1 Introduction

TURBIDIMETRY is one of the traditional methods used in microbiology to obtain bacterial growth curves. Electrical impedance has been proposed as a convenient alternative (FISTENBERG-EDEN and EDEN, 1984; FELICE et al., 1988). Our objective was to implement simultaneously both methods while separating the three components (bulk resistance, interface resistance and interface capacitive reactance) from the total impedance measured during growth between the two electrodes immersed in the medium, based on previously obtained results (FELICE et al., 1992). The only antecedent, to the best of our knowledge, is previous work (HASHIMOTO et al., 1979, 1981) which, making use of a tetrapolon technique, compared turbidimetric and impedancimetric bacterial growth curves.

2 Materials and methods

A sinusoidal voltage source was applied via an 82 kΩ series resistor, to ensure constant current, to two electrodes (stainless steel wires, Φ = 1 mm) immersed in brain heart infusion (~Merck). Everything was placed in 10 ml glass cylindrical culture cells with a diameter of 1 cm. Measurements were made at intervals of 10 min and at two frequencies, one low (20 Hz) and another high (20 KHz). The time scan permitted visualisation of the growth curve while the two frequency measurements separated the interface components (negligible above 5 KHz) from the resistive medium component, according to Warburg’s Laws (FELICE et al., 1992). The interface resistive and capacitive components were calculated using the impedance modulus, measured at 20 Hz, and the angle was obtained with a phase-sensitive circuit.

By using the well known Warburg series model, we recall that the total resistive component is equal to \( R_t = 2R_i + R_m \), with \( R_i \) = resistive interface component per electrode and \( R_m \) = resistive bulk component. In addition, \( X_t = 2X_i \), where \( X_i \) is the total capacitive reactive component and \( X_i \) stands for the contribution made by each electrode.

A green light-emitting diode (LED, 498–530 nm) was the light source to emit the beam which, after traversing the cell diametrically, was detected by the light-dependent resistor (LDR). Turbidity, which by light scattering mainly yields information about the macromolecular content (dry weight) instead of the number of cells, was defined in terms of the usual absorbance logarithmic units, i.e.

\[
A = \log \left( \frac{R_x}{R_o} \right)
\]

(2)

where \( R_x \) is the incident luminous intensity and \( \Phi \) is the transmitted luminous intensity (also called luminance). As the resistance \( R \) of the LDR is inversely proportional to \( \Phi \), the latter can be expressed as \( K/R \) which, after substitution in eqn. 1, leads to another mathematical form for the absorbance

\[
A = \log \left( \frac{R_x}{R_o} \right)
\]

(2)
where $R_c$ corresponds to the LDR resistance value at any moment of the bacterial growth and $R_m$ is its value at the initial time. According to the Beer–Lambert Law and at any moment, the measured absorbance is proportionally related to the bacterial concentration in the culture medium.

The system has three major parts; analogue/digital control, computer and culture cells. The analogue/digital block conditions the detected signal, performs the A/D conversion and transfers the data to the computer. Internal control is provided by a microprocessor*, with 2 Kb of EPROM memory and 512 bytes of RAM. External control is with a PC via an RS232 interface. The computer carries out the data processing, presenting the information on the monitor screen as time course growth curves in percentage terms for $R_m$, $R_i$, and $X_i$, and in logarithmic absorbance units (also called optical density units) for turbidity. The system is able to handle up to 64 culture cells (expandable to 512), which are placed in an oven at constant temperature ($37 \, ^\circ C \pm 0.1$). Fig. 1 displays the block diagram.

There are two main groups of programs; those resident in the EPROM memory, written in 8085 assembler language, and those resident in the PC, in Quick Basic. The first group has a modular form. Its principal functions are data acquisition and communication with the PC. The second group makes use of the concepts of modular and structured programming. Data processing is its main function.

3 Results

Calibration of the system was carried out with resistance and capacitance boxes†. In addition, the instrument contains three internal calibration resistances ($2 \times 100 - 1 \, 800 \, \Omega$, 0.1%) and a 1 $\mu F$ reference capacitor. For all ranges, the experimental errors were found below 3%. The long-term stability was tested by connecting 100 $\Omega$ in one channel and 1 $800 \, \Omega$ in another, while recording for 62 hours at ambient temperature. Drift was defined as the measured change referred to the initial value in percent. For 20 KHz, it was <1%; for 20 Hz, <0.2%.

Three growth curves were obtained with *E. coli* and *Proteus mirabilis*, in all cases separating the three impedance components and showing the turbidity pattern simultaneously. Fig. 2 displays a typical result.

4 Discussion

The microbiological results were similar to those obtained previously with an impedance bridge (FELICE et al., 1992). The system showed the possibility of running continuous measurements, day and night, without the assistance of any personnel. The system design means that it can be modified to better fit different requirements, such as the addition of new information processing modules applicable, for example, to the food industry, to complement clinical studies or to the assessment of suspect fresh waters.

The actual absorbance becomes increasingly less than the formula predicts as the concentration increases. More light shines through the suspension than expected because light scattered from one bacterium is scattered by another, so that the light is redirected back into the detector. When absorbance is greater than 0.3 and wavelengths are in the order of 500 nm or shorter, there is a deviation from Beer’s Law, a fact that may call for some kind of correction (GERHARDT, 1981). However, in our case, all the recorded absorbance values fell well below the stated level, suggesting an acceptable linear relationship.

The optical system used for turbidimetry is simple. However, it can be improved by means of a collimated light source. Simultaneous recording of turbidimetric and impedance growth curves can supply more information about the phenomenon under study. Besides, turbidity, as better accepted by microbiologists, might be used as a reference, easing the way of the impedance technique into the field of microbiology.

Another attractive advantage of the system is the automatic production of time course absorbance curves, not frequently found in microbiological research and industrial laboratories, where usually only isolated agglutination turbidimetric measurements are performed.

Time course growth curves showed the expected pattern, with lag, log and saturation phases. Fig. 2 clearly reveals approximately the same breaking point (BP) for all four curves, in this case at around 2 hours, 40 minutes, signalling the end of the lag phase and the beginning of the log phase.

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* INTEL 8085
† General Radio, Types 1432-K, 1419-A

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