CL:AIRE

EiCLaR 04
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EiCLaR bulletin

CL:AIRE's EiCLaR bulletins describe *in situ* bioremediation technology developments and tools created within the EiCLaR project. This bulletin describes the development of a bioelectrochemical remediation system to treat polluted groundwater.

# Bioelectrochemical remediation

## 1. INTRODUCTION

Biological hydrocarbon degradation requires specific microorganisms capable of breaking hydrocarbon bonds and a constant supply of electron acceptors. These acceptors can include molecular oxygen, nitrate, and sulfate (despite the risk of $H_2S$ production). While aerobic degradation is typically the fastest, it is not always the most cost-effective or easiest to implement under certain conditions. Natural attenuation of hydrocarbon-contaminated groundwater plumes is slower within the anoxic cores, where sulfate reduction and methanogenesis ($H_2S$ and $CH_4$ production, respectively) can occur.

The bioelectrochemical remediation system (BER) developed within the EiCLaR project leverages bacteria capable of anaerobic hydrocarbon degradation and electron exchange to surfaces or shuttles outside the cell. This well-coordinated biological process converts the chemical energy stored in hydrocarbons directly into electricity. The process involves a series of redox reactions: organic degraders transform complex hydrocarbons into simpler molecules, which are then oxidized by electroactive bacteria in the anaerobic anode. Electrons are transported through conductive materials to a cathode exposed to air, where oxygen is reduced to water.

BER can simultaneously remove pollutants and recover energy from the substrate. Traditionally, bioelectrochemical systems have focused on treating wastewater. However, the EiCLaR project has adapted this approach for sites polluted with mixtures of pollutants, including petroleum hydrocarbons and hexavalent chromium (site trial in China). Both electricity generation at the anode and microbial electrolysis, which uses a small amount of energy to drive reactions at the cathode, are utilized. A wide range of bioelectrochemical reactions can occur at either the anode or the cathode, including anaerobic oxidation of petroleum hydrocarbons.

The EiCLaR project launched several experiments to study factors affecting BER system performance. Initially, the goal was to maximize electric current from pollutant degradation. Various amendments, such as sewage sludge, acetate solution, and biochar, were evaluated. Microbiological community evolution in the BER system was monitored for each different system. Petroleum hydrocarbon concentrations were also measured over time, but no significant differences were observed due to matrix heterogeneity. The process performance was assessed by the electric current obtained in each experiment. Finally, field tests verified process scalability in hydrocarbon-polluted sites. A paper detailing the microbiological implications of the process is being prepared for publication.

This bulletin provides essential information on BER, its implementation, and operation. It includes a comparison of advantages and disadvantages relative to other technologies. After reading this material, readers will understand the principles of BER and the requirements for successful implementation in polluted sites.

## 2. BACKGROUND TO THE TECHNOLOGY

BER technology shares its origins with Microbial Fuel Cells (MFCs), which utilize the metabolism of microorganisms to convert chemical energy into electric signals. Using whole cells eliminates the need for enzyme purification and allows multiple enzymatic reactions to occur in near-natural conditions, with organisms regenerating necessary enzymes as part of their natural life cycle. The first MFC was developed by Davis and Yarbrough in the early 1960s, and since then, bioelectrochemical systems have evolved, with publications and patents increasing exponentially since the early 21st century.

The development of this technology has been driven by the need for sustainable energy sources. Research has focused on various parameters affecting MFC performance, including types of substrates (e.g., highly organic waste), electrode materials, and microbial populations. In MFCs, oxidation reactions are catalyzed at the anode

Enhanced and Innovative *In Situ* Biotechnologies
for Contaminated Land Remediation

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For more information on the EiCLaR Project, please visit: www.eiclar.org

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by electroactive bacteria, which transfer electrons released from degradation. These electrons pass through a resistor to the cathode, where oxygen is reduced to water (Fig. 1). Instruments like voltmeters or ammeters can measure the electrons passing through the circuit, helping to estimate the quantity of degraded compounds.

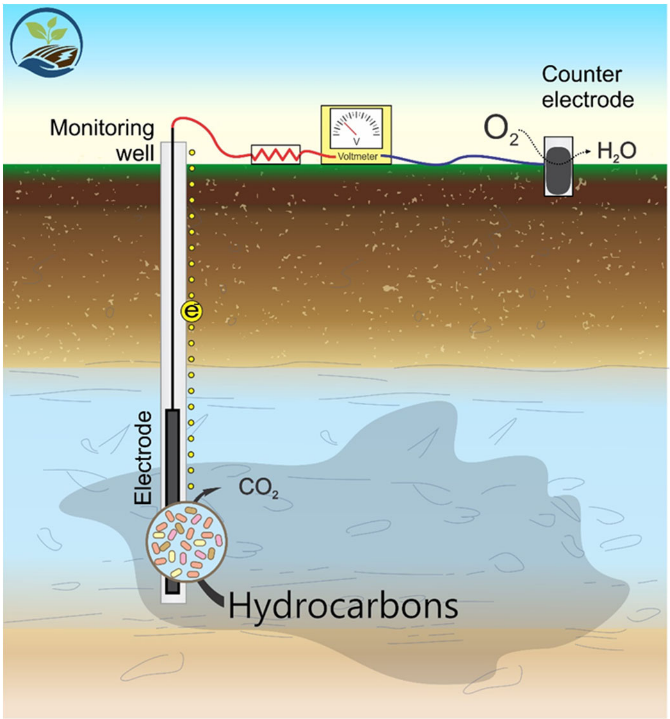

**Figure 1. Conceptual diagram of the BER technology. Hydrocarbons are degraded in the groundwater.**

Although the reaction rate in MFCs is lower compared to more aggressive technologies due to bacterial catalysis, this approach addresses the low diffusion rate of mass and energy for electron acceptors and protons in the soil, thereby accelerating the natural attenuation of pollutants.

The technology is based on the ability of electroactive bacteria to use a solid surface (electrode) as an electron acceptor. This increases the flux of electrons in anoxic groundwater where electron acceptors are scarce. Electroactive bacteria can use a range of compounds as sources of carbon and electrons including metabolites from other electron-acceptor-starved microorganisms. The thermodynamics of the electrode as an electron acceptor is enhanced by a cathode that reacts with oxygen, thus making the electrode almost as strong an electron acceptor as oxygen (minus resistance effects). The reaction at the cathode can be entirely chemical or mediated by bacteria. In addition, other electron acceptors can react with the cathode as in the case of chromium reduction from chromium VI to chromium III.

## 3. DEVELOPMENT OF THE TECHNOLOGY AT LAB-SCALE

Small- and large-scale experiments were used to determine different operating conditions such as effective distance from the well, electrical resistance, and distance to the cathode as well as confirming basic biological, electrical and hydraulic processes (Figs. 2, 3 and 4). The initial studies were in bottles in order to observe that hydrocarbons could actually drive electricity production, enhance the targeted (*Geobacter*) bacteria, and determine which compounds were degraded preferentially. The bottles with the anode in the bottle and the air cathode in contact with water on one side and air on the other are shown in Figure 2. The hydrocarbon concentrations were generally at saturation levels and therefore did not change with time. On the other hand, the slightly oxidized components of diesel such as fatty acids were degraded rapidly.

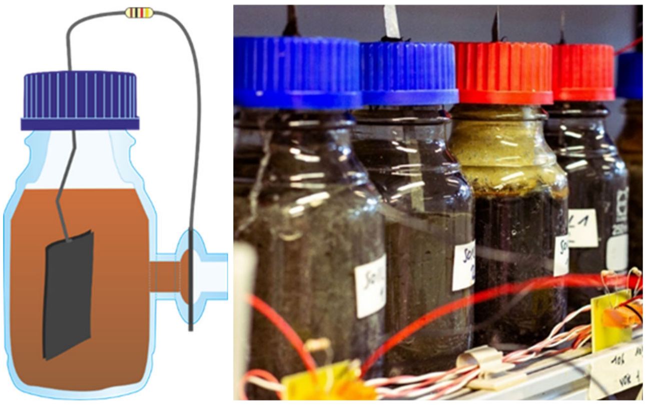

**Figure 2. Left, diagram of the bottles used to test the BER process performance in hydrocarbon (diesel, PAHs) polluted soil, the anode and the cathode are connected through a resistor. Right, actual bottles during the experiments. The soil was saturated in water and pure phase hydrocarbons were present. Controls without hydrocarbons were also tested.**

Two intermediate size reactors were constructed with the anode and cathodes separated by different distances. As the electricity production is directly linked to hydrocarbon degradation, the performance of these systems is relatively simple to evaluate. Increased distance increased resistance and slowed the production of electricity and the rate of hydrocarbon degradation.

**Figure 3. Medium-scale box for testing the BER technology.**

In the largest medium-scale reactor (Fig. 4), both the measurement of electricity as a direct indication of hydrocarbon degradation and carbon dioxide production were monitored. The mineralization of the hydrocarbons as indicated by the increase in carbon dioxide is shown in Figure 5. The electrical output is shown in Figure 6 for two of the system's (BER) biosensors. The electricity in the box increased over time (Fig. 6) while the carbon dioxide at day 100 increased along the length of the box.

Figure 4. Two medium-scale boxes used to test the performance of the BER technology at larger distances between the anode and the cathode. The box on the right has a longer distance between the anode and cathode electrodes.

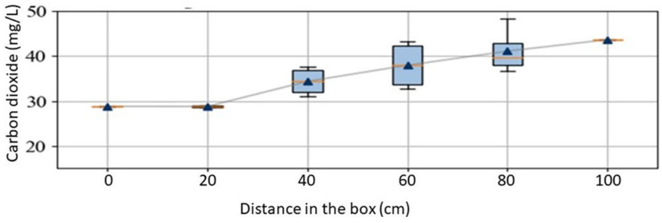

Figure 5. Production of dissolved carbon dioxide in groundwater in a BER lab reactor as hydrocarbon is degraded after 100 days. There is flow in the box from left to right.

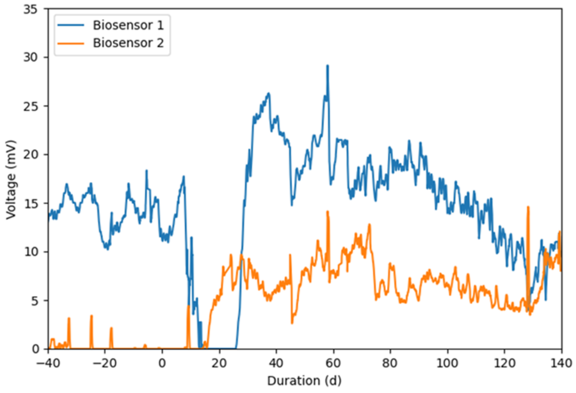

Figure 6. Electrical production in large-scale laboratory reactors. The biosensors are in two different locations. The distance between the electrode was greater for biosensor 2 (box on the right in Fig. 4).

4. DEMONSTRATION OF THE TECHNOLOGY AT FIELD-SCALE

The first pilot site (Fig. 7) is located on a former gas station property in England, known for petroleum hydrocarbon (diesel and PAHs) pollution and has been closed for over ten years. To mitigate the volatilization of these pollutants, a geotextile layer was applied to the site.

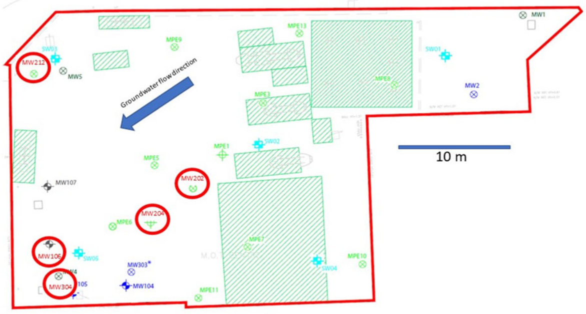

Figure 7. Map of the monitoring wells at the site in the UK.

In May 2024, a team from Université Claude Bernard Lyon 1 (CNRS) installed five electrodes in existing monitoring wells, strategically positioned according to groundwater flow. These electrodes consisted of anodes encased in perforated tubes that had been functionalized with the appropriate microbial community (Fig. 8). The installation depths of each electrode are detailed in Table 1. Counter electrodes were placed in 20 cm deep holes. Both types of electrodes were interconnected via a resistor to ensure proper functionality.

Figure 8. Installation of the electrodes at the site in the UK.

A voltmeter was utilized to measure and record the potential difference between the two electrodes every 10 minutes.

Table 1. The water level, bottom depth, and electrode depth for each well where an electrode was placed.

| | MW212 | MW106 | MW304 | MW204 | MW202 |
|---|---|---|---|---|---|
| Water level (m) | 2.63 | 2.74 | 2.82 | 3.35 | 2.9 |
| Bottom (m) | 6.35 | 8.42 | 24.45 | 8.37 | 6 |
| Electrode (m) | 4.5 | 4 | 4 | 4 | 4 |

After 24 hours of electrode installation, the voltmeters were retrieved, and the 24-hour records were reviewed for analysis. They functioned properly and were reinstalled for a two-month monitoring period (Fig. 9).

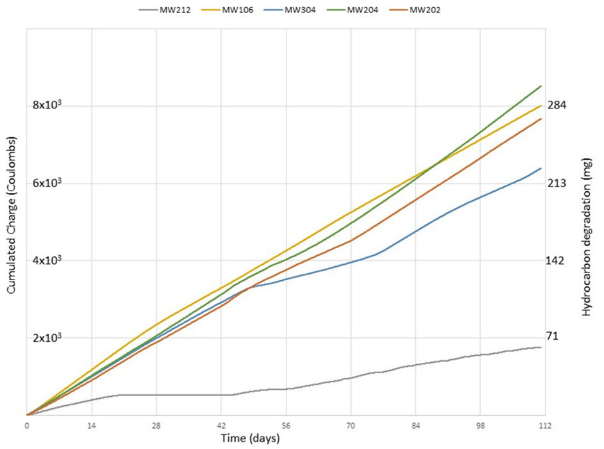

Figure 9. Cumulated charge (Coulombs) and estimated minimum hydrocarbon degradation over time in each well (MW212 was the only well out of the plume).

The installation of the electrodes marked the beginning of a series of tests to monitor the system's effectiveness. Key performance indicators included the electric current generated, changes in hydrocarbon concentration, and shifts in microbial community composition.

- **Electric current generation:** Continuous monitoring shows a steady increase in electric current, indicating active microbial degradation of hydrocarbons and efficient electron transfer.
- **Hydrocarbon concentration:** Periodic sampling and analysis will validate if a significant reduction in petroleum hydrocarbon levels occurs over the test period, demonstrating the system's capability to remediate the contaminated site. In general at about 2.5 mg hydrocarbons/L/day rate of degradation, the process needs considerable time to show an effect in highly contaminated groundwater plumes (Fig. 9).
- **Microbial community analysis:** DNA sequencing of samples from the electrodes shows an increase in populations of electroactive bacteria, confirming the successful establishment of a bioelectrochemical system.

These results collectively provide strong evidence of the BER technology's success in mitigating hydrocarbon pollution. At this site, with high residual levels of hydrocarbons, the goal was to enhance the natural attenuation. The difficulty for the consulting company is that while the plume is not getting larger, there was no evidence for the degradation. BER provides minimum quantitative degradation rates in each well.

The electricity production is the most direct and reliable measurement of hydrocarbon degradation. The wells that had the BER were compared with those that did not have the BER between two dates; May and July 2024. Due to the heterogeneity and temporal variation in hydrocarbon concentration, there was considerable variation. Two examples are shown here; one for the PAH pyrene (Fig. 10) and one for methyl tert-butyl ether (added to gasoline when lead was no longer used) – MTBE (Fig. 11).

## 5. APPLICATION OF THE TECHNOLOGY

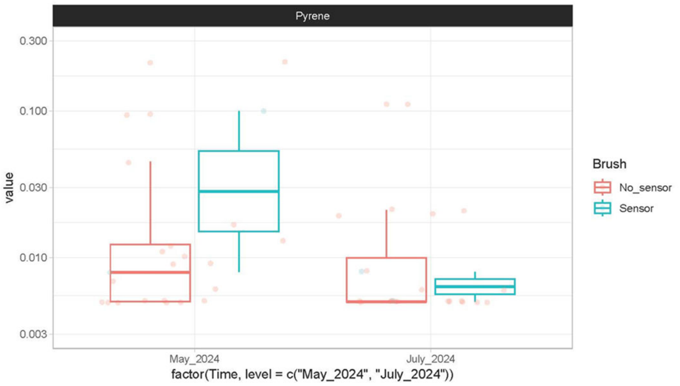

Figure 10. Comparison of the concentration (mg/L) of pyrene in monitoring wells with (green) and without (red) the BER in May and July 2024.

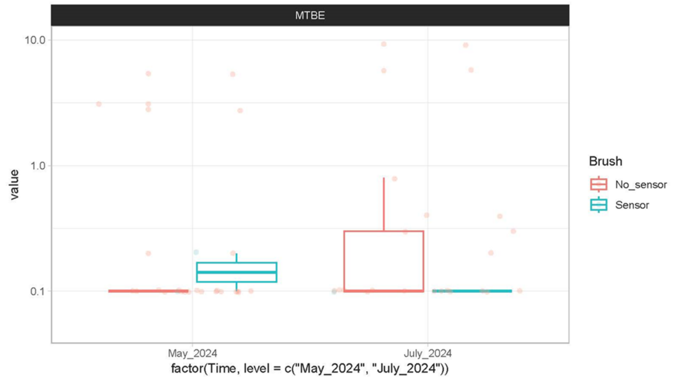

Figure 11. Comparison of the concentration (mg/L) of MTBE in monitoring wells with (green) and without (red) the BER in May and July 2024.

Some general considerations of applying the technology are provided below.

The process necessitates access to groundwater, which can be achieved through existing wells or by drilling new wells with diameters ranging from 30 to 100 mm. Electrodes must be installed below the water table and when appropriate in the screened section. Setting up each well takes about 30 minutes and requires the effort of two people. The necessary materials are easily transported and installed using basic tools, making the setup relatively straightforward.

The measurement system will be positioned at the wellhead and may need to be secured to ensure stable and accurate readings. Ensuring proper placement and stability of the measurement system is crucial for obtaining reliable data. These requirements are essential for the effective implementation of the BER process, particularly for groundwater treatment applications.

The BER process offers several potential advantages over traditional methods:

**Efficiency in low-oxygen environments:** The system functions efficiently without the need for high oxygen levels, making it highly effective in environments where oxygen is limited and aeration is challenging.
**Enhanced electron availability:** By increasing the availability of electron acceptors and enhancing electron flux, the system can achieve higher degradation rates, improving the efficiency of breaking down pollutants or other target substances.
**Minimization of undesirable by-products:** The process helps minimize the formation of undesirable by-products such as methane and sulfide, which enhances environmental safety and reduces potential hazards.
**Accessibility to difficult areas:** Capable of reaching and treating areas that are otherwise difficult to access, this technology offers a versatile solution for subsurface remediation and other applications where direct access is limited.
**Autonomous operation:** The system operates autonomously, reducing the need for continuous supervision by monitoring staff. This feature lowers labour costs and reduces the need for constant human oversight, making the technology more cost-effective and easier to manage.

There are no particular identified risks related to the installation or the operation of the system, aside from working on a polluted site. However, a potential hazard is the production of an electrical current of about 500 mVolts at 500 Ohms so for sites where electrical currents need to be controlled, some safety consideration should be assessed.

In terms of potential limitations of the technology, the following should be considered:

**Requirement for groundwater access:** The process necessitates access to groundwater, which requires either existing wells or the drilling of new wells with diameters ranging from 30 to 100 mm. This requirement can pose logistical challenges and increase initial setup costs.
**Relatively slow biodegradation rates:** The biodegradation rates in this process are relatively slow, which can extend the time required for effective treatment. This limitation means that remediation may take longer compared to some more aggressive methods.
**Need for multiple electrodes:** To ensure comprehensive coverage of the entire contaminated area, multiple electrodes must be placed. This requirement can complicate the setup, increase the overall complexity of the system, and raise the total cost of the process.
**Initial setup complexity:** The installation of multiple electrodes and the need for precise positioning below the water table add to the complexity of the initial setup. This can require more planning and expertise, potentially increasing the time and effort needed to deploy the system effectively.

There are several technology developments being investigated for future implementation. An automatic on-line real-time monitoring unit has been developed to track the performance of the process from remote locations. Different electrodes configurations have also been developed and will be implemented at future sites.

Two other pilot field tests are underway: one in the Netherlands and one in China.

## 6. CONCLUSIONS

The bioelectrochemical remediation system can be employed as an accelerated form of natural attenuation for polluted soils and groundwater. The performance depends on the microbial community established in the electrodes and the bioavailability of the pollutants. With no major risks, it can help to remediate groundwater with minimal input. While the minimal rates of hydrocarbon degradation shown here are not high in a field test (2.5 mg/L/day/well), this technology can actively aid natural attenuation and monitor the contaminant level in the groundwater. The lab studies determined the bacteria involved and provided a relationship between resistance, electrode separation distance and degradation rates. The further apart, the higher the resistance and the lower the degradation rate

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