In-situ biomass characterisation by impedance spectroscopy using a full-bridge circuit

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Abstract A full-bridge sensor system for characterising biosuspensions by impedance spectroscopy which avoids cross-sensitivity to temperature is presented. To record a large range of different kinds of cells, frequency is varied between 500 kHz and 50 MHz. The characteristic frequencies of the β-polarisation of animal cells (mouse), yeasts (Saccharomyces cerevisiae) and Escherichia coli bacteria are located in this range. The widespread applicability of this method is demonstrated by measurements on suspensions of different cells and the determination of concentration in a fermentation process. Furthermore, yeast suspensions are characterised at temperatures between \( T_1 = 24^\circ \text{C} \) and \( T_2 = 36^\circ \text{C} \) to evaluate the influence of temperature. A reduction of cross-sensitivity of about one order of magnitude compared to a single-sensor results. Changing in the conductivity of the suspension by 26% alters the measured biomass concentration by only 2.8%.

List of symbols

\( f \) frequency \([\text{s}^{-1}]\)  
\( \omega \) angular frequency \([\text{s}^{-1}]\)  
\( \tau \) characteristic time constant \([\text{s}]\)  
\( \epsilon \) dielectric number/permittivity [without dimension]  
\( \epsilon_0 \) permittivity of free space \( \epsilon_0 = 8.8542 \times 10^{-12} \, [\text{F/m}] \)  
\( \kappa \) conductivity \([\text{S/m}]\)  
\( G \) conductance \([\text{S}]\)  
\( k \) geometry factor \([\text{m}]\)  
\( C \) capacity \([\text{F}]\)  
\( C' \) capacitance per unit area \([\text{F/m}^2]\)  
\( \rho \) volume fraction [without dimension]  
\( r \) radius \([\text{m}]\)  
\( U \) voltage \([\text{V}]\)  
\( \phi \) phase angle \([\text{°}]\)  
\( T \) temperature \([\text{°C}]\)  
\( R_c \) regression coefficient

1 Introduction

For the online determination of the active biomass in biochemical reactors different methods like fluorescence spectroscopy [1], oxygen balancing [2], optical techniques [3, 4] and impedance spectroscopy [5] are used. In impedance spectroscopy, the dielectric number \( \epsilon \) and conductivity \( \kappa \) of a cell suspension are measured in the frequency range between 500 kHz and 50 MHz. The characteristic frequency of the β-polarisation of cells with average radii of 8–0.5 \( \mu \text{m} \) is located within this range. This enables the investigation of animal cells, yeasts and bacteria. To determine the complex impedance of a cell culture, the amplitude and phase angle shift are measured. Whilst the capacitance is significantly influenced by the cell concentration, the conductance strongly depends on the conductivity of the suspension. Thus, an increase in conductivity, e. g. by heating the suspension or adding salts, will reduce the phase angle, i. e. the resolution of the biomass concentration. This cross-sensitivity can be reduced by one order of magnitude if a full-bridge circuit is used.

2 Materials and methods

Cell suspensions of baker’s yeast (Saccharomyces cerevisiae) were chosen as model suspension to investigate basic properties of the sensor.

The analysed animal cells were immobilised mouse hybridoma cells (IV F19.23) from the Institute of Biotechnology I, Technical University Hamburg-Harburg.

Bacteria of the type Escherichia coli (W3110) were used to monitor the biomass during a fed-batch fermentation process (Institute of Biotechnology I, Technical University Hamburg-Harburg).

The optical densities of investigated cell suspensions were measured with the UVIKON 860 (Kontron Instruments, Neufahrn, Germany) at a frequency of \( f = 600 \, \text{nm} \).

Samples of a known volume of the suspensions were washed with distilled water to remove salts and then dried to constant mass.

The number of cells were counted in diluted suspensions in a haemocytometer (Neubauer chamber).

The impedance was measured with a vector voltmeter 8501 A (Hewlett Packard, Palo Alto, Calif.) which records amplitude and phase angle of the alternating voltage in dependence of the input voltage produced by the signal source SMY01 (Rhode & Schwarz, Munich, Germany).
3
Properties of living cells
To support the nutrient transport through the cell membrane, the surface of living cells is charged negatively by means of an ion pump effect. The membrane is extremely thin and isolating, which results in a high capacity. Mobile positive ions of the suspending medium interact with the fixed negative surface charges and a diffuse electrical double layer is built up. The relaxation of the counterions constituting the double layer causes the \( \alpha \)-dispersion. This effect is typically dominant at kHz frequencies.

Furthermore, a polarisation effect is given by the charging of the interfaces within heterogeneous materials (cell suspensions). It is a Maxwell-Wagner effect (\( \beta \)-dispersion) with a time constant \( \tau_{\beta} \). This dispersion is located in the radio frequency range.

The charging of the cell membrane capacitance \( C_m \) by ions moving within the cells is described by the following equations [6]:

\[
\omega = 2\pi \cdot f \tag{1}
\]

\[
\kappa(\omega) = \kappa_1 + (\kappa_\infty + \kappa_1) \frac{\omega^2 \cdot \tau_{\beta}^2}{1 + \omega^2 \cdot \tau_{\beta}^2} \tag{2}
\]

\[
e(\omega) = e_\infty + \frac{e_1 - e_\infty}{1 + \omega^2 \cdot \tau_{\beta}^2} \tag{3}
\]

To characterise the cell type the corresponding frequency \( f_{\beta} \) is used:

\[
f_{\beta} = \frac{1}{2\pi \cdot \tau_{\beta}} \tag{4}
\]

The subscripts 1 and \( \infty \) indicate the value for \( f< f_{\beta} \) and \( f> f_{\beta} \), respectively.

For thin membranes with negligible conductivity, the following approximated equations for cell suspensions of the concentration \( p \) in the region of the \( \beta \)-polarisation are valid [7]:

\[
e_1 \approx e_a + \frac{9}{4e_0} \cdot p \cdot r \cdot C_m \tag{5}
\]

\[
\kappa_1 \approx \kappa_a \frac{1 - p}{1 + 0.5p} \tag{6}
\]

\[
\kappa_\infty \approx \kappa_a \left[ 1 + 3p \frac{\kappa_1 - \kappa_a}{\kappa_1 + 2\kappa_a} \right] \tag{7}
\]

\[
\tau_{\beta} \approx r \cdot C_m \frac{\kappa_1 + 2\kappa_a}{2\kappa_1 \kappa_a} \tag{8}
\]

The subscript \( i \) and \( a \) indicate the values for the cytoplasm and NaCl solution, respectively.

Combining Eqs. 4 and 8 leads to a reciprocal proportionality of frequency \( f_{\beta} \) and cell radius \( r \).

4
Sensor setup
Basically, the setup of common sensors for impedance spectroscopy consists of an electrode structure, which is immersed into the cell suspension. To avoid the influence of electrode polarisation effects, the electrodes are arranged in a four-electrode arrangement [8]. Such a setup responds strongly to changes in the suspension’s temperature and conductivity. Theoretically, the influence of the temperature on the conductivity \( \kappa \) and the permittivity \( e \) of a yeast cell suspension is shown in Fig. 1 (\( \beta \)-dispersion-range). A change in temperature from \( T_1=24^\circ C \) to \( T_2=36^\circ C \) changes the conductivity of the suspending medium by 26%. The characteristic frequency is shifted from \( f_{\beta}=5.4 \) MHz to \( f_{\beta}=6.8 \) MHz. As the ratio of conductance and reactance decreases, the phase angle decreases, too. Thus, both the accuracy and the resolution of the biomass concentration, which corresponds to the maximum phase angle, are reduced as temperature rises.

To avoid this effect, an improved sensor is presented, consisting of four four-electrode structures assembled to a full-bridge circuit (Fig. 2). They are manufactured from a copper-laminated glass fibre base (2 mm in thickness) by chemical etching and covered by a titanium/gold thin film.

![Fig. 1. Conductivity and permittivity of a cell suspension (r=2.5 μm, p=0.3) in the range of the \( \beta \)-dispersion for \( T_1=24^\circ C \) and \( T_2=36^\circ C \)](image)

![Fig. 2. Full-bridge circuit with four-electrode arrangement and equivalent electric circuit (Indices: R=reference, S=suspension)](image)