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Factors influencing capacitance-based monitoring of microbial growth

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Abstract

Microbiological impedance devices are routinely used by food and manufacturing industries, and public health agencies to measure microbiological growth. Factors contributing to increases and decreases in capacitance at the culture medium-electrode interface are poorly understood. To objectively evaluate the effects of temperature, cell density and medium conductivity on capacitance, admittance values from an impedance device were standardized; capacitance was converted to susceptance to allow unit comparisons with conductance. Although increases in temperature increased susceptance, a linear relationship could not be established between the change of susceptance with temperature and conductance of the medium. Cell density by itself had no measureable effect on susceptance or conductance, indicating that cells did not impede the movement of ions in the medium or around the electrode. In a low conductivity medium, increases in conductance by the addition of ions resulted in a concomitant increase of susceptance values. However, in a high conductivity medium, increases in conductance resulted in little or no increase of susceptance values because ions saturated the electrode surface. Susceptance increased when *Escherichia coli*, *Pseudomonas aeruginosa*, *Alcaligenes faecalis* and *Staphylococcus aureus* were grown in high conductivity media because protons produced by metabolically active bacteria balance more charge on the electrode than other ions. Increases in susceptance due to bacterial growth and metabolism in low conductivity media were attributed to both increases in protons and ionic metabolites. These results indicate that capacitance may provide a better measure of microbial growth and metabolism than conductance.

Keywords: Capacitance; culture media design; Microbial growth; Susceptance

1. Introduction

Microbiological impedance devices monitor microbial metabolism in growth medium by using an electronic signal that measures the movement of ions between two electrodes (conductance) and, in some devices, the storage of charge at the electrode-medium interface (capacitance). These devices are routinely used by food (Fletcher et al., 1993; Russell, 1997, 1998; Russell et al., 1995; Smith et al., 1989) and manufacturing (Zhou and King, 1995a,b) industries to estimate product shelf-life and/or to screen for microbial contamination. These devices are also

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employed by public health facilities (Cady et al., 1978; Noble et al., 1991; Throm et al., 1977) and sanitation plants (Silverman and Munoz, 1979) to determine bacterial loads of water samples, and by medical institutions to define the growth requirements of fastidious bacteria (Dziuba et al., 1993).

The principles of impedance microbiology have been the subject of several detailed studies (Baynes et al., 1983; Bockris and Reddy, 1970; Firstenberg-Eden and Zindulis, 1984; Owens, 1985; Owens et al., 1985, 1992; Owens and Wacher-Viveros, 1986; Richards et al., 1978). These studies show that subtle changes in the ionic composition of a culture medium affect its electrical conductivity and capacitivity. This property is useful for monitoring microbial growth and metabolism because the amount of time required to cause a series of significant deviations from baseline impedance values is referred to as a detection time (DT), and this corresponds to a bacterial concentration of approximately 10^6 to 10^7 cells ml^{-1}. Since DT is inversely proportional to the bacterial concentration at the time of inoculation, this measure can be used to estimate cell counts of many different types of microbiological samples.

Although the principles of conductance microbiology are well understood, the exact mechanisms by which microbial growth and metabolism affects capacitance are not known. A hypothetical model on capacitance at the electrode-medium interface of impedance devices has recently been proposed (Noble, 1999). According to this model, measured capacitance is a function of the effective oxide capacitance (C_{ox}) at a given frequency and the Stern capacitance (C_s) (Noble, 1999). The C_{ox} refers to capacitance associated with the passivation surface layer composed of mainly chromium oxide and hydroxide (Olefjord, 1980). The C_s refers to the capacitance associated with two regions of charge separation; one region close to the electrode surface that varies linearly with distance from the electrode, and the other which forms the diffuse layer that decreases exponentially with distance from the electrode (Noble, 1999). To provide a framework to interpret changes in capacitance during microbial growth, the validity of this proposed model must be determined. Furthermore, information on factors contributing to increases or decreases in capacitance are needed so that the direction and relative rate of change of capacitance of metabolically-active microbes in culture media can be predicted. Such information is particularly useful for industrial and public health microbiologists since several studies have shown that capacitance provides a better indicator of microbial growth and metabolism than conductance (Dziuba et al., 1993; Noble, 1994; Noble et al., 1991). By knowing the factors affecting capacitance, microbiologists will be able to rationally design culture media.

The focus of this study is to identify factors affecting capacitance and to relate these findings to the model proposed by Noble, 1999. Validation of this model is needed so that guidelines to rationally design culture media can be established for industrial and public health microbiologists. Capacitance is also examined because it has been reported as insensitive and subject to random fluctuations (Richards et al., 1978); however, data collected from other studies (Dziuba et al., 1993; Firstenberg-Eden and Zindulis, 1984; Noble et al., 1991; Russell, 1997, 1998), do not support these findings. Here we examine a model which provides a framework for interpreting changes in capacitance during microbial growth and metabolism and provide guidelines for designing culture for capacitance microbiology.

2. Materials and methods

2.1. Media and buffers

Brain heart infusion broth (BHIB) (Difco, Canlab Scientific Products, Edmonton, AB), yeast carbon base (YCB) (Difco), plate count agar (PCA) (BBL Standard methods agar; Becton Dickinson Microbiology Systems, Cockeysville, MD) were prepared following the manufacturer's specifications. PCA-based broth (PCB) consisted of 5 g tryptone, 2.5 g yeast extract and 1 g glucose per liter. PCB prepared without one of the following: tryptone, yeast extract or glucose were abbreviated as PCB-T, PCB-YE and PCB-G, respectively. Glucose and yeast extract broth (GYEB) consisted of 2.5 g glucose and 0.1 g yeast extract per liter. Blood agar plates (BAP) were composed of Bacto tryptose blood agar base with yeast extract (Difco) supplemented with 5% defibrinated sheep's blood (Gibmar, Ardrossan, AB). Trypticase soy broth (TSB) contained 17 g trypticase

peptone (BBL), 3 g phytone peptone (BBL), 5 g NaCl, 2.5 g dipotassium phosphate and 2.5 g glucose per liter. Unless otherwise specified, broths were autoclaved and dispensed into 2.5 ml module wells to a volume of 1.8 ml.

Stock solutions of 0.1 and 1.0 M TRIS and HEPES buffers were prepared at pH values ranging from 6.8 to 8.4 using HCl and NaOH, respectively. Stock 0.1 M phosphate buffer was prepared at pH values ranging from 6.0 to 7.8 using monobasic and dibasic sodium phosphate (American Public Health Assoc., 1992). Stock buffered water was prepared by dissolving 34.0 g of potassium dihydrogen phosphate in 500 ml of distilled water, adjusting the pH to 7.2 with NaOH and diluting the solution to a final volume of 1 l. Buffered water consisted of 1.3 ml of the stock, 5.0 ml of magnesium chloride solution (81.1 g $MgCl_2 \cdot 6H_2O$ per liter of distilled water) in 993 ml of distilled water (American Public Health Assoc., 1992). All buffer solutions were autoclaved at 121°C for 15 min. The pH of the buffers was readjusted after autoclaving when required.

2.2. Bacteriological cultures

Bacterial strains used in this study included: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Alcaligenes faecalis* (ATCC 8750) and *Staphylococcus aureus* (ATCC 25923).

2.3. Preparation of bacterial cultures prior to inoculation

Bacterial strains were grown overnight on BAP incubated at 35°C. Several colonies were collected using a sterile cotton swab and suspended in 1 ml of sterile distilled water. Cells were washed three times with distilled water and serially diluted four-fold to a concentration of approximately 10^4 CFU ml^{-1}. Approximately 0.2 ml of the diluted suspensions were used to inoculate the module wells.

2.4. Calculation of bacterial doubling time

Bacterial doubling time (BDT) was computed using the formula: BDT $(h) = \log 2 \times (m)^{-1}$ where m refers to the slope of the regression of $\log_{10}$CFU ml^{-1} on DT (Dziuba et al., 1993). Washed bacterial cells were serially diluted ten-fold in buffered water (American Public Health Assoc., 1992) to concentrations of 10^1 to 10^7 cells per ml. Approximately 0.2 ml of each dilution were added to module wells containing PCB medium (in duplicate). Modules were incubated at a specified temperature for 48 h.

2.5. Microbiological impedance device

Impedance was measured using the Bactometer (Vitek, Hazelwood, Missouri) which has been previously described (Noble et al., 1991, Dziuba et al., 1993). This device uses a 1540 Hz sine wave (V_{p-p} = 0.265) to measure capacitance. The metal electrodes in each module well, constructed from stainless steel No. 304, had a total surface area of 0.364 cm^2. The electrodes were separated by a distance of 0.75 cm.

2.6. Standardization of the impedance device and computation of maximum slope of susceptance and conductance values

Values obtained from the impedance device were converted to SI units by establishing mathematical relationships between capacitance and conductance values of the impedance device and standardized capacitors and resistors, respectively (Noble, 1994). To compare capacitance and conductance, capacitance values were converted to susceptance (B_c) values using the formula:

$$B_c\ (\mu S) = 2 \cdot \pi \cdot f \cdot C \quad (1)$$

where f is frequency in Hz.

Where specified, susceptance values were adjusted by subtracting the lowest baseline value obtained within the first 4 h of incubation from all values. This adjustment compensated for the subtle differences between module wells and their electrodes and allowed susceptance values derived from different culture media to be compared on the same figure.

To determine the slope of susceptance and conductance values as a function of time the following formula was used:

$$\text{Slope}\ (\mu S\ h^{-1}) = (X_n - X_i)/n - i \quad (2)$$

where X refers to the average of three consecutive susceptance or conductance readings, and X_n and X_i

refers to the value of X at time n and i, respectively. The following time intervals were used to compute slope: 0.5, 1.0 and 3.0 h. Only the maximum slope obtained over 48 h of growth was considered for this study.

2.7. Data analysis

Correlation analysis was used to determine the degree of association between variables. Linear and quadratic regressions were used to estimate the relationship of one variable with another (Sokal and Rohlf, 1981). An analysis of variance was used to determine the source of variability in experimental data. Duncan's multiple range test was employed to determine if the difference between any two means in a set of means was significant (Miller, 1966). Raw data from the impedance device were transferred to the mainframe computer at the University of Alberta. Manipulation of raw data, analysis of variance and Duncan's multiple range tests were determined by using the Statistical Analysis System (SAS Institute Inc., Cary, NC).

2.8. Experimental procedures

2.8.1. Relationship between conductance and susceptance

The susceptance and conductance values of different organic and inorganic molecules, and culture media were determined by inoculating 2.0 ml volumes of sterile liquid (in quadruplicate) into module wells. The following molecules were solubilized in water, filter sterilized, and diluted to molarities ranging from 0.00001 to 0.01 M: NaF, NaBr, CsCl, KCl, NaCl, CaCl, NH_4Cl, LiCl, NH_4HCO_3, LiCl, $MgSO_4$, ammonium acetate, sodium acetate, sodium succinate, sodium lactate, sodium pyruvate, formamide and acetamide. Modules were incubated at 25°C. After 1 h of incubation, mean susceptance and conductance values from three consecutive readings were calculated.

2.8.2. Effects of incubation temperature on susceptance and conductance values

To determine the effects of incubation temperature on susceptance and conductance values, module wells were inoculated (in quadruplicate) with 2.0 ml of sterile PCB and incubated at 20, 30, 40 and 50°C. After 1 h of incubation, mean susceptance and conductance values from three consecutive readings were computed. Linear regressions were established between susceptance and conductance values, and incubation temperature.

2.8.3. Effects of ion concentration on the susceptance and conductance profiles of bacteria

Module wells containing 1.8 ml of PCB supplemented with 0, 0.5 or 1.0% NaCl were inoculated (in quadruplicate) with 0.2 ml volumes of washed bacterial suspensions of *E. coli*, *P. aeruginosa*, *A. faecalis* and *S. aureus*. Susceptance and conductance values were measured simultaneously at 1-min intervals.

2.8.4. Effects of pH buffers on the capacitance profiles of E. coli

Washed bacterial suspensions of *E. coli* were inoculated as 0.2 ml volumes (in quadruplicate) into module wells containing 1.8 ml of GYEB supplemented with either 0.01 M HEPES, TRIS and phosphate buffers at pH values of 7.2, 7.6 and 8.0. Controls for this experiment consisted of module wells containing uninoculated GYEB that were supplemented with and without buffers and module wells containing GYEB that were inoculated with 0.2 ml of washed *E. coli* cells. Susceptance and conductance values were measured simultaneously at 1-min intervals.

2.8.5. Effects of microbial cell density on susceptance and conductance values

E. coli, *P. aeruginosa*, and *S. aureus* were grown overnight at 35°C in 500 ml of PCB, centrifuged (3000 rpm$\times$5 min) and washed 3$\times$ with buffered water (American Public Health Assoc., 1992). The same protocol was carried out for *A. faecalis* except a 48 h culture was used. On the final wash, the cell pellet was resuspended with 2 ml of buffered water, vortexed for 1 min and serially diluted ten-fold to a concentration of ca. 10^1 cells ml^{-1}. One milliliter volumes were added to module wells (in duplicate) containing 0.33 ml of PCB at 4$\times$ concentration. Inoculated modules were incubated at 25°C for 2 h. The bacterial count was determined by serially diluting the sample and plating 0.1 ml (in duplicate)

onto PCA plates. Susceptance and conductance values were obtained from three consecutive readings after the samples were incubated for 1 h.

2.8.6. Effects of microbial growth on susceptance and conductance values

Washed bacterial suspensions of *E. coli*, *P. aeruginosa*, *A. faecalis* and *S. aureus* were inoculated as 0.2 ml volumes (in quadruplicate) into module wells containing 1.8 ml of TSB (high conductivity medium) and PCB (low conductivity medium). Susceptance and conductance values were measured simultaneously at 1-min intervals.

3. Results

Replicate experiments were conducted on modules from the same lot number because quality control tests revealed statistical differences in slope maxima of susceptance values by lot number ($P<0.02$, data not shown). There were no statistical differences between module lot number and DTs, or module lot number and slope maxima of conductance values (data not shown).

Microbial cell density experiments revealed that biomass did not affect susceptance and conductance values (data not shown).

3.1. Relationship between conductance and susceptance

A comparison between susceptance and conductance values of sterile growth media revealed that under specific circumstances increases in susceptance corresponded to increases in conductance (Fig. 1). When conductance of the growth medium exceeded 2.0×10^3 μS, susceptance did not change beyond a limit of 1.2×10^4 μS. In a low conductance medium such as PCB (545.3±7.7 μS), an increase in the ionic concentration by the addition of small amounts of NaCl resulted in a concomitant increase in conductance and susceptance. In a high conductance medium such as TSB (5582.2±32.7 μS) however, the addition of small amounts of NaCl increased conductance but not susceptance values. Similar results were obtained for high and low conductance solutions made from NaOH, as well as NaF, NaBr, CsCl, KCl, NaCl, CaCl, NH_4Cl, LiCl, NH_4HCO_3, LiCl, $MgSO_4$, ammonium acetate, sodium acetate, sodium succinate, sodium lactate, sodium pyruvate, formamide and acetamide. The only way susceptance increased in a high conductance medium was by adding small volumes of acid to the medium. To determine if protons increased susceptance values, 1.2 N HCl was serially diluted (ten-fold) and 10 to 70 μl volumes were added to module wells containing 2.0 ml of PCB. The data showed that the susceptance limit was increased to 1.4×10^4 μS when HCl was added to the growth medium (Fig. 1). Variability in the data was attributed to variation in the state of the passive layer that was present on a stainless steel electrode surface due to the action of HCl.

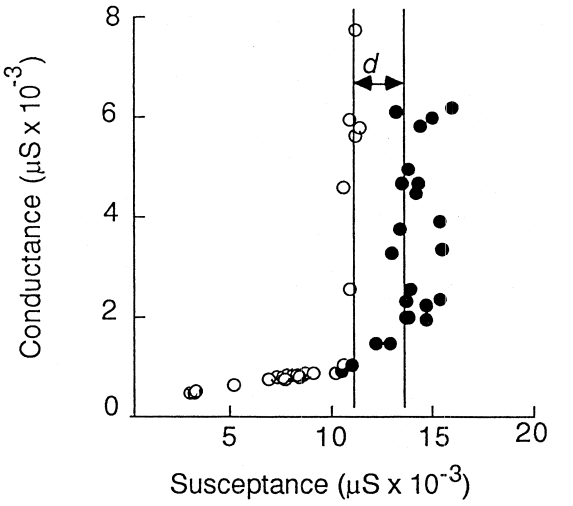

Fig. 1. Relationship between conductance and susceptance of uninoculated growth media incubated at 25°C. The parameter, *d*, represents the difference in susceptance values between growth media supplemented with (closed circle) and without HCl (open circle).

3.2. Effects of incubation temperature on susceptance and conductance values

Both susceptance and conductance were affected by temperature. Growth medium incubated at high temperatures had higher susceptance and conductance values than the same medium incubated at low temperatures (Table 1). There was a linear relation-

Table 1
Susceptance (B_c) and conductance (G) values or sterile growth medium as a function of incubation temperature

Growth medium	Admittance signal	Mean admittance (μS)[a] ± SD by temperature (°C)		
		20	30	40
PCB	B_c	6335.7±145.1	7801.3±156.6	9348.4±170.8
	G	491.5±3.7	588.4±2.0	691.0±1.7
TSB	B_c	11 026.6±261.0	11 518.1±243.7	12 170.3±406.5
	G	5279.2±29.9	6254.1±62.4	7231.5±92.4

[a] Based on three consecutive readings (in quadruplicate).

ship between both conductance and susceptance and the temperature (in °C) in all media (Table 2). The slope of this relationship showed a linear dependence on conductance (G) of the medium for the change in conductance with temperature (dG/dT). This relationship can be expressed by the equation:

$$dG/dT = (0.02 \times G) + 0.31 \quad (3)$$

which has a $R^2 = 1.0$. An increase in conductance of PCB to 1120.6 μS increased the effects of temperature by a factor of two. This means that a growth medium with a high conductance value would be more affected by changes in temperature than a medium with a low conductance value. A linear relationship could not be established between change of susceptance with temperature (dS/dT) and media conductance.

3.3. Effects of ion concentration on the susceptance and conductance profiles of type strains

Increasing the NaCl concentration of PCB medium from 0 to 0.5 and 1.0% (w/v) did not affect the slope maxima of susceptance and conductance values for *P. aeruginosa* and *S. aureus*. However, differences in slope maxima were noted for *E. coli* and *A. faecalis* grown in PCB and PCB containing 0.5 and 1.0% NaCl (Table 3). The change in conductance or susceptance with time was not strictly linear, but has been approximated using Eq. (2). A measure of the curvature is obtained by considering different slopes calculated with different time intervals. For *E. coli*, the maximum slope of the conductance signal appeared to increase with NaCl concentration providing

Table 2
Linear models for the regression between susceptance (B_c) and conductance (G) values, and incubation temperature (T (°C)) as a function of growth media

Growth medium	Mean conductivity ± SD[a] (μS)	Admittance signal	Linear model equation[b]	R^2 value
YCB	139.2±1.9	B_c	$B_c = 36.9 \times (T) + 42.9$	0.99
		G	$G = 2.5 \times (T) + 79.0$	1.00
PCB	545.3±7.7	B_c	$B_c = 158.1 \times (T) + 311.6$	1.00
		G	$G = 10.8 \times (T) + 263.0$	1.00
BIVIB	5407.3±28.3	B_c	$B_c = 79.4 \times (T) + 9537.3$	0.90
		G	$G = 103.5 \times (T) + 2874.8$	1.00
TSB	5582.2±32.7	B_c	$B_c = 82.9 \times (T) + 9169.7$	0.94
		G	$G = 101.8 \times (T) + 3214.7$	1.00

[a] At 25°C.
[b] Equations are based on admittance values collected from incubation temperatures of 20, 30, 40 and 50°C; admittance values were the mean of three consecutive readings (in quadruplicate).

Table 3
Slope maxima of susceptance (B_c) and conductance (G) values when bacteria were grown in PCB with and without NaCl for 48 h at 25°C

Bacterial species	Time (h) interval[a]	Admittance signal	Mean slope maxima ($\mu S\ h^{-1}$)±SD[b] determined with:			Level of Significance
			PCB	PCB + 0.5% NaCl	PCB + 1.0% NaCl	
E. coli	0.5	B_c	68.0±25.1	59.1±8.6	51.8±4.3	ns[c]
		G	2.9±0.1	9.1±7.4	5.8±1.8	ns[c]
	1	B_c	52.0±1.3	54.4±7.6	50.0±3.9	ns[c]
		G	2.8±0.1	5.5±2.3	5.6±1.7	ns[c]
	3	B_c	38.8±0.9	38.8±5.0	38.0±3.4	ns[c]
		G	2.1±0.1	3.4±0.7	3.8±1.3	ns[c]
A. faecalis	0.5	B_c	10.6±1.4[e]	34.0±4.1[d]	31.2±11.8[d]	$P<0.01$
		G	0.4±0.0[e]	6.6±3.8[e]	1.6±0.4[e]	$P<0.03$
	1	B_c	10.1±1.0[e]	337.0±4.4[d]	30.5±11.9[d]	$P<0.02$
		G	0.4±0.1[e]	3.7±1.7[d]	1.4±04[e]	$P<0.02$
	3	B_c	9.0±1.7[e]	28.8±4.4[d]	25.6±6.4[d]	$P<0.00$
		G	1.3±0.1[e]	1.5±0.3[d]	1.7±0.6[d]	$P<0.03$

[a] Based on Eq. (2).
[b] Maximum slopes were based on three replicate experiments that were conducted in quadruplicate.
[c] ns, not significant.
[d] Values within the same row followed by the same symbol are not statistically different ($\alpha=0.05$) as determined by Duncan's multiple range test.
[e] Values within the same row followed by the same symbol are not statistically different ($\alpha=0.05$) as determined by Duncan's multiple range test.

the time interval used to calculate slope was increased to 3.0 h ($P<0.10$). To ensure that the presence of NaCl in PCB did not affect growth rate, the BDTs of *E. coli* grown in PCB with and without NaCl were compared. The data revealed that the presence of NaCl in PCB had no measurable effect on the growth rate of *E. coli*. For *A. faecalis*, the slope maxima of susceptance and conductance values were affected by the presence of NaCl in the medium (Table 3). An increase in NaCl concentration also increased the slope maxima for susceptance and conductance ($P<0.02$). This increase could not be attributed to shorter BDTs.

3.4. Effects of buffers on the capacitance profiles of E. coli

Preliminary data showed there was no discernible difference in the capacitance profiles of *E. coli* grown in TSB or PCB medium that was supplemented with TRIS, HEPES or phosphate buffers, irrespective of the pH. However, when *E. coli* was grown in a medium having a low conductance (i.e. GYEB, 108 to 616 μS) that contained minimal nutrients and that was supplemented with TRIS (pH>7.6) and HEPES (pH 7.2 to 8.0), capacitance values increased with incubation time (Fig. 2). Such an increase was not observed when *E. coli* was grown in GYEB supplemented with phosphate buffer. Rather, capacitance values decreased with incubation time.

3.5. Effects of microbial growth on susceptance and conductance values

Regardless of the bacterial species or the growth media examined, the magnitude of susceptance values obtained during microbial growth was always greater than that of conductance values. Susceptance and conductance values, for example, increased 5004.4 and 223.8 μS, respectively, when *E. coli* was grown in PCB for 48 h (data not shown). The maximum slope of susceptance values was also greater (55.8 $\mu S\ h^{-1}$) than that of conductance values (3.0 $\mu S\ h^{-1}$).

Change in susceptance was much greater when *E.*

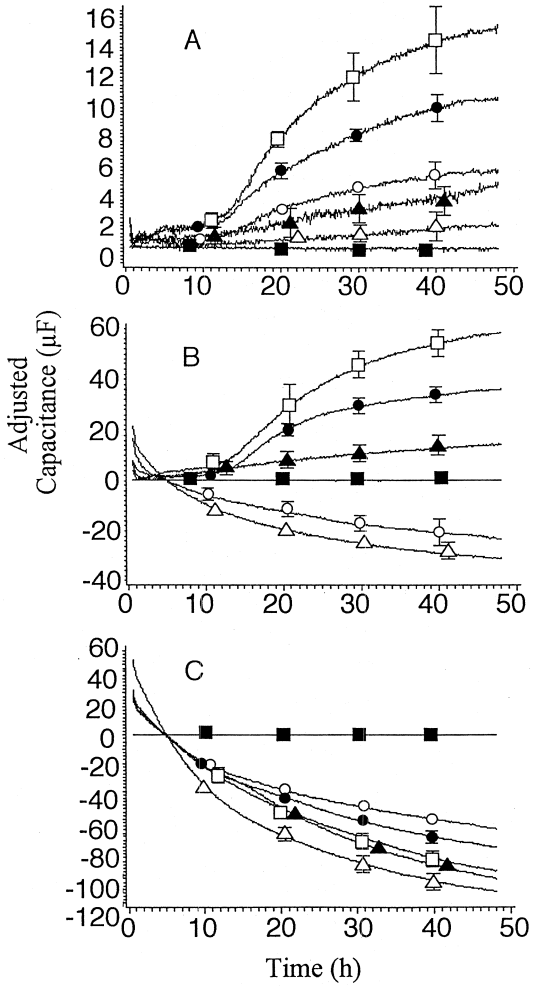

Fig. 2. Capacitance profiles of *E. coli* grown in GYEB supplemented with 0.01 M HEPES (panel A), 0.01 M TRIS (panel B), 0.01 M phosphate buffer (panel C). Mean±standard deviation are shown at 10 h intervals. Symbol, pH value: open circle, 7.2; closed circle, 7.6; open square, 8.0; closed square, no buffer; open triangle, neg. control, pH 7.2; closed triangle, neg. control, pH 8.0.

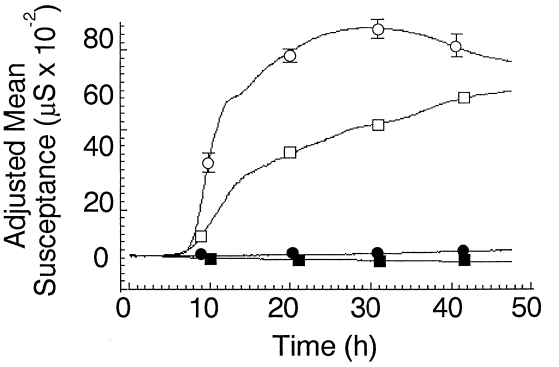

Fig. 3. Susceptance profiles of *E. coli* grown in TSB (open circle) and PCB (open square) incubated at 25°C with their corresponding negative controls (closed circle, closed square). The lines are the mean of acquired data (one reading/6 min) for four replicates. The symbols indicate the standard deviation.

coli was grown in TSB than PCB, despite the fact that the TSB medium has a much higher conductance (11 337.8±212.4 μS) than the PCB medium (6826.1±116.9 μS) (Fig. 3). These data show that increases in susceptance due to bacterial growth occurred regardless of the conductance of the growth media.

Comparisons between slope maxima of admittance values produced by growing bacteria revealed that growth medium significantly influenced slope maxima (Table 4). Bacterial strains grown in PCB-G for example, had much higher slope maxima than those grown in PCB, PCB-T or PCB-YE. However, differences in the slope maxima between PCB-G and the other media were only statistically significant for *E. coli*. With the exception of *A. faecalis*, the lowest slope maxima occurred when bacteria were grown in PCB-T. To determine if differences in slope maxima were related to the bacterial growth rate, BDTs for *E. coli* in these media were calculated. The BDT for *E. coli* grown in PCB (0.7±0.1 h), PCB-G (0.6±0.1 h), PCB-T (0.8±0.2 h) and PCB-YE (0.6±0.1 h) were not statistically significant ($P<0.24$). This finding suggests that growth rate alone cannot explain why slope maxima were higher in PCB-G than PCB.

Comparison of the slope maxima of admittance values by bacterial species showed that *E. coli* had the highest slope maxima followed by *P. aeruginosa*, *S. aureus* and *A. faecalis* (Table 4). The pH of growth medium before and after capacitance DT could not account for the differences in slope maxima. However, slope maxima appeared to be related to growth rate. When the relationship between slope maxima and BDTs from all bacteria grown in PCB were compared (Fig. 4), bacteria with short BDT had higher slope maxima than bacteria with long BDT. Moreover, the relationship between slope

Table 4
Slope maxima of susceptance (B_c) and conductance (G) values as a function of growth media and bacterial species

Bacterial species	Admittance signal	Mean slope maxima (μS h^{-1})±SD[a] by growth medium: PCB-G	PCB	PCB-T	PCB-YE	Level of significance
E. coli	B_c	55.0±5.3[c]	64.7±2.8[b]	26.2±1.8[d]	29.8±1.3[d]	$P<0.00$
	G	3.0±0.3[c]	4.2±0.2[b]	1.1±0.1[d]	1.4±0.1[d]	$P<0.00$
P. aeruginosa	B_c	25.8±4.5[b]	34.5±5.8[b]	16.5±4.1[bc]	31.4±7.7[b]	$P<0.02$
	G	1.5±0.1[b]	2.0±0.4[b]	0.8±0.2[c]	1.5±0.4[b]	$P<0.01$
A. faecalis	B_c	11.1±0.4[b]	12.6±4.7[b]	4.7±3.2[c]	3.6±0.5[c]	$P<0.01$
	G	0.4±0.0[b]	0.5±0.2[b]	0.2±0.2[bc]	0.2±0.0[c]	$P<0.05$
S. aureus	B_c	17.6±5.6	27.8±12.4	11.3±7.5	18.1±12.6	ns[d]
	G	0.9±0.4	1.6±0.6	0.5±0.3	0.8±0.5	ns[d]

[a] Based on Eq. 2 using 1.0 h time intervals; Slope maxima were based on three replicate experiments that were conducted in quadruplicate.

[b] Values within the same row followed by the same symbol are not statistically different ($\alpha=0.05$) as determined by Duncan's multiple-range test.

[c] Values within the same row followed by the same symbol are not statistically different ($\alpha=0.05$) as determined by Duncan's multiple-range test.

[d] ns, not significant.

maxima derived from susceptance and BDT was similar to the relationship between slope maxima derived from conductance and BDT. This finding suggests that growth rate influenced the slope maxima of susceptance and conductance values. A comparison of the slope maxima of admittance values and incubation temperature showed that when *E. coli* was grown in PCB medium incubated at different temperatures, slope maxima increased significantly with higher temperatures (Table 5). These data support the argument that growth rate affects slope maxima.

Preliminary studies revealed that susceptance increased with time when *A. faecalis* cells were grown in either TSB or PCB medium. Similar increases in conductance were not observed, however. To determine if differences between admittance values could be attributed to differences in inoculum size, washed *A. faecalis* cells were serially diluted tenfold and inoculated into module wells containing PCB. The admittance profiles showed that conductance values increased approximately 27 h after susceptance values increased (Fig. 5), and that this difference was independent of the inoculum size. To determine what factors were responsible for these increases, ammonia production and pH changes were monitored during the growth and metabolism of *A. faecalis*. The data showed no measurable change in ammonia production or pH when either admittance values increased.

4. Discussion

4.1. Factors that affect capacitance

Studies on the effects of temperature on admittance values indicate that both susceptance and conductance values increased with increasing temperature (Table 1). However, changes in conductance were more affected by temperature as a function of medium conductance than susceptance. These findings are in agreement with Richards et al. (1978) who found that capacitance was subject to fluctuations that could not be correlated with temperature. In a low conductance medium, the C_s portion of the hypothetical model (Noble, 1999) predicts that capacitance decreases with increasing temperature; presumably, thermal energy of mobile ions disrupts the establishment of order by electrostatic forces on the electrode surface. The data did not support this part of the proposed model. In fact, susceptance values increased with temperature (Table 1), even though a linear relationship could not be established between increases in susceptance and temperature. In a high conductance medium, the C_s portion of the

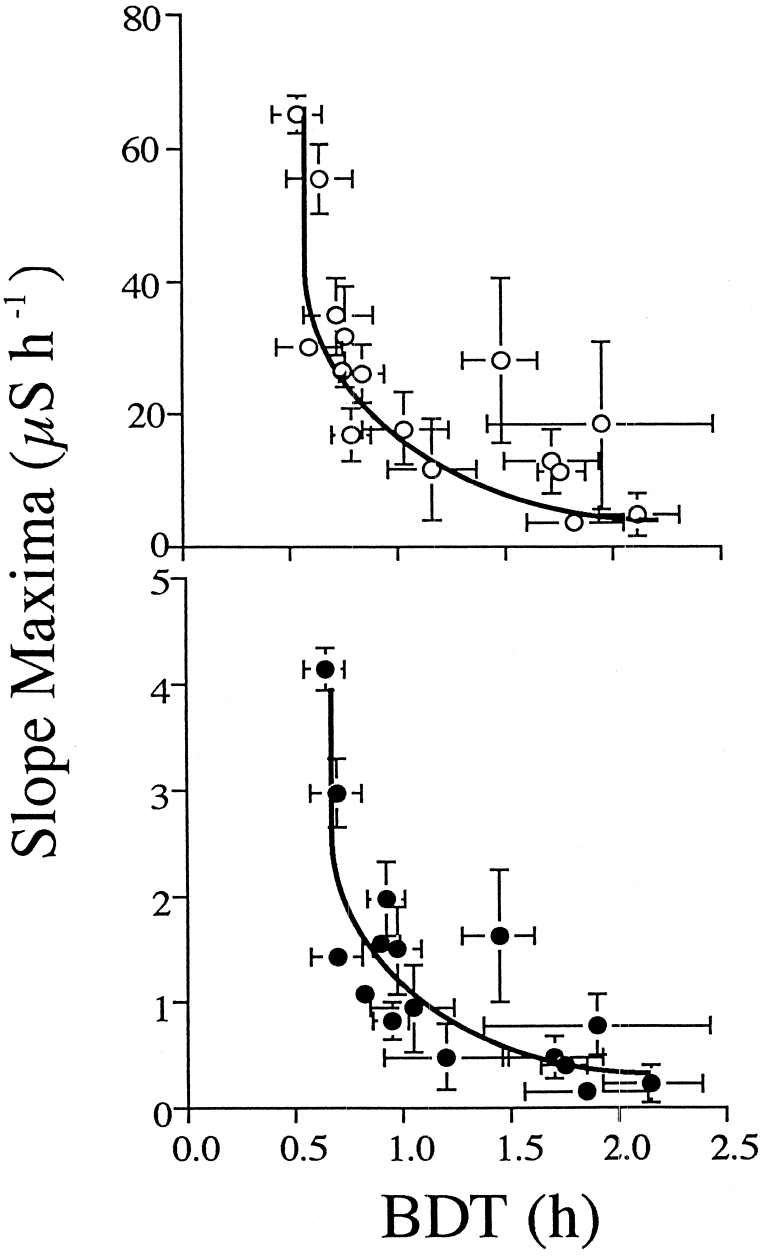

Fig. 4. Slope maxima of susceptance (open circle) and conductance (closed circle) as a function of mean BDT±standard deviation.

hypothetical model (Noble, 1999) predicts that increases in temperature should have little effect on susceptance. The effects of thermal energy on the establishment of order on the electrode surface should be small because there is always an excess of ions to balance charge on the electrode surface. The finding that conductance increased with increasing temperature can be attributed to decreases in viscosity of the growth medium (Owens, 1985). The rate of change of conductance values with temperature

Table 5
Effects of incubation temperature on slope maxima of susceptance and conductance signals obtained during the growth of *E. coli* in PCB medium

Admittance signal	Slope maxima ($\mu S\ h^{-1}$) by temperature (°C)[a]: 20	25	35	Level of significance
C	43.4±7.1[c]	65.0±6.3[c]	115.6±22.1[b]	$P<0.00$
G	1.7±0.1[d]	29±0.1[c]	6.3±0.6[b]	$P<0.00$

[a] Slope maxima were calculated using Eq. 7 using 1.0 h intervals and were based on five replicate experiments that were conducted in quadruplicate.

[b] Values within the same row followed by the same superscript letter are not statistically different ($\alpha=0.05$) as determined by Duncan's multiple-range test.

[c] Values within the same row followed by the same superscript letter are not statistically different ($\alpha=0.05$) as determined by Duncan's multiple-range test.

[d] Values within the same row followed by the same superscript letter are not statistically different ($\alpha=0.05$) as determined by Duncan's multiple-range test.

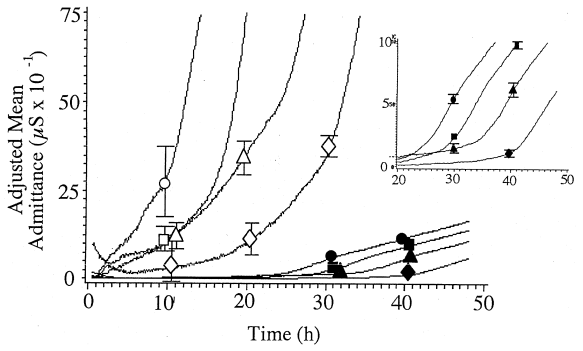

Fig. 5. Susceptance (open) and conductance (closed) profiles of *A. faecalis* grown in PCB incubated at 25°C. Four ten-fold dilutions are shown; each symbol shape represents the standard deviation of one dilution. An enlargement of the conductance profile is shown in the inset. The lines are the mean of acquired data (one reading/6 min) for four replicates.

obtained in this study (Eq. (3)) were in close agreement with those reported by Owens (1985) and Richards et al. (1978).

The effect of cell density on admittance was determined; cells, being poor conductors of electricity, might impede the movement of ions in the growth medium or cast shadows on the electrode surface (Schwan, 1963). A medium with a high bacterial load would be expected to have a lower susceptance than a medium with a low bacterial load because the former would have a smaller surface area than the latter. However, since a relationship could not be established between admittance and cell density (data not shown), cell densities in the range of 10^1 to 10^7 CFU ml^{-1} were not responsible for increases in admittance during bacterial growth.

Differences in slope maxima of susceptance values by module lot number were attributed to the surface properties of the metal electrode (data not shown). It is important to note that the surface properties of passive films are determined by the composition of the metal beneath the film (Olefjord, 1980). Therefore, subtle differences in the alloy elements nickel, molybdenum, and chromium between lot numbers would affect the reaction products formed on the metal surface as well as C_{ox}. This likely accounts for the differences in slope maxima of susceptance values by module lot number.

Studies on the relationship between conductance and susceptance showed that two different events occurred at the electrode surface when the conductance of a growth medium was increased by the addition of ions (Fig. 1). For a low conductance medium, susceptance concomitantly increased with conductance because ions added to the medium not only carried charge from one electrode to another but also balanced charge on the electrode surface. Susceptance in these media was determined by an equilibrium between electrostatic and dispersion forces, the electrostatic forces are the excess of charge residing on the electrode surface that is balanced by equal charge of opposite sign in the medium, and the dispersion forces are the thermal movement of particles in the medium (Bockris and Reddy, 1970). Susceptance reaches a maximum when Gouy-Chapman (i.e. $C_{G\text{-}C}$) portion of C_s

approaches the value of Helmholtz capacitance (Noble, 1999). Further increases in ion concentration cause no further changes in the observed capacitance. In these circumstances, susceptance can only be increased by increasing the dielectric permittivity or decreasing the distance of closest approach between the electrode surface and ions in the medium (Noble, 1999).

The finding that small quantities of HCl are able to increase susceptance beyond the limit of other ions (Fig. 1) suggests that protons are responsible for increasing susceptance values during bacterial growth. Firstenberg-Eden and Zindulis (1984) speculated that increases in capacitance might be due to newly formed metabolites changing the dielectric permittivity. However, since the dielectric permittivity of water molecules occupying 70% of the electrode surface is six and the dielectric permittivity for water molecules in the medium is 78 (Bockris and Reddy, 1970), it is unlikely that the dielectric permittivity increases as bacterial metabolism proceeds. Alternatively, a decrease in the distance of closest approach to the electrode by protons could cause an increase in the capacitance. However, since protons are more hydrated than other ions in the medium, this should have the opposite effect. It is most likely that HCl changes C_{ox} by affecting the oxide passivation layer on the steel electrodes (Epelboin et al., 1973). Protonation of metal oxide sites on the surface, or a change in the passivation layer's thickness and structure would then result in changes in the observed capacitance (Noble, 1999).

Increases in susceptance during bacterial growth and metabolism might be due to ions losing their hydration layers and adsorbing to the electrode surface as a consequence of van der Waals or chemical forces (Crow, 1979). More anions adsorb to the electrode surface than cations because loss of the hydration layer for anions is energetically favored (Bockris and Reddy, 1970). This study investigated these effects by adding different concentrations of organic and inorganic molecules to growth medium and observing whether or not susceptance values increased beyond that set by NaCl. Sodium fluoride was used as a control because adsorption of Na^+ and F^- to the electrode surface was not energetically favored (Bockris and Reddy, 1970). No differences were detected in relationship between conductance and susceptance for any of the inorganic and organic molecules investigated suggesting that adsorbance of these molecules to the electrode does not significantly contribute to increases in susceptance values.

The effects of pH buffers on capacitance profiles of *E. coli* were determined to assess the role of buffers in maximizing the change in capacitance values (Fig. 2). The incorporation of pH buffers into a high conductance medium did not significantly increase capacitance values during bacterial growth and metabolism, presumably because protons produced by *E. coli* were absorbed by the buffer. These protons were not available to interact with the passivation layer. In contrast, pH buffers incorporated into low conductance, low nutrient medium maximized changes in capacitance values during the growth and metabolism of *E. coli* because both protons and other ions assist in balancing charge on the electrode surface. These findings are significant in light of the fact that *E. coli* inoculated into GYEB without any buffers produced no changes in capacitance values, presumably because the production of protons alone was not sufficient to elicit an increase in capacitance values. These data support those of Owens (1985) that pH buffers play a significant role in maximizing conductance changes, provided that a low conductance medium is used. These results also suggest that changes in capacitance are governed by the same principles as those which govern changes in conductance.

4.2. Relationship between growth media and slope maxima

The effects of different growth media on slope maxima were examined to determine if different metabolic products affected slope maxima (Table 4). For *E. coli*, differences between slope maxima by growth media could not be attributed to growth rate, although faster growth rates appeared to increase slope maxima. One reason a direct relationship between growth rate and slope maxima could not be established is because different metabolites are produced by *E. coli* growing under different conditions. In glucose-containing media, *E. coli* ferments glucose to produce lactic, acetic and succinic acids. Not only do these products decrease the pH of the

medium, they also increase admittance values. In carbohydrate-free media containing peptone, *E. coli* hydrolyzes polypeptides to produce amino acids. These molecules do not accumulate in the medium, rather they are assimilated by rapidly growing bacteria (Owens, 1985). As a consequence, NH_4^+ ions are produced which increase admittance and pH values. It is conceivable that both the rate of metabolite production and the rate of growth account for the differences in slope maxima. Slope maxima of bacteria grown in PCB were always lower than those grown in PCB-G because glucose catabolically represses the synthesis of enzymes needed to utilize other substrates in the growth medium (Stryer, 1988). Only after most of the glucose has been utilized can enzyme production re-commence and other substrates be utilized.

A. faecalis has several characteristics that distinguish it from other bacteria employed in the study (Fig. 5). For example, *A. faecalis* produces little acid from the breakdown of carbohydrates. Growth of *A. faecalis* does not cause the pH of the medium to decrease, rather, this organism increases the pH of the medium by producing NH_3 from the degradation of peptones (Blazevic, 1976). This molecule combines with water to form NH_4^+ and hydroxide ions. It is possible that the production of hydroxide from NH_3 might account for the rise of the susceptance signal. However, the instruments used to monitor pH or NH_4^+ at the time of susceptance or conductance increase were probably not sensitive enough to detect a shift in either of the variables. Another characteristic of *A. faecalis* is that it produces organic salts and various amides as metabolic byproducts. With the exception of amides which have very low dissociation constants, any of the metabolites produced by *A. faecalis* may be responsible for the change in admittance values.

4.3. Guidelines to rationally design culture media for the enumeration of bacteria using capacitance measurements

When devising a growth medium to monitor changes in capacitance, it is desirable to direct the metabolic activities of bacteria to produce or consume protons in a low conductance medium. In this case, both protons and other ions produced by bacteria increase capacitance values (Fig. 1). However, in a high conductance medium it is desirable to direct the metabolic activities of bacteria to produce protons, as only protons increase capacitance values (Fig. 1). It is therefore counter-productive to incorporate buffers into a high conductance medium simply because buffers neutralize protons.

Investigations dealing with slope maxima were aimed at increasing capacitance values as a function of time so that definitive DT could be calculated (Fig. 3). To determine what factors affected slope maxima, studies were initiated on the effects of different module lot numbers, growth media constituents and growth rate on slope maxima. Differences in the slope maxima between module lot numbers can be attributed to differences in the passivation layer. Undoubtedly, it is desirable to have electrodes that have a consistent passivation layer because slope maxima are a function of the DT. The investigation of slope maxima using different media constituents showed that some substrates, such as glucose, reduced the slope maxima of growing bacteria, presumably due to catabolic repression. It is therefore essential to eliminate glucose from the growth medium, especially for bacterial species which are catabolically repressed by glucose, so that DTs are more definitive. Decreasing the doubling times of bacteria increased the slope maxima of capacitance and conductance values. Therefore, designing growth media to decrease the doubling time of bacteria would also provide definitive DTs.

In conclusion, this study identified factors contributing to increases and decreases in capacitance and successfully related the results to the hypothetical model proposed by Noble, 1999. Capacitance was affected by conductivity, temperature, and changes in pH. This study also showed that there were significant differences in the capacitance and conductance profiles of growing bacteria. For some bacteria, such as *E. coli*, capacitance increased concomitantly with conductance (data not shown). For other bacteria, such as *A. faecalis* (Fig. 5), increases in conductance values were found to lag 27 h behind increases in capacitance values. Further studies are required to clearly demonstrate why this is so. Capacitance, therefore, may be a more suitable

signal to monitor bacterial growth and metabolism than conductance.

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References

American Public Health Association, 1992. Standard Methods For the Examination of Water and Wastewater, 18th ed, American Public Health Association, Inc, Washington, D.C.

Baynes, N.C., Comrie, J., Prain, J.H., 1983. Detection of bacterial growth by the Malthus conductance meter. Med. Lab. Sci. 40, 149–158.

Blazevic, D.J., 1976. Current taxonomy and identification of nonfermentative gram negative bacilli. Human Pathol. 7, 265–275.

Bockris, J.O.M., Reddy, A.K.N. (Eds.), 1970. Modern Electrochemistry, Plenum Press, New York, pp. 1–1432.

Cady, P., Dufour, S.W., Lawless, P., Nunke, B., Kraeger, S.J., 1978. Impedimetric screening for bacteriuria. J. Clin. Microbiol. 7, 273–278.

Crow, D.R. (Ed.), 1979. Principles and Applications of Electrochemistry, John Wiley & Sons, New York, pp. 1–232.

Dziuba, M., Noble, P.A., Albritton, L W., 1993. A study of the nutritional requirements of a selected *Haemophilus ducreyi* strain using impedance and conventional methods. Curr. Microbiol. 27, 109–113.

Epelboin, I., Keddam, M., Lestrade, J.C., 1973. Faradaic impedances and intermediates in electrochemical reactions. Faraday Disc. Chem. Soc. 56, 264–275.

Firstenberg-Eden, R., Zindulis, J., 1984. Electrochemical changes in media due to microbial growth. J. Microbiol. Methods 2, 103–115.

Fletcher, D.L., Russell, S.M., Walker, J.M., Bailey, J.S., 1993. An evaluation of a rinse procedure using sodium-bicarbonate and hydrogen-peroxide on the recovery of bacteria from broiler carcasses. Poultry Sci. 72, 2152–2156.

Miller, R.G., 1966. Simultaneous Statistical Inference, McGraw-Hill, Inc, New York.

Noble, P.A. 1994. Theoretical and Practical Aspects of Impedance Microbiology. Ph.D. thesis, University of Saskatchewan, Saskatoon, Saskatchewan, pp. 1–201.

Noble, P.A., 1999. Hypothetical Model for Monitoring Microbial Growth by Using Capacitance Measurements – a minireview. J. Microbiol. Methods 37 (1), 45–49.

Noble, P.A., Ashton, E., Davidson, C.A., Albritton, W.L., 1991. Heterotrophic plate counts of surface water samples by using impedance methods. Appl. Environ. Microbiol. 57, 3287–3297.

Olefjord, I., 1980. The passive state of stainless steels. Mater. Sci. Eng. 42, 161–171.

Owens, J.D., 1985. Formulation of culture media for conductimetric assays: theoretical considerations. J. Gen. Microbiol. 131, 3055–3076.

Owens, J.D., Konirova, L., Thomas, D.S., 1992. Causes of conductance changes in yeast cultures. J. Appl. Bacteriol. 72, 32–38.

Owens, J.D., Miskin, D.R., Wachner-Viveros, M.C., Benge, L.C.A., 1985. Sources of conductance changes during bacterial reduction of trimethylamine oxide to trimethylammonium in phosphate buffer. J. Gen. Microbiol. 131, 1357–1361.

Owens, J.D., Wacher-Viveros, M.C., 1986. Selection of pH buffers for use in conductimetric microbiological assays. J. Appl. Bacteriol. 60, 395–400.

Richards, J.C.S., Jason, A.C., Hobbs, G., Gibson, D.M., Christie, R.H., 1978. Electronic measurement of bacterial growth. J. Phys. E. Sci. Instrum. 11, 560–568.

Russell, S.M., 1997. A rapid microbiological method for enumeration of *Pseudomonas fluorescens* from broiler chicken carcasses. J. Food Prot. 60, 385–390.

Russell, S.M., 1998. Capacitance microbiology as a means of determining the quantity of spoilage bacteria on fish fillets. J. Food Prot. 61, 844–848.

Russell, S.M., Fletcher, D.L., Cox, N.A., 1995. Comparison of media for determining temperature abuse of fresh broiler carcasses using impedance microbiology. J. Food Prot. 58, 1124–1128.

Schwan, H.P., 1963. In: Electrophysiological Methods, Part B, Physical techniques in biological research, Vol. VI, Academic Press Inc, New York.

Silverman, M.P., Munoz, E.F., 1979. Automated electrical impedance technique for rapid enumeration of fecal coliforms in effluent from sewage treatment plants. Appl. Environ. Microbiol. 37, 521–526.

Smith, P.J., Boardman, A., Shutt, P.C., 1989. Detection of *Salmonella* in animal feeds by electrical conductance. J. Appl. Bacteriol. 67, 575–588.

Sokal, R.R., Rohlf, F.J. (Eds.), 1981. Biometry, W.H. Freeman and Co, New York, pp. 1–859.

Stryer, L., 1988. Biochemistry, 3rd ed, W.H. Freeman & Co, New York.

Throm, R., Specter, S., Strauss, R., Friedman, H., 1977. Detection of bacteriura by automated electrical impedance monitoring in a clinical microbiology laboratory. J. Clin. Microbiol. 6, 271–273.

Zhou, X., King, V.M., 1995a. A rapid bactometer method for screening of biocides against sulfate-reducing bacteria. Appl. Microbiol. Biotechnol. 43, 336–340.

Zhou, X., King, V.M., 1995b. An impedimetric method for rapid screening of cosmetic preservatives. J. Ind. Microbiol. 15, 103–107.