 doi:10.20944/preprints201807.0351.v1

Development of a novel electrochemical inhibition sensor array based on bacteria immobilized on modified screen-printed gold electrodes for water pollution detection

H. Abu-Ali [a, c*], A. Nabok [a], T. Smith [b], M. Al-Shanawa [c]

[a]Materials and Engineering Research Institute, Sheffield Hallam University, UK

[b]Biomolecular Research Centre, Sheffield Hallam University, UK

[c]Faculty of Science, University of Basrah, Iraq

Abstract

The development of a novel and simple inhibition biosensor array for detection of water pollutants based on immobilized bacteria is the main goal of this work. A series of electrochemical measurements (i.e. cyclic voltammograms) were carried out on screen-printed gold electrodes with three types of bacteria, namely *Escherichia coli*, *Shewanella oneidensis*, and *Methylococcus capsulatus*, immobilized via poly L-lysine. For comparison purposes, similar measurements were carried out on bacteria samples in solutions,; also optical measurements (fluorescence microscopy, optical density, and flow cytometry) were performed on the same bacteria in both liquid and immobilized forms. The study of the effect of heavy metal ions (lead), pesticides (atrazine) and petrochemicals (hexane) on DC electrochemical characteristics of immobilized bacteria revealed a possibility of pattern recognition of the above inhibition agents in aquatic environment.

Key words: electrochemical sensor, inhibition bacteria sensor array, immobilization of bacteria, water pollution, pattern recognition.

*Corresponding author: Hisham Abu-Ali, Sheffield Hallam University, Materials and Engineering Research Institute, City Campus, Sheffield, Howards Street, S1 1WB, UK, E-mail: b4039024@my.shu.ac.uk

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 July 2018 doi:10.20944/preprints201807.0351.v1

1. Introduction

Nowadays, heavy metals, pesticides, and petrochemicals possessing serious threat to humans and living organisms are of the main concern for the environmental security. The most common sources of environmental pollution are manufacturing, automotive, agricultural, chemical, and medical industries [1]. For instance, three of the most common heavy metals released from road travel are zinc, copper, and lead, accounting for at least 90% of the total metals in road runoff [2]. These toxic agents do not remain where they originate. They can be transported to different locations in a number of different ways. Some compounds can evaporate and drift away by winds before precipitating as rainfall. In addition, runoff from agricultural and urban areas into drainage pipes and sewers also contributes to significant pollution of surface and ground water. A study from Switzerland revealed that much of the rain in Europe contains high levels of dissolved pesticides, actually 4 μg/l of 2,4-dinitrophenol, that it would be illegal to supply this water for drinking purposes [3]. A field conditions study in Hungary revealed the presence of 154 μg/l of atrazine, 89.1 μg/l of acetochlor, 47.4 μg/l of propisochlor, and 0.139 μg/l of chlorpyrifos in runoff water [4]. Another significant part of environment contamination comes from petrochemical industry. Typical petrochemical contaminants are hydrocarbons, alcohols, ketones, benzene derivatives (or BTEX), etc. Considering the adverse effects of the above pollutants on humans, animals, and wild life, the environmental agencies and World Health Organisation set quite low limits (in the 0.1-0.5 ppm range) for major heavy metals (Hg, Pb), pesticides (DDT, DDE, TDE, etc.) and petrochemicals (methyl alcohol, and BTEX) pollutants in water, food, and feed [5].

The detection of the above environmental pollutants in such low concentration is quite difficult task, though not impossible and can be achieved with the existing advanced analytical methods such as atomic absorption or atomic emission spectroscopies (AAS, AES), inductively coupled plasma mass spectroscopy (ICP-MS), cold vapours atomic fluorescence spectroscopy (CVAFS), liquid phase chromatography (HPLC). Those methods are extremely sensitive but expensive, requiring specialized laboratory conditions and highly trained personnel [6-9]. As a result, both the time and cost of analysis become very high.

An alternative approach to those high-tech methods is based on the use of biosensors, which could be much simpler, easy-to-use, and inexpensive. The main problem of biosensors, however, is the selection of bio-receptors which actually provide the function of recognition of target analyte molecules. Typical bio-receptors used in biosensors, i.e. enzymes, antibodies,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 July 2018 doi:10.20944/preprints201807.0351.v1

aptamers, peptides, can easily provide such functionality [10]. However the traditional biosensing approach may struggle with a difficult task of detecting a large number of pollutants in a complex natural environment because every analyte may require a specific receptor. As a result, a large sensor array is required to fulfil the task at least partially which may lead to a quite complex detection protocol and therefore to high cost of analysis.

One of the possible solutions to such problem is the use of inhibition biosensors, where the bio-receptor, typically enzyme, is inhibited by particular pollutants. The selectivity of this process is rather poor since the enzyme can be inhibited by different pollutants. Obviously, a single inhibition sensor cannot identify the pollutant, but a sensor array can. A good example of such inhibition sensor array was an optical enzyme sensor array which was enable of both identification and quantification of several heavy metals and pesticides [11]. Although the principle of such sensor array has been successfully proven, poor stability of enzymes used (urease and cholinesterase) was a serious drawback which prevented commercialization of such devices.

Living cells are particularly useful for detection of traces of environmentally toxic compounds because these molecules or ions interfere with one or several internal biological processes in cells and may cause modification of the cell's activity [12]. Several attempts of using whole cells as bio-receptors in inhibition sensors were reported. Another possibility explored recently was the use of microorganisms as sensitive elements [13]. Previous study of optical and electrical properties of solutions of two types of bacteria (*E. coli and D. radiodurant*) has, first, established a correlation between the optical density and electrical conductivity and the bacteria concentration in liquid samples, and then revealed a possibility of identification of the types of pollutants (heavy metals and radionuclides) by their effect on bacteria concentration [14]. Recent study was focused on the detection heavy metals and pesticides using mostly electrochemical measurements of two types of bacteria (*E. coli and S. oneidensis*) which were either free in solution or immobilized on the surface of the electrodes [15]. The results were encouraging, and the sensors with immobilized bacteria were the most promising for development of inhibition sensor array.

This work focuses on further development of electrochemical inhibition sensor array for detection of heavy metals ($PbCl_2$ salt was used here), pesticides (atrazine), and petrochemicals (hexane) which uses three channels, e.g. three electrodes with different types of bacteria (*E. coli*, *S. oneidensis*, and *M. capsulatus*) immobilized on the surface. The choice of bacteria was justified by their inhibition patterns by the analytes used; the details are given

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 July 2018 doi:10.20944/preprints201807.0351.v1

in the following section. The main detection technique in this work was cyclic voltammograms, while optical methods of optical density, fluorescence microscopy, and flow cytometry as well as cyclic voltammograms of bacteria solutions were used as complementary techniques helping to establish a correlation between the bacteria concentration and their optical and electrical properties.

2. Experimental methodology

2.1. Bacteria sample preparation

For this work the following three types of bacteria were selected: (i) *Escherichia coli* (*E. coli*, K12 strain) which belong to gram-negative bacteria type generally sensitive to different types of pollutants including heavy metals, pesticides, and hydrocarbons[16], (ii) *Shewanella oneidensis* (*S. oneidensis*, MR-1 strain) which belong to gram-positive bacteria and known to be tolerant to heavy metals [17] because of its bio-catalytic-activity towards heavy metals [25], and (iii) Methanotrophic bacteria (*Methylococcus capsulatus,* Bath strain) gram-negative bacteria which thrive in the presence of some petrochemicals [18-19] because of its bio-degradation- properties [27]. LB (Luria-Bertain) broth was used as a medium for *E. coli* [20] and *S. oniedensis* bacterial cell cultures, while *M. capsulatus* were grown in NMS (Nitrate Mineral Salts) medium [21]. All types of bacteria and respective growth media as well as phosphate buffer (PBS) were acquired from Sigma-Aldrich Co. Other chemicals, i.e. $PbCl_2$ salt, atrazine, hexane, and poly L-lysine (PLl) were also 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 agar to be used it as a bacteria source in future. In the second stage, one colony of bacteria was added into a sterile flask containing 50 ml of liquid LB broth or NMS medium for *Methylococcus capsulatus,* (Bath) strain. Lastly, the flask containing the bacterial culture was placed inside shaking incubator operating at 150 rpm shaking speed. The incubation temperatures were 30 °C for *Shewanella oneidensis* & *Methylococcus capsulatus*. While 37 °C for *E. coli*. Bacteria start growing after 16 h for *E. coli* and 24 h for *Methylococcus capsulatus & Shewanella oneidensis.* Bacteria in solution samples were then studied with optical and electrochemical methods.

Immobilization of the above mentioned bacteria on the surface of screen-printed gold electrodes was carried out via poly L-lysine (PLl) [22]. For this reason, the surface of gold was modified in a 1:1000 mixture of PLl (0.1 mg/ml) and deionised water for 1 h at 37 °C.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 July 2018 doi:10.20944/preprints201807.0351.v1

Then bacteria were immobilized by dropping stock solutions of *E. coli*, or *M. capsulatus* or *S. oneidensis* on the modified electrodes, keeping it there for 1 h, then washing out non-bound bacteria with PBS.

2.2. Experimental methodology

To study the inhibition effects on the above mentioned bacteria by selected pollutants, e.g. $PbCl_2$, atrazine, and hexane, their solutions of different concentrations (0.1, 1, 10, and 100 mM) were prepared by multiple dilution of 1 M stock solution of each analyte dissolved in deionised water. 40% ethanol solution in water was used for dissolving hexane. Liquid bacteria samples were mixed with these solutions in 1:1 ratio and kept incubated for 2 h. The samples of immobilized bacteria were treated similarly by immersing them into required solutions of pollutants for 2h.

The effect of the above pollutants on the bacterial cultures was examined and analysed using three different optical experimental techniques: fluorescence microscopy, UV–visible spectrophotometry, and flow cytometry. Flow cytometer BECTON-DICKINSON FACSCalibur instrument was used for counting the percentage of live and dead bacteria after colouring bacteria samples with L7012 Live/Dead Bacterial Viability Kit, which is a mixture of (SYTO-9) green fluorescence nucleic acid stain and the red fluorescence nucleic acid stain, propidium iodide. Fluorescence microscopy measurements were performed using Olympus-BX60 instrument using liquid bacterial samples also stained with L7012 Live/Dead (L/D) BacLight Bacterial Viability Kit [23-24]. Also, fluorescent microscopy measurements were carried out on samples of bacteria immobilized on screen printed gold electrodes. The cultivated bacteria density and changes in the live bacteria counts after exposure to pollution was monitored using optical density photometer (6715 UV/Vis Spectrophotometer JENWAY OD600).

The electrochemical measurements, i.e. cyclic voltammograms (CV), were carried out using DropSens gold screen-printed three-electrode assemblies (which include Ag/AgCl reference electrode) and DropSens microSTAT4000P potentiostat. CVs of liquid bacteria samples were recorded in a voltage range from (-0.5 V to +0.5 V). Liquid bacteria samples were studied by measuring CVs of DropSens electrodes immersed into particular bacteria solutions; the measurements were taken in liquid samples of all three bacteria before and after treatment with each analyte (pollutant) at different concentrations. The CV measurements of the

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 July 2018 doi:10.20944/preprints201807.0351.v1

electrodes with freshly immobilized bacteria were carried out in LB broth before and after treatment with each pollutant in different concentrations.

3. Experimental results and discussion

3.1 Optical characterization

Optical characterization of liquid bacteria samples is essential for studying the effect of pollutants on concentration of live bacteria in liquid samples. In contrast to our previous studies [14-15] where the methods of fluorescence microscopy and optical density (OD600) were used for characterization of liquid bacteria samples, in this work we deployed fluorescent microscopy for characterization of bacteria immobilized on the surface of screen printed gold electrode. Fluorescence microscopy images in Figure 1 show the effect of Pb^{2+} ions on *Shewanella oneidensis* bacteria immobilized on modified screen printed gold electrodes where live and dead bacteria appear as green and red spots, respectively [15]. It is clear, that the exposure to 1M solution of $PbCl_2$ salt for 2 hours reduced the number of live bacteria (green spots) and increases the dead ones (red spots). Such experiments were carried out for all three types of bacteria and all analytes used. The results of this study were presented in Table 1 as the numbers of live (green) and dead (red) bacteria on recorded images of identical dimensions.

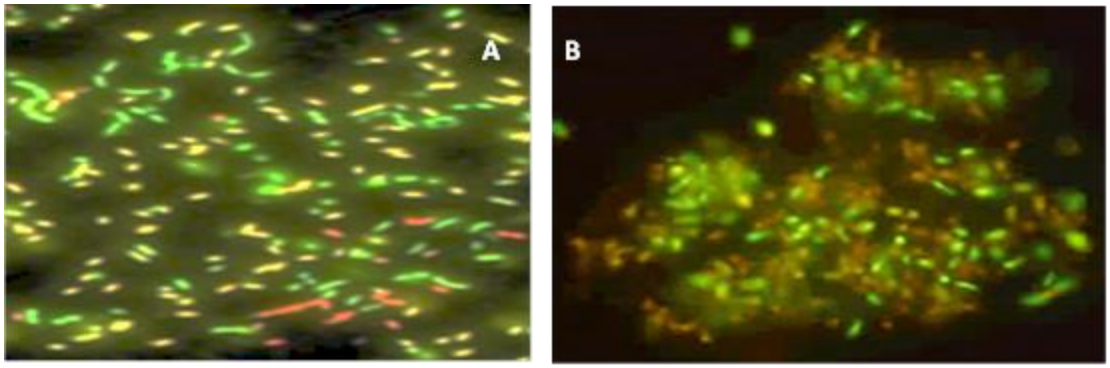

Figure 1. Fluorescence microscopy images of immobilized *Shewanella oneidensis* bacteria before (A) and after (B) treatment with $PbCl_2$ salt (1 M) for 2 hr.

Analysis of fluorescence microscopy data in Table 1 revealed that *E coli* and *M. capsulatus* are badly affected by large concentrations of Pb^{2+} ions, while *S oneidensis* are less affected. The negative effect of atrazine is dramatic and more or less similar for all three bacteria.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 July 2018 doi:10.20944/preprints201807.0351.v1

Hexane, however, did not affect *M. capsulatus*, though inhibit both *E. coli* and *S. oneidensis*. Such behaviour of immobilized bacteria is similar to those bacteria in solution [15].

Bacteria	Pollutants	before exposure		after exposure	
		Live	Dead	Live	Dead
Escherichia coli	$PbCl_2$	93	20	21	65
Shewanella oneidensis	$PbCl_2$	149	22	72	79
Methylococcus capsulatus (Bath)	$PbCl_2$	43	13	16	57
Escherichia coli	Atrazine	81	25	18	64
Shewanella oneidensis	Atrazine	79	18	15	77
Methylococcus capsulatus (Bath)	Atrazine	62	17	19	51
Escherichia coli	Hexane	69	21	20	87
Shewanella oneidensis	Hexane	57	11	28	62
Methylococcus capsulatus (Bath)	Hexane	75	19	71	14

Table 1. The numbers of live and dead bacteria immobilized on modified screen printed gold electrodes for all three bacteria before and after treatment with 1M solutions of the three pollutants for 2 hours.

The results of optical density (OD600) study of liquid bacteria samples in Figure 2 shows the bacteria counts before and after treatment with large concentrations (1 M) of $PbCl_2$. The results are similar to those of fluorescent microscopy; all bacteria appeared to be affected by $PbCl_2$ though this effect was less pronounced for *S. oneidensis*. It has to be said that the results of optical density measurements, which are based on light scattering, could be affected by different motilities of the bacteria studied.

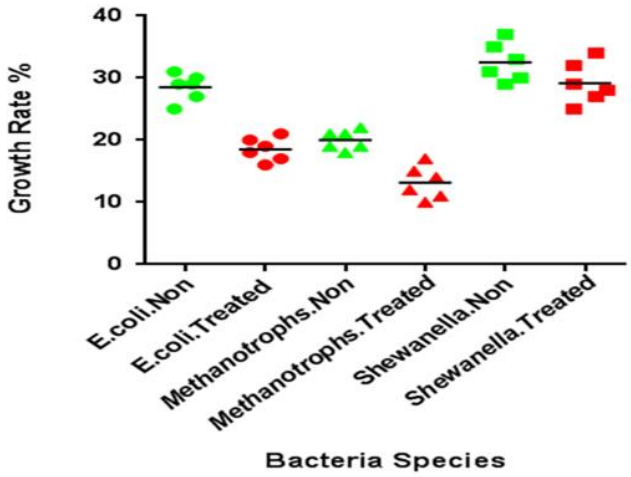

Figure 2. Optical density (OD600) data obtained for three bacteria solutions before and after treatment with 1 M solution of $PbCl_2$ for 2 hours.

The most accurate account of bacteria wellbeing can be obtained from flow cytometry measurements which combine the advantages of both fluorescence microscopy and optical density methods. Typical results of flow cytometry for all three bacteria before and after

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 July 2018 doi:10.20944/preprints201807.0351.v1

treatment with 1M solution of $PbCl_2$ are presented in Figure 3. In these experiments bacteria were stained with L7012 Live/Dead Bacterial Viability Kit and appeared on the graphs in Figure 3 as blue dots (for live bacteria) and orange dots (for dead bacteria). The increase in the dead bacteria counts after exposure to $PbCl_2$ salt (1 M concentration for 2 hours) is visually apparent for all three types of bacteria studied.

In addition to that, after $PbCl_2$ treatment, dead *E. coli* and *M. capsulatus* bacteria appear mostly in bottom-left quadrant of the graph in Figure. 3A and 3C indicating the increase in the bacteria size most-likely due to hyper atrophy of cell membrane or rapture of cell walls. Contrary, the size of *S. oneidensis* bacteria were affected much less by $PbCl_2$; dead bacteria appeared slightly enlarged since they were shifted to the bottom-left in Figure 3B.

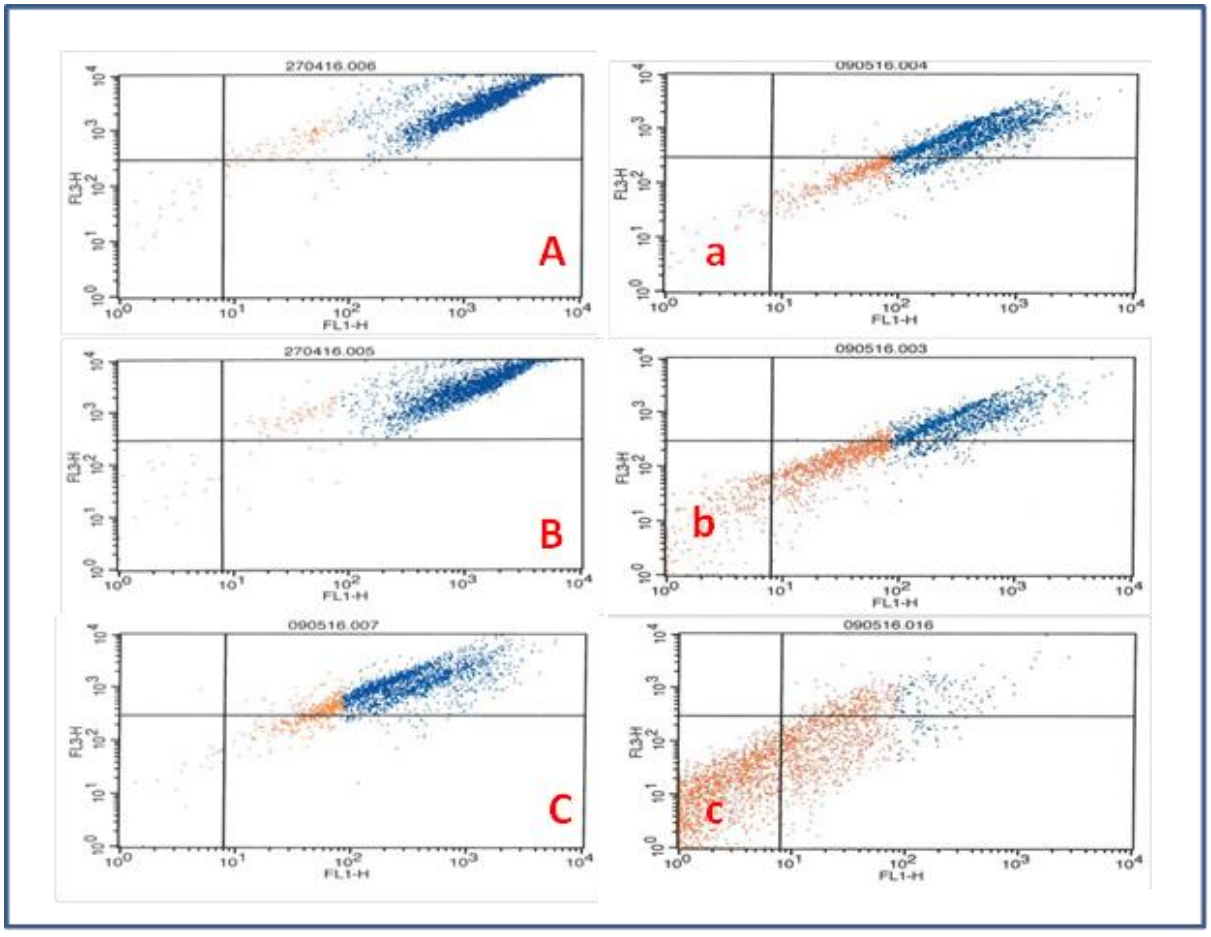

Figure 3. Flow cytometry results for *S. oneidensis* (A), *E. coli* (B), and *M. capsulatus* (C) before (left) and after (right) treatment with $PbCl_2$ (1M for 2 h).

Flow cytometry tests were carried out for the other two pollutants, e.g. atrazine and hexane, and the results as summarised in Table 2 as the percentage of live and dead bacteria.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 July 2018 doi:10.20944/preprints201807.0351.v1

Type of Bacteria	Type of Pollutants	Before		After	
		Live	Dead	Live	Dead
Escherichia coli	$PbCl_2$	61.88%	38.12%	28.11%	71.89%
Shewanella oneidensis	$PbCl_2$	74.32%	25.68%	55.68%	44.32%
Methylococcus capsulatus (Bath)	$PbCl_2$	65.49%	33.51%	36.49%	63.51%
Escherichia coli	Atrazine	78.43%	21.57%	18.43%	81.57%
Shewanella oneidensis	Atrazine	84.32%	15.68%	58.71%	41.29%
Methylococcus capsulatus (Bath)	Atrazine	77.33%	22.67%	37.33%	62.67%
Escherichia coli	Hexane	70.54%	29.46%	30.54%	69.46%
Shewanella oneidensis	Hexane	88.71%	11.29%	45.68%	54.32%
Methylococcus capsulatus (Bath)	Hexane	56.47%	43.53%	65.58%	34.42%

Table 2. Flow cytometry data: the percentage of live and dead bacteria before and after treatment with different pollutants.

Analysis of these data allowed us to conclude that *E. coli* bacteria are strongly inhibited by all three pollutants. *S. oneidensis* bacteria are less affected by Pb^{2+} ions as compared to the strong inhibition effect of atrazine and hexane. *M. capsulatus* bacteria are badly affected by Pb^{2+} ions and atrazine, while hexane/ethanol mixture even stimulates their growth.

Among the three optical methods used to determine the live and dead bacteria percentage, flow cytometry appeared to be the most reliable and not affected by different motility of *E. coli*, *M. capsulatus* and *S. oneidensis* bacteria. The dead bacteria are not motile and tend to sediment which may affect the results of static fluorescent microscopy and optical density measurements. Nevertheless, the results of optical characterization of bacteria samples provided a background for further study using much simpler electrochemical method.

3.2. Electrochemical study of bacteria in solution and immobilized bacteria samples

In this work, the effect of Pb^{2+} ions, atrazine, and hexane on cyclic voltammograms (CVs) of all three bacteria, in both bacteria solutions and immobilized bacteria was studied. Typical series of CVs recorded on *E. coli*, *S. oneidensis*, and *M. capsulatus* samples are shown in Figure. 4. The CV graphs in Figure 4 are almost featureless in the selected voltage range from -0.5 V to +0.5 V, which was chosen deliberately in order to avoid electrochemical reactions on the electrodes, with both cathodic and anodic currents just began to rise. The values of both cathodic and anodic current at -0.5 V and +0.5V, respectively, depend on the bacteria concentration in solution [14-15], however the effect on anodic current is more pronounced and it is therefore used for analysis in this work.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 July 2018 doi:10.20944/preprints201807.0351.v1

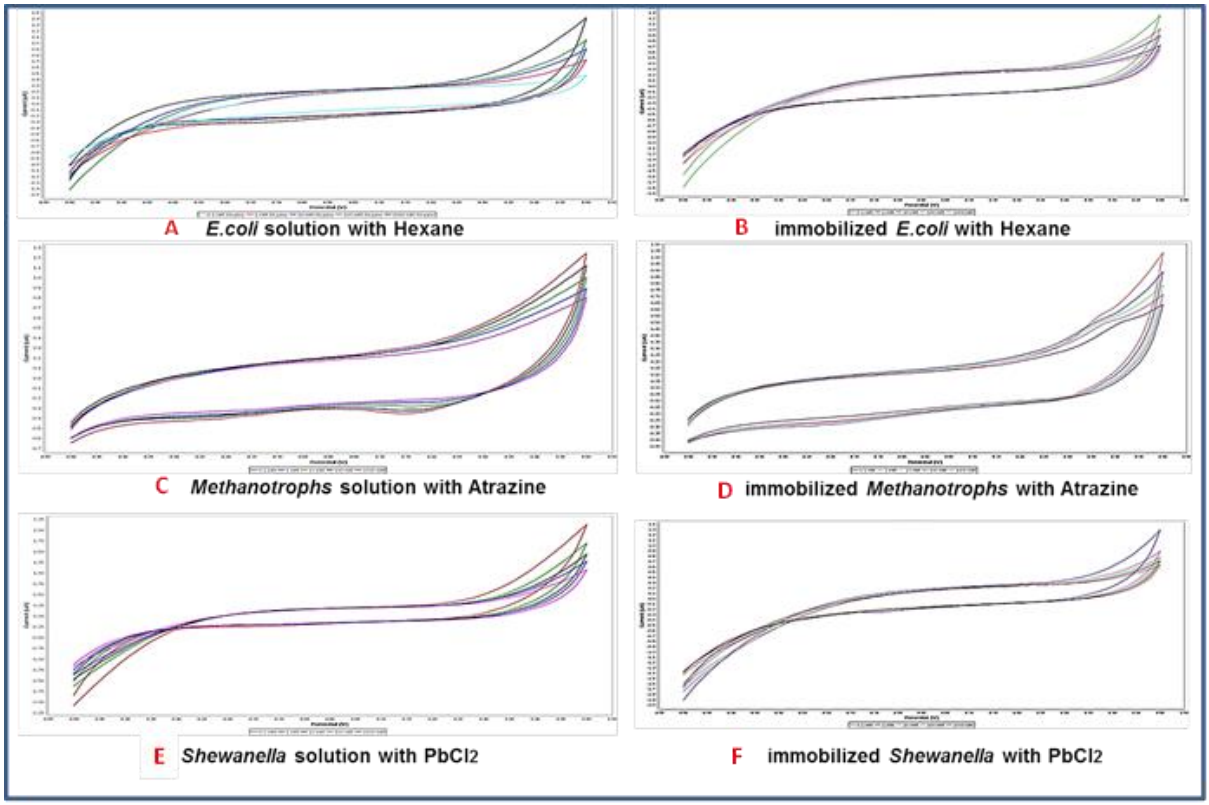

Figure 4. Cyclic voltammograms for: *E.coli* in solution (A) and immobilized *E.coli* (B) treated with hexane; *Methanotrophs* in solution (C) and immobilized *Methanotrophs* (D) treated with atrazine; and *Shewanella oneidensis* in solution (E) and immobilized *Shewanella oneidensis* (F) treated with $PbCl_2$.

In Figure 4 CV cycles appear to shift upwards upon increasing the pollutants' concentration from 0 (untreated bacteria) to 0.1mM, 1mM, 10mM, 100mM, and 1M. The characteristic parameter in this study, e.g. the value of anodic current at +0.5 V, increases with the increase in pollutant concentration for all three bacteria in both liquid and immobilized forms. This means that the electrical conductivity is controlled by bacteria adsorbed on the surface of gold electrodes and acting as insulating layer reducing the current. The correlation between bacteria concentration and the electric current (or conductivity) values is very important for further study of the effect of pollutants, and such measurements were always carried out first [14-15]. The presence of pollutants (Pb^{2+} ions, atrazine, and hexane in our case) causes the damage of bacteria cells, and therefore bacteria became less insulating, in-turn leading to the increase in the anodic current, which is observed in Figure 4.

To analyse the effect of pollutants on electrical properties of immobilized bacteria, the values of anodic current (I_A) at +0.5V from CV measurements were normalised by the currents values of uncoated electrodes in PBS with the addition of a particular pollution of particular

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 July 2018 doi:10.20944/preprints201807.0351.v1

concentrations (I_{A0}) to construct the values of relative changes of anodic current $\Delta I_A / I_{A0} = (I_A - I_{A0})/I_{A0}$. For example, for *S. oneidensis* bacteria treated with 1mM solution of $PbCl_2$ (Figure. 4F), the reference was recorded on uncoated electrodes in PBS containing 1mM of $PbCl_2$. The relative changes in anodic current are presented in Figure 5 for all three bacteria studied as concentration dependences of the three pollutants. As one can see the effects of $PbCl_2$, atrazine, and hexane on *S. oneidensis*, *M. capsulatus* and *E. coli* are completely different. *E. coli* appeared to be affected by $PbCl_2$, atrazine, and hexane even at low concentrations since the $\Delta I_A/I_{A0}$ values increase monotonically in Figures 5A, 5B, and 5C, respectively. This means that *E. coli* is equally inhibited by all three pollutants and becoming less electrically resisting. In contrast, *S. oneidensis* is almost unaffected by $PbCl_2$ at low concentrations of all pollutants up to 10mM, and then $\Delta I_A/I_{A0}$ started to increase at high concentrations of 100mM and 1M. Such behaviour of immobilized *E. coli* and *S. oneidensis* bacteria is similar to those free in liquid as reported in [15]. *M. capsulatus* respond to $PbCl_2$ (Figure. 5A) and atrazine (Figure 5B) similarly to the other two bacteria studied though the changes in $\Delta I_A/I_{A0}$ are more pronounced at high concentrations, particularly for atrazine. However, *M. capsulatus* bacteria are not affected by hexane (see Figure. 5C) even at high concentration; moreover an overall trend to small decrease in $\Delta I_A/I_{A0}$ is observed. Such behaviour was expected since *M. capsulatus* consume some hydrocarbons [18].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 July 2018 doi:10.20944/preprints201807.0351.v1

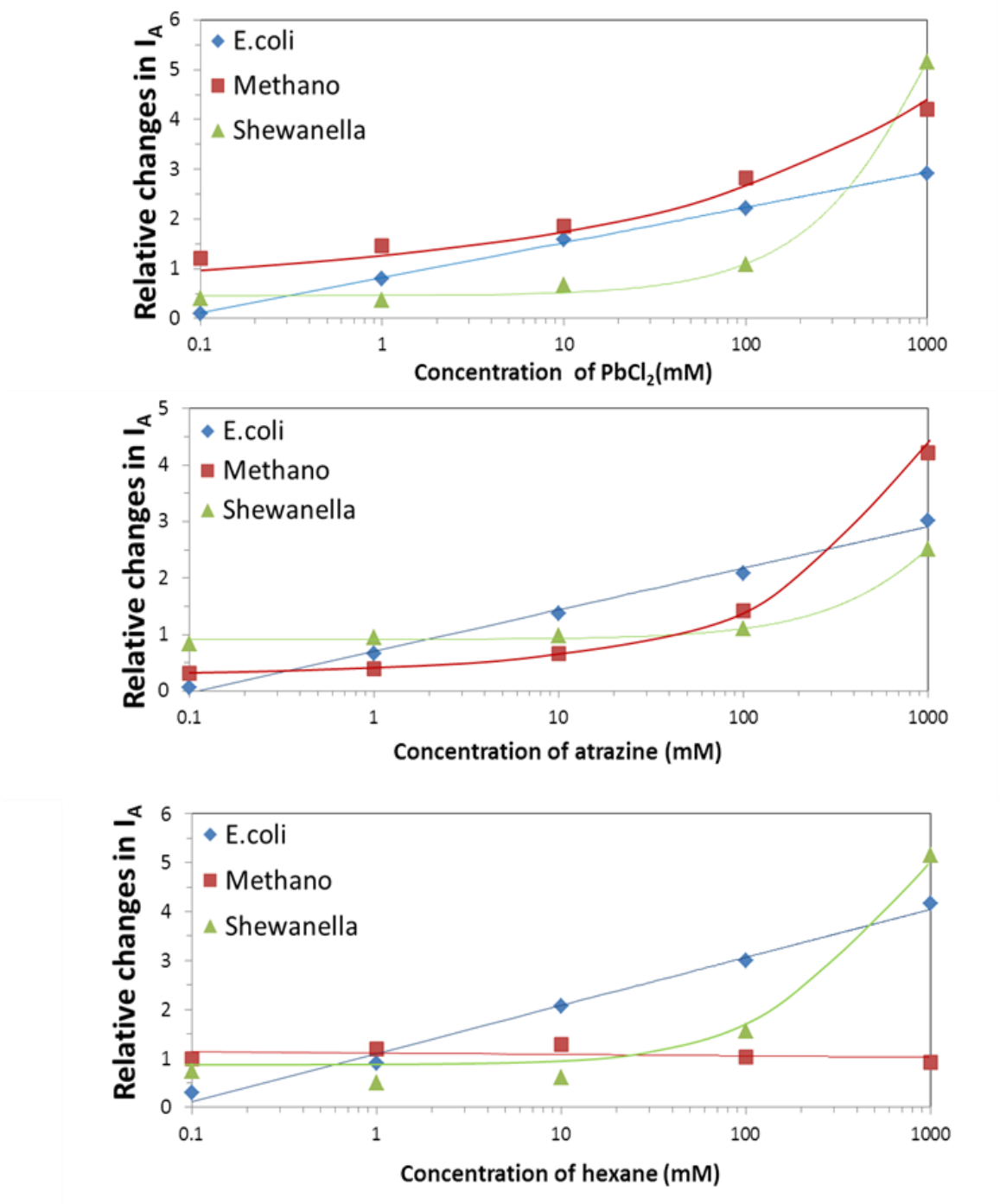

Figure 5. Comparison of relative changes of anodic current (I_A) at +0.5V of all three types immobilized bacteria samples on modified electrodes exposure to $PbCl_2$ (A), Atrazine (B), and Hexane (C).

The results presented in Figure 5 show a possibility of pattern recognition of the effect of the three pollutants studied. An attempt of pattern recognition has been done by presenting the relative responses of the three channels, e.g. three bacteria (*E.coli*, *M. capsulatus* (Bath) and

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 July 2018 doi:10.20944/preprints201807.0351.v1

Shewanella oneidensis) immobilized on three screen-printed electrodes, to the three pollutants ($PbCl_2$, atrazine, and hexane) in a pseudo-3D plot in Figure 6.

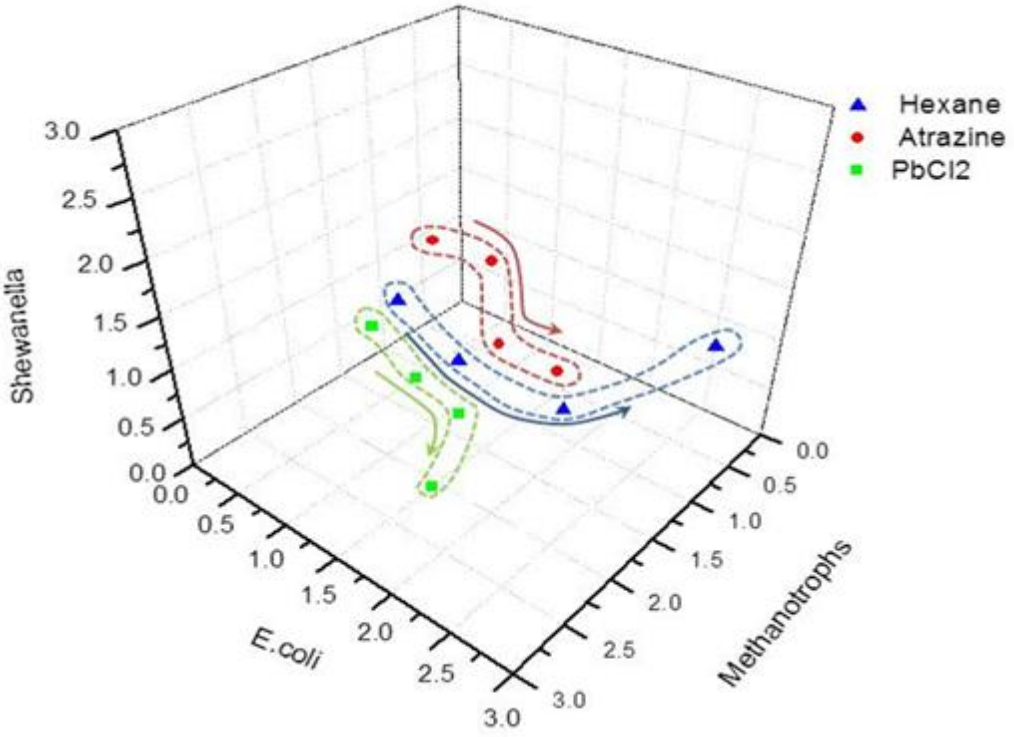

Figure 6. 3D plot of relative changes in anodic current for *E.coli*, *M. capsulatus*, and *S. oneidensis* caused by different pollutants. Arrows show the direction of the pollutants' concentration increase from 0.1mM to 100mM.

The experimental points for $PbCl_2$, atrazine, and hexane in concentrations up to 100mM shown in different colours are well-separated in this 3D graph. This is a clear indication that pattern recognition principles can be applied for identification of pollutants using different types of bacteria. The concentration of pollutants could be evaluated too using the appropriate calibration and data extrapolation.

3.3. Discussion of the results of optical and electrochemical study.

The observed effects of the above pollutants on the three selected bacteria are somehow expected. In general terms, different chemicals of both organic and inorganic origin may affect microorganisms in two possible ways, e.g. acting as either catalysers enhancing bacterial metabolism or as inhibitors having an opposite effect of reducing bacteria metabolism and even damaging bacteria membranes and causing their death.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 July 2018 doi:10.20944/preprints201807.0351.v1

In our case, *E. coli* is obviously inhibited by the pollutants used. This results in the reduction of live bacteria concentration which was confirmed by optical study. Consequently, the increased number of damaged or dead bacteria reduces their insulating properties, thus causing an increase in both anodic and cathodic currents.

Shewanella oneidensis bacteria are known to be tolerant to heavy metals in low concentration, which may have even growth stimulating (catalytic) effect [25] which can be used in water treatment [26]; high concentrations of heavy metals are damaging. This explains the observed immunity of *S. oneidensis* to heavy metals at low concentrations, while other pollutants are still acting as inhibitors. *M. capsulatus*, in contrast, are known by their abilities to use some organic chemicals (hydrocarbons, alcohols) as food [27] and therefore are used in sewage treatment [28]. In other words, *M. capsulatus* bacteria are catalysed by some petrochemicals, while heavy metals and pesticides are still acting as inhibitors. Optical and electrochemical study of both *S. oneidensis* and *M. capsulatus* showed the characteristics changes, respectively, in the live bacteria concentration and anodic current in line with their expected catalytic-inhibition patterns.

Combining the above three types of bacteria in a sensor array was logical and therefore enabled the array to identifying the type of pollutants. This could be achieved using optical methods with flow cytometry being perhaps the most suitable method for this task. However, very simple electrochemical measurements of anodic current could do a similar a job at substantially reduced cost. Screen-printed electrodes with immobilised bacteria can be prepared in advance and kept active for few weeks when stored in a fridge. Such electrical tests can be used for quick preliminary analysis of water samples; the samples indicating a presence of certain pollutants can be passed to specialised laboratories for further more detailed and accurate testing. The overall cost and time of analysis will be substantially reduced as a result.

4. Conclusions and future work

The effect of different types of pollutants, heavy metals ions (Pb^{2+}), pesticides (atrazine) and petrochemicals (hexane) on three types of bacteria, *E. coli*, *M. capsulatus* (Bath) and *S. oneidensis* was studied using three different optical techniques: fluorescent microscopy and flow cytometry which yields directly the ratio of live/dead bacteria, stained, respectively, with “green” and “red” fluorescent dyes as well as optical density measurements at 600 nm. All three optical methods are capable of detecting the effect of heavy metals, pesticides and

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 July 2018 doi:10.20944/preprints201807.0351.v1

hydrocarbons on the above bacteria, though the flow cytometry is much more reliable. Fluorescent microscopy, however, which can be also carried out on immobilized bacteria provides a very useful link to the following electrochemical study. The results obtained were encouraging, however the use of expensive and bulky optical instrumentation is not the way forward for portable and cost-effective sensor development.

Simple electrochemical tests, e.g. cyclic voltammograms, either on gold electrodes immersed into liquid bacteria samples or (even better) on modified screen printed gold electrodes with immobilized bacteria appeared to be very successful. The values of anode current was found to correlate with bacteria concentration and thus with the concentration of different pollutants acting as inhibitors for bacteria. The effect of different pollutants on the three bacteria used was different: *E. coli* is strongly inhibited, while *S. oneidensis* is practically unaffected in a wide concentration range of all pollutants used. *M. capsulatus* (Bath) is strongly inhibited by $PbCl_2$ and atrazine but completely unaffected by hexane. These facts opened a possibility of exploiting the principles of pattern recognition for identification of pollutants.

This work paves the way for the development of novel, simple, and cost effective electrochemical bacteria-based sensor array for preliminary assessment of the presents of pollutants in water. Future work which is currently underway will focus on extending the range of pollutants (different heavy metals, pesticides, and petrochemicals) and using advanced data processing tools such as (ANN) Artificial Neuron Network for analysis of real water samples.

Acknowledgements

The authors would like to thank the Iraqi Government, Ministry of Higher Education and Scientific Research and University of Basra for sponsoring the PhD project.

 doi:10.20944/preprints201807.0351.v1

References

1. Ritter, Keith Solomon, Paul Sibley, Ken Hall, Patricia Keen, Gevan Mattu, Beth Linton, L. Sources, pathways, and relative risks of contaminants in surface water and groundwater: a perspective prepared for the Walkerton inquiry. *Journal of Toxicology and Environmental Health Part A*, (2002). *65*(1), 1-142.

2. Walter, I., Martinez, F., & Cala, V. Heavy metal speciation and phytotoxic effects of three representative sewage sludges for agricultural uses. *Environmental pollution*, (2006). *139*(3), 507-514.

3. Pearce, F., & Mackenzie, D. (1999). Its raining pesticides; *NEW SCIENTIST April*, *3*

4. Konda, L. N., and Pásztor, Z. Environmental distribution of Acetochlor, Atrazine, Chlorpyrifos and Propisochlor under field conditions, *J. Agric. Food Chem*., (2001).49, 3859-3863.

5. Environment Agency, Groundwater risk assessment for your environmental permit. Website: https://www.gov.uk/guidance/groundwater-riskassessment- for-your environmental-permit#develop-your-conceptual-model ,(2017). [Accessed October 2017].

6. Evans, E. H., Day, J. A., Palmer, C. D., Price, W. J., Smith, C. M., & Tyson, J. F. Atomic spectrometry update. Advances in atomic emission, absorption and fluorescence spectrometry, and related techniques. *Journal of Analytical Atomic Spectrometry*, (2005). *20*(6), 562-590.

7. Montes-Bayon, M.; DeNicola, K.; Caruso, J.A. Liquid chromatography-inductively coupled plasma mass spectrometry. *J. Chromatography A*, (2003), 1000, 457–476.

8. Zhang, Y.; Adeloju, S.B. Coupling of non-selective adsorption with selective elution for novel in-line separation and detection of cadmium by vapour generation atomic absorption spectrometry. *Talanta* (2015), *137*, 148–155.

9. Harrington, C.F.; Clough, R.; Drennan-Harris, L.R.; Hill, S.J.; Tyson, J.F. Atomic spectrometry update. Elemental speciation. *J. Anal. At. Spectrom.* (2011), *26*, 1561–1595.

10. March, G., Nguyen, T. D., & Piro, B. Modified electrodes used for electrochemical detection of metal ions in environmental analysis. *Biosensors*, (2015).*5*(2), 241-275.

11. Nabok, A. and Haron, S., Registration of heavy metal ions and pesticides with ATR planar waveguide enzyme sensors. *Applied Surface Science*, (2004). 238(1), 423-428.

12. Osma, J. F., & Stoytcheva, M. (Eds.). *Biosensors: Recent advances and mathematical challenges*. (2014). OmniaScience.

13. Starodub, N. F., Katzev, A. M., Levkovetz, I. A., Goncharuk, V. V., Klimenko, N. A., & Vakulenko, V. F. Biosensor based on the photoluminescent bacteria and its use for express control of water contamination by some surface active substances. *TRANSDUCERS, Solid-State Sensors, Actuators and Microsystems*, 12th International Conference IEEE Sensors , (2003), 2, 1197-1200.

14. M. Al-Shanawa, A. Nabok, A. Hashim, T. Smith, S. Forder, Sensors & their applications XVII, *J. Phys. Conf. Ser.* 450 (012025) (2013)http://dx.doi.org/10.1088/1742-6596/450/1/012025.

15. H. Abu-Ali, A. Nabok, T. Smith , M. Al-Shanawa, Development of electrochemical inhibition biosensor based on bacteria for detection of environmental pollutant Sensing and Bio-Sensing Research 13 (2017) 109–114. http://dx.doi.org/10.1016/j.sbsr.2016.10.007.

16. Lăzăroaie, M. M. Multiple responses of gram-positive and gram-negative bacteria to mixture of hydrocarbons. *Brazilian Journal of Microbiology*, (2010).41(3), 649-667.

17. Ramírez-Díaz, M. I., Díaz-Pérez, C., Vargas, E., Riveros-Rosas, H., Campos-García, J., & Cervantes, C. Mechanisms of bacterial resistance to chromium compounds. *Biometals*, (2008).21(3), 321-332.

18. Leak, D. J., & Dalton, H. Growth yields of methanotrophs. *Applied microbiology and Biotechnology*, (1986). 23(6), 470-476.

19. Han, J. I., Lontoh, S., & Semrau, J. D. Degradation of chlorinated and brominated hydrocarbons by Methylomicrobium album BG8. *Archives of microbiology*, (1999). *172*(6), 393-400.

20. Sezonov, G., Joseleau-Petit, D., & D'Ari, R. *Escherichia coli* physiology in Luria-Bertani broth. *Journal of bacteriology*, (2007). *189*(23), 8746-8749.

21. Whittenbury, R., Phillips, K. C., & Wilkinson, J. F. (1970). Enrichment, isolation and some properties of methane-utilizing bacteria. Microbiology, 61(2), 205-218.

22. Suo, Z., Avci, R., Yang, X., & Pascual, D. W. Efficient immobilization and patterning of live bacterial cells. *Langmuir*, (2008). 24(8), 4161-4167.

23. Berney, M., Hammes, F., Bosshard, F., Weilenmann, H. U., & Egli, T. Assessment and interpretation of bacterial viability by using the LIVE/DEAD BacLight Kit in combination with flow cytometry. *Applied and environmental microbiology*, (2007).73(10), 3283-3290.

24. Kaprelyants, A. S., & Kell, D. B. Rapid assessment of bacterial viability and vitality by rhodamine 123 and flow cytometry. *Journal of Applied Microbiology*, (1992). *72*(5), 410-422.

25. Carmona-Martinez, A. A., Harnisch, F., Fitzgerald, L. A., Biffinger, J. C., Ringeisen, B. R., & Schröder, U. (2011). Cyclic voltammetric analysis of the electron transfer of Shewanella oneidensis MR-1 and nanofilament and cytochrome knock-out mutants. Bioelectrochemistry (Amsterdam, Netherlands), 81(2), 74-80.

26. Du, Z., Li, H., & Gu, T. (2007). A state of the art review on microbial fuel cells: a promising technology for wastewater treatment and bioenergy. Biotechnology advances, 25(5), 464-482.

27. Hanson, R. S., & Hanson, T. E. (1996). Methanotrophic bacteria. Microbiological reviews, 60(2), 439-471.

 doi:10.20944/preprints201807.0351.v1

28. Kampman, C., Hendrickx, T. L., Luesken, F. A., van Alen, T. A., den Camp, H. J. O., Jetten, M. S., ... & Temmink, H. (2012). Enrichment of denitrifying methanotrophic bacteria for application after direct low-temperature anaerobic sewage treatment. *Journal of hazardous materials*, *227*, 164-171.