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Culture Aeration in a Polythermostat

S. A. PALUMBO, J. M. BERRY, AND L. D. WITTER
Department of Food Science, University of Illinois, Urbana, Illinois

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ABSTRACT

A system for the aeration of cultures growing in a polythermostat temperature-gradient block was developed. This system provided both aeration and agitation of the cultures, while allowing the growth to be followed spectrophotometrically. The necessity for aeration of cultures growing in a polythermostat was experimentally shown.

Temperature-gradient devices of varying design, temperature range, and capacity have been described in the literature. None of those described provides for aeration of the growing cultures. Both Morita and Haight (4) and Sinclair and Stokes (8) emphasized the need for adequate aeration during the growth of aerobic organisms. Sinclair and Stokes (8) found that oxygen is rapidly depleted to growth-limiting levels in stationary cultures. Olsen and Jezeski (5) found shorter generation times when cultures of *Pseudomonas fluorescens* were aerated during growth. A method of adequately aerating polythermostat-grown cultures is described in this paper. The necessity and adequacy of the aeration system is demonstrated.

MATERIALS AND METHODS

The organisms used in this study were a psychrophilic strain of *P. fluorescens* and a mesophilic strain of *P. aeruginosa* F2.

P. aeruginosa F2 was grown in Trypticase Soy Broth (BBL). *P. fluorescens* was grown in a defined basal salts broth containing 0.6% K_2HPO_4, 0.3% KH_2PO_4, 0.1% $(NH_4)_2SO_4$, 0.02% $MgSO_4$, and 1% glucose. The glucose was autoclaved separately as a 10% solution and added to 9 ml of 1.1 × basal salts (sterilized in the culture tubes). Sterile Antifoam C (Dow Corning Corp., Midland, Mich.) was added to a level of 1,000 ppm in the culture tube after inoculation.

Growth was followed spectrophotometrically in a Spectronic-20 colorimeter (Bausch & Lomb, Inc., Rochester, N.Y.) at 340 mμ for *P. fluorescens* and at 380 mμ for *P. aeruginosa* F2. Growth curves of the change in the logarithm of the optical density with time were plotted for each of the experimental temperatures. The generation time was calculated as the length of time required for a doubling of the optical density (2). The growth rate in divisions per hour was obtained by taking the reciprocal of the generation time in hours.

Aeration of cultures in a polythermostat. The polythermostat used in these studies was similar in design to that described by Oppenheimer and Drost-Hansen (6) and had 16 rows or temperature banks with four wells per row. Each well accommodated a standard test tube (18 by 150 mm), used as the culture tube.

The aeration apparatus built for the polythermostat consisted of two parts: a manifold system for distribution of air, and a temperature equilibration-culture tube system for the growth of organisms The manifold system was similar in design to that described by Lockhart and Ecker (3).

The equilibration-culture tube system is shown in Fig. 1. In this system, moist air from the manifold was bubbled through the water in the first tube, in which the air was further humidified and brought to the temperature of the row. The equilibrated air from the first tube was then passed into a 2-ml glass syringe set in the culture tube. The barrel of the syringe was packed with cotton to sterilize the air. This sterilized air then passed into the culture broth through a bent 15-cm syringe needle attached to the syringe. The bend in the needle allowed the culture to be assayed spectrophotometrically. The needle-cork connection mounted in the end of the syringe was easily removed and allowed the growth tube to be removed from the polythermostat, to be assayed, and to be returned without contamination of the growing culture.

To insure good thermoconductivity, water was placed in the interstitial space between the culture tubes and the polythermostat block. The thermal characteristics of the equipment were evaluated before use. The temperature in the culture tubes, with and without aeration, was continuously monitored by thermocouples connected to a multichannel (Honeywell, Inc., Minneapolis, Minn.) recorder for 2 weeks. The temperature was periodically checked by reference thermometers in special wells in the polythermostat block. A linear temperature gradient was maintained in the block, the temperature in culture tubes was not altered by aeration, and the reference thermometer could be reliably used to estimate the culture tube temperature. During subsequent experimental runs, the thermocouples were removed from the culture tubes.

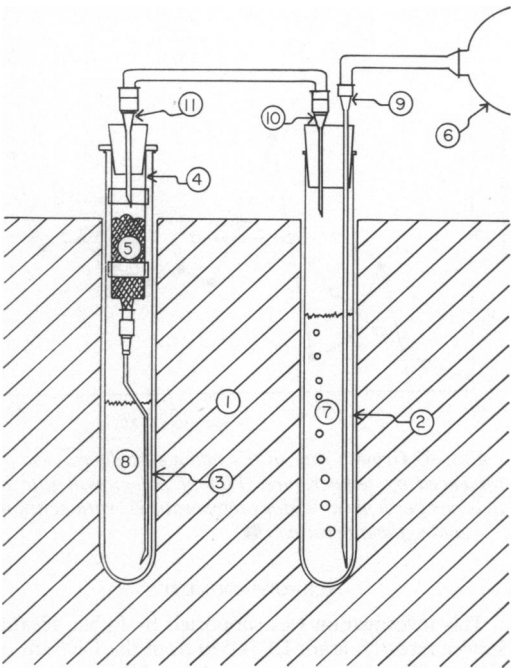

FIG. 1. *Schematic representation of the aeration system used in the polythermostat temperature-gradient block. (1) Aluminum block; (2) equilibration tube; (3) culture tube; (4) 2-ml glass syringe; (5) cotton; (6) manifold; (7) water; (8) culture broth; (9, 10, 11) syringe needles.*

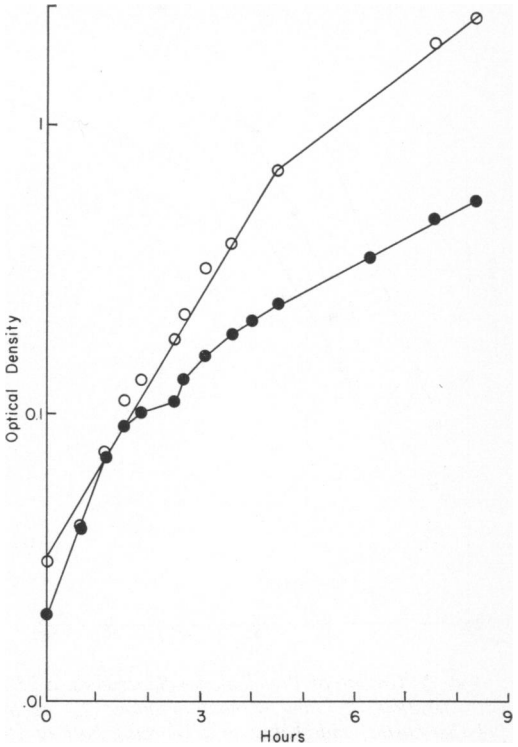

FIG. 2. *Growth of Pseudomonas aeruginosa F2 in the polythermostat at 32 C, with aeration* (○) *and without aeration* (●).

RESULTS AND DISCUSSION

The growth of a culture of *P. aeruginosa* F2 in the polythermostat with and without aeration at a temperature of 32 C is shown in Fig. 2. The growth curve of the culture without aeration had a shorter and more irregular logarithmic phase. The irregularity of the curve for the nonaerated culture makes the calculation of a generation time difficult and the comparison of the growth at various temperatures subject to considerable interpretation.

The commercially available polythermostats can be shaken to insure better thermal contact, and this shaking can also provide some degree of aeration. The adequacy of this form of aeration was examined by shaking culture tubes of *P. fluorescens* in a Warburg bath at 160 strokes per minute at 28 C. The culture grew only in the upper two-thirds of the tube, which clearly showed inadequate aeration. In Fig. 3, the growth curves of these shaken cultures are compared with an unshaken culture and a culture aerated by the system shown in Fig. 1. The aerated, the shaken, and the nonaerated cultures conveniently had generation times of 4, 5, and 6 hr, respectively.

The growth of aerobic and facultative bacteria under conditions of adequate aeration allows a more complete and efficient utilization of the energy and carbon source. With oxygen as the final electron acceptor, less of the carbon source is utilized for energy production and more is converted into cell material. Increasing the amount of oxygen supplied (aeration rate) allows the culture to grow more rapidly.

Inadequate aeration may distort the relationship of temperature to growth rate. As the temperature is lowered, there is generally a reduction in growth rate, but the increased solubility of oxygen at low temperatures may partially offset this reduction. When aeration is not adequate, it becomes a variable rather than a constant in the experimental procedure. Roth and Wheaton (7) failed to provide any aeration in attempting to determine cutoff points between the growth-temperature requirements of mesophilic and psychrophilic *Arthrobacter* strains. Davey, Miller, and Nelson (1) also failed to provide any aeration for their four test organisms (*P. fragi*, *Bacillus coagulans*, *B. stearothermophilus*, and *Streptococ-*

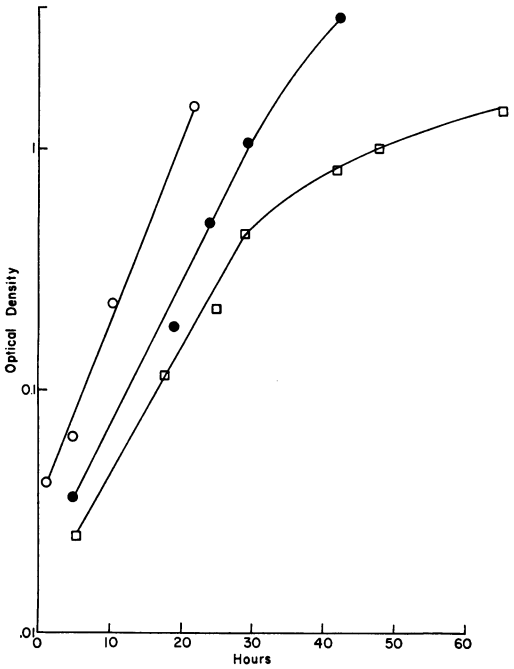

FIG. 3. *Growth of Pseudomonas fluorescens at 28 C with aeration* (○) *and without aeration* (□) *in the polythermostat, and shaken in a Warburg bath at 160 strokes per minute* (●).

cus faecalis) grown in a polythermostat with the objective of showing temperature-dependent anomalies in the growth of bacteria as related to water structure.

The growth rates of *P. fluorescens* grown in the polythermostat at various temperatures with and without aeration are given in Fig. 4. The nonaerated culture showed growth rates which were very irregular and which were generally lower than those of the aerated culture. The nonaerated cultures did not grow at 4 C and below after 12 days of incubation; the aerated cultures had an easily measurable growth rate at 0 C, the lowest temperature tested.

The influence of temperature change on the growth of bacteria cannot be properly assessed without adequate aeration. The aeration system suggested here (Fig. 1) provides such aeration for cultures grown in a polythermostat. The system minimizes evaporation of the culture media, alteration in temperature due to air addition, and contamination during optical density measurements.

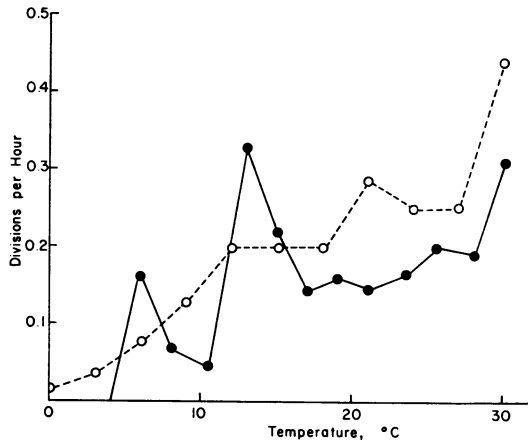

FIG. 4. *Growth rate of Pseudomonas fluorescens as influenced by temperature. Plot of the growth rate in divisions per hour at various temperatures, with aeration* (○) *and without aeration* (●).

ACKNOWLEDGMENT

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