Radio-frequency and microwave spectroscopy investigation of bacteria solutions: determination of the aggregation threshold

Mustapha Merabet

Department of Maths and Natural Sciences,
American University of Ras Al Khaimah,
Ras Al Khaimah, UAE
Email: mustapha.merabet@aurak.ac.ae

Abstract: This paper presents novel approaches to assess and characterise the biomass concentration of bacteria in a broth culture medium. It is based on the use of the colloid inductive permittivity probe, which is not affected by the electrode polarisation, and on the analysis of the distribution of relaxation processes. Specifically, the frequency-dependent dielectric responses of Lactobacilli in DMEM are investigated in the [75 kHZ – 30 MHz] and in the [130 MHz to 20 GHz] ranges. Moreover, due to the direct measurement over wide concentration ranges, the concentration threshold of the bacteria aggregation can be precisely identified. As a matter of fact, the behaviour of the amplitude of the medium-frequency relaxation process shows two distinct linear evolutions with an intersection corresponding to the aggregation threshold. The dielectric method can be easily adapted to the online monitoring of the growth of biological cells and to the control of the fermentation processes.

Keywords: aggregation; biological cells; biological process; biomass; conductivity; interface phenomena; dielectrics; dielectric measurement; dielectric spectroscopy; electromagnetic absorption; materials testing; microwave measurement; permittivity.


Biographical notes: Mustapha Merabet is an Associate Professor of Physics at the American University of Ras Al Khaimah UAE. He received his PhD in Energy Sciences from the National Institute of Scientific Research – Energy Quebec Canada, and spent 25 years in industrial research. His current research interests include electromagnetic processing, microwave and embedded systems.

This paper is a revised and expanded version of a paper entitled ‘Online measurement of biomass using colloid dielectric probe and open-ended cell: determination of the aggregation threshold’ presented at 5th International Conference on Electronic Devices, Systems and Applications ICEDSA – 2016 held at the American University of Ras Al Khaimah UAE, 6–8 December 2016.

1 Introduction

The objectives of this series of studies on bacteria in a given culture medium are to assess the concentration of the dispersed particles and to characterise the change in the water distribution between the different coexisting water states in bacteria and in the medium. The reason behind such work is to use the behaviour of the dielectric response of bacteria cultures as ‘fingerprints’ of the various strains of bacteria and in further steps to investigate the influence of the main components of the culture media on the behaviour of bacteria strains.

The fermentation and bacterial cultivation process technologies are hindered by the lack of reliable methods for the online precise and rapid monitoring of the growth profiles of the biomass without interference. The active biomass of interest in cell cultures refers to the cellular material that is able to grow and to divide and that may have some metabolic activities. Such active biomass is often mixed with inactive biomass which includes dead cells and cell fragments.

There are several means to estimate the cell concentration, but only limited techniques can be adapted to in-situ cell concentration measurements. One of the main limitations of the techniques relates to the lack of selectivity. The selectivity there refers to the ability to distinguish between living cells and all the non-active cellular material including dead cells, fragments of cells and solid and semi-solid particulates in the culture medium. The existing methods, which may be adapted to the online evaluation of the growth kinetics of cell cultures may be grouped into direct and indirect methods. The direct methods refer to techniques that measure directly one or more physical properties of the cell and its components and include fluorescence, turbidity and light scattering. The
indirect methods consists of techniques which measure parameters related to cells and their activities and include oxygen consumption, carbon-dioxide content, protein content, organic matters assimilation or excretion and pH. This group of techniques includes near-infrared spectroscopy, electrical conductivity and electrical impedance of the medium (Yardley et al., 2000; Kiviharju et al., 2001). These indirect approaches, based on mass balances or on changes induced by cells in the liquid medium, require tedious calibration procedures as they are highly dependent on the composition of the culture medium.

The dielectric spectroscopic approaches provide a number of inherent advantages (Ducommun et al., 2001; Feldman et al., 2003) such as the selectivity (living versus dead cells and fragments), rapidity of the measurement, the possibility to use the dielectric measuring cell in adverse conditions (temperature, electrolyte contents, acidic media, air bubbles and solid and semi-solid particulates in the medium). Obviously, the simultaneous realisation of all these advantages requires a specific design of the dielectric spectroscopy based method for the biomass assessment.

The dielectric response investigations are based on the identification and the analysis of the distribution of the electrical charges in the system studied and on its behaviour as a function of the frequency. Also the dielectric response can be studied as a function of other parameters such as temperature, composition or variations in the material manufacturing process. Depending on the frequency range, various physical phenomena may be identified, and the information gained would obviously relate to different scales of the studied system. In the microwave frequency range, it is mostly the dipolar nature of the system that is probed and therefore most of the obtained information relates to polar molecules or molecular aggregates. In the radio-frequency range, phenomena occurring at the interfaces between phases are tested and therefore physical structures in the nanometre-micrometre range may be characterised.

The experimental problems encountered relate mainly to the electrode polarisation due to the presence of electrolytes in the culture media and to the spurious effects of air bubbles on the measured electrical properties.

The choice of the dielectric permittivity is an intrinsic property of the material, instead of the capacitance that depends strongly on the measuring cell and on its physical state, constitutes a real advantage. Other problems associated with the electrode polarisation in the low-frequency side require a separate mathematical treatment or the use of the appropriate measuring device to avoid such spurious phenomena (Colonomos and Gordon, 1979).

The second part of this work relates to the determination of the aggregation threshold as the bacterial aggregation phenomena under adverse conditions are of great importance in biotechnology. Several techniques have been used to study the aggregation process, mainly based on the measurement of the absorbance under very specific experimental equilibrium conditions. The understanding of the relationship between the physical and the biological processes would benefit greatly from an independent determination of the aggregation threshold under adverse conditions of agitation as well as the presence of other particulates in the culture medium.

2 Theoretical background

The dielectric permittivity $\varepsilon^* = \varepsilon' - j\varepsilon''$ is a complex physical quantity that describes the interactions between a given material and an oscillating electrical field and is an intrinsic property of this material. The real part of the permittivity $\varepsilon'$ is related to the polarisation state of the medium under the action of the electrical field and indicates how much energy is stored in the medium through the polarisation which is equivalent to the elastic response in mechanics. The imaginary part $\varepsilon''$ also known as the dielectric absorption, refers to the energy dissipated within the material and lost as heat dissipation by the frictional motion of dipoles under the action of an electric field (viscous response in mechanics). By appropriately choosing the frequency range where the two properties $\varepsilon'$ and $\varepsilon''$ are investigated as function of the frequency, one can get very pertinent information on the microscopic structure of the material tested.

From a ‘dielectric’ point of view, e.g., in terms of electric interactions, cells in a liquid culture medium behave as very complex dispersions of inclusions in a liquid containing electrolytes. The shape of the inclusions may range from the sphere which is the simplest structure to the stick, which is the most complex case.

In such a case, the dielectric permittivity of the whole system consisting in a continuous liquid medium with inclusions may be described by the dielectric and conductive properties of the components. Inversely, from the dielectric permittivity of the whole system, one can derive valuable information on the dispersed phase, e.g., the cells present in the culture medium. The formalism of Maxwell-Wagner describing the interactions at the interfaces between the dispersed inclusions and the continuous phase is the most appropriate approach. Obviously, it requires the knowledge of the dielectric response in the frequency range where these electric interactions at the interfaces are the most predominant phenomena. It is in the radio-frequency range that such interfacial interactions may be measured. Also, it would be possible to investigate the dielectric response in the microwave range where the most important contribution is due to water dipoles present in both the cells and the culture medium.

2.1 Interfacial polarisation

All electro-kinetic phenomena at the interfaces between the inclusions and the continuous phase may be treated within the general formalism of the equilibrium state of the interfacial polarisation. In the present case, electro-kinetic phenomena of interest include migration of ions and of
dipolar and multipolar compounds through the surface of the dispersed inclusions, condensation of ions and of counterions at the surface of inclusions and modification of electric charge distributions across the interface. The investigation of the electric polarisation occurring at these interfaces, inform on the equilibrium state of the resulting electric charge distribution and therefore allows the characterisation of some of the mentioned electro-kinetic phenomena.

Considering the simplest case of spherical inclusions having a dielectric permittivity \( \varepsilon_B \) and conductivity \( \sigma_B \) dispersed in a continuum characterised by \( \varepsilon_B' \) and \( \sigma_B' \) as dielectric permittivity and electrical conductivity. Under the action of an electric field of angular frequency \( \omega \) \( (= 2\pi f) \) where \( f \) is the frequency in Hertz), the dielectric permittivity \( \varepsilon(\omega) = \varepsilon' - j\varepsilon'' \), and the electric conductivity \( \sigma(\omega) = \sigma' - j\sigma'' \) of the whole system, which are both related to the distribution of electric charges at the interfaces, exhibit large dispersions as function of the frequency, given by:

\[
\varepsilon^* = \varepsilon_0 + \frac{\varepsilon_1 - \varepsilon_0}{1 + j\omega \tau} + \frac{\sigma_1}{j\omega \varepsilon_0} \tag{1}
\]
\[
\sigma^* = \sigma_0 + \frac{j\omega \tau (\sigma_1 - \sigma_0)}{1 + j\omega \tau} \tag{2}
\]

where the subscripts \( l \) and \( h \) refer to low-and high-frequency limits. The resolution of the two equations, lead to the expressions of \( \varepsilon_1 \) and \( \varepsilon_0 \) of the dielectric permittivity, and to \( \sigma_1 \) and \( \sigma_0 \) of the electric conductivity. The expression relating all these 4 low and high limits of the permittivity and the conductivity, is given by:

\[
(\sigma_B - \sigma_i) \tau = (\varepsilon_i - \varepsilon_B) \tag{3}
\]

where \( \tau \) the relaxation time corresponding to the interfacial polarisation, is given by:

\[
\tau = \frac{\varepsilon_B - (1 - \phi)(\varepsilon_B - \varepsilon_D)/3\varepsilon_B}{\sigma_B - (1 - \phi)(\sigma_B - \sigma_D)/3\sigma_B} \tag{4}
\]

where \( \phi \) is the volume fraction corresponding to all inclusions that are polarised under the action of the frequency depending electric field. This parameter \( \phi \) is in fact the parameter of interest. The relaxation time \( \tau \) is experimentally determined, and the dielectric \( \varepsilon_B \) and the conductive \( \sigma_B \) properties of the continuous phase are known or may be easily measured. The volume fraction \( \phi \) of the dispersed phase is then estimated using equation (4) provided the dielectric permittivity \( \varepsilon_B \) and the conductivity \( \sigma_B \) of the dispersed inclusions are modeled adequately or assessed separately from measurements carried out in non-polar solvent constituting the continuous phase.

The relation given by equation (4), is valid over a very wide concentration range of spheroidal inclusions dispersed into a continuous phase.

Some models based on a hybrid treatment of the inclusions/medium interactions combining the dipolar and the interfacial polarisation concepts have been developed and other relations for the volume fraction \( \phi \) of the dispersed inclusions have been proposed. The most pertinent relation is given by:

\[
\Delta \varepsilon_j = \frac{9\phi R C_m}{4\varepsilon_0} \tag{5}
\]

where \( \Delta \varepsilon_j \) is the dielectric contribution of the dispersed phase corresponding to the whole permittivity from which the dielectric permittivity of the medium has been subtracted, \( R \) is the inclusion radius, \( C_m \) is the inclusion interface capacitance and \( \varepsilon_0 \) the low frequency limit of the real part of the dielectric permittivity.

For other types of inclusions such as ellipsoid, discoid or stick, equations that are similar to equation (4), have been proposed and relating the relaxation time to the electric characteristics of the components. The main differences relate to the replacement of the scalar dielectric and conductive parameters by their tensor formulations.

These approaches are relatively accurate for the estimation of the volume fraction of the dispersed inclusions. However, they are limited to mono-dispersed inclusions in the continuous phase. In case of poly-dispersed inclusions, the relaxation time corresponding to the interfacial polarisation and the inclusion radius are described by a distribution of relaxation times requiring some de-convolution procedures that are not easily carried out without affecting severely the accuracy of the computed parameters.

3 Experimental

In the first part of this work, we investigate the dielectric permittivity of different bacteria solutions in a culture medium over different and overlapping frequency ranges. Specifically, we use the Colloidal Dielectric Probe over a radio-frequency range, and an open-ended coaxial line in the microwave frequency range. We also propose a new analysis approach of the measured dielectric spectra to get pertinent information on the active biomass in the culture media. Dielectric measurements were performed in the range 130 MHz – 20 GHz using a microwave vector network analyser (Agilent 8720 D) and in the range 75 kHz – 30 MHz using a precision impedance metre (Agilent LCR 4285 A).

For the higher frequency range [130 MHz to 20 GHz], the measuring cell is of the open-ended coaxial line type. The dielectric response of the material is then derived from the total reflection of the fringing electric fields radiated from the cell.

For the lower frequency side [75 kHz to 30 MHz], the colloid measuring cell is plunged into the liquid medium. The variations of the induced electric currents in the two electromagnetic coils that are embedded into the tore forming the measuring cell are translated in terms of impedance changes and from which the dielectric properties are derived.

For both the radio-and the microwave frequency ranges, the dielectric measurements were carried out at ambient
temperature (22°C). The bacteria cultures were from the nestle collection, and consist in isolated strains of Lactobacilla La1 in a liquid growth medium DMEM from Dulbecco. The strains were grown and prepared according to standard procedures before inoculating the culture media. The bacteria culture samples were magnetically stirred at about 520 rpm for homogenisation.

The tests were carried out at 22°C for the calibration of the dielectric method, and the proportionality factors are used to estimate the concentration of each bacteria concentration culture.

4 Results and analysis

4.1 Standard characterisation of bacteria solutions

Nine (9) different solutions of La1 in DMEM were prepared via two series of dilutions, covering from $3.2 \times 10^7$ to $5.2 \times 10^8$ cfu/ml. These solutions were first tested using the plate counting and the optical density measurements at 650 nm. Both the plate counting and the optical density measurements were performed on diluted solutions in the 10E + 08 bact./ml range, and an extrapolation procedure is applied for more concentrated solutions.

The comparison between the plate counting and the optical density leads to a somehow linear curve with relatively large uncertainty. The linear regression of this curve gives a slope $b = (9.38 \pm 0.43) \times 10^7$. The residues are not uniformly distributed, while the correlation factor $R$ which is about 0.992 seems acceptable.

This linear regression analysis gives a relatively large weight to larger concentrations than for smaller ones. In order to insure that all concentrations studied contribute equally to the definition of the regression curve, the log-log plots of these results are given in Figure 1.

**Figure 1** Log-log plot of the calibration curve plate counting vs. optical density (see online version for colours)

![La1 in DMEM Calibration CFU vs OD](image)

The linear regression curve is better defined, and the regression analysis yields for the slope a value of about $b = 1.01 \pm 0.04$.

The distribution of residues is quite uniform, and the correlation factor is about 0.994, indicating a good correlation with a factor of about $1.01 \pm 0.04$ between the logarithmic values of the concentrations of La1 solutions in DMEM as determined by plate counting and by optical density at 650 nm.

To the first approximation, the optical density may be used to estimate the bacteria concentration. However, in the 108–109 u/ml concentration range which is of prime importance, the errors are relatively large and therefore this method is of very limited practical use.

4.2 Radio-frequency dielectric response

4.2.1 RF conductivity behaviour

The precision LCR metre has been set to measure automatically every 15 minutes the RF conductivity of bacteria solutions. Thirteen different concentrations of La1 in DMEM ranging from $1.947 \times 10^6$ to $9.07 \times 10^8$ units per ml, have been tested. For each concentration, 3 to 4 measurements (every 15 minutes) were performed to test the dielectric response as a function of time as a test for the stability of the measurements.

Results for the RF-conductivity as a function of time for some concentrations are given in Figure 2.

The series of plateaux corresponding each to a given concentration and tested at different times, indicate that there is quite no evolution of the dielectric response with time as the sample is vigorously stirred with a magnetic agitator during all the measurement process.

For clarity, only results for 250 kHz, 4.2 MHz and 20 MHz are reported in Figure 2. Whatever the measuring frequency, the RF-conductivity is quite constant for each concentration, indicating that the resting time before carrying out the measurement is of no significance.

**Figure 2** RF-conductivity evolution as a function of time for three different frequencies and five different concentrations of La1 (see online version for colours)

![RF-Conductivity](image)

Note: The plateaux correspond to $2.6 \times 10^6$, $6.8 \times 10^6$, $1.8 \times 10^7$, $2.1 \times 10^8$ and $5.1 \times 10^8$ u/ml concentration.
In Figure 3, the RF-conductivity amplitude in siemens-per-metre (S/m) of La1 in DMEM solutions is plotted against the concentration in units-per-millilitre (u/ml). The results of the linear regression show an acceptable correlation. The estimated slope is about 
\[ b = (7.05 \pm 0.23) \times 10^{-10} \] 
and the intercept is about 
\[ a = 1.214 \pm 0.016. \]

**Figure 3** RF-conductivity evolution as a function of concentration of La1 in DMEM (see online version for colours)

This good correlation between the RF-conductivity and the concentration is however limited to this buffer solution only, namely the DMEM.

For other buffer solutions tested, the regression parameters are different, and therefore cannot be used to predict the concentration.

### 4.2.2 Radio-frequency dielectric behaviour

In Figure 4, the behaviour of the real part of the permittivity of two La1 bacteria solution measured using the colloid dielectric probe is presented as function of the frequency. The observed dielectric response indicates that the real part of the permittivity is still increasing at lower frequencies in the audio-frequency range. As there is no contact between the electrodes and the medium under test when using the colloid dielectric probe, spurious effects due to the electrode polarisation are eliminated.

The \( \varepsilon' \) behaviour shows a large dispersion in the frequency range \([300 \text{ kHz} – 10 \text{ MHz}]\) centred about the MHz range for both concentrations.

The high-frequency limit of the dielectric permittivity is of the order of \( \varepsilon_h = 78.4 \) and is the same for both concentrations according to the graph.

The low-frequency limit of the dielectric permittivity is more difficult to estimate as after a noticeable but small plateau, the \( \varepsilon' \) is still increasing at lower frequencies. In fact, we have there an overlapping of the \( \alpha \)-dispersion and the \( \beta \)-dispersion which are associated to different relaxation phenomena.

The audio-frequency relaxation process in the KHz range known as the \( \alpha \)-dispersion is due to the relaxation of ions at the surface of bacterial cells, while the \( \beta \)-relaxation is associated to the interfacial polarisation known as Maxwell-Wagner polarisation.

For the low concentration investigated, the low-frequency limit of the permittivity \( \varepsilon_l \) is about 105 based on the experimental results only, while for the higher concentration we used simulation and extrapolation procedures in addition to the experimental data to estimate \( \varepsilon_l \) to be of the order of 126.

Equation (5) which relates the dielectric increment \( \Delta \varepsilon' \) to the volume fraction \( \phi \) of the dispersed inclusions, can be used successfully to predict \( \phi \) provided the inclusion radius \( R \) and the inclusion interface capacitance \( C_m \) are known.

In fact, the La 1 bacteria have an elongated ellipsoidal shape with a minor axis of about 0.8 μm and a major axis of about 7 μm. In such case, one should use a more complex tensor-based formula relating \( \Delta \varepsilon' \) and \( \phi \) than equation (5). However, this more rigorous approach is beyond the scope
of the present work and will not be considered as we have to estimate a number of other parameters of importance.

We will use instead equation (5) for comparison purpose only by comparing the ratio between the volume fraction $\phi$ and the ratio of the corresponding dielectric increment $\Delta \varepsilon'$. From the measures and the simulation procedures, the ratio between the dielectric increment $\Delta \varepsilon'$ of the two solutions is about 1.77 while the ratio of the two concentrations is 1.80 in quite a good agreement. We plot in Figure 5 the behaviour of the dielectric increment $\Delta \varepsilon'$ as function of the concentration in the low concentration range.

Up to $4.1 \times 10^6$ u/ml which is in the low concentration range in the present study, the dielectric increment $\Delta \varepsilon'$ is almost a linear function of the concentration.

Beyond that limiting concentration of $4.1 \times 10^6$ u/ml, the errors in the dielectric permittivity in the lower frequency range are large limiting thus the assessment reliability of the dielectric increment. For the culture medium without bacteria cells, this dielectric dispersion in the 100 kHz range is very low and of the order of the experimental errors which limit us to draw any conclusion.

4.3 Microwave-frequency dielectric behaviour

4.3.1 Experimental results

The open-ended coaxial microwave measuring cell is plunged in about 100 ml of the solutions (La1 in DMEM) where a magnetic stirrer is placed to insure the sample homogeneity and mimic the experimental conditions of an industrial fermenter. Typical dielectric spectra of DMEM and of two different concentrations of La1 in DMEM, in the 130 MHz – 20 GHz are given in Figure 6.

The real part of the dielectric permittivity $\varepsilon'$ of all the three systems DMEM and La1 in DMEM, exhibit as expected a plateau up to about 3 GHz, followed by a water-type dispersion at higher frequencies. There are slight variations in the low-frequency range values of $\varepsilon'$ between the three systems.

However, the imaginary part of the dielectric permittivity $\varepsilon''$ which is much more informative on the physical aspects, presents a very large absorption in the low-frequency range followed by a broad relaxation process. In the very high frequency range (10 GHz and higher), the dielectric absorption of the three systems is quite identical.

4.3.2 Analysis

The analysis of the dielectric spectra and their decomposition into elementary relaxation processes is carried out using two distinct methods.

Firstly, the Colonomos and Gordon method is used to determine the optimal number of elementary relaxation processes (Colonomos and Gordon, 1979; Bose et al., 1984) and secondly the Debye-Cole descriptive approach is applied to determine the characteristics of each elementary relaxation process (Bose et al., 1984).

The first method is based on a linear programming algorithm, and is independent of the type of the relaxation processes while for the second one a nonlinear curve fitting procedure is used based on the various combinations of elementary relaxation processes of Debye, Cole-Cole or Cole-Davidson types (Bose et al., 1984). In the present case, dielectric spectra as function of the frequency are described as a sum of elementary relaxation processes and a conductive contribution, as:

$$
\varepsilon' = \varepsilon'' - j \varepsilon'' = \varepsilon_\infty + \frac{\sigma}{\varepsilon_0 \omega} + \sum_{i=1}^{m} \frac{\Delta_i}{1 + (j \omega \tau_i)^{-h}}
$$

where $\sigma$ is the conductivity in [S/m], $m$ is the number of elementary relaxation processes determined using the Colonomos and Gordon approach, $\Delta_i$ and $\tau_i$ are the relative amplitude and the relaxation time of the $i^{th}$ process and $\varepsilon_\infty$ is the high-frequency limit of the real part of the dielectric permittivity. For Debye relaxation process characterised by a single relaxation time, the coefficient $h$ is zero. A Cole-Cole process ($h \neq 0$) corresponds to a finite sum of elementary relaxations about a mean relaxation process (Bose et al., 1984).

As the $\varepsilon''$ curves are more sensitive to the model used than the $\varepsilon'$ responses, the fitting procedure are applied to the $\varepsilon''$ spectra, described by:

$$
\varepsilon'' = \frac{\sigma}{\varepsilon_0 \omega} + \sum_{i=1}^{m} \frac{\Delta_i (\omega \tau_i)}{1 + (\omega \tau_i)^{-h}}
$$

Fitting results for DMEM, and for two different concentrations of La1 in DMEM are given successively in Figures 7, 8 and 9.

The quality of the fit is assessed using the Akaike criterion which is given by:

$$
AIC = n \ln (S_m) + 4m
$$

where $S_m$ is a sum of squares of difference between the experimental points and their equivalent calculated values, $n$
is the number of experimental data and \( m \) is the number of elementary relaxation processes.

The DMEM solution is better described by a conductive contribution and two simple relaxation processes of Debye type, while for the La1-DMEM solution systems, the best results correspond to a sum of three elementary relaxation processes of Debye nature and a conductivity contribution.

It is obvious from the spectral decomposition that the presence of La1 induce a new medium-frequency relaxation process to the existing low-frequency and high-frequency relaxation processes that already exist in DMEM.

For the low-frequency and the high-frequency relaxation processes, there are slight variations in both the relaxation frequency and the relaxation amplitude. They indicate clearly that the presence of La1 in the solution induce a new medium-frequency relaxation process, the amplitude of which is correlated with the concentration.

The results of these analyses are summarised in Table 1.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Results of the de-convolution of the dielectric absorption as a sum of a conductive and a series of elementary Debye relaxation processes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \sigma \ S/m )</td>
</tr>
<tr>
<td>DMEM</td>
<td>0.89</td>
</tr>
<tr>
<td>LA1 in DMEM 2.6 ( \times ) 10^6</td>
<td>1.24</td>
</tr>
<tr>
<td>LA1 in DMEM 5.1 ( \times ) 10^8</td>
<td>1.53</td>
</tr>
</tbody>
</table>

The plotting of the amplitude \( \Delta_2 \) of the medium-frequency relaxation as a function of the La1 concentration for all 13 concentrations investigated exhibit two distinct regions with different slopes as seen in Figure 10.

The low concentration range is described by a linear relation with a coefficient \( a_1 = (2.92 \pm 0.14) \times 10^{-6} \) while the higher concentration range is also best described by a linear regression with a coefficient \( a_2 = (1.19 \pm 0.29) \times 10^{-8} \).
The change of the experimental conditions such as the increase of the content of electrolytes, the change of the pH or the increase of the stirrer speed of the tested sample did not alter significantly the observed behaviour. The linear relationship between the bacteria concentration and the amplitude of the medium frequency relaxation process is evidenced provided we separate the concentration range in two distinct domains where we apply different linear regression parameters (Justice et al., 2011).

The intersection between the two linear curves corresponds to a transition from a simple type of interactions to more complex ones. From additional microscopy testing, the cell aggregation is quite spontaneous in samples collected from stirred solutions and corresponding to concentrations higher than that determined by the intersection of the two linear regression lines. On the opposite side, the cell aggregation takes excessively long times before being noticeable in samples corresponding to concentrations lower than that of the intersection.

In stagnant culture media, the aggregation of bacteria start at very low concentration and develop with time. In our series of experiment where we have a continuous agitation of the solution, the aggregation occurs after a minimal concentration as confirmed by microscopy. In the case of L.1 in DMEM, the threshold of aggregation corresponding to our experimental conditions is \((4.12 \pm 0.23) \times 10^5\) u/ml. This information is very important when designing the parameters of an industrial fermentation process as the cell aggregation may have severe detrimental effects. In other cases, the aggregation under agitation is sought after because of the synergistic effects.

Collecting all these information for large number of bacteria types and strains in various experimental conditions and storing them in large database systems would be of prime importance to the development of biotechnology (Li et al., 2015; Jagadish et al., 2015; Madaan and Chu, 2015).

5 Conclusions

This paper describes the use of two different dielectric approaches to assess and to characterise the biomass of L.1 bacteria in a culture medium. The measured dielectric spectra of the culture media are analysed with respect to the distribution of the relaxation processes associated either with the interfacial polarisation or with the dipolar relaxation. In the radio-frequency range, the dielectric response of bacteria solutions allows us to test the consistency of the results by comparing the concentration ratio and the dielectric increment ratio. In the higher frequency range, it has been shown that the presence of bacteria induces a new relaxation process characterised by a relaxation frequency in the 200 MHz range. The amplitude of this relaxation process that is specific to the bacteria in the culture medium is used to predict the concentration. The plot of the amplitude of the medium-frequency relaxation process presents a broken line as a function of the concentration. In each section the regression parameters can be used reliably to predict the bacteria concentration and the intersection point corresponds to the threshold of aggregation.

The dielectric approach based on the identification and the characterisation of the medium-frequency relaxation of the bacteria culture media can be advantageously used in monitoring bacterial growth in semi-industrial and even in industrial fermenters. The measuring cell used, which is effective under adverse conditions of pH, of chemicals, of large temperature variations and of turbidity, is combined with the uniqueness of the medium-frequency relaxation which is specific to the bacteria in the culture medium. The fast dielectric response as well as the elimination of spurious effects due to changes in physical and chemical properties, allow then the online monitoring of the bacterial growth.

Acknowledgements

The author would like to express his gratitude to the School of Graduate Studies and Research of the American University of Ras Al Khaimah, UAE for its continuous support.

References


