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Impedance microbiology: quantification of bacterial content in milk by means of capacitance growth curves☆

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Abstract

The impedancimetric method is a technique for the rapid evaluation of milk bacterial content and also of its subproducts. Several authors have made use of culture conductance changes during bacterial growth for quantitative and qualitative assessments of microbial growth. However, interface capacitance curves, C_i, have not been used. In this paper, we quantify bacteria in cow raw milk by following their growth as the above-mentioned capacitance change time course event. With it, bigger growth variations, shorter detection times and a better coefficient of correlation with the plate count method were obtained than those yielded by conductance curves. Calibration was performed by plotting initial known concentrations, IC (CFU/ml), as a function of the time detection theshold (TDT).

Keywords: Bacterial quantification; Milk contamination; Electrode–electrolyte interface; Interface capacitance; Conductance

1. Introduction

Electrical impedance, as a principle of transduction, has been applied in a wide variety of biomedical problems (Geddes and Baker, 1989). One application lies in the field of microbiology, i.e., as a means to detect, quantify and even identify bacteria. Most of the available information can be found in the classic paper of Ur and Brown (1975), in the first and only book dealing with the subject by Firstenberg-Eden and Eden (1984), in the review of Silley and Forsythe (1996), and in the interdisciplinary review by Valentinuzzi et al. (1996).

From 1975 on, and to this date, very few papers have added new basic knowledge or new ideas to the subject (Hause et al., 1981; Firstenberg-Eden and Zindulis, 1984; Felice et al., 1992; Felice, 1995). Most of the contributions of this period are mainly concerned with particular applications of the technique using existing commercial equipments (such as BACTOMETER® or MALTHUS®).

In impedance microbiology there are two main measurement techniques, direct and indirect. In the first one, the electrodes are immersed in the same cell that the inoculum is, and the changes in impedance result from changes taking place in the bulk electrolyte, in turn produced by the metabolic activity of the microorganisms (Silley and Forsythe, 1996). The indirect technique is used when the salt

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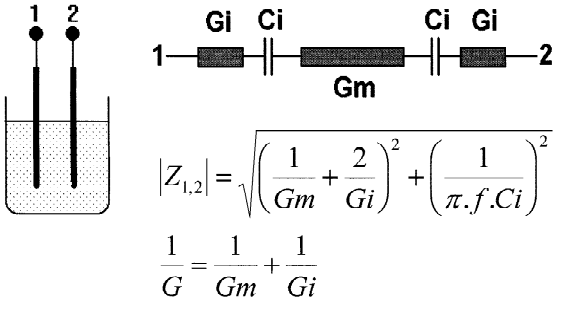
concentration of the culture broth is too high. In such case, the impedance readings are outside the normal working range of the direct impedance method. This problem can be overcome by monitoring microbial metabolism via the production of CO_2, in a chamber with electrodes and potassium hydroxide. The inoculated culture medium is in a separate and near chamber and not in contact with the electrodes or potassium hydroxide (Owens et al., 1989).

From an electrical point of view, the impedance between two electrodes can be modeled by a series circuit, as shown in Fig. 1. It includes the medium conductance G_m, the interface conductance G_i and capacitance C_i of each electrode, and also G as the total conductance. Roughly, C_i represents the double-layer capacity of the electrode–electrolyte interface (Felice, 1995).

Most of the applications are found in the detection and quantification of microorganisms in milk and dairy products. Cady (1978) was one of the first to propose impedance as an alternative method to replace the plate count for rapid screening of milk microbial content. Gnan and Luedecke (1982) used the BACTOMETER®, a commercially available system, for the same purpose. Other investigators contributed to the subject using either the above-mentioned instrument (Suhren and Heeschen, 1987; Neaves et al., 1988; Pirovano et al., 1995) or the MALTHUS® system (Richards et al., 1978; Suhren and Heeschen, 1987; Neaves et al., 1988; Visser and De-Groote, 1984), always in dairy products. The impedance technique was also applied to monitoring total microbial load in meat (Russell et al., 1994) and fish (Van Spreeken and Stekelenburg, 1986), for



$$|Z_{1,2}| = \sqrt{\left(\frac{1}{Gm} + \frac{2}{Gi}\right)^2 + \left(\frac{1}{\pi . f . Ci}\right)^2}$$

$$\frac{1}{G} = \frac{1}{Gm} + \frac{1}{Gi}$$

Fig. 1. Electrical circuit equivalence between two electrodes. $Z_{1,2}$, impedance modulus; G, total conductance; G_i, interface conductance; C_i, interface capacitance; G_m, medium conductance.

the detection and estimation of yeast in fruit juices (Deak and Beuchat, 1993) and wine (Henschke and Thomas, 1988), or to detect *Escherichia coli* in potable water (Colquhoun et al., 1995).

In this paper, we analyze a practical application of the direct impedance technique to quantify microorganisms in raw cow's milk by means of interface capacitance and total conductance time-growth curves obtained at 1000 Hz. Calibration was performed by plotting initial known concentrations, IC (in colony forming units = CFU/unit volume), which were determined by plate counting, as a function of the time detection theshold (TDT, in units of time). Each cell contained nutrient broth and raw milk in equal parts. Our results indicate that C_i time–growth curves produce larger changes, detection times somewhat shorter and a better coefficient of correlation with the plate count method, than those produced by conductance curves (see Section 3 for numerical details).

2. Materials and methods

Capacitance, C_i, and total conductance, G, were measured with a previously described laboratory custom-made equipment (Felice et al., 1991). We did not measure the interface conductance G_i because its information is redundant: at the working frequency (1000 Hz), G_i includes data from the double-layer capacitance and medium conductance, while both types are measured separately in C_i and G, respectively (Felice, 1995).

The culture cells (8 ml each) were constructed with Pyrex glass having on their top a bakelite cover which holds two identical stainless steel removable electrodes (DENTAURUM™, $\emptyset = 1$ mm, $l = 10$ mm, $A = 0.31$ cm^2). The injected current working frequency was 1000 Hz at a density not higher than $J = 1$ mA/cm^2. A thermostatic bath, containing 32 culture cells, kept their temperature within ± 0.2°C.

Before each experiment, cells were washed and rinsed with distilled water and sterilized (20 min in autoclave at 121°C); thereafter, always under sterile conditions, they were filled with 4 ml of sterilized culture medium (out of a mother solution prepared with glucose 4 g, yeast extract 20 g, tryptone 20 g, and distilled water 1000 ml) plus 4 ml of raw milk

and, finally, fitted with the electrodes. The equipment was connected to the electrodes, while another 4-ml aliquot was plate counted (72 h, at 30°C). Milk samples were collected over 2 months from different dairy farms.

The time detection theshold (TDT) corresponds to the point where there is a maximum in the second derivative of $1/G$, $1/C_i$ growth curves. We use the inverse to avoid distortion of the logarithmic initial phase in growth curves. To reduce noise or signal disturbances, air bubbles were removed and thermic transients were avoided.

The samples (240 data points), produced a collection of points or pairs of (TDT, IC) values which were obtained from the C_i, G and SPC (standard plate count) assays. Besides, they were plotted in a scattergraph calculating also the linear regression, standard error of the mean (SEM) and correlation coefficient. This relationship was used as calibration.

3. Results

Fig. 2 displays a typical growth curve as shown by the total admittance $Y(=1/Z)$ between electrodes, and also by its total conductance G and interface capacitance C_i components. In all assays, the maximal growth variations of C_i excursion in percent (150–1250%) was always larger than those displayed by G (36–150%).

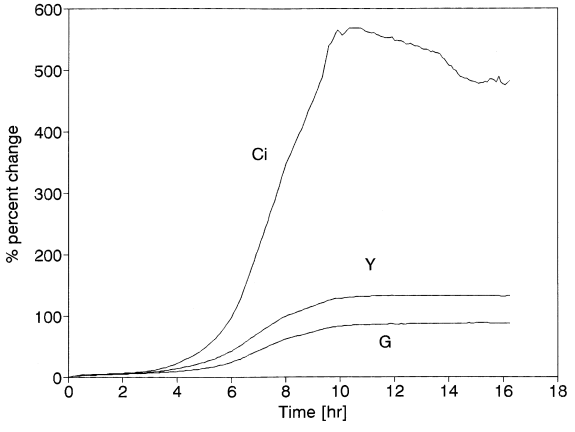


Fig. 2. Typical growth curves. Admittance modulus ($Y=1/Z$) growth curve with its conductive (G) and capacitive (C_i) parts are displayed. The sample is raw milk in culture medium at 30°C.

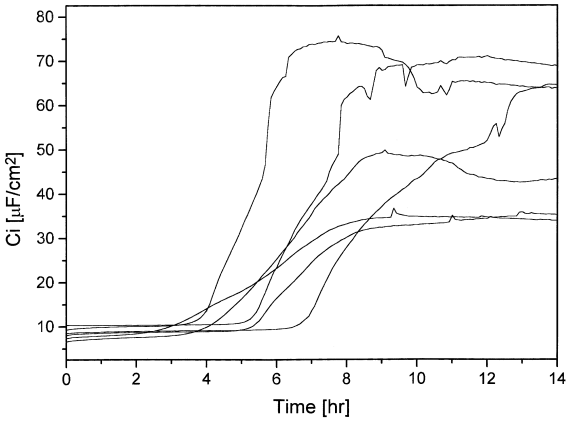


Fig. 3. Typical curves of interface capacitance C_i (in $\mu F/cm^2$). These curves are examples of those obtained during the experiments, including curves of low and high slope.

Fig. 3 illustrates a few interface capacitance curves. In them, the TDT detection algorithm included in the equipment software filtered out artifacts due to bubbles or lipid residues. The calibration regression line (Fig. 4) for C_i was,

$$IC = A + (B \times TDT) = 7.03 - 0.39 \times TDT \quad (1)$$

with a standard error of the estimate, SEE = 0.59 (68% of points lie within a ±0.59 band around the

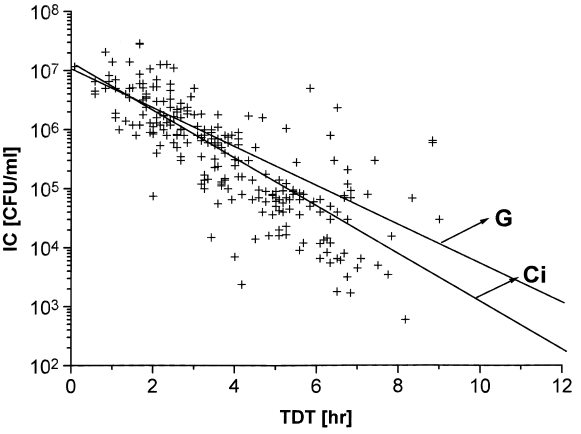


Fig. 4. Calibration curve: initial concentration IC versus threshold detection time TDT for C_i and G curves. The linear regression of the data for C_i curves gave: IC = 7.03 − 0.39 × TDT, with a correlation coefficient CC = −0.78, and a standard error of the estimate SEE = 0.6. The calculation contains 240 points that belong to raw milk assays of different dairy farms. For better clarity the data points for G curves are not shown.

straight line). The correlation coefficient was −0.78 and the standard deviations of the coefficients *A* and *B*, were 0.08 and 0.02, respectively. For *G* (Fig. 4), instead, it was,

$$IC = 7.0 - 0.33 \times TDT \quad (2)$$

with SEE = 0.63, a correlation of −0.74 and SD's 0.1 and 0.02, respectively, for the independent term *A* and the slope *B*.

The two regression lines were compared by means of the unpaired *t*-test finding a significant difference ($\alpha < 0.05$). Besides, the data indicate detection times smaller for C_i than for *G*; for example, for IC = 10 000 (CFU/ml), the C_i curve detects growth about 90 min before the conductance curve *G*. Fig. 4 displays both regressions, for C_i and *G* curves, showing how TDT for C_i curves is lower than *G* for lower initial concentrations. Another advantage is a slightly and statistically significant higher correlation (−0.78 for the former versus −0.74 for the latter).

About 10% of the experimental curves had anomalous behavior producing a TDT larger than the expected value. In other words, according to the initial concentration given by SPC, we should have obtained a shorter detection time. When the anomalous points referred to above were removed from the set, better correlation values were obtained, both for capacitance and conductance curves.

4. Discussion

The method of using C_i to quantify bacteria may be useful in a dairy plant for the quality evaluation of raw milk samples. Table 1 shows the advantages of using C_i instead of *G* curves to detect and quantify bacteria. The correlation coefficient (−0.78) can be improved by optimizing the growth temperature (Firstenberg-Eden and Eden, 1984).

Normally, changes in medium conductance G_m recognize the metabolic products generated by microorganism proliferation. By and large, weakly charged substances are transformed into highly charged terminal products, for example, the breakdown of proteins to amino acids, of lipids to acetates, or of carbohydrates to lactates. All these final metabolic products carry a larger electric charge, are smaller and, thus, show a higher mobility than the original substances (Firstenberg-Eden and Zindulis, 1984). Moreover, with bacteria, the contribution to the medium conductance changes are mostly due to proteolytic activity while carbohydrate metabolism is of less importance (Suhren and Heeschen, 1987). With yeast or moulds, instead, there are no large changes in G_m because they do not produce strongly ionized metabolites, but non-ionized end-products (such as ethanol) which tend to decrease the total conducting ionic content in the broth (Ebina et al., 1989; Silley and Forsythe, 1996).

The interface capacitance C_i herein measured is more difficult and elusive to interpret because it contains contributions due to the geometry (roughness) of the electrodes and also to the electrochemical characteristics of the interface (Felice, 1995). Monofrequency equipments are not suited to extract the interface impedance components, meaning that we should use instruments that can measure impedance in a wide low frequency spectrum (from mHz to kHz).

As a rough approximation, and using data obtained with similar material by NASA (NASA KSC-11575, 1993), we have estimated that C_i contains contributions from the double-layer capacitance and, in variable proportion, from the medium conductance. The latter component was included due to the roughness of the electrodes (Felice, 1995).

The double-layer capacity depends on ion diameter, valence, and density (Schmickler and Henderson, 1986). In a typical nutrient broth there is not a

Table 1
Comparison of C_i versus *G* curves for detection and quantification of bacteria in raw milk (advantages of using C_i over *G* curves)

	C_i curves	*G* curves
Maximum growth variation (%)	150–1250	36–150
Time detection theshold (h)(for IC = 10^4 CFU/ml)	7:46	9:05
Correlation coefficient IC-TDT	−0.78	−0.74

single kind of ion, but there is instead a complex mixture of organic and inorganic ions. When the microorganisms grow, they can modify the double-layer capacitance because bacterial metabolism induces changes in the ionic diameters, in the charges and in the ionic concentrations. The same factors affect also G_m (Firstenberg-Eden and Eden, 1984), however, in our experiments, C_i appears to be more sensitive to these kind of changes.

The above analysis is just a guide to qualitatively understand the possible events at the level of the bipolar impedance components during bacterial proliferation. Theoretical studies should go deeper, perhaps choosing as a practical goal an improvement of the method sensitivity, the latter defined as the minimum cellular concentration to elicit appreciable either resistive or reactive changes. These results point to the interface as an acceptable valid tool for that purpose.

5. Conclusions

The impedancimetric method, using the interface capacitance curves, is applicable for the detection and quantification of raw cow's milk bacterial content. With it, we can obtain greater growth variations, threshold detection times somewhat shorter, and a coefficient of correlation between IC and TDT slightly but significantly better than those obtained by total conductance curves, thus offering some advantage. Besides, we think the latter can be improved by modifying the incubation temperature.

At 1000 Hz, medium conductance represents the suspending culture broth while the capacitance is a complex mixture of medium conductance and capacitance due to the double layer. Separation of these two components requires more research.

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