- 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
groundwater ecotoxicity (Aulenta et al., 2007b). H₂ can be consumed, not only by dechlorinating bacteria, but also by other H₂ consuming microorganisms, such as methanogens, homoacetogens and sulfate reducers, thus lowering the efficiency of the process (Aulenta et al., 2007a, 2007b; Zanaroli et al., 2015). Experimental evidence, however, suggested that dechlorinating microorganisms can outcompete other H₂ consumers at low H₂ concentrations (Aulenta et al., 2008a; Smatlak et al., 1996; Yang and McCarty, 1998) (e.g. the half-velocity constants (K_s) measured for dechlorination and methanogenesis are 100 ± 50 nM and 960 ± 180 nM (Smatlak et al., 1996), respectively).

Recently, bioelectrochemical systems (BES) have been suggested as an alternative strategy to overcome some of the limitations of the current bioremediation technologies (Wang et al., 2015). The use of benthic BES has recently been reviewed (Li and Yu, 2015), however sediments are not the only environmental matrix that can be treated with a BES-based approach. BES can also be used effectively for the bioremediation of soils and water.

In this article the state of the art of this innovative approach for the bioremediation of oil spills in soil, sediment and water will be extensively reviewed. The scope is to investigate the potential of BES to remove oil spills, elucidating: (i) the key parameters that influence the process; (ii) the main advantages and limitations; (iii) the microorganisms and the biological processes involved; (iv) future research opportunities to improve understanding and field application of BES-based bioremediation approaches.

2. Bioelectrochemical systems

A BES uses microorganisms to catalyze redox reactions on or near electrodes (Logan et al., 2006). A typical reactor design consists of an anode and a cathode separated by an ion conductive matrix (Figure 3). Microorganisms can interact with the electrodes either by direct contact (e.g. microorganisms directly exchange electrons with an electrode) or via an indirect mechanism where a chemical compound acts as an electron shuttle (Figure 3). These electron shuttles can be secreted
by the microorganisms (e.g. phenazines of *Pseudomonas* spp.) (Rabaey et al., 2005) or can be added
exogenously (Logan et al., 2006).

A BES can be used to generate power (microbial fuel cell; MFC) by linking anodic oxidation of a
reduced substrate to cathodic reduction of a high potential electron acceptor (e.g. oxygen) (Logan et
al., 2006). It can also function with addition of power to drive the desired reaction (microbial
electrolysis cell; MEC) (Rozendal et al., 2006). A microbial anode in combination with a biological
or chemical cathode can be implemented to achieve production of H₂ in a MEC. This system
requires about 3 times less potential difference compared to a conventional chemical electrolysis
cell, in which the reactions at the electrodes are not mediated by microorganisms (Rozendal et al.,
2006). When operating a MEC, there are two electrical control strategies (i) operation at a fixed
potential (Rozendal et al., 2006) or (ii) operation at a fixed current (Andersen et al., 2013).

Operation at a fixed potential has the advantage that a desired reaction can be driven, or favourable
conditions for a certain (bio)catalyst can be created. The drawback is that reaction rates at the
electrode are controlled by the (bio)catalyst and not by the operator. Operation of a MEC at a fixed
current allows the operator to control the reaction rates but not the type of reaction that occurs. This
can be used, for example, when the aim is to produce large amounts of oxygen or hydrogen.

### 3. Bioelectrochemical processes for oil spill remediation

#### 3.1. Anodic oxidation and oxygen generation

The microbial mediated anodic oxidation of organic compounds in BES was initially applied as a
technology to reduce Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD)
in domestic wastewater, with concomitant energy production (Logan et al., 2006). Recently, a
number of studies have exploited BES technologies to stimulate the anaerobic oxidation of
petroleum and petroleum-derived compounds (Table 2). The anode of a BES can be used to collect
electrons produced from the oxidation of organic contaminants. It can be buried in anoxic benthic
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 (Daghoio 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.
efficiency (Radjenovic and Sedlak, 2015). Specifically, under anaerobic conditions in a marine medium, the electrochemical potential of oxygen evolution can vary depending on seawater nature (e.g. salinity, ions species and concentration), presence of other water-soluble constituents from the sediment, and environmental conditions (e.g. pH and temperature) in addition to the electrode properties. High potential electrodes can be used to deliver oxygen in the anaerobic environment where it can act as final electron acceptor for microorganisms during biodegradation of contaminants. An example of electrochemical stimulated removal of organic contaminants via oxygen evolution has been recently reported for cis-dichloroethene (cis-DCE). A polarized graphite electrode (+ 1,500 mV) was able to successfully stimulate the removal of cis-DCE (85 µmol/L) when ethene was provided as a co-metabolic substrate (Aulenta et al., 2013). Furthermore in a recently published paper, water electrolysis was driven by applying an external voltage (2 V) on Dimensionally Stable Anodes (DSA; i.e. Ti mesh covered with mixed metal oxides, primarily consisting of Ir and Ru) to produce oxygen for the stimulation of TPH removal (20 g kg$^{-1}$) in marine sediments. After 202 days of operation TPH removal in the open circuit control was 44 ± 1 %, while a slightly higher removal (i.e. 58 ± 3 %) was observed when oxygen was produced by constantly applying 2 V (Bellagamba et al., 2016).

3.2. Hydrogen generation and cathodic reduction

The reduction reactions at the cathode in a BES can be exploited to reduce a large number of oxidized compounds such as metals (Tandukar et al., 2009; Xafenias et al., 2013) or halogenated compounds (Aulenta et al., 2007a; Kong et al., 2014). Several studies have reported the stimulation of microbial dechlorination by using a cathode as a direct electron donor or by in-situ H$_2$ production (Table 3). Early studies investigated the electrochemical production of H$_2$ as a suitable strategy for the dehalogenation of 2,6-dichlorophenol (2,6-DCP) by applying currents from -1 to -15 mA (Skadberg et al., 1999). The same technology was subsequently used for trichloroethene (TCE) removal (Aulenta et al., 2008c). The use of a BES is an innovative approach to produce in-situ H$_2$,
because the energy requirement is lower compared to a purely electrochemical process (Rosenbaum et al., 2011). However, in order to control the process and avoid side reactions that consume H₂ and lead to low efficiencies, direct electron uptake from the cathode, without the production of H₂, is a preferable strategy. Pioneering studies focused on TCE removal by using a redox mediator to promote electron transfer from the cathode to the biocatalyst, resulting in complete dechlorination of TCE via cis-DCE and vinyl chloride (VC) to ethene (Aulenta et al., 2007a). Subsequent studies suggested that TCE removal can be stimulated by direct electron transfer from the cathode (Aulenta et al., 2011, 2010a, 2010b, 2009, 2008b; Verdini et al., 2015). The more oxidized tetrachloroethene (PCE) was reduced to cis-DCE by a pure culture of Geobacter lovleyi able to utilise an electrode as sole electron donor (Strycharz et al., 2008). Whereas in a separate innovative study, PCE was reduced to ethene using H₂ from water electrolysis concomitant with the electrochemically produced O₂ which was used to stimulate the microbial oxidation of the dechlorination products (Lohner and Tiehm, 2009). Other studies have focused on the dechlorination of the reduced intermediates produced during TCE removal, such as cis-DCE (Aulenta et al., 2010b; Lai et al., 2015). Furthermore, the dechlorination of 1,2-DCA was achieved in a BES reactor inoculated with a mixed culture enriched in Dehalococcoides spp. (Leitão et al., 2015).

4. Effects of materials and operational parameters

4.1. Electrode material

The choice of an appropriate anodic material affects the selectivity and efficiency of the hydrocarbon removal process (Anglada et al., 2009). The electrode material should comply with the following properties: i) high physical and chemical stability, ii) high electrical conductivity, iii) catalytic activity and selectivity for the target compounds, and iv) low cost/life ratio. Inexpensive and long life service materials should be favoured for the oxidation of hydrocarbons. Oxidation reactions may act synergistically or compete with the side reaction of oxygen evolution at the 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).
4.2. Redox potential

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

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

Oxygen evolution is a process that could affect the electrobioremediation both by stimulating the activation of hydrocarbon, or by acting as a side reaction delivering electrons to the anode which are not linked to the oxidation of the contaminant, decreasing the efficiency. In this context it is important to consider the potential of the OER when the anodic potential is selected. Cyclic
voltammetry provides information on oxidation potentials and can assist in determining the appropriate anode potential for specific applications. For example, oxygen evolution commenced at around + 750 mV on a carbon felt anode when cyclic voltammetry was run both in artificial and real seawater at neutral pH at a scan rate of 5 mV s\(^{-1}\) (unpublished data from CMET, UGent, Belgium).

Cathode potential is an important parameter that can affect the performance of e.g. dechlorination of chlorinated hydrocarbons, by controlling competing reactions, particularly methanogenesis due to \(\text{H}_2\) evolution. Cathodic potentials from - 600 mV to - 800 mV can be applied to stimulate TCE dechlorination via \(\text{H}_2\) production. The best performance was achieved around - 650 mV. However, methane production was also stimulated in the same range of potentials thus reducing the efficiency of the dechlorination process (Aulenta et al., 2008c). A strategy to enhance efficiency of microbial reductive dechlorination and to eliminate side reactions could be to supply electrons directly from the electrode. The first evidence of this process was reported with a culture of *Geobacter lovleyi* that was able to reduce PCE to cis-DCE with an electrode poised at - 300 mV serving as electron donor (Strycharz et al., 2008). A further study demonstrated that mixed cultures could also use an electrode poised at - 450 mV as sole electron donor (Aulenta et al., 2009). A higher dechlorination rate was obtained by further decreasing the cathodic potential to - 550 mV (Aulenta et al., 2010b).

An accurate study of the effect of the electrode potential was performed using a continuous flow reactor. Five cathodic potentials ranging from - 250 mV to - 750 mV were tested without exogenous nor endogenous redox mediators. At - 250 mV the TCE dechlorination rate was low (15.5 ± 1.2 \(\mu\text{mol e}^-\cdot\text{L}^-1\)) but rapidly increased when the cathodic potential was decreased to - 450 mV (58 ± 1 \(\mu\text{mol e}^-\cdot\text{L}^-1\)). Decreasing the potential increased the \(\text{H}_2\) production rate, thus increasing the dechlorination rate. However, the coulombic efficiency of the dechlorination process was nearly 100 % at - 250 mV, but decreased to less than 1 % at - 750 mV, because methanogenesis acted as an electron sink (Aulenta et al., 2011). A similar result was observed during 1,2-DCA 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$^{-2}$.
respectively. No significant effects were shown on hydrocarbon removal that were almost
completely removed regardless of the presence of exogenous redox mediators (Adelaja et al., 2015).
Studies with exogenous redox mediators have also been performed during the bioelectrochemical
removal of halogenated hydrocarbons. It was demonstrated that with an electrode poised at - 500
mV dechlorination was stimulated only when methyl viologen (MV) was in the medium (Aulenta et
al., 2007a). When the potential was further decreased to -800 mV, H₂ was produced but the
dehlorination rate did not increase (Aulenta et al., 2007a). In another study at low MV
concentration (25-750 µM) only dechlorination was stimulated, but when the MV concentration
was increased up to 5,000 µM, H₂ was produced. It was suggested that at high MV concentrations
the rate at which the electrons were transferred to the microorganisms exceeded the electron
utilization for the dehalogenation process. The electrons were thus diverted to H₂ production via a
hydrogenase (Aulenta et al., 2008b). An alternative mediator is the humic acid analogue
antraquinone-2,6-disulfonate (AQDS). Humic acids are ubiquitous redox active compounds in the
environment and several studies have demonstrated their involvement in biodegradation processes
(Van der Zee and Cervantes, 2009). AQDS was successfully used to reduce TCE to cis-DCE but
was unable to further stimulate the dechlorination to vinyl chloride (Aulenta et al., 2010a).
As artificial mediators can be toxic and inhibitory to microbial activity, mediator-free BES are
preferable during bioremediation. Artificial mediators also pose some of the same disadvantages of
the soluble electron acceptors, as they could diffuse away from the reaction area and interact with
other processes therefore decreasing the efficiency.
Endogenously produced mediators have been detected during toluene degradation. Friman and
colleagues (2012) detected the presence of a redox active compound with a potential of + 470 mV.
The authors attributed the oxidation peak to 3-methyl catechol (Friman et al., 2012). Catechol and
3-methyl catechol are typical intermediates produced during aerobic toluene degradation and their
presence could be explained by a low levels of oxygen penetrating into the reactor, however, it is
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 H₂ 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
may be attributed to variations in the configuration of the bioelectrochemical systems, or to different characteristics of the soil used.

A recent study showed that the measured radius of influence can be up to 34 cm away from the electrode over a period of 120 days. Hydrocarbon degradation was initially observed close to the anode (1 cm), but the influence of the bioelectrochemical stimulation increased over time. The authors assumed a linear correlation between the distance from the anode and the enhanced TPH removal and predicted a maximum radius of influence of 90 cm after 45 days with a BES radius of 7.5 cm, corresponding to a ratio of maximum radius of influence to radius of BES equal to 12. This estimation, however, was not supported by experimental data at a distance greater than 34 cm from the electrode (Lu et al., 2014b). Other factors, such as ohmic losses, might lead to a non-linear correlation between TPH removal enhancement and the radius of influence over long distances (Arends et al., 2014; Logan et al., 2006), thus decreasing the predicted extension of the radius of influence. However, a correlation between the radius of influence and the radius of BES can be made (Lu et al., 2014b), suggesting that, with cylindrical electrodes, the radius of influence may be extended by increasing the radius of the electrode. Further studies can be conducted to better clarify this aspect in a real field bioremediation. The mass transfer of the chemical species (e.g. groundwater flow) could extend the radius of influence of the electrode, particularly if the function of the electrode is to generate a soluble electron donor or acceptor in situ.

It was proposed that by increasing the porosity of a soil (e.g. by the addition of sand) it is possible to enhance mass transfer and promote the performance of BES-driven TPH degradation. The 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 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 higher with 2:1 soil to sand ratio (22 ± 0.5 %) compared to 5:1 soil to sand ratio (15 ± 0.1 %) or no sand (12 ± 0.4 %). Bacteria from the genus *Alcanivorax*, known obligate hydrocarbon-degrading organisms, were also strongly enriched close to the air-cathode when soil was amended with sand, indicating that sand promoted the growth of hydrocarbon degrading bacteria (Li et al., 2015).
The reported studies clearly indicate that several factors may affect the radius of influence in field applications (e.g. electrode design, water content, soil type, mass transport) and these parameters may have to be evaluated in site specific conditions in order to obtain the best treatment efficiency.

5. Microbial communities in bioelectrochemical systems during oil spill remediation

Iron reducing bacteria were first used as an inoculum in an MFC containing hydrocarbons as an energy source (Zhang et al., 2010) (Table 2). One of the first studies showed the ability of Geobacter metallireducens to use graphite electrodes as electron acceptor for the degradation of toluene (Zhang et al., 2010). Recently, the degradation of phenanthrene by Shewanella oneidensis MR1 14063 in an MFC was reported (Adelaja et al., 2014). However, strict anaerobes are not the only microorganisms studied. Pseudomonas aeruginosa NCTC 10662 has been shown to degrade phenanthrene faster (54.7 µM d⁻¹) than Shewanella oneidensis MR1 14063 (25.2 µM d⁻¹) under similar operational conditions in a MFC (Adelaja et al., 2014). Other reports seem to indicate that aerobes and facultative anaerobes are able to oxidize hydrocarbons with an anode as sole electron acceptor. Cupriavidus basilensis was able to degrade phenol with an anode via electron transfer mediated by a self-produced shuttle (Friman et al., 2013); while the presence of the catabolic intermediate catechol was hypothesized during bioelectrochemical toluene degradation by Pseudomonas putida F1 (at oxygen levels of 0.78 mg O₂ L⁻¹) (Friman et al., 2012). This finding poses open questions regarding the degradation pathway and the role of oxygen for hydrocarbon degradation by Pseudomonas sp. with an anode. Indeed, microorganisms belonging to this genus are well-described hydrocarbon degraders in aerobic conditions and the hydrocarbon activation proceeds by addition of hydroxyl groups catalyzed by an oxygenase, requiring molecular oxygen (Jindrová et al., 2002). Whether aerobes and facultative anaerobes require a low concentration of oxygen for the activation reaction to couple hydrocarbon degradation to current production in a BES is therefore an interesting and important question. A first step in hydrocarbon biodegradation catalyzed by a monooxygenase followed by further anaerobic removal of the intermediate has also been proposed.
These findings are intriguing in the context of recent studies of hydrocarbon-rich environments that are considered to be primarily anoxic and have been subject to anaerobic hydrocarbon degradation, which does not require molecular oxygen. The widespread occurrence of microorganisms that would be typically considered aerobes in these environments (An et al., 2013) (including \textit{Pseudomonas} spp.) has been interpreted either as resulting from contamination with oxygen during sampling, contamination with exogenous organisms, oxygen ingress into these environments or the existence of mechanisms that lead to \textit{in-situ} generation of reactive oxygen species (e.g. radiolytic splitting of water) (An et al., 2013; Head et al., 2014). The final possibility is that the putative aerobes observed are much more versatile in their use of electron acceptors than has hitherto been appreciated. \textit{Pseudomonas} spp., while classically thought of as catabolically versatile aerobes or facultative aerobes that can utilize NO$_3^-$ and other oxidized nitrogen species as alternative electron acceptors to oxygen, are probably more cosmopolitan in their use of electron acceptors than commonly considered. \textit{Pseudomonas aeruginosa} is known to use solid phase anodes in a MFC as an electron acceptor by using phenazine electron shuttles (Rabaey et al., 2005). The shuttles produced by \textit{Pseudomonas} could also be used by non-electroactive microorganisms, increasing thus the diversity of the microbial communities in BES.

This still poses a dilemma regarding the mechanism of hydrocarbon activation in anoxic BES by microorganisms that use molecular oxygen requiring mono- and dioxygenase systems. Some evidence related to the occurrence of hydrocarbon activation mechanisms that might be relevant to BES have been offered by studies of methanogenic crude oil and alkane degradation. In one methanogenic crude oil-degrading system inoculated with oil reservoir production water, the possibility has recently been raised that \textit{Pseudomonas} spp. may have a role in hydrocarbon fermentation coupled to methanogenesis by syntrophic interactions with methanogens (Berdugo-Clavijo and Gieg, 2014). In this study, a methanogenic oil degrading consortium, dominated by a
Smithella sp., a candidate alkane-fermenting microorganism, and a range of acetoclastic and CO$_2$-reducing methanogens, was inoculated into anoxic sand columns containing residual oil, and incubated in an anoxic chamber for over 300 days. The sand columns actively degraded crude oil hydrocarbons and generated methane. When the microbial communities in the sand columns were analysed, their composition had changed considerably with CO$_2$-reducing methanogens predominating in the community. However the most intriguing observation was that the most highly represented bacterial taxon was a *Pseudomonas* sp. (Berdugo-Clavijo and Gieg, 2014) While the authors were measured in their interpretation that this *Pseudomonas* sp. might represent the syntrophic hydrocarbon-degrading partner of the methanogens in the culture, the results do raise the intriguing possibility that some *Pseudomonas* spp. have evolved to occupy a niche whereby they are active under highly reducing conditions. This study implies that, provided that the identified *Pseudomonas* spp. strain is indeed capable of alkane fermentation, it must harbour metabolic pathways that allow activation of alkanes in the absence of oxygen. As noted above a number of *Pseudomonas* spp. have the capacity to synthesise and utilize soluble electron shuttles that could be involved in electron transfer either to methanogenic partners in syntrophic consortia, or equally, in the presence of an anode as an alternative electron acceptor, it may use the anode.

Pure cultures were not the only microbial inocula used during the bioelectrochemical oxidation of hydrocarbons. Many studies reported the anodic oxidation of hydrocarbons in a BES by using mixed cultures as microbial inocula (Table 2). Although, few studies characterized the microbial communities that developed in the reactors during the treatment, they suggest that both the presence of recalcitrant substrates and the availability of alternative electron acceptors may affect the selection of the microbial populations. Iron reducers are often described in the anodic communities that develop in BES reactors fed with readily biodegradable substrates, such as acetate (Daghio et al., 2015a; Kiely et al., 2011; Zhu et al., 2014). However, they seem to play only a marginal role during hydrocarbon degradation in BES. This implies that the recalcitrant character of the substrate drives other mechanisms than direct electron transfer to an electrode (Lu et al., 2014a; Rakoczy et
al., 2013). Morris and colleagues (2009) found that NO$_3^-$ reducing bacteria dominated the anodic community in a single chamber MFC in which diesel was degraded with simultaneous current production (Morris et al., 2009). The high abundance of bacteria capable of nitrate reduction, however, could be attributed to the MFC architecture used in this study. The air cathode used could have allowed small amount of oxygen to diffuse into the reactor, thus providing favourable conditions for facultative anaerobes such as some NO$_3^-$ reducers. In a recent study aerobes and facultative anaerobes were enriched on the anode in a single chamber MFC during diesel removal together with anaerobic bacteria (Venkidusamy et al., 2016). This observation suggested that different niches were present in the reactor. In other investigations, microbial communities dominated by microorganisms belonging to the Chloroflexi and to the Nitrospira were described during PAHs degradation (Yan et al., 2012). A recent detailed analysis performed after remediation of TPH-contaminated soil with a BES highlighted that Proteobacteria (especially Betaproteobacteria and Gammaproteobacteria) was the most abundant phylum after the treatment (Lu et al., 2014a). Among the Betaproteobacteria and Gammaproteobacteria the most abundant genera were Bordetella, Comamonas and Pseudomonas. While some species of the last two genera have been described as exoelectrogens, microorganisms belonging to the genus Bordetella are not typically reported in electroactive communities. This further indicates that in the presence of complex and recalcitrant contaminants, factors other than the ability to actively transfer electrons to the anode affect the selection of specific microbial populations. When an easily biodegradable carbon source is provided (e.g. acetate), the electrodes are colonized by microorganisms which have more efficient external electron transfer pathways; conversely, if the carbon source is recalcitrant, microorganisms possessing less efficient external electron transfer pathways, but able to oxidize the electron donor, might be favoured. This observation was highlighted in recent studies in which a key role of microorganisms involved in the sulfur cycle was suggested during hydrocarbon oxidation in marine sediments and BES (Cruz Viggi et al., 2015; Daghio et al., 2016). The role of the sulfur cycle in the process will be reviewed in the next section.
Similar to the studies on bioelectrochemical oxidation of hydrocarbons, pioneer studies on the bioelectrochemically mediated reduction of chlorinated hydrocarbons used pure cultures (Table 3). The first studies used *Geobacter lovleyi* as a biocatalyst (Aulenta et al., 2009; Strycharz et al., 2008) showing that *Geobacter* spp. are able to use electrodes both as electron acceptors and electron donors. The most extensively tested microorganisms in dechlorinating BES belong to the genus *Dehalococcoides* (Table 3). To date, *Dehalococcoides* spp. are the only known microorganisms able to completely dechlorinate TCE to ethene (Maymó-Gatell et al., 2001; West et al., 2008); indeed, the presence of these bacteria was confirmed by FISH analysis both in the communities attached to the cathode and in the medium, thus demonstrating that the cells are likely active and involved in TCE dechlorination in BES (Aulenta et al., 2010b, 2009). The presence of *Dehalococcoides* on the cathode seems to be correlated with the electrode potential. The first enzymes involved in the electron transport chain in this microorganism during chloroethene reduction were suggested to be hydrogenases (Aulenta et al., 2010a). These enzymes typically work close to the redox potential of the couple H$_2$/H$^+$ (- 414 mV at pH 7) (Armstrong et al., 2009), consistent with experimental data in a *Dehalococcoides* enriched mixed community (Aulenta et al., 2009). At higher potentials, other electron transfer pathways might be involved, leading to the enrichment of different groups, such as *Desulfitobacterium* spp. (Aulenta et al., 2010a). These findings were confirmed by the results of a recent study in which a detailed characterization of the cathodic communities involved in TCE dechlorination by CARD-FISH was performed. *Dehalococcoides* was the dominant genus in the range from - 550 to - 750 mV; conversely, an unidentified member of the *Chloroflexi* phylum outcompeted the *Dehalococcoides* when the cathode potential was higher (i.e. from - 250 to - 450 mV) (Di Battista et al., 2012). Another study showed that *Dehalococcoides* were the main planktonic bacterial cells in the cathodic chamber during dechlorination of 1,2-DCA in a bioelectrochemical system, but about 40% of the bacterial cells collected from the electrode remained unidentified (Leitão et al., 2015).
51. Effect of sulfur cycle

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

\[
\text{C}_{16}\text{H}_{34} \text{(Hexadecane)} + 12.25 \text{SO}_4^{2-} + 8.5\text{H}^+ \rightarrow 16\text{HCO}_3^- + 12.25\text{H}_2\text{S} + \text{H}_2\text{O} \quad (\text{Mbadinga et al., 2011})
\]

The bioremediation and biodegradation of spilled hydrocarbons in marine systems has been extensively studied in the aftermath of the deepest and largest offshore spill in US history which occurred in the Gulf of Mexico in April 2010 (i.e. the BP Deepwater Horizon spill) (Atlas and Hazen, 2011; Kimes et al., 2013; King et al., 2015; Rodriguez-R et al., 2015). Genes and metabolites involved in anaerobic hydrocarbon degradation were identified in a metagenomic study of cores collected near the MC-252 wellhead (Kimes et al., 2013). Many of the reads from the metagenomes were related to sequences from known anaerobic hydrocarbon degraders (e.g. *Desulfatibacillum alkenivorans* AK-01, a sulfate-reducing, hydrocarbon-degrading *Deltaproteobacterium* (So and Young, 1999)).

There is increasing evidence that the role of sulfur cycle in the anaerobic degradation of crude oil in some sulfate-containing environments is complex and that other groups of microorganisms in addition to sulfate reducing bacteria (SRB) may play a role. For example, an anaerobic microcosm approach used to investigate crude oil biodegradation under sulfate-reducing conditions showed degradation of C<sub>7</sub>-C<sub>34</sub> alkanes concomitant with sulfate removal over 300 days in oil-amended
microcosms (Sherry et al., 2013). Microbial community analysis of 16S rRNA genes in the oil-amended microcosms that were actively reducing sulfate identified sequences from *Gammaproteobacteria* most closely related to *Marinobacterium* sp. and members of the family *Peptostreptococcaceae* within the *Firmicutes* at the highest frequency, rather than conventional SRB (Sherry et al., 2013). Furthermore, a broad survey of microbial community data from a range of oil and hydrocarbon-impacted anoxic environments, demonstrated that *Firmicutes* were the most commonly detected followed by the *Gamma-, Delta- and Epsilonproteobacteria* (Gray et al., 2010).

In a further study where the focus was solely on microbial communities in petroleum reservoirs the findings were remarkably similar, with *Firmicutes* followed by *Gamma-, Epsilon- and Deltaproteobacteria* being most common (Hubert et al., 2012).

In marine sediments, it has been estimated that 80 to 90% of the sulfide produced by sulfate reduction (Equation 1) is re-oxidized to sulfate through sulfur compounds of intermediate oxidation state (Zopfi et al., 2004).

Complex interactions within the sulfur cycle have also been highlighted during the electrobioremediation process (Figure 4). In sulfide rich groundwater treated in a MFC bioreactor, aerobic hydrocarbon degraders from the order *Burkholderiales* were enriched probably due to a small amount of oxygen that diffused into the reactor and this process was speculated to be coupled with sulfide oxidation to sulfate and sulfur occurring at the anode (Rakoczy et al., 2013). In another experiment, during bioelectrochemical toluene degradation, members of the *Desulfobulbaceae* and *Desulfobacteraceae* outcompeted other microbial populations, originating from a contaminated marine sediment, because of their potential to degrade hydrocarbons while an anode and sulfate both acted as electron acceptors (Daghio et al., 2016). In parallel, sulfate in anoxic conditions is reduced to sulfide which can be oxidized to elemental sulfur on an anodic surface. Sulfide oxidation on anodes can be either a chemical process (Dutta et al., 2008) or a biological process, as suggested by the enrichment of *Desulfobulbaceae* (Daghio et al., 2016; Rakoczy et al., 2013). Indeed, some 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
where $A = \text{ampere; } C = \text{coulomb, } s = \text{second}$. The charge exchanged in the reaction $(C)$ is given by the number of electrons transferred $(n)$ and Faraday’s constant $(F; 9.64853 \times 10^4 \text{ C/mol})$ (Equation 3):

\[
C = n \times F
\]

The current into the circuit should therefore be proportional to the rate at which the electrons are transferred to the electrode by the oxidation reaction (for the anodic process), or diverted by the reduction reaction (for the cathodic process). Hypothesizing that only one metabolic process is occurring on the electrode surface, the produced current can thus be used as a real time parameter to quantify the rate of a specific metabolic process (Prevoteau et al., 2015) or a measure of the substrate concentration available for microbial degradation. Previous studies demonstrated that the current production can be correlated to COD and BOD and that a relationship can be described by Monod type kinetics (Kim et al., 2003; Kumlanghan et al., 2007; Min and Logan, 2004). Tront and colleagues found that acetate concentration in the range 0-2.3 mM was correlated with current production in a MFC inoculated with a pure culture of *Geobacter sulfurreducens*. Increasing acetate concentration above 2.3 mM did not lead to an increase in the electrical signal, probably due to limitations attributed to system design parameters (Tront et al., 2008a). The same relationship was observed in a MFC inoculated with *Shewanella oneidensis* and fed with lactate (0-41 mM) (Tront et al., 2008b). These data indicate that, in the range of concentrations tested, the substrate consumption is governed by first order kinetics. However, further studies on degradation kinetics are required, in order to apply BES based biosensors for monitoring concentrations of recalcitrant 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 H₂ delivery rate in a contaminated site can be controlled and thus the negative effects of side reactions of overdosing organics (e.g. CH₄ production) will decrease (Aulenta et al., 2011, 2010b, 2009). The
possibility of a constant electron flow implies that the electrode can serve as a virtually inexhaustible electron acceptor/donor, lowering the operational costs because continuously supplying electron acceptors/donors is not needed (Morris and Jin, 2008). Furthermore, since no chemical injection is required, the expenses and need for transport and storage are eliminated (Rabaey and Keller, 2008). Overall, this makes the bioelectrochemical approach a cleaner and cheaper process compared to traditional strategies (Aulenta et al., 2009, 2008c).

It is important to note that a complete evaluation of the cost associated to the application of BES for bioremediation is not possible yet due to the lack of experimental data on large scale plants. It is reasonable to hypothesize that the main installation cost for in-situ treatment can be associated to the cost of electrode materials which can vary between 30-50 euro m$^{-2}$ up to 500-1000 euro m$^{-2}$ if a stable anode (i.e. DSA) is used. However this cost must be considered together with the lifetime of the components, which has never been evaluated in real applications. In terms of operational costs of an in-situ bioremediation plant, the example of PCE dehalogenation to ethane can be considered. This reaction requires 8 mol of e$^{-}$ for each mol of PCE. If 1 V is needed, with 100 W a constant current of 100 A is achieved, leading to ~89 (mol e$^{-}$) d$^{-1}$ available for the dechlorination without considering possible side processes. It is thus possible to reduce ~10 (mol PCE) d$^{-1}$ (i.e. 1.66 kg d$^{-1}$) with 2.4 kWh with 2.4 kWh energy input which translates to direct operational cost of about 0.24 euro (considering 0.1 euro / kWh). If however an ex-situ treatment is needed, a considerable higher cost for pumping is incurred but these would be similar to traditional methods.

Another important advantage of BES-based approaches is the selectivity that can be achieved compared to physicochemical strategies that may lead to the formation of products with greater toxicity than the parent contaminants. For example, the conversion of nitrobenzene to aniline (a less toxic and more degradable compound) was achieved using a biocathode. The study obtained a reduction of almost 99 % of nitrobenzene to aniline without the production of nitrosobenzene, a more toxic contaminant (Wang et al., 2011) which was produced during the purely electrochemical reduction (Mu et al., 2009).
An increase in the efficiency of the process can also be reached when graphite electrodes, or electrodes, made from other carbon materials are used. Hydrocarbons can be adsorbed onto the electrode thus increasing the contaminant concentration in a highly metabolically active area that is directly associated with the electron acceptor/donor and the biocatalyst (Rabaey and Keller, 2008; Zhang et al., 2010).

In some contaminated sites biodegradation of pollutants could be limited by the lack of microorganism carrying the required metabolic pathways. In such cases the addition of selected microbial populations can be a suitable strategy (El Fantroussi and Agathos, 2005). Persistence of the inoculated microorganisms however can be limited due to competition with indigenous microbial communities, predation and/or unfavourable environmental conditions (Careghini et al., 2015; Daghio et al., 2015b). BES-based approaches may facilitate bioaugmentation by enabling microbial inoculum to persist by using electrodes pre-colonized with acclimated populations (Venkidusamy et al., 2016).

In terms of process monitoring, the electric signal generated in a BES may be used as a real time measurement of the in-situ microbial activity in order to gain information about degradation rates (Tront et al., 2008a; Williams et al., 2010). The energy harvested could also be used to power other electrical sensors for in-situ monitoring (Lovley, 2006; Shantaram et al., 2005).

As well as the advantages previously reported, the use of BES-based approaches for the removal of oil spills can also have disadvantages. The main drawback during the oxidation of hydrocarbons is that aerobic degradation is usually a faster process compared to biodegradation in the absence of O\textsubscript{2}, because of the more efficient activation of oxygenases during the first step of the pathway (Weelink et al., 2010). Furthermore, when an MFC is used to stimulate oxidation of organic contaminants the efficiency of the kinetic process at the cathode is crucial and may limit the efficiency of the device and the oxidation rate at the anode, as demonstrated by early studies on BES (Liu and Logan, 2004; Zhao et al., 2006). Conversely, when using a MEC in a marine environment, care should be taken 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

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.
BES could also be used to change the chemical equilibrium by scavenging metabolites that accumulate during the biodegradation of contaminants and which may inhibit further degradation. An example can be represented by sulfide scavenging as previously reported (Daghio et al., 2016; Dutta et al., 2008; Rakoczy et al., 2013; Zhang et al., 2014). However other mechanisms of metabolite scavenging are worthy of investigation. A worthwhile course of action may be to insert an anode, colonized for example with Geobacter spp., that is able to efficiently scavenge reducing equivalents (H₂) from a syntrophic reaction, thus removing a thermodynamic barrier (Dolfing, 2014; Sun et al., 2012).

Recently, it has been reported that bioelectrogenic bacteria on the anodes of sediment MFC can use plant root exudates as substrates leading to several beneficial effects, such as electricity production (Kaku et al., 2008; Schamphelaire et al., 2008) or reduction of greenhouse gas emissions (Arends et al., 2014). The combined treatment with the macrophyte Acorus Calamus and MFC for the degradation of pyrene and benzo[a]pyrene in contaminated sediments has also been proposed. The combination of phytoremediation and a MFC led to a higher degradation rate compared to phytoremediation or MFC treatment alone (Yan et al., 2015). This provides a promising opportunity to test the effect of a combined treatment phytoremediation-BES also with other substrates.

Another innovative opportunity to use BES for the bioremediation comes from the possibility to drive the production of organic compounds (e.g. acetate) at the cathode (Logan and Rabaey, 2012). As already mentioned, H₂ is the typical substrate for reductive dehalogenation but it was suggested that in some case also acetate may be used (Aulenta et al., 2006; He et al., 2002). Acetate production could thus be driven at the cathode, providing electron donors for the reduction of halogenated contaminants. In such strategy however, attention should be paid to side processes (e.g. acetate consumption by other microorganisms such as sulfate reducers) which may cause a low dehalogenation efficiency.

8.1. Field scale application of bioelectrochemical systems
Despite considerable scientific interest, field scale bioelectrochemical systems have not yet been tested and verified under fully representative conditions and concerns have also been raised regarding their actual scalability (Table 4). Although the scalability of a bioelectrochemical system poses some intrinsic challenges, lessons can be learned from pure electrochemical processes where hundreds of small units provide high production rates and volumes. Indeed, to date a number of consolidated electrochemical remediation technologies are commercially available and widely applied for *in-situ* treatment of a variety of inorganic and organic pollutants (Trombly, 1994). These technologies are not typically used for the direct degradation of the contaminants but apply low-voltage direct current, by means of electrode arrays, to favour the electrokinetic movement of pollutants from soils into “treatment zones” where they are removed from the water by adsorption, immobilization, or (bio)degradation. Electric current flows between pairs of anodes and cathodes suitably deployed in the contaminated subsurface environments (e.g., the vadose zone of an aquifer). In such systems, the applied current density is in the range of a few Amperes per square meter (A m\(^{-2}\)), driven by a potential difference that is typically in the order of a few Volts per centimetre (V cm\(^{-1}\)). One relevant example is the “Lasagna Process” (Ho et al., 1995), developed by a consortium of industries (DuPont, General Electrics, and Monsanto) in collaboration with US Federal Agencies (EPA; DOE), providing large-scale demonstrations of the feasibility of the technology for *in-situ* treatment of a variety of contaminants, including TCE (Ho et al., 1999a, 1999b).

In principle, similar configurations could also be adopted for bioelectrochemical remediation systems. Along this line, one of the first designs being proposed involves the use of non-corrosive carbon-based electrodes (i.e. graphite granules) placed within the contaminated aquifer to form permeable reactive barriers (PRB) intercepting (and treating) the contamination plume. Here, the granules serve both as support material for biofilm formation and as electron donors or acceptors for contaminant degradation. In theory, by placing an anodic-PRB downgradient of a cathodic-PRB it would be possible to achieve a sequential reductive-oxidative treatment (Figure 5) which is...
critical to achieve complete degradation (and detoxification) of certain classes of subsurface contaminants, such as chlorinated solvents, or when multiple contaminants are simultaneously present (e.g. metals and hydrocarbons).

This sequential (cathodic-anodic) treatment has, however, some intrinsic limitations. Indeed, the distance between cathode and anode should be kept as short as possible to minimize voltage losses due to the Ohmic resistance of groundwater. As an example, assuming a typical groundwater conductivity of 1,000 $\mu$S cm$^{-1}$ and a current density of 1 A m$^{-2}$ of electrode surface area, the Ohmic loss would be 10 V for each meter of distance between cathode and anode (Arends et al., 2014; Rozendal et al., 2008). Based on this calculation, it is obvious that electrode spacing higher than a few meters would ultimately result in unacceptable energy inputs. Alternatively, if the bioremediation process is driven by water electrolysis ($H_2$ generation at the cathode and $O_2$ generation at the anode), maintaining a certain spacing between electrodes is essential to prevent the back diffusion of oxygen to the cathode which could lead to the inhibition of anaerobic microorganisms thriving at the cathode. In principle, a better scenario would probably result from the stimulation of reductive and oxidative degradation pathways via direct electron transfer since lower power inputs would be involved, as well as, no (or minimal) $H_2$ and $O_2$ would be produced.

To overcome these limitations, a number of alternative configurations are presently being considered (e.g. involving concentric electrodes), whereby the spacing between electrodes is kept as small as possible without adversely affecting process performance.

9. Conclusions

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

Acknowledgements

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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.
Figure 5 - Scheme of a possible configuration for a sequential electro-reductive-oxidative treatment. The cathode is placed upstream the anode and it is used to stimulate the reductive dehalogenation of tetrachloroethene (PCE) and trichloroethene (TCE). The reduced compounds flow downstream to the anode where the oxidation can take place.
### Table 1 - Advantages and disadvantages of in situ bioremediation approaches for oil spill removal.

<table>
<thead>
<tr>
<th>Bioremediation approach</th>
<th>Process stimulated</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic degradation (O₂ supplying)</td>
<td>Oxidation</td>
<td>Lower cost compared to physicochemical technologies</td>
<td>High energy input</td>
<td>(Alvarez and Illman, 2006; Broden et al., 1997; Tuxen et al., 2006; Zhang et al., 2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fast growth of microorganisms and high biodegradation rate due to the high</td>
<td>Possible O₂ consumption by side reactions (e.g. Fe²⁺ oxidation, which causes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>potential of the couple O₂/H₂O (+820 mV vs. SHE)</td>
<td>aquifer clogging)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A wide range of contaminants can be attacked by oxygenases</td>
<td>O₂ can diffuse away from the reaction area</td>
<td></td>
</tr>
<tr>
<td>Use of alternative electron acceptors in anaerobic conditions</td>
<td>Oxidation</td>
<td>Lower cost compared to physicochemical technologies</td>
<td>Slower growth rate compared to aerobic degradation</td>
<td>(Alvarez and Illman, 2006; Anderson and Lovley, 2000; Rivetti et al., 2008; Zhang et al., 2010)</td>
</tr>
<tr>
<td>(e.g. NO₃⁻, SO₄²⁻)</td>
<td></td>
<td>No side reactions that can consume the electron acceptor</td>
<td>Soluble electron acceptors can diffuse away from the reactive area</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SO₄²⁻ may result in production of toxic HS⁻</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NO₃⁻ could lead to eutrophication of surface water</td>
<td></td>
</tr>
<tr>
<td>Use of H₂ releasing compounds (e.g. lactic acid, propionic</td>
<td>Reduction</td>
<td>Lower cost compared to physicochemical technologies</td>
<td>Detrimental effects on groundwater quality due to the accumulation of</td>
<td>(Aulenta et al., 2007a, 2007b; Zanaroli et al., 2015)</td>
</tr>
<tr>
<td>acid, propionic acid)</td>
<td></td>
<td>The contaminants are transformed into less toxic and more degradable</td>
<td>fermentation products (e.g. VFA)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>compounds</td>
<td>Competition of dehalogenating microorganisms and other microorganisms</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(e.g. SO₄²⁻ reducers, methanogens) for H₂</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Possible aquifer clogging due to high biomass growth</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Explosion hazards due to CH₄ accumulation</td>
<td></td>
</tr>
</tbody>
</table>
Table 2 - Summary of the key studies regarding oxidation in a BES for the remediation of oil spills.

<table>
<thead>
<tr>
<th>Compound / mixture</th>
<th>Microorganism / mixed culture</th>
<th>Redox mediator*</th>
<th>Working electrode material</th>
<th>Medium treated</th>
<th>Working electrode potential (vs. SHE)**</th>
<th>Maximum current / power / voltage</th>
<th>Removal capability</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diesel</td>
<td>Mixed culture dominated by NO₃⁻- reducing bacteria (i.e. <em>Citrobacter</em> sp., <em>Pseudomonas</em> sp., and <em>Stenotrophomonas</em> sp.)</td>
<td>Not detected</td>
<td>Stainless steel scrubber</td>
<td>Refinery wastewater and mineral medium (50:50)</td>
<td>N.A.</td>
<td>120 mW m⁻² of cathode</td>
<td>N.A.</td>
<td>(Morris and Jin, 2008)</td>
</tr>
<tr>
<td>Mixed culture</td>
<td>Not detected</td>
<td>Stainless steel scrubber</td>
<td>Phosphate buffer and diesel contaminated groundwater (1:1)</td>
<td>N.A.</td>
<td>60 ± 65mV</td>
<td>82 % removal within 21 days from 176 mg L⁻¹</td>
<td>(Morris et al., 2009)</td>
<td></td>
</tr>
<tr>
<td>Mixed culture</td>
<td>Not detected</td>
<td>Carbon fibre brush</td>
<td>Mineral medium</td>
<td>N.A.</td>
<td>114.54 mA m⁻²</td>
<td>93.5 ± 0.6 % removal from 8000 mg L⁻¹ within 30 days</td>
<td>(Venkidusamy et al., 2016)</td>
<td></td>
</tr>
<tr>
<td>Toluene</td>
<td><em>Geobacter metallireducens</em></td>
<td>Not detected</td>
<td>Unpolished graphite rod</td>
<td>Mineral medium</td>
<td>+ 500 mV</td>
<td>1 mA</td>
<td>N.A.</td>
<td>(Zhang et al., 2010)</td>
</tr>
<tr>
<td>Mixed culture</td>
<td>Not detected</td>
<td>Unpolished graphite rod</td>
<td>Sediment-seawater slurry (1:4)</td>
<td>+ 500 mV</td>
<td>N.A.</td>
<td>100 % removal from 10 µM</td>
<td>(Zhang et al., 2010)</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas putida F1</td>
<td>Not detected***</td>
<td>Graphite rod</td>
<td>Mineral medium</td>
<td>+ 325 mV</td>
<td>23 mA m⁻²</td>
<td>80 % removal within 147 hours after five additions (100 mg L⁻¹ each)</td>
<td>(Friman et al., 2012)</td>
<td></td>
</tr>
<tr>
<td>Mixed culture</td>
<td>Not detected</td>
<td>Carbon cloth</td>
<td>Mineral medium</td>
<td>N.A.</td>
<td>53.5 mV</td>
<td>100 % removal within 16.2 hours from 11.09 mg L⁻¹</td>
<td>(Lin et al., 2014)</td>
<td></td>
</tr>
<tr>
<td>Mixed culture</td>
<td>Neutral red (100-300 µM)</td>
<td>Carbon cloth</td>
<td>Mineral medium</td>
<td>N.A.</td>
<td>109.7 mV (200 µM neutral red)</td>
<td>100 % removal within 34.1 ± 0.05 hours from 11.09 mg L⁻¹</td>
<td>(Lin et al., 2014)</td>
<td></td>
</tr>
<tr>
<td>Mixed culture</td>
<td>Ferricyanide (100-2000 µM)</td>
<td>Carbon cloth</td>
<td>Mineral medium</td>
<td>N.A.</td>
<td>88.2 mV (300 µM ferricyanide)</td>
<td>100 % removal within 25.3 hours from 11.09 mg L⁻¹ (500 µM ferricyanide)</td>
<td>(Lin et al., 2014)</td>
<td></td>
</tr>
<tr>
<td>Mixed culture</td>
<td>Not detected</td>
<td>Graphite plate</td>
<td>Artificial sea water</td>
<td>+ 200 mV</td>
<td>301 mA m⁻²</td>
<td>~ 1 mg L⁻¹ d⁻¹</td>
<td>(Daghio et al., 2016)</td>
<td></td>
</tr>
</tbody>
</table>
**Mixed culture dominated by SO\(_4^{2-}\) reducers (i.e. Desulfobulbaceae and Desulfobacteraceae)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mixed culture</th>
<th>Self produced mediator (+400 mV vs SHE)</th>
<th>Graphite plate</th>
<th>Artificial sea water</th>
<th>+ 500 mV</th>
<th>431 mA m(^{-2})</th>
<th>1 mg L(^{-1}) d(^{-1})</th>
<th>(Daghi et al., 2016)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>Mixed culture</td>
<td>Not detected</td>
<td>Unpolished graphite rod</td>
<td>Sediment-seawater slurry (1:4)</td>
<td>+ 500 mV</td>
<td>N.A.</td>
<td>100 % removal from 9 µM</td>
<td>(Zhang et al., 2010)</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>Mixed culture</td>
<td>Not detected</td>
<td>Carbon cloth</td>
<td>Mineral medium</td>
<td>N.A.</td>
<td>45.2 mV</td>
<td>100 % removal within 150 hours from 10.87 mg L(^{-1})</td>
<td>(Wu et al., 2013)</td>
</tr>
<tr>
<td>TPH</td>
<td>Mixed culture</td>
<td>Not detected</td>
<td>Graphite granules</td>
<td>Contaminated groundwater</td>
<td>N.A.</td>
<td>316 mW m(^{-3})</td>
<td>80 % removal from ~15 mg L(^{-1}) with an hydraulic retention time of 27 hours</td>
<td>(Wei et al., 2015)</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>Mixed culture</td>
<td>Not detected</td>
<td>Carbon felt</td>
<td>Mineral medium</td>
<td>N.A.</td>
<td>1.15 ± 0.18 mW m(^{-2}) at 40 °C</td>
<td>510 ± 5.67 µM d(^{-1}) at 40 °C</td>
<td>(Adelaja et al., 2015)</td>
</tr>
</tbody>
</table>

**Mixed culture dominated by δ-Proteobacteria (i.e. Desulfobulbaceae, Desulfobacteraceae and Geobacteraceae)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mixed culture</th>
<th>Not detected</th>
<th>Graphite fibers</th>
<th>Benzene and sulfide contaminated groundwater</th>
<th>N.A.</th>
<th>550 µA</th>
<th>18 - 80 % removal from 150-250 µM</th>
<th>(Rakoczy et al., 2013)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>Mixed culture</td>
<td>Not detected</td>
<td>Graphite plate</td>
<td>Domestic sewage</td>
<td>N.A.</td>
<td>53.11 mW m(^{-2})</td>
<td>41 ± 3 % within 17 days</td>
<td>(Venkata Mohan and Chandrasekhar, 2011)</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>Mixed culture</td>
<td>Not detected</td>
<td>Graphite plate</td>
<td>Domestic sewage</td>
<td>N.A.</td>
<td>53.11 mW m(^{-2})</td>
<td>N.A.</td>
<td>(Chandrasekhar and Venkata Mohan, 2012)</td>
</tr>
<tr>
<td>TPH</td>
<td>Mixed culture</td>
<td>Not detected</td>
<td>Stainless steel brush</td>
<td>Contaminated sediment</td>
<td>N.A.</td>
<td>190 mV</td>
<td>24.4 % removal within 66 days from 15,958 mg kg(^{-1})</td>
<td>(Morris and Jin, 2012)</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>Mixed culture</td>
<td>Not detected</td>
<td>Carbon mesh</td>
<td>Contaminated soil</td>
<td>N.A.</td>
<td>0.85 ± 0.05 mW m(^{-2})</td>
<td>15.2 ± 0.6 % removal within 25 days</td>
<td>(Wang et al., 2012)</td>
</tr>
<tr>
<td>TPH</td>
<td>Mixed culture</td>
<td>Not detected</td>
<td>Carbon cloth</td>
<td>Contaminated soil</td>
<td>N.A.</td>
<td>73.0 ± 0.1 mA</td>
<td>73.1 % removal within 64 days from 11.46 g kg(^{-1})</td>
<td>(Lu et al., 2014a)</td>
</tr>
</tbody>
</table>

**Mixed culture dominated by β-Proteobacteria (e.g.)**

| Compound      | Mixed culture | 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*)

<table>
<thead>
<tr>
<th>Not detected</th>
<th>Biochar</th>
<th>Contaminated soil</th>
<th>N.A.</th>
<th>85.9 ± 0.1 mA m$^{-2}$</th>
<th>78.7 % removal within 64 days from 11.46 g kg$^{-1}$</th>
</tr>
</thead>
</table>

Mixed culture

<table>
<thead>
<tr>
<th>Not detected</th>
<th>Graphite granules</th>
<th>Contaminated soil</th>
<th>N.A.</th>
<th>70.4 ± 0.2 mA m$^{-2}$ (8.8 ± 0.3 mW m$^{-2}$)</th>
<th>82.1–89.7 % removal within 120 days from 12.25 ± 0.36 g kg$^{-1}$</th>
</tr>
</thead>
</table>

Mixed culture

<table>
<thead>
<tr>
<th>Not detected</th>
<th>Biochar</th>
<th>Contaminated soil</th>
<th>N.A.</th>
<th>35.2 ± 0.8 mA m$^{-2}$ (3.4 ± 0.1 mW m$^{-2}$)</th>
<th>82.1–89.7 % removal within 120 days from 12.25 ± 0.36 g kg$^{-1}$</th>
</tr>
</thead>
</table>

Mixed culture dominated by α-Proteobacteria, γ-Proteobacteria and δ-Proteobacteria

<table>
<thead>
<tr>
<th>Not detected</th>
<th>Graphite rod</th>
<th>Marine sediment</th>
<th>N.A.</th>
<th>N.A.</th>
<th>21 ± 1 % removal within 200 days from 11.9 ± 0.12 g kg$^{-1}$</th>
</tr>
</thead>
</table>

Mixed culture

<table>
<thead>
<tr>
<th>Not detected</th>
<th>Carbon mesh</th>
<th>Contaminated soil</th>
<th>N.A.</th>
<th>37 mW m$^{-2}$</th>
<th>N.A.</th>
</tr>
</thead>
</table>

Mixed culture

<table>
<thead>
<tr>
<th>Not detected</th>
<th>Carbon mesh</th>
<th>Contaminated soil</th>
<th>N.A.</th>
<th>0.282±0.015 V</th>
<th>12.5 ± 0.6 % removal within 135 days from 25.7 g kg$^{-1}$</th>
</tr>
</thead>
</table>

Mixed culture

<table>
<thead>
<tr>
<th>Not detected</th>
<th>Carbon mesh</th>
<th>Contaminated soil</th>
<th>N.A.</th>
<th>0.28 ± 0.00 mA m$^{-2}$ g$^{-1}$ soil (2.76 ± 0.07 10$^{6}$ mW m$^{-2}$ g$^{-1}$ soil)</th>
<th>22 ± 0.5 % removal within 135 days</th>
</tr>
</thead>
</table>

**Phenanthrene**

Mixed culture dominated by *Nitrospira* sp and *Chloroflexi*

<table>
<thead>
<tr>
<th>Not detected</th>
<th>Stainless steel (mesh)</th>
<th>Freshwater sediment</th>
<th>N.A.</th>
<th>17.1 ± 3.8 mV (average over 240 days)</th>
<th>0.0836 d$^{-1}$ (0-22 days) with the addition of FeOOH</th>
</tr>
</thead>
</table>

*Pseudomonas aeruginosa*

<table>
<thead>
<tr>
<th>Not detected</th>
<th>Carbon felt</th>
<th>Mineral medium</th>
<th>N.A.</th>
<th>0.19 ± 0.05 mW m$^{-2}$</th>
<th>54.70 ± 0.60 µM d$^{-1}$</th>
</tr>
</thead>
</table>

*Shewanella oneidensis*

<table>
<thead>
<tr>
<th>Not detected</th>
<th>Carbon felt</th>
<th>Mineral medium</th>
<th>N.A.</th>
<th>0.51 ± 0.03 mW m$^{-2}$</th>
<th>25.20 ± 5.15 µM d$^{-1}$</th>
</tr>
</thead>
</table>

Mixed culture

<table>
<thead>
<tr>
<th>Not detected</th>
<th>Carbon felt</th>
<th>Mineral medium</th>
<th>N.A.</th>
<th>0.37 ± 0.05 mW m$^{-2}$</th>
<th>35.70 ± 2.73 µM d$^{-1}$</th>
</tr>
</thead>
</table>

Mixed culture

<table>
<thead>
<tr>
<th>Not detected</th>
<th>Carbon felt</th>
<th>Mineral medium</th>
<th>N.A.</th>
<th>1.15 ± 0.18 mW m$^{-2}$ at 40 °C</th>
<th>320 ± 4.81 µM d$^{-1}$ at 40 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
<td>Culture Type</td>
<td>Medium</td>
<td>Redox Active Moiety</td>
<td>Potential vs SHE</td>
<td>Current Density</td>
</tr>
<tr>
<td>----------</td>
<td>--------------</td>
<td>--------</td>
<td>---------------------</td>
<td>-----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Pyrene</td>
<td>Mixed culture dominated by <em>Nitrospira</em> sp and <em>Chloroflexi</em></td>
<td>Stainless steel (mesh)</td>
<td>Freshwater sediment</td>
<td>N.A.</td>
<td>17.1 ± 3.8 mV (average over 240 days)</td>
</tr>
<tr>
<td>Phenol</td>
<td>Mixed culture</td>
<td>Not detected</td>
<td>Carbon felt</td>
<td>Waterlogged soil</td>
<td>N.A.</td>
</tr>
<tr>
<td><em>Cupriavidus basilensis</em></td>
<td>Self produced mediator (+140 mV vs SHE)</td>
<td>Graphite rod</td>
<td>Mineral medium</td>
<td>+ 325 mV</td>
<td>478 mA m⁻²</td>
</tr>
<tr>
<td>1,2-DCA</td>
<td>Enrichment form MFCs reactors</td>
<td>Not detected</td>
<td>Graphite granules</td>
<td>Mineral medium</td>
<td>NA</td>
</tr>
</tbody>
</table>

* The midpoint potential is reported in brackets
** The potential at which the working electrode was poised is reported
*** A redox active moiety (putative catechol) was detected but the midpoint potential was higher than the anodic potential
### Table 3 - Summary of the key studies targeting reduction in a BES for the remediation of oil spills.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Microorganism / mixed culture</th>
<th>Redox mediator*</th>
<th>Working electrode material</th>
<th>Medium treated</th>
<th>Working electrode potential (vs SHE)**</th>
<th>Maximum current / voltage</th>
<th>Removal capability</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCE</td>
<td>Mixed culture containing <em>Dehalococcoides</em> spp.</td>
<td>Methyl viologen (100 µM)</td>
<td>Glassy carbon</td>
<td>Mineral medium</td>
<td>- 500 mV</td>
<td>20 µA</td>
<td>N.A.</td>
<td>(Aulenta et al., 2007a)</td>
</tr>
<tr>
<td></td>
<td>Mixed culture containing <em>Dehalococcoides</em> spp.</td>
<td>Methyl viologen</td>
<td>Glassy carbon</td>
<td>Mineral medium</td>
<td>- 800 mV</td>
<td>N.A.</td>
<td>N.A.</td>
<td>(Aulenta et al., 2007a)</td>
</tr>
<tr>
<td></td>
<td>Mixed culture containing <em>Desulfotobacterium</em> sp. and <em>Dehalococcoides</em> spp.</td>
<td>Methyl viologen (25-7500 µM)</td>
<td>Glassy carbon</td>
<td>Mineral medium</td>
<td>- 450 mV</td>
<td>∼250 µA (methyl viologen 500 µM)</td>
<td>N.A.</td>
<td>(Aulenta et al., 2008b)</td>
</tr>
<tr>
<td></td>
<td>Mixed culture containing <em>Dehalococcoides</em> spp.</td>
<td>Not detected</td>
<td>Unpolished graphite rod</td>
<td>Mineral medium</td>
<td>- 650 mV</td>
<td>N.A.</td>
<td>3.73 ± 0.02 µeq h⁻¹</td>
<td>(Aulenta et al., 2008c)</td>
</tr>
<tr>
<td></td>
<td>Mixed culture containing <em>Desulfotobacterium</em> sp. and <em>Dehalococcoides</em> spp.</td>
<td>Self produced mediator (-400 mV vs SHE)</td>
<td>Carbon paper</td>
<td>Mineral medium</td>
<td>- 450 mV</td>
<td>N.A.</td>
<td>N.A.</td>
<td>(Aulenta et al., 2009)</td>
</tr>
<tr>
<td></td>
<td><em>Geobacter lovleyi</em></td>
<td>Not detected</td>
<td>Carbon paper</td>
<td>Mineral medium</td>
<td>- 450 mV</td>
<td>N.A.</td>
<td>N.A.</td>
<td>(Aulenta et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>Mixed culture containing <em>Dehalococcoides</em> spp.</td>
<td>AQDS (50-1500 µM)</td>
<td>Glassy carbon</td>
<td>Mineral medium</td>
<td>- 250mV</td>
<td>N.A.</td>
<td>180 ± 23 µeq L⁻¹ d⁻¹ (AQDS 1500 µM)</td>
<td>(Aulenta et al., 2010a)</td>
</tr>
<tr>
<td></td>
<td>Mixed culture containing <em>Desulfotobacterium</em> sp. and <em>Dehalococcoides</em> spp.</td>
<td>Self produced mediator (-550 mV vs SHE)</td>
<td>Carbon paper</td>
<td>Mineral medium</td>
<td>- 550mV</td>
<td>20 µA</td>
<td>22.4 µeq L⁻¹ d⁻¹</td>
<td>(Aulenta et al., 2010b)</td>
</tr>
<tr>
<td></td>
<td>Mixed culture dominated by <em>Chloroflexi</em> (unidentified <em>Chloroflexi</em> and <em>Dehalococcoides</em> spp.)</td>
<td>Not detected</td>
<td>Graphite granules</td>
<td>Mineral medium</td>
<td>- 250mV</td>
<td>15.0 ± 0.8 µA</td>
<td>15.5 ± 1.2 µeq L⁻¹ d⁻¹</td>
<td>(Aulenta et al., 2011; Di Battista et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>Mixed culture dominated by <em>Chloroflexi</em> (unidentified <em>Chloroflexi</em> and <em>Dehalococcoides</em> spp.)</td>
<td>Not detected</td>
<td>Graphite granules</td>
<td>Mineral medium</td>
<td>- 450mV</td>
<td>N.A.</td>
<td>58 ± 1 µeq L⁻³ d⁻¹</td>
<td>(Aulenta et al., 2011; Di Battista et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>Mixed culture dominated by <em>Chloroflexi</em> (<em>Dehalococcoides</em> spp.)</td>
<td>Not detected</td>
<td>Graphite granules</td>
<td>Mineral medium</td>
<td>- 550 mV (average)</td>
<td>266 ± 5 µA</td>
<td>62 ± 2 µeq L⁻³ d⁻¹</td>
<td>(Aulenta et al., 2011; Di Battista et al., 2012)</td>
</tr>
<tr>
<td>Culture Type</td>
<td>Mediator</td>
<td>Alg. Surface</td>
<td>Medium</td>
<td>E (mV)</td>
<td>I (mA/cm²)</td>
<td>µmol L⁻¹ d⁻¹</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>------------------------------------------------</td>
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<td>---------------------</td>
<td>--------</td>
<td>------------</td>
<td>--------------</td>
<td>------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Mixed culture dominated by Chloroflexi (Dehalococcoides spp.)</td>
<td>Not detected</td>
<td>Graphite granules</td>
<td>Mineral medium</td>
<td>-650</td>
<td>N.A.</td>
<td>N.A.</td>
<td>(Aulenta et al., 2011; Di Battista et al., 2012)</td>
<td></td>
</tr>
<tr>
<td>Mixed culture dominated by Chloroflexi (Dehalococcoides spp.)</td>
<td>Not detected</td>
<td>Graphite granules</td>
<td>Mineral medium</td>
<td>-750</td>
<td>N.A.</td>
<td>N.A.</td>
<td>(Aulenta et al., 2011; Di Battista et al., 2012)</td>
<td></td>
</tr>
<tr>
<td>Mixed culture containing Dehalococcoides spp.</td>
<td>Not detected</td>
<td>Graphite granules</td>
<td>Mineral medium</td>
<td>-250</td>
<td>N.A.</td>
<td>~71 % removal from 35 µM (flow rate 0.4 mL min⁻¹)</td>
<td>(Verdini et al., 2015)</td>
<td></td>
</tr>
<tr>
<td>Mixed culture containing Dehalococcoides spp.</td>
<td>Not detected</td>
<td>Graphite granules</td>
<td>Mineral medium</td>
<td>-350</td>
<td>N.A.</td>
<td>~97 % removal from 35 µM (flow rate 0.4 mL min⁻¹)</td>
<td>(Verdini et al., 2015)</td>
<td></td>
</tr>
<tr>
<td>Mixed culture containing Dehalococcoides spp.</td>
<td>Not detected</td>
<td>Graphite granules</td>
<td>Mineral medium</td>
<td>-450</td>
<td>N.A.</td>
<td>~90 % removal from 35 µM (flow rate 0.4 mL min⁻¹)</td>
<td>(Verdini et al., 2015)</td>
<td></td>
</tr>
<tr>
<td>PCE Geobacter lovleyi</td>
<td>Not detected</td>
<td>Unpolished graphite rod</td>
<td>Mineral medium</td>
<td>-300</td>
<td>N.A.</td>
<td>~25 µmol d⁻¹ from 100 µmol</td>
<td>(Strycharz et al., 2008)</td>
<td></td>
</tr>
<tr>
<td>Mixed culture containing Desulfotobacterium sp. and Dehalococcoides spp.</td>
<td>Not detected</td>
<td>Stainless steel mesh</td>
<td>Mineral medium</td>
<td>NA</td>
<td>0.05 mA cm⁻² (applied)</td>
<td>~23 µmol d⁻¹ from 24 - 45 µmol L⁻¹</td>
<td>(Lohner and Tiehm, 2009)</td>
<td></td>
</tr>
<tr>
<td>Mixed culture containing Desulfotobacterium sp. and Dehalococcoides spp.</td>
<td>Not detected</td>
<td>Stainless steel mesh</td>
<td>Mineral medium / Contaminated groundwater</td>
<td>NA</td>
<td>0.5 mA (applied)</td>
<td>100 % removal with a load of 1.5 µmol d⁻¹ in mineral medium***</td>
<td>(Lohner et al., 2011)</td>
<td></td>
</tr>
<tr>
<td>cis-DCE Mixed culture containing Desulfotobacterium sp. and Dehalococcoides spp.</td>
<td>Self produced mediator (-550 mV vs SHE)</td>
<td>Carbon paper</td>
<td>Mineral medium</td>
<td>-550</td>
<td>2 µA</td>
<td>1.5 µeq L⁻¹ d⁻¹</td>
<td>(Aulenta et al., 2010b)</td>
<td></td>
</tr>
<tr>
<td>Mixed culture</td>
<td>Not detected</td>
<td>Graphite granules</td>
<td>Contaminated groundwater</td>
<td>-550</td>
<td>N.A.</td>
<td>N.A.</td>
<td>(Lai et al., 2015)</td>
<td></td>
</tr>
<tr>
<td>Mixed culture</td>
<td>Not detected</td>
<td>Graphite granules</td>
<td>Contaminated groundwater</td>
<td>-650</td>
<td>N.A.</td>
<td>N.A.</td>
<td>(Lai et al., 2015)</td>
<td></td>
</tr>
<tr>
<td>Mixed culture</td>
<td>Not detected</td>
<td>Graphite granules</td>
<td>Contaminated groundwater</td>
<td>-750</td>
<td>N.A.</td>
<td>4.89 ± 0.46 µM residual concentration (14.2 ± 0.7 µM influent concentration)</td>
<td>(Lai et al., 2015)</td>
<td></td>
</tr>
<tr>
<td>1,2-DCA Mixed culture containing Dehalococcoides spp.</td>
<td>Not detected</td>
<td>Graphite rods</td>
<td>Mineral medium</td>
<td>-300</td>
<td>N.A.</td>
<td>10 ± 4 µeq L⁻¹ d⁻¹</td>
<td>(Leitão et al., 2015)</td>
<td></td>
</tr>
<tr>
<td>Culture Type</td>
<td>Detection</td>
<td>Electrode Material</td>
<td>Medium</td>
<td>Applied Potential</td>
<td>Activity</td>
<td>References</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------------</td>
<td>-----------------</td>
<td>--------------------</td>
<td>---------------------</td>
<td>-------------------</td>
<td>-------------------</td>
<td>----------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed culture containing <em>Dehalococcoides</em> spp.</td>
<td>Not detected</td>
<td>Graphite rods</td>
<td>Mineral medium</td>
<td>- 500 mV</td>
<td>N.A.</td>
<td>N.A.</td>
<td>(Leitão et al., 2015)</td>
<td></td>
</tr>
<tr>
<td>Mixed culture containing <em>Dehalococcoides</em> spp.</td>
<td>Not detected</td>
<td>Graphite rods</td>
<td>Mineral medium</td>
<td>- 600 mV</td>
<td>N.A.</td>
<td>N.A.</td>
<td>(Leitão et al., 2015)</td>
<td></td>
</tr>
<tr>
<td>Mixed culture containing <em>Dehalococcoides</em> spp.</td>
<td>Not detected</td>
<td>Graphite rods</td>
<td>Mineral medium</td>
<td>- 700 mV</td>
<td>N.A.</td>
<td>24.3 ± 17.5 µeq L⁻¹ day⁻¹</td>
<td>(Leitão et al., 2015)</td>
<td></td>
</tr>
<tr>
<td>Mixed culture containing <em>Dehalococcoides</em> spp.</td>
<td>Not detected</td>
<td>Graphite rods</td>
<td>Mineral medium</td>
<td>- 900 mV</td>
<td>N.A.</td>
<td>37 ± 10 µeq L⁻¹ d⁻¹</td>
<td>(Leitão et al., 2015)</td>
<td></td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>Mixed culture</td>
<td>Carbon cloth</td>
<td>Mineral medium</td>
<td>0.5 V (applied)</td>
<td>0.135 ± 0.015 h⁻¹ (with 2.78 mM of glucose)</td>
<td>Over 99 % from 0.5 mM</td>
<td>(Wang et al., 2011)</td>
<td></td>
</tr>
<tr>
<td>Mixed culture</td>
<td>Not detected</td>
<td>Carbon cloth</td>
<td>Mineral medium</td>
<td>0.3 V (applied)</td>
<td>0.135 ± 0.015 h⁻¹ (with 2.78 mM of glucose)</td>
<td>Over 99 % from 0.5 mM</td>
<td>(Liang et al., 2014)</td>
<td></td>
</tr>
</tbody>
</table>

* 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
Table 4 - Summary of the advantages and the disadvantages of electrobioremediation for oil spill removal.

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>The energy level and the flux of electrons can be set and maintained constant</td>
<td>Anaerobic degradation is usually slower compared to aerobic degradation</td>
</tr>
<tr>
<td>The process is clean</td>
<td>When an MFC is used the cathodic reaction may limit the anodic reaction</td>
</tr>
<tr>
<td>The operational cost is lower</td>
<td>Chlorine gas can be produced in marine environments</td>
</tr>
<tr>
<td>High selectivity towards the target compounds</td>
<td>The scale-up of the technology is challenging</td>
</tr>
<tr>
<td>Hydrocarbons can be adsorbed on the electrodes when graphite (or carbon) is used</td>
<td>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)</td>
</tr>
<tr>
<td>Electrodes can be used to improve the bioaugmentation efficiency</td>
<td></td>
</tr>
<tr>
<td>The electrical signal can be used as a monitoring tool</td>
<td></td>
</tr>
</tbody>
</table>