Detection of γ-radiation and heavy metals using electrochemical bacterial-based sensor

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Abstract. The main aim of this work is to develop a simple electrochemical sensor for detection of γ-radiation and heavy metals using bacteria. A series of DC and AC electrical measurements were carried out on samples of two types of bacteria, namely *Escherichia coli* and *Deinococcus radiodurans*. As a first step, a correlation between DC and AC electrical conductivity and bacteria concentration in solution was established. The study of the effect of γ-radiation and heavy metal ions (Cd^{2+}) on DC and AC electrical characteristics of bacteria revealed a possibility of pattern recognition of the above inhibition factors.

1. Introduction

This work is a part of ongoing research targeting the development of novel, simple, and cost effective methods for monitoring environmental pollutants, particularly radionuclides and heavy metals being common contaminants of water resources [1, 2]. It is known that micro-organisms such as bacteria are very sensitive to γ-radiation produced by radionuclides as well as to heavy metals [3, 4]. The use of micro-organisms for assessment of general toxicity of aqueous environment was reported previously [5]. Identification of the types of pollutants in the environment and the evaluation of their concentration is much more difficult task which is impossible to solve using a single sensor. However, the sensor array approaches [6] utilising several types of bacteria being inhibited differently by different types of pollutants could solve the above problem.

Our early experiments with *Escherichia coli* (*E. coli*) established a correlation between optical properties of liquid bacteria samples and bacteria concentration [7]. The effect of γ-radiation on concentration of live E coli bacteria was studied using three different optical techniques: (i) fluorescent microscopy with which yields directly the ratio of live/dead bacteria, stained, respectively, with "green" and "red" fluorescent dyes, (ii) optical density measurements at 600 nm, and (iii) fluorescent spectroscopy. The results obtained were encouraging however, the use of expensive and bulky optical instrumentation limits the sensor development.

In this work, we used simple electrical (electrochemical) measurements for the same purpose, i.e. first, establishing the correlation between electrical properties (conductivity) of liquid bacteria samples and live bacteria counts, after that studying the effect of γ-radiation and heavy metal ions (Cd^{2+}) on bacteria. Electrochemical measurements were successfully used for studying electrical properties of cells deposited on metal electrodes and showed great prospects of using such cell-based sensors for detection of various analytic [8, 9]. In our study, the principles of cell-sensors were extended further to more complex objects such as bacteria. In this work, in addition to *E.coli* bacteria, we used another

type of bacteria, *Deinococcus radiodurans* known by its high resistance to γ-radiation [10]. The use of two types of bacteria may lead to pattern recognition of inhibition factors, in our case γ-radiation and Cd^{2+} ions.

2. Experimental methodology

2.1. Bacteria sample preparation
Two types of bacteria were selected for this work: Gram-negative *Escherichia coli* (*E. coli*) and Gram-positive *Deinococcus Radiodurans* (*D. Radiodurans)*. Non-pathogenic HD5α strain of *E. coli* was used in LB (Luria-Bertain) broth [11] as a medium for *E. coli* cell culture. Anderson R1 strain of *D. Radiodurans,* which is extremely resistant to ionizing radiation, ultraviolet light, desiccation, oxidizing and electrophilic agents [10] was used in this work with 1-Nutrient agar (oxoid cm3). Both types of bacteria and respective growth media were acquired from SIGMA-ALDRICH CO. and OXOID LTD. Other chemicals, i.e. $CdCl_2$, salt were purchased from SIGMA-ALDRICH CO.
Cultivation of bacteria was performed in several stages. The first step was to cultivate a specific strain of bacteria in Petri dish containing solid broth agar, in order to use it as a bacteria source in future. In the second stage, one colony of bacteria was added into a sterile flask containing 50ml of liquid broth. Finally, the flask containing the bacterial culture was placed inside shaking incubator operating at 150 rpm shaking speed. The incubation temperatures were 30 C° for *D. Radiodurans* and 37 C° for *E. coli*. Bacteria start growing after 16 hours for *E. coli* and 24 hours for D. *Radiodurans*.

2.2. Experimental procedures
The cultivated bacteria density and changes in the live bacteria counts after exposed to pollution were recorded with Optical Density Photometer (6715 UV/Vis Spectrophotometer JENWAY OD600).
Solid radioactive source (Co-57) with the activity of 330 MBq was used for irradiation of bacteria samples. This source emits Gamma radiation (136, 14) keV with the equivalent dose of about 2000 mSv/h and the half-life 271.79 days [12]. The radiation dose (for bacteria samples) was varied using different exposure times from 1h up to 360h. $CdCl_2$ salt (from Sigma Aldrich) was used for testing bacteria samples. Solutions of different concentrations of $CdCl_2$ (down 0.1mM) were prepared by multiple dilution of 1M stock solution of $CdCl_2$ in de-ionised water (MQ Elga). Bacteria samples were mixed with $CdCl_2$ solutions in 1:1 ratio and kept incubated from 1 hour till 384 hours.
All electrical measurements were performed in a simple, sandwich-type, two-electrode cell. The cell schematically shown as inset in Figure 1 has PTFE body (1) and contains platinum counter electrode (2); gold-coated glass slide acting as working electrode (3) was sealed against the cell via rubber O-ring (4), the cell has inlet and outlet tubes (5, 6) allowing the injection of liquid medium containing bacteria. DC electrical test were performed using 6517A Keithley electrometer in voltage range ±0.5V. It has to be noted that preliminary electrochemical measurements were made in a standard three-electrode cell using DropSens microSTAT200 equipment (results are not presented here) and showed no difference to the measurements done in two-electrode cell in the ±0.5V voltage range which was selected in order to avoid oxidation and reduction electrochemical reactions on metal electrodes. AC electrical measurements were performed using (hp-4284A PRECISION LCR METER) in the frequency range from 20 Hz to 1 MHz with the amplitude of AC voltage of 100mV and no DC bias applied. The spectra of two parameters Gp and Cp corresponding to a parallel connection of conductance and capacitance were recorded.

3. Experimental results and discussion

3.1. DC electrical measurements
Typical I-V characteristics of E-coli samples having different concentrations of bacteria are shown in Figure1. Cathode current appeared to be much higher than anode current. The increase in bacteria concentration in solution leads to decrease of cathode current. This could be explained by adsorption

of bacteria on the working electrode surface and thus the reduction in DC conductivity. Samples of
D. radiodurans bacteria showed very similar DC characteristics. For detection purposes, the values of
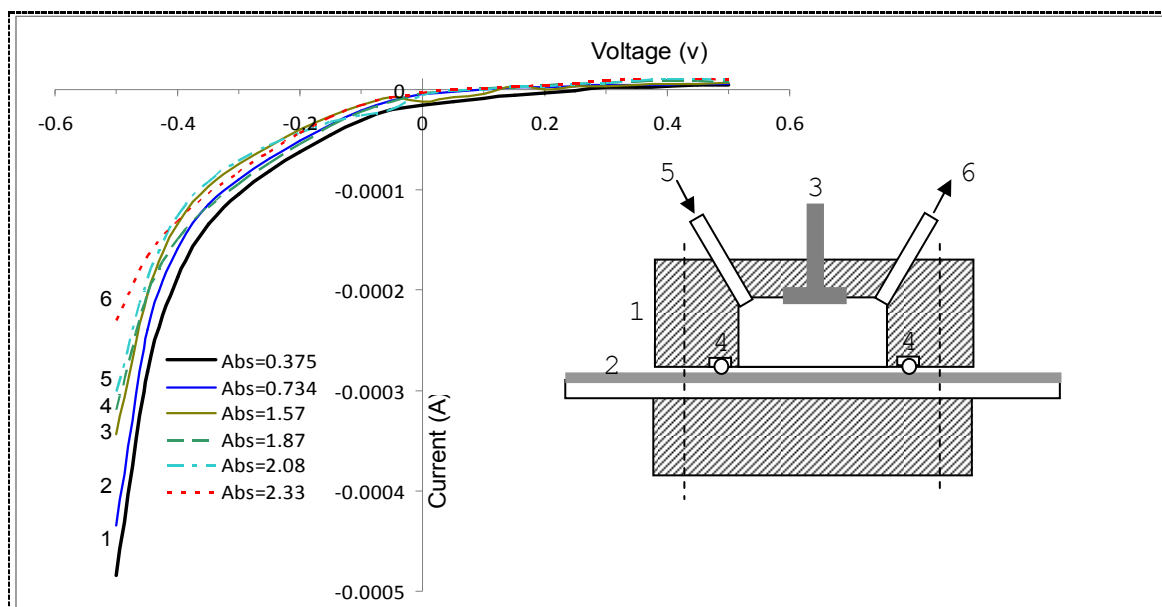cathode current at –0.5V will be discussed throughout this paper.

Figure 1. Typical DC I-V characteristics of E-coli samples of different concentrations measured
in Abs units of optical density (OD_{600}): (1) 0.375, (2) 0.734, (3) 1.57, (4) 1.87, (5) 2.08, (6) 2.33
Inset shows the design of two-electrode cell.

The effect of γ-radiation and heavy metal ions (Cd^{2+}) on cathode DC current is illustrated in Figures 2
and 3. As one can see in Fig.2, DC conductivity of *E. coli* samples goes down exponentially with the
radiation dose increase, while for *D. radiodurans* DC conductivity increases a little at low doses then
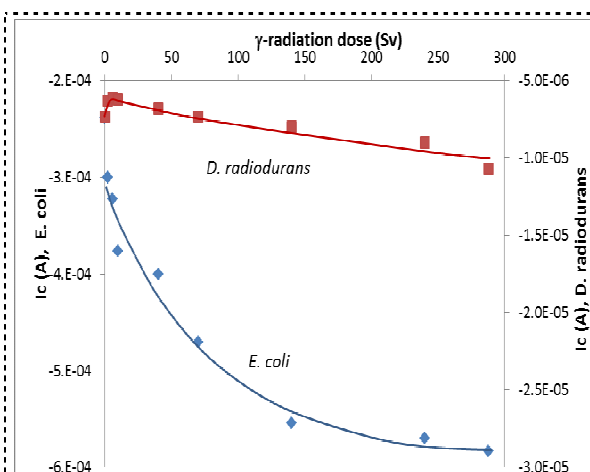decreases linearly at high doses. The effect of Cd^{2+} ions is similar on both bacteria (see Fig.3).

Figure 2. Dependence of cathode current of
E. coli and *D. radiodurans* bacteria samples
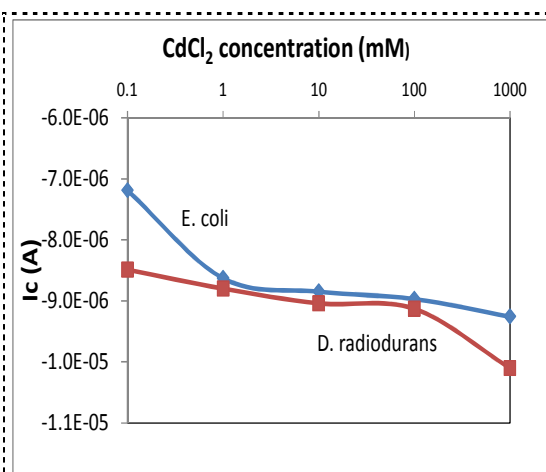on γ-radiation dose.

Figure 3. Dependence of cathode current of
E. coli and *D. radiodurans* bacteria samples
on $CdCl_2$ concentration

3.2. AC electrical measurements

Typical spectra of AC conductance (Gp) and capacitance (Cp) for bacteria samples are shown in Figure 4. The conduction values increase with the frequency and reach saturation at about 900kHz; Such behaviour is similar to that observed for cells [1] and can be explained by the dominant contribution from the material (bacteria in our case) absorbed on the surface of metal electrode. For sensing purposed, it would be useful to analyse the values of Gp at the saturation, i.e. at 900 kHz.
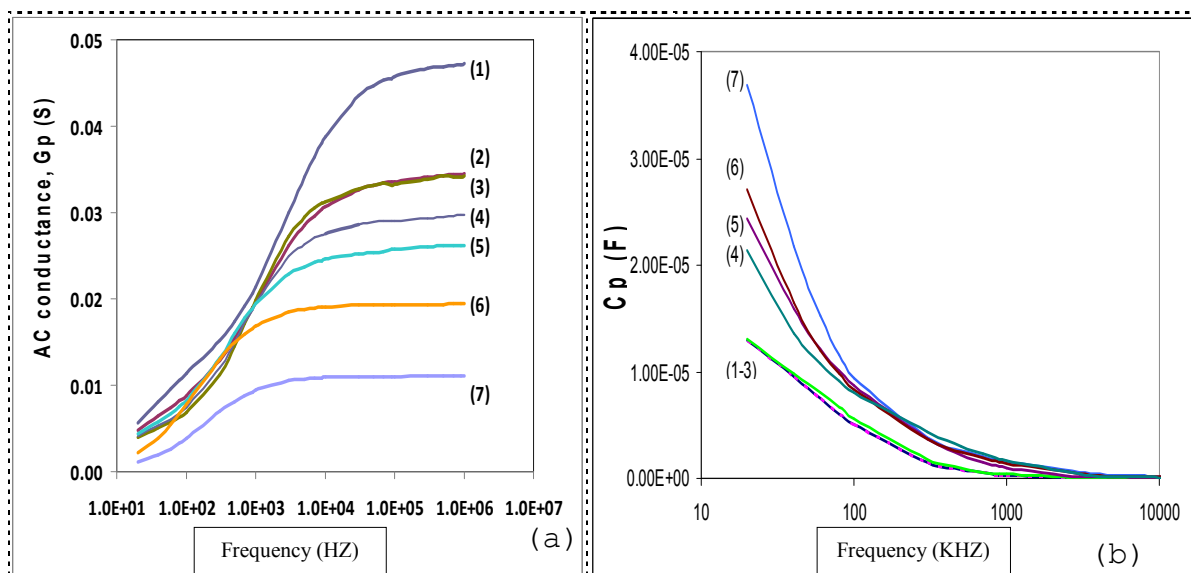

Figure 4. Spectra of Gp (a) and Cp (b) for E. coli bacteria samples of different concentrations presented in optical density Abs units: (1) clear broth, Abs =0.0, (2) Abs =0.237, (3) Abs =0.532, (4) Abs =0.801, (5) Abs =1.39, (6) Abs =1.57, (7) Abs =2.08

The capacitance, in contrary, maximal at low frequency and decreases in 4-5 orders of magnitude at 1MHz. The increase in bacteria concentration leads to larger capacitance values. These facts are a complimentary proof of the key role of bacteria adsorbed on the surface of metal.
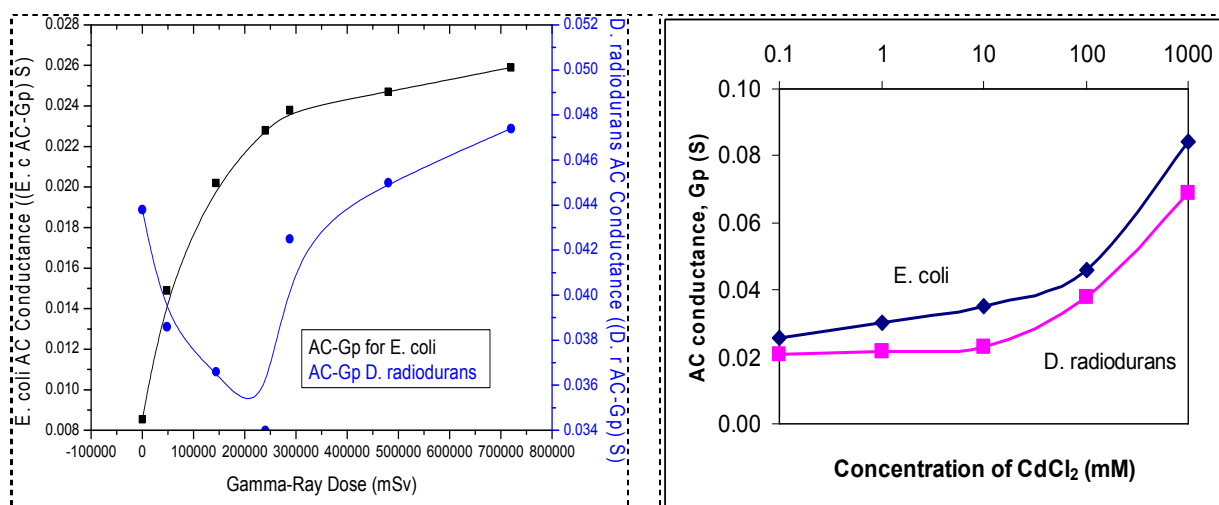

Figure 5. The effect of γ-radiation on Gp values at 900 kHz for *E. coli* and *D. radiodurans* bacteria samples.

Figure 6. The effect of $CdCl_2$ on Gp values at 900 kHz for *E. coli* and *D. radiodurans* bacteria samples.

The effect of γ-radiation and $CdCl_2$ salt on Gp values at 900 kHz is demonstrated in Figures 5 and 6. AC conduction of *E. coli* increases with the increase in radiation dose which correlate well with the decrease in bacteria concentration. In case of *D. radiodurans,* Gp decreases at low radiation doses and increases at high doses. Cd^{2+} ions cause similar effect on both *E. coli* and *D. radiodurans* bacteria with Gp rising on the increase of $CdCl_2$ concentration. Detailed analysis of AC data (which is currently underway) should be based on a simple equivalent circuit model consisting of parallel conductance and capacitance most-likely associated with adsorbed layer of bacteria on the metal surface.

3.3. Data analysis: Pattern recognition of inhibiting factors
The data of DC and AC electrical measurement correlate and complement each other; they are also in a good agreement with the results of optical study [7]. The difference in responses of *E. coli* and *D. radiodurans* bacteria to γ-radiation and heavy metal ions (Cd^{2+} in our case) allows the application of principle of pattern recognition solving the "reverse problem", i.e. identification and quantification of pollutants in the unknown environment. Figure 7 shows the plot of relative DC responses (Ic current values were normalised by Ic_0 corresponding to untreated bacteria samples) of *E. coli* and *D. radiodurans* bacteria as X- and Y- axes respectively. The "open square" data points correspond to
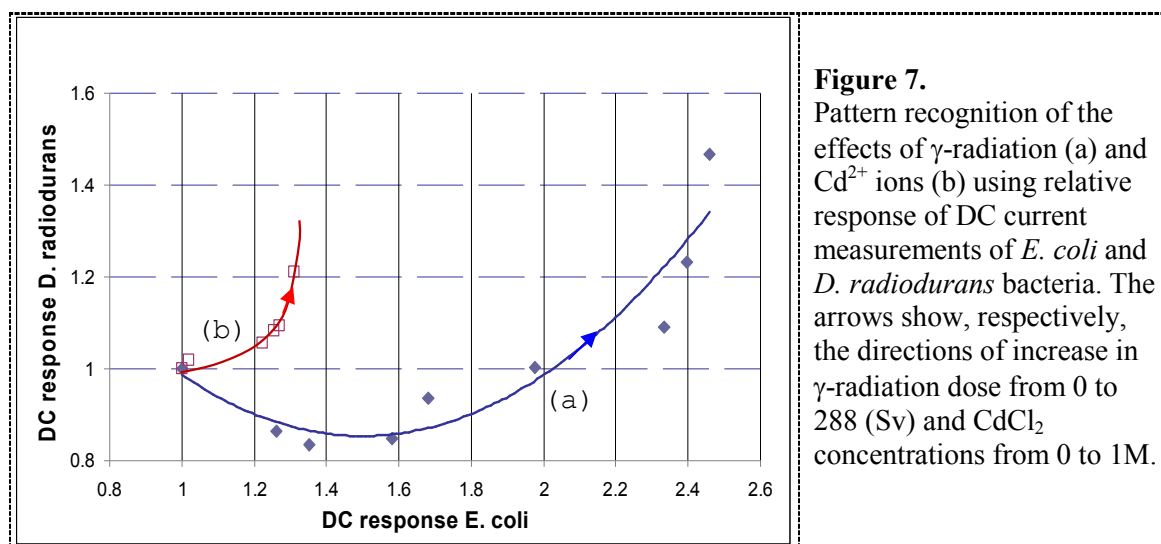

Figure 7. Pattern recognition of the effects of γ-radiation (a) and Cd^{2+} ions (b) using relative response of DC current measurements of *E. coli* and *D. radiodurans* bacteria. The arrows show, respectively, the directions of increase in γ-radiation dose from 0 to 288 (Sv) and $CdCl_2$ concentrations from 0 to 1M.

$CdCl_2$ exposure, while "filled diamond" points correspond to γ-radiation exposure. The separation of the two curves is clear which offers great possibility of pattern recognition of the above inhibiting factors.

4.   Conclusions

DC and AC electrical study of two types of bacteria *E. coli* and *D. radiodurans* was a success. The obtained data correlated between themselves as well as with the data of optical study carried out previously. The measurements of DC current and Gp and Cp spectra can be used for quantification of live bacteria concentrations and thus the effect of γ-radiation and heavy metal ions on bacteria. Moreover, the difference in responses of *E. coli* and *D. radiodurans* bacteria to γ-radiation and heavy metal ions allows the application of principle of pattern recognition for identification and quantification of pollutants. This work proved the concept of a simple and cost effective electrical bacteria-based sensor and sensor array for preliminary assessment of the presents of toxins in water.

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