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- Electrobioremediation is an innovative approach for contaminants removal
- Petrochemical compounds can be successfully removed by electrobioremediation
- Several parameters (e.g. electrode, potential, mediators) influence the process
- Microbiological processes can be complex and sulfur cycle has an important role
- The scale up of the technology is the future challenge

Electrobioremediation of oil spills

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Abstract

Annually, thousands of oil spills occur across the globe. As a result, petroleum substances and petrochemical compounds are widespread contaminants causing concern due to their toxicity and recalcitrance. Many remediation strategies have been developed using both physicochemical and biological approaches. Biological strategies are most benign, aiming to enhance microbial metabolic activities by supplying limiting inorganic nutrients, electron acceptors or donors, thus stimulating oxidation or reduction of contaminants. A key issue is controlling the supply of electron donors/acceptors. Bioelectrochemical systems (BES) have emerged, in which an electrical current serves as either electron donor or acceptor for oil spill bioremediation. BES are highly controllable and can possibly also serve as biosensors for real time monitoring of the degradation process. Despite being promising, multiple aspects need to be considered to make BES suitable for field applications including system design, electrode materials, operational parameters, mode of action and radius of influence. The microbiological processes, involved in bioelectrochemical contaminant degradation, are currently not fully understood, particularly in relation to electron transfer mechanisms. Especially in sulfate rich environments, the sulfur cycle appears pivotal during hydrocarbon oxidation. This review provides a comprehensive analysis of the research on bioelectrochemical remediation of oil spills and of the key parameters involved in the process.

1. Introduction

Thousands of accidents occur every year that lead to crude oil being spilled and entering the environment (Figure 1). Crude petroleum is a complex mixture containing more than 17,000 identified chemical components. Saturated and aromatic hydrocarbons represent the majority of the non-polar fraction (Head et al., 2006), halogenated hydrocarbons are not found in crude oil but are typically derived from petroleum hydrocarbons and include a halogen such as F, Cl, Br, and I. Both halogenated and non-halogenated hydrocarbons can be released into the environment and contaminate soil and water (Moran et al., 2007; Poulsen et al., 1992). These compounds have a

broad range of detrimental effects on the environment (Badawi et al., 2000; Durmusoglu et al., 2010), and therefore need to be removed efficiently.

The technologies to remove contaminants from a polluted site can be grouped into two main categories and applied individually or in synergy: physicochemical technologies, and biological technologies (bioremediation) (Alvarez and Illman, 2006). Physicochemical technologies can include physical removal (i.e. excavation of soil and sediment or groundwater pumping), washing by co-solvents or surfactants, thermal desorption, electrokinetic movement of contaminants and oxidation or reduction via chemical agents (Alvarez and Illman, 2006; Trombly, 1994).

Bioremediation exploits the vast metabolic diversity of microorganisms to degrade organic contaminants by using the pollutant as a source of energy and carbon (Alvarez and Illman, 2006). It is often inexpensive compared to physicochemical methods, and allows the complete mineralization of the pollutants. However, it typically requires more time to achieve full remediation (Atlas, 1995). Remediation technologies can be divided into *ex-situ* (involving the extraction of the contaminated matrix for treatment *on-site*, or *off-site*) and *in-situ* (which does not involve extraction of the contaminated matrix). *In-situ* microbial mediated reactions have been successfully used for the reduction and oxidation of petroleum derived contaminants (Alvarez and Illman, 2006). The goal of bioremediation is to stimulate the removal of contaminants by overcoming the limitations to microbial metabolism that would otherwise prevent contaminant removal. A common approach is to supply electron donors to stimulate the degradation (i.e. reduction) of halogenated compounds, or electron acceptors to stimulate the degradation (i.e. oxidation) of non-halogenated compounds (Alvarez and Illman, 2006) (Table 1).

Aerobic metabolism is stimulated by adding oxygen (e.g. by air sparging, Figure 2) (Farhadian et al., 2008), which has the benefit of faster rates of hydrocarbon removal compared to anaerobic bioremediation strategies (Weelink et al., 2010). Furthermore, even though the oxidation of hydrocarbons can occur in anaerobic environments (Weelink et al., 2010), oxygen is an important reactant for hydrocarbon breakdown (Baldwin et al., 2009). However, oxygen solubility in water is

low and it can be consumed by unwanted side reactions with reduced species (e.g. Fe^{2+} or Mn^{2+}) which are usually abundant in contaminated matrices (Broden et al., 1997; Tuxen et al., 2006). Anaerobic microbial metabolism can be effectively enhanced with the addition of chelators, which solubilize Fe^{3+}, or with the addition of soluble electron shuttles (e.g. humic substances) able to promote electron transfer to insoluble electron acceptors, such as Fe^{3+} or Mn^{4+} oxides (Lovley et al., 1996; Lovley et al., 1994). Anaerobic biodegradation can be stimulated also by adding sulfate or nitrate (Coates et al., 1996; Mihelcic and Luthy, 1988; Vaiopoulou et al., 2005; Weiner et al., 1998). The main drawback of the strategies mentioned above is that the supplemented reagents are consumed rapidly and naturally migrate away from the contaminated area. Continuous amendment with the depleted reagents or electron acceptors is therefore required, and this increases the cost (Zhang et al., 2010).

Halogenated hydrocarbons can be effectively remediated by reductive dehalogenation by microbes that use them as a terminal electron acceptors during anaerobic respiration, and are thus reduced to less halogenated, or non-halogenated, compounds which can be more biodegradable (de Bruin et al., 1992; Seshadri et al., 2005). Stimulation of microbial reduction can be achieved by supplying electron donors (Table 1). The typical electron donor for the dehalogenation is hydrogen (H_2), although some studies suggest that acetate may also be used (Aulenta et al., 2006; He et al., 2002). H_2 can be delivered directly or by passive dissolution using hollow fibre membranes (Fang et al., 2002; Ma et al., 2003). Another strategy to indirectly supply H_2 for the reductive dechlorination is by using organic substrates, such as butyric acid, ethanol or lactic acid that can be fermented at low H_2 partial pressure (Aulenta et al., 2005; Fennell et al., 1997; Panagiotakis et al., 2007). However, these approaches can be costly due to the need for continuous supply of water-soluble electron donors. Furthermore, controlling the supply rate of the electron donors can be a crucial step to avoid unwanted side reactions and the accumulation of fermentation products (e.g. volatile fatty acids; VFA) with deterioration of water quality. For example, VFA and dissolved metals accumulated in microcosms amended with lactate during reductive dechlorination and as a result increased

104 groundwater ecotoxicity (Aulenta et al., 2007b). H_2 can be consumed, not only by dechlorinating
105 bacteria, but also by other H_2 consuming microorganisms, such as methanogens, homoacetogens
106 and sulfate reducers, thus lowering the efficiency of the process (Aulenta et al., 2007a, 2007b;
107 Zanaroli et al., 2015). Experimental evidence, however, suggested that dechlorinating
108 microorganisms can outcompete other H_2 consumers at low H_2 concentrations (Aulenta et al.,
109 2008a; Smatlak et al., 1996; Yang and McCarty, 1998) (e.g. the half-velocity constants (K_s)
110 measured for dechlorination and methanogenesis are 100 ± 50 nM and 960 ± 180 nM (Smatlak et
111 al., 1996), respectively).
112 Recently, bioelectrochemical systems (BES) have been suggested as an alternative strategy to
113 overcome some of the limitations of the current bioremediation technologies (Wang et al., 2015).
114 The use of benthic BES has recently been reviewed (Li and Yu, 2015), however sediments are not
115 the only environmental matrix that can be treated with a BES-based approach. BES can also be used
116 effectively for the bioremediation of soils and water.
117 In this article the state of the art of this innovative approach for the bioremediation of oil spills in
118 soil, sediment and water will be extensively reviewed. The scope is to investigate the potential of
119 BES to remove oil spills, elucidating: (i) the key parameters that influence the process; (ii) the main
120 advantages and limitations; (iii) the microorganisms and the biological processes involved; (iv)
121 future research opportunities to improve understanding and field application of BES-based
122 bioremediation approaches.
123

124 **2. Bioelectrochemical systems**

125 A BES uses microorganisms to catalyze redox reactions on or near electrodes (Logan et al., 2006).
126 A typical reactor design consists of an anode and a cathode separated by an ion conductive matrix
127 (Figure 3). Microorganisms can interact with the electrodes either by direct contact (e.g.
128 microorganisms directly exchange electrons with an electrode) or via an indirect mechanism where
129 a chemical compound acts as an electron shuttle (Figure 3). These electron shuttles can be secreted

130 by the microorganisms (e.g. phenazines of *Pseudomonas* spp.) (Rabaey et al., 2005) or can be added
131 exogenously (Logan et al., 2006).
132 A BES can be used to generate power (microbial fuel cell; MFC) by linking anodic oxidation of a
133 reduced substrate to cathodic reduction of a high potential electron acceptor (e.g. oxygen) (Logan et
134 al., 2006). It can also function with addition of power to drive the desired reaction (microbial
135 electrolysis cell; MEC) (Rozendal et al., 2006). A microbial anode in combination with a biological
136 or chemical cathode can be implemented to achieve production of H_2 in a MEC. This system
137 requires about 3 times less potential difference compared to a conventional chemical electrolysis
138 cell, in which the reactions at the electrodes are not mediated by microorganisms (Rozendal et al.,
139 2006). When operating a MEC, there are two electrical control strategies (i) operation at a fixed
140 potential (Rozendal et al., 2006) or (ii) operation at a fixed current (Andersen et al., 2013).
141 Operation at a fixed potential has the advantage that a desired reaction can be driven, or favourable
142 conditions for a certain (bio)catalyst can be created. The drawback is that reaction rates at the
143 electrode are controlled by the (bio)catalyst and not by the operator. Operation of a MEC at a fixed
144 current allows the operator to control the reaction rates but not the type of reaction that occurs. This
145 can be used, for example, when the aim is to produce large amounts of oxygen or hydrogen.
146

147 **3. Bioelectrochemical processes for oil spill remediation**

148 *3.1. Anodic oxidation and oxygen generation*

149 The microbial mediated anodic oxidation of organic compounds in BES was initially applied as a
150 technology to reduce Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD)
151 in domestic wastewater, with concomitant energy production (Logan et al., 2006). Recently, a
152 number of studies have exploited BES technologies to stimulate the anaerobic oxidation of
153 petroleum and petroleum-derived compounds (Table 2). The anode of a BES can be used to collect
154 electrons produced from the oxidation of organic contaminants. It can be buried in anoxic benthic
155 sediment, or in a contaminated aquifer, and electrically connected to a cathode placed in the

overlying water (Bond et al., 2002; Williams et al., 2010). The anode can be pre-inoculated or readily colonized by members of the resident microbiota with electron transfer abilities. Electrons collected from the anaerobic oxidation of the contaminant, flow through an electrical connection to the cathode in the aerobic water column, were they can be used to reduce oxygen (Lovley and Nevin, 2011). A similar set-up could be used to stimulate bioremediation in hydrocarbon contaminated aquifers. Indeed it has been demonstrated that a borehole anode can serve as an electron acceptor with a cathode embedded at the ground surface, meters above (Williams et al., 2010). A simpler configuration can be obtained by using so-called "electrochemical snorkels". In this strategy, the electrode material is a conductive rod which spans the aerobic and the anaerobic zones, functioning both as a cathode and as an anode. With this configuration, however, electrical power is not harvested nor can activity be monitored (Cruz Viggi et al., 2015; Lovley, 2011).

Early studies in this field reported the use of complex mixtures of highly contaminated refinery wastewater and diesel contaminated groundwater as suitable electron donors in MFCs, coupling hydrocarbon removal with power production (Morris et al., 2009; Morris and Jin, 2008). Using an anode as electron acceptor, Diesel Range Organics (DRO) removal was 82%, whereas in the open circuit control only 31% was removed (Morris et al., 2009). Both alkanes and aromatic hydrocarbons can be degraded in BES. Toluene is the easiest degradable component of BTEX compounds (Benzene, Toluene, Ethylbenzene and Xylenes), and its degradation has been studied at a variety of anode potentials, both with pure cultures and consortia (Daghio et al., 2016; Friman et al., 2012; Lin et al., 2014; Zhang et al., 2010). Benzene was degraded in the anode of a BES by mixed cultures enriched from contaminated sediments (Zhang et al., 2010), groundwater (Rakoczy et al., 2013; Wei et al., 2015), wastewater (Wu et al., 2013) and anaerobic sludge (Adelaja et al., 2015). Polycyclic aromatic hydrocarbons (PAHs) degradation has also been reported in several studies (Adelaja et al., 2015, 2014; Yan et al., 2012; Zhang et al., 2010). Phenol has been bioelectrochemically degraded both by mixed cultures and a pure culture of *Cupriavidus basilensis* (Friman et al., 2013; Huang et al., 2011). In contaminated sites, however, mixtures of different

hydrocarbons rather than single compounds are present. Total petroleum hydrocarbons (TPH) degradation has been extensively assessed in different studies (Chandrasekhar and Venkata Mohan, 2012; Cruz Viggi et al., 2015; Li et al., 2015, 2014, Lu et al., 2014a, 2014b; Morris and Jin, 2012; Venkata Mohan and Chandrasekhar, 2011; Wang et al., 2012; Zhang et al., 2015). Although there are increasing numbers of studies regarding the oxidation of non-halogenated hydrocarbons in BES, biodegradation of widespread mixtures, such as gasoline, still remains to be assessed. The oxidation of halogenated hydrocarbons has also been investigated and bioelectrochemical oxidation of 1,2-dichloroethane (1,2-DCA) with different microbial inocula has been shown to be an attractive technology for the bioremediation of chlorinated compounds (Pham et al., 2009).

A major bottleneck of electrochemical bioremediation of hydrocarbons under anaerobic conditions is initiation of the degradation pathway (Bertrand et al., 2011). In aerobic conditions the first step of the degradation pathway involves an oxygenase that catalyzes the addition of hydroxyl groups. Under anaerobic conditions less efficient processes than aerobic activation usually take place. The initial biodegradation step can occur either by fumarate addition or by carboxylation for *n*-alkanes and either by fumarate addition, hydroxylation or carboxylation for aromatic compounds (Fuchs et al., 2011; Head et al., 2014; Widdel and Rabus, 2001). Presence of a limited amount of oxygen could benefit and initiate the process of primary hydrocarbon bio-oxidation (Baldwin et al., 2009; Weelink et al., 2010) and such strategies have already been presented (Table 1). From this perspective, an anode can not only serve as alternative electron acceptor during hydrocarbon biodegradation (Bond et al., 2002; Cruz Viggi et al., 2015; Lovley and Nevin, 2011; Williams et al., 2010), but also contribute to the initiation phase by oxygen production and altering the pH in its proximity. Despite known standard oxygen potential values (i.e + 1,230 mV *vs* SHE, all potentials are relative to SHE unless otherwise stated), in real applications, oxygen evolution overpotential depends on the electrode material (Anglada et al., 2009). Oxygen evolution through water oxidation usually results in a decrease in pH that may impose further effects on biodegradation. Type, design and electrode material are determining factors of oxygen evolution potential and treatment

208 efficiency (Radjenovic and Sedlak, 2015). Specifically, under anaerobic conditions in a marine
209 medium, the electrochemical potential of oxygen evolution can vary depending on seawater nature
210 (e.g. salinity, ions species and concentration), presence of other water-soluble constituents from the
211 sediment, and environmental conditions (e.g. pH and temperature) in addition to the electrode
212 properties. High potential electrodes can be used to deliver oxygen in the anaerobic environment
213 where it can act as final electron acceptor for microorganisms during biodegradation of
214 contaminants. An example of electrochemical stimulated removal of organic contaminants via
215 oxygen evolution has been recently reported for *cis*-dichloroethene (*cis*-DCE). A polarized graphite
216 electrode (+ 1,500 mV) was able to successfully stimulate the removal of *cis*-DCE (85 µmol/L)
217 when ethene was provided as a co-metabolic substrate (Aulenta et al., 2013). Furthermore in a
218 recently published paper, water electrolysis was driven by applying an external voltage (2 V) on
219 Dimensionally Stable Anodes (DSA; i.e. Ti mesh covered with mixed metal oxides, primarily
220 consisting of Ir and Ru) to produce oxygen for the stimulation of TPH removal (20 g kg^{-1}) in marine
221 sediments. After 202 days of operation TPH removal in the open circuit control was 44 ± 1 %,
222 while a slightly higher removal (i.e. 58 ± 3 %) was observed when oxygen was produced by
223 constantly applying 2 V (Bellagamba et al., 2016).
224
225 *3.2. Hydrogen generation and cathodic reduction*
226 The reduction reactions at the cathode in a BES can be exploited to reduce a large number of
227 oxidized compounds such as metals (Tandukar et al., 2009; Xafenias et al., 2013) or halogenated
228 compounds (Aulenta et al., 2007a; Kong et al., 2014). Several studies have reported the stimulation
229 of microbial dechlorination by using a cathode as a direct electron donor or by *in-situ* H_2 production
230 (Table 3). Early studies investigated the electrochemical production of H_2 as a suitable strategy for
231 the dehalogenation of 2,6-dichlorophenol (2,6-DCP) by applying currents from -1 to -15 mA
232 (Skadberg et al., 1999). The same technology was subsequently used for trichloroethene (TCE)
233 removal (Aulenta et al., 2008c). The use of a BES is an innovative approach to produce *in-situ* H_2,

234 because the energy requirement is lower compared to a purely electrochemical process (Rosenbaum
235 et al., 2011). However, in order to control the process and avoid side reactions that consume H_2 and
236 lead to low efficiencies, direct electron uptake from the cathode, without the production of H_2, is a
237 preferable strategy. Pioneering studies focused on TCE removal by using a redox mediator to
238 promote electron transfer from the cathode to the biocatalyst, resulting in complete dechlorination
239 of TCE via *cis*-DCE and vinyl chloride (VC) to ethene (Aulenta et al., 2007a). Subsequent studies
240 suggested that TCE removal can be stimulated by direct electron transfer from the cathode (Aulenta
241 et al., 2011, 2010a, 2010b, 2009, 2008b; Verdini et al., 2015). The more oxidized tetrachloroethene
242 (PCE) was reduced to *cis*-DCE by a pure culture of *Geobacter lovleyi* able to utilise an electrode as
243 sole electron donor (Strycharz et al., 2008). Whereas in a separate innovative study, PCE was
244 reduced to ethene using H_2 from water electrolysis concomitant with the electrochemically
245 produced O_2 which was used to stimulate the microbial oxidation of the dechlorination products
246 (Lohner and Tiehm, 2009). Other studies have focused on the dechlorination of the reduced
247 intermediates produced during TCE removal, such as *cis*-DCE (Aulenta et al., 2010b; Lai et al.,
248 2015). Furthermore, the dechlorination of 1,2-DCA was achieved in a BES reactor inoculated with
249 a mixed culture enriched in *Dehalococcoides* spp. (Leitão et al., 2015).
250

251 **4. Effects of materials and operational parameters**

252 *4.1. Electrode material*

253 The choice of an appropriate anodic material affects the selectivity and efficiency of the
254 hydrocarbon removal process (Anglada et al., 2009). The electrode material should comply with the
255 following properties: i) high physical and chemical stability, ii) high electrical conductivity, iii)
256 catalytic activity and selectivity for the target compounds, and iv) low cost/life ratio. Inexpensive
257 and long life service materials should be favoured for the oxidation of hydrocarbons. Oxidation
258 reactions may act synergistically or compete with the side reaction of oxygen evolution at the
259 anode, depending on the choice of material. In real applications, the Oxygen Evolution Reaction

overpotential (OER; i.e. difference between the value of the voltage at which the oxidation of water actually begins to take place, and the theoretical thermodynamic value (Chen, 2004)) depends mainly on the electrode material (Anglada et al., 2009). Anodes with low OER overpotential, such as graphite or platinum, are characterized by a high electrochemical activity towards the OER and low chemical reactivity toward oxidation of organics. Thereafter, low current densities are applied at such anodes to drive pollutant oxidation; at higher current densities, current efficiency is expected to decrease due to the production of oxygen. In contrast, anodes with a high OER overpotential, such as a DSA, higher current densities can be applied without the concern that current efficiency is reduced due to occurrence of the OER.

For stimulation of microbial metabolism with anodes serving as electron acceptors, stainless steel electrodes (Morris et al., 2009; Morris and Jin, 2012, 2008; Yan et al., 2012) or conductive carbon and graphite have been used (Table 2). A comparison of anodic materials used in different studies, in terms of removal efficiencies, is difficult, because factors other than the electrode material may affect the degradation rate. A slightly higher removal efficiency (78.7%) was observed with a biochar anode compared to a carbon cloth anode (73.1%) over 64 days of operation both starting from an initial TPH concentration of 11.46 g $(kg\ soil)^{-1}$. The authors attributed the higher removal efficiency of the biochar to the higher sorption capabilities that facilitated hydrocarbon diffusion in the anode (Lu et al., 2014a). In a separate study, a biochar electrode was also compared with a graphite granule anode. The two materials showed similar performances in terms of TPH removal, but the graphite granule anode generated a higher current density (70.4 ± 0.2 mA m^{-2}) compared to the biochar anode (35.2 ± 0.8 mA m^{-2}) (Lu et al., 2014b).

Adsorption of hydrocarbons on carbon and organic phases in soil and sediment is known to reduce their bioavailability, however adsorption on the electrode surface does not appear to negatively affect biodegradation of hydrocarbon contaminants. Studies with [^{14}C]-toluene and [^{14}C]-benzene and isotopic analysis during benzene degradation showed that contaminants adsorbed onto the electrode could still be metabolized (Rakoczy et al., 2013; Zhang et al., 2010).

286

287 *4.2. Redox potential*

288 The anode in a BES is the final electron acceptor in microbial metabolism. The energy gain for the
289 microorganisms is higher using electron acceptors with a more positive potential (Madigan et al.,
290 2011), thus it is reasonable to hypothesize that a more positive anodic potential can enhance
291 hydrocarbon oxidation in BES. However, results correlating anodic potential to bioelectrochemical
292 oxidation of organic substrates are controversial and do not confirm this expectation. With easily
293 biodegradable substrates (e.g. acetate) a positive correlation between anode potential and current
294 production was observed, but other studies have shown the opposite trend (Aelterman et al., 2008;
295 Wagner et al., 2010). Recently, it was observed that polarizing an anode at a positive potential (> +
296 397 mV) resulted in a lower current production with a pure culture of *Shewanella oneidensis* MR-1,
297 because electron transfer proteins were damaged at higher potentials (TerAvest and Angenent,
298 2014).

299 Most studies related to hydrocarbon degradation in BES used an MFC configuration, without
300 controlling the anodic potential. Toluene degradation was studied with an anode poised at potentials
301 from + 275 to + 700 mV using a pure culture of *Pseudomonas putida* F1 and current production
302 increased with increasing anodic potential (Friman et al., 2012). Another study tested the ability of
303 mixed cultures enriched from a contaminated marine sediment to degrade toluene, and confirmed
304 the correlation between current and potential, but no effect on the degradation rate was observed in
305 a potential window from + 200 mV to + 500 mV (Daghio et al., 2016). Thus, in the range of + 200
306 till + 700 mV data are scarce and further investigation is warranted to reach solid conclusions about
307 the role of anode potential on biodegradation of recalcitrant compounds.

308 Oxygen evolution is a process that could affect the electrobioremediation both by stimulating the
309 activation of hydrocarbon, or by acting as a side reaction delivering electrons to the anode which
310 are not linked to the oxidation of the contaminant, decreasing the efficiency. In this context it is
311 important to consider the potential of the OER when the anodic potential is selected. Cyclic

312 voltammetry provides information on oxidation potentials and can assist in determining the
313 appropriate anode potential for specific applications. For example, oxygen evolution commenced at
314 around + 750 mV on a carbon felt anode when cyclic voltammetry was run both in artificial and
315 real seawater at neutral pH at a scan rate of 5 mV s^{-1} (unpublished data from CMET, UGent,
316 Belgium).
317 Cathode potential is an important parameter that can affect the performance of e.g. dechlorination of
318 chlorinated hydrocarbons, by controlling competing reactions, particularly methanogenesis due to
319 H_2 evolution. Cathodic potentials from - 600 mV to - 800 mV can be applied to stimulate TCE
320 dechlorination via H_2 production. The best performance was achieved around - 650 mV. However,
321 methane production was also stimulated in the same range of potentials thus reducing the efficiency
322 of the dechlorination process (Aulenta et al., 2008c). A strategy to enhance efficiency of microbial
323 reductive dechlorination and to eliminate side reactions could be to supply electrons directly from
324 the electrode. The first evidence of this process was reported with a culture of *Geobacter lovleyi*
325 that was able to reduce PCE to cis-DCE with an electrode poised at - 300 mV serving as electron
326 donor (Strycharz et al., 2008). A further study demonstrated that mixed cultures could also use an
327 electrode poised at - 450 mV as sole electron donor (Aulenta et al., 2009). A higher dechlorination
328 rate was obtained by further decreasing the cathodic potential to - 550 mV (Aulenta et al., 2010b).
329 An accurate study of the effect of the electrode potential was performed using a continuous flow
330 reactor. Five cathodic potentials ranging from - 250 mV to - 750 mV were tested without exogenous
331 nor endogenous redox mediators. At - 250 mV the TCE dechlorination rate was low (15.5 ± 1.2
332 μmol e^- L^{-1}) but rapidly increased when the cathodic potential was decreased to - 450 mV (58 ± 1
333 μmol e^- L^{-1}). Decreasing the potential increased the H_2 production rate, thus increasing the
334 dechlorination rate. However, the coulombic efficiency of the dechlorination process was nearly
335 100 % at - 250 mV, but decreased to less than 1 % at - 750 mV, because methanogenesis acted as
336 an electron sink (Aulenta et al., 2011). A similar result was observed during 1,2-DCA
337 dechlorination with cathodic potentials from - 300 mV to - 900 mV. The dechlorination rate

increased linearly by decreasing the cathodic potential whereas the coulombic efficiency was near 70% at - 300 mV and decreased to less than 2% for potentials lower than - 600 mV (Leitão et al., 2015). The study of the dechlorination of *cis*-DCE in a flow reactor at potentials between - 550 and - 750 mV, showed that nitrate reduction and sulfate reduction can also represent an electron sink at more negative potentials (below - 550 mV), decreasing the coulombic efficiency from more than 90% at - 550 mV to 60% at - 750 mV (Lai et al., 2015). Recently, it was reported that the applied cathodic potential has to be considered together with the velocity of groundwater flow (Verdini et al., 2015). TCE dechlorination rate increased almost linearly with the flow velocity (from 0.3 m d^{-1} to 1.7 m d^{-1}) at more reducing potentials (- 450 mV) but the influence of the mass transport decreased at higher potentials (- 350 mV and - 250 mV). This observation suggests that the effect of flow velocity at the lower potential was probably due to the influence of electrolytically generated hydrogen. Hydrogen was transported with the water flow and sustained the dehalogenation process, while only direct extracellular electron transfer occurred at the higher potential (Verdini et al., 2015).

4.3. Redox mediators

Redox mediators enable electron transfer to and from an electrode. Neutral red (100-300 μM) and ferricyanide (100-2000 μM) were used as redox mediators in the anodic chamber in order to investigate their influence on toluene degradation. It was found that toluene removal decreased with both the mediators compared to a control without redox mediators (Lin et al., 2014). The negative effect was probably due to the toxicity of the mediators (Lin et al., 2014; Smolinská and Takáčová, 2012). The effect of riboflavin and anthraquinone-2-sulfonate (AQS) on the degradation of phenanthrene and benzene in an MFC was also investigated. Riboflavin (30 μM) highly improved the power density and reached 26.17 ± 0.08 mW m^{-2} compared to AQS (30 μM) and to the control without the addition of mediators that reached 0.57 ± 0.05 mW m^{-2} and 0.47 ± 0.01 mW m^{-2}

363 respectively. No significant effects were shown on hydrocarbon removal that were almost
364 completely removed regardless of the presence of exogenous redox mediators (Adelaja et al., 2015).
365 Studies with exogenous redox mediators have also been performed during the bioelectrochemical
366 removal of halogenated hydrocarbons. It was demonstrated that with an electrode poised at - 500
367 mV dechlorination was stimulated only when methyl viologen (MV) was in the medium (Aulenta et
368 al., 2007a). When the potential was further decreased to -800 mV, H_2 was produced but the
369 dechlorination rate did not increase (Aulenta et al., 2007a). In another study at low MV
370 concentration (25-750 μM) only dechlorination was stimulated, but when the MV concentration
371 was increased up to 5,000 μM, H_2 was produced. It was suggested that at high MV concentrations
372 the rate at which the electrons were transferred to the microorganisms exceeded the electron
373 utilization for the dehalogenation process. The electrons were thus diverted to H_2 production via a
374 hydrogenase (Aulenta et al., 2008b). An alternative mediator is the humic acid analogue
375 antraquinone-2,6-disulfonate (AQDS). Humic acids are ubiquitous redox active compounds in the
376 environment and several studies have demonstrated their involvement in biodegradation processes
377 (Van der Zee and Cervantes, 2009). AQDS was successfully used to reduce TCE to cis-DCE but
378 was unable to further stimulate the dechlorination to vinyl chloride (Aulenta et al., 2010a).
379 As artificial mediators can be toxic and inhibitory to microbial activity, mediator-free BES are
380 preferable during bioremediation. Artificial mediators also pose some of the same disadvantages of
381 the soluble electron acceptors, as they could diffuse away from the reaction area and interact with
382 other processes therefore decreasing the efficiency.
383 Endogenously produced mediators have been detected during toluene degradation. Friman and
384 colleagues (2012) detected the presence of a redox active compound with a potential of + 470 mV.
385 The authors attributed the oxidation peak to 3-methyl catechol (Friman et al., 2012). Catechol and
386 3-methyl catechol are typical intermediates produced during aerobic toluene degradation and their
387 presence could be explained by a low levels of oxygen penetrating into the reactor, however, it is
388 unlikely that this redox active compound could have acted as a mediator for electron transfer in this

system, since the anode was poised at + 325 mV, a potential considerably lower than the midpoint potential of the detected redox active molecule.

The production of a redox active moiety involved in electron transfer to an anode polarized at + 500 mV was also described in a mixed culture dominated by sulfate-reducing bacteria during toluene degradation. The midpoint potential of the redox active site was around + 400 mV, but the nature of the redox mediator was not identified (Daghio et al., 2016).

Further studies are needed to elucidate the role of redox mediators when hydrocarbons compounds other than toluene are oxidized. So far only one study on other petrochemicals reported the presence of an unidentified mediator (+ 140 mV) self-produced by a pure culture of *Cupriavidus basilensis* with an anode polarized at + 325 mV during bioelectrochemical oxidation of phenol (Friman et al., 2013).

Recent advances in the description of cathodic electron transfer mechanisms have revealed that some dechlorinating bacteria are able to accept electrons directly from a graphite electrode, without the addition of external mediators (Aulenta et al., 2010b, 2009; Strycharz et al., 2008). An unidentified redox active moiety with a midpoint potential around - 400 mV was detected in the supernatant during TCE dechlorination. This molecule was not detected when the culture was grown with H_2 as electron donor, therefore it was possible to hypothesize that it had a role in the electron transfer from the solid electron donor (Aulenta et al., 2009).

Experimental evidence suggests that in the environment natural molecules may also play an important role in the electron transfer to solid electron acceptors. Humic acids can act as electron shuttles in MFCs (Milliken and May, 2007) as well as acting as electron acceptors in their own right (Lovley et al., 1996a). Several reviews discuss the role of natural mediators during the bioremediation of contaminants (Hong and Gu, 2009; Martinez et al., 2013; Van der Zee and Cervantes, 2009). For example, anaerobic toluene degradation linked to reduction of humic compounds has been reported (Cervantes et al., 2001). Also the more recalcitrant benzene was degraded using AQDS as sole electron acceptor by two microbial consortia (Cervantes et al., 2011)

and by a pure culture of *Geobacter* (Zhang et al., 2012). Humic substances have formal potentials, at pH 7, of approximately + 740 mV and thus are potentially very good electron acceptors (Struyk and Sposito, 2001). The redox active component of the humic acid is thought to be quinone moieties (Scott et al., 1998). In crude oil the asphaltene fraction can contain up to around 5% oxygen. The oxygen is present in a number of functional groups including possibly quinones (Speight, 2014). While asphaltenes (and associated resin fractions of crude oils), are much less functionalised than humic acids it is possible that these more polar components of crude oils could provide an organic, macromolecular electron shuttle for anaerobic microbial metabolism. The intriguing possibility therefore exists that, in environments contaminated with complex mixtures of petroleum hydrocarbons such as crude oil, humic acids in soils and sediments or asphaltenes in crude oils might themselves act as electron shuttles promoting bioelectrochemical oxidation coupled to anodes provided in the soil/sediment. Electrodes in BES may serve to recycle the natural mediators, which can be reoxidized at the anode, providing a continuous source of electron acceptors for the degradative microorganisms.

4.4. Radius of influence

The extension of the radius of influence of an electrode is one of the most important aspects to address before applying BES-based technologies for the bioremediation of soil and sediment.
A first attempt at evaluating the radius of influence during TPH degradation in soil was conducted using a saline soil (conductivity 8.32 mS cm^{-1}). A high conductivity decreases the electrical resistance, but in spite of that, the degradation rate was enhanced only in samples collected close to the electrode (<1 cm) after 25 days of incubation, while the removal rate farther from the anode (1-2 cm and 2-3 cm) was similar to the removal rate in open circuit controls (Wang et al., 2012). In another study, TPH degradation was enhanced during 64 days of operation both at <1 cm and also at 5 cm from the anode, compared to the controls (Lu et al., 2014a). The difference in the results

440 may be attributed to variations in the configuration of the bioelectrochemical systems, or to
441 different characteristics of the soil used.
442 A recent study showed that the measured radius of influence can be up to 34 cm away from the
443 electrode over a period of 120 days. Hydrocarbon degradation was initially observed close to the
444 anode (1 cm), but the influence of the bioelectrochemical stimulation increased over time. The
445 authors assumed a linear correlation between the distance from the anode and the enhanced TPH
446 removal and predicted a maximum radius of influence of 90 cm after 45 days with a BES radius of
447 7.5 cm, corresponding to a ratio of maximum radius of influence to radius of BES equal to 12. This
448 estimation, however, was not supported by experimental data at a distance greater than 34 cm from
449 the electrode (Lu et al., 2014b). Other factors, such as ohmic losses, might lead to a non-linear
450 correlation between TPH removal enhancement and the radius of influence over long distances
451 (Arends et al., 2014; Logan et al., 2006), thus decreasing the predicted extension of the radius of
452 influence. However, a correlation between the radius of influence and the radius of BES can be
453 made (Lu et al., 2014b), suggesting that, with cylindrical electrodes, the radius of influence may be
454 extended by increasing the radius of the electrode. Further studies can be conducted to better clarify
455 this aspect in a real field bioremediation. The mass transfer of the chemical species (e.g.
456 groundwater flow) could extend the radius of influence of the electrode, particularly if the function
457 of the electrode is to generate a soluble electron donor or acceptor *in situ*.
458 It was proposed that by increasing the porosity of a soil (e.g. by the addition of sand) it is possible
459 to enhance mass transfer and promote the performance of BES-driven TPH degradation. The
460 charge output increased from 2.5 C g^{-1} soil (no sand) to 2.9 C g^{-1} soil (soil to sand content of 5:1
461 *w/w*) and 3.5 C g^{-1} soil (soil to sand content of 2:1 *w/w*) over 135 days. Similarly, TPH removal was
462 higher with 2:1 soil to sand ratio (22 ± 0.5 %) compared to 5:1 soil to sand ratio (15 ± 0.1 %) or no
463 sand (12 ± 0.4 %). Bacteria from the genus *Alcanivorax*, known obligate hydrocarbon-degrading
464 organisms, were also strongly enriched close to the air-cathode when soil was amended with sand,
465 indicating that sand promoted the growth of hydrocarbon degrading bacteria (Li et al., 2015).

466 The reported studies clearly indicate that several factors may affect the radius of influence in field
467 applications (e.g. electrode design, water content, soil type, mass transport) and these parameters
468 may have to be evaluated in site specific conditions in order to obtain the best treatment efficiency.
469

470 **5. Microbial communities in bioelectrochemical systems during oil spill remediation**

471 Iron reducing bacteria were first used as an inoculum in an MFC containing hydrocarbons as an
472 energy source (Zhang et al., 2010) (Table 2). One of the first studies showed the ability of
473 *Geobacter metallireducens* to use graphite electrodes as electron acceptor for the degradation of
474 toluene (Zhang et al., 2010). Recently, the degradation of phenanthrene by *Shewanella oneidensis*
475 MR1 14063 in an MFC was reported (Adelaja et al., 2014). However, strict anaerobes are not the
476 only microorganisms studied. *Pseudomonas aeruginosa* NCTC 10662 has been shown to degrade
477 phenanthrene faster (54.7 μM d^{-1}) than *Shewanella oneidensis* (25.2 μM d^{-1}) under similar
478 operational conditions in a MFC (Adelaja et al., 2014). Other reports seem to indicate that aerobes
479 and facultative anaerobes are able to oxidize hydrocarbons with an anode as sole electron acceptor.
480 *Cupriavidus basilensis* was able to degrade phenol with an anode via electron transfer mediated by
481 a self-produced shuttle (Friman et al., 2013); while the presence of the catabolic intermediate
482 catechol was hypothesized during bioelectrochemical toluene degradation by *Pseudomonas putida*
483 F1 (at oxygen levels of 0.78 mg O_2 L^{-1}) (Friman et al., 2012). This finding poses open questions
484 regarding the degradation pathway and the role of oxygen for hydrocarbon degradation by
485 *Pseudomonas* sp. with an anode. Indeed, microorganisms belonging to this genus are well-described
486 hydrocarbon degraders in aerobic conditions and the hydrocarbon activation proceeds by addition of
487 hydroxyl groups catalyzed by an oxygenase, requiring molecular oxygen (Jindrová et al., 2002).
488 Whether aerobes and facultative anaerobes require a low concentration of oxygen for the activation
489 reaction to couple hydrocarbon degradation to current production in a BES is therefore an
490 interesting and important question. A first step in hydrocarbon biodegradation catalyzed by a
491 monooxygenase followed by further anaerobic removal of the intermediate has also been proposed

492 during bioelectrochemical benzene removal from groundwater fed reactors (Rakoczy et al., 2013;
493 Wei et al., 2015).
494 These findings are intriguing in the context of recent studies of hydrocarbon rich environments that
495 are considered to be primarily anoxic and have been subject to anaerobic hydrocarbon degradation,
496 which does not require molecular oxygen. The widespread occurrence of microorganisms that
497 would be typically considered aerobes in these environments (An et al., 2013) (including
498 *Pseudomonas* spp.) has been interpreted either as resulting from contamination with oxygen during
499 sampling, contamination with exogenous organisms, oxygen ingress into these environments or the
500 existence of mechanisms that lead to *in-situ* generation of reactive oxygen species (e.g. radiolytic
501 splitting of water) (An et al., 2013; Head et al., 2014). The final possibility is that the putative
502 aerobes observed are much more versatile in their use of electron acceptors than has hitherto been
503 appreciated. *Pseudomonas* spp., while classically thought of as catabolically versatile aerobes or
504 facultative aerobes that can utilize NO_3^- and other oxidized nitrogen species as alternative electron
505 acceptors to oxygen, are probably more cosmopolitan in their use of electron acceptors than
506 commonly considered. *Pseudomonas aeruginosa* is known to use solid phase anodes in a MFC as
507 an electron acceptor by using phenazine electron shuttles (Rabaey et al., 2005). The shuttles
508 produced by *Pseudomonas* could also be used by non-electroactive microorganisms, increasing thus
509 the diversity of the microbial communities in BES.
510 This still poses a dilemma regarding the mechanism of hydrocarbon activation in anoxic BES by
511 microorganisms that use molecular oxygen requiring mono- and dioxygenase systems. Some
512 evidence related to the occurrence of hydrocarbon activation mechanisms that might be relevant to
513 BES have been offered by studies of methanogenic crude oil and alkane degradation. In one
514 methanogenic crude oil-degrading system inoculated with oil reservoir production water, the
515 possibility has recently been raised that *Pseudomonas* spp. may have a role in hydrocarbon
516 fermentation coupled to methanogenesis by syntrophic interactions with methanogens (Berdugo-
517 Clavijo and Gieg, 2014). In this study, a methanogenic oil degrading consortium, dominated by a

518 *Smithella* sp., a candidate alkane-fermenting microorganism, and a range of acetoclastic and CO_2-
519 reducing methanogens, was inoculated into anoxic sand columns containing residual oil, and
520 incubated in an anoxic chamber for over 300 days. The sand columns actively degraded crude oil
521 hydrocarbons and generated methane. When the microbial communities in the sand columns were
522 analysed, their composition had changed considerably with CO_2-reducing methanogens
523 predominating in the community. However the most intriguing observation was that the most highly
524 represented bacterial taxon was a *Pseudomonas* sp. (Berdugo-Clavijo and Gieg, 2014) While the
525 authors were measured in their interpretation that this *Pseudomonas* sp. might represent the
526 syntrophic hydrocarbon-degrading partner of the methanogens in the culture, the results do raise the
527 intriguing possibility that some *Pseudomonas* spp. have evolved to occupy a niche whereby they are
528 active under highly reducing conditions. This study implies that, provided that the identified
529 *Pseudomonas* spp. strain is indeed capable of alkane fermentation, it must harbour metabolic
530 pathways that allow activation of alkanes in the absence of oxygen. As noted above a number of
531 *Pseudomonas* spp. have the capacity to synthesise and utilize soluble electron shuttles that could be
532 involved in electron transfer either to methanogenic partners in syntrophic consortia, or equally, in
533 the presence of an anode as an alternative electron acceptor, it may use the anode.
534 Pure cultures were not the only microbial inocula used during the bioelectrochemical oxidation of
535 hydrocarbons. Many studies reported the anodic oxidation of hydrocarbons in a BES by using
536 mixed cultures as microbial inocula (Table 2). Although, few studies characterized the microbial
537 communities that developed in the reactors during the treatment, they suggest that both the presence
538 of recalcitrant substrates and the availability of alternative electron acceptors may affect the
539 selection of the microbial populations. Iron reducers are often described in the anodic communities
540 that develop in BES reactors fed with readily biodegradable substrates, such as acetate (Daghio et
541 al., 2015a; Kiely et al., 2011; Zhu et al., 2014). However, they seem to play only a marginal role
542 during hydrocarbon degradation in BES. This implies that the recalcitrant character of the substrate
543 drives other mechanisms than direct electron transfer to an electrode (Lu et al., 2014a; Rakoczy et

544 al., 2013). Morris and colleagues (2009) found that NO_3^- reducing bacteria dominated the anodic
545 community in a single chamber MFC in which diesel was degraded with simultaneous current
546 production (Morris et al., 2009). The high abundance of bacteria capable of nitrate reduction,
547 however, could be attributed to the MFC architecture used in this study. The air cathode used could
548 have allowed small amount of oxygen to diffuse into the reactor, thus providing favourable
549 conditions for facultative anaerobes such as some NO_3^- reducers. In a recent study aerobes and
550 facultative anaerobes were enriched on the anode in a single chamber MFC during diesel removal
551 together with anaerobic bacteria (Venkidusamy et al., 2016). This observation suggested that
552 different niches were present in the reactor. In other investigations, microbial communities
553 dominated by microorganisms belonging to the *Chloroflexi* and to the *Nitrospira* were described
554 during PAHs degradation (Yan et al., 2012). A recent detailed analysis performed after remediation
555 of TPH-contaminated soil with a BES highlighted that *Proteobacteria* (especially
556 *Betaproteobacteria* and *Gammaproteobacteria*) was the most abundant phylum after the treatment
557 (Lu et al., 2014a). Among the *Betaproteobacteria* and *Gammaproteobacteria* the most abundant
558 genera were *Bordetella*, *Comamonas* and *Pseudomonas*. While some species of the last two genera
559 have been described as exoelectrogens, microorganisms belonging to the genus *Bordetella* are not
560 typically reported in electroactive communities. This further indicates that in the presence of
561 complex and recalcitrant contaminants, factors other than the ability to actively transfer electrons to
562 the anode affect the selection of specific microbial populations. When an easily biodegradable
563 carbon source is provided (e.g. acetate), the electrodes are colonized by microorganisms which have
564 more efficient external electron transfer pathways; conversely, if the carbon source is recalcitrant,
565 microorganisms possessing less efficient external electron transfer pathways, but able to oxidize the
566 electron donor, might be favoured. This observation was highlighted in recent studies in which a
567 key role of microorganisms involved in the sulfur cycle was suggested during hydrocarbon
568 oxidation in marine sediments and BES (Cruz Viggi et al., 2015; Daghio et al., 2016). The role of
569 the sulfur cycle in the process will be reviewed in the next section.

570 Similar to the studies on bioelectrochemical oxidation of hydrocarbons, pioneer studies on the
571 bioelectrochemically mediated reduction of chlorinated hydrocarbons used pure cultures (Table 3).
572 The first studies used *Geobacter lovleyi* as a biocatalyst (Aulenta et al., 2009; Strycharz et al., 2008)
573 showing that *Geobacter* spp. are able to use electrodes both as electron acceptors and electron
574 donors. The most extensively tested microorganisms in dechlorinating BES belong to the genus
575 *Dehalococcoides* (Table 3). To date, *Dehalococcoides* spp. are the only known microorganisms
576 able to completely dechlorinate TCE to ethene (Maymó-Gatell et al., 2001; West et al., 2008);
577 indeed, the presence of these bacteria was confirmed by FISH analysis both in the communities
578 attached to the cathode and in the medium, thus demonstrating that the cells are likely active and
579 involved in TCE dechlorination in BES (Aulenta et al., 2010b, 2009). The presence of
580 *Dehalococcoides* on the cathode seems to be correlated with the electrode potential. The first
581 enzymes involved in the electron transport chain in this microorganism during chloroethene
582 reduction were suggested to be hydrogenases (Aulenta et al., 2010a). These enzymes typically work
583 close to the redox potential of the couple H_2/H^+ (- 414 mV at pH 7) (Armstrong et al., 2009),
584 consistent with experimental data in a *Dehalococcoides* enriched mixed community (Aulenta et al.,
585 2009). At higher potentials, other electron transfer pathways might be involved, leading to the
586 enrichment of different groups, such as *Desulfitobacterium* spp. (Aulenta et al., 2010a). These
587 findings were confirmed by the results of a recent study in which a detailed characterization of the
588 cathodic communities involved in TCE dechlorination by CARD-FISH was performed.
589 *Dehalococcoides* was the dominant genus in the range from - 550 to - 750 mV; conversely, an
590 unidentified member of the *Chloroflexi* phylum outcompeted the *Dehalococcoides* when the
591 cathode potential was higher (i.e. from - 250 to - 450 mV) (Di Battista et al., 2012). Another study
592 showed that *Dehalococcoides* were the main planktonic bacterial cells in the cathodic chamber
593 during dechlorination of 1,2-DCA in a bioelectrochemical system, but about 40% of the bacterial
594 cells collected from the electrode remained unidentified (Leitão et al., 2015).
595

596 *5.1. Effect of sulfur cycle*

597 Sulfate, one of the most abundant electron acceptors in marine sediments (in seawater up to 28 mM)
598 (Thauer et al., 2007), has been estimated to support the mineralization of about 50% of the organic
599 matter deposited in continental shelves (Jørgensen, 1982). During the last two decades, hydrocarbon
600 degradation under sulfate-reducing conditions gained interest due to several *in vitro* and *in-situ*
601 studies suggesting the potential of this process for the removal of hydrocarbons in the marine
602 environment (Coates et al., 1997; Hayes et al., 1999; Widdel et al., 2010) (Equation 1).

603

604 Equation 1

605 $C_{16}H_{34}$ (Hexadecane) + 12.25 SO_4^{2-} + 8.5H^+ → 16HCO_3^- + 12.25H_2S + H_2O (Mbadinga et al.,
606 2011)

607

608 The bioremediation and biodegradation of spilled hydrocarbons in marine systems has been
609 extensively studied in the aftermath of the deepest and largest offshore spill in US history which
610 occurred in the Gulf of Mexico in April 2010 (i.e. the BP Deepwater Horizon spill) (Atlas and
611 Hazen, 2011; Kimes et al., 2013; King et al., 2015; Rodriguez-R et al., 2015). Genes and
612 metabolites involved in anaerobic hydrocarbon degradation were identified in a metagenomic study
613 of cores collected near the MC-252 wellhead (Kimes et al., 2013). Many of the reads from the
614 metagenomes were related to sequences from known anaerobic hydrocarbon degraders (e.g.
615 *Desulfatibacillum alkenivorans* AK-01, a sulfate-reducing, hydrocarbon-degrading
616 *Deltaproteobacterium*) (So and Young, 1999).
617 There is increasing evidence that the role of sulfur cycle in the anaerobic degradation of crude oil in
618 some sulfate-containing environments is complex and that other groups of microorganisms in
619 addition to sulfate reducing bacteria (SRB) may play a role. For example, an anaerobic microcosm
620 approach used to investigate crude oil biodegradation under sulfate-reducing conditions showed
621 degradation of C_7-C_{34} alkanes concomitant with sulfate removal over 300 days in oil-amended

622 microcosms (Sherry et al., 2013). Microbial community analysis of 16S rRNA genes in the oil-
623 amended microcosms that were actively reducing sulfate identified sequences from
624 *Gammaproteobacteria* most closely related to *Marinobacterium* sp. and members of the family
625 *Peptostreptococcaceae* within the *Firmicutes* at the highest frequency, rather than conventional
626 SRB (Sherry et al., 2013). Furthermore, a broad survey of microbial community data from a range
627 of oil and hydrocarbon-impacted anoxic environments, demonstrated that *Firmicutes* were the most
628 commonly detected followed by the *Gamma*-, *Delta*- and *Epsilonproteobacteria* (Gray et al., 2010).
629 In a further study where the focus was solely on microbial communities in petroleum reservoirs the
630 findings were remarkably similar, with *Firmicutes* followed by *Gamma*-, *Epsilon*- and
631 *Deltaproteobacteria* being most common (Hubert et al., 2012).
632 In marine sediments, it has been estimated that 80 to 90% of the sulfide produced by sulfate
633 reduction (Equation 1) is re-oxidized to sulfate through sulfur compounds of intermediate oxidation
634 state (Zopfi et al., 2004).
635 Complex interactions within the sulfur cycle have also been highlighted during the
636 electrobioremediation process (Figure 4). In sulfide rich groundwater treated in a MFC bioreactor,
637 aerobic hydrocarbon degraders from the order *Burkholderiales* were enriched probably due to a
638 small amount of oxygen that diffused into the reactor and this process was speculated to be coupled
639 with sulfide oxidation to sulfate and sulfur occurring at the anode (Rakoczy et al., 2013). In another
640 experiment, during bioelectrochemical toluene degradation, members of the *Desulfobulbaceae* and
641 *Desulfobacteraceae* outcompeted other microbial populations, originating from a contaminated
642 marine sediment, because of their potential to degrade hydrocarbons while an anode and sulfate
643 both acted as electron acceptors (Daghio et al., 2016). In parallel, sulfate in anoxic conditions is
644 reduced to sulfide which can be oxidized to elemental sulfur on an anodic surface. Sulfide oxidation
645 on anodes can be either a chemical process (Dutta et al., 2008) or a biological process, as suggested
646 by the enrichment of *Desulfobulbaceae* (Daghio et al., 2016; Rakoczy et al., 2013). Indeed, some
647 members of the family *Desulfobulbaceae* are the so called cable bacteria, which are able to oxidize

sulfide in marine sediment and to deliver the electrons over long distances (Pfeffer et al., 2012; Schauer et al., 2014). Sulfur may then be reduced again to sulfide which can be reoxidized and this cycle can go on and on, enhancing the current production (Dutta et al., 2009). Another possible mechanism is the back oxidation of sulfur to sulfate (Zhang et al., 2014), which provides a continuous source of electron acceptors for SRB. In the above mentioned processes sulfide can serve as an electron shuttle to deliver the electrons from the microorganisms to the electrode (Dutta et al., 2009; Rakoczy et al., 2013).

These observations open new possibilities for the application of the electrobioremediation processes. Sulfide present in solution can be oxidized on an anode to elemental sulfur which can be further oxidized to sulfate (Dutta et al., 2008; Zhang et al., 2014) leading to a reduction in toxicity due to sulfide removal. Experimental data suggest that competition between the anode and iron minerals as a sink for sulfide has to be considered. Recent results indicated that when using an anode as electron acceptor, iron sulfide as well as elemental sulfur deposition occurred during toluene removal in a sulfate rich environment (Daghio et al., 2016). Further research is required to determine if the use of electrodes for sulfide removal (Figure 4) can be a useful strategy in contaminated environments and to what degree the electrode competes with sulfide removal via a reaction with iron minerals.

6. *In-situ* monitoring and sensing

The use of BES technology as a monitoring tool in the field of bioremediation provides an interesting outlook in addition to the stimulation of contaminants removal. Many studies have tried to assess the correlation between the electrical output and the bioelectrochemical degradation of organic matter. The electrical current is proportional to the number of electrons that flow into the circuit per unit time (Equation 2):

Equation 2

674 1A = 1C / 1s

675

676 where A = ampere; C = coulomb, s = second. The charge exchanged in the reaction (C) is given by
677 the number of electrons transferred (n) and Faraday's constant (F; 9.64853 x 10^4 C/mol) (Equation
678 3):

679

680 Equation 3

681 C = n x F

682

683 The current into the circuit should therefore be proportional to the rate at which the electrons are
684 transferred to the electrode by the oxidation reaction (for the anodic process), or diverted by the
685 reduction reaction (for the cathodic process). Hypothesizing that only one metabolic process is
686 occurring on the electrode surface, the produced current can thus be used as a real time parameter to
687 quantify the rate of a specific metabolic process (Prévoteau et al., 2015) or a measure of the
688 substrate concentration available for microbial degradation. Previous studies demonstrated that the
689 current production can be correlated to COD and BOD and that a relationship can be described by
690 Monod type kinetics (Kim et al., 2003; Kumlanghan et al., 2007; Min and Logan, 2004). Tront and
691 colleagues found that acetate concentration in the range 0-2.3 mM was correlated with current
692 production in a MFC inoculated with a pure culture of *Geobacter sulfurreducens*. Increasing acetate
693 concentration above 2.3 mM did not lead to an increase in the electrical signal, probably due to
694 limitations attributed to system design parameters (Tront et al., 2008a). The same relationship was
695 observed in a MFC inoculated with *Shewanella oneidensis* and fed with lactate (0-41 mM) (Tront et
696 al., 2008b). These data indicate that, in the range of concentrations tested, the substrate
697 consumption is governed by first order kinetics. However, further studies on degradation kinetics
698 are required, in order to apply BES based biosensors for monitoring concentrations of recalcitrant
699 compounds (e.g. hydrocarbons). A recent study showed that a MFC based biosensor was an

effective alternative to measure the extent of bioremediation *in-situ*. Graphite anodes were installed in an aquifer where U(VI) bioreduction was stimulated by supplying acetate. The current was correlated with uranium removal and the indigenous microorganisms in the aquifer were able to colonize the electrode responding quickly to the change in the electron donor concentration (Williams et al., 2010).

BES based biosensors could easily be installed in existing monitoring well networks which would decrease both the number of analyses and the time required to assess microbial activity (Tront et al., 2008a). However, the presence of a background signal generated by the oxidation of carbon sources other than the contaminant should be considered, which could be addressed by placing control biosensors outside of the contaminated area. An alternative solution to eliminate the interference of side reactions could be the development of enzymatic biosensors with a high selectivity for the target contaminants.

An innovative approach for monitoring TPH removal by resistivity survey in sandy soil treated with BES has been recently suggested. A decrease in TPH concentration was linked to an increase in soil conductivity (Mao et al., 2016). It is thus possible to hypothesize that a complementary monitoring approach with geoprobes and BES based biosensors can be a successful strategy.

7. Advantages of bioelectrochemical processes

There are several advantages of BES-based approaches for the stimulation of *in-situ* bioremediation (Table 4). One of the main benefits lies in the fact that the conversion of contaminants can be manipulated by altering the potential of the anode or of the cathode. The energy levels can be set, thus setting favourable thermodynamic conditions for the reaction and adapting to *in-situ* circumstances. Furthermore, the flux of electrons can be maintained stably for extended time periods. For example during the stimulation of reductive dechlorination with BES, the H_2 delivery rate in a contaminated site can be controlled and thus the negative effects of side reactions of overdosing organics (e.g. CH_4 production) will decrease (Aulenta et al., 2011, 2010b, 2009). The

726 possibility of a constant electron flow implies that the electrode can serve as a virtually
727 inexhaustible electron acceptor/donor, lowering the operational costs because continuously
728 supplying electron acceptors/donors is not needed (Morris and Jin, 2008). Furthermore, since no
729 chemical injection is required, the expenses and need for transport and storage are eliminated
730 (Rabaey and Keller, 2008). Overall, this makes the bioelectrochemical approach a cleaner and
731 cheaper process compared to traditional strategies (Aulenta et al., 2009, 2008c).
732 It is important to note that a complete evaluation of the cost associated to the application of BES for
733 bioremediation is not possible yet due to the lack of experimental data on large scale plants. It is
734 reasonable to hypothesize that the main installation cost for *in-situ* treatment can be associated to
735 the cost of electrode materials which can vary between 30-50 euro m^{-2} up to 500-1000 euro m^{-2} if a
736 stable anode (i.e. DSA) is used. However this cost must be considered together with the lifetime of
737 the components, which has never been evaluated in real applications. In terms of operational costs
738 of an *in-situ* bioremediation plant, the example of PCE dehalogenation to ethane can be considered.
739 This reaction requires 8 mol of e^- for each mol of PCE. If 1 V is needed, with 100 W a constant
740 current of 100 A is achieved, leading to ~ 89 (mol e^-) d^{-1} available for the dechlorination without
741 considering possible side processes. It is thus possible to reduce ~ 10 (mol PCE) d^{-1} (i.e. 1.66 kg d^{-1})
742 with 2.4 kWh with 2.4 kWh energy input which translates to direct operational cost of about 0.24
743 euro (considering 0.1 euro / kWh). If however an *ex-situ* treatment is needed, a considerable higher
744 cost for pumping is incurred but these would be similar to traditional methods.
745 Another important advantage of BES-based approaches is the selectivity that can be achieved
746 compared to physicochemical strategies that may lead to the formation of products with greater
747 toxicity than the parent contaminants. For example, the conversion of nitrobenzene to aniline (a less
748 toxic and more degradable compound) was achieved using a biocathode. The study obtained a
749 reduction of almost 99 % of nitrobenzene to aniline without the production of nitrosobenzene, a
750 more toxic contaminant (Wang et al., 2011) which was produced during the purely electrochemical
751 reduction (Mu et al., 2009).

752 An increase in the efficiency of the process can also be reached when graphite electrodes, or
753 electrodes, made from other carbon materials are used. Hydrocarbons can be adsorbed onto the
754 electrode thus increasing the contaminant concentration in a highly metabolically active area that is
755 directly associated with the electron acceptor/donor and the biocatalyst (Rabaey and Keller, 2008;
756 Zhang et al., 2010).
757 In some contaminated sites biodegradation of pollutants could be limited by the lack of
758 microorganism carrying the required metabolic pathways. In such cases the addition of selected
759 microbial populations can be a suitable strategy (El Fantroussi and Agathos, 2005). Persistence of
760 the inoculated microorganisms however can be limited due to competition with indigenous
761 microbial communities, predation and/or unfavourable environmental conditions (Careghini et al.,
762 2015; Daghio et al., 2015b). BES-based approaches may facilitate bioaugmentation by enabling
763 microbial inoculum to persist by using electrodes pre-colonized with acclimated populations
764 (Venkidusamy et al., 2016).
765 In terms of process monitoring, the electric signal generated in a BES may be used as a real time
766 measurement of the *in-situ* microbial activity in order to gain information about degradation rates
767 (Tront et al., 2008a; Williams et al., 2010). The energy harvested could also be used to power other
768 electrical sensors for *in-situ* monitoring (Lovley, 2006; Shantaram et al., 2005).
769 As well as the advantages previously reported, the use of BES-based approaches for the removal of
770 oil spills can also have disadvantages. The main drawback during the oxidation of hydrocarbons is
771 that aerobic degradation is usually a faster process compared to biodegradation in the absence of O_2,
772 because of the more efficient activation of oxygenases during the first step of the pathway (Weelink
773 et al., 2010). Furthermore, when an MFC is used to stimulate oxidation of organic contaminants the
774 efficiency of the kinetic process at the cathode is crucial and may limit the efficiency of the device
775 and the oxidation rate at the anode, as demonstrated by early studies on BES (Liu and Logan, 2004;
776 Zhao et al., 2006). Conversely, when using a MEC in a marine environment, care should be taken
777 not to produce chlorine gas which is potentially more toxic than the original contaminant, and can

chemically react with organic and inorganic compounds producing toxic chemicals, similar to the generation of disinfection by products that are known to be produced during drinking water treatment (Richardson et al., 2007).

Other issues, such as the choice of appropriate materials and potentials, or the effect of the radius of influence and limitations during scale-up should not be overlooked during electrobioremediation feasibility studies.

Although the discussion in this review is more focused on *in-situ* application of BES for electrobioremediation of oil spills, it could be possible to develop bioelectrochemical reactors for *ex-situ* treatment (e.g. groundwater can be extracted and treated above ground). *Ex-situ* treatment typically allows a better control of the conditions and a faster removal of the contaminants, nevertheless the operational cost increases due to energy consumption for the extraction of the contaminated matrix (Alvarez and Illman, 2006), likely decreasing the advantages of BES technology.

8. Future research perspectives

Future research directions should perhaps be directed towards adjustment of physicochemical conditions *in-situ* to promote the activity of organisms involved in the bioelectrochemical removal of contaminants. Bacterial activity is affected by pH and most microorganisms have an optimum at circum-neutral pH (Alvarez and Illman, 2006), however in contaminated sites acidic or alkaline conditions may occur (Bamforth and Singleton, 2005). In such environments, adjusting the pH could be a strategy to improve the bioremediation process. Caustic or acid can be generated *in-situ* by water electrolysis however, proton and oxygen generation occur simultaneously as well as hydroxyl and hydrogen gas production (Lin et al., 2016). Changes in pH can also affect the availability of alternative electron acceptors such as Fe^{3+} which may affect bioelectrochemical anode reduction reactions.

803 BES could also be used to change the chemical equilibrium by scavenging metabolites that
804 accumulate during the biodegradation of contaminants and which may inhibit further degradation.
805 An example can be represented by sulfide scavenging as previously reported (Daghio et al., 2016;
806 Dutta et al., 2008; Rakoczy et al., 2013; Zhang et al., 2014). However other mechanisms of
807 metabolite scavenging are worthy of investigation. A worthwhile course of action may be to insert
808 an anode, colonized for example with *Geobacter* spp., that is able to efficiently scavenge reducing
809 equivalents (H_2) from a syntrophic reaction, thus removing a thermodynamic barrier (Dolfing,
810 2014; Sun et al., 2012).
811 Recently, it has been reported that bioelectrogenic bacteria on the anodes of sediment MFC can use
812 plant root exudates as substrates leading to several beneficial effects, such as electricity production
813 (Kaku et al., 2008; Schamphelaire et al., 2008) or reduction of greenhouse gas emissions (Arends et
814 al., 2014). The combined treatment with the macrophyte *Acorus Calamus* and MFC for the
815 degradation of pyrene and benzo[*a*]pyrene in contaminated sediments has also been proposed. The
816 combination of phytoremediation and a MFC led to a higher degradation rate compared to
817 phytoremediation or MFC treatment alone (Yan et al., 2015). This provides a promising opportunity
818 to test the effect of a combined treatment phytoremediation-BES also with other substrates.
819 Another innovative opportunity to use BES for the bioremediation comes from the possibility to
820 drive the production of organic compounds (e.g. acetate) at the cathode (Logan and Rabaey, 2012).
821 As already mentioned, H_2 is the typical substrate for reductive dehalogenation but it was suggested
822 that in some case also acetate may be used (Aulenta et al., 2006; He et al., 2002). Acetate
823 production could thus be driven at the cathode, providing electron donors for the reduction of
824 halogenated contaminants. In such strategy however, attention should be paid to side processes (e.g.
825 acetate consumption by other microorganisms such as sulfate reducers) which may cause a low
826 dehalogenation efficiency.
827
828 *8.1. Field scale application of bioelectrochemical systems*

829 Despite considerable scientific interest, field scale bioelectrochemical systems have not yet been
830 tested and verified under fully representative conditions and concerns have also been raised
831 regarding their actual scalability (Table 4). Although the scalability of a bioelectrochemical system
832 poses some intrinsic challenges, lessons can be learned from pure electrochemical processes where
833 hundreds of small units provide high production rates and volumes. Indeed, to date a number of
834 consolidated electrochemical remediation technologies are commercially available and widely
835 applied for *in-situ* treatment of a variety of inorganic and organic pollutants (Trombly, 1994). These
836 technologies are not typically used for the direct degradation of the contaminants but apply low-
837 voltage direct current, by means of electrode arrays, to favour the electrokinetic movement of
838 pollutants from soils into "treatment zones" where they are removed from the water by adsorption,
839 immobilization, or (bio)degradation. Electric current flows between pairs of anodes and cathodes
840 suitably deployed in the contaminated subsurface environments (e.g., the vadose zone of an
841 aquifer). In such systems, the applied current density is in the range of a few Amperes per square
842 meter (A m^{-2}), driven by a potential difference that is typically in the order of a few Volts per
843 centimetre (V cm^{-1}). One relevant example is the "Lasagna Process" (Ho et al., 1995), developed by
844 a consortium of industries (DuPont, General Electrics, and Monsanto) in collaboration with US
845 Federal Agencies (EPA; DOE), providing large-scale demonstrations of the feasibility of the
846 technology for *in-situ* treatment of a variety of contaminants, including TCE (Ho et al., 1999a,
847 1999b).
848 In principle, similar configurations could also be adopted for bioelectrochemical remediation
849 systems. Along this line, one of the first designs being proposed involves the use of non-corrosive
850 carbon-based electrodes (i.e. graphite granules) placed within the contaminated aquifer to form
851 permeable reactive barriers (PRB) intercepting (and treating) the contamination plume. Here, the
852 granules serve both as support material for biofilm formation and as electron donors or acceptors
853 for contaminant degradation. In theory, by placing an anodic-PRB downgradient of a cathodic-PRB
854 it would be possible to achieve a sequential reductive-oxidative treatment (Figure 5) which is

855 critical to achieve complete degradation (and detoxification) of certain classes of subsurface
856 contaminants, such as chlorinated solvents, or when multiple contaminants are simultaneously
857 present (e.g. metals and hydrocarbons).
858 This sequential (cathodic-anodic) treatment has, however, some intrinsic limitations. Indeed, the
859 distance between cathode and anode should be kept as short as possible to minimize voltage losses
860 due to the Ohmic resistance of groundwater. As an example, assuming a typical groundwater
861 conductivity of 1,000 $\mu S\ cm^{-1}$ and a current density of 1 $A\ m^{-2}$ of electrode surface area, the Ohmic
862 loss would be 10 V for each meter of distance between cathode and anode (Arends et al., 2014;
863 Rozendal et al., 2008). Based on this calculation, it is obvious that electrode spacing higher than a
864 few meters would ultimately result in unacceptable energy inputs. Alternatively, if the
865 bioremediation process is driven by water electrolysis (H_2 generation at the cathode and O_2
866 generation at the anode), maintaining a certain spacing between electrodes is essential to prevent the
867 back diffusion of oxygen to the cathode which could lead to the inhibition of anaerobic
868 microorganisms thriving at the cathode. In principle, a better scenario would probably result from
869 the stimulation of reductive and oxidative degradation pathways via direct electron transfer since
870 lower power inputs would be involved, as well as, no (or minimal) H_2 and O_2 would be produced.
871 To overcome these limitations, a number of alternative configurations are presently being
872 considered (e.g. involving concentric electrodes), whereby the spacing between electrodes is kept as
873 small as possible without adversely affecting process performance.
874

875 **9. Conclusions**

876 The electrobioremediation of oil spills is a rapidly growing field. The potential of this innovative
877 technology for the bioremediation of a variety of hydrocarbons, both halogenated and non-
878 halogenated compounds, is revealed by several studies. The main issue to be faced in the near future
879 will be the scale-up of this technology from lab scale reactors to field scale systems. This will allow

880 a better comparison of the electrobioremediation strategies with current technologies used for *in situ*
881 bioremediation.
882
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891
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Figure captions

Figure 1 – Oil fate after a spill in marine environments. A) The non-soluble compounds form a non-aqueous phase on the surface. The light compounds evaporate. The soluble fraction slowly dissolve in water, is dispersed in the water column and the heavy compounds reach the seafloor where can be degraded by microorganisms. In the oxic zone (water column and sediment surface) aerobic degradation takes place. Deeper in the sediment anoxic and anaerobic biodegradation (becomes the prevalent mechanisms (red arrows). B) Detail of microbial metabolisms in the sediment. The electron acceptors with higher redox potential are consumed close to the sediment surface. The order in which the electron acceptors are consumed in an idealised system is $O_2 > NO_3^- > Fe^{3+} > SO_4^{2-} > CO_2$.

Figure 2 - Hydrocarbon contaminated groundwater treated by air sparging. Air is injected below the water table to supply oxygen and to stimulate aerobic biodegradation.

Figure 3 - Scheme of a typical BES. In the anodic compartment a non-halogenated hydrocarbon is oxidized to CO_2. In the cathodic chamber an halogenated hydrocarbon is reduced. The two chambers are separated by a Cation Exchange Membrane (CEM). The electron exchange is reported with the flash, the metabolic reactions that lead to the oxidation/reduction of the contaminants are reported with the dashed lines. The electron transfer mechanisms with the electrode involve both abiotic reactions (shuttles and H_2 production) or biotic reactions (use of pili or outer membrane cytochromes).

Figure 4 - Possible role of sulfur cycle during hydrocarbon degradation in BES. A) Sulfate reducers oxidize hydrocarbons and reduce S_xO_y to HS^-. B) HS^- can be oxidized to S_0 on the anodic surface. C) S_0 can then be reduced to HS^- or D) back oxidized to S_xO_y forming a cycle.

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1449 Figure 5 - Scheme of a possible configuration for a sequential electro-reductive-oxidative treatment.
1450 The cathode is placed upstream the anode and it is used to stimulate the reductive dehalogenation of
1451 tetrachloroethene (PCE) and trichloroethene (TCE). The reduced compounds flow downstream to
1452 the anode where the oxidation can take place.

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1454 **Tables**

1455

1456 Table 1 - Advantages and disadvantages of *in situ* bioremediation approaches for oil spill removal.

Bioremediation approach	Process stimulated	Advantages	Disadvantages	Reference
Aerobic degradation (O_2 supplying)	Oxidation	Lower cost compared to physicochemical technologies Fast growth of microorganisms and high biodegradation rate due to the high potential of the couple O_2/H_2O (+820 mV *vs*. SHE) A wide range of contaminants can be attacked by oxygenases	High energy input Possible O_2 consumption by side reactions (e.g. Fe^{2+} oxidation, which causes aquifer clogging) O_2 can diffuse away from the reaction area	(Alvarez and Illman, 2006; Broden et al., 1997; Tuxen et al., 2006; Zhang et al., 2010)
Use of alternative electron acceptors in anaerobic conditions (e.g. NO_3^-, SO_4^{2-})	Oxidation	Lower cost compared to physicochemical technologies No side reactions that can consume the electron acceptor	Slower growth rate compared to aerobic degradation Soluble electron acceptors can diffuse away from the reactive area SO_4^{2-} may result in production of toxic HS^- NO^{3-} could lead to eutrophication of surface water	(Alvarez and Illman, 2006; Anderson and Lovley, 2000; Rivett et al., 2008; Zhang et al., 2010)
Use of H_2 releasing compounds (e.g. lactic acid, propionic acid)	Reduction	Lower cost compared to physicochemical technologies The contaminants are transformed into less toxic and more degradable compounds	Detrimental effects on groundwater quality due to the accumulation of fermentation products (e.g. VFA) Competition of dehalogenating microorganisms and other microorganisms (e.g. SO_4^{2-} reducers, methanogens) for H_2 Possible aquifer clogging due to high biomass growth Explosion hazards due to CH_4 accumulation	(Aulenta et al., 2007a, 2007b; Zanaroli et al., 2015)

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1458 Table 2 - Summary of the key studies regarding oxidation in a BES for the remediation of oil spills.

Compound / mixture	Microorganism / mixed culture	Redox mediator*	Working electrode material	Medium treated	Working electrode potential (*vs*. SHE)**	Maximum current / power / voltage	Removal capability	Reference
Diesel	Mixed culture dominated by NO_3^- - reducing bacteria (i.e. *Citrobacter* sp., *Pseudomonas* sp.and *Stenotrophomonas* sp.)	Not detected	Stainless steel scrubber	Refinery wastewater and mineral medium (50:50)	N.A.	120 mW m^{-2} of cathode	N.A.	(Morris and Jin, 2008)
	Mixed culture	Not detected	Stainless steel scrubber	Phosphate buffer and diesel contaminated groundwater (1:1)	N.A.	60 - 65mV	82 % removal within 21 days from 176 mg L^{-1}	(Morris et al., 2009)
	Mixed culture rich in γ-*Proteobacteria*	Not detected	Carbon fibre brush	Mineral medium	N.A.	114.54 mA m^{-2}	93.5 ± 0.6 % removal from 8000 mg L^{-1} within 30 days	(Venkidusamy et al., 2016)
Toluene	*Geobacter metallireducens*	Not detected	Unpolished graphite rod	Mineral medium	+ 500 mV	1 mA	N.A.	(Zhang et al., 2010)
	Mixed culture	Not detected	Unpolished graphite rod	Sediment-seawater slurry (1:4)	+ 500 mV	N.A.	100 % removal from 10 µM	(Zhang et al., 2010)
	Pseudomonas putida F1	Not detected***	Graphite rod	Mineral medium	+ 325 mV	23 mA m^{-2}	80 % removal within 147 hours after five additions (100 mg L^{-1} each)	(Friman et al., 2012)
	Mixed culture	Not detected	Carbon cloth	Mineral medium	N.A.	53.5 mV	100 % removal within 16.2 hours from 11.09 mg L^{-1}	(Lin et al., 2014)
	Mixed culture	Neutral red (100-300 µM)	Carbon cloth	Mineral medium	N.A.	109.7 mV (200 µM neutral red)	100 % removal within 34.1 ± 0.05 hours from 11.09 mg L^{-1}	(Lin et al., 2014)
	Mixed culture	Ferricyanide (100-2000 µM)	Carbon cloth	Mineral medium	N.A.	88.2 mV (300 µM ferricyanide)	100 % removal within 25.3 hours from 11.09 mg L^{-1} (500 µM ferricyanide)	(Lin et al., 2014)
	Mixed culture dominated by SO_4^{2-} reducers (i.e.	Not detected	Graphite plate	Artificial sea water	+ 200 mV	301 mA m^{-2}	~ 1 mg L^{-1} d^{-1}	(Daghio et al., 2016)

	Desulfobulbaceae and *Desulfobacteraceae*)							
	Mixed culture dominated by SO_4^{2-} reducers (i.e. *Desulfobulbaceae* and *Desulfobacteraceae*)	Self produced mediator (+400 mV *vs* SHE)	Graphite plate	Artificial sea water	+ 500 mV	431 mA m^{-2}	~ 1 mg L^{-1} d^{-1}	(Daghio et al., 2016)
Benzene	Mixed culture	Not detected	Unpolished graphite rod	Sediment-seawater slurry (1:4)	+ 500 mV	N.A.	100 % removal from 9 µM	(Zhang et al., 2010)
	Mixed culture dominated by δ-*Proteobacteria* (i.e. *Desulfobacteraceae*, *Desulfobulbaceae* and *Geobacteraceae*)	Not detected	Graphite fibers	Benzene and sulfide contaminated groundwater	N.A.	550 µA	18 - 80 % removal from 150-250 µM	(Rakoczy et al., 2013)
	Mixed culture	Not detected	Carbon cloth	Mineral medium	N.A.	45.2 mV	100 % removal within 150 hours from 10.87 mg L^{-1}	(Wu et al., 2013)
	Mixed culture	Not detected	Graphite granules	Contaminated groundwater	N.A.	316 mW m^{-3}	80 % removal from ~15 mg L^{-1} with an hydraulic retention time of 27 hours	(Wei et al., 2015)
	Mixed culture	Not detected	Carbon felt	Mineral medium	N.A.	1.15 ± 0.18 mW m^{-2} at 40 °C	510 ± 5.67 µM d^{-1} at 40 °C	(Adelaja et al., 2015)
Naphthalene	Mixed culture	Not detected	Unpolished graphite rod	Sediment-seawater slurry (1:4)	+ 500 mV	N.A.	~ 100 % removal within 9 days from 100 µM	(Zhang et al., 2010)
TPH	Mixed culture	Not detected	Graphite plate	Domestic sewage	N.A.	53.11 mW m^{-2}	41 ± 3 % within 17 days	(Venkata Mohan and Chandrasekhar, 2011)
	Mixed culture	Not detected	Graphite plate	Domestic sewage	N.A.	53.11 mW m^{-2}	N.A.	(Chandrasekhar and Venkata Mohan, 2012)
	Mixed culture	Not detected	Stainless steel brush	Contaminated sediment	N.A.	190 mV	24.4 % removal within 66 days from 15,958 mg kg^{-1}	(Morris and Jin, 2012)
	Mixed culture	Not detected	Carbon mesh	Contaminated soil	N.A.	0.85 ± 0.05 mW m^{-2}	15.2 ± 0.6 % removal within 25 days	(Wang et al., 2012)
	Mixed culture dominated by β-*Proteobacteria* (e.g.	Not detected	Carbon cloth	Contaminated soil	N.A.	73.0 ± 0.1 mA	73.1 % removal within 64 days from 11.46 g kg^{-1}	(Lu et al., 2014a)

	Bordetella sp.) and γ-*Proteobacteria* (e.g. *Pseudomonas*)							
	Mixed culture dominated by β-*Proteobacteria* (e.g. *Comamonas* sp.) and γ-*Proteobacteria* (e.g. *Pseudomonas*)	Not detected	Biochar	Contaminated soil	N.A.	85.9 ± 0.1 mA m^{-2}	78.7 % removal within 64 days from 11.46 g kg^{-1}	(Lu et al., 2014a)
	Mixed culture	Not detected	Graphite granules	Contaminated soil	N.A.	70.4 ± 0.2 mA m^{-2} (8.8 ± 0.3 mW m^{-2})	82.1–89.7 % removal within 120 days from 12.25 ± 0.36 g kg^{-1}	(Lu et al., 2014b)
	Mixed culture	Not detected	Biochar	Contaminated soil	N.A.	35.2 ± 0.8 mA m^{-2} (3.4 ± 0.1 mW m^{-2})	82.1–89.7 % removal within 120 days from 12.25 ± 0.36 g kg^{-1}	(Lu et al., 2014b)
	Mixed culture dominated by α-*Proteobacteria*, γ-*Proteobacteria* and δ-*Proteobacteria*	Not detected	Graphite rod	Marine sediment	N.A.	N.A.	21 ± 1 % removal within 200 days from 11.9 ± 0.12 g kg^{-1}	(Cruz Viggi et al., 2015)
	Mixed culture	Not detected	Carbon mesh	Contaminated soil	N.A.	37 mW m^{-2}	N.A.	(Li et al., 2014)
	Mixed culture	Not detected	Carbon mesh	Contaminated soil	N.A.	0.282±0.015 V	12.5 ± 0.6 % removal within 135 days from 25.7 g kg^{-1}	(Zhang et al., 2015)
	Mixed culture	Not detected	Carbon mesh	Contaminated soil	N.A.	0.28 ± 0.00 mA m^{-2} g^{-1} soil (2.76 ± 0.07 10^{-4} mW m^{-2} g^{-1} soil)	22 ± 0.5 % removal within 135 days	(Li et al., 2015)
Phenantrene	Mixed culture dominated by *Nitrospira* sp and *Chloroflexi*	Not detected	Stainless steel (mesh)	Freshwater sediment	N.A.	17.1 ± 3.8 mV (average over 240 days)	0.0836 d^{-1} (0-22 days) with the addition of FeOOH	(Yan et al., 2012)
	Pseudomonas aeruginosa	Not detected	Carbon felt	Mineral medium	N.A.	0.19 ± 0.05 mW m^{-2}	54.70 ± 0.60 μM d^{-1}	(Adelaja et al., 2014)
	Shewanella oneidensis	Not detected	Carbon felt	Mineral medium	N.A.	0.51 ± 0.03 mW m^{-2}	25.20 ± 5.15 μM d^{-1}	(Adelaja et al., 2014)
	Mixed culture	Not detected	Carbon felt	Mineral medium	N.A.	0.37 ± 0.05 mW m^{-2}	35.70 ± 2.73 μM d^{-1}	(Adelaja et al., 2014)
	Mixed culture	Not detected	Carbon felt	Mineral medium	N.A.	1.15 ± 0.18 mW m^{-2} at 40 °C	320 ± 4.81 μM d^{-1} at 40 °C	(Adelaja et al., 2015)

Pyrene	Mixed culture dominated by *Nitrospira* sp and *Chloroflexi*	Not detected	Stainless steel (mesh)	Freshwater sediment	N.A.	17.1 ± 3.8 mV (average over 240 days)	0.1363 d^{-1} (0-22 days) with the addition of FeOOH	(Yan et al., 2012)
Phenol	Mixed culture	Not detected	Carbon felt	Waterlogged soil	N.A.	2.1 mA	0.390 d^{-1}	(Huang et al., 2011)
	Cupriavidus basilensis	Self produced mediator (+140 mV *vs* SHE)	Graphite rod	Mineral medium	+ 325 mV	478 mA m^{-2}	0.36 mg L^{-1} h^{-1}	(Friman et al., 2013)
1,2-DCA	Enrichment form MFCs reactors	Not detected	Graphite granules	Mineral medium	NA	0.17 ± 0.02 mA	45.6 ± 0.5 mg L^{-1} d^{-1}	(Pham et al., 2009)

* The midpoint potential is reported in brackets
** The potential at which the working electrode was poised is reported
*** A redox active moiety (putative cathecol) was detected but the midpoint potential was higher than the anodic potential

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1461 Table 3 - Summary of the key studies targeting reduction in a BES for the remediation of oil spills.

Compound	Microorganism / mixed culture	Redox mediator*	Working electrode material	Medium treated	Working electrode potential (*vs* SHE)**	Maximum current / voltage	Removal capability	Reference
TCE	Mixed culture containing *Dehalococcoides* spp.	Methyl viologen (100 µM)	Glassy carbon	Mineral medium	- 500 mV	20 µA	N.A.	(Aulenta et al., 2007a)
	Mixed culture containing *Dehalococcoides* spp.	Methyl viologen	Glassy carbon	Mineral medium	- 800 mV	N.A.	N.A.	(Aulenta et al., 2007a)
	Mixed culture containing *Desulfitobacterium* sp. and *Dehalococcoides* spp.	Methyl viologen (25-7500 µM)	Glassy carbon	Mineral medium	- 450 mV	~250 µA (methyl viologen 500 µM)	N.A.	(Aulenta et al., 2008b)
	Mixed culture containing *Dehalococcoides* spp.	Not detected	Unpolished graphite rod	Mineral medium	- 650 mV	N.A.	3.73 ± 0.02 µeq h^{-1}	(Aulenta et al., 2008c)
	Mixed culture containing *Desulfitobacterium* sp. and *Dehalococcoides* spp.	Self produced mediator (-400 mV *vs* SHE)	Carbon paper	Mineral medium	- 450 mV	N.A.	N.A.	(Aulenta et al., 2009)
	Geobacter lovleyi	Not detected	Carbon paper	Mineral medium	- 450 mV	N.A.	N.A.	(Aulenta et al., 2009)
	Mixed culture containing *Dehalococcoides* spp.	AQDS (50-1500 µM)	Glassy carbon	Mineral medium	- 250mV	N.A.	180 ± 23 µeq L^{-1} d^{-1} (AQDS 1500 µM)	(Aulenta et al., 2010a)
	Mixed culture containing *Desulfitobacterium* sp. and *Dehalococcoides* spp.	Self produced mediator (-550 mV *vs* SHE)	Carbon paper	Mineral medium	- 550mV	20 µA	22.4 µeq L^{-1} d^{-1}	(Aulenta et al., 2010b)
	Mixed culture dominated by *Chloroflexi* (unidentified *Chloroflexi* and *Dehalococcoides* spp.)	Not detected	Graphite granules	Mineral medium	- 250mV	15.0 ± 0.8 µA	15.5 ± 1.2 µeq L^{-1} d^{-1}	(Aulenta et al., 2011; Di Battista et al., 2012)
	Mixed culture dominated by *Chloroflexi* (unidentified *Chloroflexi* and *Dehalococcoides* spp.)	Not detected	Graphite granules	Mineral medium	- 450mV	N.A.	58 ± 1 µeq L^{-1} d^{-1}	(Aulenta et al., 2011; Di Battista et al., 2012)
	Mixed culture dominated by *Chloroflexi* (*Dehalococcoides* spp.)	Not detected	Graphite granules	Mineral medium	- 550 mV	266 ± 5 µA (average)	62 ± 2 µeq L^{-1} d^{-1}	(Aulenta et al., 2011; Di Battista et al., 2012)

	Mixed culture dominated by *Chloroflexi* (*Dehalococcoides* spp.)	Not detected	Graphite granules	Mineral medium	- 650 mV	N.A.	N.A.	(Aulenta et al., 2011; Di Battista et al., 2012)
	Mixed culture dominated by *Chloroflexi* (*Dehalococcoides* spp.)	Not detected	Graphite granules	Mineral medium	- 750 mV	N.A.	N.A.	(Aulenta et al., 2011; Di Battista et al., 2012)
	Mixed culture containing *Dehalococcoides* spp.	Not detected	Graphite granules	Mineral medium	-250 mV	N.A.	~71 % removal from 35 µM (flow rate 0.4 mL min^{-1})	(Verdini et al., 2015)
	Mixed culture containing *Dehalococcoides* spp.	Not detected	Graphite granules	Mineral medium	-350 mV	N.A.	~97 % removal from 35 µM (flow rate 0.4 mL min^{-1})	(Verdini et al., 2015)
	Mixed culture containing *Dehalococcoides* spp.	Not detected	Graphite granules	Mineral medium	-450 mV	N.A.	~90 % removal from 35 µM (flow rate 0.4 mL min^{-1})	(Verdini et al., 2015)
PCE	*Geobacter lovleyi*	Not detected	Unpolished graphite rod	Mineral medium	- 300 mV	N.A.	~ 25 µmol d^{-1} from 100 µmol	(Strycharz et al., 2008)
	Mixed culture containing *Desulfitobacterium* sp. and *Dehalococcoides* spp.	Not detected	Stainless steel mesh	Mineral medium	NA	0.05 mA cm^{-2} (applied)	~ 23 µmol d^{-1} from 24 - 45 µmol L^{-1}	(Lohner and Tiehm, 2009)
	Mixed culture containing *Desulfitobacterium* sp. and *Dehalococcoides* spp.	Not detected	Stainless steel mesh	Mineral medium / Contaminated groundwater	NA	0.5 mA (applied)	100 % removal with a load of 1.5 µmol d^{-1} in mineral medium***	(Lohner et al., 2011)
cis-DCE	Mixed culture containing *Desulfitobacterium* sp. and *Dehalococcoides* spp.	Self produced mediator (-550 mV *vs* SHE)	Carbon paper	Mineral medium	- 550 mV	2 µA	1.5 µeq L^{-1} d^{-1}	(Aulenta et al., 2010b)
	Mixed culture	Not detected	Graphite granules	Contaminated groundwater	-550 mV	N.A.	N.A.	(Lai et al., 2015)
	Mixed culture	Not detected	Graphite granules	Contaminated groundwater	-650 mV	N.A.	N.A.	(Lai et al., 2015)
	Mixed culture	Not detected	Graphite granules	Contaminated groundwater	-750 mV	N.A.	4.89 ± 0.46 µM residual concentration (14.2 ± 0.7 µM influent concentration)	(Lai et al., 2015)
1,2-DCA	Mixed culture containing *Dehalococcoides* spp.	Not detected	Graphite rods	Mineral medium	- 300 mV	N.A.	10 ± 4 µeq L^{-1} d^{-1}	(Leitão et al., 2015)

	Mixed culture containing *Dehalococcoides* spp.	Not detected	Graphite rods	Mineral medium	- 500 mV	N.A.	N.A.	(Leitão et al., 2015)
	Mixed culture containing *Dehalococcoides* spp.	Not detected	Graphite rods	Mineral medium	- 600 mV	N.A.	N.A.	(Leitão et al., 2015)
	Mixed culture containing *Dehalococcoides* spp.	Not detected	Graphite rods	Mineral medium	- 700 mV	N.A.	24.3 ± 17.5 μeq L^{-1} day^{-1}	(Leitão et al., 2015)
	Mixed culture containing *Dehalococcoides* spp.	Not detected	Graphite rods	Mineral medium	- 900 mV	N.A.	37 ± 10 μeq L^{-1} d^{-1}	(Leitão et al., 2015)
Nitrobenzene	Mixed culture	Not detected	Carbon cloth	Mineral medium	N.A.	0.5 V (applied)	Over 99 % from 0.5 mM	(Wang et al., 2011)
	Mixed culture	Not detected	Carbon cloth	Mineral medium	N.A.	0.3 V (applied)	0.135 ± 0.015 h^{-1} (with 2.78 mM of glucose)	(Liang et al., 2014)

* The midpoint potential is reported in brackets
** The potential at which the working electrode was poised is reported
*** A sequential reductive – oxidative treatment was performed

1463 Table 4 - Summary of the advantages and the disadvantages of electrobioremediation for oil spill

1464 removal.

Advantages	Disadvantages
The energy level and the flux of electrons can be set and maintained constant	Anaerobic degradation is usually slower compared to aerobic degradation
The process is clean	When an MFC is used the cathodic reaction may limit the anodic reaction
The operational cost is lower	Chlorine gas can be produced in marine environments
High selectivity towards the target compounds	The scale-up of the technology is challenging
Hydrocarbons can be adsorbed on the electrodes when graphite (or carbon) is used	The process may be affected by pH changes that can occur in a contaminated site or close to the electrodes (e.g. H^+ production at the anode and H^+ consumption at the cathode)
Electrodes can be used to improve the bioaugmentation efficiency	
The electrical signal can be used as a monitoring tool	

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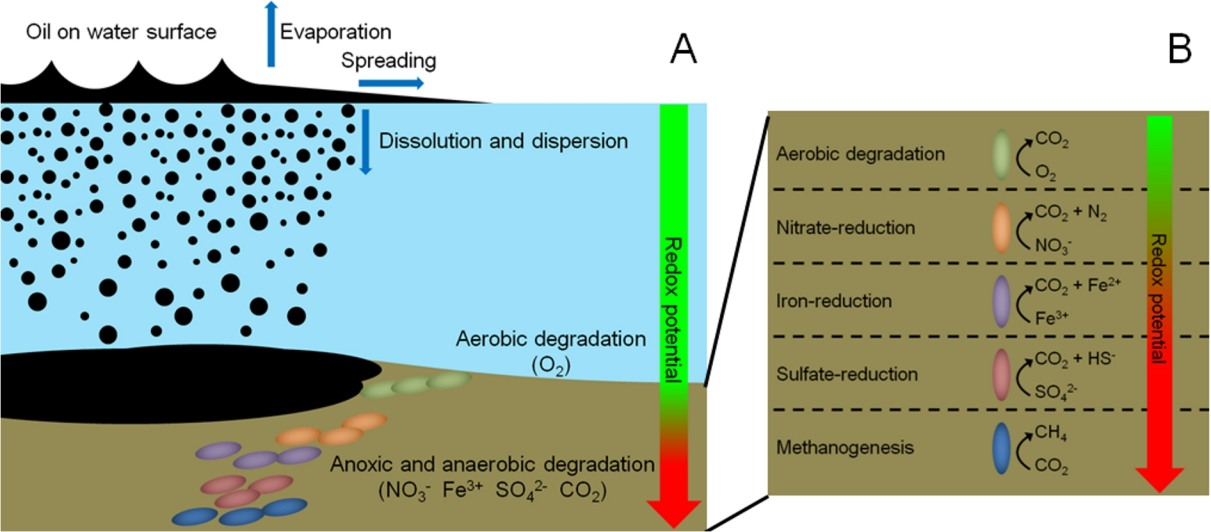
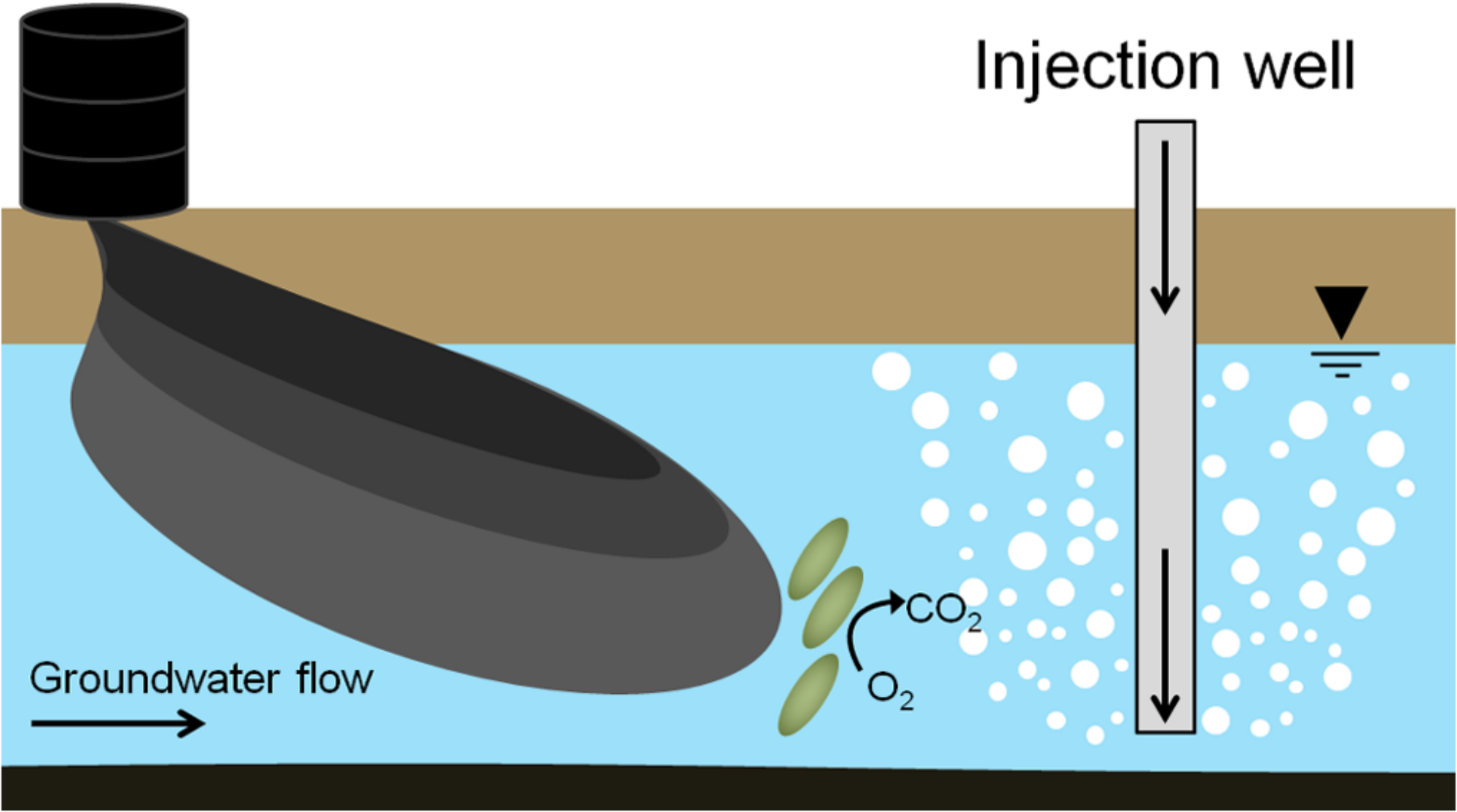
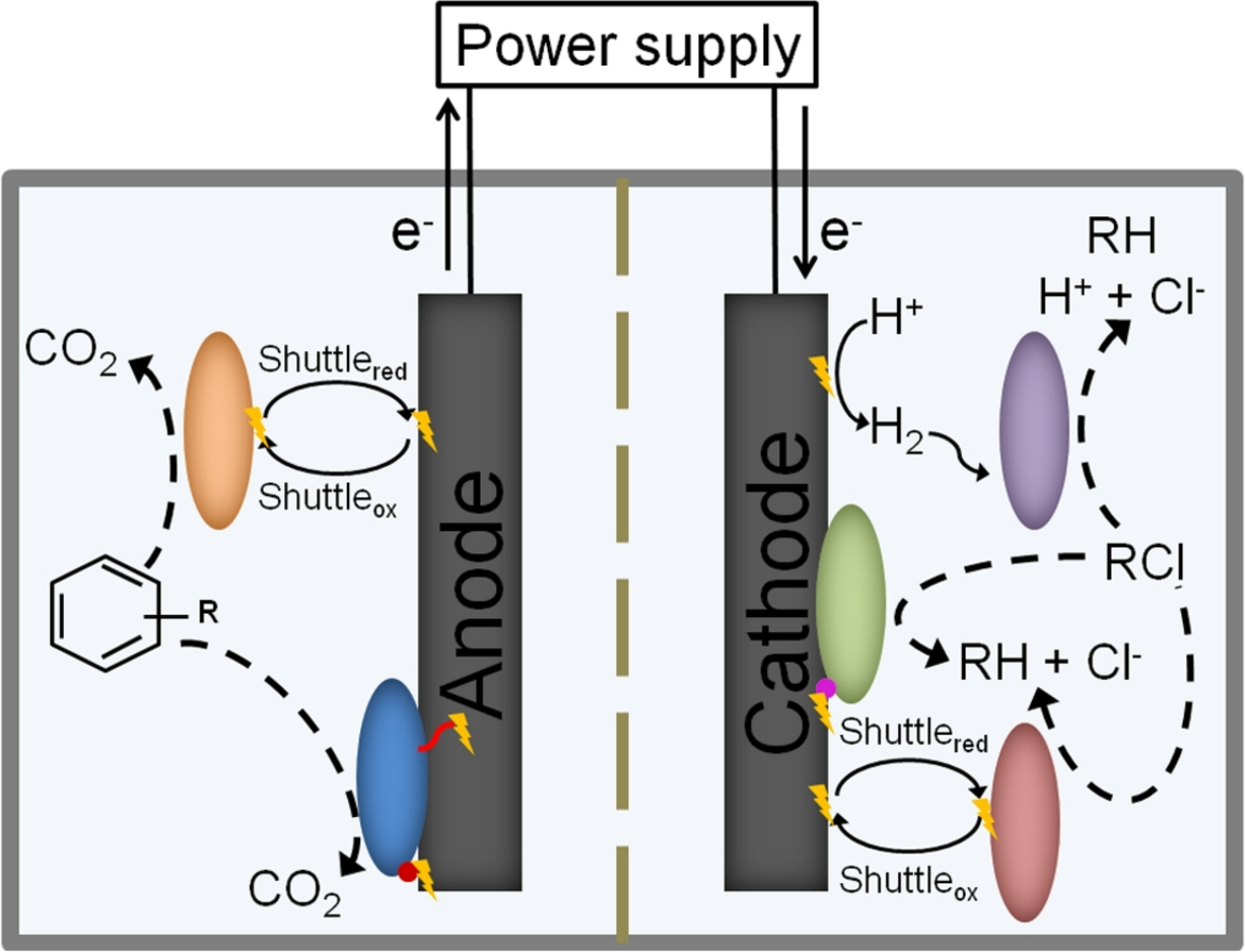
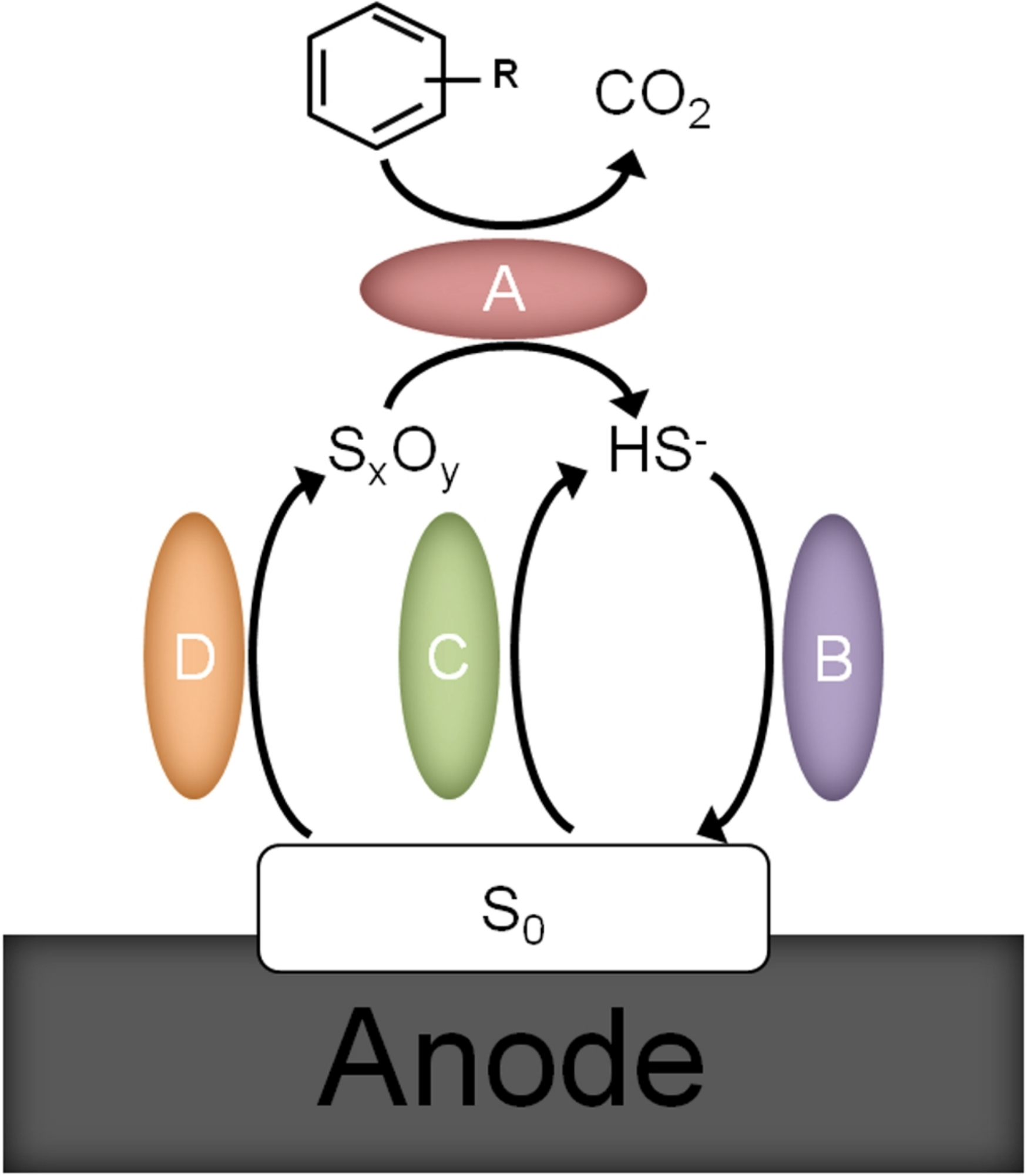
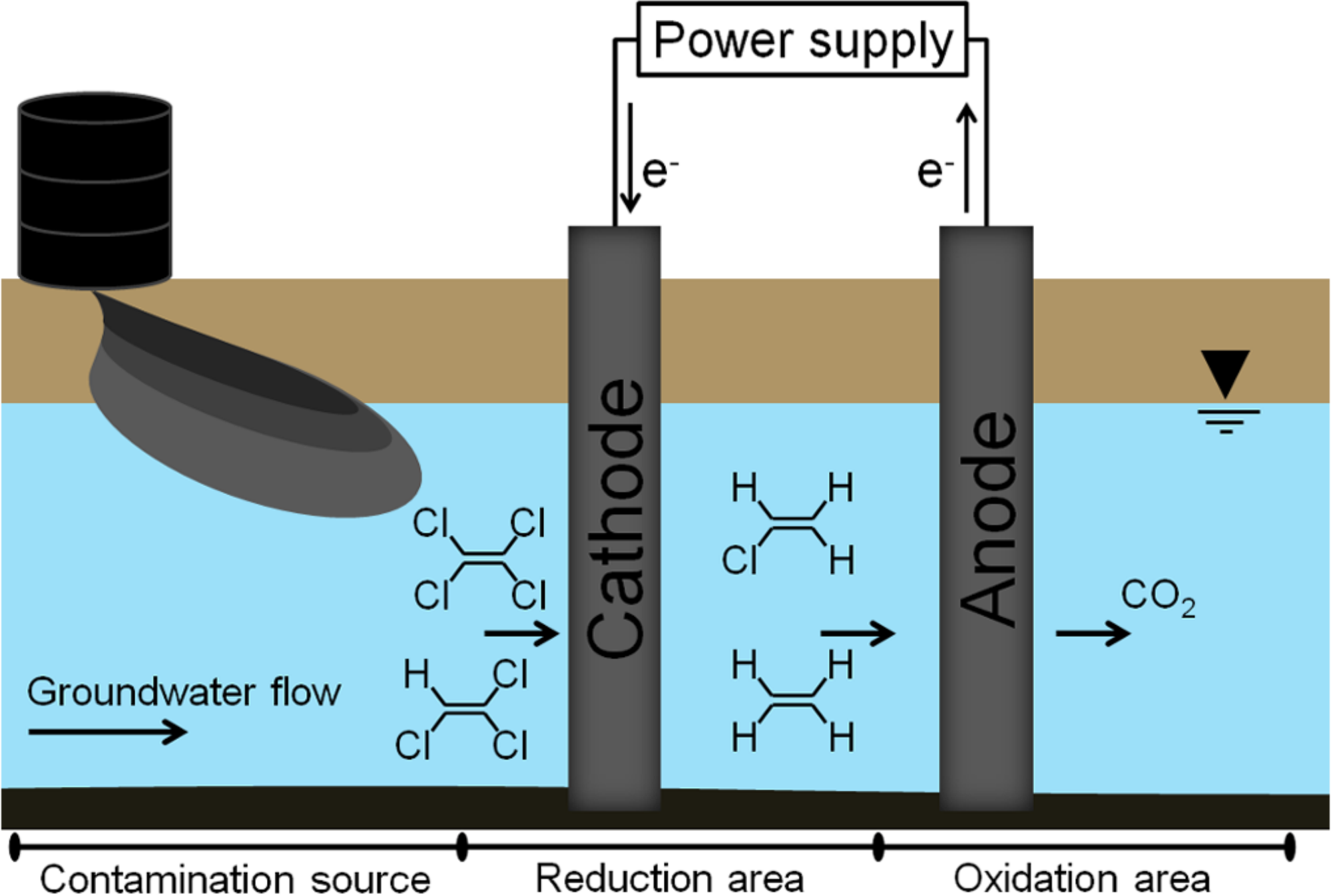
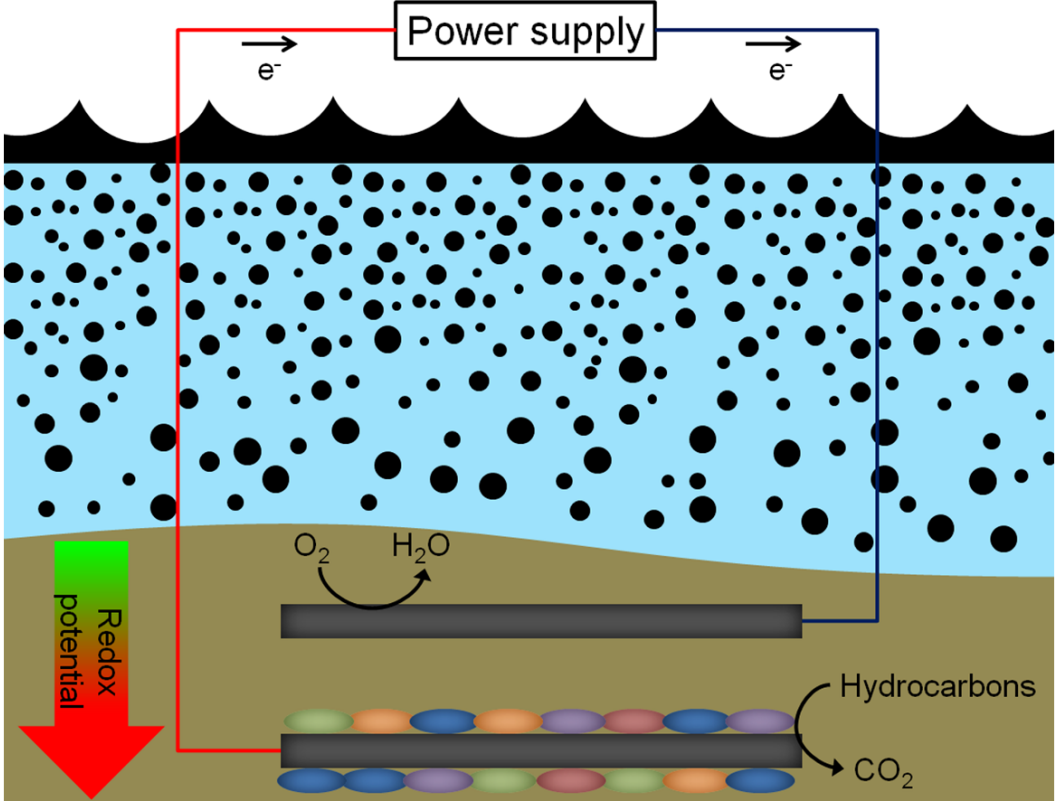

Oil on water surface
Evaporation
Spreading
A
Dissolution and dispersion
Aerobic degradation
(O_2)
Anoxic and anaerobic degradation
(NO_3^- Fe^{3+} SO_4^{2-} CO_2)
Redox potential
B
Aerobic degradation
CO_2
O_2
Nitrate-reduction
$CO_2 + N_2$
NO_3^-
Iron-reduction
$CO_2 + Fe^{2+}$
Fe^{3+}
Sulfate-reduction
$CO_2 + HS^-$
SO_4^{2-}
Methanogenesis
CH_4
CO_2
Redox potential


Injection well
CO_2
O_2
Groundwater flow


Power supply
e-
e-
Anode
Cathode
CO2
Shuttlered
Shuttleox
R
CO2
H+
H2
RH
H+ + Cl-
RCl
RH + Cl-
Shuttlered
Shuttleox


R
CO_2
A
S_xO_y
HS^-
D
C
B
S_0
Anode


Power supply
e-
e-
Cathode
Anode
Groundwater flow
CO2
Contamination source
Reduction area
Oxidation area


Power supply
e-
e-
O2
H2O
Redox potential
Hydrocarbons
CO2