopted that would have statistical significance. Day, time of day, flow rate, and sampler position (at a given temperature) were combined for the purposes of balancing the effects of unknown factors. Four days of testing were required for a complete study at each temperature setting.

During each test, air samples were collected before the sterilizer and at three stations along the retention tube. Air samples also were taken to determine room air contamination. A Brown potentiometer continuously recorded temperature every 45 seconds at 6 points along the retention tube.

The general procedure followed for all tests was identical. Prior to each test series the controlling thermostat was set and adjusted to control air temperatures within the limits desired. Six series of tests were run at control temperatures as follows: two each at 600 to 650 F, one each at 550 to 600 F, 500 to 550 F, 450 to 500 F and 400 to 450 F. Air flow was varied four times during each test (10, 25, 50, and 75 cfm). In addition to a control sample, five air samples were taken at each air flow. The post-Trent samples were taken in one of the five positions of the sampling tube illustrated in figure 4.

Each day's test was conducted in the same manner. The Trent sterilizer control system was placed into operation. All recording instruments were properly calibrated and the air flow was dampered to the desired flow rate.

After the system reached equilibrium (15 minutes), a control test was made. A suspension of Bacillus globigii spores which had been heat shocked at 80 C for 10 minutes and cooled rapidly was then introduced into the nebulization chamber, using a Chicago-type nebulizer. Five-minute air samples were taken previous to and after the Trent as previously described. This procedure was followed during each air flow test.

RESULTS

Figure 6 shows graphically the per cent penetration at sampling stations 1, 2, and 3 at average temperatures of 425 to 625 F for air flows of 10 and 25 cfm. No significant penetration occurred 9 feet from the Trent outlet at these air flows above the control temperature setting of 475 F. At temperatures above this setting, the recovery was spotty and followed no definite trend. The presence of the test organism at these temperatures may be the result of air contamination during plating, as the test organism is normally found in air. The average penetration (0.00002 per cent) recorded represents a recovery of only 1 organism from 1 of 20 5-cubic-foot air samples. At a controlled temperature averaging from 450 to 500 F at the 10 cfm flow an average penetration of 0.00012 per cent occurred at station 1 and 0.00005 per cent at station 2. For this flow, the retention time varied from 3.1 seconds (sampling station 1) to 23.8 seconds (sampling station 3).

Significant penetration occurred at average control temperatures of 425 F at an air flow of 25 cfm.
penetration at station 1, 2, and 3 was 0.00365, 0.00479, and 0.00085 per cent. Little significance can be attached to the recovery of the test organism at average constant temperature settings above 475 F. The penetration recorded again represents the recovery of only one organism.

Figure 7 shows that if the air flow is increased to 50 cfm, significant penetration occurs at 475 F. Retention times corresponding to stations 1, 2, and 3 were 0.6, 2.3 and 4.8 seconds. At control settings of 475 F penetrations averaged approximately 0.02 per cent at station 1 and 0.001 per cent at station 2. When the control temperature was decreased 50 F, an appreciable increase in penetration occurred.

This figure also illustrates the penetration that occurs at air flows of 75 cfm. The retention times at stations 1, 2, and 3 were 0.4, 1.5 and 3.2 seconds. It was statistically concluded that at an average control temperature of 575 F, 0.0001 per cent or less penetration would occur in approximately 3 seconds. Test results show, however, that if a minimum retention time of 3 seconds is maintained at an average control temperature of 625 F, a greater factor of safety is produced.

These two figures show that minimum penetration occurs when the control temperature and retention time are increased. Maximum penetration occurs when retention time and control temperature are decreased.

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SUMMARY
The results of this investigation indicate that virtual sterilization, 99.9999 per cent or more corresponding to 0.0001 per cent or less penetration of Bacillus globigii spores, may be achieved at the following conditions:

1) 425 F — 24 seconds retention time
2) 475 F — 10 seconds retention time
3) 525 F — 5 seconds retention time
4) 575 F — 3 seconds retention time

An even greater degree of safety can be obtained if the air is maintained at 625 F for 3 seconds. Knowing that 425 F and 24 seconds retention give the desired sterilization, an approximate rule that may be used in determining the desired temperature and retention time combination is that a 50-F increase in temperature is approximately as effective as tripling the retention time.

REFERENCE