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Apparatus for Independent Control of Aeration Rates in a Series of Cultures[1]

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It has been demonstrated repeatedly that oxygen tension is a significant factor in culturing microorganisms. Availability of oxygen influences growth rates of eubacteria (Dagley *et al.*, 1953), actinomycetes (Webley, 1954), and protozoa (Browning *et al.*, 1952). The capacity for motility depends in some cases on oxygen supply (Sherris *et al.*, 1957). Metabolic patterns, and the end-products of metabolism, may be related qualitatively or quantitatively to oxygen tension in cultures of bacteria (Dagley *et al.*, 1950; Pirt, 1957; Finn, 1954) and molds (Rolinson and Lumb, 1953; Finn, 1954).

It thus becomes imperative that oxygen tension be rigidly controlled in most experiments, and often that its influence be quantitatively assessed. Although oxygen supplies may be controlled and held constant in a variety of ways (Finn, 1954), most aeration equipment provides little flexibility for measuring the effect of variations in oxygen tension. Oxygen availability is held constant in any one experiment, but there is no provision for comparing simultaneously a number of cultures growing at different, independently controlled aeration rates. The apparatus described here has proved useful for rapid, laboratory-scale screening of response to varied oxygen tension over a considerable range.

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DESCRIPTION OF APPARATUS

Essentially, the apparatus consists of a series of needle valves, monitored by individual flow-rate meters and connected by a manifold inlet to a controlled source of air under pressure. The valve-controlled outlets are in turn connected to a series of growth vessels. A schematic diagram of the apparatus is presented in figure 1.

The air source is a laboratory compressed air line. Any good air pump would probably serve as well. Heavy-walled rubber tubing connects the air line to a filter made by packing a length of metal pipe with Pyrex wool. The filter, in turn, is connected to a dia-

phragm valve (No. 695 air pressure regulator[2]) which will maintain a constant downstream pressure; such a valve is necessary because compressed air lines are often subject to rather large pressure fluctuations. A pressure gauge is then provided to monitor the valve setting. Since pressures below this valve usually will be considerably lower than line pressure, it is best to provide some sort of release device to prevent "back-pressure" from rupturing the rubber tubing which connects various parts of the apparatus. A simple closed metal sleeve fitting over an upright length of pipe, open at the top, serves this purpose very well. A small metal can is soldered to the top of the sleeve and filled with enough lead shot so that air will escape only when the pressure in the system exceeds 20 lb.

The air at controlled pressure enters a manifold and is distributed to a series of flowmeters. The outlet from each meter is provided with a needle valve which permits precise control of air flow rates. For use with laboratory-scale cultures, small plastic "purge meters" are relatively inexpensive and reasonably accurate. These are available in banks of 6 meters with individual needle valve outlets and a manifold inlet (S2006-V Brooks-Mite 6-unit flowmeters[3]). The apparatus consists of 2 such banks of meters, providing 12 outlets in all. The diaphragm valve, pressure gauge, and flowmeters with valves are mounted together in a metal panel, with all pipe connections behind the panel and nipples for connections to rubber tubing projecting from the panel below each flowmeter.

[2] Fischer and Porter Co., Hatboro, Pennsylvania.

[3] Brooks Rotameter Co., Lansdale, Pennsylvania.

Although growth vessels of many types may be used with this apparatus, we have found 18 by 150 mm culture tubes containing 10 ml of medium to be most convenient for small-scale experiments. The tubes are covered with loose-fitting aluminum caps through the tops of which small holes have been drilled. A short piece of rubber tubing is fitted into the hole, and a length of glass tubing then placed through the cap as shown in figure 1. The length of aeration tubes is kept constant; 14 cm from the cap to the lower end of the tube is convenient. A disc of membrane filter material (nylon-impregnated MF, type HA[4]), is cemented (MF cement[4]) over the upper end of the aeration tube. Air passes through these filters with no measurable impedance, although they provide quantitative removal of bacteria from the air stream. Sterility controls have remained uniformly negative. The earlier filtration through Pyrex wool lowers bacterial and gross particulate contamination to a point where there is little or no danger of overloading or clogging the filters on individual aeration tubes. Another short length of rubber tubing fits over the upper end of the aeration tube, protecting the filter from accidental damage. Rubber tubing connections from the flowmeter outlets are provided with short pieces of glass tubing at the ends; these are slipped into the rubber tips of individual aeration tubes.

Culture media are sterilized in tubes with ordinary aluminum caps, and the aeration caps, with filters in place, are placed in empty tubes and sterilized separately by autoclaving. It is not necessary to attempt

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

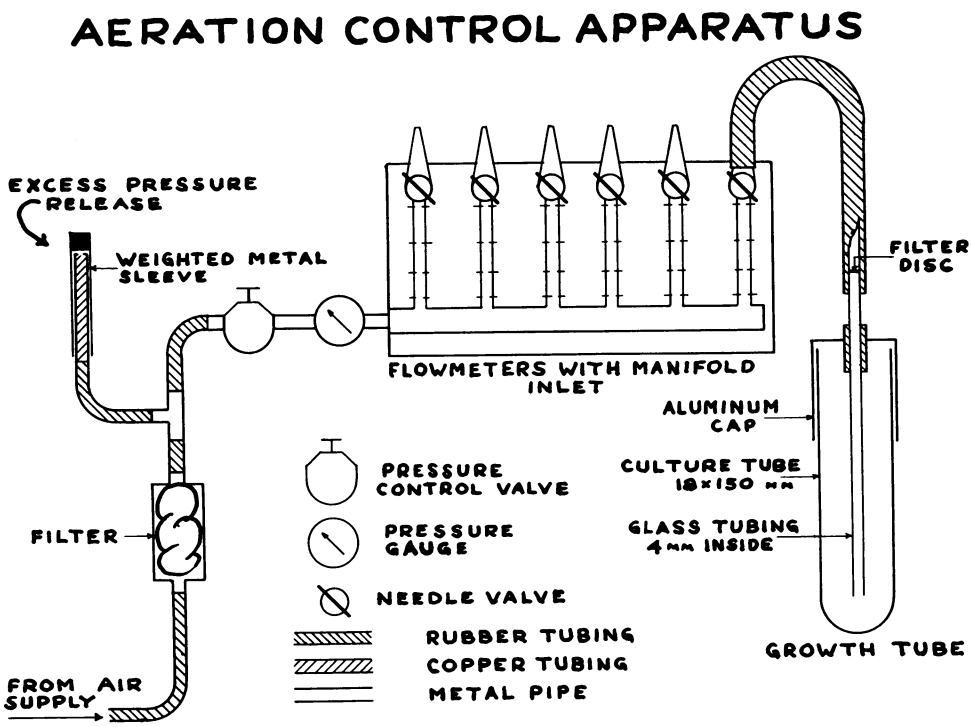

Figure 1. Diagram of culture apparatus described in text

sterilization of the entire flowmeter panel. Growth tubes are inoculated, placed in racks in a water bath set at the desired incubation temperature, and the caps replaced with aeration tubes which are then connected to individual outlets on the flowmeter panel. With the diaphragm valve completely open, the compressed air supply is turned on until the pressure gauge holds steady at 15 lb. The valve is then closed until the desired air pressure is reached in the apparatus (1 lb pressure is usually sufficient). Needle valves are now adjusted individually to the desired flowmeter settings. At these pressures, changes in individual valve settings do not affect the pressure gauge reading or the flow rates delivered from adjacent valves.

A number of measures of microbial response to aeration rates in the various tubes are now possible. For turbidimetric measurements, optically calibrated growth tubes are used. Tubes are disconnected from the apparatus when readings are to be made, and placed directly in a colorimeter. Caps may be lifted slightly so that aeration tubes clear the light path, or aeration tubes may be replaced temporarily by ordinary caps. As many as three holes may conveniently be drilled in caps fitting tubes of this size, so that it is possible to

TABLE 1

*Growth of Escherichia coli strain K12 in nutrient broth at various aeration rates**

Air flow rate, ml per min	15.5	20.5	23.5	30.5	36.0	42.5	46.5	59.0	70.5	77.0	89.5	105.0
Oxygen absorption coefficient†	5	7	8	10	14	16	17	18	20	22	23	27
Incubation Time (hr)	Optical Densities‡											
0	.00	.00	.00	.00	.00	.00	.00	.00	.00	.01	.01	.00
1	.01	.01	.02	.02	.02	.03	.02	.03	.02	.02	.02	.01
2	.07	.06	.06	.07	.06	.08	.06	.07	.06	.06	.07	.06
3	.26	.22	.25	.25	.22	.28	.22	.25	.26	.21	.21	.19
4	.44	.40	.48	.52	.48	.53	.48	.53	.52	.52	.50	.50
5	.56	.58	.62	.68	.67	.71	.71	.72	.73	.73	.73	.73
6	.70	.70	.74	.78	.77	.85	.82	.87	.89	.87	.87	.89
7	.76	.79	.83	.90	.94	.95	.96	1.00	1.01	1.02	1.03	1.02
8	.83	.92	.89	.96	.98	.97	.98	1.05	1.00	1.00	1.02	1.00
9	.88	.91	.95	.98	1.00	1.00	1.00	1.00	1.00	1.05	1.02	1.07
10	.91	.90	.94	.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.07
11	.90	.90	.94	.98	1.00	1.00	1.00	.98	1.00	.98	1.00	1.05
12	.90	.90	.94	.98	1.00	.98	.98	.98	1.00	.98	1.00	1.00

* Series of culture tubes each containing 10 ml of broth and inoculated with 0.1 ml from the same 24-hr broth culture. Tubes were connected to the aeration device described in the text and aerated, without sparging, at 37 C.

† Millimoles O_2/L/hr, determined by the method of Cooper *et al.*, (1944).

‡ 2-Log G, determined in a spectronic 20 colorimeter at 625 mμ.

TABLE 2

Comparison of the growth of two strains of Micrococcus pyogenes in nutrient broth at various aeration rates

Strain	*M. pyogenes* var. *aureus*, strain H					*M. pyogenes* var. *aureus*, strain S2			
Air flow rate, ml per min	20.5	42.5	77.0	105.0		20.5	42.5	77.0	105.0
Oxygen absorption coefficient	7	16	22	27		7	16	22	27
Incubation Time (hr)	Optical Densities				Incubation Time (hr)	Optical Densities			
0	.00	.01	.00	.00	0	.00	.00	.00	.00
1	.02	.03	.01	.03	4	.01	.00	.01	.01
2	.11	.06	.02	.04	5	.02	.01	.02	.03
3	.42	.09	.02	.06	6	.05	.02	.04	.05
4	.90	.36	.04	.08	7	.07	.04	.07	.09
5	1.11	.74	.14	.09	8	.11	.05	.12	.15
6	1.16	.92	.42	.26	9	.19	.10	.24	.27
7	1.26	1.10	.63	.47	10	.38	.24	.46	.50
8	1.26	1.16	.82	.62	11	.54	.42	.62	.74
9	1.26	1.22	.96	.73	12	.70	.68	.85	.96
10	1.30	1.22	1.07	.89	13	.80	.84	.98	1.10
11	1.30	1.26	1.13	.98	14	.87	1.00	1.10	1.22
12	1.26	1.22	1.16	1.02	15	.92	1.10	1.19	1.30

Experimental conditions as in table 1.

provide a sampling port, or to fit caps with platinum electrodes and KCl-agar bridges for determinations of redox potentials during growth. Larger growth vessels may be employed if it is desirable to remove larger samples.

Calibration of the Apparatus

Although flowmeters of this type are provided with a uniform scale calibrated in cu ft per hr or ml per min, individual meters vary considerably and the manufacturer's calibration is not always accurate. Flow rates may be determined experimentally by water displacement, but flow rate alone is of little importance. It is much better to attach some arbitrary scale to the meters and to determine individual oxygen absorption coefficients at various settings of each flowmeter, using either polarigraphic (Wise, 1951) or sulfite oxidation determinations (Cooper *et al.*, 1944). As Finn (1954) has pointed out, aeration data recorded in this form are more meaningful and may more readily be "scaled up" to larger volumes if the need arises. With our apparatus, oxygen absorption coefficients are obtainable at values between 5 and 50 mM per L per hr. Since the air supply only bubbles gently through the medium in the growth tubes, considerably higher aeration values may be obtained by sparging the air flow. This can be accomplished by fitting the lower end of the aeration tube with a small glass sparger, or by cementing over the lower end of the tube a piece of membrane filter material of rather large pore size (these filters are available in a number of porosities). If it is necessary to work with much larger volumes of culture, or at extremely high aeration rates, it would probably be advisable to use more expensive flowmeters capable of accurate measurements at more rapid flow rates.

Experimental Results

The results of a typical growth experiment with *Escherichia coli* are shown in table 1. It will be noted that at aeration values below 16 mM of oxygen per L per hr growth rate as measured by optical density depends on the rate of oxygen supply. The growth may vary with the medium used and other factors such as size of inoculum, but is characteristic and reproducible for a particular strain or species. Strain H of *Micrococcus pyogenes* var. *aureus*, for example, responds differently. Advanced aeration rates caused lengthened lag periods, slower growth and lower total densities (table 2). Table 2 shows also that strain S2, a mutant of H which is abnormally sensitive to the toxic effects of stilbestrol, differs markedly from the parent strain in its pattern of response, requiring a higher rate of aeration for maximum growth. The significance of these and other observations has yet to be determined, but they serve to illustrate the importance of careful measurement and control of oxygen supply.

Summary

An apparatus has been described which provides for growing a series of cultures at independently varied and measurable aeration rates. Aside from its utility in screening experiments to determine the optimum aeration conditions for various fermentations, the apparatus is useful for study of any system in which oxygen tension may exert a differential influence.

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