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Hypothetical model for monitoring microbial growth by using capacitance measurements – a minireview

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Abstract

Microbiological impedance devices are used routinely by food and manufacturing industries, and public health agencies to measure microbial growth and metabolism. In this paper a hypothetical model explaining the effects of microbial growth and metabolism on capacitance at electrode-medium interfaces, that can be supported by fundamental theories and principles of electrochemistry, is presented. This model provides a framework to interpret changes in capacitance during microbial growth and metabolism and can be used to generate and test hypotheses on factors (i.e., temperature, microbial cell density, microbial growth and medium conductivity) contributing to increases or decreases in capacitance.

Keywords: Capacitance; Impedance; 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 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 bacterial growth because a relationship can be established between the ionic composition of the growth medium and bacterial metabolism. That is, as large uncharged organic molecules in the culture medium are metabolized by bacteria, there is an increase in the quantity of ionized metabolites in the medium.

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Both conductance and capacitance values of the culture medium concomitantly change with the accumulation of these charged end-products. Impedance devices monitor both the accumulation of charge at the electrode-medium interface and the increase in ions in the medium by comparing baseline values obtained within the first hour of incubation to values obtained at specified time periods. 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. 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 principle of impedance microbiology appears to be simple and straightforward, it was not until 1985 that it became possible to anticipate whether a particular microorganism–medium combination would increase or decrease the conductivity of a medium. Owens (1985) described the basic theory of electrolytic conductivity in culture medium and proposed a model which allowed the direction and relative rate of change of conductivity in cultures to be predicted. His paper was the first comprehensive study on changes in conductance during bacterial growth.

Changes in capacitance at the culture medium–electrode interface during microbial growth are poorly understood. A hypothetical model explaining the relationship between culture medium conductivity and capacitivity at electrode surface–culture medium interfaces is needed so that hypotheses on factors contributing to increases or decreases in capacitance can be generated and tested. Such information would be useful for industrial and public health microbiologists since several studies have shown that capacitance signals provide a better indicator of microbial growth than conductance signals (Dziuba et al., 1993; Noble, 1994; Noble et al., 1991). Moreover, by knowing factors affecting capacitance, microbiologists will be better able to rationally design culture media.

The focus of this review is to present a hypothetical model explaining the effects of microbial growth on capacitance that can be supported by fundamental theories and principles of electrochemistry. I assert that the principles of capacitance microbiology need to be clarified so that hypotheses on factors, such as temperature, microbial cell density, microbial growth, and medium conductivity, contributing to increases or decreases in capacitance can be generated and tested. 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; Russell, 1997, 1998; Noble et al., 1991; Firstenberg-Eden and Zindulis, 1984), do not support these findings. To date, technical literature on the physical, chemical and electrical aspects of capacitance at the electrode–medium interface is difficult to understand for most nonspecialists. Here I present a model which provides a framework to interpret changes in capacitance during microbial growth for microbiologists.

2. Theoretical concepts

2.1. Relationship between impedance, capacitance, susceptance, and conductance

Impedance (Z) is a complex entity composed of two distinct components: resistance and reactance (Kell and Davey, 1990; Schwan, 1963). The resistive component of impedance is inversely related to conductance (G), while the reactive component is inversely related to capacitance (C). The formula that describes this relationship for a capacitor and resistor in series is given by

$$Z^2 = (1/G)^2 + [1/(2\pi fC)]^2 \quad (1)$$

where f is frequency. In this model, the resistance is due to the solution and the capacitance is due to the electrode–medium interface. The inverse of impedance is admittance (Y) and this entity is related to conductance and capacitance. Susceptance (B_c) is equivalent to capacitance except it is dependent upon frequency and has the same units as conductance (i.e., B_c (μS) $= 2\pi fC$. One can determine the contribution of resistance and reactance to impedance by comparing the observed current waveform in response to application of a sinusoidally-modulated

voltage waveform (Kell and Davey, 1990). If the impedance is due entirely to the resistance, the phase angle between current and voltage would be 0. On the other hand, if impedance is due to the reactance, the phase angle between current and voltage would be 90°. With both resistive and reactive components present, the phase angle varies between these extremes and can be used to determine both resistance and capacitance.

Microbiological impedance devices monitor the phase angle between voltage and current by using two metal electrodes that are immersed in a culture medium. Application of a small DC current through the electrodes causes cations and anions in the medium to move toward negatively- and positively charged electrodes, respectively. Application of a small AC through these electrodes causes ions to move from one electrode to another in a frequency-dependent manner (Hause et al., 1981). Impedance devices measure resistance and reactance at timed intervals using an AC at a specified frequency. Mathematical algorithms compare impedance values obtained at different times to determine whether or not a significant change has occurred. A series of significant changes in impedance values is reported as a DT.

2.2. Electrode–medium interface

The environment in close proximity to the electrode is quite different from that of the bulk culture medium. Helmholtz (1879) described this interface as two sheets of rigidly fixed charge, one sheet being the electrode surface and the other being the medium. Since the charge densities on each sheet are equal in magnitude but opposite in charge, the relationship between the electrode surface and the medium is not unlike a parallel-plate capacitor which can mathematically be expressed as:

$$C_{\mathrm{H}} = \epsilon\,(4\pi d)^{-1} \tag{2}$$

where C_{H} refers to the Helmholtz capacitance, ϵ is the dielectric permittivity (i.e., a measure of the ability of the medium to reduce the charge on the electrode) and d is the distance between plates (Bockris and Reddy, 1970). This equation predicts that changes in capacitance are due to changes in the dielectric permittivity or the distances between the plates. However, observed data suggest otherwise (Bockris and Reddy, 1970) and has consequently led to the development of alternative models.

Rather than consider the interface between an electrode and a liquid as two sheets of rigidly fixed charge, Gouy (1909) and Chapman (1913) proposed that an electrode submerged in a liquid is enveloped by an ionic cloud. In this model, not only are ions affected by the electrode charge, but they are affected by the thermal buffeting of particles in solution. The ionic atmosphere around the electrode diffuses exponentially into the liquid medium with increasing distance from the electrode (Bockris and Reddy, 1970). The mathematical equation used to explain this relationship is

$$C_{\mathrm{G\text{-}C}} = [(\epsilon z^2 e_{\mathrm{o}}^2\, n)\cdot(2\pi kT)^{-1}]^{1/2}\cosh(ze_{\mathrm{o}}\psi)\cdot(kT)^{-1} \tag{3}$$

where $C_{\mathrm{G\text{-}C}}$ refers to the Gouy-Chapman capacitance, ϵ is the dielectric permittivity, z is the valence of ion species, e_{o} is the electronic charge, n is the ion concentration, ψ is the potential (v), k is the Boltzmann constant and T is the temperature (°K). This equation predicts that there is a concomitant increase in capacitance with ion concentration. The Gouy-Chapman model provides a reasonable prediction of capacitance in solutions providing a very low number of ions (e.g., <0.001 M NaCl). However, a comparison between predicted and observed capacitance in high salt solutions (e.g., 1 M NaCl) shows that Eq. (3) overestimates the capacitance that is actually observed (Bockris and Reddy, 1970).

Stern (1924) united the Helmholtz and the Gouy-Chapman models by stating that there are 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 (Bockris and Reddy, 1970). These layers are in series and can be mathematically expressed as

$$(C_{\mathrm{S}})^{-1} = (C_{\mathrm{H}})^{-1} + (C_{\mathrm{G\text{-}C}})^{-1} \tag{4}$$

where C_{S} is Stern capacitance and C_{H} and $C_{\mathrm{G\text{-}C}}$ are from Eqs. (2) and (3), respectively. The significance of Eq. (4) is that in a dilute liquid medium, the

diffuse layer has greater effects on capacitance than the two sheets of rigidly-fixed charge proposed by the Helmholtz model. In a concentrated liquid medium, the reverse is true. That is, the Helmholtz model has greater effects on capacitance simply because the $C_{G\text{-}C}$ component in Eq. (4) becomes insignificant.

The electrodes commonly used in microbiological impedance devices are made of stainless steel on which a passivation surface layer composed of mainly chromium oxide and hydroxide is formed by aging in NaOH solution (Olefjord, 1980). The passivation layer, which is only a few atoms thick, lowers the dissolution rates of the metal and protects the metal from corrosion. There is a capacitance associated with the oxide layer C_{ox} and a resistance of the oxide which are in series with the Stern capacitance. This leads to a capacitance associated with the oxide layer C_{ox} and a resistance of the oxide which are in series with the Stern capacitance. The measured capacitance is then

$$(C)^{-1} = (C_{ox})^{-1} + (C_s)^{-1} \quad (5)$$

where C_{ox} is the effective oxide capacitance at a given frequency and the contribution from the oxide resistance has been neglected (Morrison, 1980). The passivation layer can change due to solution conditions such as pH, and this will change C_{ox}. Consequently, pH changes may influence the observed C, although this will depend on conditions.

3. Synthesis and conclusions

The hypothetical model describing capacitance in microbial impedance devices is presented in Eq. (5). Although the C_s (Eq. (4)) component of the model (Eq. (5)) indicates that conductivity of the culture medium affects capacitivity, it is difficult to ascertain whether these affects will be significant or entirely negligible. The effects of conductivity on capacitance are further complicated by temperature. In low conductance medium, the Gouy-Chapman (Eq. (3)) portion of C_s (Eq. (4)) predicts that capacitance should decrease with increasing temperature. However, in high conductance medium, changes in temperature should be negligible since temperature is not accounted by the Helmholtz (Eq. (2)) portion of the C_s model (Eq. (4)) and the Gouy-Chapman (Eq. (3)) portion of C_s (Eq. (4)) is insignificant. Inclusion of C_{ox} in the model (Eq. (5)) implies that changes in the thickness and structure of the passivation layer of the metal electrodes may also significantly affect capacitance. This complicates interpreting what causes changes in capacitance in impedance devices since protons affect both C_s and C_{ox}.

The hypothetical model (Eq. (5)) presented in this review warrants experimentation to determine its validity. Experiments focused on the relationship between medium conductivity and capacitivity will enable us to determine how increases in ions affect capacitance of stainless steel electrodes immersed in culture media . For example, the model predicts that at pH 7, increases in medium conductivity would increase the measured capacitance until the dielectric permittivity is maximized and/or the distance of closest approach between the electrode surface and bulk medium is minimized [as accounted for by C_H (Eq. (2))]. In such conditions, the C_{ox} component of the model would not be affected by increased conductivity. However, decreases in pH of culture media would likely affect both C_s and C_{ox} of the model. In the model, protons affect C_H and $C_{G\text{-}C}$ components of C_s as well as the surface of the passivation layer (i.e., C_{ox}). The validity of the model can be determined by examining how capacitance changes with respect to pH and conductivity. In addition, several factors (i.e., temperature and cell densities) may also affect the conductivity of the growth medium as well as capacitivity. Determining which of these factors significantly contribute to changes in capacitance is essential for anticipating whether a particular microorganism–medium combination will increase or decrease capacitance. Such an approach will enable microbiologists to rationally design culture media for monitoring specific microbes.

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