

An Aerobic-Bacteria Culture Apparatus Employing a New Foambreaker

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January 20, 1967



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ABSTRACT

An aerobic-bacteria culture apparatus incorporating a novel mechanical foambreaker has been developed. The culture vessel consists of a 30-in. length of glass pipe four inches in diameter oriented vertically and closed at each end with a stainless steel plate. The bottom plate holds air spargers, and the top is bored to admit an air outlet and, in the center, the foambreaker. The foambreaker consists of a vertical stainless steel tube closed at its lower end with a cap which leaves a one-mil slit between it and the bottom edge of the tube. When the tube is pressurized with compressed air, a high-velocity sheet of air emerges 360 degrees around the tube and effectively breaks foam rising from the aerated medium. The addition of chemical antifoaming agents is thus obviated. Relatively high yields of such bacteria as Bacillus megaterium, Escherichia coli and Serratia marcescens have been obtained.

PROBLEM STATUS

This is a final report on one phase of the problem. Work on other phases is continuing.

AUTHORIZATION

NRL Problem C05-21
Project RR 001-01-43-4757

Manuscript submitted September 23, 1966.

AN AEROBIC-BACTERIA CULTURE APPARATUS EMPLOYING A NEW FOAMBREAKER

INTRODUCTION

In order to provide bacteria in sufficient quantity for exploratory studies on the physicochemical characteristics of bacterial surfaces, we have found it necessary to develop a fairly routine method for growing aerobic bacteria in relatively large quantities, i.e., for several grams or more. Descriptions of such apparatus abound (1,2), but most of them have a common disadvantage: they require the addition of chemicals to control the copious foam usually resulting from the vigorous aeration rates used. Since chemical antifoams are necessarily surface-active agents, one might reasonably expect them to be adsorbed on microbial surfaces, under some conditions at least, and thus alter the character of the structure of primary interest and about which information is being sought. Furthermore, it has been noted by Edebo, et al. (3) that the addition of silicone antifoam to a culture of Bacillus megaterium strain M completely stopped reproduction. These workers also found that antifoam addition during the later stages of growth decreased yields of other organisms. Deindorfer and Geden (4) as well as Solomons and Perkin (5) have shown that the addition of relatively small amounts of chemical antifoams to a given medium severely inhibits oxygen transfer. In view of these findings, it is not unreasonable to expect that antifoams might alter normal biosynthesis of the cell wall and therefore result in an abnormal structure and/or chemical composition. The culture apparatus described in this report makes it unnecessary to resort to chemical antifoams.

DESIGN OF THE CULTURE APPARATUS

General Description

The culture apparatus (Fig. 1) was designed to contain a volume of 4.5 liters of medium. A standard Pyrex pipe 30 in. in length and four inches inside diameter was used as the culture vessel. Each end of the Pyrex pipe was sealed with specially fabricated stainless steel plates by means of flanges and bolts. A leakproof seal was provided by means of an "O" ring or gasket between each plate and the groove in the end of the pipe. Swagelok stainless steel fittings, adapted to the stainless steel plates, were used for the various inlet and outlet connections of the culture apparatus. The stainless steel foambreaker nozzle described here was mounted in the top center section of the culture apparatus.

Foam Control

The foambreaker nozzle, fabricated of stainless steel (Fig. 2; see also Ref. 6) consists of a short section of thick-walled tubing of one inch outside diameter. This section mates with a rod having a one-inch-diameter cap to form a one-mil slit between the tube and cap. In practice the slit end of the nozzle is located four to five inches above the medium, with the slit parallel to the surface of the medium. Compressed, filtered air fed in at the upper end of the nozzle tube escapes through this narrow slit in a horizontal direction 360 degrees around the nozzle. When the nozzle is pressurized to 8 to 10 psi, the emerging sheet of air effectively prevents the foam from rising up through it. Thus, the problems

attending the use of chemical antifoam such as the contamination of bacterial surfaces and unfavorable effects on growth or reproduction are avoided.

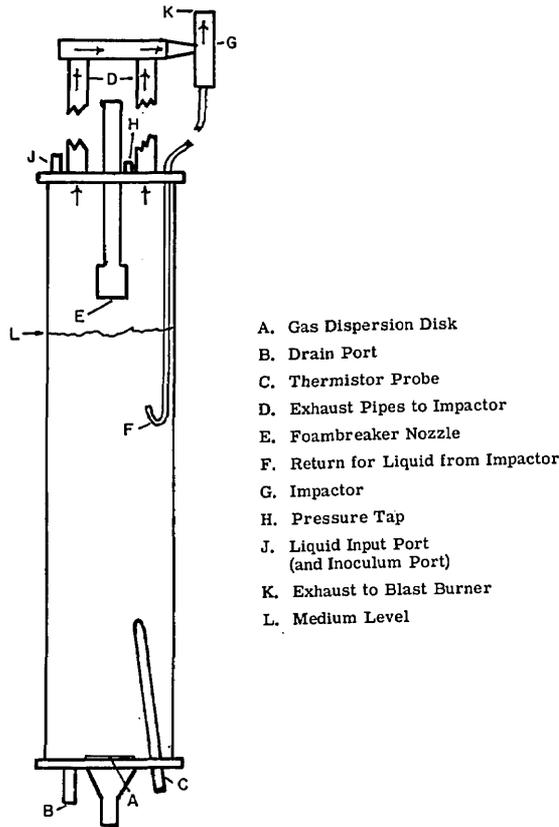


Fig. 1 - Schematic of culture apparatus

The foambreaker nozzle is held in place in the center of the top plate of the culture apparatus by a union-type fitting which has a silicone rubber gasket between it and the nozzle. As the union is tightened, the gasket is compressed around the nozzle tube. This arrangement permits the nozzle to be held firmly in position, in addition to sealing the connection. Thus leakage of bacterially contaminated air to the surroundings is prevented.

Prevention of leakage is important, because the nozzle generates a dilute aerosol as it destroys foam. To help prevent much of this bacteria-laden material from escaping, a jet impactor (7) is placed in the exhaust duct of the culture vessel. The jet impactor is a device having a small orifice with a plate located within a critical distance from the outlet of the orifice. Liquid particles of a given velocity are either impacted on the plate or remain airborne, depending on their mass. The impacted material is returned to the culture apparatus by means of a small stainless steel tube, which terminates below the surface of the culture medium. This return tube must extend sufficiently high above the liquid level in the apparatus to prevent medium from being forced out through the tube by the internal pressure. Beyond the impactor the exhaust air passes through a 3/4-in. I.D. rubber hose and into a blast burner located in a fume hood, where incineration of any remaining airborne bacteria occurs.

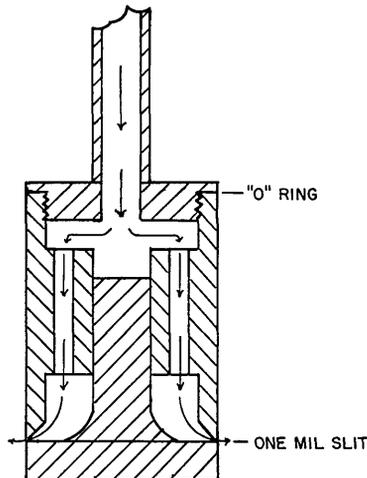


Fig. 2 - Foambreaker nozzle, cross section

Incoming air for the nozzle, usually ranging from 15 to 30 lpm, is cleaned by passing it through a compressed-air filter, an activated carbon bed, and then through a two-inch-diameter glass fiber filter paper. This glass fiber filter paper has an aerosol filtration efficiency of 99.98 percent removal for a monodisperse liquid DOP aerosol of 0.3 micron diameter. Filtration of the air serves the twofold purpose of providing clean air for the nozzle as well as removing bacteria or other microorganisms which would otherwise contaminate the culture apparatus.

Aeration

Aeration is accomplished by forcing air through a dispersing device which is a porous disk of sintered stainless steel two inches in diameter (Gelman Instrument Company filter holder). To simplify installation, the entire male portion of the filter holder, with its sintered metal disk, is incorporated into the lower stainless steel plate of the culture vessel.

Aeration air is passed through a Navy gas mask canister (C-1), which removes particulate matter, including bacteria, and foreign gases or vapors that may have been present in the compressed air.

Coarse and fine air-pressure regulators are employed to control the compressed air. A needle valve is used as well as a rotameter to control and measure aeration air flow. Generally, aeration air volume for the 4.5-liter-capacity culture vessel is supplied at the rate of one to two liters per minute.

Volume and pH Control

To maintain approximate starting liquid volume during the experimental run, a syringe-type metering pump is used to keep the medium in the culture vessel at a reasonably constant level. This arrangement is used to compensate for the relatively large evaporative loss caused by the intimate mixing of dry compressed air from the foambreaker nozzle and wet foam, and also the much smaller loss due to aerosol, some of which passes through the jet impactor. To make up this loss, it is necessary to add approximately one liter of solution during an 18-hour culture period.

Approximate pH control (within one unit or less) is achieved by adjusting the hydrogen-ion concentration of the sterile solution of the makeup liquid. This method requires that one know in advance the direction and approximate magnitude of the change in the hydrogen-ion concentration as growth occurs. Approximate pH variations are determined during preliminary runs in shake-flask cultures.

Temperature Control

To maintain proper temperature, a Model 63 Thermistemp Temperature Controller (Yellow Springs Instrument Company, Inc.), consisting of a thermistor probe and an electronic controller, is used. Heat is provided by a one-inch-wide, six-foot-long heating tape ("Briskeat," Briscoe Mfg. Co.) which is wrapped around the outside of the culture vessel. It is rated at 110 volts and 576 watts capacity, but is operated through a Variac at 65 watts. The temperature variation within the culture vessel with this arrangement is within $\pm 0.5^{\circ}\text{C}$.

EXPERIMENTAL METHOD AND RESULTS

The growth of Bacillus megaterium, strain KM, and Escherichia coli, strain K-12, in the apparatus was studied in some detail. A few additional runs were made with Escherichia coli, strain Crookes, Serratia marcescens, strain 8 UK, and Micrococcus lysodeikticus.

The usual routine followed was to subculture the chosen organism in broth tubes in an incubator at controlled temperature for several consecutive days. The microbiological medium used in the precultures was always the same as that in the 4.5-liter culture vessel. After the adaptive period, a log-phase culture of four to five milliliters was used to inoculate 500 ml of broth for shake-flask culture at controlled temperature. After 8 to 12 hours incubation, this seed culture was used as the inoculum for the four liters of sterile broth in the culture vessel.

After the addition of the medium and the inoculum to the sterile culture vessel, the thermostat, air for aeration, and the foambreaker air were turned on in that order. With the ratio of nozzle air to aeration air properly adjusted, the apparatus could be left unattended overnight. In practice, nozzle air to aeration air ranged from as low as 10 to 1 up to 30 to 1, depending upon the foaming properties of the medium used. Make-up liquid was metered in at the required rate. Initial make-up liquid input was usually timed to coincide with the end of the lag phase of growth, which generally occurred about four to six hours from time of inoculation.

Klett-Summerson photometer readings (red filter) and pH measurements were taken at appropriate intervals during trial runs. At the completion of a run, the cells were harvested by centrifuging. Yields were then determined by measuring the wet-packed volume after centrifuging for ten minutes at $12,000 \times g$ and/or, more reliably, by drying an aliquot of microbial suspension at 105°C to constant weight, with a correction for the weight of buffer salts when present.

Klett readings for two different, typical organisms in the culture apparatus are shown in Fig. 3. Usually within about six hours the exponential phase was underway. Experimental runs were usually terminated after 18 hours, except in cases where the period was purposely shortened. In the case of Bacillus megaterium, strain KM, for example, cell harvest after ten to twelve hours furnished final cell-wall preparations substantially free of poly- β -hydroxybutyric acid storage granules. These small, water-insoluble, intracellular polymer granules are found to exist in greater and consequently more troublesome quantities in late log-phase cultures (8). Hence, early harvesting is better from the standpoint of good cell-wall preparation, because many of the granules tend to remain trapped within the isolated cell wall.

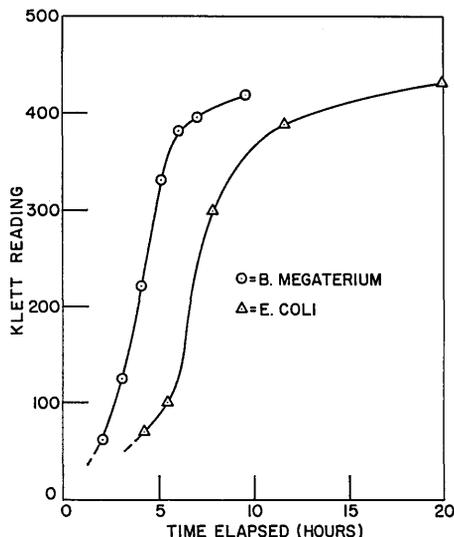


Fig. 3 - Typical growth curves

Typical yields are shown in Table 1. Dry weights varied from 6.6 grams for Micrococcus lysodeikticus to 16.2 grams per run for Serratia marcesens, strain 8 UK.

Table 1
Yields for Typical Aerobic Bacteria

Organism	Dry Weight (grams*)
<u>Bacillus megaterium</u> , strain KM	10.8
<u>Escherichia coli</u> , strain K-12	10.4
<u>Serratia marcesens</u> , strain 8 UK	16.2
<u>Micrococcus lysodeikticus</u>	6.6
<u>Escherichia coli</u> , strain Crookes	7.8

*Per run basis (4.5 liters)

DISCUSSION

The most novel feature of this particular culture apparatus is the mechanical foam-breaker. This foambreaker nozzle has been used with several types of culture media and has been found to function efficiently and reliably, provided the nozzle-to-aeration-air ratio is large enough. Another important consideration is container geometry in the immediate region of the nozzle. Although vessels with shapes different from the cylindrical type reported here could be used, it is necessary that the rising foam be channeled to the foambreaker (i.e., within about one and a half inches of its periphery) in order to obtain foam control.

In spite of the usefulness of the nozzle as an effective destroyer of foam, there are inherent disadvantages in using it. Principal among them is the generation of aerosol when the nozzle breaks up the wet foam. This action inevitably gives rise to airborne

bacteria within the culture vessel. Consequently measures must be taken to make the container vessel leaktight, particularly since it is under positive pressure with respect to the surrounding air. In addition, the exhaust air must be freed of bacteria. In this apparatus jet impaction and the blast burner for the exhaust air have proven effective in decontamination of the effluent air.

Another shortcoming is evaporative loss of liquid due to the intimate contact between the dry nozzle air from the compressed-air source and wet foam. To compensate for this loss, volume has been kept approximately constant by the addition of make-up liquid.

At least three improvements could be made in the culture apparatus. These are (a) automatic pH control, (b) mechanical stirring, and (c) better level control of the medium. None of these improvements were made because of the complications involved, a main problem being space in which to add these features.

There are possible research applications for the device. One conceivable use would be the characterization of aerosols produced by the action of the nozzle on foams from various solutions and suspensions. With the slight modification of putting the foambreaker nozzle in a separate foam-overflow container, this apparatus could serve very well as a foam-fractionation device.

CONCLUSIONS

The bacteriological-culture apparatus described in this report incorporates a mechanical foambreaker of novel design which eliminates the need for chemical antifoam agents. Chemical antifoaming agents, in some cases, have the disadvantage of affecting the life processes of bacteria. The foambreaker provides a circular sheet of high-velocity air which effectively breaks up any foam without the need for growth-inhibiting anti-foam chemicals.

The culture apparatus has been shown to be useful for obtaining relatively large quantities of aerobic bacteria. After experimental runs usually of 18 hours duration, or less, bacteria yields amounted to dry-weight quantities of 6.6 to 16.2 grams.

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DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author) Naval Research Laboratory Washington, D.C. 20390		2a. REPORT SECURITY CLASSIFICATION Unclassified	
		2b. GROUP --	
3. REPORT TITLE AN AEROBIC-BACTERIA CULTURE APPARATUS EMPLOYING A NEW FOAMBREAKER			
4. DESCRIPTIVE NOTES (Type of report and inclusive dates) An interim report on the problem.			
5. AUTHOR(S) (First name, middle initial, last name) William H. Echols			
6. REPORT DATE January 20, 1967	7a. TOTAL NO. OF PAGES 9	7b. NO. OF REFS 8	
8a. CONTRACT OR GRANT NO. NRL Problem C05-21	9a. ORIGINATOR'S REPORT NUMBER(S) NRL Report 6494		
b. PROJECT NO. RR 001-01-43-4757	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)		
c.			
d.			
10. DISTRIBUTION STATEMENT Distribution of this document is unlimited.			
11. SUPPLEMENTARY NOTES		12. SPONSORING MILITARY ACTIVITY Department of the Navy (Office of Naval Research)	
13. ABSTRACT <p>An aerobic-bacteria culture apparatus incorporating a novel mechanical foambreaker has been developed. The culture vessel consists of a 30-in. length of glass pipe four inches in diameter oriented vertically and closed at each end with a stainless steel plate. The bottom plate holds air spargers, and the top is bored to admit an air outlet and, in the center, the foambreaker. The foambreaker consists of a vertical stainless steel tube closed at its lower end with a cap which leaves a one-mil slip between it and the bottom edge of the tube. When the tube is pressurized with compressed air, a high-velocity sheet of air emerges 360 degrees around the tube and effectively breaks foam rising from the aerated medium. The addition of chemical antifoaming agents is thus obviated. Relatively high yields of such bacteria as <u>Bacillus megaterium</u>, <u>Escherichia coli</u> and <u>Serratia marcescens</u> have been obtained.</p>			

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Foambreaker Aerobic bacteria Microorganisms Culture apparatus Foam fractionation						