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ADVOCATE bulletin

CL:AIRE's ADVOCATE bulletins describe practical aspects of research which have direct application to the characterisation, monitoring or remediation of contaminated soil or groundwater. This bulletin describes laboratory experiments using bioelectrochemical systems to enhance the bioremediation of contaminated groundwater.

Enhancing bioremediation of groundwater by microbial interaction with a solid state electrode: proof-of-concept

1. Introduction

Plumes of organic chemicals in groundwater are frequently anaerobic due to limitations in the availability and aqueous solubility of oxygen. As biodegradation rates for anaerobic processes, which use electron acceptors such as nitrate or sulphate for respiration, are usually slower than for aerobic processes, plumes of organic contaminants may persist in groundwater (Atlas and Philp, 2005). A novel, but under-developed, approach to enhance the anaerobic bioremediation of organic compounds in groundwater is to couple the exchange of electrons between bacteria and solid state electrodes. In this case, electrodes inserted into the subsurface serve as an inexhaustible electron acceptor for microbial metabolism of organic compounds (Zhang *et al.*, 2010). Bioelectrochemical systems (BES) with one electrode in contact with the organic contaminant can be constructed to enhance the bioremediation of groundwater while generating small amounts of electrical energy. This technology, if developed for *in situ* application, could offer an environmentally sustainable option to manage groundwater contaminated with organic compounds.

In principle, an electrode installed in the subsurface can be constructed as a permeable reactive barrier (PRB) made of conductive graphite granules (Figure 1) (Aulenta and Majone, 2010). Groundwater bacteria, which naturally attach to the graphite, degrade contaminants as they pass through and transfer the electrons to the graphite, which functions as an anode. The electrons are then transported to a cathode placed in nearby uncontaminated oxygenated groundwater.

The energy that bacteria gain from the biodegradation of organic compounds is proportional to the electrochemical potential of the final electron acceptor. Oxygen is the most favourable electron acceptor in terms of energy gain for microorganisms. There are two possible ways to regulate the potential of the anode. Firstly, it can develop naturally, according to the metabolic properties of the microbial community and electrical resistance of the load. The second possibility is to use a small amount of electrical energy to fix the anode potential and control the microbial metabolism and biodegradation rate (Logan, 2008; Wagner *et al.*, 2010).

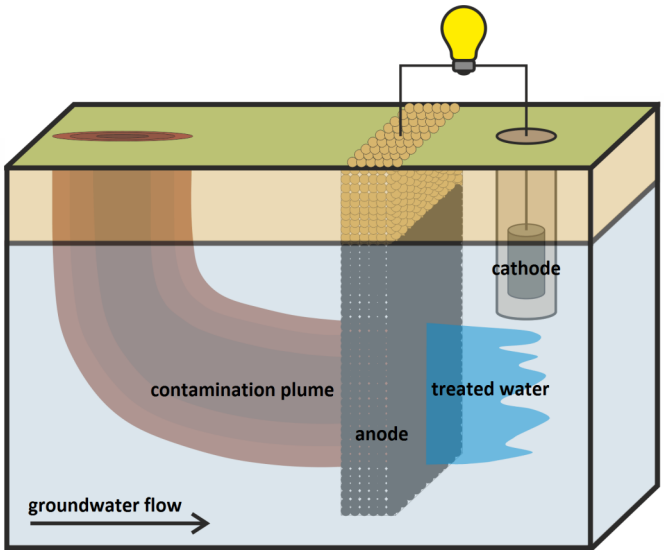

Figure 1: Conceptual model of a bioelectrochemical system (BES) with the anode constructed as permeable reactive barrier.

2. Proof-of-concept Experiment

Recent laboratory studies report that bioremediation of organic pollutants such as petroleum hydrocarbons, phenol, benzene, naphthalene, phenanthrene and pyrene can be enhanced by the electron transfer to an electrode in BES (Luo *et al.*, 2009; Morris *et al.*, 2009; Zhang *et al.*, 2010; Huang *et al.*, 2011; Mohan and Chandrasekhar, 2011; Morris and Jin, 2012; Wang *et al.*, 2012; Yan *et al.*, 2012). However, research on this technology has to date been undertaken in BES reactors at the laboratory level and its application at the field-scale is still under-developed.

Laboratory experiments to date have used groundwater amended with nutrients (Morris and Jin, 2008; Morris *et al.*, 2009), but this fails to represent the *in situ* conditions in most aquifers. This technology has also not been examined in detail for the bioremediation of phenols, which may be released to groundwater as

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complex organic mixtures from manufactured gas plants, coking works, wood treatment facilities and other industrial sites. However, previous BES research has only evaluated phenol biodegradation (Luo *et al.*, 2009; Huang *et al.*, 2011).

In this study, the feasibility of using BES to enhance the bioremediation of coal tar-contaminated groundwater, containing a mixture of phenolic compounds, was examined without any addition of nutrients. This laboratory experiment serves as a proof-of-concept analysis for potential field-scale application in subsequent phases.

3. Experimental Design

The laboratory experiment was run in an H-type BES with 1 kΩ resistor (Figure 2). The electrodes made of carbon cloth were placed in different chambers separated by a semi-permeable membrane (Nafion 117), to simulate conditions in the aquifer, i.e the anode and cathode in the contaminated and uncontaminated groundwater, respectively (Figure 1).

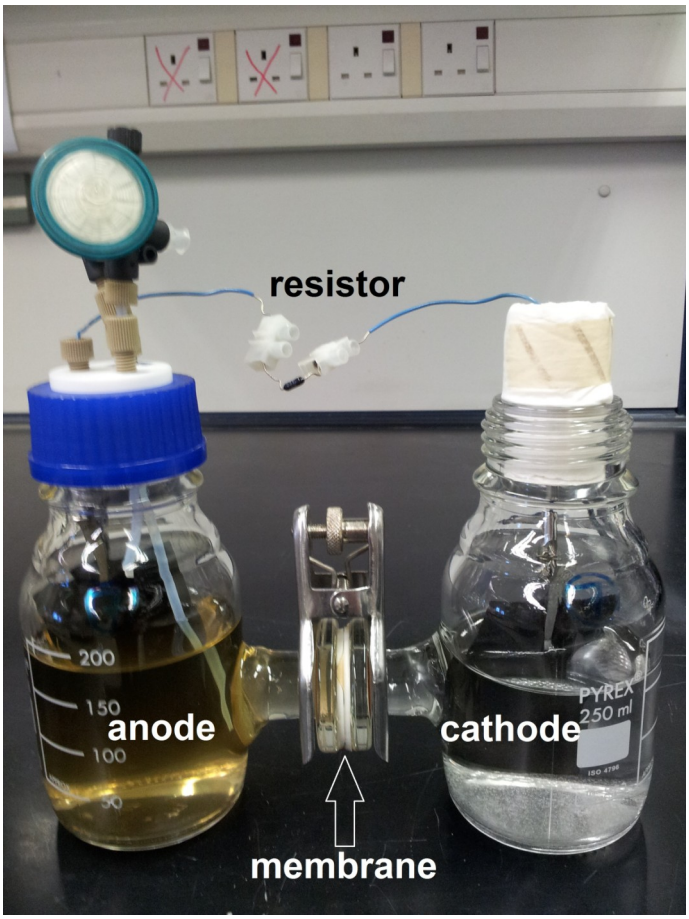

Figure 2: H-type BES used in the experiment.

Groundwater containing a mixture of phenolic compounds was used in the experiments. Three different BES reactors were set up, a closed circuit BES (CC-BES), an open circuit control BES (OC-BES) and a biologically inactive control BES ("sterile"-BES, S-BES) (Table 1). The biodegradation rate should be enhanced in the CC-BES, where the closed circuit enables the transfer of electrons from the anode to the cathode. The system with the open circuit, OC-BES, corrects for background biodegradation rates without an operational BES. The S-BES systems identifies any changes in chemistry or electricity production caused by abiotic processes in the system.

Table 1: Experimental set up.

Type of BES	Resistor	Anode chamber	Cathode chamber
CC-BES	1 kΩ	Contaminated groundwater	Uncontaminated groundwater autoclaved
OC-BES	None	Contaminated groundwater	Uncontaminated groundwater autoclaved
S-BES	1 kΩ	Contaminated groundwater with sodium azide	Uncontaminated groundwater autoclaved

All BES were operated for 24 days, only in one replicate. The closed circuit voltage (across the resistor) was monitored daily. The resistor was mounted on the OC-BES only for the purposes of measuring the closed circuit voltage. The maximum power produced by the BES was measured when the closed circuit voltage, i.e. electricity production by BES, reached its maximum value.

Samples for chemical analysis were taken every 5-7 days from the anode and cathode chamber and filter-sterilized. The concentration of the phenolic compounds was determined by high-performance liquid chromatography (HPLC). The detection limit was 1 mg/l and the precision of this analysis was ±7%.

4. Electricity Production

The closed circuit voltage (Figure 3) started increasing in biologically active BES after 3 days of operation, reaching a maximum value of 82 mV in the CC-BES and 29 mV in the OC-BES. Electricity production decreased to 14 mV in the CC-BES after 16 days (Fig. 3).

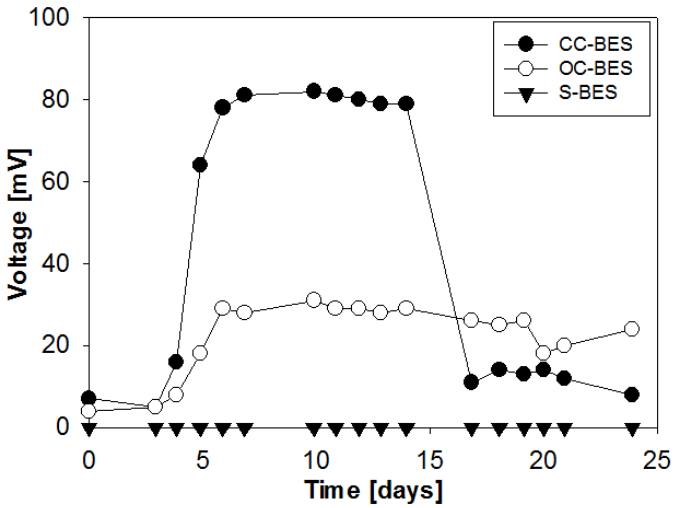

Figure 3: Voltage production for different BES systems used in the experiment.

The measured voltage of the control sample S-BES remained at 0 mV. The maximum power produced by the CC-BES reached 2.0 mW/m^2 of the anode surface area, while the OC-BES produced only 1.2 mW/m^2.

The results demonstrate that no electricity can be produced in the absence of bacterial activity. In this study, the closed circuit voltage production and maximum power show that the CC-BES can produce more electricity than the OC-BES. This is likely due to the adaptation of bacteria in the CC-BES for electricity generation. The closed circuit creates a selection pressure in the CC-BES microbial community, which can lead to a community adapted to the transfer of electrons to the anode (Yang *et al.*, 2013).

5. Biodegradation of Phenolic Compounds

Phenolic compounds diffused from the anode chamber to the cathode chamber through the semi-permeable membrane (Figure 4). This decreased the concentration of total phenols in the anode chamber from 158 mg/l to 93 mg/l at the end of the experiment. The concentration of phenolic compounds in the cathode chamber of the abiotic system, S-BES, increased to 72 mg/l. The diffusion coefficient for the total phenols was calculated from these results to be (1.280±0.017)·10^{-6} cm^2/s.

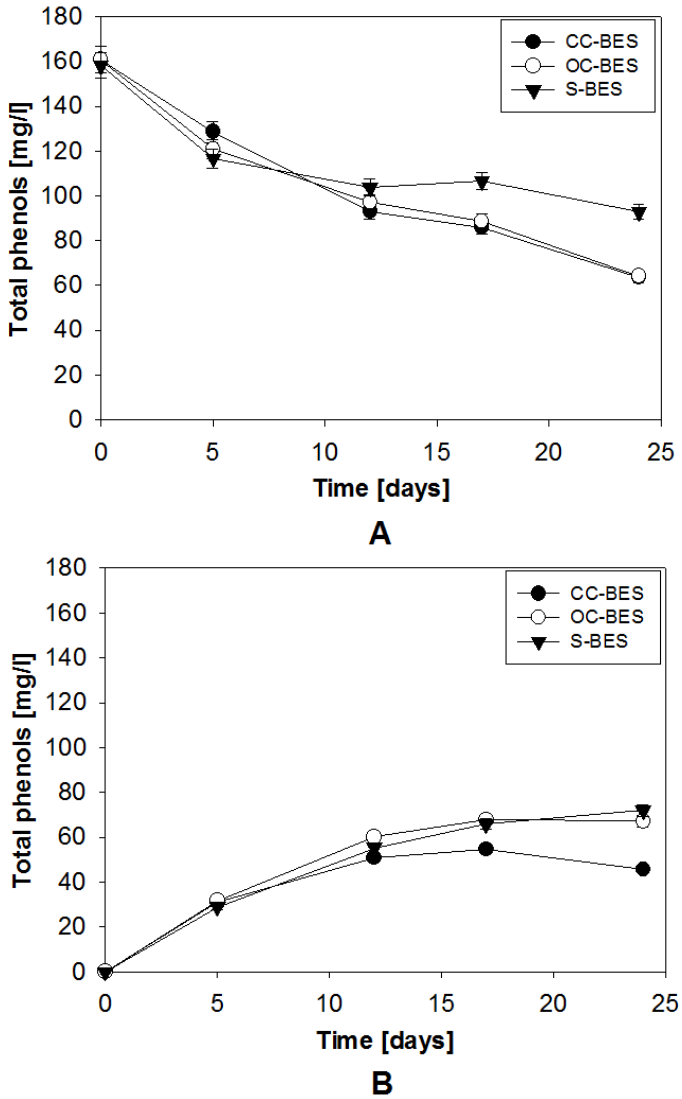

Figure 4: Concentration of total phenols in the anode (A) and cathode (B) chamber. The error bars show the precision of the HPLC analysis.

The concentration of phenolic compounds in the anode chamber of the OC-BES decreased from 161 mg/l to 64 mg/l, due to diffusion into the cathode chamber and biodegradation. The final concentration of total phenols in the cathode chamber is similar to the abiotic system, S-BES (67 mg/l). It was previously reported that oxygen can be transported from the cathode chamber via the Nafion membrane to the anode chamber (Kim *et al.*, 2007). It is likely that oxygen served as the main electron acceptor for biodegradation of the phenolic compounds in the OC-BES. Total phenols in the anode chamber of the CC-BES decreased from 161 mg/l to 64 mg/l. This change was also caused by diffusion and biodegradation. Considering the concentration of phenolic compounds in the anode chamber, there is no significant difference between the CC-BES and OC-BES. However, the concentration of total phenols in the cathode chamber of the CC-BES starts decreasing after 16 days of operation and reaches 46 mg/l at the end of the experiment. It is likely that the phenols were transported back to the anode chamber and then biodegraded, supporting further electricity production.

The microbial community in the CC-BES used two types of electron acceptors: oxygen transported to the anode chamber and the anode electrode. The presence of the anode electrode as an electron acceptor enhanced the biodegradation of the phenolic compounds by 17% compared with the OC-BES.

6. Conclusions

Bioremediation of groundwater contaminated by phenolic compounds was enhanced by the presence of a solid state electrode, which served as an electron acceptor for microbial metabolism. In this case, the biodegradation of total phenols was enhanced by 17%. The results presented in this bulletin are preliminary, only from one replicate and more measurements are needed to confirm this conclusion. Phenolic compounds diffused through the membrane separating the two chambers, which must be considered when interpreting the biodegradation rate and performance of the BES design. The power generated by the BES in this experiment was only 2 mW/m^2 of the anode surface area and is unlikely to be sufficient for practical use. However, when compared with traditional *in situ* bioremediation technologies such as biosparging, BES provide an opportunity to save energy.

Future research will explore the mass transfer processes, electron acceptors and microbial community present in the system in more detail. Further experimentation should involve development of a continuous-flow BES reactor with a PRB electrode that simulates field conditions, including the regulation of the anode potential for better bioremediation performance.

7. Acknowledgements

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