In situ Groundwater Treatment with Bioelectrochemical Systems (BES): Critical Review and Future Perspectives

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Abstract

Groundwater contamination is an ever-growing environmental issue, that has attracted much and undiminished attention for the past half century. Groundwater contamination originates from anthropogenic (e.g. hydrocarbons), natural compounds (e.g. nitrate and arsenic), or both; to tackle these contaminants different technologies have been tested during the years. Recently, bioelectrochemical systems (BESs) have emerged as a potential treatment for groundwater contamination, with in situ applications reported, that showed promising results. Nitrate and hydrocarbons (toluene, phenanthrene, benzene, BTEX and light PAHs) have been successfully removed, due to the interaction of microbial metabolism with poised electrodes, other than physical migration due to the electric field generated in BES. The selection of proper BESs relies on several factors and problems such as complexity of the groundwater, scale-up and energy requirements that need to be taken into account. Modelling efforts could help predict case scenarios and choose an ideal design and approach to solve these issues. In this review, we critically analyze in situ BES applications for groundwater remediation, focusing in particular on the different setups proposed, and we identify and discuss the existing research gaps in the field.

Keywords: bioelectrochemical systems; groundwater remediation; bioelectroremediation; denitrification; in situ treatment
1. Introduction

Groundwater is susceptible to pollutants and its contamination can cause it to become unsafe and unfit for human use. Contamination might be due to natural causes, for example arsenic (As) or nitrate (NO$_3^-$)-containing rocks (Menció et al., 2016; Tabelin et al., 2018) or more frequently by anthropic activities. Hence, a large variety of contaminants can be found in groundwater: aromatic compounds and chlorinated hydrocarbons (Logeshwaran et al., 2018), inorganic metallic compounds (Galitskaya et al., 2017; Luu et al., 2009), and nutrients (Chen et al., 2016). Recently, pharmaceutically active compounds (PhACs) and contaminants of emerging concern (CECs) have also been identified in groundwater as solutes (Lopez et al., 2015).

Different treatment technologies have been applied to groundwater remediation: physical, chemical and biological, with variable rate of success (Callegari et al., 2018; Dong et al., 2019; O’Connor et al., 2018; Sarkar and Paul, 2016). Application of Pump & Treat (P&T) schemes is among the most diffused strategies, whereby groundwater is extracted and subsequently treated outside of the aquifer; this type of solution allows for a better control of the process (i.e. directly observable by the operator), however this approach may be waste- and energy-intensive when the extracted groundwater is not in need of immediate use (Majone et al., 2015). Therefore, much research is focusing on the development of in situ treatments, which are considered to be more sustainable for the general protection of this resource.

Bioelectrochemical systems (BESs) have steadily emerged in the last 15 years as a versatile and promising technology. BESs have been employed in different ways for a variety of tasks: (1) microbial fuel cells (MFC), degrading organic matter and producing electrical energy (Capodaglio et al., 2013), (2) microbial electrolysis cells (MEC), producing valuable hydrogen gas at the cathode (Miller et al., 2019), (3) microbial desalination cells (MDC), providing desalinated water from seawater or brackish water (Brastad and He, 2013), and (4) microbial electrosynthesis systems (MES), synthetizing value-added chemicals and commodities using a poised biocathode (Wang and Ren, 2013). Additionally, BESs have been integrated with other technologies such as membrane
bioreactors, algal photobioreactors and capacitive deionization, in hybrid system configurations to increase overall performance, both in terms of energy consumption/production and contaminant removal (Xiao et al., 2012; Yuan et al., 2012).

Amongst the different applications of BESs, in situ groundwater bioelectroremediation, remediation using bioelectrochemical systems, showed to be a promising niche for these systems, due to their peculiar characteristics. These include the possibility to use different redox environments both at the anode and cathode, to work at different set potentials, and the flexibility of the technology (Modin and Aulenta, 2017). In addition, the combination of anodic and cathodic redox environment together with the microbial metabolism paves the way to a variety of intriguing and beneficial removal pathways.

Modin and Aulenta (2017) earlier reviewed the challenges and opportunities of in situ bioelectroremediation, mainly focusing on the general process and the biological mechanisms of electron-electrode transfer; however, in this review we shift the focus on different aspects of in situ bioelectroremediation. We first focus in depth on different in situ applications of bioelectrochemical systems for groundwater remediation, then we discuss the challenges for bioelectroremediation and finally also identify current research gaps and potential future research directions.

2. Practical hurdles in groundwater remediation suggesting BESs as possible solutions

An aquifer is undisputedly a challenging environment, therefore, due to the intrinsic nature of the surrounding environment, groundwater remediation will face several hindrances. Biological water/wastewater treatment may require electron donors (in case reduction is required) and acceptors (in case oxidation is required). In a scenario of hydrocarbon contaminated groundwater, for instance, limited presence of electron acceptors (NO$_3^-$, oxygen, and sulfate) may limit contaminant oxidation. In addition, limited mixing within an aquifer allows replenishment of electron acceptors only at the physical boundary of a contaminated plume, where diffusion and dispersion are the predominant mixing factors (Li and Yu, 2015). Due to normally low concentration of organic matter in
groundwater, a similar behavior can be observed for reduction reactions, such as denitrification, where the limiting electron donor may reduce the possible reaction rates (Shen et al., 2015). In addition, metabolism and growth of microorganisms may be slowed down in these circumstances since the majority of them is attached to soil particles or located in the sediments, where diffusion of electron acceptors and donors might be particularly difficult (Li and Yu, 2015).

Based on these premises, in situ bioremediation may become particularly challenging; the application of BESs may become a solution to the problem or at least to a large part of it. The lack of natural onsite electron acceptors and donors can be substituted by insertion of electrodes, acting as virtually inexhaustible electron acceptors (anode) or donors (cathode), thus supporting microbial metabolism (Aulenta et al., 2011). In some cases, addition of chemicals as electron donors/acceptors is chosen, requiring periodical replacement of the spent agents, which is not needed in the case of electrodes. In addition, when reduction reactions are involved, it is possible to set the desired cathodic electrode potential at a reductive level that simply cannot be reached by the sole addition of chemical reagents (Li and Yu, 2015; Williams et al., 2010).

Furthermore, water electrolysis could generate oxygen and hydrogen, which may serve respectively as additional electron acceptor and donor. Electrokinetic enhancement was reported to enhance mixing and mass transport, due to the electric field generated in proximity of the electrodes (Gill et al., 2014). Also, electro-osmosis can induce water displacement with subsequent resuspension of immobilized bacteria and contaminants. This leads to an increase of contact between substrate and bacteria, independently from the hydraulic conductivity of the porous medium (Gill et al., 2014; Jones et al., 2011; Li and Yu, 2015; Lohner et al., 2008; Xu et al., 2010).
Figure 1: Main advantages of *in situ* bioelectroremediation.

Li and Yu (2015) reported other advantages of BESs application for groundwater *in situ* remediation, namely: electrokinetic enhancement of nutrient delivery and microbial metabolism connected to higher bioavailability, higher bacterial enrichment and adsorption due to large electrodes’ surface.

3. *In situ* bioelectroremediation: the quest for the ideal setup

In the last decade, due to the multidisciplinary and faceted nature of BESs, different setups based on BES technology have been developed. The majority of applications have focused on groundwater denitrification and removal of petroleum hydrocarbons (Table 1). Petroleum hydrocarbons are usually removed in BES by oxidation in an anodic compartment; NO$_3^-$ can be reduced in BESs to nitrogen gas via autotrophic denitrification or autohydrogenotrophic denitrification (at the cathode) or via heterotrophic denitrification (at the anode); the presence of organic matter is necessary in the latter case.
<table>
<thead>
<tr>
<th>Target</th>
<th>Name</th>
<th>Removal pathway(s)</th>
<th>Applied potential/voltage/driving force</th>
<th>Initial conc.</th>
<th>Rem. rate</th>
<th>η</th>
<th>Inoculum</th>
<th>Prevalent microbial species</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate</td>
<td>BES</td>
<td>Anodic heterotrophic denitrification</td>
<td>0.8 V (between electrodes)</td>
<td>25 mg NO₃⁻·N L⁻¹</td>
<td>208.2 ± 13.3 gNO₃⁻·N m⁻³ d⁻¹</td>
<td>90.50%</td>
<td>Digested sludge</td>
<td>-</td>
<td>Tong and He (2013)</td>
</tr>
<tr>
<td>Nitrate</td>
<td>SMDDC</td>
<td>Cathodic autotrophic denitrification</td>
<td>Voltage generated by OM oxidation at the anode</td>
<td>20 mg NO₃⁻·N L⁻¹</td>
<td>0.483 kgNO₃⁻·N m⁻³°CV d⁻¹</td>
<td>90.5% in 12 hrs</td>
<td>Gammaproteobacteria (Shewanella) (anode); Alphaproteobacteria and Sphingobacteria (cathode)</td>
<td>Zhang and Angeldaki (2013)</td>
<td></td>
</tr>
<tr>
<td>Nitrate</td>
<td>BES</td>
<td>Physical migration in a concentrating chamber &amp; successive anodic heterotrophic denitrification</td>
<td>0.8 V (between electrodes)</td>
<td>21.4 mgNO₃⁻·N L⁻¹</td>
<td>N/A</td>
<td>55% in 17 hrs</td>
<td>Anaerobic sludge</td>
<td>-</td>
<td>Tong and He (2014)</td>
</tr>
<tr>
<td>Nitrate</td>
<td>Biocathode buried in simulated aquifer</td>
<td>Cathodic autotrophic denitrification</td>
<td>-0.7 V vs SHE</td>
<td>50 mg NO₃⁻·N L⁻¹</td>
<td>322.6 mg m⁻² d⁻¹</td>
<td>-</td>
<td>Anaerobic sludge</td>
<td>Thiothrix, Paracoccus</td>
<td>Nguyen et al. (2016a)</td>
</tr>
<tr>
<td>Nitrate</td>
<td>Biocathode buried in sand or gravel</td>
<td>Cathodic autotrophic denitrification</td>
<td>-0.303 V vs SHE</td>
<td>30 mg NO₃⁻·N L⁻¹</td>
<td>35.35 mgNO₃⁻·N m⁻² d⁻¹</td>
<td>97%</td>
<td>Parent biocathode</td>
<td>-</td>
<td>Ceccone et al. (2019a)</td>
</tr>
<tr>
<td>Nitrate</td>
<td>Biocathode buried in sand or gravel</td>
<td>Cathodic autotrophic denitrification</td>
<td>1.0 V (between anode and cathode)</td>
<td>30 mg NO₃⁻·N L⁻¹</td>
<td>36.23 mgNO₃⁻·N m⁻² d⁻¹</td>
<td>100%</td>
<td>Parent biocathode</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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**Table 1**: Specifications of BES performing in situ groundwater treatment

| Nitrate | SMFC - BER | Cathodic autotrophic denitrification | 0.27 mA, powered by a SMFC | 30 mg NO$_3^-$ N L$^{-1}$ | 3.87 mgN L$^{-1}$ h$^{-1}$ | Groundwater | *Hyphomicrobium, Terrimicrobium, Teridipaera, Prosthecobacter* | Liu et al. (2019) |
| Phenantrene and benzene | MFC | Anodic oxidation | 100 ppm (phenantrene), 2000 ppm (benzene) | - | >80% (phenantrene), >90% (benzene) | Parent MFC | - | Adelaja et al. (2017) |
| Phenol | Bioelectric well | Anodic phenol oxidation | +0.2 V vs SHE (anode potential) | 25 mg L$^{-1}$ | 59 ± 3 mg L$^{-1}$ d$^{-1}$ | 99.5 ± 0.4% | Refinery wastewater | *Geobacter* | Palma et al. (2018a) |
| Toluene | Bioelectric well | Anodic toluene oxidation | +0.2 V vs SHE (anode potential) | 25 mg L$^{-1}$ | 67.2 ± 5.7 mg L$^{-1}$ d$^{-1}$ | 100% | Refinery wastewater | *Geobacter* | Palma et al. (2018b) |
| Benzenes, toluene, ethylbenzene, xylene | Bioelectric well | Anodic oxidation | +0.2 V vs SHE (anode potential) | 5 mg L$^{-1}$ (benzene), 14 mg L$^{-1}$ (toluene), 2 mg L$^{-1}$ (ethylbenzene), 4 mg L$^{-1}$ (xylene) | 3.3±0.1 mg L$^{-1}$ d$^{-1}$ (ethylbenzene), 4.5±0.2 mg L$^{-1}$ d$^{-1}$ (xylene) | - | Refinery wastewater | *Geobacter* | Palma et al. (2019) |
| Light PAHs | GAC-BES | Anodic oxidation and adsorption on GAC | MFC setup | 1546 mg L$^{-1}$ | N/A | 99% | Contaminated groundwater | *Betaproteobacteria* (in particular *Pseudomonadaceae*) | Kirmizakis et al. (2019) |

Tong and He (2013) developed a BES, placed within the aquifer medium, able to attract NO$_3^-$ in the anode chamber, and remove it by heterotrophic denitrification (Figure 2A); tests were carried...
out with both synthetic and real groundwater. Anode and cathode chambers consisted of separated porous tubes wrapped by anionic (AEM) and cationic (CEM) exchange membranes respectively. The anode chamber was fed with synthetic/real groundwater while the cathodic one with buffer solution. The system was operated in both MFC and MEC modes; application of 0.8 V potential between anode and cathode led to the best results, obtaining $\text{NO}_3^-$ removal rates up to $208.2 \pm 13.3 \, \text{gNO}_3^- \cdot \text{m}^3 \cdot \text{d}^{-1}$. Competition between ion exchange and electricity-driven migration was observed, leading to $\text{NO}_3^-$ removal rate of $158.2 \pm 4.2 \, \text{gNO}_3^- \cdot \text{m}^3 \cdot \text{d}^{-1}$ in open circuit condition. In this case, electricity generation prevented the migration of undesired ions into groundwater by ion exchange inhibition. Higher current densities were recorded when the system was operated with real groundwater, due to the natural presence of other ions that enhanced ion movement and assisted electricity generation (Tong and He, 2013). In a follow-up study by Tong and He (2014), the current flow generated by a tubular BES induced $\text{NO}_3^-$ migration out of groundwater, accumulating it in a concentration chamber, defined by the presence of CEM and AEM. The BES setup was similar to that of a tubular MDC (Figure 2C). Electrons generated by organic matter oxidation at the anode flowed to the cathode, while cations migrated to the concentration chamber from the anode. Simultaneously, anions, including the target pollutant $\text{NO}_3^-$, reached the concentration chamber through the AEM, where they were retained due to the presence of the CEM preventing their intrusion into the anode chamber. In this case, rather than biological denitrification, $\text{NO}_3^-$ removal from groundwater was due mainly to the physical migration induced by the generated electric current, with a subsequent treatment needed to denitrify the concentrated feed afterwards (Tong and He, 2014). Zhang and Angelidaki (2013) proposed a modification of the MDC setup: bioelectricity was used to attract $\text{NO}_3^-$ into the anodic chamber through an AEM, then $\text{NO}_3^-$ was transferred to the cathode chamber, where it was reduced via autotrophic denitrification (Figure 2B). $\text{NO}_3^-$ removal rate of 90.5% was obtained with HRT of 12 hours; in this case the ionic strength of groundwater was a limiting factor for $\text{NO}_3^-$ removal. The addition of a nitrification step in the loop transferring $\text{NO}_3^-$ from anode to cathode was beneficial to both bioelectricity production and $\text{NO}_3^-$ attraction, removing ammonia ($\text{NH}_3/\text{NH}_4^+$) that appeared in
the anode chamber due to the lack of aeration. The setup proved to be versatile, and capable, with minor modifications, to remove NH$_3$ from anaerobic reactors, at the same time balancing NH$_3$ inhibition (Zhang and Angelidaki, 2015a, 2015b).

Denitrification processes using biocathodes buried in a simulated aquifer have been investigated by Nguyen et al. (2016a), showing that NO$_3^-$ removal rates depend on the burial percentage of the electrode; the electrodes were immersed in sand at a variable submersion percentage (10, 50 and 100%, plus a control without sand). 30% decrease in NO$_3^-$ removal rates was observed and reported when recirculation was impeded by the addition of sand at the bottom of the cathode chamber (Nguyen et al., 2016a) (Figure 2D).

The need for local recirculation was discussed by Jain and He (2018), where, in order to achieve good BES performances, recirculation was indicated as an essential step to promote contact between substrate and biomass, while decreasing overpotentials. The required energy contribution of stirring in small reactors, or pumping in bigger reactors, cannot be however neglected, and may massively affect the overall energy balance of the application (Jacobson et al., 2015; Zou and He, 2018). Recently, Cecconet et al. (2019a) confirmed the decrease in NO$_3^-$ removal rates reported by Nguyen et al. (2016a) by operating buried biocathodes completely immersed in sand and gravel, assisted by a potentiostat or power supply. The biocathodes operated in gravel achieved better results due to greater possibility of water movement compared to the one with sand. In addition, higher accumulation of intermediate nitrogen species was found in biocathodes operated by potentiostat, compared with those operated by power supply. Increased accumulation of intermediate nitrogen species, e.g. nitrous oxide (N$_2$O), has been shown in other studies to be a potential route of nitrogen loss in bioelectroremediation systems (Srinivasan et al., 2016; Van Doan et al., 2013; Vilar-Sanz et al., 2013). This is likely due to the intrinsic behavior of biofilms and their microbial stratification, interactions, substrate gradients and interaction allowing for biotic and abiotic formation of N$_2$O (Sabba et al., 2018). The results of Cecconet et al. (2019a) and Nguyen et al. (2016a) suggest that the
insertion of electrodes in a porous medium, even though feasible and simple, presents some limitations that could be corrected and eliminated with a more dedicated design (Figure 2D).

<table>
<thead>
<tr>
<th>Target</th>
<th>Setup</th>
<th>Pros</th>
<th>Cons</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO₃⁻</td>
<td><img src="https://example.com/diagramA.png" alt="Diagram A" /></td>
<td>Heterotrophic denitrification (higher kinetics compared to autotrophic)</td>
<td>Necessity of power supply despite OM oxidation; wastewater as anolyte may contaminate the aquifer</td>
<td>Tong and He (2013)</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td><img src="https://example.com/diagramB.png" alt="Diagram B" /></td>
<td>Versatile</td>
<td>Use of wastewater as anolyte may contaminate the aquifer</td>
<td>Zhang and Angelidaki (2013)</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td><img src="https://example.com/diagramC.png" alt="Diagram C" /></td>
<td>Tubular, adapt to be used in wells</td>
<td>N physically concentrated, not biologically removed</td>
<td>Tong and He (2014)</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td><img src="https://example.com/diagramD.png" alt="Diagram D" /></td>
<td>Simulation of an aquifer remediation</td>
<td>Membrane use not feasible, low performances</td>
<td>Nguyen et al. (2016a); Ceconet et al. (2019a)</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td><img src="https://example.com/diagramE.png" alt="Diagram E" /></td>
<td>Synergic remediation of sediment and groundwater</td>
<td>No need of wastewater or power supply to fuel the system</td>
<td>Liu et al. (2019)</td>
</tr>
<tr>
<td>Phenanthrene and benzene</td>
<td><img src="https://example.com/diagramF.png" alt="Diagram F" /></td>
<td>High performances in PAH removal</td>
<td>Use of bromate as catholyte is not sustainable</td>
<td>Adelaja et al. (2017)</td>
</tr>
<tr>
<td>Phenol, toluene, BTEX</td>
<td><img src="https://example.com/diagramG.png" alt="Diagram G" /></td>
<td>High performances, internal recirculation, absence of expensive membrane</td>
<td>Granular graphite may possess non-scalable properties</td>
<td>Palma et al. (2018a, 2018b, 2019)</td>
</tr>
</tbody>
</table>
**Figure 2:** Advantages and disadvantages of different BES setups for *in situ* groundwater denitrification

A BES setup, known as “bioelectric well”, was proposed for *in situ* remediation of hydrocarbon contaminated groundwater (Palma et al., 2018b) (Figure 2G). This setup consisted of a granular graphite anode and a stainless steel mesh cathode; physically separated by a polyethylene mesh, while hydraulic connection was maintained. The system, operated at the set anode potential of +0.2 V vs Standard Hydrogen Electrode (SHE), obtained nearly complete (99.5%) phenol removal. This BES showed an average degradation rate of 59 ± 3 mg L\(^{-1}\) d\(^{-1}\) when inoculated with refinery wastewater; lower performances, i.e. 23±1 mg L\(^{-1}\) d\(^{-1}\), were recorded when municipal activated sludge was used as inoculum. In both cases, *Geobacter* species were predominant in the mature biofilm on the surface of the graphite granules at the anode.

In a follow-up study, Palma et al. (2018a) using the same, previously applied anode potential for phenol removal, tested the bioelectric well during long term operation to remove toluene, achieving the highest toluene removal rate reported so far for anaerobic toluene oxidation (67.2 ± 5.7 mg L\(^{-1}\) d\(^{-1}\)) (Figure 2G). *Geobacter* species acted as catalyst for the oxidation, initiated by fumarate addition, a common removal pathway for hydrocarbon-degrading anaerobic microorganisms (Palma et al., 2018a). Recently, a bioelectric well showed to successfully remove mixtures of benzene, toluene, ethyl-benzene and xylenes (BTEX) from groundwater (Palma et al., 2019). Compared to other applications previously described, the bioelectric well has, due to its vertical design, the advantage of being easily adaptable for placement in existing groundwater wells. In addition, the setup could be easily scalable, and built to include internal recirculation. However, the use of granular graphite as electrode material might require some attention due to the material’s non-scalable properties, as reported by other researchers (Rozendal et al., 2008; Zhou et al., 2011), and the tendency to form dead volumes in the bed with subsequent loss in performance (Cecconnet et al.,
205 2018b); therefore, the application of other 3-D, scalable electrode materials (e.g., carbon or graphite
206 foam) should be further investigated.

207 Recently, Kirmizakis et al. (2019) proposed a BES for gasworks groundwater in situ
208 remediation, designed with a graphite electrode chamber (in place of the conventional non-conductive
209 material) coupled with granular activated carbon (GAC) to increase available anode surface area. A
210 latex membrane was used to divide the anode from the cathode chamber. This GAC-BES showed 99%
211 removal of aliphatic and aromatic compounds with rapid bacterial colonization. The main class of
212 bacteria found in the system was betaproteobacteria with specific PAH-degrading
213 Pseudomonadaceae, commonly detected in gasworks-contaminated groundwater (Kirmizakis et al.,
214 2019).

215 In situ treatment of phenanthrene and benzene contaminated groundwater with a MFC was
216 reported by Adelaja et al. (2017), where a tubular MFC was designed with carbon felt anode exposed
217 to the contaminated groundwater and used for long term operation (about 155 days) (Figure 2F). The
218 system was able to remove up to 90% petroleum hydrocarbons at the anode and up to 79% bromate
219 (BrO$_3^-$) at the cathode (added as catholyte). The MFC was tested under copiotrophic (high
220 concentration of organic C $\simeq$ 1500 ppm for benzene and 100 ppm for phenanthrene) and oligotrophic
221 (low concentration of organic carbon $\simeq$ 50 ppb for both the considered contaminants) conditions. The
222 highest (0.76 mW m$^{-2}$) and lowest (0.01 mW m$^{-2}$) power densities were achieved in copiotrophic and
223 oligotrophic conditions respectively, while contaminants removal remained constantly high (higher
224 than 80% for benzene and phenantrone in copiotrophic conditions).

225 Despite these interesting results, due to the toxicity of BrO$_3^-$ (a known carcinogen, Hutchinson
226 et al., 1997) and the possibility of leaks, the use of BrO$_3^-$ as catholyte in in situ applications should
227 be avoided, and should be strictly restricted to ex situ treatment. The presence of BrO$_3^-$ in groundwater
228 has been reported (Butler et al., 2005), originating mainly as byproduct of potabilization processes
229 (Butler et al., 2006). Ex situ treatments would allow a combined treatment of both contaminants,
230 maintaining separate streams.
Recently, a 3-chamber BES was proposed by Liu et al. (2019), where a sediment MFC was used to drive NO$_3^-$ reduction in a bioelectrochemical reactor, whose electrodes were connected to the sediment MFC’s electrodes (Figure 2E). The connection of the two systems enhanced both performances, allowing a 66% decrease of organic matter content in the sediment and an observed denitrification rate of 3.7 mgN L$^{-1}$ h$^{-1}$. These results were achieved in both simulated groundwater and at even higher levels with real groundwater. The presence of the microcurrent positively influenced establishment of naturally occurring groundwater denitrifying microorganisms (Liu et al., 2019).

Based on existing literature analysis, the main characteristics of an optimal BES for in situ groundwater treatment can be identified as shown in Figure 3. These include the possibility to be placed in existing wells or trenches, avoiding expensive excavations; the ability to intercept groundwater flow; the lack of membranes to reduce maintenance and costs; internal recirculation to allow proper contact between biomass and substrate; large surface electrodes to allow biofilm growth; use of a power supply or potentiostat to set a desired potential and avoid limitations linked to anodic organic matter oxidation rate; easily scalable and/or modular setup.

**Figure 3:** BES characteristics for in situ bioelectroremediation
4. Discussion and future perspectives

4.1. Evolution of permeable reactive barriers: a future for BES development?

Among the options for remediation of contaminated groundwater plumes, the use of permeable reactive barriers (PRB), and in particular because of their biological decline, biobarriers have been promoted and applied in the past. A PRB is built by introducing reactive media orthogonally to the established trajectory of the contaminated groundwater plume; driven by the natural hydraulic gradient, the plume passively migrates through the PBR, allowing contact of the transported contaminants with the reactive material within the PBR, and thus leading to their fixation, transformation or precipitation to a neutral or less environmentally harmful form (Obiri-Nyarko et al., 2014).

Integration of PRBs with microbial metabolism allows the creation of “biobarriers”, or bio-PRBs. These consist of materials that enhance, support and stimulate microbial metabolism, allowing in situ bioremediation of contaminated groundwater (Obiri-Nyarko et al., 2014). Usually, microbial populations, necessary for degradation of target compound, are already present in the contaminated area (Careghini et al., 2013), while in their absence, properly enriched, preadapted bacteria can be introduced (Sarkar et al., 2017).

Several contaminants could be removed by biobarriers: reduced compounds, such as petroleum hydrocarbons, whereby oxygen (serving as terminal electron acceptor) is added via air sparging, or by addition of oxygen-releasing compounds such as CaO₂, MgO₂, H₂O₂ (Careghini et al., 2013); oxidized compounds, whereby low-cost organic matter (woodchips, alfalfa, leaves, sawdust, composted municipal sewage sludge, mulch) is added into the biobarrier (Liu et al., 2013), acting as electron donor.

Advantages of using PRBs and biobarriers are numerous and include the passivity of the technology, the possible combinations of multiple barriers to remove different specific contaminants, the avoidance of groundwater extraction (and related energy consumption), the possibility of using on site above ground areas for other purposes (Obiri-Nyarco et al., 2014; Careghini et al., 2013).
of the disadvantages conversely, is that reactive materials ought to be periodically removed/ replaced for perduring operation, and the need for long-term monitoring.

The integration of BES with reactive permeable barriers have been proposed by Palma et al. (2018b), as a possible application of their bioelectric well (Figure 4A). Such setup could provide an efficient treatment for groundwater, while saving energy by using natural groundwater flow to achieve contact between substrate and bioelectrodes, without the need for a dedicated pumping systems to attract groundwater. The aquifer flow may also (completely or partially) substitute the need for recirculation, with an additional decrease in the overall system energy consumption.

3-D electrodes built with new materials such as carbon foam or granular graphite could be a good option for these applications, as they offer a porous medium that allows water to flow, while still offering ample surface for biofilm growth, combined with an excellent electrical conductivity. Microbial populations able to perform direct or indirect electron-electrode transfer, necessary for the establishment of a BES, have been already reported to naturally occur in groundwater and used as inoculum, meaning that the addition of exogenous bacteria may not be necessary (Kirmizakis et al., 2019; Liu et al., 2019; Yang et al., 2015).

Figure 4: Scheme of the bioelectrochemical barriers proposed by Palma et al. (2018b).
One advantage of the integration of BES with PRBs rely on both anode and cathode acting as virtually inexhaustible electron sink and donor, respectively. Therefore, no replacement of the reactive materials/chemicals is needed. However, a thick biofilm could change the conductivity of such electrodes which may create the necessity of washing or replacing them after some time. On the other hand, Czurda and Haus (2002) reported that the integration of electrochemical processes in biobarriers may reduce the fouling induced by excessive microbial growth, and remove undesired biomolecules and precipitates. Based on this idea, the existing permeable barriers may be retrofitted and offer the possibility of oxidation and reduction of a variety of contaminants.

Palma et al. (2018a, 2018b) proposed the combined use of several units of their tubular-designed bioelectric well. An upgrade of this system could be the use of possible setups able to intercept the groundwater flow, similarly to the diffused funnel and gate or open channel design of PRBs (Fig. 4B).

4.2. Energy consumption: an often-neglected parameter

Zou and He (2018) recently analyzed the energy sustainability of bioelectrochemical systems for multiple purposes (desalination, wastewater treatment, hydrogen production) considering the additional energy costs due to the recirculation and feeding/extraction of influents/effluents. A similar analysis of in and ex situ treatment with MFC and power supply-assisted biocathodes was conducted by Cecconet et al. (2018c), showing that in situ denitrification using MFC can be energy-positive. The performances of the poised biocathodes were far higher than the MFC’s in terms of nitrogen removal rates (around 30% higher). Based on that calculation, it is possible to consider the use of MFC for long-term groundwater denitrification (or biocathodic reduction of other chemicals). Electrons produced by anodic organic matter oxidation could be used for cathodic reduction, and the limitation of removal rates would be acceptable for an in situ treatment, where the spatial dimension of the contamination is high. One issue to be tackled is the use of organic matter as anolyte, and the need to avoid further contamination. On the other hand, in an ex situ configuration the use of a poised
biocathode would benefit from higher removal rates, being able to ensure higher flow rates for different purposes. More attention should be given to the actual energy consumption of BESs, by means of a life cycle assessment (LCA) of the technology, where all the different aspects such as recirculation, pumping, etc. are considered.

Recently, the microbial electrochemical snorkel emerged as a novel BES type (Figure 5). A snorkel is a short-circuited MFC, where a microbial anode is directly coupled with a biotic or abiotic cathode. The snorkel does not produce (or require) power, but it works at the maximum current sustainable by the system, and at the same time, hence, at the maximum electrochemical reaction rate (Hoareau et al., 2019). This system is characterized by its extreme simplicity, as it may be constituted by a single rod (graphite, steel or carbon), exposed to two different redox environments (Viggi et al., 2017, 2015). Snorkels have been used to degrade organic matter from wastewater (Aguirre-Sierra et al., 2016) and NO$_3^-$ from low-organic wastewater, in addition to remediating hydrocarbon-contaminated sediments (Yang et al., 2015).

The application of a snorkel for groundwater remediation would allow treatment where no external energy is provided, and where simplicity would suggest its use for \textit{in situ} applications. However, the snorkel is a relatively new type of BESs, and some challenges might be unsolved, such as the inability of fully controlling the electrode potential, the lack of accurate delimitation of anodic and cathodic zones, and the possible presence of oxygen in the anodic zone. Some solutions, including numerical and mathematical modelling and use of anocathodophilic biofilms have been proposed (Hoareau et al., 2019).
**Figure 5**: Microbial electrochemical snorkel. (A) Oxygen and (B) nitrate as electron acceptor.

### 4.3. Complex groundwater with multiple contaminants

Different and/or concomitant anthropic activities (i.e. agriculture, industry) may contaminate groundwater with different pollutants (Bartzas et al., 2015; Han et al., 2016; Venkatramanan et al., 2016). Therefore, working on single contaminants can be interesting and useful to understand the single component removal pathway and its related kinetics but it might not be useful for real applications.

Earlier attempts of using BESs for the removal of multiple contaminants from groundwater have been reported: Butler et al. (2010) first studied the interaction and competition of NO$_3^-$ and perchlorate as terminal electron acceptors (these compounds are often associated in groundwater) in a BES biocathode; Xie et al. (2014) showed the inhibition of perchlorate reduction in presence of 2.1 mM of NO$_3^-$, and a slowed reduction at lower NO$_3^-$ concentrations. Nguyen et al. (2016b) carried out research on the As oxidation at the anode and denitrification at the cathode of BES, maintaining a strict separation of the streams (i.e. the anodic and cathodic influents). The competition between
vanadium and chromium in BES cathodes has also been investigated (Zhang et al., 2012) while Lai et al. (2015) performed reductive BES-based dechlorination of cis-dichloroethylene (cis-DCE) in NO$_3^-$ and sulfate contaminated real groundwater with a simultaneous reduction of all three compounds.

In order to enhance sustainability of treatment, the multi-contaminant approach should be implemented, considering industry-originated organic carbon sources or organic contaminants commonly detected in groundwater (e.g., petroleum hydrocarbons). Potential inhibition effects due to the combination of different contaminants should also be assessed. The use of BESs with separated chambers should be carefully evaluated, as the separation of reduced and oxidized contaminants in groundwater is not as feasible as in lab setups. A flow-through setup, similar to the one described by Pous et al. (2017), may represent an option: in this case the contaminated stream would first be exposed to the anode, allowing oxidation processes, and then to the cathode where reduction would occur. Another option is the use of anocathodophilic bacteria that employ electrodes as both electron acceptors and donors based on redox conditions; some examples have been reported for biofilms capable of catalyzing organic matter oxidation and NO$_3^-$ or chromium reduction (Beretta et al., 2018; Molognoni et al., 2017; Pous et al., 2016).

4.4. Need for additional modeling efforts

Modeling MFC operation has advanced extensively with the possibility to predict both organic matter removal and energy production (Capodaglio et al., 2017; Gadkari et al., 2018; Pinto et al., 2010). In addition, MEC and MDC processes have been successfully modeled (Ping et al., 2014; Pinto et al., 2011) and latter models evaluated integration of BESs with membrane bioreactors and algal photobioreactors (Li and He, 2016; Luo et al., 2017). Statistical methods have also been applied to BES technology to improve knowledge on the operation (Cecconet et al., 2018a; Luo et al., 2016).

Up to date, only a single modeling effort has been reported for groundwater treatment using BES. Srinivasan et al. (2016) developed a model for groundwater denitrification based on the model
proposed by Pan et al. (2013), showing a competition between NO$_3^-$ and nitrite for electrons in the biocathode of an MFC. Based on existing models, the removal of the considered contaminants in groundwater could be simulated, taking into account competition between different electron acceptors.

4.5. Scaling-up

BES applications at full scale for wastewater treatment have been reported (Table 2), but no data are yet available for full scale applications for groundwater treatment. Most of the data for such BES applications are still at laboratory scale. Recently, Wang and He (2019) discussed the dimension that a BES should reach in order to be referred to as “pilot scale”, concluding that a large part of the examples of pilot scale BES reactors available in literature should not be considered as such, based on their practical flow or hydraulic capacity: a pilot reactor should have between 0.1 and 5% of the full scale application flow rate. This concept is difficult to be applied in the case of in situ groundwater bioelectroremediation due to the impossibility to identify a flow-rate for the reactors. In contrast, a range of flow rate values can be calculated for ex situ on site applications. In the case of drinking water treatment plants built to serve small or medium communities (with a flow rate in the 500-5000 m$^3$ d$^{-1}$ range), no reported BES was able to meet the 0.5-250 m$^3$ d$^{-1}$ required flow-rate. A different scenario should be considered for decentralized applications: if we consider an isolated household housing four people, a daily water consumption of 400 L d$^{-1}$ can be calculated based on the World Health Organization (WHO) requirement of 100 L d$^{-1}$ per capita as the minimum daily water intake necessary to avoid any level of health concern connected to the available water quantity (Howard and Bartram, 2003). Therefore, a pilot scale BES reactor for such a decentralized household should guarantee a flow rate higher than 0.4-20 L d$^{-1}$. This condition has been met by BES performing groundwater treatment, with flow rate values exceeding 12 L d$^{-1}$ in the denitrifying BES operated by Pous and co-workers (2017). Based on that, it is possible to state that BES for groundwater treatment have reached the pilot scale level (even though limited to decentralized applications) and are no longer confined to laboratory settings.
Recently, application of small scale BESs in series has been proposed as an alternative to the increase in size for technology upscaling (Greenman and Ieropoulos, 2017). Series application of several small-scale BESs may be a feasible option to both remove different contaminants (e.g., biocathodes poised at different potential focused each on a different contaminant, similar to the setup reported by Huang et al. (2015) and increase energy production. This sequential approach was tested by Ceccone et al. (2019b) for groundwater denitrification. The sequential coupling of two denitrifying biocathodes showed to be particularly promising in terms of energy sustainability: the specific energy consumption (SEC) of the system decreased at the increase of the NO$_3^-$ load, showing that such a system was more energy-efficient when operated at low HRTs (advantageous aspect for a full-scale treatment technology).

The possibility of connecting two or more BES units represent another aspect of the flexibility of the technology; applications of stacked MFCs have been reported (Kim et al., 2017; Vilajeliu-Pons et al., 2017; Wu et al., 2016), as also the applications of MFC to supply enough voltage to allow MEC processes (Choi et al., 2014; Liu et al., 2016).

<table>
<thead>
<tr>
<th>BES type</th>
<th>Dimension (L)</th>
<th>Influent</th>
<th>Modularized?</th>
<th>N° of modules</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFC</td>
<td>1000</td>
<td>Brewery WW</td>
<td>Yes</td>
<td>12</td>
<td>Logan (2010)</td>
</tr>
<tr>
<td>MFC</td>
<td>90</td>
<td>Brewery WW</td>
<td>Yes</td>
<td>5</td>
<td>Dong et al. (2015)</td>
</tr>
<tr>
<td>MFC</td>
<td>200</td>
<td>Municipal WW</td>
<td>Yes</td>
<td>96</td>
<td>Ge and He (2016)</td>
</tr>
<tr>
<td>MFC</td>
<td>250</td>
<td>Municipal WW</td>
<td>No</td>
<td>-</td>
<td>Feng et al. (2014)</td>
</tr>
<tr>
<td>MEC</td>
<td>130</td>
<td>Urban WW</td>
<td>Yes</td>
<td>10</td>
<td>Baeza et al. (2017)</td>
</tr>
<tr>
<td>MFC</td>
<td>300</td>
<td>Urine</td>
<td>Yes</td>
<td>432</td>
<td>Ieropoulos et al. (2016)</td>
</tr>
</tbody>
</table>
Few examples of commercial application of the BES technology have been reported so far (e.g. Plant-e, spinoff of Wageningen University, Netherlands; Cambrian water), mainly related to the production of bioenergy using MFCs, or hydrogen production using MECs. The cost of the materials (membrane and electrode mainly) is still a hard-to-overcome issue (Foley et al., 2010; Pant et al., 2011), and only large-scale commercialization of BESs could lower those costs. An alternative is represented by the application of natural materials in place of membranes and electrodes (Goglio et al., 2019), but these still offer far lower performance than engineered or conventional materials. Based on the above, major cuts on the materials’ costs (i.e. membranes) are needed in order to allow BES to become an established technology for groundwater denitrification.

### 4.6. Contaminants of emerging concern

CECs are a class of substances used for a variety of purposes: personal care, food production, human and animal health (pharmaceuticals), industrial manufacturing and fire suppression (Richardson and Kimura, 2017). CECs have been detected worldwide in groundwater: their presence has been reported in Europe (Jurado et al., 2012; Stuart et al., 2012), Americas (Montes-Grajales et al., 2017), Asia (Lapworth et al., 2018), Africa (Arukwe et al., 2012) and Oceania (Sui et al., 2015), in developed and developing countries alike. Their occurrence in groundwater is to be ascribed to anthropic activities (Lapworth et al., 2012); therefore, CECs can be used as tracers to identify groundwater contamination.
due to wastewater infiltration (McCance et al., 2018). As most of the world relies on the use of groundwater for drinking water production, and CECs’ regulations have not been issued yet (at least in the European Union), much debate on the matter is going on (Lapworth et al., 2019).

BESs proved to be able to remove CECs with high performance, in some cases higher than conventional water and wastewater treatments (e.g., biological process), scoring interesting results in particular in the removal of recalcitrant contaminants due to the combination of microbial metabolisms with different redox conditions offered by anode and cathode, respectively (Cecconet et al., 2017). Therefore, the investigation on the removal of CECs from groundwater should be intensified, taking into consideration the particularities of groundwater (general low conductivity, and low concentration of nutrients and organic matter); in addition, the removal of those contaminants should be considered in combination with the presence of other contaminants that may occur in the aquifer, especially if there are orders of magnitude of difference between their concentrations.

**4.7. Biosensing**

Recently, much attention has been attracted by the development of BES-based biosensors for environmental monitoring (Ivars-Barceló et al., 2018). Therefore, some researchers have shifted their focus on applications for contaminants detection and monitoring in groundwater. Velasquez-Orta and co-workers (2017) designed an MFC-based biosensor for the online monitoring of fecal and organic pollution in shallow groundwater wells, obtaining responsive increases in the current produced; the system was sensitive to temperature fluctuations but not to changes in salinity or modifications of the external resistance (and thus to longer wiring for the connections of the electrodes). Field tests highlighted the influence of water level oscillations in the wells causing air exposition at the cathode (Velasquez-Orta et al., 2017). Organic matter presence in an aquifer undergoing bioremediation was ascertained by the increase in current density in a BES-based biosensor; current quickly dropped when organic matter presence was discontinued, suggesting that the system was able to monitor subsurface microbial activity during in situ bioremediation (Williams et al., 2010). The electrodes
were able to produce detectable current despite the long separation of anode and cathode (6 m), and
electron transfer was attributed to *Geobacter* species (Williams et al., 2010).

Bio-current generated by a bioanode poised at +0.2 V vs SHE was reported being linearly
correlated with the increase in concentration of biogenic Fe(II), serving thus as indicator; Fe(II) is
widely used chemical in groundwater remediation, and the system could monitor the concentration
of the compound in an effective way (Feng et al., 2013). A BES-based biosensor for As was
developed by Webster et al. (2014) using an engineered *Shewanella oneidensis* strain, allowing an
arsenite detection limit of 40 µM and a linear range up to 100 µM. A BES-based biosensor able to
monitor NO$_3^-$ concentrations in real-time was proposed by Su et al. (2019), but designed for
monitoring secondary effluents of wastewater treatment plants, and thus requiring organic matter to
properly work; such design could not be adapted to groundwater monitoring. Biosensors developed
to monitor microbial activity in anoxic sediments (Wardman et al., 2014) could, with some
modifications in the setup, be applied to groundwater monitoring.

The development of BES-based biosensors is of extreme interest to the research community,
due to the possibility of operating in off-grid and decentralized applications, and the suitability for *in situ*
and on site testing, other than requiring less time and advanced technological skills compared to
conventional analytical techniques (Grattieri et al., 2017).

5. Conclusions

Amongst the different technologies applicable for *in situ* treatment of contaminated groundwater,
BESs showed to be a suitable and feasible option. The analysis of the different setups reported so far
in literature highlighted that it is crucial to properly design a BES to be operated in a harsh and
challenging environment such as the aquifer, and that the simple adaptation of *ex situ* BES setups
may not be sufficient to achieve the desired results.

Research in the field mainly focused on denitrification and hydrocarbons removal, showing
excellent results due to the interaction of microbial metabolism and poised electrodes. In the near
future research should focus on emerging topics such as the interaction between multiple contaminants (both reduced and oxidized), accurate estimation of the energy consumption for in situ treatment with BES, development of reliable models to simulate and predict the processes and possible integration of BES with PRBs for the interception and treatment of contaminated plumes. The integration of these research gaps with the existing BES technology could lead to the development of reliable and resilient BES for in situ bioelectroremediation.

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**Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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