Electrical Impedance Measurements: Rapid Method for Detecting and Monitoring Microorganisms

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A conceptually simple and easy-to-use technique is described that uses continuous impedance measurements for automated monitoring of microbial growth and metabolism. The method has been applied to a wide range of microorganisms. Optical clarity is not required. The sensitivity and reproducibility of the method are demonstrated. The mechanism whereby microbial growth alters the impedance of the medium is discussed, as well as potential applications of the method to clinical microbiology.

Impedimetric methods in microbiology have received increasing attention since the presentation of two impedance-monitoring systems at the First International Symposium on Rapid Methods and Automation in Microbiology in June of 1973 (2, 11), and the description of a third system at the 75th Annual Meeting of The American Society for Microbiology, 1973 (13).

Since that time, impedance-monitoring instrumentation has been used in blood (4-6) and urine cultures (3, 5).

Although very sensitive and highly automated impedance monitoring is a relatively new procedure, the concept dates back to the last century (9). It has long been known that the medium supporting the growth of microorganisms changes its chemical composition with time as nutrients are consumed and metabolic end products are produced. These changes in medium composition are associated with changes in the impedance—the resistance to the flow of an alternating current conducted through the medium. The use of a reference chamber containing uninoculated medium allows one to compensate for changes in impedance brought about by physical and chemical factors not related to microbial growth such as fluctuations in temperature, absorption of gases, enzymatic and spontaneous changes in chemical composition of the medium, etc.

This paper describes some of the basic features of microorganism impedance changes monitored by a BACTOMETER 32 microbial monitoring system (Bactomatic, Inc., Palo Alto, Calif.), a 32-channel automated impedance bridge designed especially for use with microbial cultures. The magnitude, shape, and reproducibility of microbial impedance responses for a wide variety of microorganisms and media were studied. Also discussed are the organism concen-

trations necessary to produce impedance changes and the factors that influence the response. Finally, the potential for using impedance measurements to automate various microbiological procedures of importance to the clinical microbiologist is reviewed.

MATERIALS AND METHODS

Instrumentation. The impedance-measuring instrument (and accessories) used in all experiments was the BACTOMETER 32 microbial monitoring system (Bactomatic) (Fig. 1). This instrument has been described by Hadley and Senyk (5). It has two chambers for each measurement, a sample chamber and a reference chamber, both of which are filled with media but only one of which is inoculated with microorganisms. The instrument measures the ratio, $Z_R/(Z_R + Z_S)$, where Z_S and Z_R are the impedances in the sample and reference chambers, respectively. By measuring this ratio, the instrument cancels out many factors, such as fluctuations in temperature, which affect the impedance of both sample and reference chambers simultaneously.

A universal serial interface was constructed to enable data measured by the instrument to be processed or stored by a Data General Nova 1220 minicomputer (Data General, Southboro, Mass.) equipped with a Ball Computer Products (Sunnyvale, Calif.) dual-disk drive-memory capable of 5.0-Mbytes storage. This latter feature greatly facilitated the accumulation and interpretation of very large quantities of data. In addition, the instrument was modified so that the frequency of the applied sinusoidal signal could be varied to certain set values between 200 Hz and 30 kHz on command from the computer.

Electrodes. Unless otherwise noted, experiments were performed at 2 kHz, using clusters of impedance chambers known as modules (Fig. 2). These contained a pair of vertical stainless-steel electrodes arising from a stainless-steel lead frame embedded in plastic and projecting into each chamber. A module consists of eight sample and eight reference chambers made of styrene acrylonitrile copolymer plastic. The modules

FIG. 1. Impedance-monitoring instrument (BAC-TOMETER 32 microbial monitoring system) and strip chart recorder.

FIG. 2. Drawing of impedance sample chambers (module) with cut-away showing vertical stainless-steel electrodes arising from embedded stainless-steel lead frame.

were sterilized by either ethylene oxide or by radiation. The chambers of the modules were sealed with tape.

Impedance measurements. Sterile modules were aseptically filled with 1.0 ml of sterile medium in the reference chamber and an equal amount of inoculated medium in the sample chamber. All chambers were sealed, and the module was inserted into the connector within the incubator portion of the instrument. Impedance ratios were automatically recorded every 96 s on the strip chart recorder or sent in digital form to the computer by means of the interface.

Changes in impedance ratio have been converted to changes in the impedance of the sample for all data presented in this paper.

Detection of microbial growth. Impedance changes due to microbial growth accelerate with time and thus can be distinguished from noise and drift arising from nonmicrobial factors such as a fluctuations in temperature. The impedance change due to microbial growth is defined as an accelerating change in the impedance that is equal to or greater than 0.8% of the base line impedance when the measuring signal is at 2,000 Hz. This relatively large change in impedance was chosen to clearly distinguish microbial responses from noise and drift.

Cultures and media. *Escherichia coli* and *Pro-*

teus vulgaris cultures were derived from American Type Culture Collection cultures 12015 and 6380, respectively. All other cultures were clinical isolates. Commercially dehydrated or concentrated media were used and reconstituted according to the manufacturer's directions. Brain heart infusion broth (BHI), Trypticase soy broth (TSB), Trypticase soy agar, and Columbia broth were obtained from Baltimore Biological Laboratory, Cockeysville, Md. Medium 199, with Hanks balanced salts solution 10-times concentrated without NaHCO_3 (Microbiological Associates) was reconstituted according to the manufacturer's directions.

Plate counts. Plate counts were taken from duplicate samples incubated in identical modules in the same incubator from which no measurements were being recorded. The duplicate modules avoided the brief disturbance in impedance measurements that follow sample taking and allowed more frequent and larger portions of the sample to be taken. All plate counts were done in triplicate on Trypticase soy agar plates incubated at 35°C.

RESULTS AND DISCUSSION

(i) Spectrum of microorganisms causing impedance change. The basis of impedance monitoring is assumed to be changes in chemical composition of the medium brought about by metabolic processes occurring within the microbial cell, or its surface, or by exogenous microbial enzymes in the medium. These processes bring about a corresponding change in the conductivity of the medium and, to a smaller degree, a change in the capacitive reactance of the medium. In addition, there is a change in the composition of the double layer of charged materials adsorbed onto the electrodes that has a very strong effect upon the surface capacitive reactance of the electrode. Since impedance is a complex entity made up of a resistive or conductive component and a reactive component, all of these processes could contribute to the impedance change measured during microbial growth (3, 8). Any organism that alters medium composition by its growth or metabolism should produce a corresponding change in the impedance measured by electrodes in the medium.

To test this hypothesis, a wide range of microorganisms was tested for the ability to produce impedance change when growing in a variety of media. These organisms are shown in Table 1, where it can be seen that aerobic and anaerobic bacteria, yeasts, molds, and mycoplasma are represented. To date, every microorganism that showed macroscopic evidence of growth throughout a broth culture has been shown to produce an impedance change as well. Organisms that produced microcolonies or grew only on the medium surface may have produced weak or undetectable impedance changes even though

TABLE 1. *Microorganisms capable of producing an impedance change in a variety of media*

Bacteria	Bacteria cont.
<i>Alcaligenes faecalis</i>	<i>P. diminuta</i>
<i>Acinetobacter calcoaceticus</i>	<i>Salmonella enteritidis</i>
<i>Bacillus stearothermophilus</i>	<i>S. typhimurium</i>
<i>B. subtilis</i>	<i>Serratia marcescens</i>
<i>Bacteroides clostridioformis</i>	<i>Shigella sonnei</i>
<i>B. fragilis</i>	<i>S. flexneri</i>
<i>B. melaninogenicus</i>	<i>S. dysenteriae</i>
<i>B. ochraceus</i>	<i>Staphylococcus aureus</i>
<i>Citrobacter diversus</i>	<i>S. epidermidis</i>
<i>C. freundii</i>	<i>Streptococcus faecalis</i>
<i>Clostridium bifermentans</i>	<i>S. pyogenes</i>
<i>C. perfringens</i>	<i>Streptomyces griseus</i>
<i>C. sporogenes</i>	<i>Zymomonas anaerobia</i>
<i>C. tyrobutyricum</i>	
<i>Enterobacter aerogenes</i>	Mycoplasma
<i>E. cloacae</i>	<i>Acholeplasma laidlawii</i>
<i>Escherichia coli</i>	<i>Mycoplasma fermentans</i>
<i>Eubacterium limosum</i>	<i>M. hominis</i>
<i>Fusobacterium nucleatum</i>	<i>M. pneumoniae</i>
<i>F. symbiosum</i>	<i>M. pulmonis</i>
<i>Haemophilus influenzae</i>	<i>Ureaplasma urealyticum</i>
<i>Klebsiella ozaenae</i>	
<i>K. pneumoniae</i>	Yeasts
<i>Lactobacillus brevis</i>	<i>Candida albicans</i>
<i>L. casei</i>	<i>C. parapsilosis</i>
<i>Mycobacterium phlei</i>	<i>Saccharomyces cerevisiae</i>
<i>Pediococcus cerevisiae</i>	<i>Torulopsis glabrata</i>
<i>Peptococcus prevotii</i>	
<i>Peptostreptococcus anaerobius</i>	
<i>Proteus mirabilis</i>	Mold
<i>P. vulgaris</i>	<i>Aspergillus niger</i>
<i>Pseudomonas aeruginosa</i>	

macroscopic growth was evident. The list in Table 1 is not intended to be inclusive, but rather illustrative.

Typical impedance changes recorded for various microorganisms are shown in Fig. 3, which shows the percent decrease in impedance produced by cultures of *Klebsiella pneumoniae*, *E. coli*, and *Staphylococcus aureus* growing in TSB.

(ii) **Threshold of response.** A certain concentration of microorganisms, known as the threshold concentration, was necessary before

there was a detectable impedance change. Concentrations of organisms greater than threshold produced an immediate impedance change. However, low initial concentrations of organisms could be detected by allowing them to replicate to threshold concentrations. The threshold concentration is a function of the microorganism, the medium in which the organism was growing, and the electrodes used. Furthermore, thresholds were altered by the frequency of the signal being used—lower thresholds were observed at lower frequencies. Finally, the threshold is very much a function of how much impedance change must take place to satisfy the definition of detection. For most practical purposes, we defined detection as an accelerating change in impedance of 0.8%. This is a generous measure, readily seen and unlikely to be caused by nonmicrobial causes. By this definition, thresholds generally range between 10^6 and 10^7 organisms per ml. Some representative thresholds defined in this way are shown for various organisms in Table 2.

(iii) **Detection times.** Microorganism detection times, i.e., the times required for the organisms to grow to threshold concentrations, are a function of the initial concentration of microorganisms, the generation time of the organisms or population of organisms, and the lag phase in a particular medium. For a given organism or population in a given medium, detection times increase as the initial concentration becomes smaller. Detection times graphed against the log of the initial concentration generally fall along a straight line (Fig. 4) due to the exponential growth of the organisms. This relationship between detection time and initial concentration enables one to classify samples according to bacterial levels (early detection times indicating high bacterial concentrations and late detection

FIG. 3. Representative impedance curves for three common microorganisms. Percent impedance decrease is graphed against time in hours for *K. pneumoniae* (solid curve), *E. coli* (long dashed curve), and *S. aureus* (short dashed curve).

TABLE 2. Typical generation times and thresholds for a variety of microorganisms^a

Organism	Medium ^b	Generation time (min) ^c	Approx. threshold (per ml) ^d
Bacteria			
<i>Escherichia coli</i>	TSB	20	2×10^7
<i>E. coli</i> (SS)	BHI	20	2×10^7
<i>Proteus vulgaris</i>	TSB	20	2×10^7
<i>P. vulgaris</i> (SS)	BHI	25	4×10^7
<i>Salmonella enteritidis</i>	BHI	20	10^7
<i>Klebsiella pneumoniae</i> (SS)	BHI	25	4×10^7
<i>Pseudomonas aeruginosa</i>	BHI	25	5×10^6
<i>P. aeruginosa</i> (SS)	BHI	25	3×10^7
<i>Staphylococcus aureus</i>	BHI	35	4×10^6
<i>Streptococcus pyogenes</i>	Todd-Hewitt broth	60	4×10^6
<i>Lactobacillus brevis</i> (32°C)	Universal beer broth	90	5×10^7
<i>Pediococcus cerevisiae</i> (32°C)	Universal beer broth	240	5×10^7
<i>Neisseria gonorrhoeae</i>	Thayer-Martin broth	45	2×10^7
Fungi			
<i>Saccharomyces cerevisiae</i> (32°C)	GPYE broth	90	10^6
<i>Candida albicans</i> (32°C)	TSB + 2 % glucose	45	4×10^6
<i>Aspergillus niger</i> (32°C)	GPYE broth	70	10^7
Mycoplasma			
<i>Acholeplasma laidlawii</i>	PPLO broth	210	10^7
<i>Ureaplasma urealyticum</i>	Urea medium	150	2×10^6

^a Unless otherwise indicated, all organisms were grown without agitation at 35°C. All organisms were monitored with gold-plated printed-circuit board electrodes except organisms noted with (SS) where vertical stainless-steel electrodes were used. This table is from A. N. Sharpe and P. S. Clarke (ed.), *Mechanizing microbiology*, 1978. Courtesy of Charles C Thomas, Publisher, Springfield, Ill. (3).

^b GPYE, Glucose-peptone-yeast extract.

^c Determined by plate count.

^d Thresholds correspond to detection defined as 0.8% impedance change and have a standard deviation of roughly 0.2 log when the same electrodes, organism strains, and media are used.

FIG. 4. Detection times in hours graphed against initial concentration of microorganisms for *E. coli* ($\blacktriangle$) and *S. aureus* ($\circ$) both growing in TSB at 35°C. The solid lines represent least-squares linear fits to the data points for each organism. The slope of the line reflects the organisms' generation times (26 min for *E. coli* and 38 min for *S. aureus*). The correlation between log initial concentration and detection time was 0.95 for *E. coli* and 0.94 for *S. aureus*.

times signaling low concentrations), or to obtain rough estimates of the microorganisms' generation time (by dividing the delay between detections of two different dilutions by the number of times the population would have to double to increase from the smaller to the larger initial concentration).

(iv) **Replicate agreement.** Figure 5 shows the change in impedance with time when two concentrations (1×10^3 and 1×10^5 organisms per ml) of *E. coli* are grown in TSB at 35°C. The curve represents the mean impedance change of 15 replicates of each dilution. The vertical bars represent ± 1 standard deviation from the mean of the impedance change from base line at the times indicated. The base line is established after the first 0.5 h of measurement, since there is considerable nonmicrobial impedance change occurring initially as the module comes to thermal equilibration with the incubator and the electrodes equilibrate with the medium. The arrows denote the mean detection times, and the horizontal bars represent 1 standard deviation before and after the detection time. These standard deviations (approximately 10% of the mean

FIG. 5. Percent impedance decrease with time when *E. coli* in two concentrations (10^3 and 10^5 organisms per ml) is grown in TSB at 35°C . The curve represents the mean of 15 replicates. The vertical bars represent 1 standard deviation above and below the mean. Detection times, defined as the time when 0.8% accelerating change in impedance has taken place, are depicted by the vertical arrows. The horizontal bars represent the detection time standard deviation before and after the mean.

response and 0.3 h for the detection times) indicate the high degree to which the impedance responses from these two dilutions of *E. coli* can be discriminated from each other and from the base line.

(v) **Relationship of impedance change to change in microorganism concentration.** Figure 6 shows a comparison of impedance change with viable plate counts for *E. coli* growing in medium 199 and in Columbia broth. These media were selected to represent a completely defined although complex medium on the one hand, and a medium rich in peptones but ill-defined chemically on the other hand. For both media, the cumulative impedance change (upper graphs) increases roughly exponentially with about the same doubling time as the viable count (lower graph). In fact, the correlation coefficients between the log of the cumulative impedance change and the log of the viable counts ranged between 0.96 and 0.99 for the four pairs of curves (two dilutions in two media) shown in Fig. 6.

Upon closer examination, however, it can be seen that there are some significant differences between the viable count and impedance curves. In the Columbia broth, for example, the cumulative impedance change shows a small plateau,

flattening out for about an hour when the viable counts reach 10^8 colony-forming units per ml, and then resuming their previous rate of increase. There is no evidence of such a plateau in the viable count curves.

It should also be noted that the impedance stops changing before the microorganisms reach stationary phase. This, together with data from other organism/medium combinations in which the impedance continues to change long after the organisms are in stationary phase (5), serves to remind one that it is most probable that it is metabolic activity rather than cell numbers that is being recorded by impedance changes. To the extent that the rate of microbial metabolism parallels microbial growth, impedance change may serve to estimate growth rates. However, this relationship must be established for each organism, medium, and type of electrode under consideration.

Finally, observe that in Fig. 6 the onset of impedance change coincides with concentrations of microorganisms well below the threshold levels (corresponding to 0.8% impedance change). The practice of defining detection in terms of a substantial (0.8%) change reduces the chance of confusing drift and noise with a microbial response at the expense of a higher threshold.

FIG. 6. Comparison in two media, Columbia broth (COL) and medium 199 (M 199), of impedance decrease with time and microorganism growth with time. The upper two graphs depict the percent impedance change with time in hours. The lower two graphs depict the number of organisms determined by plate count (©) using the same time axis. *E. coli* was grown at two initial concentrations. The higher is shown by a solid line. The lower by a dashed line. Both percent impedance change and the number of organisms are graphed along logarithmic axes of the same scale to allow comparison of the slopes.

(vi) Characteristic impedance responses.

There is some evidence that the shape of the impedance response may be, to some degree, characteristic of the organism, medium, and electrodes used to produce it. For example, Fig. 7 shows impedance responses from two strains each of *E. coli*, *K. pneumoniae*, *P. vulgaris*, and *Pseudomonas aeruginosa*, all growing at 35°C in BHI.

There are clearly some features common to all these organisms and some features which suggest that there may be characteristic profiles for each species (especially *P. aeruginosa*) as

has been suggested for microcalorimetric thermograms (1, 7). More data is needed with a greater number of strains of each species to confirm this point.

(vii) **Mechanisms of impedance change.** Which chemical events taking place during microbial growth are responsible for the impedance change measured in these experiments? Changes in pH, although a frequent concomitant of microbial growth, cannot be evoked, since strong impedance decreases are noted with acid producers such as *E. coli* growing in TSB and ammonia producers such as *P. mirabilis* growing in urea broth. In the former case, the pH is decreasing, in the latter, it is increasing. Yet the impedance decreases in both cases. Changes in the conductivity of the medium may be a more likely contributor to impedance change, however. Conductivity changes have been frequently reported to accompany microbial growth (3, 5), since uncharged nutrients become converted by metabolic processes into charged end products. Changes in conductivity are changes in the resistive component of impedance. The relationship is given by:

$$Z^2 = R^2 + X_c^2$$

where R is the resistance and X_c is the capacitive reactance; and $X_c = 1/(2 \pi fC)$, where f is the frequency and C denotes the capacitance. By measuring the impedance at different frequencies, the relative contributions of the capacitive reactance and resistance can be obtained. To test the hypothesis that it is the conductance (or resistance) which changes in the medium, *E. coli* was grown in TSB at 35°C and the frequency of the applied signal varied from 400 Hz to 30 kHz. The impedance response at these frequencies for a single culture is shown in Fig. 8. There is a considerable increase in the impedance change at lower frequencies and a corresponding reduction in threshold as well. Furthermore, these data show that although there are measurable changes in conductivity, it is primarily the reactive component which is altered during microbial growth. These data are confirmed as well by variable cell-length measurements (as discussed by Schwan [8]). We speculate that the observed change in reactance results from the charged double layer on the surface of the electrodes which is altered during microbial growth. The exact mechanism remains to be elucidated.

(viii) **Applications.** Impedance measurements provide a convenient way of monitoring microorganism growth. In addition, they provide a method for the automation of tests of sterility, such as blood and spinal fluid cultures, and for

FIG. 7. Rate of impedance decrease graphed against time for two strains each of *E. coli*, *K. pneumoniae*, *P. vulgaris*, and *P. aeruginosa*. All organisms were grown at 35°C in BHI and monitored with stainless-steel electrodes and a frequency of 2,000 Hz.

FIG. 8. Percent impedance decrease graphed against time for *E. coli* growing in TSB at 35°C and monitored at frequencies of 400, 2,000, and 30,000 Hz.

rapid screening for high bacterial concentrations, such as in urine cultures or food-quality assurance. By the use of multiple samples, rapid automated antibiotic susceptibility testing and bacterial identification may be possible. Imped-

ance measurements lend themselves readily to automation; many samples can be processed at one time without mechanical movement of sample holder or sensor. Optical clarity is not a requirement, and thus opaque samples are mon-

itored as easily as optically clear samples. Finally, there is little or no sample preparation required, and the equipment is simple to use.

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