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## Electrochemical changes in media due to microbial growth

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### Summary

Microbial metabolism affected the electrical impedance parameters of a two terminal-measuring cell-containing growth media. The relationship between microbial growth and relative changes in both the capacitive and resistive parts of impedance was examined. Both components of impedance were shown to be indicative of bacterial growth. In low conductivity media the change in the conductance of the media ( $G_{sol}$ ) clearly correlated to bacterial growth. In more conductive media the relative changes in  $G_{sol}$  were smaller, and in these media measurements of the changes of polarization capacitance ( $C_{pol}$ ) were useful for monitoring bacterial growth.

Yeast growth in two media resulted in large changes in  $C_{pol}$  (20–100%) while the changes in  $G_{sol}$  were very small (1–4%). This result indicated that, for some combinations of microorganisms and media, measuring  $C_{pol}$  might be preferable over  $G_{sol}$  for the detection of microbial growth.

Microbial metabolism resulted in a change of 2–2.5 units in pH. This pH change resulted in a 40% change in  $C_{pol}$  but less than a 14% change in  $G_{sol}$ .

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Key words: *Capacitance – Conductance – Impedance – Yeast monitoring*

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### Introduction

#### *Use of impedance in monitoring microbial growth*

The need for rapid automated methods in modern microbiology is well recognized, as demonstrated by the many symposia devoted to this topic in recent years. Different physical and chemical effects of bacterial growth are employed in the different automated systems currently available. The use of electrical effects, especially the impedance change associated with microbial metabolism, is reported to

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have significant advantages for the detection of microorganisms in complex substances, such as food and cosmetics [1, 2] over other methods currently used.

Impedance is the resistance to flow of an alternating current through a conducting material. Impedance is a complex entity composed of a resistive component and a reactive component. Changes in impedance due to microbial growth can be measured by placing an inoculated growth medium into a container equipped with two metal electrodes which contact the liquid. The impedance of such a measuring cell may be represented as a series combination of the bulk impedance of the electrolyte and the interface or polarization impedance of the electrodes. A schematic description of such a system is shown in Fig. 1. For the frequency range of 400 Hz–25 KHz, the bulk impedance is mainly resistive (equal to the reciprocal of the conductance), but the polarization impedance has both resistive and capacitive parts ( $R_{pol}$  and  $C_{pol}$ , respectively) (Fig. 1), which are frequency dependent [3, 4].

There are a few commercial systems which detect the effect of microbial metabolism on the electrical impedance of a two terminal measuring cell [5–7]. The commercial instruments do not use complex impedance directly but rather some function of it. The correlation between microbial growth and impedance has been shown previously [1, 7, 8].

#### *Effect of microbial growth on conductance*

The changes in conductance are associated with changes in the solution or in the bulk electrolyte. As microorganisms metabolize, they create new end products in the medium. Generally uncharged or weakly charged substances are transformed to highly charged end products. For example, the transfer of proteins to amino acids, of lipids to acetate or of carbohydrates to lactate, all create smaller, more

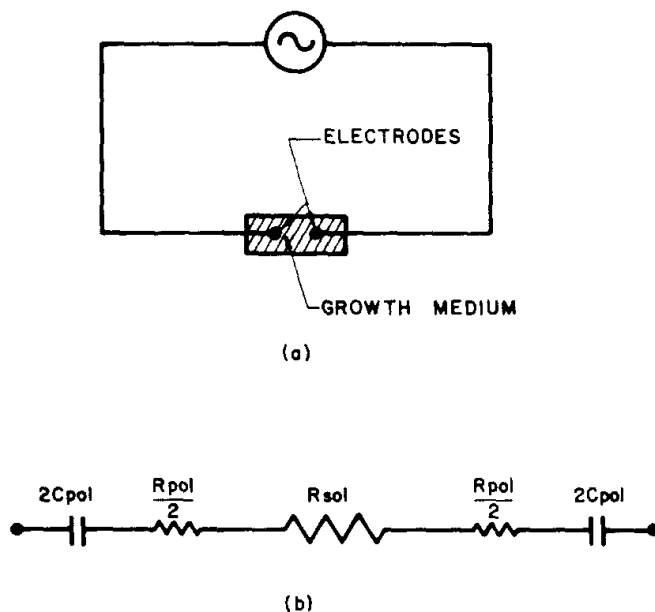


Fig. 1. Sample cell with growth medium between electrodes (a) and equivalent circuit (b).

charged end products. In addition, the molecules formed are smaller and therefore more mobile.

#### *Effect of microbial growth on capacitance*

When a metallic electrode is immersed in a growth medium, a DC-boundary potential is found to exist between the electrodes and the fluid establishing contact with the electrodes. If an alternating current is passed through the electrode into the contact fluid, this DC-contact polarization potential becomes modulated with an alternating potential [9]. The interface region, considered as the two sides together, is electrically neutral with a potential difference across the interface. This arrangement of charged and oriented dipoles in the interface region is called the electrical double layer.

Most attention has been given to the effects of microbial growth on the conductance of the medium. Very little attention has been given to the effect of microbial growth on the electrical double layer or on the electrode polarization. Capacitance of polarization can change due to microbial metabolism since smaller molecules could result in smaller distances between the capacitor plates. The smaller ions formed can also increase the surface of the plates by increasing the concentration of ions in close proximity to the electrode. Richards et al. [6] stated that the polarization capacitance is relatively insensitive to the changes accompanying bacterial growth, and also, it is subject to random fluctuations which are of the same magnitude as those ascribed to the growth of bacteria. However, these results were based on the growth of one microorganism (*E. coli*) in one medium. The purpose of this research was to explore further the relationships between microbial growth, change in polarization capacitance, and change in medium conductivity.

### **Materials and Methods**

#### *Microbiological procedures*

*Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were grown in Plate Count Broth (Difco, PCB), Brain Heart Infusion Broth (Difco, BHI) and, in some cases, Tryptic Soy Broth (Difco, TSB). *Streptococcus pyogenes* (ATCC 19615) and *Staphylococcus epidermidis* (ATCC 12228) were grown only in TSB. The yeasts *Saccharomyces cerevisiae* (ATCC 4097), *Candida utilis* (ATCC 9226) and *Kloeckera apiculata* (ATCC 36356) were grown in TSB and Yeast Carbon Base (Difco, YCB). *C. utilis* was also grown in Orange Serum Broth (Difco). The cultures were incubated in a 100 ml blood culture bottle modified to allow samples to be withdrawn periodically and counted by the standard pour plate method or by the spiral plater (Spiral Systems, Inc., Model C). The bottles were fitted with pairs of 0.64 mm diameter  $\times$  5 mm long stainless steel (type 304, passivated) electrodes spaced 1.0 cm apart for the measurement of electrical impedance.

#### *Impedance measurements*

Impedance measurements were made using a transformer type admittance

bridge similar to those described by Cole [10] and Berberian [11], using a high sensitivity null detector with a tracking filter to eliminate harmonic responses. Admittance (impedance<sup>-1</sup>) was measured at 0.5, 1, 2, 5, 10, 20 and 50 KHz. The admittance readings, which were in terms of parallel conductance and capacitance, were transformed to the equivalent series resistance and capacitance circuit. Then the (bulk) solution resistance (i.e., growth medium resistance) was separated from the polarization resistance by the method given by Schwan [9] which is based on the frequency dependence of the polarization resistance ( $R_{pol}$ ) and the frequency independence of the solution resistance ( $R_{sol}$ ). This method involves the following steps: (a) making an estimate of  $R_{sol}$  from which  $R_{pol}$  is calculated as a function of frequency, (b) plotting  $\log R_{pol}$  against  $\log$  frequency and noting the curvature in the plot at the high frequency end. If the graph curves upward  $R_{sol}$  was estimated too low. The estimate is then adjusted and the process repeated. This procedure was programmed on an Apple II computer. The solution conductance  $G_{sol}$  is simply the reciprocal of  $R_{sol}$  and is related to the solution conductivity  $\sigma$ , by  $\sigma = k G_{sol}$  where  $k$  is the cell constant in  $m^{-1}$ . The cell constant was not calibrated in this work because we were interested in changes in  $C_{pol}$  and  $G_{sol}$  as opposed to their absolute values.

#### *Comparison of capacitance change and plate counts*

A cell suspension of *Saccharomyces cerevisiae* was diluted in 0.1% peptone to  $10^{-6}$ . A total of 38 subsamples of the intermediate dilutions were tested impedimetrically and enumerated by standard methods. As a consequence of the data obtained with the admittance bridge, the Bactometer<sup>®</sup> *Microbial Monitoring System* Model M-120 described previously [5] was modified to allow separate measurements of capacitance and conductance. The  $C_{pol}$  detection times, defined as the onset of acceleration in the capacitance curves, were correlated to Log CFU on Yeast extract-malt extract agar (30°C, 5 days).

#### *Gas detection and pH measurement*

Gas evolution was detected and measured by growing a parallel culture in a 100 ml bottle vented to a simple gas collection apparatus. Experiments involving pH were conducted using TSB, BHI, PCB and YCB. The media pH changes were forced with acetic acid, tartaric acid, citric acid or ammonium hydroxide. Different amounts of 15% acid solution (in the same medium) were added to bottles (fitted with electrodes) containing 50 ml of the appropriate medium. The solution was thoroughly mixed and 2.0 ml aliquots were taken for pH measurements. Impedance was measured after the electrodes had equilibrated. More acid was added and the procedure was repeated.

## **Results**

#### *Impedance change in sterile media*

An initial equilibration period of 0.5–1.5 h was required for both the polarization capacitance and the (bulk) medium conductance. After this stabilization period,

little change was observed in either  $G_{\text{sol}}$  or  $C_{\text{pol}}$  in sterile media. The main factor affecting  $G_{\text{sol}}$  equilibration was temperature.  $G_{\text{sol}}$  had a temperature coefficient of 1.5–2.0%/°C. The equilibration time depended on both the thermal time constant of the test cell and the initial temperature difference between the cell and the incubator. Polarization capacitance had a temperature constant of about 1%/°C. The equilibration of  $C_{\text{pol}}$  involved an electrochemical equilibration in addition to the temperature equilibration. It was observed that agitating the bottle after equilibration caused a second equilibration period of approximately 1 h.

#### *Effect of microbial growth on impedance*

The growth of *E. coli* in a low conductivity medium (PCB + 0.4% glucose) gave a clear change (about 35%) of the conductance which was well correlated with the growth curve (Fig. 2), after the bacterial concentration reached  $10^6$ – $10^7$ /ml. Polarization capacitance also showed a marked response (about 38%), but continued to rise throughout the duration of the experiment, even when the rate of bacterial growth was decreasing.

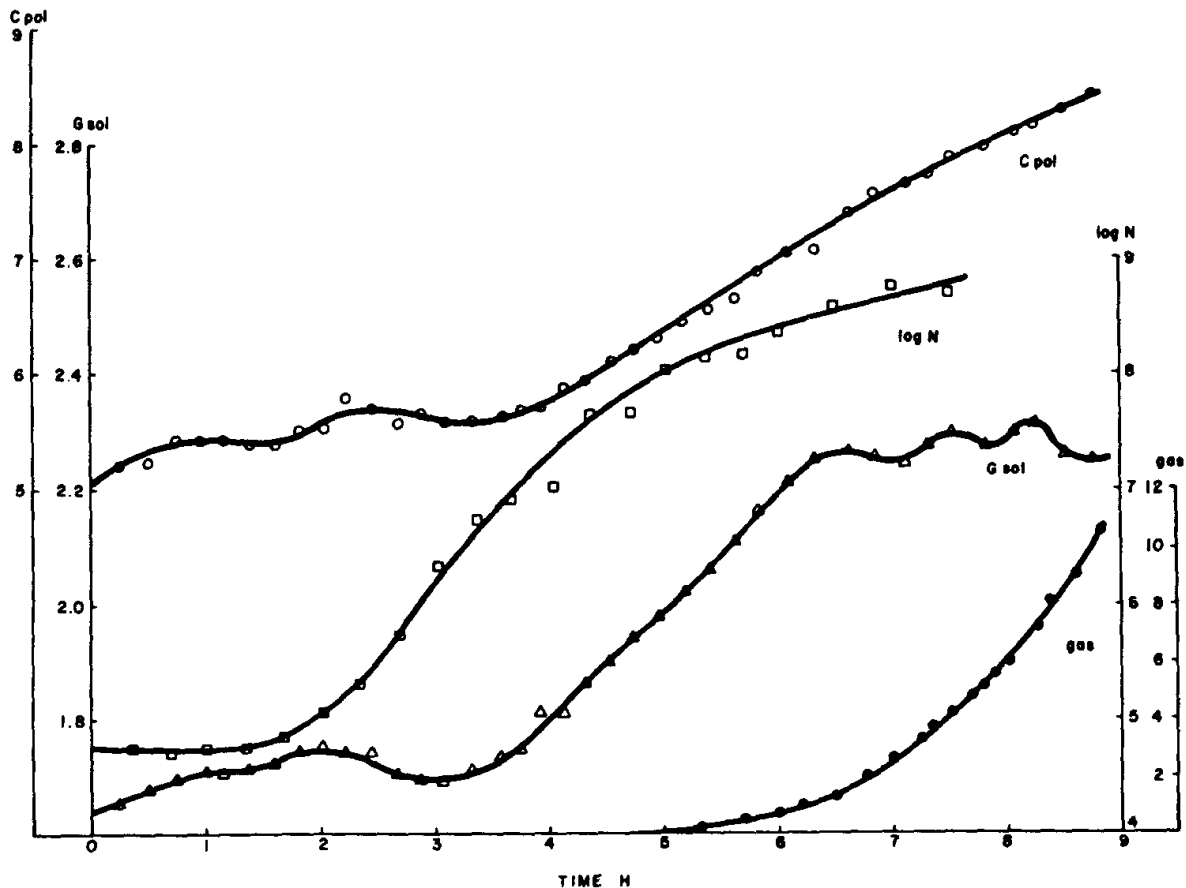


Fig. 2. The effect of growth of *E. coli* in PCB + 0.4% glucose on  $C_{\text{pol}}$ ,  $G_{\text{sol}}$ ,  $\log N$ , and gas evolution.  $N$  is number of bacteria per ml; gas in ml;  $C_{\text{pol}}$  in  $\mu\text{F}$ ;  $G_{\text{sol}}$  in mS.

In all media examined, the evolution of gas was shown by erratic changes in the solution conductance caused by bubble formation near the electrodes. The extent of gas formation was medium-dependent.

When *E. coli* was grown in a high conductivity medium (BHI), the fractional change in medium conductivity was less (6%) and extended over a shorter period of time before the erratic behavior associated with gas evolution commenced (Fig. 3). The change in polarization capacitance was about 31% and was relatively undisturbed by the gas evolution. Repeating experiments showed the same trend in  $C_{pol}$ . The greatest change in  $C_{pol}$  was observed when new electrodes were used. With every subsequent re-use of electrodes, the percent change observed in  $C_{pol}$  diminished.

Bacteria showed various responses in  $C_{pol}$  and  $G_{sol}$  in different media (Table 1). *Pseudomonas aeruginosa* and *E. coli* showed similar  $G_{sol}$  changes in PCB and BHI, similar  $C_{pol}$  changes in PCB, and different  $C_{pol}$  changes in BHI. The fractional changes in  $G_{sol}$  due to growth of *E. coli* in the three media inversely correlated to the conductivity of these media. The growth of *Streptococcus pyogenes* and *Staphylococcus epidermidis* in TSB resulted in slightly higher changes in  $G_{sol}$  than did the growth of *E. coli*.

The growth of all the yeasts tested resulted in large changes in  $C_{pol}$  and relatively

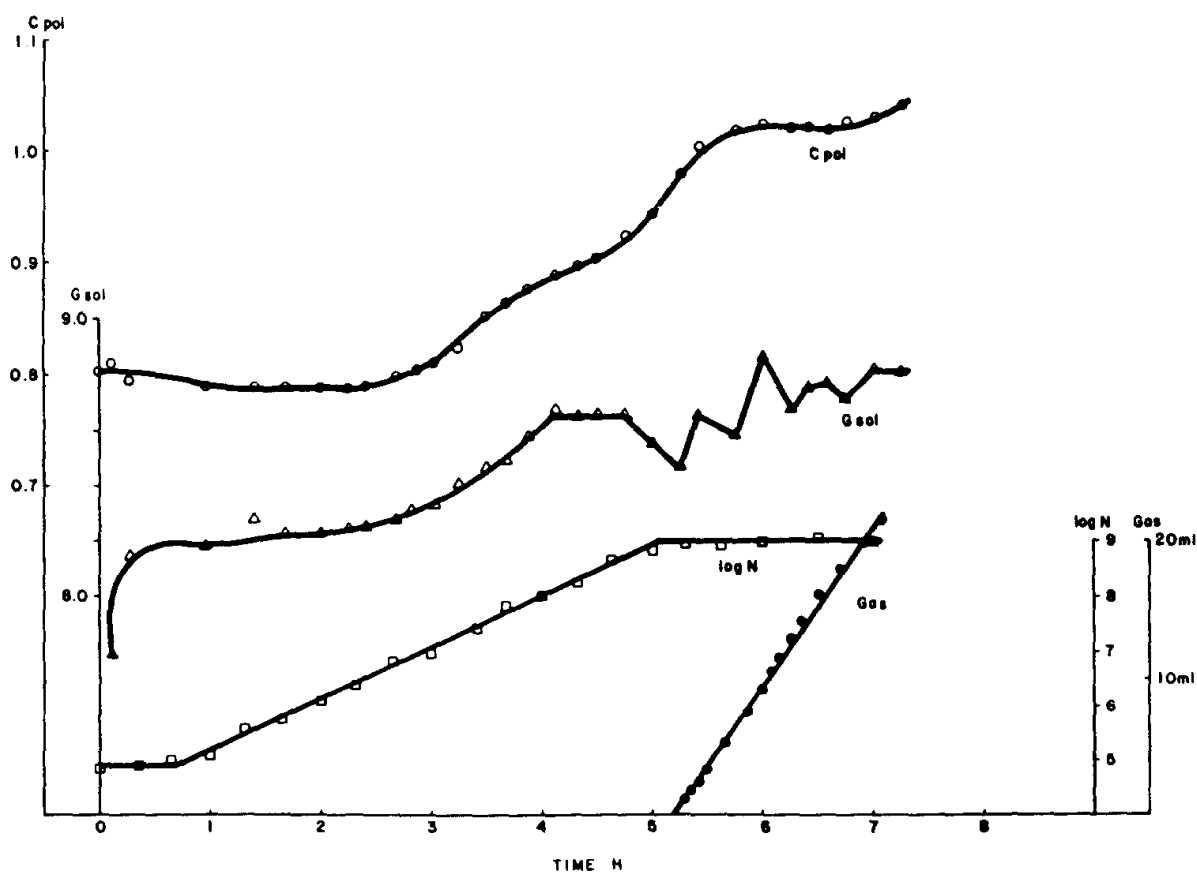


Fig. 3. The effect of growth of *E. coli* in BHI on  $C_{pol}$ ,  $G_{sol}$ , log  $N$  and gas evolution.  $N$  is number of bacteria per ml; gas in ml;  $C_{pol}$  in  $\mu F$ ;  $G_{sol}$  in mS.

TABLE 1  
CHANGES IN  $C_{pol}$  AND  $G_{sol}$  DUE TO MICROBIAL GROWTH

Organism	Medium	Medium conduc- tance (mS)	Percent change in $C_{pol}$	Percent change in $G_{sol}$
<i>E. coli</i>	PCB <sup>1</sup>	1.3	56	35
<i>E. coli</i>	PCB + 0.4% Glucose	1.7	56	35
<i>E. coli</i>	BHI <sup>2</sup>	8.0	70	7
<i>E. coli</i>	TSB <sup>3</sup>	6.9	90	10
<i>Pseudomonas aeruginosa</i>	PCB	1.4	55	40
<i>Pseudomonas aeruginosa</i>	BHI	7.9	23	10
<i>Streptococcus pyogenes</i>	TSB	6.7	42	16
<i>Staphylococcus epidermidis</i>	TSB	6.8	52	13
<i>Saccharomyces cerevisiae</i>	TSB	7.0	103	4
<i>Saccharomyces cerevisiae</i>	YCB <sup>4</sup>	4.5	30	2
<i>Candida utilis</i>	TSB	6.7	20	1
<i>Kloeckera apiculata</i>	YCB	4.7	60	1

<sup>1</sup> PCB, plate count broth.

<sup>2</sup> BHI, brian heart infusion.

<sup>3</sup> TSB, tryptic soy broth.

<sup>4</sup> YCB, yeast carbon base.

small changes in  $G_{sol}$  (Table 1). The growth of *Saccharomyces cerevisiae* in TSB changed  $G_{sol}$  by only 4%, while at the same time  $C_{pol}$  exhibited over 100% increase (Fig. 4). When *C. utilis* was grown in orange serum broth,  $G_{sol}$  decreased due to the yeast growth rather than increased (Fig. 5).

#### Detection times ( $C_{pol}$ ) versus colony forming units

Detection times ( $C_{pol}$ ) correlated highly ( $r = -0.989$ ,  $n = 38$ ) with log of colony forming units/ml of *Saccharomyces cerevisiae* (Fig. 6). A change in the slope of the line was observed when the cell number approached and exceeded  $10^5$  CFU/ml. The dispersion of points around the line increased when the cell number dropped below  $10^2$  CFU/ml.

#### Effect of pH

Bacterial metabolism usually caused a drop of 1.5–2.5 pH units. The growth of yeasts in TSB did not cause the pH of the medium to fall very far (1.0 pH unit). However, in YCB most yeasts caused a drop in pH of 2–2.5 units. In order to assess the contribution of these pH changes to the change of  $C_{pol}$  and  $G_{sol}$ , pH was plotted versus polarization capacitance in Fig. 7 and versus solution conductance in Fig. 8.

A drop in pH of 2 units resulted in ~ 40% increase in  $C_{pol}$  in all media tested with all three types of acids. The addition of base resulted in smaller changes in  $C_{pol}$ . The increase in  $G_{sol}$  due to the drop in 2 units of pH was less than 14% (Fig. 8). In media with low ionic concentration (PCB and YCB) a further drop in pH

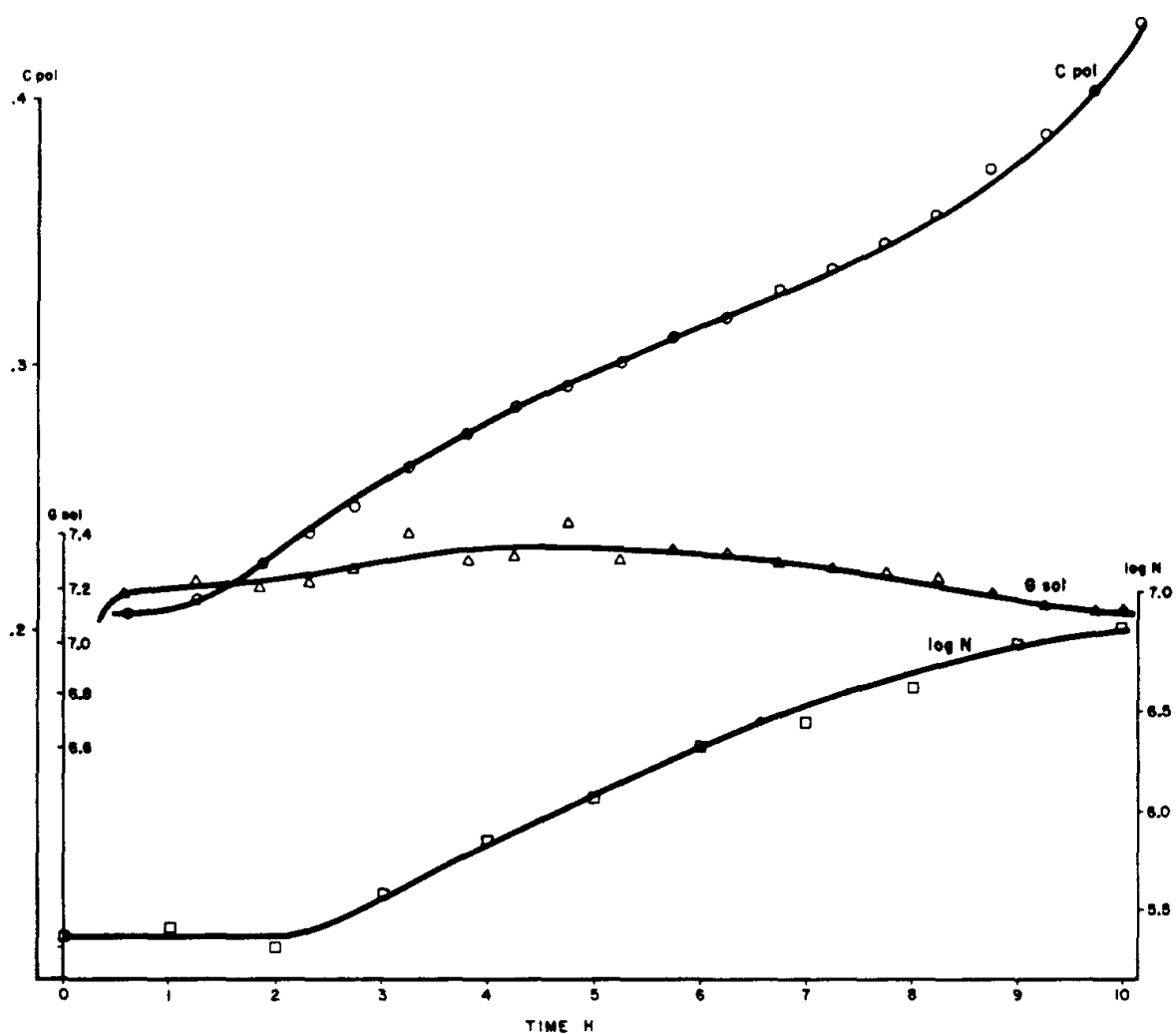


Fig. 4. The effect of growth of *S. cerevisiae*, in TSB on  $C_{pol}$ ,  $G_{sol}$  and  $\log N$ .  $N$  is number of yeasts per ml; gas in ml;  $C_{pol}$  in  $\mu F$ ;  $G_{sol}$  in mS.

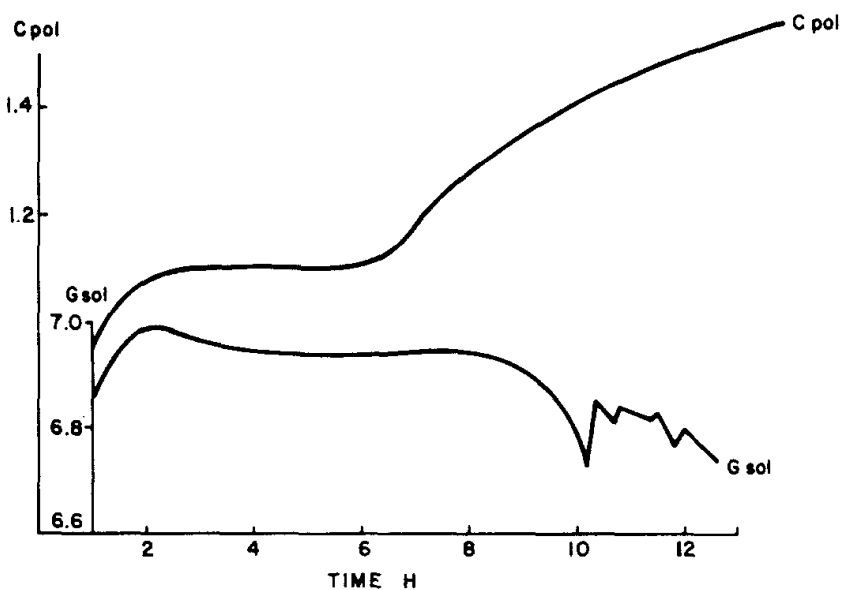


Fig. 5. The effect of growth of *C. utilis*, in Orange Serum Broth on  $C_{pol}$  and  $G_{sol}$ .  $C_{pol}$  in  $\mu F$  and  $G_{sol}$  in mS.

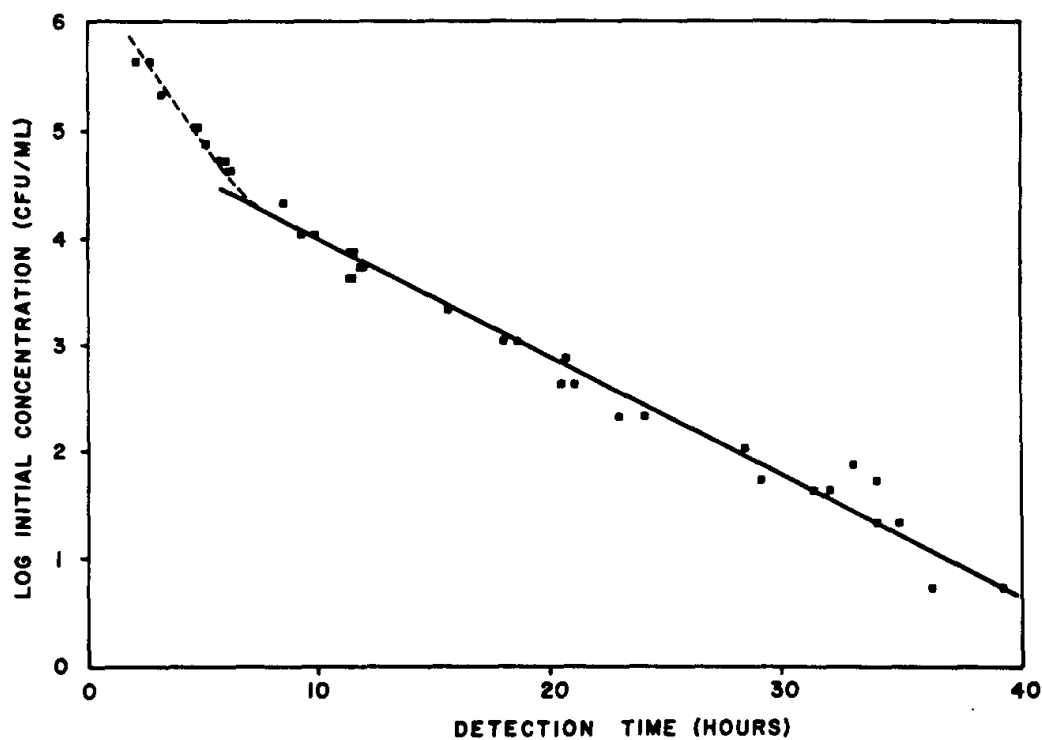


Fig. 6. Correlation of impedance detection times and log initial cell concentration (CFU/ml) of *Saccharomyces cerevisiae* in yeast carbon base.

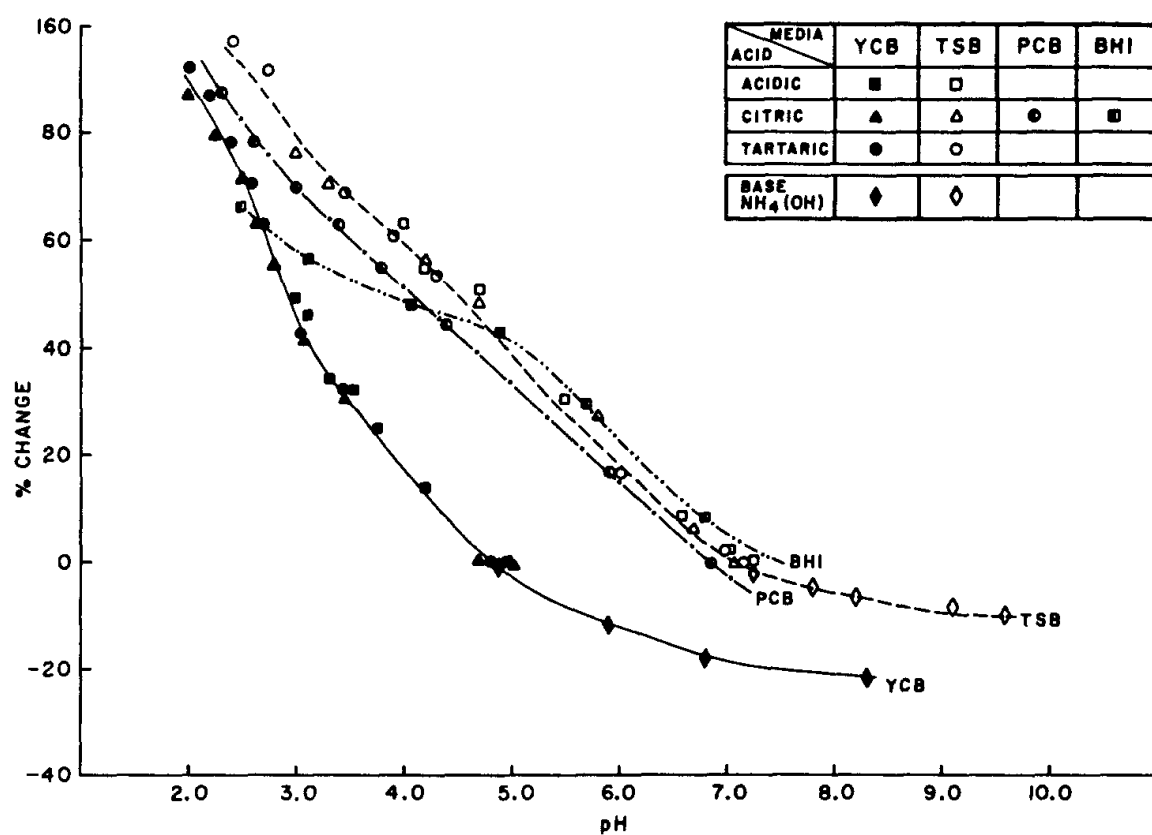


Fig. 7. The effect of pH on  $C_{pol}$  ( $\mu F$ ).

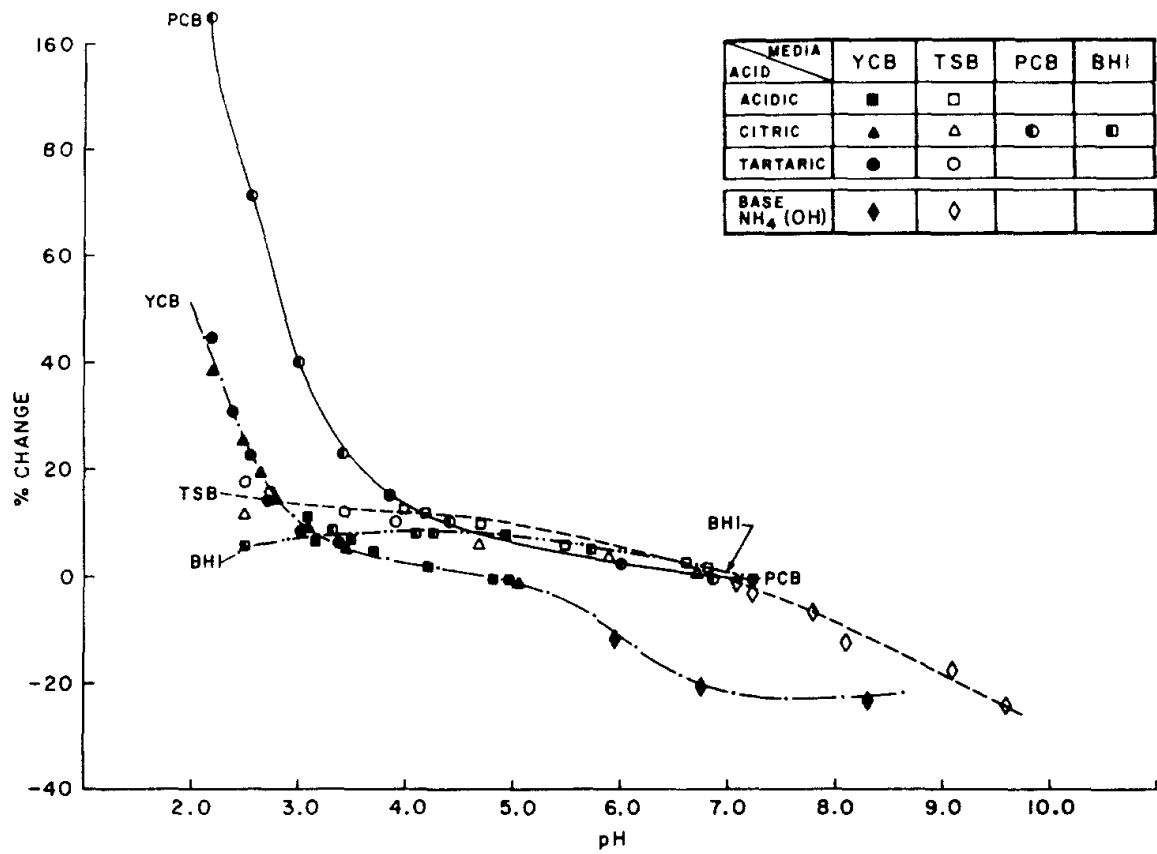


Fig. 8. The effect of pH on  $G_{sol}$  (mS).

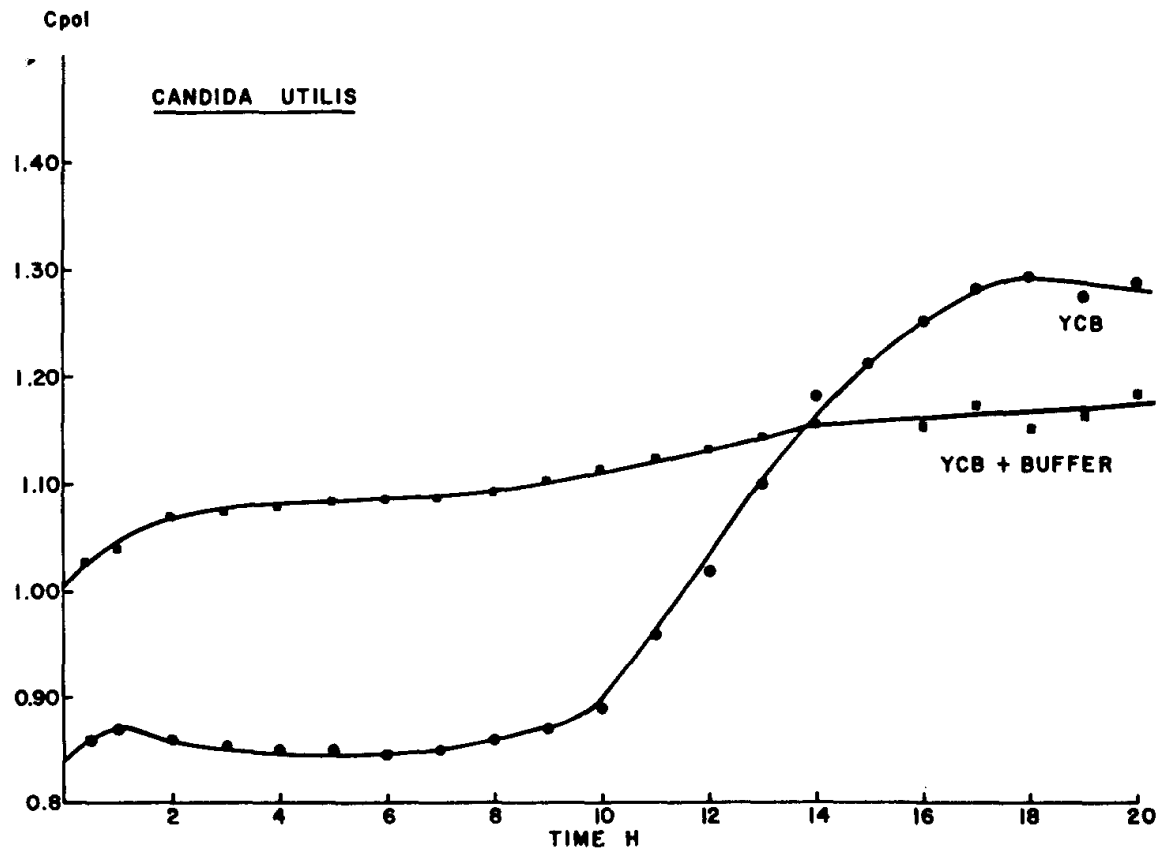


Fig. 9. The effect of the addition of buffer to YCB on the percent change in  $C_{pol}$  due to growth of *C. utilis*.  $C_{pol}$  in  $\mu F$ .

resulted in larger changes in  $G_{\text{sol}}$ , while in media with high ionic concentration (BHI and TSB) no further change in  $G_{\text{sol}}$  was observed. The influence of pH drop on the increase in  $C_{\text{pol}}$  was also demonstrated when *C. utilis* was grown in YCB with and without 0.1 M phosphate buffer (Fig. 9). In the buffered medium the total change in  $C_{\text{pol}}$  was less than 25% of that observed in the unbuffered medium.

## Discussion

### *Comparison of $C_{\text{pol}}$ and $G_{\text{sol}}$ as indicators of microbial growth*

In the low conductivity media, such as PCB, bacterial metabolism resulted in clearly detectable changes in  $G_{\text{sol}}$  due to the accumulation of ionizable metabolic end products and due to the greater mobility of the ions formed. The measurement of  $G_{\text{sol}}$  by itself was sufficient to detect bacterial metabolism in such media. In the more conductive media such as TSB and BHI, the fractional change in  $G_{\text{sol}}$  was much smaller than in PCB (Table 1). Therefore, the use of  $G_{\text{sol}}$  as the only impedance parameter for bacterial growth might require very sensitive instrumentation.

In their impedimetric measurement of cultures of *E. coli* in a low conductivity medium, Richards et al. [6] found that while  $G_{\text{sol}}$  was related to bacterial growth,  $C_{\text{pol}}$  "was most unlikely to be a reliable means of observing bacterial growth". In the present study changes in  $C_{\text{pol}}$  due to the growth of *E. coli* were not random, and correlated to growth in all media tested (Figs. 2 and 3). In a higher conductivity medium (Fig. 3)  $C_{\text{pol}}$  followed bacterial growth up to the stationary phase and was a better indicator of growth than  $G_{\text{sol}}$ .

When yeasts were examined, a finding contrary to that of Richards et al. [6] with *E. coli* was observed, i.e., a good correlation of  $G_{\text{sol}}$  with growth did not occur, but a clear relationship between  $C_{\text{pol}}$  and growth was found (Figs. 4 and 5). The small change in  $G_{\text{sol}}$  obtained with yeasts might be due to either the fact that the yeasts did not produce strongly ionized metabolites under the conditions of these experiments or due to the ability of yeasts to take up ions from the solution [12]. The uptake of ions might have caused the observed downward trend in  $G_{\text{sol}}$  for *C. utilis* in Orange Serum Broth (Fig. 5).

### *Effect of gas production*

The effect of gas generation on  $G_{\text{sol}}$  was to cause random, noise-like variations. The effect of  $C_{\text{pol}}$  was much smaller. It was observed on the admittance bridge null setting but was not visible at the scale factors used for the figures. The fluctuations in the  $G_{\text{sol}}$  signal were caused by the random release of the bubbles from the electrode surface and the bursting of the bubble at the surface. The bubbles had more effect at the electrodes because they can adhere for a time to the surface of the electrode, hence decreasing the effective area of the electrodes. However, in the bulk solution they quickly floated to the top.

The break-up in  $G_{\text{sol}}$  signal could be used to detect gas production by microorganisms. The fermentation of sugar to acid will result in smooth increasing  $G_{\text{sol}}$  curves while gas production accompanying fermentation will produce a visible disruption of the signal.

### *Effect of pH on polarization capacitance and solution conductance*

The change in pH which accompanied microbial growth can be expected to affect both  $C_{\text{pol}}$  and  $G_{\text{sol}}$ . In the experiments with bacteria, the pH decreased from about 7–4.5. The results showed that changes in pH were not the main cause for the change in  $G_{\text{sol}}$  in any of the media tested. Alternatively, changes in  $C_{\text{pol}}$  due to a decrease in pH accounted for 20–40% of the total increase in  $C_{\text{pol}}$  with bacterial growth, and for 20–70% in  $C_{\text{pol}}$  with yeast growth. Therefore, microbially promoted pH changes appeared to be an important factor in the change of  $C_{\text{pol}}$ . This fact could also be demonstrated by the diminished change in  $C_{\text{pol}}$  when buffer was added to YCB (Fig. 9). One might take advantage of this result by devising media that allows large pH changes due to growth of selected groups of genera of microorganisms. One would then expect large  $C_{\text{pol}}$  changes to occur in these media, thereby facilitating the detection of these organisms.

### *The relationship between $C_{\text{pol}}$ and microbial growth*

Capacitance changes due to microbial growth are related to changes in ionic composition in close proximity to the electrodes. Capacitance is affected by the dielectric constant of the solution between the electrodes and the double layer ( $E$ ), the area of the double layer ( $A$ ), and the thickness of the dielectric ( $d$ ) as follows:

$$C = E \frac{A}{4\pi d}$$

Microbial growth generating smaller ions can decrease the distance between the capacitor plates. The smaller ions can also increase the effective surface area of the plate by increasing the concentration of ions in close proximity to the electrode. This effect may also occur due to the charged surfaces of the cells themselves depending on their reproduction and motility. Finally, the newly formed metabolites of the microorganisms may have a different dielectric constant. The reason that pH decrease causes large changes in  $C_{\text{pol}}$  might be due to the effectiveness of the  $\text{H}^+$  ions formed to simultaneously increase the area ( $A$ ) and decrease the thickness of the double layer.

This work has presented the first evidence that  $C_{\text{pol}}$  could serve as an effective tool for monitoring microbial growth. Furthermore, this work suggests that  $C_{\text{pol}}$  may be the only impedance component that is useful for monitoring yeast growth. The linear relationship found between detection times ( $C_{\text{pol}}$ ) and log CFU/ml for *S. cerevisiae* demonstrates the fact that the changes in capacitance are due to yeast growth. The high correlation obtained suggests that monitoring  $C_{\text{pol}}$  changes could be used as a new method for the estimation of numbers of yeasts and their activity. This relationship became non-linear when high numbers ( $> 10^5$ ) or low numbers ( $< 10^2$ ) of yeast were present, as previously shown with impedance [5]. At a high cell concentration it is believed that the impedance threshold is reached before equilibration occurred. The dispersion of points at low counts was attributed to uneven distribution of cells in the test containers as well as to error in the plate count.

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