AUTOMATIC RECORDING OF THE GROWTH RATES OF CONTINUOUSLY CULTURED MICROORGANISMS*

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Continuous culture techniques in which the growth rate of the microorganism is the independent variable have been described by Myers and Clark (1) and Bryson (2) and "chemostatic" methods, in which the input rate of a growth factor is the independent variable, by Novick and Szilard (3) and Monod (4). The theory on which the present work is based may be summarized briefly as follows. Let the total volume of a culture be held constant at V and let nutrient solution be added at such a rate, w volume units per unit time, that the mass of protoplasm per unit volume is held constant at $m = m_r$. Then the rates of synthesis and removal of protoplasm are equal and we may write $V\ dm/dt = m_r w$ (1). Differentiation of (1) with respect to time gives the general relation $V\ d^2m/dt^2 = m_r\ dw/dt$ (2). In the special case of logarithmic growth, Equation 1 becomes $w/V = \alpha$ (3) in which α is the growth constant.

It is clear that if w is measured and recorded continuously as a function of time, a continuous record of the growth rate, and rate of change of the growth rate, is obtained and that by this means a technique which has heretofore been purely preparative in scope may be converted into an analytical method for studying the kinetics of cell growth (1) before the steady state is reached (selection, adaptation), (2) during the steady state regime, and (3) after exposure of the steady state culture to a physical, chemical, or viral agent. The development of a method for realizing these possibilities was the objective of the present work. For convenience, and as a means of emphasizing its distinguishing characteristics, we refer to our apparatus as a "microbial auxanometer."

Scheme of Design

The essential features of a single unit of the auxanometer are shown in Fig. 1. The control section is similar to turbidostatic devices reported previously (1, 2). An increase in protoplasmic density deflects the galvanometer beam to T_3, opens the valve, and adds nutrient solution until the reference density is restored. A syphon removes a volume of culture equal to that of the solution added. Automatic recording of $w(t)$ is accomplished by counting the drops of

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solution added in fixed, arbitrarily adjustable time intervals and registering the counts on a commercial traffic counter. Calibration, in terms of volume per drop, is carried out during routine operation by measuring the volume of effluent culture collected during a given recorder count.

Constructional Details

The principal requirements to be met by the growth tube, Fig. 2, were (1) elimination of filming on those parts of the tube wall which are traversed by

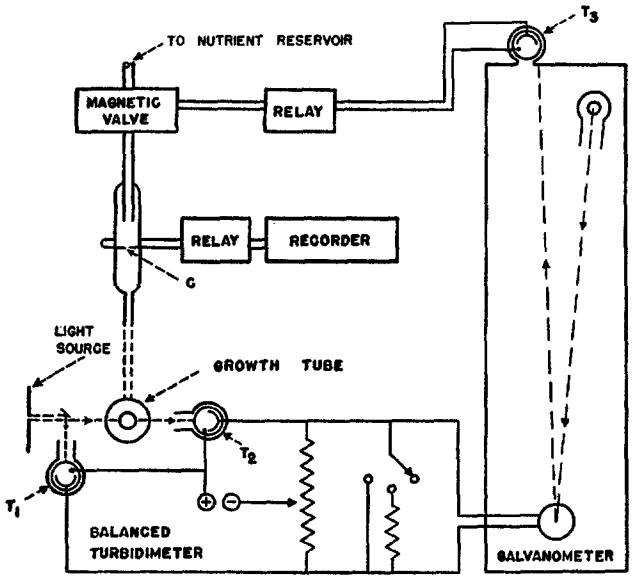

FIG. 1. Schematic diagram of complete auxanometer. The turbidimeter, photorelay, and valve maintain a constant culture density by dropwise addition of nutrient solution. The drop count for intervals adjustable from 1 to 60 minutes is registered by Pt contactor *C* and the automatic reset, print-out recorder. $T_1 = T_2 = T_3$ = RCA 927 vacuum phototubes.

the light beam, and (2) adequate aeration without disturbance of the light beam. The "windshield wiper" arrangement, adopted after it was found that the most vigorous mechanical stirring did not prevent slow deposition of a light-absorbing film, complicates the construction but has survived numerous changes in tube design because of its reliability. The scrapers are cut from ¼ inch surgical rubber tubing. Air is introduced at a pressure of 30 to 40 cm. of water close to the wall of the growth vessel and through a tube which is constricted to an internal diameter of *ca.* 0.5 mm. near its end and bent to point vertically upward. This design produces a stream of small bubbles which do not wander into the light beam, and vigorous circulatory stirring of the entire

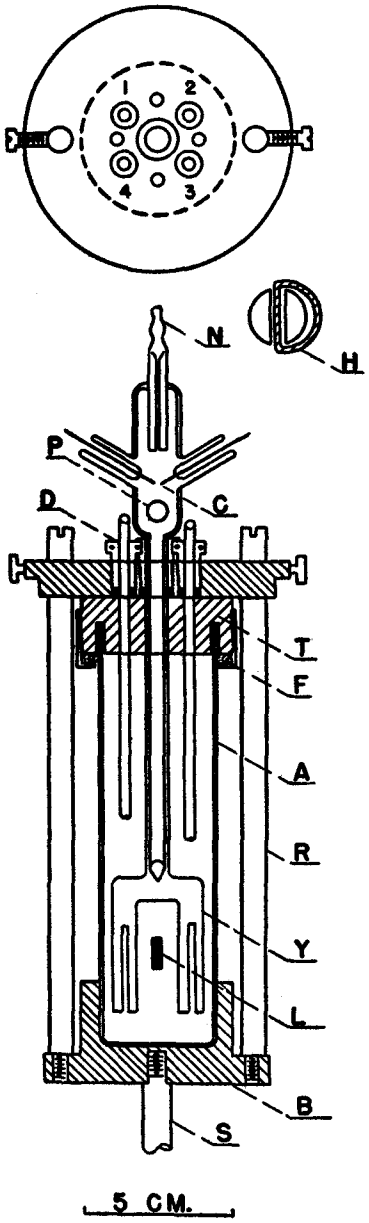

FIG. 2. Growth tube assembled for autoclaving. Tie rods R are removed after mounting. The 38 mm. culture tube A, cemented in dural cup B, is rotated by spindle S. Surgical tubing H held by glass forks Y prevents deposition on walls. T = teflon cap. F = felt gasket. Air input (1), air output (2), syphon tube (3), and thermocouple well (4) are secured by dural bushings D bearing on koroseal washers. P = inoculation port. C = Pt drop-counting contacts. L = light beam. N = nutrient medium input.

culture. The thermocouple well allows continuous recording of the true temperature on a potentiometric recorder. The teflon cap is so designed that vapor condensate cannot come into contact with the metal parts of the assembly.

Freedom from long term drift and high sensitivity in the turbidimeter are obtained by the use of intense parallel illumination[1] with voltage stabilization, vacuum phototubes in a compensating circuit, and a high sensitivity (5×10^{-10} amp.) galvanometer. The potentiometer which forms two arms of the bridge, a 10 turn helipot with duodial, is chosen to match the critical damping resistance of the galvanometer. The photorelay which operates the valve is of the conventional forward connected type in which phototube and amplifier operate on a common D. C. supply. Any form of magnetic valve which permits the nutrient supply tube to be inserted without separation of the tube is suitable.

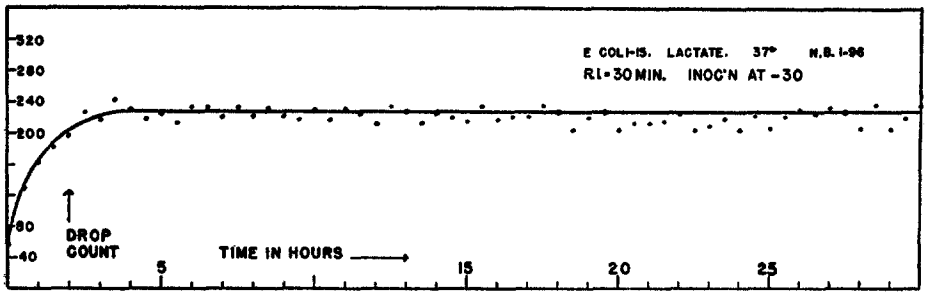

FIG. 3. Growth rate recording for a culture which maintains a single steady state. Points are observed drop counts per 30 minute recording interval. The horizontal curve represents an ideal steady state of mean generation time 46.4 minutes. Inoculation was at −30 minutes.

In the recording section, the relay must be of the type which holds long enough to operate the recorder after actuation by a momentary contact; the Type CK Farmer relay[2] is satisfactory. The recorder is a Type SCI Streeter-Amet relay-rack register with built-in microflex timer adjustable from 0 to 120 minutes, and tape feed-out.[3] It resets to zero automatically after printing each count.

The auxanometer now in use in our laboratory is a three channel instrument containing three growth tubes, each with its own turbidimeter, mounted in a single metal box over 0.4 R. P. M. rotators driven from a common shaft. This box, the solution reservoirs, and valves are contained in a thermostated angle iron–plexiglas cabinet. The syphons are so arranged that the effluent culture can be collected aseptically in interchangeable tubes or passed into waste

[1] Partial assemblies of the 6 volt microscope illuminators supplied by Erb and Gray, Los Angeles.

[2] Farmer Electric Co., Waltham, Massachusetts.

[3] Streeter-Amet Co., Chicago.

bottles. The entire instrument is A. C.-operated. All D. C. potentials come from a single stabilized power supply (Lambda Electronics No. 28). The air supply is humidified and sterilized. The nutrient reservoirs, 8 liter pyrex serum bottles, are fitted with glass grinds carrying a syphon delivery tube, pressure equalizing port, and 10 mm. UF fritted glass filters for addition of solutions sensitive to autoclaving.

Performance

A typical growth rate curve, plotted directly from the recorder tape, is reproduced in Fig. 3. This run was made at a cell count of about 10^7. At this count the culture and nutrient solution differ only slightly in optical density and hunting is relatively pronounced.

Potential sources of error are as follows. (1) Variation of drop volume with drop delivery rate. Excessive differences between the drop rates employed in calibration and operation should be avoided. (2) Inadequate aeration. This error is eliminated by increasing the air supply until the recorder count shows no dependence on aeration rate. (3) Trapped cells. Cells adhering at points not reached by the scrapers may contribute to the observed growth without contributing to the turbidity. Growth curve abnormalities apparently attributable to trapping have been observed in several runs, generally after 30 hours or more of continuous operation. The time required for completing measurements on a culture will ordinarily be short relative to the time at which the effects of trapping become significant. More complete suppression of trapping appears to be feasible and is to be attempted if the need for it arises.

SUMMARY

A procedure which establishes steady states in cultures of microorganisms and also provides continuous automatic recording of their growth rates is described.

REFERENCES

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