Inhibition Biosensor Based on DC and AC Electrical Measurements of Bacteria Samples.

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Inhibition Biosensor Based on DC and AC Electrical Measurements of Bacteria Samples

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Abstract

The main idea of this project is to utilise microorganisms (bacteria) as sensing elements in the inhibition type biosensors for detection of heavy metals and pesticides which are dissolved in water. This work focuses on developing novel, cost-effective sensing technologies for detection of environmental pollutions. Two types of bacteria (*E. coli and S. oneidensis*) which are inhibited by heavy metals were used in this work. The bacteria density or concentration was characterised first with optical techniques of fluorescence microscopy, optical density OD600, and flow cytometry. Then a series of electrochemical measurements were carried out on the same bacteria samples. The study of the effect of heavy metals on the above bacteria revealed a possibility of pattern recognition of the above pollutants.

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1. Introduction

Heavy metal pollution is a problem associated with areas of intensive industry. Zinc, copper, and lead are three of the most common heavy metals released from road travel. Lead concentrations, however, have been decreasing consistently since leaded gasoline was discontinued [1]. The existing high-tech methods of their detection are usually expensive and laboratory based. This work is a part of ongoing research targeting the development of novel, simple, and cost effective methods for monitoring environmental pollutants, particularly pesticides and heavy metals being common contaminants of water resources [2]. It is known that micro-organisms such as bacteria are very sensitive to heavy metals [3];[4]. The use of micro-organisms for assessment of general toxicity of aqueous environment was reported previously. 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 utilising several types of bacteria being inhibited differently by different types of pollutants could solve the above problem [2]. Our early experiments with *Escherichia coli* (*E. coli*) established a correlation between optical properties of liquid bacteria samples and bacterial density. In this work, we used simple electrochemical measurements for establishing the correlation between conductivity of liquid bacteria samples and

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live bacteria counts, and studying the effect of heavy metal ions (Hg^{2+}) on bacteria. In addition to *E.coli* bacteria, we used another type of bacteria, *Shewanella oneidensis* known by its high resistance to heavy metals. The use of two types of bacteria may lead to pattern recognition of inhibition factors, in our case pesticides and heavy metals.

2. Results and Discussions

The numbers of live and dead bacteria were determined with Fluorescence Microscopy and OD600 similarly to described in [2]. Live and dead bacteria are stained in green and red, respectively. The L7012 Live/Dead Bacterial Viability Kit for flow cytometry was also used for counting the percentage of live and dead *E. coli* & *S. oneidensis* bacteria after coloring bacteria samples. Fig. 1 (A) and (B) show the results of flow cytometry as the percentage of live (blue) and dead (red) bacteria. The electrochemical measurements bacteria samples were carried out using DropSens pontentiostat and screen printed gold electrodes. Potential was recorded against Ag/AgCl reference electrode. Typical Cyclic voltammograms are shown in Fig. (C) for *E. coli*; the bacteria appeared to act as an insulator reducing the current. The value of cathode current (Ic) is correlated with bacteria density. Fig. (D) Shows different effects of heavy metal (Hg) on Ic for *E. coli* and *S. oneidensis,* which could be used for pattern recognition of pollutants.

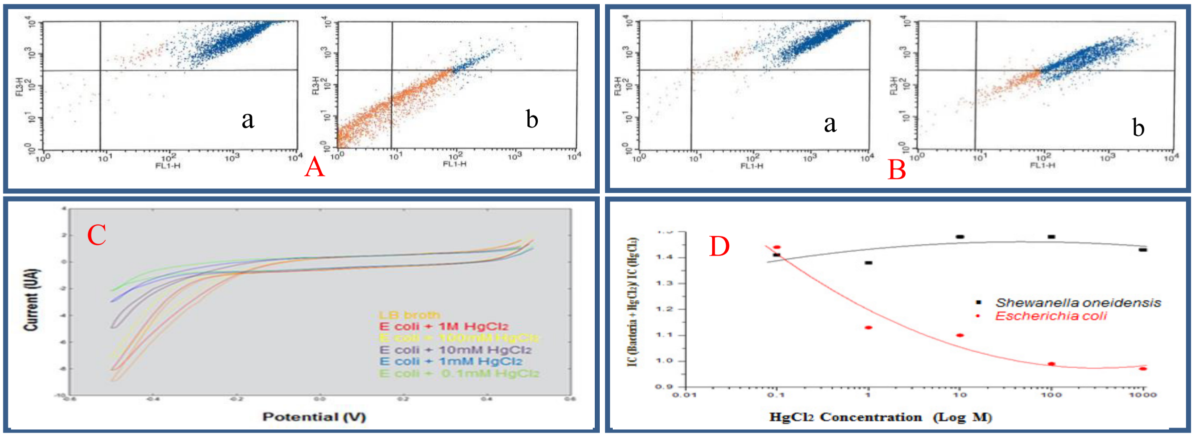

Figure1. Flow cytometry results for **(A)** *E.coli* and **(B)** *S. oneidensis* bacteria before (a) and after (b) treatment with 1M $HgCl_2$ solution; **(C)** CV curves recorded on LB broth and *E. coli* samples treated with $HgCl_2$ of different concentrations; **(D)** comparison of relative changes in Ic at -0.5V of *E.coli* and *S. oneidensis* bacteria samples on exposure to $HgCl_2$.

3. Conclusions

The results obtained proved the concept of a simple bacteria-based electrochemical sensor array which is enable to distinguish water pollutants using pattern recognition principles.

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