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Production of Low Concentration Particulate Aerosols By A Sonic Disseminator Technique

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December 1966

ABSTRACT

This report describes a technique by which an ultra-sonic vibrator may be used to produce particulate aerosols with concentration levels below 5000 particles per cubic foot in an enclosed volume with good repeatability.

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Introduction and Purpose

With the advent of planetary quarantine and spacecraft sterilization programs, there has arisen a need for a mechanism which can be used to produce a stable, low concentration, dry bacterial spore aerosol. This aerosol is needed so that the settling properties of these fine particles may be studied and so that techniques for removing these viable particles from various classes of surfaces may be developed. Several types of devices have previously been available that are capable of producing predictable aerosols of medium to heavy concentrations. However, to study settling-out on surfaces which should yield a very low contamination level, as in preparation for a sterile space flight situation, some means had to be devised which would produce bacterial aerosols of concentrations below 5000 particles per cubic foot within an enclosed volume.

The purpose of this report is to describe one mechanism which has been developed to perform this low concentration, viable particle dissemination service.

Background

Dry *Bacillus subtilis* var. *niger* spores have been used extensively in tests and experiments performed to study and determine the aerosol properties of this type of bacteria and to facilitate the development of devices which can be used for bacterial spore discovery and assay. Due to the small size and mass of these bacteria, on the order of 10^{11} to 10^{12} bacteria per gram may be obtained in dry bulk quantities. Therefore, the problem of placing approximately 2000 bacteria in a cubic foot of aerosol can be very difficult if the total volume of the enclosed area which is to be contaminated is less than one thousand cubic feet. For example, a room with a total volume of 1000 cubic feet would only require 2×10^6 spores to achieve the loading of 2000 spores per cubic foot. If the sample from which the spores were taken contained 2×10^{11} viable spores per gram, the total weight of the aerosolized material would only be 10^{-5} grams. The difficulty in repeatably aerosolizing this small amount of material can readily be appreciated when one considers doing this a number of times to obtain approximately the same aerosol concentration for a series of tests. Another very trying difficulty is breaking up this small amount of material into individual viable particles. The following pages describe a basic method of using energy in the near ultra-sonic range to accomplish the tasks mentioned and to overcome, to a large extent, the difficulties which are encountered.

Description of a Sonic Disseminator

Energy in the near ultra-sonic spectrum was found to be a very useful tool to solve the problem of producing low concentration aerosols repeatably. This energy not only tends to separate particles from a surface, but also it breaks up groups of particles into individual particles with a reputable efficiency. Figure 1 shows the basic set-up which has been used. The Branson Model LS-75 Sonifer is the device used to deliver the sonic energy. To obtain the most accurate and repeatable results, a piece of sterile aluminum foil with dimensions of 1 1/2" x 3/4" can be weighed, and then a calculated weight of the desired aerosol contaminant can be transferred to the aluminum foil strip using a sterile cotton swab and a good set of microbalance scales. The approximate weight of material to be added to the strip may be determined by multiplying the total volume of the already clean area which is to be contaminated by the desired level of contamination per cubic foot. This number of spores or particles obtained in this manner usually must then be multiplied by a constant k to obtain the more exact material weight which is necessary. The constant k takes into account primarily particle loss and, to some small extent, particle clumping. It must be determined separately for each different experimental environment and each set of contributing factors.

The piece of aluminum foil which is prepared in this manner is then taped to the tip of the Branson Sonifer using a piece of two-sided adhesive tape. When loading the foil strip, a 3/4" x 3/4" section is used for material deposition so that another 1/2" x 3/4" section is left for handling the strip and for fastening it to the tip of the Sonifer. With the strip thus loaded and attached, the power to the mixing fan and the Sonifer may be turned on in sequence and left on for some pre-determined length of time. In all cases when loading a 385 cubic foot clean room, a period of 20 seconds with a power setting of three on the Branson Sonifer was used to clear the particles from the aluminum foil strip. A total running time of 80 seconds is allowed for the mixing fan in this size room. This is sifficient to produce a very homogeneously distributed aerosol. The very rapid vibration of the Sonifer probe tip imparts a sufficient amount of energy into the aluminum foil strip to liberate the particles into the surrounding air.

Since the frequency of vibration of the foil strip may approach 20 kHz, the majority of the particles are removed from any clump formations and are liberated in an individual form. However, a small percentage of dry spores may escape in clumps which may vary in size from 5 to 25 microns in the mean diameter and which may contain from 2 to 100 individual spores. To provide further assistance in breaking up these particle clumps and to insure against the aerosolization of the larger clumps, a cover of the form shown in Figure 2 may be fitted over the probe tip after the loading procedure is complete. This cover serves several purposes. First, the indirect path which the aerosolized particles must take to escape the confines of the cover guards against large particles being ejected from the loaded strip into the air immediately upon turning on the sonifer power. Secondly, the confines of the cover provide a form of resonant vibration chamber which further tends to break up clumps when the probe power is turned on. Finally, the stream of clean nitrogen entering the cover evenly mixes the aerosolized contamination and liberates the mixture into the desired volume.

In many cases the extremely close regulation of the procedures described above may not be needed or desired. When this situation occurs, a much easier and simpler procedure is available. This procedure consists of only using the Sonifer probe tip for the loading mechanism. The probe tip itself is loaded with the contaminant material by brushing on a very thin layer with a cotton swab. With a small amount of experimentation, the relationship between the aerosol concentration and the area of the probe tip which must be coated to obtain the given concentration can be determined. The hood and nitrogen line then may or may not be used, depending only on the criticality of removing some larger particles from the aerosol. One disadvantage of the hood is that it contributes to particle loss to some extent.

Experimental Results

All of the work done in testing and using the Sonic Disseminator at Sandia Laboratory has been done in a class 100 laminar flow clean room facility. This facility is reasonably air tight so that it can first be cleaned by operating the room until the room air is free of airborne particles.

Then the room is turned off and is contaminated with the desired particulate aerosol using the Sonic Disseminator.

An example of using the Sonic Disseminator by weighing out a quantity of bacterial spores follows. A quantity of 20×10^{-6} grams of dry Bacillus subtilis var. niger spores was obtained from a bulk quantity which contained 5.4×10^{11} viable spores per gram. The 20×10^{-6} gram quantity was weighed using a Mettler Microbalance and was deposited on the aluminum foil strip as described. The loaded strip was attached to the Disseminator assembly while the clean room was in operation. After completing the loading procedure, the clean room power was turned off, and the air in the room was allowed to become static. The Sonifer and mixing fan were then turned on for periods of 20 seconds and 80 seconds, respectively. With the room thus loaded, the aerosol was sampled at a rate of 300 cc/min. for a period of 30 minutes counting all particles 0.5 micron and larger in their mean diameter with a Royco Particle Counter. The average count for a one-minute sampling period was 56.1 particles. The extremes of the counts were 45 per minute and 65 per minute. Using the average count of 56.1 counts per minute at a rate of 300 cc per minute gives an average of 5600 particles per cubic foot. Multiplying this loading by the total room volume of 385 cubic feet gives a total loading of 2.16×10^{6} particles. This compares very well with the value obtained by multiplying the weight of the strip loading by the spore density in the bulk material. This operation gives 10.8×10^{6} total particles.

The stability of the aerosol cloud obtained using this type of Disseminator in a clean room of this sort is very good as can be seen by observing the tabulated data (listed below) for one particular loading.

Time Elapsed After Loading, Minutes	Royco Count for Particles ≥ 0.5 Microns With 1 Minute Cycle Time	Variation from The Mean Count of 32 Counts Per Minute
1	31	-1
3	33	+1
5	29	-3
7	34	+2
9	20	-12
11	38	+6
13	23	-9
15	44	+12
17	26	-6
19	28	-4
21	41	+9
23	33	+1
25	34	+2
27	30	-2
29	31	-1

The size distribution of the particles in the aerosol can also be obtained with the Royco Particle Counter. Using _Bacillus subtilis_ var. _niger_ spores with a median diameter of 1.9 microns and a quantity with 90.0% of the spores less than 5 microns in diameter, the following size distribution was obtained:

Particle Size	Number Counted During One Minute Royco Count at 300 cc/min.
.5	17
.6	24
.8	17
1.0	21
1.2	14
1.5	14
2.0	12
3.0	15
4.0	10
5.0	5
6.0	11
8.0	1
10.0	0

An idea of the evenness of loading of a particular aerosol may be obtained by using bacterial spores as the aerosol contaminant and by allowing these spores to settle on to glass petri dishes filled with nutrient agar. The colonies which grow in the agar represent the number of particles which have settled on the surface at some point in the room. A definite correlation then exists between the number of particles which impinge at any point and the average aerosol loading directly above the point considered. Figure 3 shows an example of this technique. The circles represent the 10 centimeter diameter petri dishes, and the number within the circle represents the number of spores which settled on the surface. For this particular experiment the dissemination procedure was as described and a 30 minute settling period was allowed before once again turning on the clean room air flow to clean the room air.

Using the technique described in the preceding paragraph a curve representing the number of particles which settle out of the aerosol on to agar filled Petri dishes versus the aerosol loading for a given settling period may be determined. A typical curve is shown in Figure 4. To obtain this curve dry Bacillus subtilis var. niger spores were aerosolized and allowed to settle for 30 minutes. For this particular set of conditions the curve could be fit very nicely by curve fitting methods to the algebraic equation:

$$y = k\,x^2$$

where y = colonies/cm^2 on petri dishes

x = particles/cm^3 in the aerosol

$k = 4.8 \times 10^{-3}$

This equation is then readily available to calculate the necessary aerosol loading for any desired surface loading. Finally, the approximate weight of material to be aerosolized can be calculated from the necessary aerosol loading. This is not meant to imply that all relationships between the airborne concentrations and the settled concentrations will be described by an equation of the form $y = k\,x^2$. This equation simply defined the situation for one set of environmental conditions and one settling period.

The repeatability with which a given concentration may be obtained on a surface for a given settling period can be seen by observing the following data. The situation which existed and which was the controlling factor for the following sets of data was that a very evenly spread distribution of Bacillus subtilis var. niger spores were needed on several 600 square centimeter aluminum surfaces with concentrations approximately the same after each loading sequence. Based on previous experimental data the correct approximate loading for the disseminator to give an average loading between 0.5 and 1.5 spores per square centimeter on agar filled petri dishes after a 30 minute settling period was determined. The aerosol and surface loadings which were obtained with these specifications are given below:

No. of Particles $\geq$ 0.5 Micron Per Cubic Foot of Aerosol Based on Royco Count	Average Number of Spores Per Square Centimeter of Petri Dish
Exp.#1 4660	1.416
Exp.#2 4139	0.908
Exp.#3 4003	0.973

Conclusion

For situations which require a study of some particulate aerosol with a concentration below 5000 particles per cubic foot, a sonic vibration technique may be very useful. The Sonic Disseminator is capable of performing this task for either viable or non-viable particulate aerosols and is capable of aerosolizing the contaminant particles in their individual states.

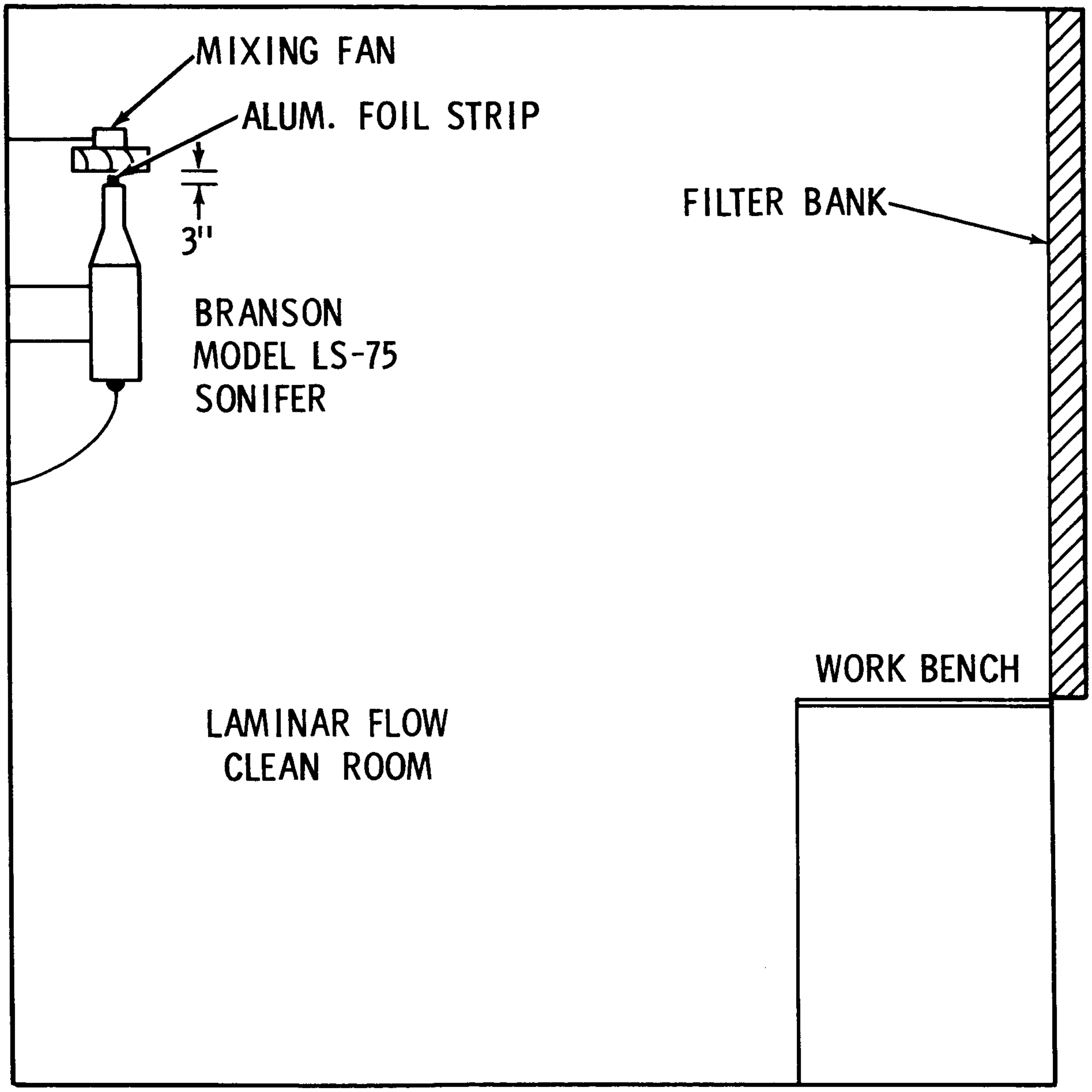

FIGURE 1 BASIC SONIC DISSEMINATOR OPERATION

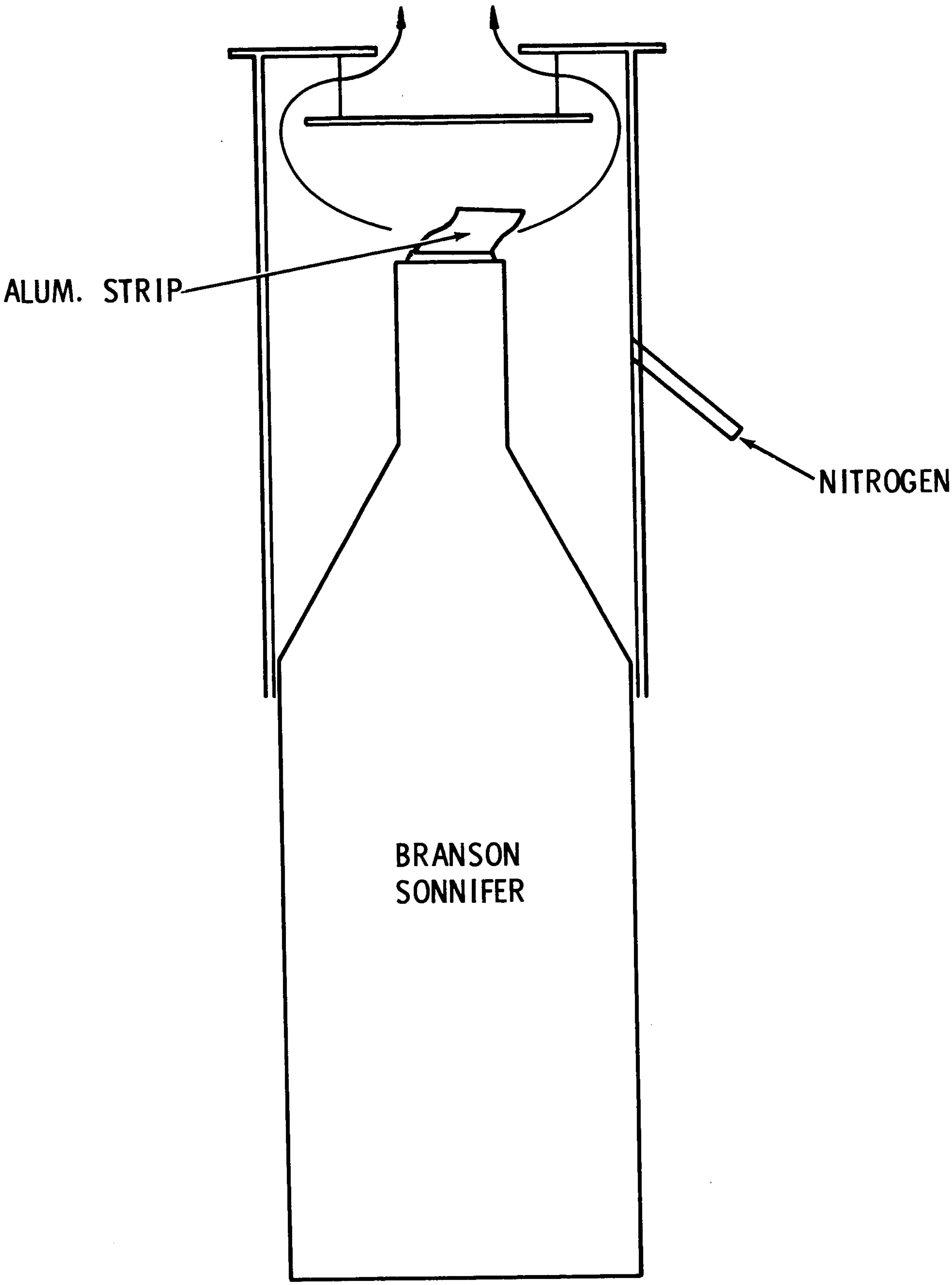

FIGURE 2 DISCRIPTION OF SONIC DISSEMINATOR WITH COVER

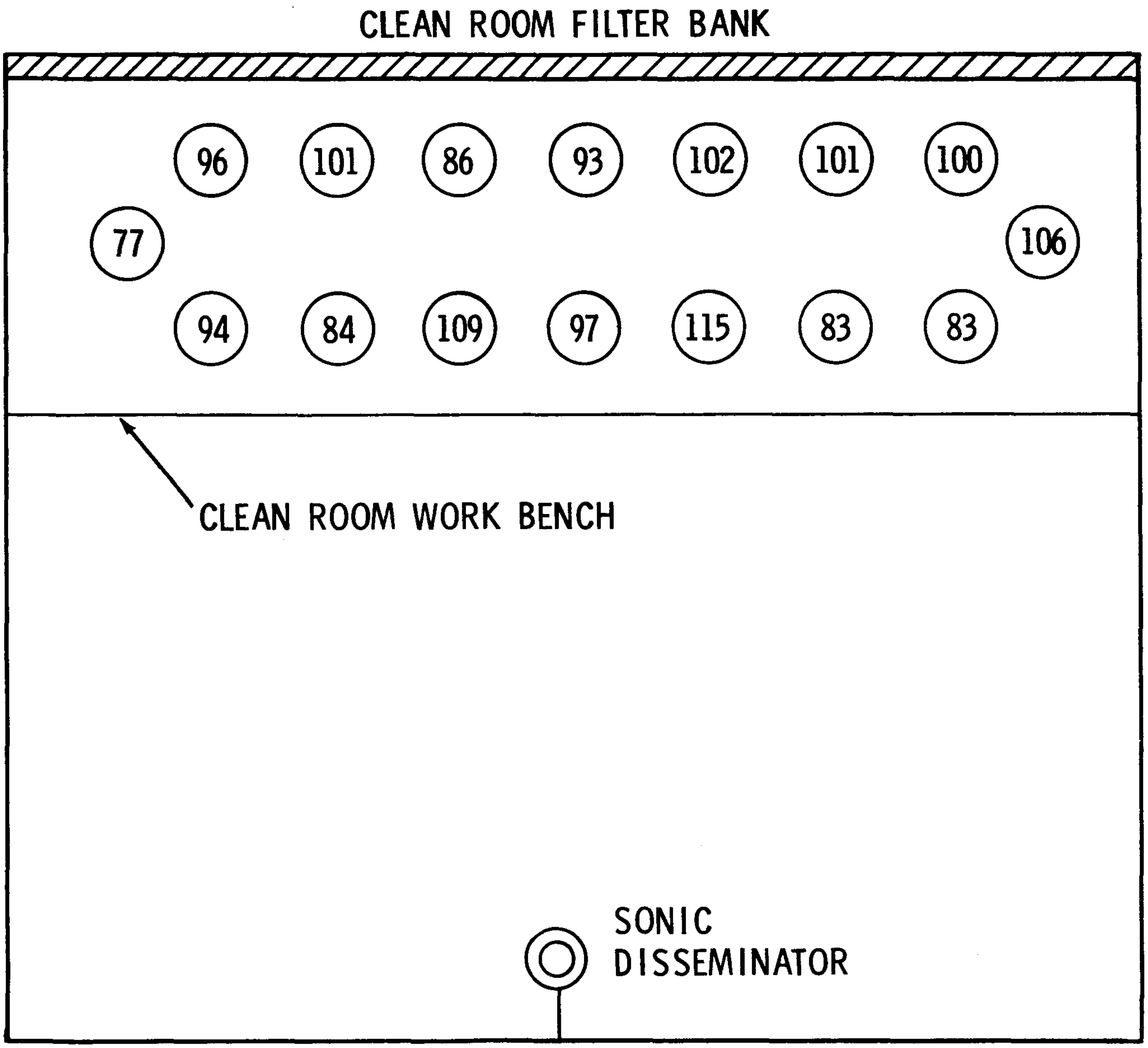

FIGURE 3 DISTRIBUTION OF SPORES WHICH HAVE SETTLED OUT OF THE AEROSOL

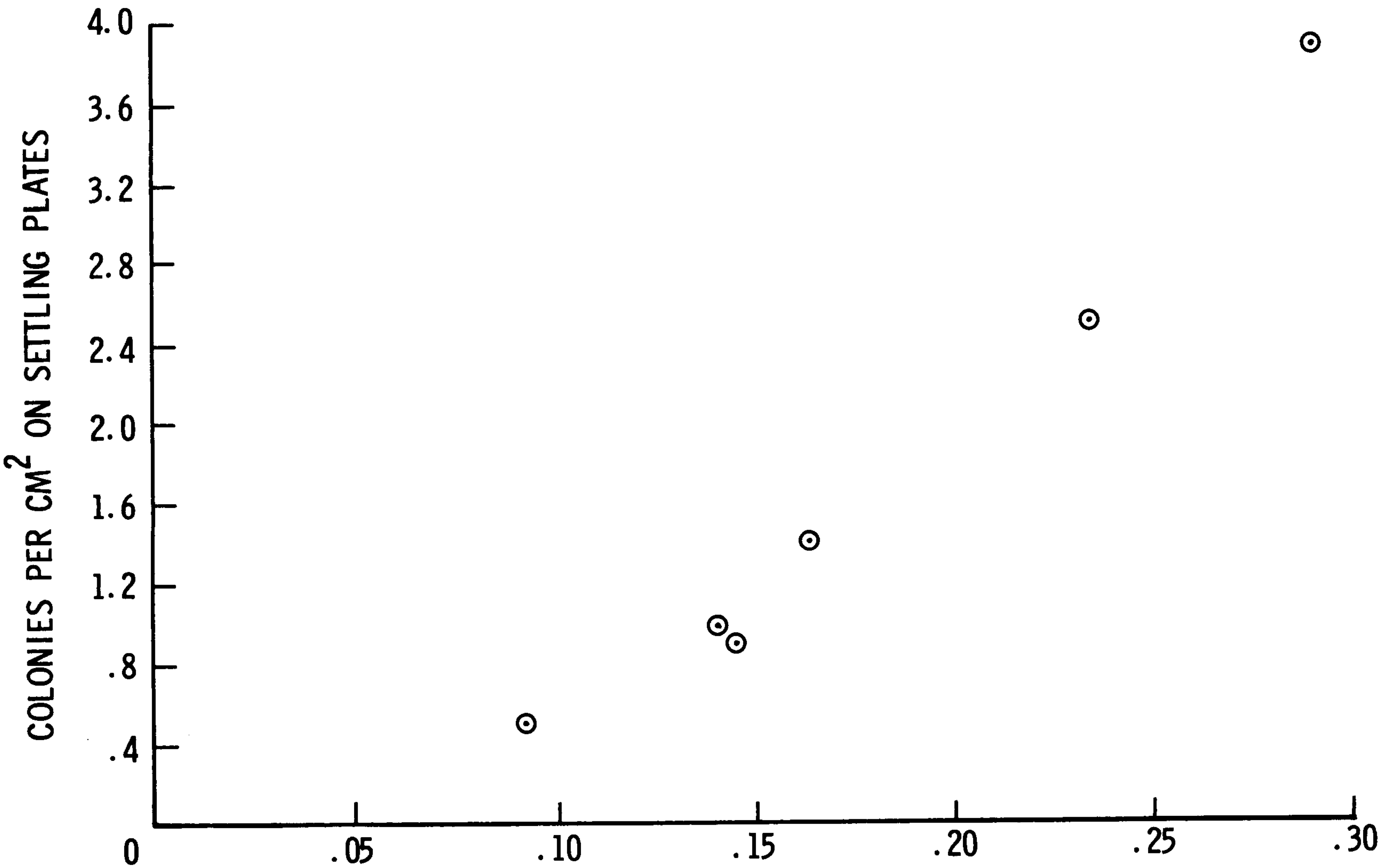

FIGURE 4 NUMBER OF PARTICLES PER CM^3 OF AEROSOL WITH MEAN DIAMETER $\geq$ 0.5 MICRONS

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