A Method Detecting Bacteria in Culture Medium by Simultaneous Measurement of Electrical Impedance and Turbidity

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Abstract

Simultaneous measurement of impedance and turbidity changes in culture medium was proposed. The impedance change was measured by means of a four-electrode method. Two characteristic parameters for *Escherichia coli* and *Staphylococcus epidermidis* grown in Brain Heart Infusion broth were obtained. The growth speed α (inverse of generation time) and the delay time T of impedance change were computed as characteristic parameters from measured quantities. Since bacteria had inherent α and T, they were tentatively represented in term of a characteristic vector with two components α and T. Two bacteria were clearly discriminated on the two dimensional space.

Introduction

The automatic and rapid detection of bacteria is an urgent problem in biological routine test. Many authors have proposed, as a powerful method, to measure the electrical impedance change of bacterial culture medium[2,3,4,5,12,14]. Impedance change may be brought by bacterial growth and metabolism[3]. This method is useful for detecting bacteria[4], but not so fruitful for getting parameters characterizing bacteria so far. On the other hand, optical measurement can give another information of growing bacteria[8,11]. The transmittance or turbidity of culture medium is proportional to the concentration of bacteria[11]. Impedance and turbidity changes, therefore, may introduce two different physical or physicochemical properties.

The authors proposed a simultaneous measurement of impedance and turbidity changes of culture medium[6,7]. Impedance change showed similar time course to that reported in other papers[4,5,12]. However, the change was not so reproducible and different from expected results in our phenomenological model dealing with impedance and turbidity changes[6,7]. This reason was dominantly due to the surface effect of electrodes[2,5]. Four-electrode method is known as a conventional method to exclude the effects[9]. The present paper describes the simultaneous measurement of turbidity and impedance changes by means of four-electrode method. Impedance change progresses after some delay and in parallel way with turbidity change. From the data recorded on the chart two characteristic parameters, i.e. the delay time (T) and growth speed (α) are computed for *Escherichia coli* (*E. coli*) and *Staphylococcus*

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epidermidis (*S. epidermidis*) grown in BHI broth. The bacteria are tentatively represented by a characteristic vector of the components T and α. From the characteristic population on the two dimensional space, two bacteria are well discriminated.

Experimental method and materials

Impedance measurement

The transparent styrol cells (W × H × L = 3.2 × 1.6 × 6.4 cm³) were used for simultaneously measuring impedance and turbidity changes. Four stainless steel wires (0.7 mmϕ) or gold wires (0.7 mmϕ) were attached to the lid as shown in Fig. 1. Fig. 2
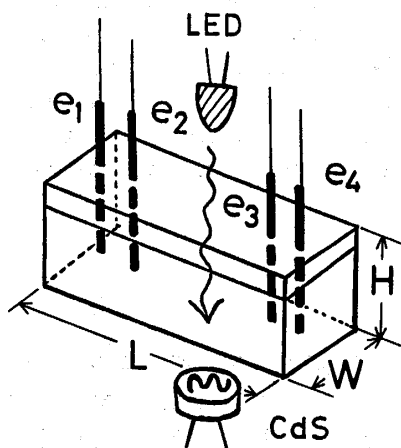

Fig. 1   Schematic view of cell configuration. Thick lines e_1, e_2, e_3, and e_4 are stainless steel or gold electrodes. An a.c.-current is applied across e_1 and e_4, and impedance change is observed by measuring potential between e_2 and e_3. Transmitted light of light emission diode (LED) through the culture cell is detected by photosensor element of CdS-cell.
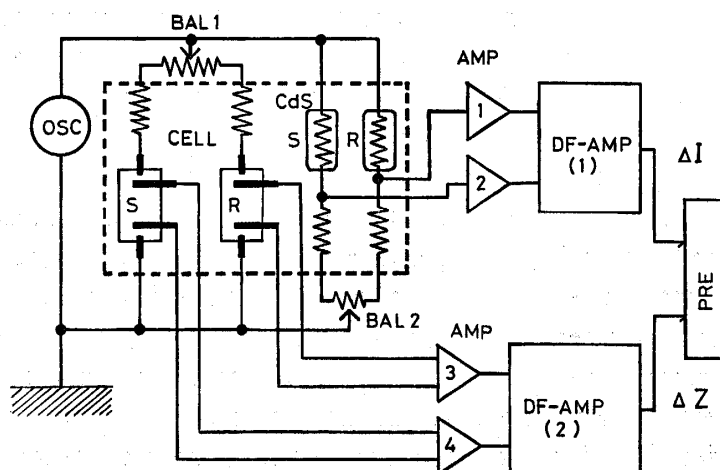

Fig. 2   Block diagram of measuring system. OSC supplies sinusoidal current of 1 KHz to the bridge circuit composed by culture-cell and CdS-cell. AMP1, AMP2, AMP3 and AMP4 are d.c.-amplifiers with high input impedance. The output signal from the electrical circuit DF-AMP(1) and DF-AMP(2) are proportional to the changes ΔI and ΔZ, respectively, and recorded on the chart of pen-recorder (PRE). BAL1 and BAL2 are balancing variable resistors.

exhibits the block diagram of measuring system. The alternating current of 1 KHz (OSC) was applied to current electrodes e_1 and e_4. The magnitude of the current was about 60 μA. Impedance change was monitored by measuring the potential between e_2 and e_3. To increase the magnitude of impedance change, the distance between e_2 and e_3 was made as large as possible (4 cm). The differential signal was picked up by a pair of d.c.-amplifiers (AMP3, AMP4; imput impedance $\doteqdot$ 100 MΩ). Thus we have measured the potential without current through the potential electrodes. The output signal from each amplifier was led to the electrical circuit (DF–AMP(2)) composed of a narrow band pass filter ($Q \doteqdot 80$ at 1 KHz), average detector, low pass filter, and differential d.c.-amplifier. The smoothed output signal was recorded on the chart of two-pen recorder (PRE).

To compare the data of four-electrode method with those of two-electrode method, impedance change was also measured between current electrodes e_1 and e_4, when the potential electrodes e_2 and e_3 were allowed to stand.

Turbidity measurement

The light emission diode (LED) of red was used as a conventional light source. The light was vertically irradiated from the top of the cell (see Fig. 1). The resistance change of CdS due to the change in the intensity of transmitted light was detected by a bridge circuit as shown in Fig. 2. The difference signal between sample element (S) and reference element (R) was amplified by a pair of amplifiers (AMP1 and AMP2). Its output was led to the circuit (DF–AMP(1)) which was the same composition as DF–AMP(2). The rectified signal was recorded on the chart (PRE) together with impedance change. When the change in the intensity of transmitted light was small, the change (ΔI) was proportional to that of CdS resistance. The turbidity τ was given in the usual form as

$$\tau = \log (I_0/(I_0 - \Delta I)) = \Delta I/I_0, \tag{1}$$

in which I_0 was the initial intensity of transmitted light and the relation of $\Delta I/I_0 \ll 1$ was assumed.

Preperation of materials

The culture broth was Brain Heart Infusion (BHI) (DIFCO), which was reconstituted from dehydrated media according to manifacture directions. The test bacteria samples were *E. coli* and *S. epidermidis* which were isolated from clinical specimens and identified as described usual manner in the clinical laboratory of Medical School of Yamaguchi University. The test bacteria were the culture of overnight grown in BHI.

Practical procedure of measurement

The cells were soaked in germicidal liquid soap for about one hour and washed for several hours in water. The sterilized BHI was poured into the sample cell (S) and reference cell (R). After the sample cell was inoculated, the cell was sealed and set in

the box surrounded by dotted square as shown in Fig. 2. The box was controlled within 0.1°C at 37°C by thermo-modules. Bridge balances (BAL1 and BAL2) were taken after about 30 minutes to allowing thermal equilibrium. The bacterial growth was estimated to be negligible during that time.

Two traces on the chart was proportional to the turbidity and the impedance change, respectively. Turbidity was corresponded to Eq. (1), and impedance change ratio was represented in the following;

$$\delta Z = \varDelta Z / Z_0, \qquad (2)$$

in which Z_0 was the initial value of impedance.

Results

Firstly, we compared two- and four-electrode methods. Fig. 3A and Fig. 3B show typical examples measured by fresh and re-used electrodes, respectively (gold electrodes). The curves E2 and E4 were transcribed from the measured by two- and four-electrode methods, respectively. In the case of two-electrode method, the ratio δZ changes monotonically in fresh electrodes as Fig. 3A, but that does not reproduce in the second try (re-used electrodes) as shown in Fig. 3B. Two phasic change is observed. In contrast to this, the reproducible and monotonic change can be found in four-electrode method (E4), although δZ is smaller than two-electrode method (E2).

Figs. 4A and 4B exhibit typical δZ and τ computed from the data measured simultaneously using four-electrode method (stainless steel electrodes). About 10^5 cells per ml of $E.\ coli$ and $S.\ epidermidis$ were inoculated in BHI. In the case of $E.\ coli$
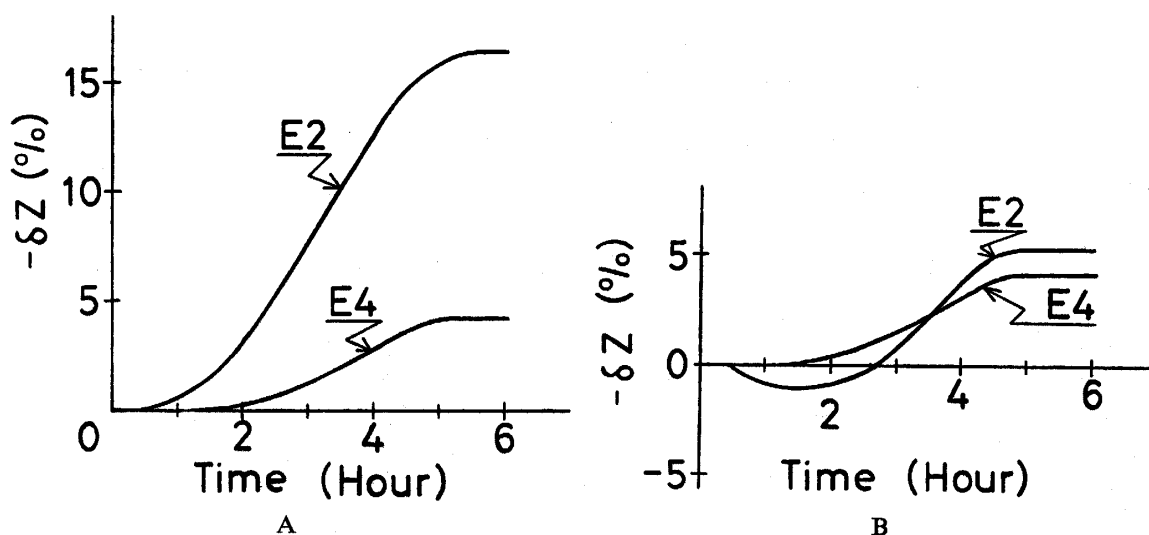

Fig. 3   Time course of impedance decrease reduced by the initial impedance value. Curves E2 and E4 are the changes measured by means of two- and four-electrode methods, respectively. 10^5 cells/ml of $E.\ coli$ are inoculated in BHI broth.

A: Curves measured by fresh electrodes.
B :   Curves measured by re-used electrodes.

(Fig. 4A), an appreciable change of $\tau(\risingdotseq 10^{-3})$ can be found at about one hour after inoculation (curve TR). This appearance time was inversely proportional to the initial concentration of bacteria. In the logarithmic scale, τ increases linearly with time up to the stationary phase of about 4 hours as shown in Fig. 4A. The curve shifted to the side of earlier or later time without changing the slope of the linear part or shape, when we increased or decreased the initial concentration of bacteria (10^3–10^7 cells per ml). It supports that τ is proportional to the bacterial concentration. The slope represents the growth speed α (or the inverse of the generation time) of bacteria. The values of τ at stationary phase were the same in regardless of the initial concentration.
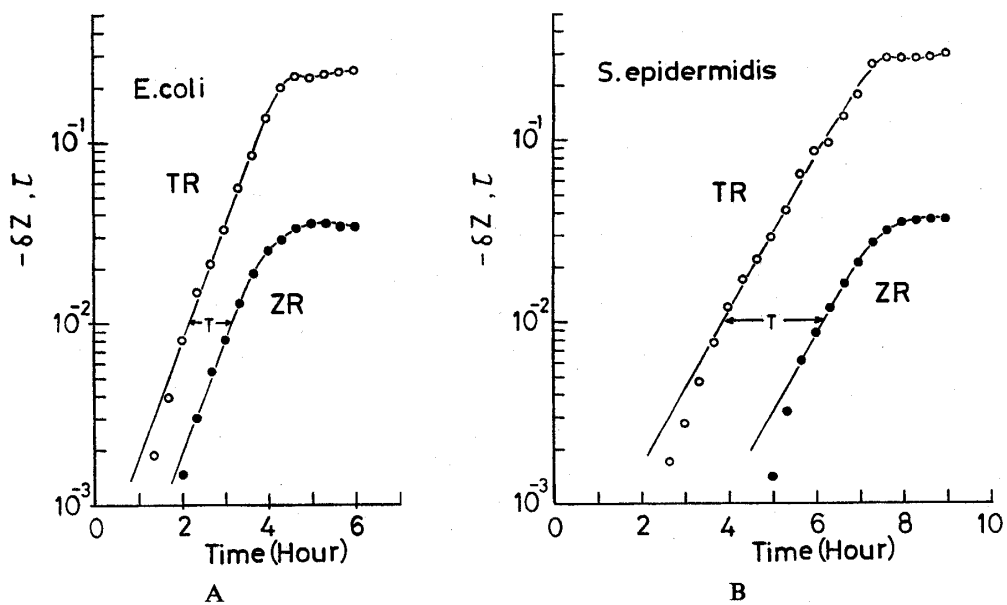

Fig. 4 Turbidity and impedance changes by simultaneous measurement. The curve ZR indicates the reduced impedance decrease δZ computed from the data on the chart. The curve TR is that for turbidity τ.

A: About 10^5 cells of $E.$ $coli$ are inoculated in BHI broth.
B : About 10^5 cells of $S.$ $epidermidis$ are inoculated in BHI broth.

The impedance change ratio δZ is plotted on the semi-log graph (curve ZR) as Fig. 4A. The gradual change of δZ is completely different from other papers or our previous reports, which were used two-electrode method in impedance measurement[5,6]. The δZ begins to rise on the line of 10^{-3} at about 2 hours after inoculation. This time is rather earlier than the previous reports[7], in which the time was about 2.5 hours for this concentration. The δZ behaves in parallel way with τ and increases linearly on the logarithmic scale with the progress of time. The linear part has the same slope as τ. The time interval between two parallel lines is illustrated by the symbol T. In this case T is about one hour. From the linear part of TR and ZR, we obtain about 20 minutes for the generation time (α^{-1}) of $E.$ $coli$. This value agrees with the published data[13].

Similar change of τ and δZ are also seen for $S.$ $epidermidis$ (Fig. 4B). The increase

of τ is slower than *E. coli* and its appreciable change is found at about 2.5 hours. With increasing or decreasing the initial concentration, the curves of τ and δZ showed the same behaviors as *E. coli*. From the slope of the linear part, the generation time is estimated to be about 50 minutes and 2.5 times longer than *E. coli*. *T* is about 2.5 hours in this case for *S. epidermidis*. This wide linearity of ZR at log phase was not found previously[6,7]. The parallel change of δz and τ means that four-electrode method is very superior in quantitatively measuring the impedance change of culture medium to detecting bacteria.

Discussion

By four-electrode method, we could get impedance change going in parallel way to turbidity change. The reproducibility of impedance was better than two-electrode method. The impedance had only the resistive component for the frequency range of 10 Hz–10 KHz. This result implies that impedance change measured by four-electrode method comes dominantly from the resistance change of the medium. In fact, in the usual two-electrode method, large impedance change was verified to be due to a large reactive component (unpublished data), and the component became larger in lower frequencies. One reason is that the electric double layer is build up on the surface of electrodes[2,5]. Four-electrode method can exclude such surface effects[9].

Based on a simple phenomenological theory, let us consider the curves TR and ZR in Fig. 4A and 4B. Suppose that the metabolic period is the same for individual bacteria at log phase and the produced materials by metabolic process is accumulated in the culture medium. The ratio δZ is approximately expressed by

$$\delta Z \propto n_0 2^{\alpha(t-T-t_1)} \tag{3}$$

where n_0 is the bacterial concentration at $t = t_1$, α is the growth speed and the delay time T is proportional to metabolic time. The parameter T will be shown later to be the time interval T introduced in the preceding Figs. 4A and 4B. The τ is approximated as

$$\tau = \Delta I / I_0 = n_0 2^{\alpha(t-t_1)} \tag{4}$$

Eq. (3) is different from Eq. (4) by a numerical factor $2^{\alpha T}$. Two parallel lines are obtained by plotting τ and δZ versus time t on the semi-log graph. The lines seem to coincide with the linear part of curves TR and ZR in Figs. 4A and 4B excepting deviation from the lines at early time. This deviation is due to the smallness of differences ΔZ and ΔI.

To determine t_1 and n_0, the bacterial growth was examined by performing simultaneously conventional plate count and measurement of τ. The count number was well distributed around the curve of τ shown in Fig. 5. This confirms the proportionality of τ to the bacterial concentration. The linear part of τ was extrapolated to the axis of $t=0$, then time t_1 estimated to be near to zero and the crossing point gave the initial turbidity corresponding to n_0. The n_0 could be evaluated by taking into
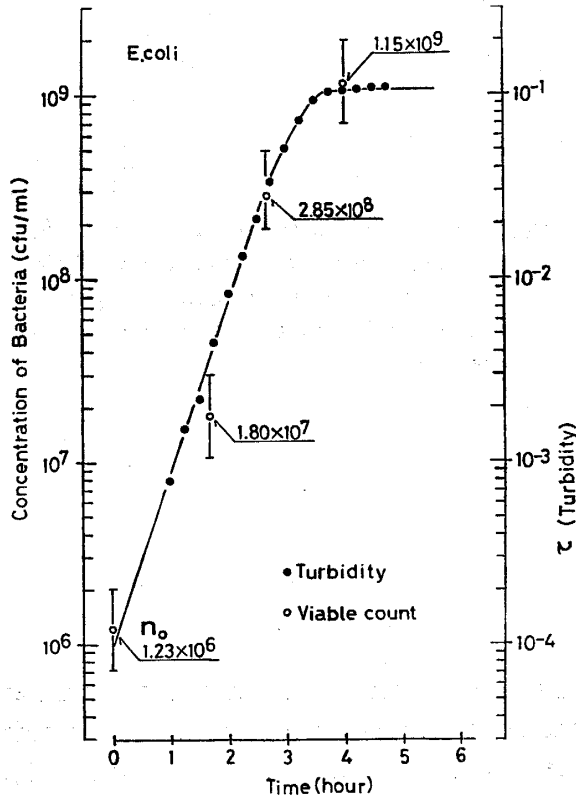
Fig. 5 Comparison of turbidity change and bacterial concentration evaluated by conventional plate count method for *E. coli* grown in BHI broth. Filled circles are computed turbidity from the data, and open circles are the number of bacteria counted by plate count method.

account of the maximum of the concentration, for example, about 10^9 cells per ml for *E. coli*. The evaluated values agreed well with the inoculated concentration.

We used the current of about 60 μA for measuring the impedance. There should be anxious about the effect of current on the growth of bacteria. To examine this, the turbidity change was studied under the current of 100 μA and 0 μA. However, the difference in turbidity change could not be observed for these currents, so we concluded that 60 μA was safe.

We obtained two parameters α and T. The parameters should have the proper values for bacteria under growing conditions such as temperature, pH, medium, antibiotics or drugs. The parameters T and α have not been established as characteristic ones so far. The T has the dimensions of time and is expected to proportion to the metabolic time of individual bacteria. Another parameter α has the inverse dimension of time and expresses the growth speed of individual bacteria. Although much discussion are needed for T and α as characteristic parameters identifying bacteria, we tentatively propose to use a characteristic vector which is composed of two components T and α in a characteristic space. The bacteria are assumed to be expressed by the location of the top of the vector in the two dimensional space. Simultaneous measurements were carried out several times for *E. coli* and *S. epidermidis*. The parameter T could be determined by measuring the time interval at the same value of τ and δZ ($= 10^{-2}$ in Figs. 4A and 4B). Computed parameters are plotted on a two dimensional graph as Fig. 6. The filled triangles are those for *E. coli* and open triangles are for *S. epidermidis*. Although the range of distribution of parameters is a little wide, these
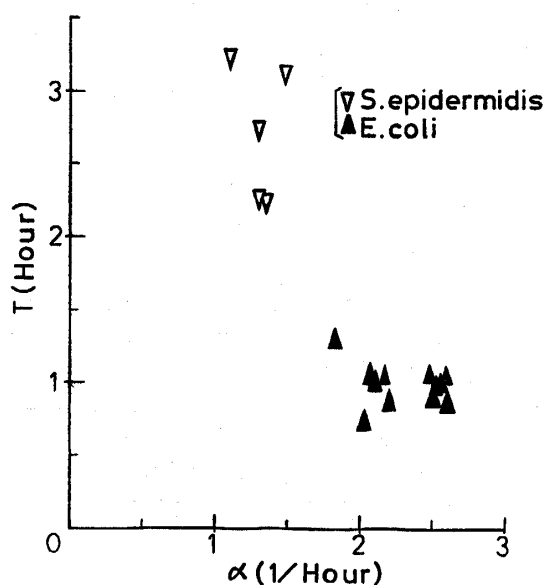
Fig. 6   Plot of evaluated α and T on a two-dimensional characteristic space. Filled triangles and open inversed triangles are those for *E. coli* and *S. epidermidis*, respectively.

two bacteria are well separated.

In the previous sections, rather large cells were described. In clinical use, smaller cells will be favorable. We tried experiments several times for several small cells with the volume 2 to 4 m*l*. The distance between e_2 and e_3 was 1 to 2 cm (see Fig. 1). The electrodes were gold, platinum, and stainless steel wires of 0.7 mmϕ, and *E. coli* was grown in BHI broth. We could get the similar results as Fig. 4A with gold and platinum electrodes. However, the base line drifted sometimes with stainless steel ones. The origins of the fluctuation are not clear at the present stage. This may indicate as other papers that gold or platinum are superior even for small cells[12].

Acknowledgments

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