Construction and Use of a Continuous Recording Nephelometer

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Received for publication October 30, 1958

The measurement of media turbidity by transmitted or scattered light has been widely used as a function of microbial growth. Currently available photoelectric and spectrophotometric instruments require that an operator perform the manual task of placing a tube in the instrument and recording the results. This procedure results in a good deal of tube handling, questionable efficiency in reading the results, and, many times, failure to record data during nonworking hours. Recently, considerable interest has been expressed in converting the entire procedure of reading and recording media turbidity to an automatic process and two such instruments have been described (Hutchison and Coutlas, 1957; Harris, 1958). This paper describes a third instrument which has such features as the automatic handling of up to 300 test tubes and the automatic recording of data measured. In spite of this considerable capacity, the machine is small, less than two feet square, portable, and capable of being wheeled readily into a moderate size incubator.


Materials and Methods

Apparatus

A front and top view of the instrument are shown in figures 1 and 2. The essentials of this instrument are two separate racks, each holding 10 test tubes. As each tube passes in front of the optical system, the turbidity is recorded on a card and the cycle is completed every 10 hr. The removable card on each rack is divided equally into ten 1 by 2½ in. spaces with appropriate grid lines for turbidity and time (figure 3). The component parts are described in detail.

Optical, electronic, and recording systems. The light source is a 32 CP, 6 to 8 v prefocused base automobile headlight bulb. This source is very compact, has high output and good life and the filament is precisely oriented with respect to the base.

Surrounding the lamp is a slotted cylinder driven at 3600 rpm by a synchronous motor. This constitutes a chopping mechanism which delivers light interrupted 420 times per sec in opposite directions across the cylinder with the light beams passing through respective slits in each direction. One beam goes through a lens system and heat absorbing filter into the test tube.
Light reflected at 90 degrees from microorganisms in the tube is reflected again from a tiny mirror and passes through a lens system into a "sample" photocell. The output from this photocell is fed into a summing amplifier. The other beam passes directly from the chopper and slit into a "reference" photocell. The output of this photocell is amplified and applied both to a clipper circuit and to a precision ten turn "Helipot" potentiometer, thence from the "Helipot" slider to the summing amplifier. A "Twin-Tee" adjusted to 420 cycles is connected in a feedback circuit around the summing amplifier. By means of this arrangement, unwanted signals caused by ambient light, hum, and so forth, are attenuated approximately 30 db. The filtered signal is then fed into another clipper followed by a power amplifier. The power amplifier also incorporates a "Twin-Tee" filter to further attenuate unwanted signals. The output of the power amplifier drives the control phase of a two-phase servomotor.

The output of the preamplifier which goes directly to a clipper circuit is then passed into a power amplifier essentially the same as the one used for the control phase. The second amplifier drives the fixed phase of the same two-phase motor.

The servomotor drives the "Helipot" to a point where the inputs to the summing amplifier are balanced resulting in a null in the amplifier output. The servomotor also rotates a vertical screw which carries a stylus on supporting nut to the proper height. The nut is prevented from rotating by the tines of a spring-loaded fork attached to the traveling nut and straddling vertical support rod. In this manner, the servomotor quickly drives the stylus and potentiometer to an equilibrium position determined by the reflected light entering the sample photocell. The stylus is then energized through a cam operated switch and pricks a hole in the card recording the datum point. Provision is made to use either a linear potentiometer or a 21/2 cycle log.

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Figure 1. Front view showing (A) the control panel, (B) optical and recording system, (C) racks containing tubes, and (D) cards on which turbidity is recorded.

Figure 2. Top view, showing (A) the control panel, (B) the optical system, (C) the recording stylus, and (D) the racks containing tubes. Arrows indicate the direction of travel as the racks proceed through each 2-hr cycle.

Figure 3. Card from rack showing 2-hr turbidity readings. The tubes contain the following bacteria: (1) Escherichia coli, (2) Salmonella typhosa, (3) Proteus vulgaris, (4) Micrococcus pyogenes var. aureus (5) Proteus rettgeri, (6) Streptococcus hemolyticus (pyogenes), (7) Streptococcus faecalis, (8) broth control.
CONTINUOUS RECORDING NEPHELOMETER

Mechanical moving of the tubes. Recordings of turbidity are made against time. The time shift of the stylus is produced by a 1 revolution per day (rpm) clock motor. This motor drives a single revolution scroll cam of 1-in. travel. The entire stylus, potentiometer, and servomotor assembly is mounted on a pair of vertical flat springs and is shifted horizontally from left to right by this cam at the rate of 1 in. in about 23½ hr. In the remaining ½ hr, the cam returns the stylus to the origin and the cycle repeats.

The 300 test tubes are lightly held by springs in 30 racks. These racks contain 10 tubes and on the front side hold a special printed blotter on which the stylus marks the individual records of each of the 10 tubes. On the bottom of each rack is a series of 10 rectangular holes spaced precisely 1 in. apart. These holes serve to advance the rack from 1 tube to the next. At the top of the ends of each rack are located 2 small plastic rollers that support the racks during their movement from the front of the machine to the back.

To return each tube to the reading position every 2 hr, it is necessary that all racks be pushed to the back of the machine, progress across the back in a manner the reverse of their travel in the front, and then be brought forward to complete the "cycle" in a sort of "do-se-do" operation. The progression of each rack along the back is accomplished by a cam and mechanical links extending along the machine under the racks. Similarly, a cam engages and disengages a pin at the back by means of a long push rod extending down the center below the other mechanisms.

The rack replacement shift refers to the shift from one rack to the next. This requires a simultaneous movement of 1¼ in. forward to the racks on the left side and an equal movement to the rear of the racks on the right side.

Four hook arms are used to pull the two groups of racks to the back and to the front. The right hand pair of hooks is directly linked through a crank to an oscillating shaft. The left hand hooks are connected to the other end of this shaft by a pair of intermediate direction-changing linkages. The shaft is driven by a cam on a small, synchronous motor. All four hooks are of sheet metal and slide on ways located at the sides and middle. The hook tips are spring loaded to snap over the ends of the rack at the conclusion of the last reading of each rack.

Stirring of cultures, media, and reading of results. It became readily apparent after a few preliminary experiments that it was necessary to stir the contents of each tube. This was accomplished by a magnetic stainless steel rod, ¼ in. diameter by ½ in. in length, placed in each tube and a motor-driven Alnico magnet in close proximity to the underside of the test tube adjacent to the reading station. In separate studies, no apparent difference was noted when the cultures were vigorously shaken by hand and compared to results obtained when the cultures were stirred by the magnetic bar. Also, viable counts indicated that the stainless steel rod did not stimulate or suppress the growth of the organisms used in this study.

The selection of culture media was not critical and the instrument performed equally well with synthetic as well as nonsynthetic media. However, as is characteristic of any nephelometer, only cultures producing an even, homogenous growth could be used. Optical matched tubes were used throughout these studies.

During the course of a 24-hr period, 11 datum points were obtained for each tube from which the growth curves were composed. As the size of these curves was relatively small, approximately 1 by 1¼ in., they were enlarged to 8 by 11 in. by means of a simple opaque projector. In order to have a point of reference, standard curves were produced which related turbidity readings to viable counts for each test organism. The large capacity of this instrument allowed the use of a sufficient number of replicates so that the data could be evaluated statistically.

RESULTS

The instrument described in this paper was used in a variety of experiments with a wide range of common bacterial species. Satisfactory growth curves were obtained with strains of micrococci, streptococci, and various members of the enteric group including coliforms and proteus. The bacteria whose growth curves are presented in figure 3 were grown in brain heart infusion broth with the exception of *Streptococcus hemolyticus (pyogenes)* which was grown in a semisynthetic medium. The constituents of this medium, referred to as 7 AW, are presented elsewhere (Wilkins et al., 1956). All of the tubes contained approximately 1 × 10⁶ cells per ml at zero time and grew to a maximum number of approximately 1 × 10⁸ cells per ml. Certain aspects of these curves are worth considering at this time. Of interest is the uniform rate of growth of *S. hemolyticus* in the semisynthetic medium as compared to the growth curves of the other bacteria in a nonsynthetic medium. Also, there was a marked increase in the growth of *Salmonella typhosa*, *Proteus rettgeri* and *Streptococcus faecalis* after 2 hr of incubation at 37 C which was not evident with the other organisms.

This instrument was used in a wide variety of experiments, however, only a few of its applications will be described. It was extremely valuable in determining the effect of antibiotics on bacterial growth especially at sublethal levels (Wilkins et al., 1956). In other experiments, the antibiotic was added at different stages of growth in which turbidity readings plus viable counts

*Indiana Steel Products Corporation, Valparaiso, Indiana.*
provided much useful information on the mode of action of the drug.

In studies on antibiotic combinations, turbidity readings obtained with this instrument indicated that a combination of novobiocin and penicillin was synergistic when tested against strains of micrococci and proteus (Barbiers and Lewis, 1957). This nephelometer was also used on studies of the rate of development of resistance by various bacteria in the presence of one or more antibiotics. Numerous nutritional studies on micrococci were conducted in which turbidity readings were correlated with growth response. Also, the instrument was used for routine procedures such as bioassays in which the large capacity of this instrument permitted the testing of numerous samples at one time.

SUMMARY

This paper describes the construction and use of a continuous recording nephelometer. The salient features of this instrument are the automatic handling of up to 300 test tubes and the recording of data measured every 2 hr, the instrument is small, less than 2 ft square and portable, and it has been successfully used in various fields of bacteriology including growth rate studies, the action of antibacterial agents, antibiotic combinations, and nutritional studies.

REFERENCES


Efficient Utilization of Fatty Oils as Energy Source in Penicillin Fermentation

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Received for publication December 15, 1958

It is well known that the use of fatty oils, that is, animal and vegetable oils, in a corn steep-lactose-CaCO₃ medium accelerates the penicillin production (Stefaniak et al., 1946; Goldschmidt and Koffler, 1950; Ishida and Isono, 1952; Anderson et al., 1956). These oils have been shown to be readily metabolized by the fungus (Rollinson and Lumb, 1952; Anderson et al., 1956) and can serve as the main energy source for the fermentation (Perlman and Langylkke, 1953). No true advantage over lactose has, however, been demonstrated when the latter was completely replaced by these oils (Perlman and Langylkke, 1953). Several years ago we undertook a study of this problem, trying to find any possible process whereby the use of oil might be of real advantage. The results of this study are reported here.

MATERIALS AND METHODS

The fermentation was carried out in shake flasks incubated at 25°C on a rotary shaker (280 rpm, 2 in. stroke)


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