An Electric Incinerator for Sterilization of Small Volumes of Air

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The use of microbial aerosols in the study of infectious diseases requires safe disposal of air exhausted from the aerosol chamber or from the apparatus employed for respiratory challenge of animals. This also applies to small fermentors used for mass cultivation of infectious microorganisms, and to other similar devices requiring a continuous airflow. An ultraviolet air sterilizer (Miller *et al.*, 1955) has proved effective, but it has the disadvantage that the UV lamps must be cleaned and tested frequently.

Our experience with small electric incinerators has been that those made of glass are easily broken, and that, if resistance wires are used as heating elements, they are prone to burn out. The electric air sterilizer described in this communication eliminates most of these disadvantages. Its sturdy, simple construction should assure years of service without maintenance.

Materials and Methods

Apparatus. Details of the incinerator and the testing apparatus are shown in figures 1 and 2. Figure 1 shows the incinerator to be essentially a resistance type heating coil which also serves as a channel for passage of air. The coil is formed from a single 10 foot length of type 347 stainless steel tubing $\frac{3}{8}$ of an in. in diameter, with a wall thickness of $\frac{1}{32}$ of an in. The ends of this coil are connected across the secondary of a 750 watt transformer, which is wound to develop a current of about 80 amperes at 9 volts through the coil. The coil is enclosed in an insulating jacket and a thermocouple pyrometer indicates the temperature of the exhaust air.

The apparatus is designed to sterilize air at a flow rate of 1 cubic foot per min (cfm). When the entering air is at an ambient temperature of 75 F, a maximum exhaust temperature of 900 F is reached after a warm up period of 20 min. The apparatus operates from a 115 volt AC line.

Experimental methods. Spore suspensions of *Bacillus subtilis* var. *niger* were used to determine the sterilizing efficiency of the incinerator at varying temperatures with an air flow of 1 cfm entering the incinerator. The organism was grown in a casein partial hydrolyzate medium and was heat shocked at 60 C for 80 min. The heat shocked stock suspension was diluted 1:10 with

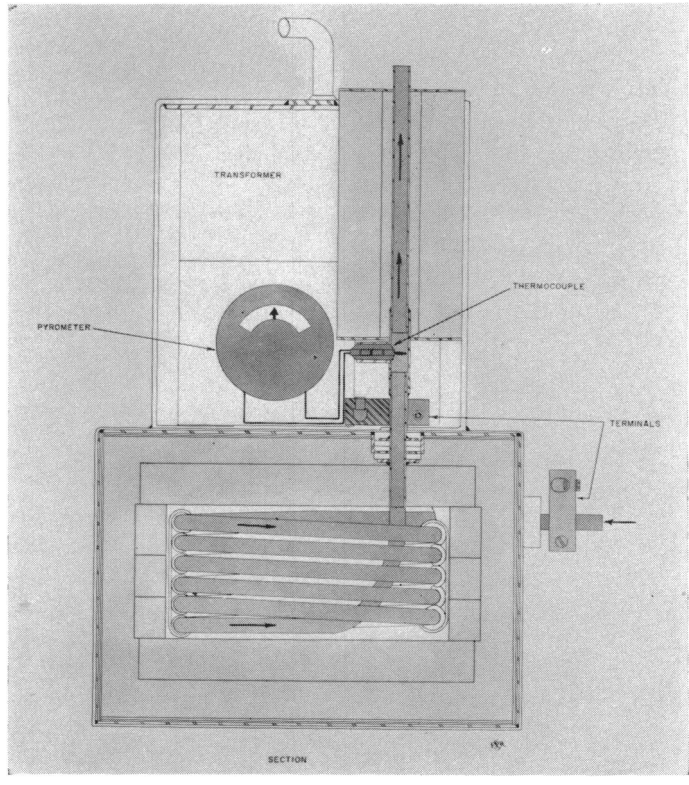

Figure 1. Cross sectional drawing of 1 cfm air incinerator

distilled water before use. For each test, the temperature of the incinerator was regulated by means of a variable transformer, and the air flow was adjusted to 1 cfm. Bacterial aerosols were generated with a Chicago type glass nebulizer (Rosebury, 1947) and passed through the sterilizer. Air entering the device was sampled by means of liquid impingers containing 20 ml Bacto tryptose broth with two drops of olive oil added to counteract foaming. These samples were diluted in distilled water and streaked onto duplicate plates of corn steep agar.[1] The exit air was cooled by passing the exhaust air line through a water bath. The air was then passed through membrane filters[2] (Type AA) to test for sterility. The filters were placed on corn steep agar plates and all plates were incubated at 37 C for 48 hr. A total of 90 tests was conducted, representing a combined sampling time of 900 min during which aerosols were continually passed through the apparatus.

[1] Black strap molasses, 10 g; treated corn steep liquor, 30 g; agar, 20 g; distilled water, 1000 ml; adjust pH to 7.

Results

The results of these experiments are summarized in table 1. These data show that air containing aerosol concentrations of 1×10^8 spores per cubic foot was sterilized when the temperature at the thermocouple was 400 F or above and when the air flow entering the sterilizer was adjusted to 1 cfm. This is the limiting temperature for sterilization of the spore concentrations used. Below this temperature sterilization was not obtained.

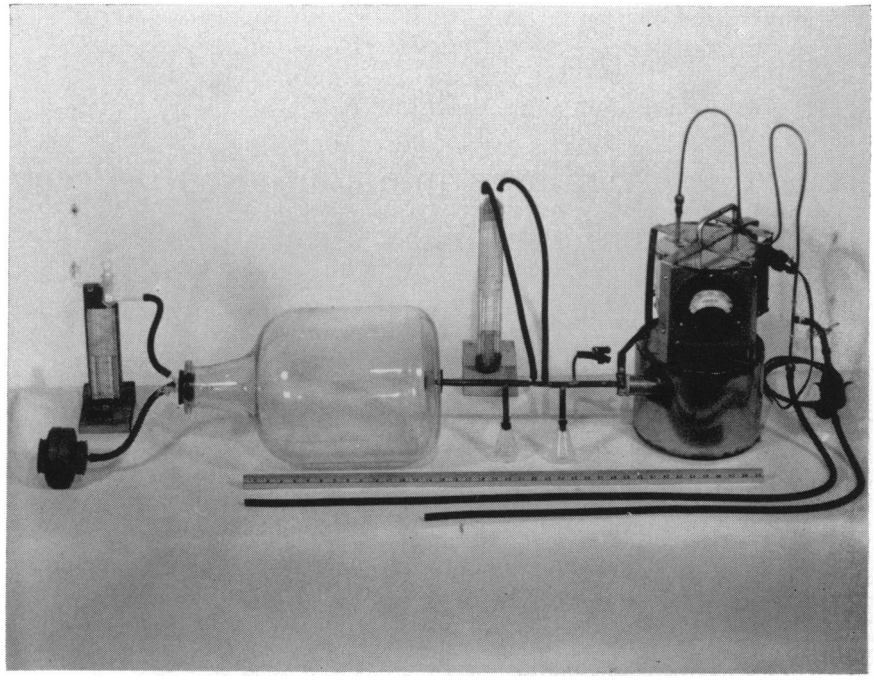

Figure 2. Air incinerator and aerosol chamber used for testing

TABLE 1

Sterilization of air containing spores of Bacillus subtilis

Temperature	Total Min of Sampling Time	Total Organisms Per Cu Ft Recovered From Exit Air
F		
800	100	0
750	100	0
700	100	0
650	60	0
600	60	0
550	60	0
500	60	0
450	60	0
400	60	0
350	180	0.44
300	60	TNTC

Concentration of spores per cu feet of air entering incinerator = 1×10^8. Air flow entering incinerator = 1 cfm. TNTC = Too numerous to count.

Readings taken from various points along the coil showed that the temperature of the air varies considerably as it proceeds through the coil, and that the peak temperature is greater than that indicated by the pyrometer. Although a detailed study of this relationship was not made, it is clear that the pyrometer reading is not a true index of the temperatures to which the aerosol is exposed during its passage through the coil.

Discussion

The deliberate production of infectious microbial aerosols is one of the most hazardous laboratory operations. However, many basic factors concerning infectivity, aerosol stability, and decay cannot be determined in any other way. Infectious aerosols may also

[2] Millipore Filter Corp., Watertown, Massachusetts.

result from other techniques, such as those requiring aeration of cultures. The exhaust air from such operations must be sterilized and the most reliable means of doing this is incineration. The 1 cfm capacity of the incinerator described above is sufficient for most laboratory scale operations.

If provision is made for cooling the exit air, the apparatus may be employed as a source of sterile air which is sometimes needed for certain laboratory procedures.

Although the minimum sterilizing temperature was 400 F, the incinerator is routinely operated at 500 F or above. This stipulation allows for variations which may occur in the air flow and provides a safety factor in case of power failure during operation. This operating temperature will also permit a reduction in the size of the transformer used on future models.

SUMMARY

A small-volume air incinerator is described which is capable of sterilizing air at flow rates up to 1 cfm. The apparatus is a resistance-type heater utilizing a 750 watt transformer and a coil of stainless steel tubing as the resistance and air channel. The incinerator can be used for sterilizing air that is contaminated by some laboratory techniques or may be used to produce sterile air for various laboratory procedures.

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Chemically Defined Media Supplemented with Precursors for Synnematin Production

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Synnematin has been shown to have many of the attributes of a valuable antibiotic. It has an acute toxicity of only one-seventh as much as penicillin (Hwang and Primack, 1956–7). It is effective *in vivo* against a variety of experimental infections (Hobby *et al.*, 1957; Olson and Jennings, 1954; and Rickher *et al.*, 1957) and is also effective clinically (Benavides *et al.*, 1955). Abraham *et al.* (1955) found cephalosporin N to be the same as synnematin B. Therefore, the characteristics of cephalosporin N (Newton and Abraham, 1954) apply to synnematin also. The reasons for the present lack of commercial availability are most closely related to cost and difficulty of isolation and purification. Published data (Olson *et al.*, 1954) show that the only medium which would allow synnematin production of 400 units per ml or more is one which contains 4 per cent corn meal, 4 per cent soybean meal, 1 per cent calcium carbonate, 0.1 per cent ammonium sulfate, and 0.1 per cent antifoam (lard oil with 3 per cent octadecanol). This medium becomes so heavy with growth that some industrial fermentors are not capable of providing sufficient aeration. Also, the harvested filtrate obtained with this medium contains a high percentage of impurities, thus adding to the difficulty of recovering synnematin. To obviate some of these difficulties a study was initiated to develop media with the following characteristics: (a) Sufficient chemical definition to allow the evaluation of substances for stimulation of synnematin production and selection of substances which might be precursors of the synnematin molecule; (b) less viscous, so that the less efficient industrial fermentors could be used; and (c) lower percentage of solids in the harvested filtrate with high antibiotic potency in order to facilitate recovery.

MATERIALS AND METHODS

Culture. Cephalosporium salmosynnematum MDH 3590AUV48B3 was used in all experiments. The genus of this culture has now been changed by Grosklags and Swift (1957) to *Emericellopsis*. The culture was maintained on Czapek-Dox agar slants and transferred every 30 days.

Seed production. A portion of mycelium approximately 50 sq mm removed from an agar slant was used to start the seed flask. The seed flasks were grown 2 days in a natural medium which consisted of 2 per cent soybean meal, 2 per cent corn meal, 0.5 per cent calcium carbonate, and 0.1 per cent antifoam (lard oil plus 3 per cent octadecanol) (Olson *et al.*, 1954). After 2 days of incubation, 0.5 ml was used to seed 50 ml of the medium B of Pisano *et al.* (1954) and allowed to grow 24 hr before use. All seed flasks were shaken at 30 C.

Fermentation. All natural media used were autoclaved