Evaluation of *Pseudomonas* sp. growth in culture medium using electrical impedance spectroscopy with two bipolar geometries

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**Abstract.** The electrical properties of microbial culture have been studied by electrical impedance spectroscopy. Electrochemical changes in microbial systems are facilitated by the metabolic and proteolytic action of microorganisms. In this study, the growth of isolated bacteria compatible with genus *Pseudomonas* was evaluated by direct technique using electrical impedance spectroscopy with two electrode geometries. In these experiments a minimal salt medium with glucose as the energy source was used. The data of electrical parameters were correlated by counting viable cells using cetrimide agar. Cell growth in the culture medium was increased in capacitance, clearly describing the exponential phase of bacterial growth. The correlation of the viable cell count compared with the capacitance showed a high Pearson coefficient (>0.9). The bacterial growth can be estimated in culture medium using electrical parameters, such as capacitance using parallel plates electrode and parallel circuit equations.

1. Introduction

The study of electrical properties has been used to understand physiological, biophysical and biotechnological phenomena [1, 2]. These properties can be measured using techniques such as electrical impedance spectroscopy and dielectric spectroscopy. Both techniques analyze electrical attributes such as conductivity and permittivity of a sample (biological or non-biological) based on a response of electrical fields applied to material. Some applications in impedance microbiology have been detection and quantification of *Alicyclobacillus acidoterrestris* in apple juice [3]; a positive correlation was observed between the classical microbiology and the impedance methods.

Impedance spectroscopy is a technique that allows detection, quantification and even identification of microorganisms, especially bacteria [4, 5], using electrodes such as bipolar, tetrapolar or micro; all measurements can be taken using both direct and indirect techniques [6]. The analyzing principle is based on equivalent circuit, in which the culture medium is considered a resistance and the electrode-electrolyte phase as the capacitance. These two contributing components can be distinguished by changing the frequency. The electrode impedance dominated at low frequency (<100 Hz), while impedance measured at 10000 Hz was dominated by the medium effect [5].

Electrical properties of biological cells and their relationship with bacterial growth have been extensively studied. From some electrical properties such as capacitance, resistance and conductivity [5, 7, 8], Felice et al [9] determined that capacitance is more efficient than conductivity to quantify bacterial growth in milk. Other studies evaluate growth, quantification or bacterial detection such as *Escherichia*...
coli [5], Vibrio cholerae or Salmonella [10], demonstrating the importance of electrochemical processes mediated by cellular metabolism. In this study, the microbial growth of Pseudomonas sp. in minimal salt medium with glucose as the energy source was monitored with two electrode geometries, using electrical impedance spectroscopy, by analyzing the curves of capacitance as a function of time at a frequency range of 42 Hz to 5 MHz for parallel circuits.

2. Methodology

2.1. Bacteria, medium and culture conditions
This study was undertaken in Caldas, Colombia. Five morphocolonies were extracted from two soil samples at a depth of 20 cm. Bacterial isolation of genus Pseudomonas was carried out on cetrimide agar with incubation at 36 ºC for 72 hours. The bacteria were described according to their morphology and gram stain. Each colony was preserved at 7 ºC in nutritive broth (Difco™ Nutrient broth) and mineral oil for further analysis.

For electrical study of microbial growth, a colony of 0.8 mm diameter was cultured in minimal salt medium with glucose (0.03 molar), as the carbon source and viable cell counts were made on the incubated nutrient agar at 30 ºC for 24 to 48 hours, using the conventional method. Furthermore, a growth curve was performed evaluating turbidity of the culture medium, measuring absorbance at 600 nm in a Helios γ spectrophotometer.

2.2. Electrode geometries
The cell-type electrodes were built using polymethyl methacrylate (PMM) and stainless steel AISI 304. A bipolar configuration, with cylindrical electrodes, was used.

![Electrode configurations](image)

**Figure 1.** (a) Coplanar electrode (b) parallel plate electrode.

The first configuration is coplanar, included within a 2.66 mm diameter cylinder and with a pair of 0.51 cm² electrodes placed in the bottom of the container at a distance from each other of 0.44 mm (Figure 1a). The second configuration is of parallel plates, with a cubic geometry. The two electrodes have a cross-sectional area of 0.50 cm² each, at a distance of 1.55 cm from each other (figure 1b). The electric cell characterization was carried out in sterile distilled water and saline solution at 0.9% weight/volume (data not shown).

2.3. Impedance measurements
Capacitances were measured following the direct method used by Felice et al [5, 9]. Bacterial culture media were measured with an HIOKI 3532-50 LCR impedance meter, performing a frequency sweep from 42 Hz to 5 MHz, recording 50 measurements and applying current of 0.1 mA. Before each experiment, both electrode cells were sterilized with 3% glutaraldehyde; culture medium, saline solution and distilled water were sterilized in autoclave at 124 ºC and 17.1 Psi for 15 minutes. For each
measurement, the electrode cell was filled with 6 ml of solution in order to be analyzed, recording measurements at average room temperature of 22.7 °C ± 0.53 for 10 hours (culture media, saline solution, distilled water) and 33 hours (inoculated culture media).

![Figure 2. Measurements configuration.](image)

2.4. Data analyses
The Kruskal-Wallis test was used statistically, to establish if there were significant differences in the measuring times of the culture medium. The inoculated and uninoculated culture medium at 5 kHz was analyzed statistically using the Mann-Whitney test. To determine correlation between conventional method and impedance spectroscopy, regression models were used. The statistical analysis was done with a significance level of 5% and R software was used.

3. Results
Bacterial growth monitoring by methods such as spectrophotometry and viable cell count was calculated. Under environmental conditions and starting from a bacterial concentration of approximately 1.0x10^3 CFU/mL, the latency phase was estimated around the first three hours. After this time, microbial growth is reached up to an average of 25 hours, with a final concentration of 1.0x10^10 CFU/mL. The exponential phase of growth was estimated at between three and 15 hours (Figure 3).

According to bacterial kinetics of the evaluated sample, cell division is estimated every 1.4 hours per generation, that is, each hour has 0.72 generations. Inoculated medium and culture medium showed statistical differences in electrical measures (p <0.05); the Mann-Whitney test presented a p-value of 0.00 for capacitance. As for the frequency range (42 Hz to 5 MHz), 5 kHz best-represented bacterial growth. The capacitance monitored during bacterial growth has better results with parallel plate electrodes with the correlation coefficient at 0.94 between the capacitance and cell count (Figure 3). Coplanar electrode correlation was near zero.

4. Discussions and Conclusions
The capacitance of culture medium using parallel plate electrodes has variable behavior between two and ten hours, with an average of 2.80x10^{-7}F. The inoculated culture medium registered significant variations of capacitance during the experimentation process, as a result of bacterial proliferation in the culture medium. Bacterial growth established an increase in capacitance. At low frequencies (<10 kHz), the double layer capacitance reflects impedance behavior [6]. Given the frequency of 5 kHz analyzed in growth of Pseudomonas, it can be established that impedance is determined by reactance, indicating that capacitance of medium increases as impedance decreases and, given the direct relationship between capacitance and permittivity, data obtained are consistent with the theoretical foundations [10]. The impedance measurement at several frequencies between 18 Hz and 18 kHz, by which medium impedance was obtained at high frequencies (>5 kHz) and interface impedance, was negligible at such high frequencies [5].

Theoretically, the capacitance of bacterial medium increases with bacterial proliferation [6]. Results shown in this study (Figure 3), using an electrolyte characteristic culture medium and electrode
configuration, establish an increase in capacitance. Research such as Felice et al [5, 9], of bacterial growth in conventional culture medium, established that bacterial growth has a negative effect on the impedance of the system [11]. The electrochemical principle of these investigations is based on ionic characteristic of metabolic compounds, that increase the conductivity system and decrease the impedance.

![Graph](image_url)

**Figure 3.** Relationship between the capacitance and viable cell count (CFU/mL).

However, the behavior parameter analyzed in this study for growth of *Pseudomonas* can be explained by the relationship of the adhesion effect into surface electrodes. *Pseudomonas* have the ability to bind their cells by flagella that have S exoenzymes and membrane proteins [11], forming microcolonies that become biofilms [12]. This grouping of bacteria with adhesion capacity can constitute a barrier for the passage of electrical signal.

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