Chapter 12
Bio-electrochemical Remediation of Petroleum Hydrocarbons

Anna Espinoza-Tofalos, Pablo Alviz-Gazitua, Andrea Franzetti, and Michael Seeger

Abstract Bioelectrochemistry and, more specifically, microbial electrochemistry are research fields that establish their fundaments on the molecular and electrochemical link between microbes (also known as exoelectrogens or, focusing only on bacteria, electrochemically active bacteria) and electrodes. Bioelectrochemistry can be used as a strategy in bioremediation when traditional bioremediation is not an option due to the lack of suitable electron acceptors, and in which bioelectrochemical systems (BESs) are used for the removal of pollutants from the environment. For example, in subsurface hydrocarbon-polluted water, the absence of final electron acceptors may limit the biodegradation rate. Therefore, bioelectrochemical systems can be used as a sustainable remediation technology. Moreover, microbial metabolism can be stimulated in a BES when overpotential is applied, increasing the rate of pollutant degradation. BES has been studied for the remediation at laboratory and pilot scale of water, soil, and sediments affected by organic pollutants, such as hydrocarbons (aliphatic, aromatic) and chlorinated compounds. In addition, BES can be exploited as biosensors to detect organic pollutants in environmental matrices and remote sites. One of the main challenges in this field is to scale up the technology towards the commercial BES remediation applications.

12.1 Introduction

12.1.1 Petroleum Hydrocarbons as Pollutants

During the last century, the world economy has been based on petroleum and its refined products, using it as the main manufacturing and energy source for industry and people (Varjani 2017). Due to a growing economy, during 2015, the increase of

A. Espinoza-Tofalos · A. Franzetti
Department of Earth and Environmental Sciences, University of Milano-Bicocca, Milan, Italy

P. Alviz-Gazitua · M. Seeger (✉)
Department of Chemistry & Center of Biotechnology, Universidad Técnica Federico Santa Maria, Valparaiso, Chile

P. Kumar, C. Kuppam (eds.), *Bioelectrochemical Systems*,
https://doi.org/10.1007/978-981-15-6868-8_12

oil requests in the world was 1.54 million barrels per day higher than the previous year, especially in non-OECD countries (OPEC 2015). Environmental petroleum release frequently occurs when oil is extracted or during the processes of refining, transportation, and storage (Okoh 2006; Das and Chandran 2011; Fuentes et al. 2014; Varjani 2017). Spills in marine environments constitute less than 10% of total hydrocarbon releases. Ninety percent of total discharge to the environment is represented by routine activities (Ivshina et al. 2015). A review on polluted areas in Europe identified around 1,170,000 possible contaminated sites (PCSs) and 127,000 contaminated sites of which around 45% have already been remediated (Panagos et al. 2013).

Benzene, toluene, ethylbenzene, and xylenes (BTEX), polycyclic aromatic hydrocarbons (PAHs), phenols, minerals, oil, and chlorinated hydrocarbons (CHC) are petroleum components or derivatives. The distribution of contaminants in groundwater shows two main classes of pollutants: hydrocarbons and heavy metals, where petroleum pollution contributes jointly to 54.4% of groundwater contamination (Fig. 12.1b) (Panagos et al. 2013). The discharge of these compounds into the environment is the principal reason for water and soil contamination (Holliger et al. 1997; Das and Chandran 2011). Even small oil spills into surface and subsurface waters can cause high concentrations of hydrocarbons that often overpass the limits dictated by the law (Spence et al. 2005).

The fate and distribution of hydrocarbons in the environment depend on several biotic (Acton and Barker 1992) and abiotic factors as physical processes related to weathering (Galt et al. 1991). It has been reported that petroleum components cause mutations and death of water and soil biota (Couillard et al. 2005) due to their high toxicity (Tang et al. 2011). Specific oil components have carcinogenic and neurotoxic properties, such as benzene, toluene, xylenes, naphthalene and *n*-hexane (Ritchie et al. 2010). Petroleum spills in water that prevents sunlight to pass through it affect not only the biota but also physical and chemical processes. Hydrocarbon-polluted waters, soils, and sediments should not be used for agriculture, urbanization, and as water source for people and animals. The removal of hydrocarbon components from the environment involves physical, chemical, and biological processes (Okoh 2006; Fuentes et al. 2014).

12.1.2 Remediation of Petroleum Hydrocarbon Contaminated Sites

The removal of pollutants from the environment is a requirement for sustainable development. Remediation technologies are applied in situ or ex situ. Physicochemical and biological processes have been applied to the clean-up of contaminated environments (Tyagi et al. 2011; Fuentes et al. 2014; Daghio et al. 2017). Physical strategies include extraction, thermal desorption, soil washing, and filtration techniques; while chemical treatments involve the addition of strong oxidant or reducing

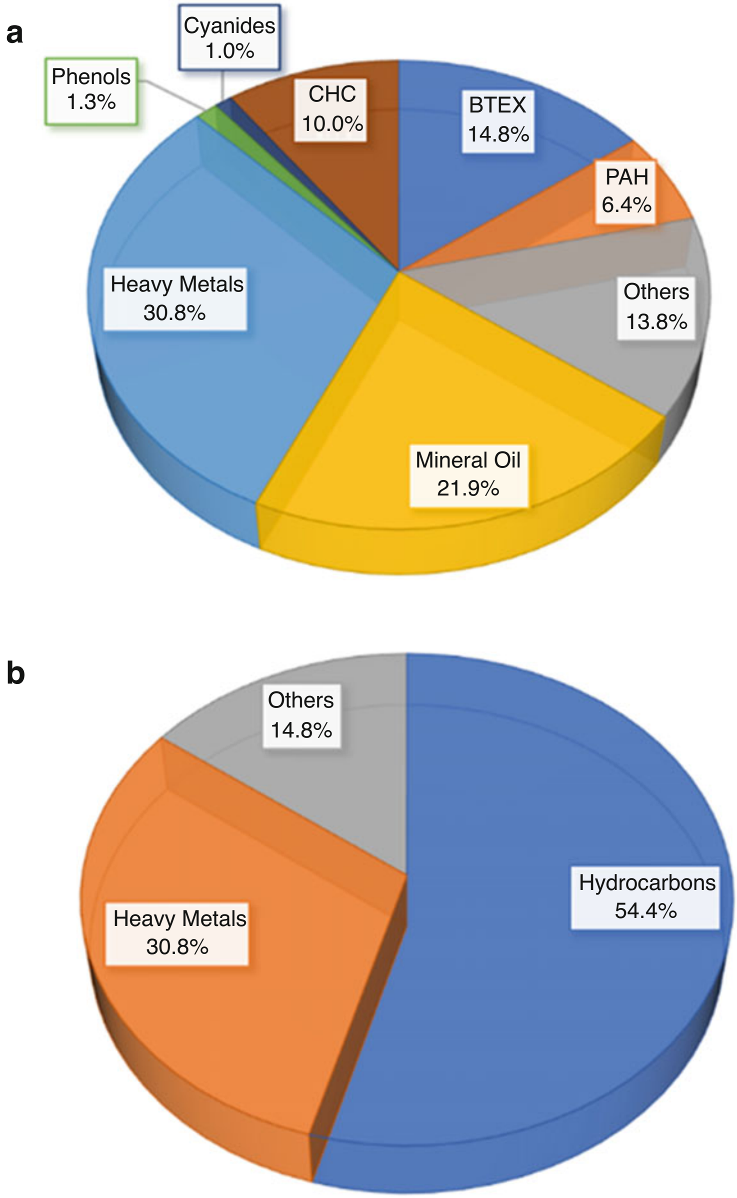

Fig. 12.1 General distribution of contaminants affecting soil and groundwater in Europe (**a**) and grouped by type of contaminants (**b**). Adapted from Panagos et al. (2013)

agents to lower the toxicity of the pollutants. Bioremediation is an attractive technology for the restoration of polluted waters and soils (Rojas et al. 2011; Fuentes et al. 2014; Orellana et al. 2018). Bioremediation is an efficient, cost-effective, and eco-friendly technique that relies on the microbial capabilities to metabolize the pollutants into harmless or less-toxic compounds, causing minimal ecological effects (Atlas 1995; Morgante et al. 2010; Saavedra et al. 2010; Méndez et al. 2017; Orellana et al. 2018; Durán et al. 2019). The most common strategies in bioremediation are biostimulation and bioaugmentation. Biostimulation consists in the stimulation of indigenous microorganisms with degradative capabilities through the addition of nutrients and/or electron acceptors. Bioaugmentation is the application of microorganisms that possess selective metabolic capabilities (Mrozik and Piotrowska-Seget 2010; Fuentes et al. 2014). To bioremediate hydrocarbon contaminated water, the most common biostimulation approaches are bioventing, water circulation systems, air sparging, and biobarriers. Bioventing is used mainly to stimulate aerobic degradation processes by pulling air above the watercourse. In water circulation systems, water is extracted and amended with electron acceptors and nutrients and back injected into groundwater. During air sparging, compressed air is injected, and oxygen is provided to enhance the natural aerobic microbial degradation of pollutants. Biobarriers involve a permeable and biologically active fence located perpendicularly to the plume, creating a zone of high microbial activity (Alvarez and Illman 2005). Microorganisms have been vastly used to bioremediate hydrocarbon-polluted environments (Tyagi et al. 2011), including soils (Rivelli et al. 2013; Fuentes et al. 2016), sediments (Militon et al. 2015), and water (Acton and Barker 1992; Farhadian et al. 2008). Pollutants can be used by microorganisms as carbon and energy sources, leading to their complete degradation (mineralization) or are converted through detoxification processes into harmless compounds (Rivelli et al. 2013). Microorganisms are the main biocatalysts for hydrocarbon bioremediation (Fuentes et al. 2014). Diverse microorganisms are capable to metabolize a wide range of hydrocarbons through evolved mechanisms that activate these compounds and generate metabolic intermediates that are funneled into central catabolic pathways (Méndez et al. 2011; Fuentes et al. 2014; Agulló et al. 2017; Durán et al. 2019; Espinoza-Tofalos et al. 2020).

12.2 BES for the Remediation of Hydrocarbons

Biological strategies for the remediation of environmental matrices have several advantages in comparison with physicochemical technologies. However, biological techniques may have also drawbacks. For example, in bioaugmentation the fate of added microorganisms is difficult to predict and in biostimulation, the addition of nutrients and electron acceptors might present some disadvantages (e.g., the formation of toxic intermediates, the elevated cost of continuously insufflating air). Moreover, when air is injected in soil or underground water, the probability that most volatile hydrocarbons will be stripped is high. Thus, to trap the volatile pollutants filters that may imply high cost should be used.

These limitations might be overcome by the application of bioelectrochemical systems (BESs) for the remediation of hydrocarbons from underground water, soils, and sediments (Table 12.1). Figure 12.2 illustrates a BES for hydrocarbon bioremediation. BES uses the redox gradient between a buried electrode and the hydrocarbons. Microorganisms that colonize the electrode surface oxidize these organic pollutants in absence of oxygen using the electrode as a non-exhaustible electron acceptor. Then, electrons are remotely transferred by the electrode to oxygen or other thermodynamically favorable electron acceptor (Lovley 2011; Morris and Jin 2012; Lu et al. 2014a, b).

BES-based technologies are advantageous compared with traditional bioremediation methods: (1) the electrode acts as an inexhaustible electron acceptor/donor and (2) the co-localization of pollutants, microbes and electron acceptor, enhance the removal of the contaminants (Lovley 2011; Wang et al. 2015).

Morris and Jin (2008) used a BES to couple hydrocarbons removal with electric power production. BESs have been applied to study the electrochemical-driven biodegradation of hydrocarbons in water (Morris et al. 2009; Franzetti et al. 2017; Espinoza-Tofalos et al. 2018; Palma et al. 2018a, b, 2019), soils (Wang et al. 2012, 2019), and sediments (Morris and Jin 2012; Cruz Viggi et al. 2015).

12.2.1 BES for the Remediation of Hydrocarbon-Polluted Water

The remediation of several classes of hydrocarbons has been studied in BES: single compounds (Rakoczy et al. 2013; Wei et al. 2015; Palma et al. 2018a, b), mixtures (Adelaja et al. 2017; Palma et al. 2019), and wastewater (Morris et al. 2009; Majumder et al. 2014; Srikanth et al. 2016; Daghio et al. 2017; Roustazadeh Sheikhyousefi et al. 2017; Mohanakrishna et al. 2018; Espinoza-Tofalos et al. 2020).

Benzene degradation by microbial communities has been studied in microbial fuel cells (MFC) and polarized BES. The limitations of these systems have been studied, providing special attention to the cathodic abiotic reaction. Oxygen is the most studied and used electron acceptor on the cathodic chamber (Rakoczy et al. 2013; Wei et al. 2015; Liu et al. 2018). However, also ferricyanide (Wu et al. 2013) and anoxic cathodes have been employed in BES for the removal of aromatic hydrocarbons (Daghio et al. 2018; Palma et al. 2018a, b, 2019). Air-cathodes in MFC configuration often lead to oxygen diffusion through the cation exchange membrane (Rabaey and Verstraete 2005; Morris et al. 2009; Adelaja et al. 2015). Oxygen diffusion from the cathodic chamber to the anodic one in BES for benzene removal has been reported by compound-specific isotope analysis, revealing that monohydroxylation is the benzene activation step (Rakoczy et al. 2013; Wei et al. 2015). To study the BES-based technology for in situ remediation, a mixed culture from a polluted site or refinery wastewater should be used as inoculum due to the high abundance of hydrocarbon-degrading microorganisms. A novel bioelectrochemical reactor configuration, “the bioelectric well,” revealed higher phenol removal when the bioelectrochemical reactor was re-inoculated with refinery

Table 12.1 BES-based technologies applied in hydrocarbon bioremediation

	Hydrocarbon source (ppm)	Environmental matrix	Microbial inoculum	BES system (L)	Poised potential (V)	Removal efficiency (%)		Study
						Closed circuit	Control/ open circuit	
Water	*n*-Alkanes and aromatics mixture (203.5)	Refinery groundwater	Native microbial community	Dual chamber MFC (0.01–246.5)		[a]		Morris and Jin (2008)
	Diesel (300)	Refinery groundwater	Native microbial community	Dual chamber MFC (0.9)		82	31	Morris et al. (2009)
	Benzene (11.7–19.5)	Sulfide contaminated groundwater	Native microbial community	Dual chamber MFC (1)		18–80		Rakoczy et al. (2013)
	Benzene (10.9–21.7)	Artificial polluted water	Oil cracking wastewater	Dual chamber MFC (1.3)		100		Wu et al. (2013)
	Toluene (69)	Artificial polluted water	Oil cracking wastewater	Dual chamber MFC (0.5)		100		Lu et al. (2014a, b)
	PAH (2213)	Refinery wastewater	*Pseudomonas putida* BCRC 1059	Dual chamber MFC (0.25)		30		Majumder et al. (2014)
	Phenanthrene and benzene (20–30)	Artificial polluted water	Sewage treatment anaerobic sludge	Dual chamber MFC (0.25)		92		Adelaja et al. (2015)
	Benzene (15)	Polluted groundwater	Native microbial community	Dual chamber MFC (0.32)		80		Wei et al. (2015)
	Aliphatics and phenol (60–90)	Refinery wastewater	Enriched culture	Dual chamber MFC (0.25)		81		Srikanth et al. (2016)
	Aliphatics (2000)	Refinery wastewater	Native microbial community	Single chamber MFC (0.125)		97		Roustazadeh Sheikhyousefi et al. (2017)

	Phenol (25)	Artificial polluted water	Refinery wastewater	Bioelectric well (0.25)	0.2	99.5		Palma et al. (2018b)
	BTEX mixture (8–20)	Artificial polluted water	Refinery wastewater	Polarized BES (0.12)	0.8	~100		Daghio et al. (2018)
	TPH (1090)	Refinery wastewater	Domestic wastewater	Single chamber MFC (0.35)	0.5	92	45	Mohanakrishna et al. (2018)
	Benzene (60)	Polluted groundwater	Petrochemical wastewater consortium	Tubular MFC (5.7)		100		Liu et al. (2018)
	Toluene (45)	Artificial polluted water	*Cupriavidus metallidurans* CH34	Dual chamber MFC (0.32)		87	10	Espinoza Tofalos et al. (2018)
	BTEX (25)	Artificial polluted water	Native microbial community	Bioelectric well (0.25)	0.2	94		Palma et al. (2019)
Sediments	Toluene (140)	Artificial polluted marine sediment	*Geobacter metallireducens* ATCC 53774 and DSM 7210	Dual chamber MFC (0.94)	0.3	~100	14–77	Zhang et al. (2010)
	TPH (16000)	Beach sediment	Native microbial community	Sediment MFC (0.05)		24	2	Morris and Jin (2012)
	IFO180 (11.9)	Artificial polluted marine sediment	Native microbial community	Oil spill snorkel (0.12)		80		Cruz-Varjani (2017)
	Toluene (~35)	Artificial polluted marine sediment	Refinery wastewater	Bioelectric well (0.25)	0.2	~100		Palma et al. (2018a)
	TPH (~14,000)	Marine sediment	Sandy marine sediments	Sediment MFC (1)	2	58–59	36–44	Bellagamba et al. (2017)
	PAH (50)	Artificial polluted sediment	Native microbial community	Sediment MFC[a]		31–36		Sherafatmand and Ng (2015)
	PAH (0.2–0.5)	Marine sediment	Native microbial community	Sediment MFC (0.9)		94		Hamdan et al. (2017)
	PAH (54–420)	River sediment	Native microbial community	Single chamber MFC (390)		74		Li et al. (2017)

(continued)

Table 12.1 (continued)

	Hydrocarbon source (ppm)	Environmental matrix	Microbial inoculum	BES system (L)	Poised potential (V)	Removal efficiency (%)		Study
						Closed circuit	Control/ open circuit	
	Toluene (40)	Artificial polluted marine sediment	Native microbial community	Polarized BES (0.25)	0.3 V	100		Daghio et al. (2016)
Soils	Phenol (80)	Hydrocarbon contaminated soil	Native microbial community	Soil MFC (0.25)		90		Huang et al. (2011)
	TPH (28,300)	Hydrocarbon contaminated soil	Native microbial community	U tube MFC (2.736)		a		Xin et al. (2012)
	TPH (114,600)	Hydrocarbon contaminated soil	Native microbial community	Soil MFC (3)		48–79	38–45	Lu et al. (2014a)
	TPH (12,250)	Hydrocarbon contaminated soil	Native microbial community	Soil MFC (50)		82–90	68	Lu et al. (2014b)
	PAH (82–103)	Hydrocarbon contaminated soil	Native microbial community	Soil MFC[a]		27–54	24–34	Yu et al. (2017)
	n-Alkanes (23–48,207)	Hydrocarbon contaminated soil	Native microbial community	Soil MFC (0.324)		13		Zhang et al. (2014)

BTEX benzene, toluene, ethylbenzene, xylenes; *PAH* polyaromatic hydrocarbons, *IFO180* intermediate fuel oil, *TPH* total petroleum hydrocarbons
[a]Data non-reported

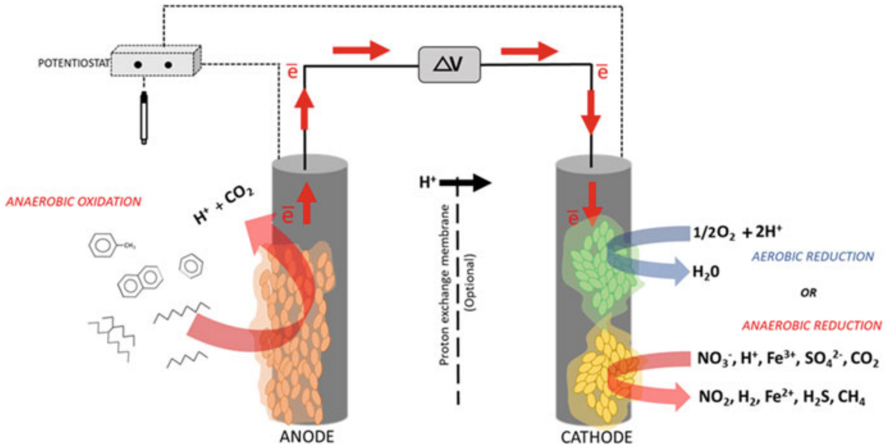

Fig. 12.2 General scheme of BES for petroleum hydrocarbons remediation. BES remediation of hydrocarbons uses the redox gradient between electrodes. Under anaerobic conditions, electroactive microorganisms use hydrocarbons as electron donors towards an anode that acts as a virtually inexhaustible electron acceptor. In ex situ systems, electroneutrality is maintained by ions transport through an ion-permeable medium or membrane. Electrons travel via an external circuit to the cathode, where they are finally transferred to a suitable electron acceptor. Electricity can be an output product of this process. In addition microbial metabolism may be stimulated by applying an external overpotential through a power source (two electrodes configuration; a voltage difference is applied between electrodes) or with a potentiostat (three electrodes configuration illustrated in this figure; a selected voltage may be imposed on the working electrode)

wastewater compared with municipal activated sludge inoculation (Palma et al. 2018b). In this study, the anode was potentiostatically set at +0.2 V versus SHE and the cathode was maintained anoxic. The application of an external voltage may be advantageous because it stimulates microbial metabolism (Wagner et al. 2010). This is related to the fact that the main factor that determines the optimal metabolic conditions in a reactor is the potential of the terminal respiratory proteins used by exoelectrogenic bacteria (Wagner et al. 2010). Therefore, the optimal imposed voltage should be tested depending on the inoculum and the type of pollutants. Two and three electrodes configurations have been studied. When the anode was potentiostatically polarized, applied voltages ranged between +200 mV and +500 mV in studies for the removal of toluene, phenol, and BTEX (Zhang et al. 2010; Daghio et al. 2016; Palma et al. 2018a, b). However, microbial metabolism can be stimulated also by applying a voltage difference between anode and cathode (two electrodes configuration). This set-up presents the advantage to require less sophisticated instrumentation (especially if an in situ application is required), because just a power supply is needed but not a potentiostat. However, the disadvantage is that the working potential is no longer controlled and varies depending on the redox conditions of the medium.

12.2.2 BES for the Remediation of Hydrocarbon-Polluted Sediments

Sediments are environmental matrixes particularly suitable to be treated with microbial electrochemical technologies for two main reasons: (1) sediments are anoxic, thus optimal for bioelectrochemical oxidation on the anode surface, and (2) sediments are water-saturated, hence electrolytic conditions are guaranteed (especially marine sediments). Due to the favorable conditions for their development, sediment microbial fuel cells (SMFC) have been studied for the degradation of hydrocarbons.

Different configurations have been tested, from the double chamber where processes can be well controlled (Zhang et al. 2010; Daghio et al. 2016; Bellagamba et al. 2017), to the single chamber that reproduces better an in situ application (Morris and Jin 2012; Cruz Viggi et al. 2015; Sherafatmand and Ng 2015; Hamdan et al. 2017; Li et al. 2017; Palma et al. 2018b). In single chamber SMFC, the anode is buried into the sediment and the cathode can be placed on the overlying aerobic water or completely submerged (which does not guarantee oxic reactions on the cathode surface). In studies with aerated cathodes, phenanthrene removal reached 89% (Hamdan et al. 2017), whereas PAHs (including benzo(a)pyrene, benzo(k)-fluoranthene, and benzo(a)fluoranthene) were efficiently removed up to 94% (Li et al. 2017). Interestingly, in a study that compared aerated vs anoxic cathodes, SMFCs achieved 42% naphthalene, 31% acenaphthene, and 36% phenanthrene removal when an aerobic cathode was operated, and 77%, 53%, and 37% removal, respectively, when the cathode was placed under anaerobic conditions (Sherafatmand and Ng 2015). This demonstrates that both configurations can be used, depending on the overlying water oxygen concentrations and/or operational requirements. An innovative set-up, the so-called "oil-spill snorkel" simplifies the system set-up, by burring part of a single conductive material (the snorkel) in the sediment (that acted as an anode) and leaving the other half on the overlying O_2-containing water (oxic zone) (Cruz Viggi et al. 2015). Even if this design showed lower performances than other similar studies (21% TPH removal within 22 days), it is an inexpensive and simple alternative for the removal of hydrocarbons from sediments.

12.2.3 BES for the Remediation of Hydrocarbon-Polluted Soils

The remediation of hydrocarbon-polluted soils with BES-based technologies has not been extensively investigated. However, since Huang et al. (2011) proposed this technology to remediate phenol-contaminated soil in a MFC, the use of this technology has found a new field of application.

Soils polluted with phenol (Huang et al. 2011) but mainly soils contaminated by petroleum hydrocarbon were studied in soil MFC (Xin et al. 2012; Lu et al. 2014a, b;

Zhang et al. 2014; Yu et al. 2017; Wang et al. 2019). Unlike BES technologies for water remediation, soil studies have been focused in MFC-based designs that stimulate the microbial metabolism without external polarization, by using potentiostatically controlled buried anodes with air-cathodes, the so-called soil microbial fuel cells. Water content (Xin et al. 2012; Wang et al. 2019), the distance between anodes (Lu et al. 2014a, b; Yu et al. 2017), or both factors (Xin et al. 2012; Wang et al. 2019) are the most studied variables, but also reactor design (e.g., U-shape) (Xin et al. 2012), electrodes arrangement (horizontal or vertical) (Zhang et al. 2014), electrodes materials (Lu et al. 2014a), and soil texture (Lu et al. 2014a; Wang et al. 2019).

Water content is indeed a key parameter for the successful remediation of hydrocarbon-polluted soils. High water contents (possibly up to saturation) are needed to favor mass transport phenomena and to lower the internal resistance (Xin et al. 2012). In a study with saturated vs unsaturated conditions, at the end of the experiment (248 days) a maximum of 59% and 45% TPH were removed in saturated sandy and clay soils, respectively, which was approximately 48% (sandy soil) and 55% (clay soil) higher than under unsaturated conditions.

Most studies indicate that the radius of influence (ROI) is a key factor and that TPH removal rates decrease with the distance from the anode (Yu et al. 2017) due to less microbial electrochemical activities and mass transfer phenomena. However Lu et al. (2014a) concluded that the TPH degradation rates in BESs were higher than those in control reactors operated at open circuits, suggesting that bioelectrochemical stimulation had a positive influence on the pollutants removal, even at a certain distance from the electrodes. In any case, water content and distance from electrodes are highly linked. Wang et al. (2019) reported that TPH removal was not enhanced when measured 35 cm far from the anode (in comparison with open circuit controls) when the soil was unsaturated. However, under saturation conditions, at 35 cm of distance, an increase in toluene removal (11%) was observed in saturated sandy soils. Interestingly, saturated soils may inhibit classical aerobic bioremediation of hydrocarbons but enhance bioelectrochemical bioremediation. In a report focused on the study of ROI, the authors concluded that the TPH degradation rate was highly dependent on the radius of influence during the first samplings (days 5 and 15) but became a less significant variable with longer incubation times. On day 120, a maximum of 90% (68% in control) of TPH was removed from soil, and the soil TPH fraction was independent on the distance from the anodes (Lu et al. 2014b). By correlating the amount of TPH degradation and the radial distance from the BES anodes, it was possible to predict the ROI after a specific time of treatment. However, the ROIs may be influenced by some soil characteristics such as water content, matrix permeability, and porosity, besides the type of pollutant.

The structure of microbial communities is indeed influenced by the use of an electrode as an electron acceptor. Lu et al. (2014a) reported that *Proteobacteria* was the most abundant phylum in the polluted soils treated with MFC technology. However, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Acidobacteria* were also observed. Differences in composition of bacterial communities were observed in non-contaminated (mainly *Proteobacteria* and *Actinobacteria*) and hydrocarbon-contaminated soils (higher levels of *Proteobacteria*). *Proteobacteria* increased by conventional and BES bioremediation. Interestingly, an increase of *Firmicutes* was observed specifically in BES hydrocarbon remediation. Mainly *Proteobacteria* were observed at the carbon cloth anode, whereas *Firmicutes* showed an increase in the biochar anode. Wang et al. (2019) reported that the dominant genus on the bioanodes was *Geobacter* (~27%), which is a model electroactive and hydrocarbon-degrading bacterium.

12.3 Challenges

Remediation using BES has been studied at laboratory and pilot scale. One of the main challenges is to scale up remediation technology using BES towards commercial applications. To improve the remediation efficiency by BES, critical physico-chemical parameters should be determined and modulated, and the radius of influence of electrodes in matrices should be increased. Proteobacteria and specifically *Geobacter* genus have been associated with BES. Nevertheless, most of the electrode organisms in BES are unknown and have not yet been cultivated. Therefore, the microbial communities involved in BES remediation processes should be further characterized. Next-generation sequencing technologies and metagenomic approaches will be useful to determine the main microbial players in BES involved in the removal of the petroleum hydrocarbons in different matrixes, and the mechanisms involved in the degradation and extracellular electron transfer. Main microbes should be cultivated, and their metabolism characterized. These studies will be useful to increase the knowledge of the process for the design of improved remediation processes using BES towards knowledge-driven engineering for commercial applications.

12.4 Conclusions and Future Perspectives

BES has been applied for the remediation of several classes of hydrocarbons from water, soil, and sediments in different polluted scenarios. Degradation of hydrocarbons by microbial communities has been studied in MFC and polarized BES. Oxygen is the most used electron acceptor on the cathodic chamber, but also anoxic cathodes have been employed in BES for hydrocarbon bioremediation. The application of an external voltage through polarization by a potentiostat of the anode (from +200 to +500 mV vs SHE) could stimulate microbial degradation of diverse hydrocarbons, but other voltages can be studied depending on the characteristics of the matrix including the microbial community. MFC in different configurations (double and single chambers) have been used for the degradation of hydrocarbons in sediments. In addition, few studies reported bioelectrochemical treatment of polluted soils. The saturation of soil with water is critical for successful bioelectrochemical remediation of hydrocarbon-polluted soils. The microbial communities are key players for BES bioremediation, but the degrading microorganisms are largely unknown. The main challenge of remediation using BES is to scale up the process for commercial applications and in situ bioremediation. BES remediation processes represent an attractive alternative to develop a robust and sustainable technology for the clean-up of petroleum hydrocarbon-polluted waters, sediments, and soils for a circular economy.

Acknowledgements MS and PAG acknowledge financial support by Fondecyt 1200756 and ANID PIA Genomics and Applied Microbiology for Bioremediation and Bioproducts (GAMBIO) Ring, ACT172128 Chile grant.

References

Acton, D. W., & Barker, J. F. (1992). In situ biodegradation potential of aromatic hydrocarbons in anaerobic groundwaters. *Journal of Contaminant Hydrology, 9*, 325–352.

Adelaja, O., Keshavarz, T., & Kyazze, G. (2015). The effect of salinity, redox mediators and temperature on anaerobic biodegradation of petroleum hydrocarbons in microbial fuel cells. *The Journal of Hazardous Materials, 283*, 211–217.

Adelaja, O., Keshavarz, T., & Kyazze, G. (2017). Treatment of phenanthrene and benzene using microbial fuel cells operated continuously for possible in situ and ex situ applications. *International Biodeterioration and Biodegradation, 116*, 91–103. https://doi.org/10.1016/j.ibiod.2016.10.021

Agulló, L., Romero-Silva, M. J., Domenech, M., & Seeger, M. (2017). *p*-Cymene promotes its catabolism through the *p*-cymene and the *p*-cumate pathways, activates a stress response and reduces the biofilm formation in *Burkholderia xenovorans* LB400. *PLoS ONE, 12*, e0169544. https://doi.org/10.1371/journal.pone.0169544

Alvarez, P. J. J., & Illman, W. A. (2005). *Bioremediation and natural attenuation: Process fundamentals and mathematical models*. Hoboken: Wiley.

Atlas, R. M. (1995). Bioremediation of petroleum pollutants. *International Biodeterioration & Biodegradation, 35*, 335–336. https://doi.org/10.1016/0964-8305(95)90041-1

Bellagamba, M., Cruz Viggi, C., Ademollo, N., Rossetti, S., & Aulenta, F. (2017). Electrolysis-driven bioremediation of crude oil-contaminated marine sediments. *New Biotechnology, 38*, 84–90. https://doi.org/10.1016/j.nbt.2016.03.003

Couillard, C. M., Lee, K., Légaré, B., & King, T. L. (2005). Effect of dispersant on the composition of the water-accommodated fraction of crude oil and its toxicity to larval marine fish. *Environmental Toxicology and Chemistry, 24*, 1496–1504. https://doi.org/10.1897/04-267r.1

Cruz Viggi, C., Presta, E., Bellagamba, M., Balijepalli, S. K., Zanaroli, G., Papini, M. P., Rossetti, S., & Aulenta, F. (2015). The "Oil-Spill Snorkel": An innovative bioelectrochemical approach to accelerate hydrocarbons biodegradation in marine sediments. *Frontiers in Microbiology, 6*, 1–11. https://doi.org/10.3389/fmicb.2015.00881

Daghio, M., Aulenta, F., Vaiopoulou, E., Franzetti, A., Head, I. M., Arends, J. B. A., Sherry, A., Su, A., Bestetti, G., & Rabaey, K. (2017). Electrobioremediation of oil spills. *Water Research, 114*, 351–370. https://doi.org/10.1016/j.watres.2017.02.030

Daghio, M., Espinoza Tofalos, A., Leoni, B., Cristiani, P., Papacchini, M., Jalilnejad, E., Bestetti, G., & Franzetti, A. (2018). Bioelectrochemical BTEX removal at different voltages: Assessment of the degradation and characterization of the microbial communities. *Journal of Hazardous Materials, 341*, 120–127. https://doi.org/10.1016/j.jhazmat.2017.07.054

Daghio, M., Vaiopoulou, E., Patil, S. A., Suárez-Suárez, A., Head, I. M., Franzetti, A., & Rabaey, K. (2016). Anodes stimulate anaerobic toluene degradation via sulfur cycling in marine sediments. *Applied and Environmental Microbiology, 82*, 297–307. https://doi.org/10.1128/AEM.02250-15

Das, N., & Chandran, P. (2011). Microbial degradation of petroleum hydrocarbon contaminants: An overview. *Biotechnology Research International, 2011*, 941810. https://doi.org/10.4061/2011/941810

Durán, R. E., Méndez, V., Rodríguez-Castro, L., Barra-Sanhueza, B., Salvà-Serra F., Moore, E. R. B., Castro-Nallar, E., & Seeger, M. (2019). Genomic and physiological traits of the marine bacterium *Alcaligenes aquatilis* QD168 isolated from Quintero Bay, Central Chile, reveal a robust adaptive response to environmental stressors. *Frontiers in Microbiology, 10*, 528. https://doi.org/10.3389/fmicb.2019.00528

Espinoza-Tofalos, A., Daghio, M., Gonzalez, M. M., Papacchini, M., Franzetti, A., & Seeger, M. (2018). Toluene degradation by Cupriavidus metallidurans CH34 in nitrate-reducing conditions and in Bioelectrochemical Systems. *FEMS Microbiology Letters, 365*, 1–8. https://doi.org/10.1093/femsle/fny119

Espinoza-Tofalos, A., Daghio, M., Palma, E., Aulenta, F., & Franzetti, A. (2020). Structure and functions of hydrocarbon-degrading microbial communities in Bioelectrochemical systems. *Watermark, 12*, 20343. https://doi.org/10.3390/w12020343

Farhadian, M., Vachelard, C., Duchez, D., & Larroche, C. (2008). In situ bioremediation of monoaromatic pollutants in groundwater: A review. *Bioresource Technology, 99*, 5296–5308. https://doi.org/10.1016/J.BIORTECH.2007.10.025

Franzetti, A., Daghio, M., Parenti, P., Truppi, T., Bestetti, G., Trasatti, S. P., & Cristiani, P. (2017). Monod kinetics degradation of low concentration residual organics in membraneless microbial fuel cells. *Journal of the Electrochemical Society, 164*, 3091–3096. https://doi.org/10.1149/2.0141703jes

Fuentes, S., Barra, B., Caporaso, J. G., & Seeger, M. (2016). From rare to dominant: A fine-tuned soil bacterial bloom during petroleum hydrocarbon bioremediation. *Applied and Environmental Microbiology, 82*, 888–896. https://doi.org/10.1128/AEM.02625-15

Fuentes, S., Méndez, V., Aguila, P., & Seeger, M. (2014). Bioremediation of petroleum hydrocarbons: Catabolic genes, microbial communities, and applications. *Applied Microbiology and Biotechnology*. https://doi.org/10.1007/s00253-014-5684-9

Galt, J., Lehr, W. J., & Payton, D. L. (1991). Fate and transport of the Exxon Valdez oil spill. *Environmental Science & Technology, 25*, 202–209.

Hamdan, H. Z., Salam, D. A., Hari, A. R., Semerjian, L., & Saikaly, P. (2017). Assessment of the performance of SMFCs in the bioremediation of PAHs in contaminated marine sediments under different redox conditions and analysis of the associated microbial communities. *Science of the Total Environment, 575*, 1453–1461. https://doi.org/10.1016/j.scitotenv.2016.09.232

Holliger, C., Gaspard, S., Glod, G., Heijman, C., Schumacher, W., Schwarzenbach, R. P., & Vazquez, F. (1997). Contaminated environments in the subsurface and bioremediation: Organic contaminants. *FEMS Microbiology Reviews, 20*, 517–523.

Huang, D. Y., Zhou, S. G., Chen, Q., Zhao, B., Yuan, Y., & Zhuang, L. (2011). Enhanced anaerobic degradation of organic pollutants in a soil microbial fuel cell. *Chemical Engineering Journal, 172*, 647–653. https://doi.org/10.1016/j.cej.2011.06.024

Ivshina, I. B., Kuyukina, M. S., Krivoruchko, A. V., Elkin, A. A., Makarov, S. O., Cunningham, C. J., Peshkur, T. A., & Atlas, M. (2015). Environmental science processes & impacts oil spill problems and sustainable response strategies through new technologies. *Environmental Science. Processes & Impacts, 33*, 1201–1219. https://doi.org/10.1039/C5EM00070J

Li, H., He, W., Qu, Y., Li, C., Tian, Y., & Feng, Y. (2017). Pilot-scale benthic microbial electrochemical system (BMES) for the bioremediation of polluted river sediment. *Journal of Power Sources, 356*, 430–437. https://doi.org/10.1016/j.jpowsour.2017.03.066

Liu, S. H., Lai, C. Y., Ye, J. W., & Lin, C. W. (2018). Increasing removal of benzene from groundwater using stacked tubular air-cathode microbial fuel cells. *Journal of Cleaner Production, 194*, 78–84. https://doi.org/10.1016/j.jclepro.2018.05.138

Lovley, D. R. (2011). Live wires: direct extracellular electron exchange for bioenergy and the bioremediation of energy-related contamination. *Energy & Environmental Science, 4*, 4896. https://doi.org/10.1039/c1ee02229f

Lu, L., Huggins, T., Jin, S., Zuo, Y., & Ren, Z. J. (2014a). Microbial metabolism and community structure in response to bioelectrochemically enhanced remediation of petroleum hydrocarbon-contaminated soil. *Environmental Science & Technology, 48*, 4021–4029. https://doi.org/10.1021/es4057906

Lu, L., Yazdi, H., Jin, S., Zuo, Y., Fallgren, P. H., & Ren, Z. J. (2014b). Enhanced bioremediation of hydrocarbon-contaminated soil using pilot-scale bioelectrochemical systems. *Journal of Hazardous Materials, 274*, 8–15. https://doi.org/10.1016/j.jhazmat.2014.03.060

Majumder, D., Maity, J. P., Tseng, M. J., Nimje, V. R., Chen, H. R., Chen, C. C., Chang, Y. F., Yang, T. C., & Chen, C. Y. (2014). Electricity generation and wastewater treatment of oil refinery in microbial fuel cells using *Pseudomonas putida*. *International Journal of Molecular Sciences, 15*, 16772–16786. https://doi.org/10.3390/ijms150916772

Méndez, V., Agulló, L., González, M., & Seeger, M. (2011). The homogentisate and homoprotocatechuate central pathways are involved in 3- and 4-hydroxyphenylacetate degradation by *Burkholderia xenovorans* LB400. *PLoS ONE, 6*, e17583. https://doi.org/10.1371/journal.pone.0017583

Méndez, V., Fuentes, S., Morgante, V., Hernández, M., González, M., Moore, E., & Seeger, M. (2017). Novel hydrocarbon-degrading and heavy metal-resistant *Acinetobacter*, *Kocuria* and *Pseudomonas* strains isolated from a crude oil polluted soil in Central Chile. *Journal of Soil Science and Plant Nutrition, 17*, 1074–1087. https://doi.org/10.4067/S0718-95162017000400017

Militon, C., Jézéquel, R., Gilbert, F., Corsellis, Y., Sylvi, L., Cravo-Laureau, C., Duran, R., & Cuny, P. (2015). Dynamics of bacterial assemblages and removal of polycyclic aromatic hydrocarbons in oil-contaminated coastal marine sediments subjected to contrasted oxygen regimes. *Environmental Science and Pollution Research, 22*, 15260–15272. https://doi.org/10.1007/s11356-015-4510-y

Mohanakrishna, G., Abu-Reesh, I. M., Kondaveeti, S., Al-Raoush, R. I., & He, Z. (2018). Enhanced treatment of petroleum refinery wastewater by short-term applied voltage in single chamber microbial fuel cell. *Bioresource Technology, 253*, 16–21. https://doi.org/10.1016/j.biortech.2018.01.005

Morgante, V., López-López, A., Flores, C., González, M., González, B., Vásquez, M., Rosselló-Mora, R., & Seeger, M. (2010). Bioaugmentation with *Pseudomonas* sp. strain MHP41 promotes simazine attenuation and bacterial community changes in agricultural soils. *FEMS Microbiology Ecology, 71*, 114–126. https://doi.org/10.1111/j.1574-6941.2009.00790.x

Morris, J. M., & Jin, S. (2008). Feasibility of using microbial fuel cell technology in bioremediation of hydrocarbons in groundwater. *Journal of Environmental Science and Health, Part A: Environmental Science and Engineering, 43*, 18–23.

Morris, J. M., & Jin, S. (2012). Enhanced biodegradation of hydrocarbon-contaminated sediments using microbial fuel cells. *Journal of Hazardous Materials, 213–214*, 474–477. https://doi.org/10.1016/j.jhazmat.2012.02.029

Morris, J. M., Jin, S., Crimi, B., & Pruden, A. (2009). Microbial fuel cell in enhancing anaerobic biodegradation of diesel. *Chemical Engineering Journal, 146*, 161–167. https://doi.org/10.1016/j.cej.2008.05.028

Mrozik, A., & Piotrowska-Seget, Z. (2010). Bioaugmentation as a strategy for cleaning up of soils contaminated with aromatic compounds. *Microbiological Research, 165*, 363–375. https://doi.org/10.1016/j.micres.2009.08.001

Okoh, A. I. (2006). Biodegradation alternative in the cleanup of petroleum hydrocarbon pollutants. *Biotechnology and Molecular Biology Reviews, 1*, 38–50.

OPEC. (2015). *Annual report 2015*. Vienna: OPEC.

Orellana, R., Macaya, C., Bravo, G., Dorochesil, F., Cumsille, A., Valencia, R., Rojas, C., & Seeger, M. (2018). Living at the frontiers of life: extremophiles in Chile and their potential for bioremediation. *Frontiers in Microbiology, 9*, 2309. https://doi.org/10.3389/fmicb.2018.02309

Palma, E., Daghio, M., Espinoza Tofalos, A., Franzetti, A., Cruz Viggi, C., Fazi, S., Petrangeli Papini, M., & Aulenta, F. (2018a). Anaerobic electrogenic oxidation of toluene in a continuous-flow bioelectrochemical reactor: process performance, microbial community analysis, and biodegradation pathways. *Environmental Science: Water Research & Technology, 2018*, 2136–2145. https://doi.org/10.1039/C8EW00666K

Palma, E., Daghio, M., Franzetti, A., Petrangeli Papini, M., & Aulenta, F. (2018b). The bioelectric well: A novel approach for in situ treatment of hydrocarbon-contaminated groundwater. *Microbial Biotechnology, 11*, 112–118. https://doi.org/10.1111/1751-7915.12760

Palma, E., Espinoza Tofalos, A., Daghio, M., Franzetti, A., Tsiota, P., Cruz Viggi, C., Papini, M. P., & Aulenta, F. (2019). Bioelectrochemical treatment of groundwater containing BTEX in a continuous-flow system: Substrate interactions, microbial community analysis, and impact of sulfate as a co-contaminant. *New Biotechnology, 53*, 41–48. https://doi.org/10.1016/j.nbt.2019.06.004

Panagos, P., Van Liedekerke, M., Yigini, Y., & Montanarella, L. (2013). Contaminated sites in Europe: Review of the current situation based on data collected through a European network. *Journal of Environmental and Public Health, 2013*, 158764.

Rabaey, K., & Verstraete, W. (2005). Microbial fuel cells: Novel biotechnology for energy generation. *Trends in Biotechnology, 23*, 291–298. https://doi.org/10.1016/j.tibtech.2005.04.008

Rakoczy, J., Feisthauer, S., Wasmund, K., Bombach, P., Neu, T. R., Vogt, C., & Richnow, H. H. (2013). Benzene and sulfide removal from groundwater treated in a microbial fuel cell. *Biotechnology and Bioengineering, 110*, 3104–3113. https://doi.org/10.1002/bit.24979

Ritchie, G. D., Still, K. R., Alexander, W. K., Nordholm, A. F., Wilson, C. L., Rossi III, J., & Mattie, D. R. (2010). A review of the neurotoxicity risk of selected hydrocarbon fuels. *Journal of Toxicology and Environmental Health. Part B, Critical Reviews, 4*, 223–312. https://doi.org/10.1080/10937400118874

Rivelli, V., Franzetti, A., Gandolfi, I., Cordoni, S., & Bestetti, G. (2013). Persistence and degrading activity of free and immobilised allochthonous bacteria during bioremediation of hydrocarbon-contaminated soils. *Biodegradation, 24*, 1–11. https://doi.org/10.1007/s10532-012-9553-x

Rojas, L. A, Yáñez, C., González, M., Lobos, S., Smalla, K., & Seeger, M. (2011). Characterization of the metabolically modified heavy metal-resistant *Cupriavidus metallidurans* strain MSR33 generated for mercury bioremediation. *PLoS One, 6*, e17555. https://doi.org/10.1371/journal.pone.0017555

Roustazadeh Sheikhyousefi, P., Nasr Esfahany, M., Colombo, A., Franzetti, A., Trasatti, S. P., & Cristiani, P. (2017). Investigation of different configurations of microbial fuel cells for the treatment of oilfield produced water. *Applied Energy, 192*, 457–465. https://doi.org/10.1016/j.apenergy.2016.10.057

Saavedra, M., Acevedo, F., González, M., & Seeger, M. (2010). Mineralization of PCBs by the genetically modified strain *Cupriavidus necator* JMS34 and its application for bioremediation of PCBs in soil. *Applied Microbiology and Biotechnology, 87*, 1543–1554. https://doi.org/10.1007/s00253-010-2575-6

Sherafatmand, M., & Ng, H. Y. (2015). Using sediment microbial fuel cells (SMFCs) for bioremediation of polycyclic aromatic hydrocarbons (PAHs). *Bioresource Technology, 195*, 122–132. https://doi.org/10.1016/j.biortech.2015.06.002

Spence, M. J., Bottrell, S. H., Thornton, S. F., Richnow, H. H., & Spence, K. H. (2005). Hydrochemical and isotopic effects associated with petroleum fuel biodegradation pathways in a chalk aquifer. *Journal of Contaminant Hydrology, 79*, 67–88. https://doi.org/10.1016/j.jconhyd.2005.06.003

Srikanth, S., Kumar, M., Singh, D., Singh, M. P., & Das, B. P. (2016). Electro-biocatalytic treatment of petroleum refinery wastewater using microbial fuel cell (MFC) in continuous mode operation. *Bioresource Technology, 221*, 70–77. https://doi.org/10.1016/j.biortech.2016.09.034

Tang, J., Wang, M., Wang, F., Sun, Q., & Zhou, Q. (2011). Eco-toxicity of petroleum hydrocarbon contaminated soil. *Journal of Environmental Sciences, 23*, 845–851. https://doi.org/10.1016/S1001-0742(10)60517-7

Tyagi, M., da Fonseca, M. M. R., & de Carvalho, C. C. R. (2011). Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes. *Biodegradation, 22*, 231–241. https://doi.org/10.1007/s10532-010-9394-4

Varjani, S. J. (2017). Microbial degradation of petroleum hydrocarbons. *Bioresource Technology, 223*, 277–286. https://doi.org/10.1016/j.biortech.2016.10.037

Wagner, R. C., Call, D. F., & Logan, B. E. (2010). Optimal set anode potentials vary in bioelectrochemical systems. *Environmental Science & Technology, 44*, 6036–6041. https://doi.org/10.1021/es101013e

Wang, Huan, Lu, L., Mao, D., Huang, Z., Cui, Y., Jin, S., Zuo, Y., & Ren, Z. J. (2019). Dominance of electroactive microbiomes in bioelectrochemical remediation of hydrocarbon-contaminated soils with different textures. *Chemosphere, 235*, 776–784. https://doi.org/10.1016/j.chemosphere.2019.06.229

Wang, H., Luo, H., Fallgren, P. H., Jin, S., & Ren, Z. J. (2015). Bioelectrochemical system platform for sustainable environmental remediation and energy generation. *Biotechnology Advances, 33*, 317–334. https://doi.org/10.1016/j.biotechadv.2015.04.003

Wang, X., Cai, Z., Zhou, Q., Zhang, Z., & Chen, C. (2012). Bioelectrochemical stimulation of petroleum hydrocarbon degradation in saline soil using U-tube microbial fuel cells. *Biotechnology and Bioengineering, 109*, 426–433. https://doi.org/10.1002/bit.23351

Wei, M., Harnish, F., Vogt, C., Ahlheim, J., Neu, T. R., & Richnow, H. H. (2015). Harvesting electricity from benzene and ammonium-contaminated groundwater using a microbial fuel cell with an aerated cathode. *Royal Society of Chemistry, 5*, 5321–5330. https://doi.org/10.1039/C4RA12144A

Wu, C. H., Lai, C. Y., Lin, C. W., & Kao, M. H. (2013). Generation of power by microbial fuel cell with ferricyanide in biodegradation of benzene. *Clean: Soil, Air, Water, 41*, 390–395. https://doi.org/10.1002/clen.201200198

Xin, W., Zhang, C., Qixing, Z., Zhineng, Z., & Cuihong, C. (2012). Bioelectrochemical stimulation of petroleum hydrocarbon degradation in saline soil using U-tube microbial fuel cells. *Biotechnology and Bioengineering, 109*, 426–433.

Yu, B., Tian, J., & Feng, L. (2017). Remediation of PAH polluted soils using a soil microbial fuel cell: Influence of electrode interval and role of microbial community. *Journal of Hazardous Materials, 336*, 110–118. https://doi.org/10.1016/j.jhazmat.2017.04.066

Zhang, T., Gannon, S. M., Nevin, K. P., Franks, A. E., & Lovley, D. R. (2010). Stimulating the anaerobic degradation of aromatic hydrocarbons in contaminated sediments by providing an electrode as the electron acceptor. *Environmental Microbiology, 12*, 1011–1020. https://doi.org/10.1111/j.1462-2920.2009.02145.x

Zhang, Y., Wang, X., & Li, X. (2014). Horizontal arrangement of anodes of microbial fuel cells enhances remediation of petroleum hydrocarbon-contaminated soil. *Environmental Science and Pollution Research, 22*, 2335–2341. https://doi.org/10.1007/s11356-014-3539-7