APPLIED MICROBIOLOGY, May 1974, p. 874-877

Vol. 27, No. 5
Printed in U.S.A.

A High-Precision and Large-Capacity Laser Biophotometer

A. MARCHAND, J. J. PIAUD, AND P. DU PASQUIER

Centre de Recherche Paul Pascal, Domaine Universitaire, 33405-Talence, France, and Laboratoire de Virologie, Hôpital Pellegrin, 33-Bordeaux, France

Received for publication 30 October 1974

A new type of biophotometer is described. Very precise and reproducible measurements of bacterial concentration can be performed at 3-s intervals for each of 32 simultaneously developing bacterial cultures. The large number of results is handled with modern data acquisition and computer-processing techniques.

High precision and reliability in bacterial growth studies can only be achieved, when required, through time-consuming techniques. But most often only a rough estimate of the microbial concentration is needed; in such cases the biophotometer is a very convenient apparatus. The biophotometer makes use of the absorbance and scattering of light by bacteria suspended in a broth: the intensity of the transmitted light is measured, and a calibration curve reveals the bacterial concentration.

Most currently available biophotometers, however, are not suitable for accurate determinations of the growth rate constants and, generally speaking, for a detailed analysis of the kinetics of the growth of a culture.

The primary shortcoming of commercially available apparatus is poor reproducibility in the complex movements of the single light source or photoelectric receptor, or both, so that between two succesive measurements with the same microbial culture cell, there is usually some possibility of change in the position of the light beam relative to the culture cell. Another shortcoming of commercial apparatus is that they have capacity for only a small number of simultaneous cultures, so that very little information can be gained in a single experiment when various media or growth conditions are studied. Thirdly, they use "white" light; the relation between optical density and bacterial concentration of the cultures is not a linear one, so that careful calibration is necessary if the transmitted intensity measurements are to be translated accurately in terms of bacterial concentrations. And finally, they have a poor temperature stabilization.

We describe here a new type of biophotometer that is built to meet these shortcomings. The new type of biophotometer was constructed to obtain precise and reproducible in situ measurements of microbial concentrations, in a reasonably short time, for a moderate or large number of simultaneously developing bacterial cultures.

MATERIALS AND METHODS

The new type of biophotometer was constructed in accordance with the following principles. (i) The light source is a laser, so that the optical density of the cultures is a linear function of the bacterial concentration (monochromatic light); the intensity of the incident light is always sufficient for the accurate measurement of the transmitted intensity, even with strongly absorbing cultures; and the optics of the whole apparatus are extremely simple, because the light beam is perfectly parallel along its entire path. (ii) The light source and receptor, as well as the microbial culture cuvettes, are kept at fixed positions, which eliminates the numerous errors due to the usual movements of the light source in relation with the cuvettes. (iii) The transmitted intensity measurements are repeated at a high rate. This permits the study of fast processes. Moreover, increased accuracy is gained by statistical treatment of the large amount of data thus obtained. This implies the use of modern techniques for data acquisition and preparation for computer processing.

A schematic drawing of the apparatus is presented in Fig. 1. The light source is a Spectra-Physics type 132 laser (He-Ne; power 1 mW). The light beam (632.8 nm) has a diameter of 0.8 mm. The laser axis (vertical) is a symmetry axis for the whole apparatus.

Thirty-two culture cells (Hellma cuvettes) (20 by 20 by 50 mm), made with optical glass, are placed along a circle 35 cm in diameter, whose axis coincides with the laser axis. Each cell housing is provided with a rotating magnet that moves a small Teflon-encased iron strirring-rod in the cell.

The light beam from the laser is first reflected at a right angle by a set of prisms (P_1), situated on the symmetry axis and rotating around it. The horizontal light beam is rotated and thus successively passes through each culture cell; after reflexions on P_2 and P_3, it is again reflected at a right angle by another set of prisms (P_4). It then coincides once more with the

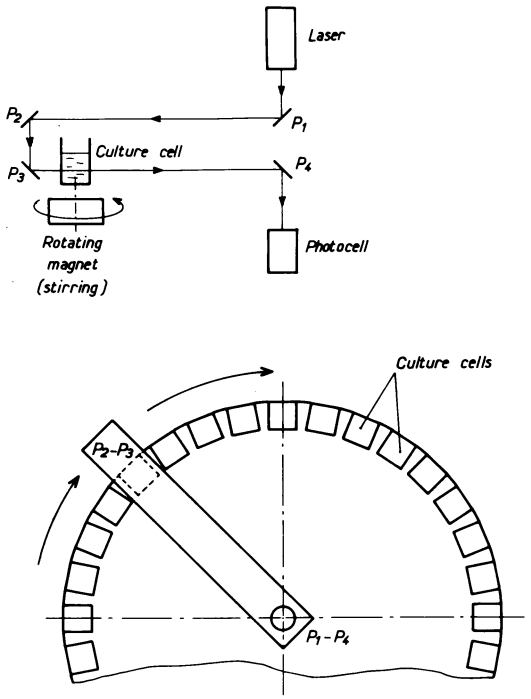

FIG. 1. *Schematic structure of the biophotometer.*

laser axis and, after a suitable attenuation, falls upon a photoelectric cell (Radiotechnique type 150 CV, spectral response S1, giving a current of 50.10^{-9} *A* at full scale). The rotation period of the light beam is 3 s: this is the shortest possible time between two successive measurements on the same culture cell.

Measurements. The measuring process of the transmitted intensity is triggered at the precise time when the light beam runs within 0.1 mm of the center of each culture cell. This is achieved with an encoding disk that rotates with the beam around the axis of the apparatus. This encoding device, which was especially designed and built in our laboratory, also "reads" the number of the cell.

Exposure of the photocell during 100 μs is sufficient to obtain a signal, which is first memorized by a "sample and hold" amplifier. During the 0.1-s time-lapse between measurements on two successive cells, the signal is passed to an analogue to digital converter; the resulting 11-bit figure, together with the cell number, is punched on a paper tape. The tape perforator may be programmed to punch only the results for a fraction of the culture cells or for every n^{th} measurement ($1 \leq n \leq 99$).

A numeric display (solid state) shows the results of the transmitted intensity measurements performed on any one of the culture cells.

Temperature stabilization is achieved with resistors (above room temperature, up to 70 C) and with a cooling fluid circuit (below room temperature, down to 0 C). The temperature can be kept constant for hours at 37 $\pm$ 0.1 C.

Computer processing. The large amount of data from a single experiment may be either fed directly from the punched tape to a computer, or transferred to a magnetic tape before processing. The choice of a suitable program will then depend upon the kind of scientific output that is expected from the experiment.

RESULTS AND DISCUSSION

This new biophotometer was extensively tested during the last year. The ease of utilization, speed, and precision of measurement are at the expected level. Figure 2 shows, for instance, the growth curve of a culture of *Escherichia coli* K-12 in a tryptone broth (Difco); the transmitted intensity (lower curve) is plotted as a function of time. Each experimental point represents the average of 10 successive measurements at 30-s intervals (the rate of measurements could be 10 times faster if necessary).

From the transmitted intensity I, and the incident intensity I_0, the optical density $D = -\ln (I/I_0)$ is computed and plotted on Fig. 2 (upper curve). Because monochromatic light is used, D would be

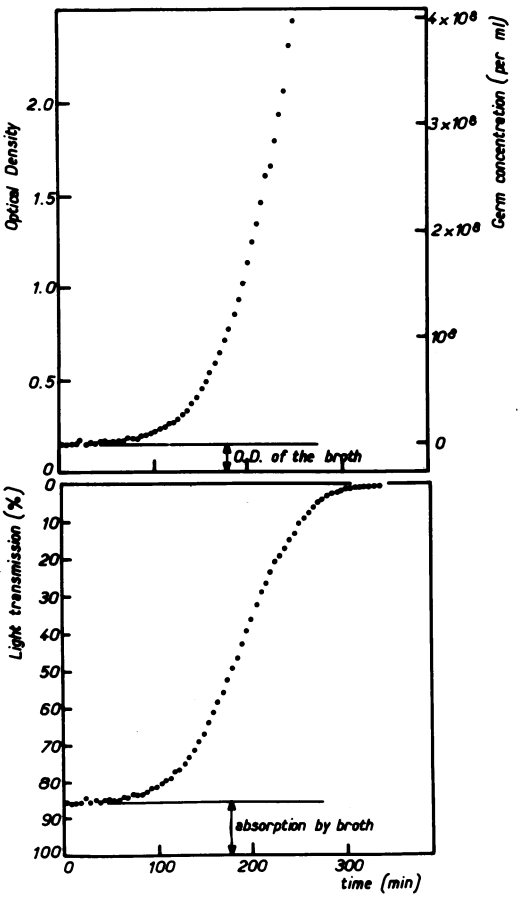

FIG. 2. *Growth curve of a culture of E. coli K-12. Each point represents the average of 10 successive measurements at 30-s intervals. Up to 32 such cultures can be simultaneously studied.*

directly proportional to the bacterial concentration (number of cells per milliliter) except that part of the light is also absorbed by the nutrient broth. The bacterial concentration C is actually only a linear function of optical density D. This was confirmed experimentally by plotting D versus C (obtained by conventional counting techniques). A straight line was obtained, and thereafter this calibration was used to compute C from the measured D values. The precision of the C versus D relationship is limited by the accuracy of the counting technique and not by that of the optical density measurement.

Transmitted intensity I can be measured from O to I_0. Table 1 shows some characteristic values of I/I_0, of the standard deviation S_{I/I_0}, of the optial density D, and its standard deviation S_D, as well as the approximate corresponding bacterial concentrations (most tests were performed with *E. coli*).

A total of 10^6 cells (*E. coli*) per ml contribute approximately 25×10^{-4} to the optical density. At very low bacterial concentrations the broth optical density itself is much larger than this value, and the cell concentration is determined with very poor precision. A total concentration of 10^6/ml gives a "signal" that is only twice the standard deviation S_D; it can be easily detected but not properly measured.

But when the cell concentration is 10^7/ml, it can be determined with a better than 10% precision. At concentration larger than 10^8/ml the precision is better than 1%.

Reproducibility tests have shown that for identical cultures simultaneously developing in the biophotometer, the results are also reproducible within the precision limit of 10^6 bacteria per ml.

The possibility of studying 32 cultures simultaneously is a very important time-saving factor. A single run of the biophotometer is sufficient to study growth kinetics and determine growth rate constants in a variety of media, temperature, and pH conditions, etc., or to make extensive tests of the effects of drugs on the growth rates of microbes.

Figure 3 gives such an example. A number of *E. coli* K-12 cultures (10-ml volume and similar to those of Fig. 2) were simultaneously growing in the apparatus;

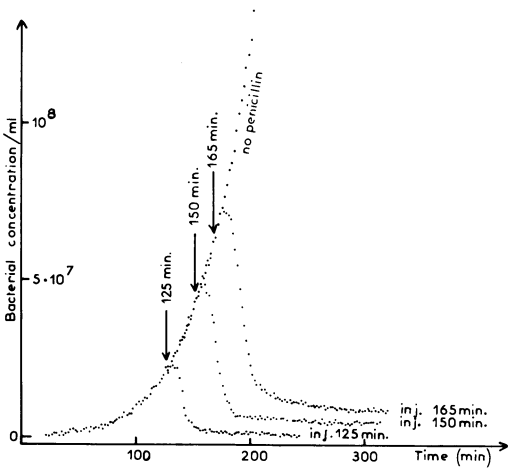

FIG. 3. *Simultaneous E. coli K-12 cultures (10-ml volume) after 10^4 U of penicillin were injected into the cultures at various times. Each point represents the average of 10 successive measurements at 12-s intervals.*

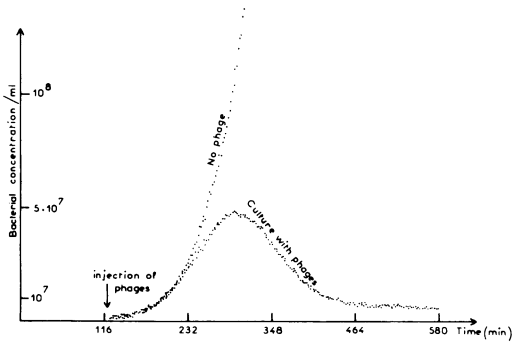

FIG. 4. *Effect of injection of 2.10^4 bacteriophages per ml in E. coli K-12 cultures. Each point represents the average of 10 sucessive measurements at 12-s intervals. The initial phage: bacteria ratio is very low, and a number of rounds of phage multiplication take place (about six to eight) before the effect of phage injection starts being noticeable. By that time the production of phages is no longer synchronous throughout the culture, and no discrete "bursts" are detected. The curve levels off around 500 min at a "cell concentration" of 10^7/ml, which has no real meaning because of the presence of dead cells and various debris.*

TABLE 1. *Some characteristic values of transmitted intensity, optical density, their standard deviations, and corresponding cell concentrations of E. coli cultures*

Determination[a]	Transmitted intensity				
$I:I_0$	0	0.02	0.2	0.8	1
S_{I/I_0}		3×10^{-4}	12×10^{-4}	12×10^{-4}	12×10^{-4}
$S_{I/I_0}:I/I_0$		0.015	0.006	0.0015	
D		3.9	1.6	0.22	
S_D		0.015	0.006	0.0015	
$S_D:D$		0.004	0.004	0.007	
Approximate bacterial concn (*E. coli*/ml)		65×10^7	30×10^7	10^7	

[a] Abbreviations: I, transmitted intensity; I_0, incident intensity; S, standard deviation; D, optical density.

0.05 ml of broth containing 10^4 U of penicillin was added to the various cultures at the times indicated. The accuracy and reproducibility of the curves allow a detailed analysis of the effect of penicillin on growth kinetics.

Similar experiments were performed with a bacteriophage. Figure 4 shows the results of the injection of 2×10^4 phages per ml into a K-12 culture containing approximately 10^6 bacteria per ml. A number of such curves have been obtained in various conditions. A quantitative interpretation is possible.

Improvements of the existing apparatus are being made to provide for in situ irradiation of the cultures and built-in data processing equipment.