Evaluation of *Pseudomonas aeruginosa* biofilm formation using Quartz Tuning Forks as impedance sensors

T. Piasecki\(^a\), G. Guł\(^b\), K. Nitsch\(^a\), K. Waszczuk\(^a\), Z. Drulis-Kawa\(^b\), T. Gotszalk\(^a\)

\(^a\)Faculty of Microsystem Electronics and Photonics, Wroclaw University of Technology, Z. Janiszewskiego 11/17, 50-372 Wroclaw, Poland

\(^b\)Institute of Genetics and Microbiology, University of Wroclaw, S. Przybyszewskiego 63/77, 51-148 Wroclaw, Poland

**Abstract**

Shape of metallisation on Quartz Tuning Forks (QTFs) allowed to use them as impedance sensors for monitoring biofilm formation of *Pseudomonas aeruginosa* strains. Modelling using electrical equivalent circuits was used. It allowed to detect which electrical properties of sensor change with biofilm formation. Characteristic frequencies of 20 Hz and 1 MHz were found as suitable for simple detection of biofilm state. Resistance measured at 1 MHz allowed to detect changes in media conductivity caused by biofilm growth. It was in accordance with results obtained using another methods. In addition, the conductance at 20 Hz was suitable for detection of biofilm destruction caused by the lack of nutrients.

**1. Introduction**

Quartz Tuning Forks (QTFs) apart from typical use as frequency reference may be used in many other applications, for example to detect the formation of biofilm on its surface by measuring the shift of resonance frequency caused by the mass of adhered biofilm [1]. On the other hand the shape of the metallization pattern on the QTF surface (Figure 1) is similar to the interdigitated electrodes (IDE) on typical impedance sensors used for fast bacteria culture growth monitoring [2,3].
Motivation of this work was to determine whether the QTF may be used as an impedance sensor to detect the formation of biofilm. The positive outcome of such research would lead to the development of combined mass and impedance biofilm detection method.

Bacterial biofilm represent the most prevalent type of microbial growth in nature and are involved for example in food contamination during production, preparation or storage, therefore bacterial consortia may be a serious and costly problem in the industry [4].

Biofilms are also crucial to the development of clinical infections. They can be a source of diseases and are often associated with high-level antimicrobial resistance of the associated organisms [5]. Resistance to antibiotics or complement are possible by producing extracellular matrix by bacteria themselves in which biofilm cells are embedded. Matrix consists of different types of extracellular polymeric substances (EPS) (proteins, lipids, exopolysaccharides, extracellular DNA) and is responsible for adhesion to biotic and abiotic surfaces. Microorganisms that produce EPS could colonize indwelling medical devices to form biofilm. Device-associated infections result in substantial morbidity and mortality, and potentially represent a large increase in the cost of caring for patients [6].

2. Experiment and results

*Pseudomonas aeruginosa* PAO1 (ATCC 15692) and ATCC 27853 strains were used as model biofilm forming bacteria. Cultures were suspended in Muller Hinton Broth (MHB) with start density of $5 \cdot 10^5$ CFU/ml in 8 wells of 24-well titrate plate.

QTFs were sterilised in autoclave and mounted in 8-channel switch (Figure 2a). Their impedance spectra were measured quasi-simultaneously using Solartron 1260 impedance analyser in frequency range from 0.1 Hz to 1 MHz. Amplitude of measurement signal was 25 mV. During the experiment impedance spectra were measured repeatedly while the cultures were incubated in 37°C and at 100% Rh to minimize the effect of water evaporation on the results. Impedance spectra obtained during measurements were analysed using equivalent circuit presented in Figure 2b.

![Figure 1. Quartz Tuning Fork: photograph (a), the 3D model (b) and partial development of the surface (c). Letters A, B, C, D mark sides of the QTF.](image)

![Figure 2. Photograph of measurement setup: 8-channel switch and 24-well titrate plate (a) and the equivalent circuit used for impedance spectra modelling (b).](image)
Equivalent circuit consisted of $R_{med}$ related to the measured resistivity of medium surrounding the sensor and three elements related to the phenomena occurring at the surface of the sensor: $CPE_{surf}$ modelling the polarization of electric double layer and aluminium oxide layer at the electrodes, $R_{surf}$ related to the charge transfer resistance through these layers and $CPE_d$ modelling the Warburg diffusion impedance [7]. Modelling was performed using Scribner ZView™ software.

Exemplary impedance spectra obtained during the experiment with *P. aeruginosa* PAO1 strain were shown in Figure 3a. The equivalent circuit modelling allowed to determine which parameters of this circuit change with time in another way in the bacterial strain and MHB. Following differences were found: drop in the $R_{med}$ between 6 and 12 h of incubation (Fig. 4a, red arrows) with simultaneous rise of capacitance of $CPE_{surf}$ (Fig. 4b) and rise of parameter $Q$ of $CPE_d$ after several days (Fig. 4c).

Decomposition of exemplary impedance spectra (Figure 3b) showed that these characteristic parameters may be determined by measuring impedance at specific frequencies. The value of $R_{med}$ determines the shape of the impedance spectra above 100 kHz while the $R_{surf}$-$CPE_d$ branch was most significant at low frequencies, below 20 Hz.

Results of such simplified measurement done during the experiment in which there were 3 wells with *P. aeruginosa* PAO1 strain, 3 wells with *P. aeruginosa* ATCC 27853 strain and 2 wells with MHB were shown in Figure 5. Due to the differences in the cell constant of the QTFs acting as the impedance sensors the value of resistance measured at 1 MHz were normalised. Plots were similar to these obtained by equivalent circuit modelling.
3. Conclusions

Lowering of $R_{med}$ after few hours was caused by the presence of bacteria on the surface of the sensor and due to the excretion of metabolites. Results were consistent with previously published [1] obtained using classical standard growth curve method and by mass measurements using QTFs and colorimetric assay.

After 48 h the conductivity at low frequencies rose significantly (Figure 4b). Again it was observed only at wells with bacteria. Most probably it was caused by the change of electric properties of biofilm matrix at the moment of nutrients deprivation in liquid medium and the matrix itself become its source [5].

Results allowed to conclude that QTFs may be used as impedance sensors of Pseudomonas aeruginosa biofilm formation thus combined mass and impedance measurement systems may be built.

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References