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Review

Electro-bioremediation of wastewater: Transitioning the focus on pure cultures to elucidate the missing mechanistic insights upon electro-assisted biodegradation of exemplary pollutants

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ABSTRACT

Electro-bioremediation of exemplary water pollutants such as nitrogenous, phosphorous, and sulphurous compounds, hydrocarbons, metals and azo dyes has already been studied at a macro-scale level using mixed cultures. The technology has been generally established as a proof of concept at the technology readiness level (TRL) of 3, and there are already specific cases where the technology reached TRL 5. However, this technology is less utilized compared to traditional approaches. Although, mixed cultures result in high electro-biodegradation efficiency, their use hinders process' mechanistic insights which are better determined through pure cultures studies. This knowledge can lead to improved technologies. Therefore, this manuscript focuses on the specific pollutants' electro-biodegradation by pure cultures, assessing the availability of information regarding genes, enzymes, proteins and metabolites involved. Furthermore, studies characterizing the dominant genera or species are assessed, in which the available information at molecular level is evaluated. In total, less than 40 studies were found which were predominantly focused on the electro-biodegradation potential rather than the mechanistic insights. This highlights a gap in the field featuring a motivation to transitioning the focus on the study of pure cultures to unravel the mechanistic insights. Therefore, specific actions are suggested. Characterization of the mixed cultures followed by microorganisms' isolation is crucial. Thus, electroactive and biodegradation characteristics will be revealed using omics, genome annotation and transcriptional kinetics. This can lead to optimization at the microbiological level through genetic engineering, synthetic biology, mathematical modelling and strategic building of co-cultures. This research focus offers new avenues for sustainable wastewater treatment.

1. Introduction

Traditional bioremediation

Over the past decades, the discharge of pollutants into the environment has become a severe cause for concern. At present, environmental pollutants are dispersed across various ecosystems, including air, water, soil, and marine sediments. Households and industries are responsible for generating wastewater containing a wide range of pollutants. Groundwater, the primary source of fresh and drinking water, is particularly vulnerable to these pollutants (Puggioni et al., 2021). The ongoing trends of population growth, urbanization, and industrialization are expected to contribute to increased wastewater generation and with that, groundwater pollution. Consequently, addressing the reduction of water pollution and wastewater treatment is essential (Varjani, 2022).

Most inorganic and organic pollutants have the potential to undergo chemical and/or biological transformations, resulting in non-toxic or less toxic forms. Nevertheless, the conversion of pollutants through natural processes is rather slow due to the restricted availability of

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electron donors or acceptors necessary for the biotic or abiotic reduction or oxidation of pollutants. To address this challenge and manage pollutants in water and wastewater matrices, advanced processes and technologies have been employed. These include thermo- and chemical-physical techniques, such as electrodialysis, adsorption, precipitation, ion exchange, microbiological methods, phytoremediation, nanotechnology, membrane filtration, ultrasonic irradiation and advanced oxidation processes (Wang et al., 2020b; Al-Qodah et al., 2014, 2019). However, most of these processes are chemical and energy-demanding, cost-intensive and occasionally time-consuming.

Microbial electrochemistry to overcome the limitations of traditional bioremediation technologies

In the past two decades, technologies derived from microbial electrochemistry, such as Bioelectrochemical Systems (BES), have been proposed as an alternative approach to overcome the limitations of traditional remediation technologies (Wang et al., 2020b). These technologies employ electroactive microorganisms (EAM) that act as biocatalysts to facilitate oxidation and reduction reactions on solid electrodes. BES technologies can be separated into the following categories: (a) microbial fuel cells (MFC) that generate electricity (Logan et al., 2006), (b) microbial electrolysis cells (MEC) for the production of H_2 (Logan et al., 2008), (c) microbial electrosynthesis cells (MES) to produce added-value compounds *via* CO_2 reduction, and (d) microbial desalination cells (MDC) that separate charged ions from saline water (Cao et al., 2009) *via* electricity generation.

Although this concept was proposed in 1911 by Potter M.C. (Potter, 1911), it was not until nearly 100 years later that BES were employed in wastewater bioremediation (Logan et al., 2006). Various BES technologies, ranging from laboratory to *in-site* scales, have already been successfully used for the bioremediation of pollutants in various environments, including soil and sediments, groundwater, water, and wastewater matrices (Rozendal et al., 2008; Sun et al., 2020; Wang et al., 2020b). Indeed, this is visible in the rise of published reviews within the field, as illustrated in Fig. 1. These studies have been crucial in providing insights into the technology, enabling the establishment of a technology readiness level (TRL) of at least 3 and even further scale-up optimization strategies related to BES's conditions and design. However, parameters at the microbiological level remain predominantly unchanged.

To date, only MFC and MEC have been used for the removal of pollutants in wastewater (Fig. 2). Other utilization of BES involves the potential integration with constructed wetlands (CW). CW utilize substrates from the wastewater under treatment and supports the growth of microorganisms and plants, leading to the bioremediation of wastewater. To enhance the efficiency of CW, the integration of BES has proven successful in facilitating the exchange of electrons with electrically conductive materials (Prado de Nicolás et al., 2022). Furthermore, it was demonstrated that BES form low amounts of waste sludge during wastewater bioremediation (Brown et al., 2015), which is a beneficial advantage compared to conventional bioremediation technologies.

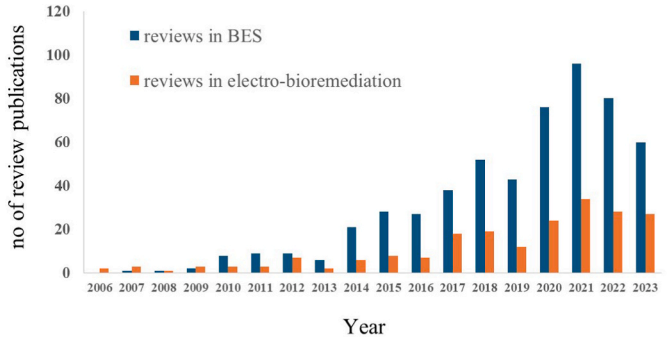


Fig. 1. Number of review publications in BES technologies and electro-bioremediation from 2006 until 2023 based on web of science.

Extracellular electron transfer (EET) in bioelectrochemical systems (BES)

The process through which EAM maintain their metabolism by either donating or releasing electrons to solid donors or acceptors is known as extracellular electron transfer (EET) (Paquete et al., 2022a). In BES, electrodes function as either electron donors or acceptors, facilitating electron transfer through either a direct or indirect manner. EAM can be exoelectrogens or electrotrophs. Exoelectrogens are microorganisms that grow on the anode and transfer electrons produced by oxidizing organic substrates to it. On the other hand, electrotrophs can receive electrons from the cathode and use them in their metabolism (Logan et al., 2019; Varjani, 2022). Multiheme *c*-type cytochromes play a crucial role in these processes, although in some cases, electron mediators produced by the microorganisms can also assist in EET mechanisms (Paquete, 2020). Over 100 microorganisms covering the three domains of life have been found as electroactive (Logan et al., 2019).

Macro-scale in BES for bioremediation: studies using mixed cultures

Mixed cultures are primarily used in BES technologies, contributing positively to the bioremediation of pollutants and EET processes with the electrode. Indeed, mixed cultures present in activated sludge, aerobic or anaerobic sludge, and acclimatized inoculums perform better in BES given the diverse metabolic versatility and EET processes of the different organisms (Cao et al., 2019; Prathiba et al., 2022) compared to pure cultures. However, the use of mixed cultures prevents the elucidation of the mechanisms, gene regulation, and metabolic pathways behind electro-assisted biodegradation steps, as well as differentiating between direct and indirect EET mechanisms performed by the different organisms (Schröder, 2018). This is due to the metabolic diversity and complicated metabolic pathways existing in a microbial community. The metabolic diversity as well as the metabolism followed for electroactivity in EAM is not yet determined clearly (Koch and Harnisch, 2016).

Study of pure cultures to elucidate the mechanistic insights involved in biodegradation and electron transfer to/from electrodes

To transition the focus towards this direction, studying pure cultures at the laboratory and, thus, micro-scale level can shed light on the metabolic pathways involved in degradation and electron transfer to and from electrodes upon pollutants electro-biodegradation. Mechanistic insights of electroactivity and biodegradation deserves systematic research, as it can lead to significant breakthroughs in the understanding of microbial systems involved in generation of alternative electricity with simultaneous pollutants biodegradation (Mahidhara et al., 2017). The most important advantage of pure cultures compared to mixed cultures is that they enable the study of EET pathways and metabolic processes performed by specific organisms (Luo et al., 2015). The most characteristic example is the systematic study using *Geobacter sulfurreducens* (for instance (Bond and Lovley, 2003; Holmes et al., 2006; Steidl et al., 2016)) and *Shewanella oneidensis* (for instance (Gorby et al., 2006; Marsili et al., 2008; Kouzuma et al., 2018)) strains as pure cultures which revealed the proteins and genes involved in EET mechanisms. However, upon the use of other known electroactive microbial species, little is known about the genes, proteins/enzymes, and metabolites responsible for those mechanisms (Kracke et al., 2015; Verma et al., 2023). This is true despite the fact that the anodic (Paquete et al., 2022a) or cathodic (Huang et al., 2011) EET mechanisms have been suggested. In reality, more than 50 pure cultures have been studied to date in BES using conventional substrates, including glucose, peptone, sediments,

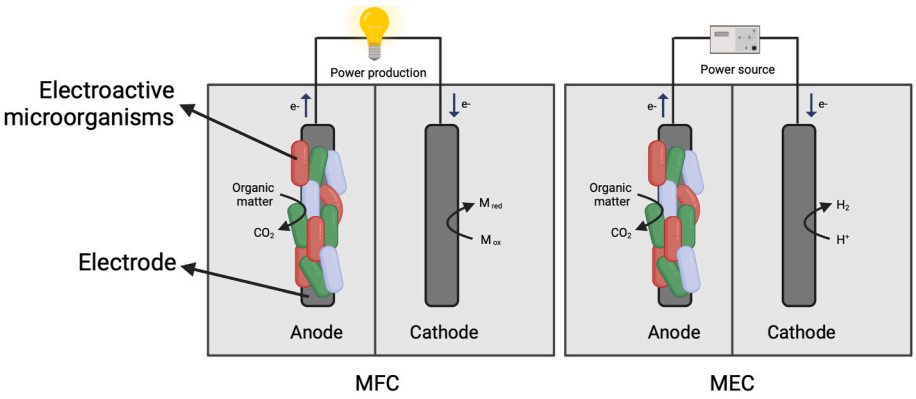


Fig. 2. Type of BES technologies used for removal of organic pollutants: microbial fuel cell (MFC) and microbial electrolysis cell (MEC).

activated sludge, acetate, or lactate (Luo et al. 2015, 2023) and still without focusing on the molecular level of the bioprocess. In the electro-assisted biodegradation of pollutants, there has been even more limited research on the utilization of pure cultures to study the mechanistic insights of the process focusing solely on the biodegradation and electricity generation potential. The genes and proteins for exemplary pollutants degradation and EET identified so far, are only a tiny part of the broader spectrum of molecules that actively participate in the process. This underscores the significance of investigating pollutants electro-assisted biodegradation properties by pure cultures to uncover novel proteins, enzymes and metabolites possessing favourable characteristics and functionalities. To date, there are no review papers showing the study of electro-biodegradation of specific pollutants from wastewater using pure cultures. On top of this, no review papers exist gathering information about the mechanistic insights of biodegradation and EET using those specific pollutants in wastewater by pure cultures.

Consequently, in this review, electro-bioremediation performed by EAM in efficiently removing pollutants from contaminated water, focusing particularly on six major pollutant categories: nitrogenous, phosphorous and sulphurous compounds, heterocycle compounds, metals, and azo-dyes, is explored. This review will offer insights into the spectrum from the macro to the laboratory-scale and hence, micro-scale level, focusing particularly on existing research concerning pure cultures and identifiable dominant genera or species responsible for pollutant degradation and EET. This review will further highlight the known genes, proteins, enzymes and metabolites involved in electro-assisted biodegradation of those pollutants. Consequently, the perspective on the existing gaps in current literature will be identified, encouraging further research at the molecular level.

2. From mixed to pure cultures upon electro-assisted biodegradation of nitrogenous pollutants

Electro-bioremediation of nitrogen compounds

The presence of nitrogen contamination, primarily associated with ammonium (NH_4^+) and nitrate (NO_3^-) species in wastewater, remains a critical environmental concern due to its potential to cause water pollution and ecological damage of aquatic systems. In response, electro-bioremediation has emerged as a sustainable approach for treating nitrogen-contaminated water.

Studies of mixed cultures: examples of technology readiness level establishment

Recent research has demonstrated the conversion of ammonium into dinitrogen gas (N_2) through electricity-driven processes, utilizing various microorganisms such as Anammox bacteria (Shaw et al., 2020), feammox (Ruiz-urigüen et al., 2019), autotrophic nitrifying bacteria (Vilajeliu-pons et al., 2018; Osset-Alvarez et al., 2022), and heterotrophic nitrifying microorganisms (Pous et al., 2023). While these studies have established the proof-of-concept (TRL: 3), further research should prioritize enhancing ammonium removal rates in these processes.

A more streamlined approach involves the electro-bioremediation of nitrate-contaminated water (Sevda et al., 2018). The denitrifying biocathode is a complex system that integrates a microbial community with electrochemical reactions. Consequently, a diverse range of microorganisms have been identified in denitrifying biocathodes, with the majority belonging to the β-Proteobacteria class (Rogińska et al., 2023). Currently, this process is advancing towards scaling up, and it has reached a TRL of 5, indicating its validation in a relevant environment. The intensification of this process has led to significant improvements in nitrate removal rates, achieving up to 1.2 kg $N/m^3 \cdot d$ (Ceballos-escalera et al., 2021) along with the development of compact technologies designed for short hydraulic retention times (0.5h) (Pous et al., 2017).

Studies of pure cultures or dominant genera/species

While processes for the removal of both ammonium and nitrate have received extensive attention in terms of their applicability, including removal rates, efficiencies, and yields, there is a noticeable scarcity of studies that concentrate on characterizing the microbial communities involved in these processes and assessing the biodegradation and EET mechanisms. Cyclic voltammetry (CV) has traditionally been employed as a technique to provide additional characterization of the electrogenic (in the case of ammonium oxidation) or electrotrophic (in the case of nitrate reduction) activities of the bioanode/biocathode. Electrochemical characterization *via* CV can be performed either *in situ* on flat-plate electrodes or *ex situ* on fixed-bed electrodes composed of granular graphite (Korth et al., 2022). In the latter scenario, granules are extracted for CV analysis using specialized sampling units. These granules are securely held in place with an e-clamp grab hook and subsequently transferred directly to four-neck round-bottom flasks equipped with an integrated three-electrode setup (Quejigo et al., 2021).

Shaw and colleagues (Shaw et al., 2020) elucidated the electron transfer mechanisms underlying the anaerobic oxidation of **ammonium** by anammox bacteria, including ***Candidatus Brocadia, Candidatus Scalindua,*** *and* ***Kuenenia stuttgartiensis*** cultures. Their study revealed the electrochemical activity of these cultures, offering compelling evidence for electrode-dependent anaerobic NH_4^+ oxidation by phylogenetically diverse anammox bacteria. Notably, this EET-dependent anammox process can take place at relatively low voltages, typically within the range of 0.3–0.6 V vs. SHE. In addition, it was noted that genes encoding for ammonium transporter (AmtB), hydroxylamine oxidoreductase and hydrazine dehydrogenase (HDH) enzymes as well as the metabolite hydroxylamine were involved in the process. Therefore, it would worth studying the strains as pure cultures in order to understand the electro-assisted biodegradation mechanism of each strain

involved.

In another study by Pous and colleagues (Pous et al., 2023), electrochemical characterization was conducted on a heterotrophic nitrifying microorganism, specifically an ***Alcaligenes* strain HO-1** culture. Their investigation revealed that the presence of a polarized electrode actively supported the metabolic functions of *Alcaligenes* strain HO-1, enabling the simultaneous oxidation of succinate and ammonium. In this context, the authors postulate that hydroxylamine, an intermediate in the conversion of ammonium into dinitrogen gas, may play a role in the observed electrochemical signal, a hypothesis that had been previously suggested with nitrifying-enriched communities (Vilajeliu-pons et al., 2018). These findings underscore the potential of integrating electrodes into bioprocesses to enhance overall system performance as well as study in detail *Alcaligenes* strain HO-1 attached on electrodes at molecular level, which has not been performed yet.

The information regarding EET-dependent mechanisms is more extensive in denitrifying biocathodes. Pous and colleagues (Pous et al., 2014) identified two distinct formal potentials at −0.1 V and −0.5 V vs. SHE (pH 8) in a biocathode primarily comprised of the β-proteobacterium ***Thiobacillus* sp**. Other studies have reported electroactive redox sites at −0.174 V vs. SHE (pH 7) for a denitrifying biofilm predominantly colonized by ***Geobacter*** (Pous et al., 2016), as well as −0.288 V and −0.206 V vs. SHE (pH 7) in the case of a microbiome consisting of ***Pseudomonas nitroreducens*** and ***Paracoccus versutus*** (Korth et al., 2022). Furthermore, Ceballos-Escalera et al. (Ceballos-Escalera et al., 2021) observed a derived formal potential for EET related to nitrate reduction at -0.02 ± 0.01 V (pH 7.75 ± 0.2) in her denitrifying biofilm, primarily composed of ***Sideroxydans lithotrophicus* ES-1**, which was used to treat contaminated groundwater containing nitrate and arsenite. These potentials correspond to the presence of one or multiple redox cofactors and are integral to the transfer of electrons from the cathode to the subsequent binding and reduction of nitrate within cells, a crucial step that is recognized as the limiting factor in this process. However, it was noticed that although the description of the EET-dependent biodegradation mechanisms involved either in microbial communities with dominant genus or pure cultures is exhaustive, the information regarding genes, enzymes/proteins and metabolites involved is missing.

3. From mixed to pure cultures upon electro-assisted biodegradation of phosphorous pollutants

Electro-bioremediation of phosphorous compounds

Besides being essential for life, phosphorous derivatives have a wide range of applications, ranging from their use in agriculture to industry. This is why phosphorous is a significant contaminant found in wastewater, and treating it is a critical step in wastewater treatment plants. Various methods, such as chemical precipitation, biological nutrient removal, and advanced treatment technologies, are widely used to reduce phosphorous levels (Akpor and Muchie, 2010; Kelly and He, 2014). Recovery of this compound from wastewater is fundamental for environmental pollution control, but even more significant for the exploitation of secondary phosphorous resources, such as for use as fertilizer (Wang et al., 2022), because phosphate rock, which is a natural phosphorous resource, is limited in supply (Yu et al., 2022). In some countries, regulations have been put in place for wastewater treatment plants to not only reduce the amount of residual phosphate in treated water but also to recover the accumulated phosphorous from microbial biomass or from the ash that remains after biomass incineration (Blank, 2023). Phosphorous recovery with BES has been tested in both MFC and MEC.

Studies of mixed cultures: examples of technology readiness level establishment

A 10 L dual-chamber MFC stack with a volume of 1.2 L per unit fed with urine for three months, allowed this technology to reach the TRL 4 for electro-bioremediation. The pH increase in the cathode chamber was utilized to recover phosphate and ammoniacal nitrogen as struvite in an external reactor. This was crucial to achieve an efficiency of 90% for phosphate recovery and 46% for ammoniacal nitrogen recovery (Sharma et al., 2021). The effect of the pH increase was further validated in studies involving swine wastewater and mixed cultures in an air-cathode single-chamber MFC (Ichihashi and Hirooka, 2012) and a single-chamber MFC (Cusick and Logan, 2012). However, it still raised questions, particularly regarding the involvement of electroactive microorganisms in this process.

Zhang and colleagues explained that the hydrogen produced in MEC increased the localized pH adjacent to the cathode electrode, and this is the most important factor for struvite precipitation (Zhang et al., 2012b). Struvite precipitation was the predominant method to recover phosphorus in cathodes. However, in the presence of other cations, like Ca^{2+}, various phosphorus minerals, including calcium phosphate, may form and accumulate near the cathode in response to the rise of pH. Calcium phosphate is commercially appealing and can be obtained without the need for an external calcium source, as calcium is abundant in wastewater (Wang et al. 2022, 2024). Using this procedure, Wang and colleagues were able to reach 74% of phosphorus removal (Wang et al., 2024).

Studies of pure cultures or dominant genera/species

A two-chamber MFC has been used to produce electricity and reduce iron phosphate ($FePO_3$) into immobilized orthophosphates (H_3PO_4, HPO_4^-, HPO_4^{2-} and PO_3^{-4}) on the cathode using digested sewage sludge. The mobilized phosphate reacted with stoichiometric amounts of $MgCl_2$ and NH_4OH and precipitated struvite ($MgNH_4PO_4^-6H_2O$). In this setup, $FePO_4$ was reduced through the metabolic activity of ***Escherichia coli*** in the anode. The process yielded up to 82% recovery (600 mg l^{-1}) of struvite (Fischer et al., 2011). Although in this process, the microorganism used was known, the EET-assisted molecular mechanisms in the anode leading to the cathode phosphorus precipitation were not studied.

Struvite precipitation is typically reported in BES on cathodes, or by combined anode-mediated oxidation and cathodic reduction. Phosphorus recovery in wastewater can occur without the addition of chemicals *via* the establishment of a localized high-pH environment near the cathode. But BES can also be integrated with other treatment processes, such as biological phosphorus removal and chemical precipitation. However, this combined process still needs to be explored as a synergistic approach to achieve comprehensive phosphorus removal and recovery (Wang et al. 2022, 2024).

4. From mixed to pure cultures upon electro-assisted biodegradation of chlorinated aliphatic hydrocarbons pollutants

Electro-bioremediation of chlorinated aliphatic hydrocarbons pollutants

In the past decades, chlorinated aliphatic hydrocarbons (CAHs) such as trichlorethene (TCE), perchloroethene (PCE) and 1,2-dichloroethane (1,2-DCA) have been used as traditional solvents in industry, dry-cleaning and degreasing agents (Lai et al., 2017). Today, these CAHs, along with their transformation products, like cis-1,2-dichloroethene (cDCE) and vinyl chloride (VC), are commonly detected in groundwater. They tend to accumulate in the lower layers of aquifers, resulting in groundwater contamination (Dell'Armi et al., 2022b). Given their toxicity and carcinogenic properties, it has become imperative to remove CAHs from natural ecosystems (Dell'Armi et al., 2022b). In electro-bioremediation, the biodegradation of CAHs can take place both aerobically and anaerobically (Tiehm and Schmidt, 2011). Under anaerobic conditions, organohalide respiring bacteria (OHRB) facilitate reductive dechlorination (RD), reducing the highly chlorinated parent

compounds into less-chlorinated transformation products (Lai et al., 2017). This process can occur in the presence of electron donors, which can be either organic compounds or hydrogen (Dell'Armi et al., 2022b).

Studies of mixed cultures: examples of technology readiness level establishment

Many studies use anaerobic sludge or mixed cultures as inoculum to perform dechlorination. These studies have established a TRL of 3. The transformation products of CAHs, cDCE and VC, can be biodegraded through oxidative pathways. Therefore, the combination of RD upstream and oxidative dechlorination (OD) downstream can be a feasible design for biodegrading CAHs from groundwater (Tiehm and Schmidt, 2011). Towards this direction, a bio-anode has been polarised to oxidise cDCE (Aulenta et al., 2013) and MECs have been successfully adopted in the last years for the stimulation of the sequential reductive and oxidative dechlorination of CAHs (Zeppilli et al., 2021). This process has been validated under laboratory conditions, which were crucial to achieve a TRL of 4.

A sequential reductive and oxidative environment has been obtained by the utilization of different MEC designs, such as the use of a flow-through cathode-anode bio-electrochemical reactor with an anode made of different materials. In this system, RD of TCE occurred in the cathode at potential −0.65 V vs. SHE and with an initial organic load rate of TCE of 20 mM d^{-1} (Lai et al., 2017). The reactor exhibited its best performance when the anode comprised mixed metal oxide rods inserted into a bed of silica beads, with methane serving as a co-metabolite. This design resulted in 18.40 ± 0.73 mM TCE removal from the cathode. On the anode, nearly 95% of TCE and its daughter compounds originating from the cathode were oxidised.

In another sequential reductive/oxidative BES system, employing two membrane-less MEC reactors connected in series and utilizing real groundwater, PCE was fully transformed to VC in the first MEC at – 0.45 V vs SHE, while 92 ± 2 % VC was oxidised in the second MEC (Dell'Armi et al., 2022b).

Studies of pure cultures or dominant genera/species

Several OHRB bacteria, including *Dehalobacter*, can transform PCE and TCE to cDCE (Lai et al., 2017). However, among OHRB, only ***Dehalococcoides mccartyi*** belonging to *Dehalococcoides* species have the capacity to fully reduce CAHs to ethene, which is harmless. The reductive process involves key enzymes known as reductive dehalogenases (RDase), which are encoded by the specific genes including *bvcA*, *pceA*, *tceA*, *vcrA* (Matturro et al., 2021; Dell'Armi et al., 2022b). When this microorganism dominates within microbial consortia, its reductive dehalogenases genes can serve as biomarkers for monitoring the RD process, applicable in both laboratory and *in site* applications (Matturro et al., 2021). In the RD reaction of *D. mccartyi*, the removal of one chlorine atom from the CAHs carbon skeleton occurs through the introduction of two electrons and one hydrogen atom (Dell'Armi et al., 2022a; Dell'Armi et al., 2022b). It is proposed that the potential mechanism might involve indirect electron transfer, however, the use of molecular hydrogen *in site* is not feasible. Therefore, it has been predicted that the fermentation of more complex substrates, such as ethanol, lactic, and volatile fatty acids (VFAs) can serve as a source of hydrogen (Dell'Armi et al., 2022a). Even in the presence of mixed cultures, *D. mccartyi* has been identified as the key contributor to the biological RD occurring within the reductive compartment of BES (Di Franca et al., 2022). However, as of now, this microorganism has not been recognized as electroactive (Matturro et al., 2021). Although, the main genetic steps of *D. mccartyi* responsible for fully reducing CAHs to harmless compounds is known, further research towards revealing the genetic/enzymatic mechanisms of other microorganisms involved in dechlorination in BES is required. In particular, the combination of microorganisms in a community can lead to electro-assisted dechlorination. Therefore, studying the genetic/enzymatic dechlorination steps of isolated species of the community is necessary. For instance, on a biocathode of a BES where acclimatized anaerobic sludge was used, PCE, 1,2-DCA, and TCE were dechlorinated revealing the electron fluxes during the dechlorination of CAHs using different carbon substrates. In this process, the biocathode reached >99% dechlorination efficiency at −0.26 V vs. SHE compared to open circuit and abiotic cathode. All the carbon substrates resulted in microbiomes enriched with cathode-respiring bacteria and dechlorinators. For example, when glucose was the substrate, ***Lactococcus*** and ***Anaeroarcus*** were the dominant genus, while when acetate was used as the substrate, ***Geobacter*** and ***Pseudomonas*** dominated the culture. The presence of sodium bicarbonate led to ***Bacillus*** domination (Chen et al., 2018). Following the identification of the dominant microorganisms with different substrates, quantification of the functional genes *pceA*, *tceA*, *rdhA1* encoding for typical reductive dehalogenases reducing PCE/TCE to cis- DCE, TCE to cis-DCE and 1,2-DCA to ethene, respectively, and one encoding for the outer membrane multi-heme *c*-type cytochrome (OmcX) was performed. The DNA expression of those genes was higher when glucose was the substrate followed by acetate and then sodium bicarbonate. Thus, providing mechanistic insights and, also biomonitoring of the effectiveness of the dechlorination process focusing on Desulfobulbaceae and *Dehalobacter* species which are contained in the communities and are basic dechlorinators. However, mechanistic insights regarding ***Lactococcus Anaeroarcus***, ***Geobacter***, ***Pseudomonas*** and ***Bacillus*** species and, more specifically the related strains participating in the electro-assisted dechlorination, as well as their syntrophic partners, at molecular level have not been performed yet.

5. From mixed to pure cultures upon electro-assisted biodegradation of polycyclic aromatic hydrocarbons, total petroleum hydrocarbons, cyclic hydrocarbons and diesel range organics pollutants

Electro-bioremediation of hydrocarbon pollutants derived from petroleum industries

The toxic and persistent pollutants of polycyclic aromatic hydrocarbons, total petroleum hydrocarbons (TPHs), cyclic hydrocarbons, such as benzene, toluene, ethylbenzene and xylene (BTEX) as well as diesel range organics are present in solid waste or wastewater derived from petroleum industries. These wastes can be classified as produced water, formation water, petroleum refinery water (PRW), and petroleum sludge (Mohanakrishna et al., 2018).

Most of the hydrocarbons are biodegraded aerobically. However, the aeration strategy has several constraints including high energy consumption, production of volatile contaminants, clogging issues near oxygen/air injection points due to high microbial growth yield, and limited efficiency of oxygen consumption due to the presence of reduced mineral substances (e.g. Fe(II) and HS). To overcome these issues, over the last years, BESs operated under anaerobic conditions have been used for hydrocarbon bioremediation (Palma et al., 2018).

Studies of mixed cultures: examples of technology readiness level establishment

In electro-bioremediation, the treatment of such complex refinery wastewater streams, has been achieved mainly with mixed cultures, that have better biodegradation efficiency in comparison with pure cultures. Most of these studies use anaerobic, aerobic, activated sludge, acclimatized inoculum, or mixed consortia (Kronenberg et al., 2017; Srikanth et al., 2018; Cabrera et al., 2021; Kondaveeti et al., 2023). Since most of these studies have been validated in laboratory settings, they have advanced to achieve a TRL of 4. For instance, when domestic wastewater was used as the inoculum in an air-cathode MFC for PRW biodegradation with a total COD of 970 ± 6 mg L^{-1}, the COD removal reached 84%

and the power density was 280 mW m^{-2} (Zhang et al., 2014). Similarly, in a double chamber MFC utilizing activated sludge and granule graphite as packing material for PRW treatment, with COD and oil content of 450 ± 50 mg L^{-1} and 50 ± 5 mg L^{-1}, respectively, the removal efficiency of COD and oil content was of 64% and 84%, respectively. Concurrently, the power density reached 330 mW m^{-2} (Guo et al., 2016).

• Studies of pure cultures or dominant genera/species

To study the involvement of specific species and strains, the bio-electrochemical degradation of phenanthrene was explored using ***Shewanella oneidensis* MR-1 14063**, ***Pseudomonas aeruginosa* NCTC 10662**, an undefined anaerobic consortium, and their combinations. This study aimed to enhance both the biodegradation efficiency and electricity generation in a dual chamber MFC (Adelaja et al., 2014). It was revealed that *P. aeruginosa* strain was able to remove COD more efficiently and presented the highest degradation rate of phenanthrene reaching 60.61 ± 1.69% and 54.7 ± 0.6 μM d^{-1}, respectively, achieving a maximum power density of 0.19 ± 0.05 mW m^{-2}. The undefined anaerobic consortium augmented with *P. aeruginosa* had the best overall performance achieving the highest COD removal (65.6 ± 5.52%) of phenanthrene, degradation rate (27.30 ± 1.10 μM d^{-1}) and maximum power density (0.11 mW m^{-2}). Under MFC conditions, the *Shewanella oneidensis* MR-1 EET mechanistic steps and the electron mediators produced by *Pseudomonas aeruginosa* are already known. However, further mechanistic insights studies regarding the electro-assisted biodegradation of hydrocarbons by these strains have not been performed yet.

In another research study, the bio-electrochemical degradation of PRW in phosphate buffer was examined using an H-type double chamber MFC, using an initial COD value of 450 ± 30 mg L^{-1} and oil concentration of 18.5 ± 0.5 mg L^{-1} (Guo et al., 2014). The ***Microbacterium* sp.**, ***Paenibacillus* sp.** and ***Deinococcus* sp.** strains were studied as pure cultures and as mixtures revealing synergistic and antagonistic effects. The COD removal efficiency and oil content removal of the treatment were 85.96 ± 1.10%, 86.67 ± 1.10%, 85.00 ± 1.10% and 80.52 ± 1.10%, 84.01 ± 1.10%, and 77.19 ± 1.10%, respectively, for each strain. The power density achieved was 73.86 mW m^{-3}, 85.41 mW m^{-3}, and 67.78 mW m^{-3}, for each strain respectively. *Paenibacillus* sp. and *Deinococcus* sp. strains demonstrated synergistic behaviour achieving COD and oil removal efficiency of 87 ± 1.10% and 85.56 ± 1.10% respectively. The maximum power density attained was 103 mW m^{-3}. Therefore, it's worth further studying of the electroactive and biodegradation characteristics at molecular level to be able to understand the developed co-culture's characteristics.

Utilizing a pure culture of wild-type ***Pseudomonas putida* BCRC 1059** in an air-cathode chamber MFC for the treatment of PRW, with an initial COD of 990 mg L^{-1}, resulted in power density of only 0.005 mW cm^{-2} and COD removal efficiency of 30%, respectively (Majumder et al., 2014). However, no mechanistic insights regarding the electro-assisted biodegradation of *P. putida* have been revealed yet. In a separate study, the electroactivity of a pure culture of ***Pseudomonas citronellolis* 620C** was investigated on a dual chamber MFC for the treatment of oily wastewater originating from drilling operations, with an initial COD of 43.625 mg L^{-1}. The power density and COD removal efficiency reached 0.05 mW m^{-2} and 83.6 ± 0.1%, respectively (Varnava et al., 2024). This study established that pyocyanin served as the electron mediator, providing, for the first time, insights into the EET mechanism operating for biodegradation purposes of this strain. Further studies at molecular level would shed light to the electro-assisted biodegradation mechanism involved.

The ability of ***Rhodopseudomonas palustris* RP2** to degrade petroleum hydrocarbons (PH) at diesel range (C9−C36) of 800 mg L^{-1} initial concentration in a cubic MFC was assessed producing a maximum power density of 720 ± 7 μW m^{-2}. The concomitant removal reached 47.4 ± 2.7% (Venkidusamy and Megharaj, 2016). Cyclic voltammetry studies showed that, when acetate was the fuel, the electrons may be transferred to the anode electrode through membrane-bound proteins. The reveal of the electro-assisted biodegradation mechanism involved would offer mechanistic insights of the process.

BTEX removal has also been studied in MFCs using pure cultures and microbial communities which have been characterized. Toluene oxidation was studied in a dual chamber MFC under external voltage using ***Pseudomonas putida* F1** (Friman et al., 2012). At 500 mV, the optical density at 600 nm (OD_{600}) reached 0.75 and the maximum current density was 94 mA m^{-2}. Cyclic voltammetry experiments suggested toluene oxidation of up to 78%. Proteomic analysis revealed that iron–sulphur subunit, cytochrome *c* oxidase, ubiquinol-cytochrome *c* reductase, cbb_3-type subunit III, and non-electron transport proteins such as outer membrane ones play a key role in electron transfer of *P. putida* F1 to the anode electrode.

***Cupriavidus metallidurans* CH34** was studied on a MEC to assess toluene removal (Tofalos et al., 2018). In this study, toluene was removed by 73 % (initial toluene 60 mg L^{-1}) in a total of 17 days, while the maximum current density achieved was 48 mA m^{-2}. However, further studies at molecular level regarding the electro-assisted biodegradation of toluene by this microorganism have not been performed yet.

In a mixed culture employed in a MFC to evaluate toluene oxidation, the removal efficiency reached 88% (initial toluene at 300 mg m^{-3}) (Zhang et al., 2018). The microbial community analysis suggested that ***Chryseobacterium*** and ***Zoogloea*** were the dominant species in the MFC. Cyclic voltammetry analysis demonstrated peaks that may correspond to the presence of menaquinones or outer membrane cytochromes (OMC), such as OmcZ or OmcS. Therefore, it was proposed that a direct electron transfer pathway from the microorganisms to the anode electrode could exist in this setup.

The study of the anaerobic treatment of synthetic groundwater containing toluene (25 mg L^{-1}) was performed on a continuous-flow bioelectrochemical reactor (Palma et al., 2018). At an anode potential of +0.2 V vs. SHE and recycle flow rate at 75 mL min^{-1}, the electro-assisted toluene oxidation rate was of 67.2 ± 5.7 mg L^{-1} day^{-1} with a current of 5.1 ± 0.1 mA. In this study, toluene degradation was attributed to the dominant ***Geobacter*** species. It was demonstrated that toluene oxidation was initiated due to fumarate addition, due to the presence of benzylsuccinate (BS) as metabolite.

Electro-assisted biodegradation of BTEX mixtures, such as toluene and benzene in synthetic groundwater, has also been investigated in a tubular-MFC (Lin et al., 2022). Both contaminants were completely degraded in 6 days with a power density of 15.6 W m^{-3}. In the anode biofilm, the main species were identified as the EAM ***Geobacter*** and ***Thermincola*,** while ***Rhodocyclaceae***, which can degrade toluene, was found to be the dominant species in the anode suspension. Studies of the individual species and, more specifically, the strains involved could reveal the mechanistic insights of each microorganism involved in the process.

In a dual chamber MFC, the removal of toluene, ethylbenzene, and *o*-xylene was evaluated, achieving a removal efficiency of 90% when all the contaminants were present in the anode (Zhang et al., 2020). Microbial community analysis showed that when the feeding gas changed from toluene to another contaminant, the abundance of EAM ***Chryseobacterium* sp.**, ***Hydrogenophaga* sp.**, and ***Geobacter* sp.** decreased. Whereas, the presence of *o*-xylene resulted in the domination of its degrader, ***Alicycliphilus* sp.** Furthermore, scanning electron microscopy and cyclic voltammetry analysis revealed the presence of nanowire/pili, suggesting a direct electron transfer process between the bacteria and the electrode. However, no further information is available regarding the mechanistic insights of the relevant strains.

Therefore, it was realised that in many studies, there is still no evidence about the genes, proteins and enzymes involved in the EET-assisted biodegradation under BES conditions. Even though, *Pseudomonas* genus is often identified in an inoculum for efficient degradation

of aliphatic and aromatic hydrocarbons and is a well-known genus for the degradation of hydrocarbons. Thus, more in-depth studies at the molecular level to enhance our knowledge are necessary during electro-assisted biodegradation of hydrocarbons.

6. From mixed to pure cultures upon electro-assisted biodegradation of metals pollutants

Electro-bioremediation of metals

The presence of heavy metals in the environment is widely recognized as a significant ecological issue. Most of these metals, originating from activities of mining, metallurgical operations, electroplating, cement production, leather tanning, and manufacturing industries that are metal-based, pose severe threats to both humans and other forms of life (Tchounwou et al., 2012). Common heavy metals found in wastewater include Arsenic (As), Lead (Pb), Mercury (Hg), Cadmium (Cd), Chromium (Cr), Copper (Cu), Nickel (Ni), and Zinc (Zn) (Qasem et al., 2021). While some of these metals are essential trace elements necessary for living organisms, many, especially at elevated concentrations can cause acute and chronic toxicity (Tchounwou et al., 2012).

The removal of metals from wastewater and aqueous streams although can be accomplished using physical and chemical techniques (Fu and Wang, 2011; Yadav et al., 2021), suffers from drawbacks as these methods are both environmentally harmful and expensive. For this reason, in recent years, BESs have been explored as a versatile solution for extracting metals from wastes and aqueous streams (Nancharaiah et al., 2019; Kumbhar et al., 2021). These processes can take place in a single or double chamber BES, and they can involve either a biotic or abiotic cathode. While numerous metals can be used effectively as terminal electron acceptors in MFC operations due to their high positive reduction potential (e.g. cobalt, chromium, gold, selenium, silver, copper), some metals require energy or the involvement of microorganisms to be efficiently reduced.

Studies of mixed cultures in the anode and abiotic cathodes: examples of technology readiness level establishment

The BES technologies for metal removal have already reached TRL 4, with some cases being validated in the laboratory with real wastewater. For example, cobalt can be an efficient terminal electron acceptor for the operation of MFC. This is because of the high positive potential of the Co (III)/Co(II) couple (+1.61 V vs SHE) when present as $LiCoO_2$ particles (i.e. used as cathode materials in lithium ion batteries) in the cathode compartment (Huang et al., 2013). In this study, MFC was used to effectively reduce Co(III), where synergetic interactions between the current generation and conventional chemical process for complete recovery of cobalt and recycling of spent lithium ion batteries have been observed. However, Co(II) can only be removed from biocathodes on a MEC. A self-driven MFC-MEC system has been used to completely recover cobalt from wastewaters, allowing it to achieve a leaching rate of 3.2 mg Co L^{-1} h^{-1} and 3.0 mg Co L^{-1} h^{-1} on a series and parallel setup, respectively (Huang et al., 2014). In another study, the removal and recovery of Co ions were achieved by the electromigration, electrostatic adsorption of granular activated carbon, and chemical precipitation of cobalt hydroxide and cobalt carbonate in a 3D-MFC (Huang et al., 2019) demonstrating that efficient cobalt removal can be achieved with BES.

In the environment, the most stable forms of chromium are hexavalent (Cr(VI) and trivalent (Cr(III)). While Cr(VI) is soluble in water and extremely toxic to living organisms, Cr(III) is less toxic, and can precipitate in water at moderately acidic to alkaline pHs. Microbial reduction of Cr(VI) was first observed in a MFC by Wang and colleagues where acetate was used as the electron donor in the anode chamber and chromate was used as the electron acceptor in the cathode compartment (Wang et al., 2008). Since then, the removal of chromium has been achieved in numerous other studies (Liu et al., 2011; Zhang et al., 2012a; Gangadharan and Nambi, 2015; Wang et al., 2016).

The spontaneous cathodic reduction of copper (Cu(II) to Cu^0) can also take place in a MFC. This process occurs when the MFC utilizes acetate or glucose as an electron donor, achieving a net theoretical voltage of 0.49 or 0.69 V vs SHE, respectively, by using copper as the terminal electron acceptor. Indeed, copper from waste streams can be recovered in its metallic form at the MFC's cathode (Heijne et al., 2010; Tao et al., 2011; Rodenas Motos et al., 2015). In this setup, the energy required for metal reduction is derived from the oxidation of organic material at the anode. This process not only enables the recovery of copper at a rate exceeding 99% but also has the potential to generate additional electricity. Recently, it was demonstrated that MFC can be integrated with micro-electrolysis to enhance copper removal (Wang et al., 2020a).

Removal of other metals, including gold (Au(III)) (Choi and Hu, 2013; Hubenova et al., 2023), silver (Ag(I)) (Choi and Cui, 2012; Tao et al., 2012; Almatouq et al., 2022), selenium (Se(IV)) (Catal et al., 2009; Wang et al., 2013; Sravan et al., 2020), and mercury (Hg(II)) (Wang et al., 2011; Kumar et al., 2020) was also demonstrated in MFC. In all these situations, the reduction potential of the metals is positive enough to be reduced by the abiotic cathode. However, some metal ions cannot be reduced as their redox potential is lower than the anode potential. In this situation an external power supply is required, to transfer the electrons to the cathode for reduction. For example, silver could be recovered from an aqueous solution as Ag^0 by microbial electrochemical snorkel (Mitov et al., 2022). In this study, different types of cathodes were tested to remove silver from an $AgNO_3$ solution, demonstrating that the operation in microbial electrochemical snorkel mode is more advantageous than that in MFC mode in terms of both silver removal and recovery. Also, the removal of selenite and selenate was observed in a MEC experimental setup, reaching >99% removal over the course of the experiment (Jugnia et al., 2021).

Studies of pure cultures or dominant genera/species in biotic biocathodes

The use of biocathodes is important in the development of higher biopotentials when compared with abiotic cathodes. This is because the potential gradient between the anode and cathode chamber is higher allowing the metal reduction at the cathode to occur (Wang et al., 2023). Examples of this are the reduction of chromium at the cathode by different bacteria (Hsu et al., 2012; Wu et al., 2015), including ***Shewanella oneidensis* MR-1** (Xafenias et al., 2013) which resulted in a decrease of 9 mg L^{-1} Cr(VI) within 4 h. In another study, the reduction of Cr(VI) was achieved by a microbial community dominated by ***Trichococcus pasteurii*** and ***Pseudomonas aeruginosa*** (Tandukar et al., 2009). In this work, a relatively fast reduction of Cr(VI) could be achieved at initial concentrations below 80 mg L^{-1}, reaching a maximum reduction rate of 0.46 mg Cr(VI) VSS $^{-1}$ h^{-1}. However, mechanistic insights of the process have not been assessed yet.

Removal of Cu(II) can also be achieved in a biocathode. Shen and colleagues (Shen et al., 2017) evaluated four indigenous electroactive bacteria ***Stenotrophomonas maltophilia* JY1**, ***Citrobacter* sp. JY3**, ***Pseudomonas aeruginosa* JY5** and ***Stenotrophomonas* sp. JY6** for Cu (II) reduction. They demonstrated that, in addition to abiotic reduction, a plausible mechanism for Cu(II) reduction involves a direct electron transfer process (Shen et al., 2017). In another study, it was shown that the Cu(II) concentration influences the microbial activity on the biocathode (Lin et al., 2021). These findings motivate further research at molecular level for every strain involved to understand the co-culture characteristics.

The simultaneous removal of different metals using MFC has also been recently explored. The utilization of multiple-reactor coupling MFC and MEC has enabled the reduction of Cr(VI), Cu(II) and Cd(II) (Huang et al., 2015). Additionally, the presence of a mixed community with

Ochrobactrum, ***Halomonas***, and ***Achromobacter*** as dominant genera, has shown effectiveness in removing Cd(II) and Ni(II) (Singh and Kaushik, 2021). In the conducted study, a removal efficiency of 87% for Cd and 92% for Ni, was achieved when the concentration of distillery wastewater was 10 mg L^{-1}. Furthermore, recently the removal of Cu(II), Pb(II), Zn(II) and Cd(II) has been accomplished on the biocathode of a MEC, where ***Sphingomonas***, ***Azospira*** and ***Cupriavidus*** showed stronger tolerance to heavy metals and became the dominant genera (Shi et al., 2023). However, the genes, proteins and enzymes by which these organisms are able to effectively treat heavy metal wastewater in BES have not been elucidated.

Metals can also be recovered from waste in the form of nanoparticles using bacteria. Examples of these are palladium, platinum, and gold (Corte et al., 2012; Jahnke et al., 2019; Liang et al., 2022). Different studies have demonstrated that nanoparticles present in anodes can increase power density in BES (Quan et al., 2015; Wu et al., 2018; Asghary et al., 2019).

7. From mixed to pure cultures upon electro-assisted biodegradation of sulphurous pollutants

Electro-bioremediation of sulphurous pollutants

Produced water (PW), characterized by high salt concentrations, constitutes the largest waste stream in oil and gas exploration. PW contains several components acting as pollutants including suspended solids, sodium chloride, magnesium and calcium carbonates, emulsified oils, petroleum hydrocarbons, and sulphate (Jain et al., 2017). PW can either be reinjected into the oil and gas reservoir or disposed of into the environment. However, before disposal, PW must undergo treatment due to the presence of sulphate-reducing bacteria (SRB), which can proliferate in PW due to the presence of sulphate. Failure to treat PW appropriately can result in the clogging of pipelines and storage tanks, microbially influenced corrosion (MIC), and contamination of groundwater, water, and soil ecosystems. Usually, sulphate is transformed to sulphide through anaerobic digestion. Sulphide can then be oxidised to sulphur which is less toxic and can be recovered. For sulphate and hydrocarbons removal, conventional PW treatment methods include physical, chemical, biological, and membrane-based processes, which can be employed individually or in combination (Jain et al., 2017). While physical technologies are effective, they often form secondary waste, require significant land, or space, and have a long retention time. On the other hand, chemical processes may be energy intensive and produce high concentrations of secondary chemical waste, posing challenges for treatment (Vlasopoulos et al., 2006). In addition, they have high operational and chemical expenses, along with significant sludge production. One of the main disadvantages of using biological technologies is the formation of sludge and long retention time. To overcome this limitation, membrane bioreactors have been employed (Shannon et al., 2008), and membrane-based technologies have also been used independently (Shaffer et al., 2013). However, a significant drawback of membrane use is the cost and fouling. The large volumes of PW generated render most physical or membrane-based processes unviable. Furthermore, the mixture of pollutants of PW hinders the chemical and biological treatments (Fakhru'l-Razi et al., 2009). Therefore, there is a growing interest within both industrial and scientific communities to develop innovative treatment solutions for such specific wastewater. BESs can balance the energy and operational costs of wastewater treatment (Logan and Rabaey, 2013), while the properties of PW make them suitable for BES. The effectiveness of BESs in sulphide removal with simultaneous electricity generation has been established since 2006 (Rabaey et al., 2006).

Studies of mixed cultures: examples of technology readiness level establishment

Several studies are using mixed cultures for sulphate removal using sulphate-reducing bacteria (SRB) and sulphide-oxidizing bacteria (SOB) in BES (Coma et al., 2013; Rodrigues and Leão, 2020). For instance, when single and double-chamber MFCs were used for hydrocarbons and sulphate removal from PW (Mohanakrishna et al., 2019), the active anodic biofilm came from sewage sludge, so it was a mixed consortium. Both MFCs' operation conditions were similar. Among the components of the medium, Na_2SO_4 and commercial oil were 2.0 g L^{-1} and 0.8 g L^{-1}, respectively. However, double chamber MFC showed higher sulphate and hydrocarbons removal efficiency reaching 93.9% and 53.1% removal efficiency of sulphate and TPHs, respectively, while power reached 1089 mW m^{-2}.

Studies of pure cultures or dominant genera/species

Some microorganisms have been investigated as pure cultures in BES for sulphate management providing mechanistic insights. For instance, the formation of biofilm on the cathode by ***Desulfovibrio desulfuricans*** **ATCC 27774** was examined at a potential of −0.400 V vs. SHE using lactate as the substrate in a medium containing SO_4^{-2} (Cordas et al., 2008). Sulphide concentration was maximized, while lactate was completely degraded. The electroactivity of ***D. caledoniensis*** on pyrolysis graphite electrodes when sulphate was reduced to sulfide using lactate as substrate was also investigated (Yu et al., 2011). In this scenario, the biofilm might acquire electrons from values lower than −0.610 V vs. SHE to produce hydrogen. Aulenta and colleagues (Aulenta et al., 2012) employed lactate and sulphate in a biocathode where ***D. paquesii*** was growing, accepting electrons from a graphite electrode for hydrogen production at a potential more reducing than −0.9 V vs. SHE. Hydrogen is a key product in the metabolism of the *Desulfovibrio* species. It can either be used to complement sulphate reduction or produced when sulphate is not present (Carepo et al., 2002). In sulphate and lactate media, hydrogenase involved in the hydrogen cycle is responsible for catalyzing hydrogen oxidation and evolution (Odom and Peck, 1981; Singh et al., 2021). In the cathode, sulphate reduction involves an eight-electron reaction that includes the reduction of sulphate to sulphite, and then the subsequent reduction to sulphide. Within the microbial cell, the presence of sulphate activates Adenosine Triphosphate (ATP) sulphurylase, which forms adenosine 5′ -phosphosulfate (APS). APS is then converted to sulphite by APS reductase (AprBA) as catalyzing a two-electron reaction. Sulphite is subsequently reduced to sulphide with the dissimilatory sulphite reductase complex (Dsr) serving as the catalyst. In the final reaction, an energy-conserving membrane complex, DsrMKJOP or DsrMK, is also involved (Agostino and Rosenbaum, 2018).

When CO_2 is the sole inorganic substrate, ***Acidithiobacillus ferrooxidans***, ***Desulfosporosinus orientis***, ***Thiobacillus denitrificans***, ***Sulfurimonas denitrificans*** and ***Desulfovibrio piger*** can grow on a biocathode reducing sulphate to sulphide at a potential of −0.5 V vs. Ag/AgCl (Rodrigues and Rosenbaum, 2014). ***Desulfopila corrodens*** **IS4** using CO_2 as the carbon source can take up electrons directly from elemental iron (Beese-Vasbender et al., 2015). While this can lead to severe corrosion, it also resulted in rapid sulphate reduction and hydrogen generation at a potential of −0.4 V vs. SHE. ***Desulfobacterium autotrophicum*** **HRM2** is another electrotrophic microorganism capable of using CO_2 as a substrate for acetogenesis (Zaybak et al., 2018) The peak current uptake occurred at a potential of −0.243 V vs SHE.

In the anode, Rabaey and colleagues (Rabaey et al., 2006) demonstrated sulphide oxidation to sulphur using a square MFC closed to the air and a tubular MFC with the cathode open to the air. In the tubular MFC, 514 mg of sulphide L^{-1} in the net anodic compartment (NAC) was eliminated with an initial sulphide flow of 0.60 g L^{-1} NAC day^{-1}. Additionally, the maximum power output reached 101 mW L^{-1} NAC. The suspension was identified as either ***Alcaligenes*** **sp.** or ***Paracoccus*** **sp**, with the latter being able to grow when sulphide and nitrate are the electron donor and acceptor, respectively. However, studies to assess the

mechanistic insights have not been performed yet.

D. desulfuricans has also been used in the anode of an air-single chamber MFC with continuous flow for simultaneous sulphate removal and electricity generation. Sulphate removal reached 99%, while the maximum power achieved was of 0.51 mW cm^{-2} (Zhao et al., 2008). Furthermore, Kang and colleagues (Kang et al., 2014) used a mediator-less MFC with *D. desulfuricans*, reaching a maximum current density of 233 ± 24.2 mA m^{-2}. It was proposed by the authors that direct electron transfer included cytochromes *c3* and conductive bacterial appendages. They showed that hydrogen and/or peptide bonds facilitated the attachment of cytochrome *c3* to the treated electrode.

In another study of a dual chamber MFC, where the anode contained raw landfill leachate, the microbial community consisted mainly of ***Tetrathiobacter***, ***Desulfovibrio*** and ***Aeromonas*** species which are electroactive SRB, SOB, and fermenters. COD removal was complete after 3 days and maximum power and current density reached 9.15 W m^{-3} and 16.17 A m^{-3}, respectively (Kumar et al., 2017).

8. From mixed to pure cultures upon electro-assisted biodegradation of azo-dyes pollutants

Electro-bioremediation of azo-dyes pollutants

Azo dyes represent more than 60% of total dye-related compounds, making them the most commonly used dye classification (Benkhaya et al., 2020). Approximately 10–15% of wastewater containing azo dyes is released into the environment, posing an environmental hazard (Cui et al., 2021). Therefore azo-dyes wastewater treatment is imperative. The conventional technologies used, such as physical and (electro) chemical result in secondary waste, high energy and operational costs, and partial degradation; thus, hindering wide applicability at wastewater treatment facilities. Furthermore, the anaerobic biological process has a slow rate, low electron employment, and requires exogenous electron donors; thus, resulting in high maintenance and operational costs. Hence, enhanced technologies for biological treatment are necessary. Over the last years, it has been determined that bioremediation can be enhanced through the application of an electrical circuit thanks to the electroactivity of some microbes (Cui et al., 2021).

In electro-bioremediation, azo dyes serve as electron acceptors and can be used at the anode along with other organic substrates. It is widely acknowledged that co-substrates are needed at the anode to activate degradation mechanisms and facilitate electricity generation through exoelectrogens or other bacteria. Alternatively, azo dyes can also be reduced in a (bio)cathode. When azo dyes function as electron acceptors in the cathode, it is noteworthy that with the use of co-substrates such as organic substrates (e.g. glucose), they may be partially reduced by non-EAM through the use of electrons that are not derived from the cathode but from the organic substrates (Cui et al., 2021).

Studies of mixed cultures: examples of technology readiness level establishment

Numerous studies are focused on assessing decolorization efficiency by employing mixed cultures, utilizing both aerobic and anaerobic sludges, rather than concentrating on specific bacteria. Most of these studies have determined experimental proof-of-concept (TRL 3). For instance, Oon and colleagues studied the products of New Coccine (NC) decolorization in a double chamber MFC using mixed culture from sludge (Oon et al., 2018). The concentration of NC increased from 25 mg L^{-1} to 200 mg L^{-1} with an almost stable acetate concentration resulting in over 90% decolorization efficiency at every concentration with an external resistance of 1000 Ohms. As NC concentration increased, power density was reduced from 20.13 ± 0.37 W m^{-3} to 10.83 ± 1.21 W m^{-3}. Sun and colleagues studied active brilliant red X-3B (ABRX3) decolorization in an aerobic biocathode dual chamber MFC where glucose was the fuel in the anode (Sun et al., 2011). The aerobic biocathode can successfully further treat the effluent from the anaerobic MFC used to decolorize azo dyes. The initial concentration of the ABRX3 was 1500 mg L^{-1}, and over 90% of the colour was removed in the anaerobic MFC. The effluent was then fed to the biocathode chamber. This configuration resulted in an increase in power density from 50.74 mW m^{-2} to 213.93 mW m^{-2} and a further reduction of COD. They supported that the intermediates of the decolorization process of ABRX3 may function as redox mediators for the electron transfer from the cathode to the oxygen.

Studies of pure cultures or dominant genera/species

Ilamathi and colleagues investigated the decolorization of reactive orange 16 (RO-16) and reactive black 5 (RB-5), at 0.5 mM each, by two ***Pseudomonas*** strains in single chamber MFCs with modified cathode using glucose as fuel (Ilamathi et al., 2019). The concentration of each azo dye was reduced by more than 94% by each strain at the end of the process. The authors revealed that although the biodegradation mechanisms relied in azo reductase and oxidative laccase, the biodegradation efficiency of ***P. aeruginosa*** was higher compared to ***P. fluorescens*** as the strains present distinct enzymes' activity. We also believe that phenazine-based compounds produced by *P. aeruginosa* may enhance EET. In the MFC of *P. aeruginosa* the maximum power density reached 2887 ± 13 μW m^{-2} and 1906 ± 7 μW m^{-2} when RO-16 and RB-5 were used, respectively. While in the MFC of *P. fluorescens* the maximum power density was lower reaching 1896 ± 15 μW m^{-2} and 1028 ± 9 μW m^{-2}, when RO-16 and RB-5 were used, respectively. However, studies at the molecular level have not been performed yet to reveal further information.

The electroactive bacterium ***G. sulfurreducens* PCA** facilitates extracellular decolorization of azo dyes through the involvement of extracellular cytochromes *c*. Specifically, the outer-membrane proteins OmcB, OmcC, and OmcE are the main cytochromes responsible for the extracellular reduction of the azo dye methyl orange (MO) (Liu et al., 2017). It was found that, in the anode, 40 mg L^{-1} of MO are fully degraded within 5 h with acetate as the electron donor. It was also demonstrated that ***Shewanella oneidensis* MR-1** uses the outer-membrane cytochromes to reduce MO (Louro et al., 2023). In this study, it was shown that the perturbation of the heme environment of OmcA can abolish the capacity of *S. oneidensis* to reduce MO and other electron acceptors. Metabolic and transcriptional studies in this bacterium also revealed that dioxygen can compete with MO as an electron acceptor (Cao et al., 2017).

***Shewanella marisflavi* EP1** can decolorize Xylidine Ponceau 2R (XP2R) and 2, 4-Xylidine-an intermediate of XP2R (Xu et al., 2016) at high salt concentrations. 50 mg L^{-1} of dye, 20 mM lactate, and 50 mM fumarate were used. It has been observed that EP1 had high decolorization efficiencies (>94.5%) for 0–6% NaCl after 17 h cultivation. It has also been observed that the decolorization occurs extracellularly, accompanied by bio-flocculation mechanisms at high salt concentrations. These findings suggest that the flocculation of azo dyes can occur biologically, potentially eliminating the need for chemical interventions in this process. The latter in another interesting observation upon studying of pure cultures.

***Klebsiella* sp. C NCIM 5546** is another pure culture studied for azo dye decolorization (Holkar et al., 2018). 50–800 mg L^{-1} reactive blue 19 (RB19) dye in synthetic textile wastewater was used. The decolorization efficiency was better at 50 mg L^{-1} reaching 90% within 24 h, generating up to 84 mW m^{-2}, under pH 7, 37 °C, 6 g L^{-1} glucose, and 60 glucose: microbe ratio. The intermediates detected are oxalic acid, indene-1,3-dione, and hydroquinone.

Until now, most studies of pure cultures have been performed in the anode. The proposed three pathways for bio-electrochemical decolorization occurring at the anode are (a) due to the production of azo reductase and other useful enzymes and flavins intracellularly; (b) the production of cytochromes *c* extracellularly; and (c) the direct or

indirect induction of redox mediators (Cui et al., 2021). However, the genes, proteins, enzymes and metabolites involved in those processes for most of EAM and degraders used in azo-dyes biodegradation are missing.

9. Overview – Bridging knowledge gaps and future research directions in electro-bioremediation of wastewater

Up to now, studies involved in electro-bioremediation of exemplary pollutants in wastewater using BES have mainly employed enriched microbial communities, with only a few studies being implemented with pure cultures; thus, hindering the discovery of the molecular processes involved in EET and bioremediation (Table 1). In this review, we identified only 39 studies that employed both pure cultures and mixed cultures, where the dominant species were identified, in electro-assisted biodegradation. Furthermore, we realised that, even though pure cultures offer the unique advantage to study the process at the molecular level, the research up to now is mainly focused on the electro-biodegradation potential of the microorganisms, with only a few studies presenting mechanistic insights (Table 1). In addition, despite the potential in terms of operating expenses (OPEX) costs for BES technologies in electro-bioremediation, which have reached TRL 5, they continue to be less utilized compared to traditional bioremediation approaches, due to various constraints such as capital costs, the need for biocompatible electrodes and limited achievable current density at an acceptable voltage. One of the main limitations lies in the insufficient understanding at the microbiological level. This knowledge is imperative in many different scientific aspects as explained below.

In a microbial community of a cell, it is known that the majority of biodegraders are not necessarily electroactive or are weak electrogens/electrotrophs, but are important in the electro-assisted biodegradation process due to microbial interactions (Doyle and Marsili, 2018). Knowing the dominant species/genus of a mixed culture is not enough. Therefore, their identification followed by understanding of their participation in the electro-assisted biodegradation of specific pollutant (s) using pure cultures would be crucial. This knowledge will allow us to understand the role of each species in the microbial community during electro-bioremediation or build effective co-cultures. It will also guide us in intensifying the process to meet the standard for successful business cases. Therefore, the transition to pure cultures study in BES, at micro-scale level, of biodegraders, weak and strong electrogens/electrotrophs to get the mechanistic insights, would provide new research avenues and opportunities.

In particular, studies of pure cultures can enhance knowledge about quorum sensing (QS) which is essential for biofilm formation on electrodes (Paquete et al., 2022b; Islam et al., 2023). The mechanistic insights related to QS in pure cultures could be revealed upon electro-biodegradation of pollutants. Then, since the QS mechanistic insights of single cultures would have been studied, the QS mechanisms in a known microbial community of a mixed culture could be better understood. Another critical aspect of studying the mechanistic insights of pure cultures upon pollutants' electro-biotransformation lies in the interpretation of direct and/or indirect EET in a microbial community. In the anode, EAM can coordinate the electrons acquired from the biotransformation of the organic pollutant. In the cathode, similarly to the anode, the biotransformation of the specific pollutant could be due to acquired electrons. Therefore, upon study of pure cultures of EAM, the electron transfer pathway in a microbial community would be better understood and studied in further detail. Finally, the fate of some pollutants of wastewater used as carbon sources significantly influences BES performance. Current methods often measure substrate degradation through chemical oxygen demand (COD) removal, which is a rather black-box approach. Therefore, revealing the gene regulatory mechanisms involved in the biotransformation of such pollutants in BES is crucial for effective monitoring of the electro-assisted biodegradation process. When gene regulation is known, monitoring of specific gene expressions responsible for encoding the enzymes/proteins participating in electro-assisted biodegradation can act as biomarkers and biosensors, offering insights into the effectiveness of the process.

To study single species, several methodologies and approaches can be used and are suggested as future directions. An important methodology which should be rendered mandatory when studying water pollutants electro-bioremediation is next-generation sequencing (NGS) of mixed cultures when they are acclimatized to pollutant(s) environment. NGS, also called metabarcoding, is a gene-based approach, such as 16S rRNA genes amplicon sequencing (Kouzuma et al., 2018), and could be beneficial not only to determine the genera that are responsible for the electro-assisted biodegradation in the bioanode or biocathode, but also the species and strains of the process. Then, further culture-dependent studies of the microbial strains, as pure cultures, focusing on the molecular level, could reveal the gene regulatory network involved in the electro-assisted biodegradation of pollutants, as proposed in Fig. 3. The pollutant(s) of interest will be the primary carbon source and reason of microbial growth.

Following that, genetic and metabolic products should be analysed to determine electroactive and biodegradation characteristics of pure cultures, both intra- and extra-cellularly (Fig. 3). There are several methodologies to find genes, enzymes/proteins and metabolites responsible for biodegradation and electron transfer (Fig. 4). One of great magnitude is omics analysis, in terms of transcriptomics, proteomics, or metabolomics. Indeed, omics is a powerful tool to reveal information about gene regulatory processes, as well as metabolic pathways involved in the process which has not been widely applied in BES yet and, especially, in electro-bioremediation. Proteomics has been used to study some pure cultures (Pereira-Medrano et al., 2013; Yu et al., 2018; Feng et al., 2022). While transcriptomics (Nevin et al., 2009; Wang and Sheng, 2024) and metabolomics (Mahidhara et al., 2017) are still in their infancy. An emerging field in BES is the meta-omics analysis such as metagenomics and metatranscriptomics. Metagenomic is a term for shotgun-sequencing analysis of a whole metagenome (Kouzuma et al., 2018), such as the mixed cultures of BES in electro-bioremediation. Metagenomics presents a promising avenue for discovering novel genes encoding for pollutant-degrading and EET responsible proteins and enzymes, proposing a comprehensive approach to exploring the mixed cultures biological basis reservoir. Metatranscriptomic analysis (mRNA sequencing for whole microbial communities) is also possible for recognising metabolic reactions of the microbial community at a time of analysis (Kouzuma et al., 2018). However, although, meta-omics studies may provide mechanistic insights of the community, it's the unveiling of the gene regulatory network of each weak and strong EAM and their syntrophic partners, through pure cultures' studying, that can effectively explain the meta-omics findings.

Another route to gain mechanistic insights upon pure cultures study is through comparison with similar pathways of the same genus/species when the same type of pollutant is used. This can be achieved through whole genome sequencing of the studied microorganisms followed by genes/proteins annotation using EGGNOG, BLAST or other tools (Fig. 4). Hence, the metabolic pathway of biodegradation and EET could be revealed performing genetic engineering to validate the existence of the genes.

Knowing the gene sequences, gene quantification could be performed to understand the expression of the gene. Transcriptional kinetics could also be studied. mRNA expression is many times assumed as a preparatory step for enzyme/protein production. This could assist in gaining insight into the dynamic behaviour of specific key genes participating in the electro-assisted biodegradation (Tsipa et al., 2016). Confirmation can then be achieved by expressing the specific genes during the process and studying the phenotype through genetic engineering approaches.

The adoption of non-invasive methods, exemplified by the eClamp methodology (Ceballos-Escalera et al., 2024), is crucial for understanding electrochemical analysis without interrupting the metabolic

Table 1
Details of bio-electrochemical degradation processes, known pathways, genes/enzymes/proteins and metabolites/electrical connectors of exemplary pollutants using pure cultures, and dominant species and genera.

Objective	Reactor microbiome	WE compartment/ Electrode material	Electrical Operation	Eapp (V vs SHE, Ag/AgCl)	Maximum power/ current/ voltage	Removal efficiency/rate	Removal process	Genes/ enzymes/ proteins involved	Metabolites/ electrical connectors involved	Medium	Reference	Molecular mechanism
Anaerobic ammonium oxidation (Anammox)	*Candidatus Brocadia*, *Candidatus Scalindua*, *Kuenenia stuttgartiensis*	**Anode**/ graphite rods	Potentiostatic (+0.6 V vs. SHE)	0.3–0.6 V vs. SHE	n.a	n.a	Electrode-dependent anaerobic NH_4^+ oxidation	Genes encoding for ammonium transporter (AmtB), hydroxylamine oxidoreductase and hydrazine dehydrogenase (HDH)	Hydroxylamine	Inorganic medium rich in ammonium	Shaw et al. (2020)	n.a.
Direct ammonium oxidation to nitrogen gas (Dirammox)	*Alcaligenes strain HO-1*	**Cathode**/ carbon cloth	Potentiostatic (+0.2 V vs. Ag/ AgCl)	n.a	100 mA m^{-2}	48 mg N L^{-1} d^{-1}	Electro-assisted hydroxylamine conversion	n.a.	Hydroxylamine: an intermediate in the conversion of ammonium into dinitrogen gas	Synthetic medium rich in succinate	Pous et al. (2023)	n.a.
Nitrate reduction	*Pseudomonas nitroreducens* and *Paracoccus versutus*	**Cathode**/ Titanium rods connected to titanium meshes	Potentiostatic (−0.1V vs SHE)	−0.288 V and −0.206 V vs. SHE (pH 7)	n.a	34 g N m^{-3} day^{-1}	Direct electron transfer	n.a	n.a	Synthetic aquaculture effluent	Korth et al. (2022)	n.a.
Nitrate reduction	*Sideroxydans lithotrophicus* sp. (70-50%), *Denitratisoma oestradiolicum* sp. (5-4%) and *Achromobacter agilis* sp. (4-2%)	**Cathode**/ granular graphite	Potentiostatic (−0.320V vs Ag/ AgCl)	−0.225 ± 0.007 V vs. Ag/ AgCl sat. KCl at pH 7 and 25 °C.	12.5 A m^{-2}	0.9 ± 0.1 kg NO_3^- m^{-3} d^{-1}	Direct electron transfer	n.a	n.a	Synthetic groundwater solution	Ceballos-Escalera et al. (2024)	n.a.
Phosphate reduction	*Escherichia coli* in the anode	**Cathode**/ carbon felt	External resistance (2530 Ω)	Not applicable	3.1 μW	Up to 82%	Probably electrons-protons transfer from the anode to the cathode and through membrane, respectively	n.a	n.a	Pure $FePO_4$ .H_2O or sewage sludge, 0.1–0.3 mM Methylene Blue	Fischer et al. (2011)	n.a.
Reductive dichlorination of CAHs followed by oxidative dichlorination	*Dehalococcoides mccartyi* dominated	**Cathode**/ **anode**/ graphite granules	Potentiostatic/ Galvanostatic (+15 mA)	−0.45 V vs SHE	n.a	RD: PCE and TCE decreased from 35 to 5 μg L^{-1} and from 47 to 11 μg L^{-1}, respectively. OD: VC and cis-DCE removal efficiencies of 92 ± 2 and 100 ± 6 %, respectively	Probably indirect electron transfer	Reductive dehalogenases (RDase) encoded by the corresponding genes including *bvcA*, *pceA*, *tceA*, *vcrA*	n.a	Real groundwater	Dell' Armi et al. (2022b)	The removal of one chlorine atom from the CAHs carbon skeleton occurs through the introduction of two electrons and one hydrogen atom (Dell'Armi et al., 2022a; Dell'Armi et al., 2022b)
Reductive dechlorination of PCE, 1,2-DCA, and TCE	-*Lactococcus* and *Anaeroarcus* -*Geobacter* and *Pseudomonas* -*Bacillus*	**Cathode**/ graphite fiber brushes	-Glucose -Acetate -Sodium bicarbonate	−0.26 V vs. SHE	n.a	RD in 24 h: −93.0 ± 1.7% with glucose −74.6 ± 4.3% with acetate −53.9 ± 1.5%	Probably both direct and indirect electron transfer	Reductive dehalogenases encoded by *pceA*, *tceA*, *rdhA1*, outer membrane multi-heme *c*-	n.a	-Glucose -Acetate -Sodium bicarbonate	Chen et al. (2018)	n.a.

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Table 1 (*continued*)

Objective	Reactor microbiome	WE compartment/ Electrode material	Electrical Operation	Eapp (V vs SHE, Ag/AgCl)	Maximum power/ current/ voltage	Removal efficiency/rate	Removal process	Genes/ enzymeç/ proteins involved	Metabolites/ electrical connectors involved	Medium	Reference	Molecular mechanism
						with sodium bicarbonate		type cytochrome (OmcX).				
Oxidation of phenanthrene	-*Shewanella oneidensis* MR1 14063 -*Pseudomonas aeruginosa* NCTC 10662 -undefined anaerobic consortium	**Anode**/ carbon felt	External resistance (1000 Ω)	Not applicable	1.25 mW m^{-2}	−12,122 mg L^{-1} removed using *P. aeruginosa* −13,12 mg L^{-1} removed using the undefined anaerobic consortium and *P. aeruginosa* (Initial phenanthrene:20 mg L^{-1})	Probably electro-assisted biodegradation using electron mediators	n.a	n.a	Phenanthrene with defined minimal medium	Adelaja et al. (2014)	n.a.
Oxidation of PRW	-*Microbacterium* sp -*Paenibacillus* sp -*Deinococcus* sp	**Anode**/ graphite rod	External resistance (1000 Ω)	Not applicable	−73.86 mW m^{-3} −85.41 mW m^{-3} −67.78 mW m^{-3}	−386,8 mg L^{-1} COD removed using *Microbacterium* sp −390,9 mg L^{-1} COD removed using *Paenibacillus* sp −382,5 mg L^{-1} COD removed using *Deinococcus* sp (Initial PRW COD: 450 mg L^{-1})	Bio-electrochemical degradation	n.a	n.a	Phosphate buffer	Guo et al. (2014)	n.a.
Oxidation of PRW	*Pseudomonas putida* BCRC 1059	**Anode**/ carbon material	External resistance (1000 Ω)	Not applicable	0.005 mW cm^{-2}	664 mg L^{-1} COD removed (30%) (Initial COD of PRW: 2213 mg L^{-1})	Probably electro-assisted biodegradation using electron mediators	n.a	n.a	Nutrient broth media containing peptic digest of animal tissue, sodium chloride, beef extract, yeast extract	Majumder et al. (2014)	n.a.
Oxidation of OW	*Pseudomonas citronellolis* 620C	**Anode**/ carbon veil	External resistance (4500 Ω)	Not applicable	0.05 mW m^{-2}	Removal of 83.6 ± 0.1% (Initial COD of OW: 43,625 mg L^{-1}) (in 7 days)	Indirect electro-assisted biodegradation	n.a	(phenazine-based) Pyocyanin	M9 minimal salts medium	Varnava et al. (2024)	n.a.
Oxidation of PH (C9−C36)	*Rhodopseudomonas palustris* RP2	**Anode**/ carbon paper or graphite fiber brushes	External resistance (1000 Ω)	Not applicable	21 ± 3 mA m^{-2} and 720 ± 7 μW m^{-2}	Concomitant removal of 47.4 ± 2.7% (initial PH at 800 mg L^{-1})	Probably indirect electron transfer	Membrane-bound proteins detected	n.a	Acetate in phosphate-buffered saline buffer	Venkidusamy and Megharaj (2016)	n.a.
Oxidation of toluene	*Pseudomonas putida* F1	**Anode**/ graphite rod	External voltage (75, 125, 175, 250 and 500 mV)	Not applicable	94 mA m^{-2}	Maximum removal of 78% (initial toluene: 100 mg L^{-1}) (in 147 h)	Electro-assisted biodegradation	Key proteins: iron–sulphur subunit, cytochrome *c* oxidase, ubiquinol-cytochrome *c* reductase, cbb_3-type subunit III and outer membranes	n.a	Mineral medium	Friman et al. (2012)	n.a.
Oxidation of Toluene	*Cupriavidus metallidurans* CH34	**Anode**/ graphite rods	External voltage (800 mV)	Not applicable	48 mA m^{-2}	Removal of 73% (initial toluene	Electro-assisted biodegradation	n.a	n.a	M9 minimal medium	Tofalos et al. (2018)	n.a.

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Table 1 (*continued*)

Objective	Reactor microbiome	WE compartment/ Electrode material	Electrical Operation	Eapp (V vs SHE, Ag/AgCl)	Maximum power/ current/ voltage	Removal efficiency/rate	Removal process	Genes/ enzymeç/ proteins involved	Metabolites/ electrical connectors involved	Medium	Reference	Molecular mechanism
						60 mg L^{-1}) (in 17 days)						
Oxidation of Toluene	*Geobacter, Chryseobacterium* and *Zoogloea* sp	**Anode**/ carbon brush	External resistance (1000 Ω)	Not applicable	0.164 V	Removal of 88% (initial toluene at 300 mg m^{-3})	Proposed direct electron transfer	Outer membrane cytochromes	Menaquinone	Mineral salt solution medium	Zhang et al. (2018)	n.a.
Oxidation of toluene	*Geobacter* species	**Anode**/ graphite granules	Not applicable	+0.2 V vs. SHE	5.1 ± 0.1 mA	Removal rate of 67.2 ± 5.7 mg L^{-1} day^{-1} at anode recycle flow-rate of 75 mL min^{-1} (initial toluene: 25 mg L^{-1}	Probably direct electron transfer	n.a	Fumarate addition, the presence of benzylsuccinate (BS) as metabolite	Synthetic groundwater	Palma et al. (2018)	n.a.
Oxidation of toluene and benzene	*Geobacter, Thermincola* and *Rhodocyclaceae*	**Anode**/ carbon rod	External resistance (1000 Ω)	Not applicable	15.6 W m^{-3}	Complete removal (Initial toluene and benzene: 100 mg L^{-1}, respectively) (in 6 days)	Electro-assisted degradation	n.a	n.a	Nutrient solution	Lin et al. (2022)	n.a.
Oxidation of toluene, ethylbenzene and *o*-xylene (TE(*o*-x))	*Chryseobacterium* sp., *Hydrogenophaga* sp., *Geobacter* sp and *Alicycliphilus* sp	**Anode**/ carbon cloth and Pt plate	External resistance (1000 Ω)	Not applicable	3.2 mW m^{-2}	Removal of 90% (Initial TE(o-x) at 760 ± 30 mg m^{-3})	Probably direct electron transfer	n.a	Nanowire/pili	Sodium acetate medium	Zhang et al. (2020)	n.a.
Reduction of Co(III) to Co(II)	Biotic anode: *Geobacter sulfurreducens* *Bacteroidetes* sp. *Pseudomonas* sp. dominated	**Cathode**/ graphite felt	External resistance (200 Ω) Potentiostatic (Ag/AgCl sat. KCl, 0.222 V vs. SHE)	Not applicable	258 ± 9 mW m^{-3}	50 mg L^{-1} (in 48h)	Abiotic reduction	n.a	n.a	$LiCoO_2$ and KCl in the cathode and acetate medium in the anode	Huang et al. (2013)	n.a.
Reduction of Cr(VI)	*Shewanella oneidensis* MR-1	**Cathode**/ graphite felt	External resistance (1000 Ω)/Potentiostatic (−0.5 V vs Ag/ AgCl)	Not applicable	32.5 mA m^{-2}	9 mg L^{-1} (in 4 h)	Biotic reduction	n.a	n.a	Lactate medium	Xafenias et al. (2013)	n.a.
Reduction of Cr(VI)	*Trichococcus pasteurii* and *Pseudomonas aeruginosa* dominated	**Cathode**/ graphite plate	External resistance (1000 Ω)	Not applicable	55.5 mW m^{-2}	0.46 mg g VSS· h^{-1}	Biotic reduction	n.a	n.a	Medium containing sodium bicarbonate	Tandukar et al. (2009)	n.a.
Reduction of Cu(II)	-*Stenotrophomonas maltophilia* JY1 -*Citrobacter* sp. JY3 -*Pseudomonas aeruginosa* JY5 -*Stenotrophomonas* sp. *JY6*	**Cathode**/ graphite felt	External resistance (510 Ω)/Potentiostatic	−0.36 V and +0.46 V vs. SHE	n.a.	Initial Cu(II) of 5 mg L^{-1} and 20 mg L^{-1}: 0.82 ± 0.05 and 1.11 ± 0.01 mg L^{-1} h^{-1} removal, respectively	Biotic reduction and direct electron transfer	n.a	n.a	Medium containing sodium bicarbonate	Shen et al. (2017)	n.a.
Removal of Cd and Ni	*Ochrobactrum, Halomonas, Achromobacter* dominated	**Cathode**/ graphite brush	External resistance (200 Ω) Potentiostatic (−0.262 V for Ni and −0.403 V for Cd vs SHE)	Not applicable	722 mW m^{-3}	87% removal of Cd and 92% removal Ni (at 10 mg L^{-1})	Biotic reduction	n.a	n.a	Distillery wastewater	Singh and Kaushik (2021)	n.a.
Removal of Cu (II), Pb (II), Zn(II) and Cd(II)	*Sphingomonas, Azospira and Cupriavidus* dominated	**Cathode**/ carbon felt	Potentiostatic (0.9 V vs SHE)	−1.2–1.2 V vs. SHE at	208.60 ± 5.24 μA cm^{-2}	Removal of: 98.76 ± 0.32% of Cu, 98.01 ± 0.76% of Pb, 73.58 ± 4.83% of	Biotic reduction	n.a	n.a	CH_3COONa, KCl, NH_4Cl, mineral solution, and vitamin	Shi et al. (2023)	n.a.

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Table 1 (*continued*)

Objective	Reactor microbiome	WE compartment/ Electrode material	Electrical Operation	Eapp (V vs SHE, Ag/AgCl)	Maximum power/ current/ voltage	Removal efficiency/rate	Removal process	Genes/ enzymeç/ proteins involved	Metabolites/ electrical connectors involved	Medium	Reference	Molecular mechanism
						Zn, 84.39 ± 5.95% of Cd (at 20 mg L^{-1})						
Sulphate reduction	*Desulfovibrio desulfuricans* ATCC 27774	**Cathode**/254 SMO or graphite plates	Potentiostatic	−0.4 V vs. SHE	−270 mA m^{-2}	n.a.	Electro-assisted biodegradation	Hydrogenase, Adenosine Triphosphate (ATP)	Sulphide	Lactate medium	Cordas et al. (2008)	Eight-electron reactions for the reduction of sulphate to
Sulphate reduction	*Desulfovibrio caledoniensis*	**Cathode**/ pyrolytic graphite coupons	Potentiostatic ((Lower than −0.61 V vs SHE)	0.74 to −0.76 V vs SHE	n.a.	n.a.	Electro-assisted biodegradation	sulfurylase, adenosine 5′ -phosphosulfate (APS) reductase		Lactate medium	Yu et al. (2011)	sulfite and then sulfide. sulphate presence activates
Sulphate reduction	*Desulfovibrio paquesii*	**Cathode**/ graphite rod	Potentiostatic (−0.9 V vs. SHE)	0.2 to −1 V vs. SHE	n.a.	n.a.	Electro-assisted biodegradation (probably *c*-type cytochromes)	(AprBA), sulfite reductase complex (Dsr) and membrane		Lactate medium	Aulenta et al. (2012)	Adenosine Triphosphate (ATP)
Sulphate reduction	*Acidithiobacillus ferrooxidans, Desulfosporosinus orientis, Thiobacillus denitrificans, Sulfurimonas denitrificans, Desulfovibrio piger*	**Cathode**/ graphite rod	Potentiostatic	−0.5 V vs. Ag/AgCl	−50.5 mA cm^{-2} −4 mA cm^{-2} −2.5 mA cm^{-2} −2.5 mA cm^{-2} −1.6 mA cm^{-2}	n.a.	Electro-assisted biodegradation	complex DsrMKJOP or DsrMK(Agostino and Rosenbaum, 2018)		CO_2 medium	Rodrigues and Rosenbaum (2014)	sulfurylase which forms adenosine 5′ -phosphosulfate (APS). APS is transformed to sulfite with APS reductase (AprBA) as catalyst in a
Sulphate reduction	*Desulfopila corrodens* IS4	**Cathode**/ graphite rod	Potentiostatic (−0.4 V vs. SHE)	–0.15 V to −0.5 V vs. SHE	n.a.	n.a.	Electro-assisted biodegradation		n.a	CO_2 medium	Beese-Vasbender et al. (2015)	reaction of two-electrons. Then sulfite is reduced
Sulphate reduction	*Desulfobacterium autotrophicum* HRM2	**Cathode**/acid pretreated graphite plates	Potentiostatic (−0.243 V vs SHE)	−0.4 V and −0.5 V vs SHE	−14.2 ± 1.5 mA m^{-2}	n.a.	Electro-assisted biodegradation		n.a	CO_2 medium	Zaybak et al. (2018)	to sulfide with the dissimilatory sulfite reductase complex (Dsr) as catalyst. In the last reaction, an energy-conserving membrane complex (DsrMKJOP or DsrMK) is also involved (Agostino and Rosenbaum, 2018).
Sulphide removal	*Alcaligenes* sp. or *Paracoccus* sp	**Anode**/ granular graphite	External resistance (100 Ω)	−0.150 V vs SHE (2 days), then at −0.100 V vs SHE	101 mW L^{-1} net anodic compartment (NAC)	Removal of 514 mg sulfide L^{-1} NAC (initial sulfide flow: 0.60 g L^{-1} NAC day^{-1}	Electro-assisted biodegradation	n.a	n.a	Glucose and sulphate medium	Rabaey et al. (2006)	n.a.
Sulphate reduction	*Desulfovibrio desulfuricans* Essex 6 (DSM 642)	**Anode**/ activated carbon cloths	External resistance: Not known	Not applicable	0.51 mW cm^{-2}	Removal of 99% (Initial sulphate: 3.03 g dm^{-3})	Electro-assisted biodegradation	n.a	n.a	Lactate medium	Zhao et al. (2008)	n.a.
Sulphate reduction	*Desulfovibrio desulfuricans* (ATCC 27774)	**Anode**/ graphite felt	External resistance: (100 Ω)	Not applicable	233 ± 24.2 mA m^{-2}	COD removal up to 98%	Direct electron transfer	Cytochrome *c3*, conductive bacterial appendages	Sulphide	Lactate medium	Kang et al. (2014)	n.a.

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Table 1 (*continued*)

Objective	Reactor microbiome	WE compartment/ Electrode material	Electrical Operation	Eapp (V vs SHE, Ag/AgCl)	Maximum power/ current/ voltage	Removal efficiency/rate	Removal process	Genes/ enzymeç/ proteins involved	Metabolites/ electrical connectors involved	Medium	Reference	Molecular mechanism
Sulphate reduction	*Desulfovibrio, Aeromonas, Tetrathiobacter*	**Anode**/ graphite sheets	External resistance (1100, 300, 100 and 200 Ω)	Not applicable	9.15 W m^{-3} and 16.17 A m^{-3}	-complete COD removal (initial COD = 3300 mg L^{-1}) (in 3 days)	Direct electron transfer	n.a	n.a	Raw landfill leachate medium	Kumar et al. (2017)	n.a.
Decolorization of RO-16 and RB-5	-*Pseudomonas aeruginosa* MTCC 2582 -*Pseudomonas fluorescens* MTCC 2269	**Anode**/ graphite block	External resistance (220 Ω)	Not applicable	−2887 ± 13 μW m^{-2} (RO-16) and 1906 ± 7 μW m^{-2} (RB-5) and −1896 ± 15 μW m^{-2} (RO-16) and 1028 ± 9 μW m^{-2} (RB-5)	0.47 mM	Electro-assisted decolorization	Azo reductase and oxidative laccase,	n.a	Glucose modified minimal salt medium	Ilamathi et al. (2019)	n.a.
Decolorization of MO	*Geobacter sulfurreducens* PCA	Not applicable	Not applicable	Not applicable	Not applicable	40 mg L^{-1} (in 5h)	Direct electron transfer	OmcB, OmcC, and OmcE are the main cytochromes responsible	n.a	Acetate medium	Liu et al. (2017)	n.a.
Decolorization of XP2R and 2, 4-Xylidine	*Shewanella marisflavi* EP1	Not applicable	Not applicable	Not applicable	Not applicable	47.25 mg L^{-1}	extracellular reduction	n.a	n.a	Lactate, fumarate medium	Xu et al. (2016)	n.a.
Decolorization of RB19	*Klebsiella* sp. C NCIM 5546	**Anode**/ graphite	External resistance	Not applicable	84 mW m^{-2}	45 mg L^{-1} (in 24h)	Electro-assisted decolorization	n.a	Oxalic acid, indene- 1, 3-dione and hydroquinone	Synthetic wastewater with RB19, M9 minimal medium and Glucose	Holkar et al. (2018)	n.a.
Decolorization of MO	*Shewanella oneidensis MR-1*	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Electro-assisted decolorization (probably direct electron transfer)	Outer membrane cytochromes, OmcA	n.a	Not applicable	Louro et al. (2023)	n.a.

*N.a: non available, CAH: chlorinated aliphatic hydrocarbons, PCE: perchloroethene, 1,2 DCA: 1,2-dichloroethane, TCE: trichlorethene, RD: reductive dichlorination, OD: oxidative dichlorination, PRW: petroleum refinery water, OW: oily wastewater, PH: petroleum hydrocarbons, XP2R: Xylidine Ponceau 2R, RO: reactive orange, RB: reactive blue, MO: methyl orange.

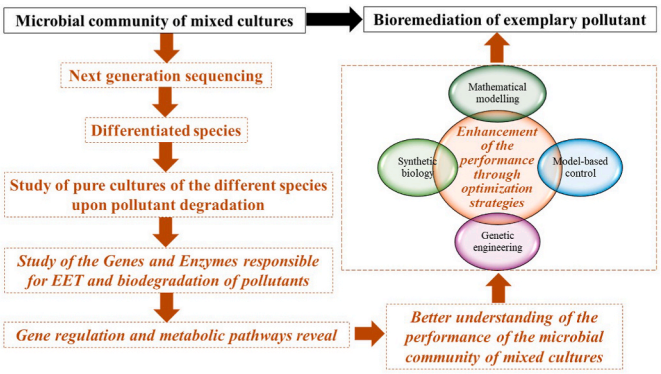


Fig. 3. The proposed concept to close the gaps in electro-bioremediation of contaminated waters through studying pure cultures at a micro-scale level.

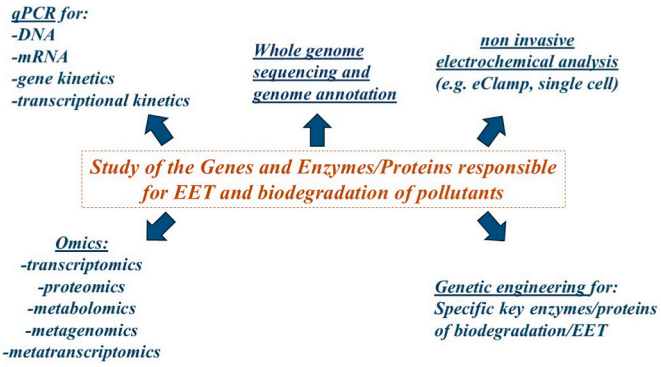


Fig. 4. The proposed methodologies to study the genes and enzymes/proteins responsible for EET and/or biodegradation of pollutants using pure cultures.

activity of the community/pure culture (Fig. 4). These methods enable the screening of operational parameters without disrupting ongoing operations. This approach provides insights at the granule level, offering guidance for engineering bio-electrochemical reactors and improving treatment rates. However, further exploration is needed to adapt the electrochemical analysis for non-granular reactor configurations without affecting the microbial community/pure culture. Another non-invasive *in situ* electrochemical method could be single-cell electrochemistry. The latter could enhance the knowledge about the dynamic metabolic activities of single live unicellular microorganisms (Xie and Wang, 2023) such as bacteria, which are commonly used in BES (Fig. 4).

When the gene regulation of microorganisms is known, optimization strategies, at microbiological level, are possible. These strategies include mathematical modelling through microbial growth kinetics models, genetic engineering, and synthetic biology approaches (Fig. 3). Mathematical modelling in BES can play a key role in predicting biodegradation rates, biomass growth, and biofilm formation, among other parameters (Tsipa et al., 2021). In these models, empirical equations, such as Monod and Monod-type, are typically used (Jadhav et al., 2021). However, these equations are traditionally applied under a narrow range of conditions, and often lack fit, while ignoring transcriptional regulation (Kovárová-Kovar and Egli, 1998). Therefore, the connection of gene regulation to microbial growth kinetics models could lead to accurate mathematical modelling to predict bio-performance (Tsipa et al. 2018, 2021).

Modelling gene regulation can also lead to model-based gene control which can enhance the performance of the process through enhanced biodegradation rates (Tsipa et al., 2018). Similarly, if a gene-regulating EET pathway is model-based-controlled, the performance of the system, and, subsequently, the process can be enhanced (Tsipa et al., 2018). Furthermore, the key mechanistic information of the biodegradation and EET pathways in BES could be used in genetic engineering to overexpress or knock out genes to increase substrate biodegradation, electron availability, and biomass growth, among other purposes; thus, enhancing the performance of the system and leading to system optimization at the microbiological level. Similarly, when key metabolic steps are known, synthetic biology using microbial cell factories could take place to accelerate pollutant biodegradation, EET, and biofilm formation rates resulting in overall enhanced performance of the process (Teravest and Ajo-Franklin, 2016; Dong et al., 2022) and microbial optimization (Fig. 3).

The above points should be revealed through a multidisciplinary approach including engineering, molecular biology, biochemistry, bioinformatics, and structural biology knowledge.

10. Conclusions

In this review, we were able to detect 39 studies of electro-assisted biodegradation of exemplary water pollutants using pure cultures and identified mixed cultures. We realised that, despite the wide use of mixed cultures and established TRL of at least 3, the specific species primarily accountable for the pollutant removal and EET transfer remain unidentified. Even when identification of the species was performed, the electro-assisted mechanisms of the process were still not known in many cases. Furthermore, the study of pure cultures at laboratory and, hence, micro-scale level is not only limited but, in most cases, restricted to studying merely the electroactivity and biodegradation potential of the culture. However, study of pure cultures cannot drive the electro-biodegradation process efficiency, but, instead, it can reveal mechanistic insights at molecular level. Therefore, we suggest a transition from macro-to micro-scale and from mixed to pure cultures to determine the genes, enzymes and proteins, and metabolites responsible for the electro-assisted biodegradation. NGS followed by microorganisms' isolation is the first step towards this direction. Then pure cultures can be studied using non-invasive methods to perform whole genome sequencing, omics, transcriptional kinetics and genetic engineering. Only with this information, it will be possible to advance sustainable wastewater treatment at microbiological level through genetic engineering, synthetic biology, mathematical modelling and strategic building of co-cultures.

Finally, studying the molecular mechanisms of pure cultures with electroactive properties for other pollutants of emerging concern such as polyfluoroalkyl substances (PFAS), microplastics, and micro-pollutants (i.e. trace pharmaceutical products or antibiotics) is essential.

CRediT authorship contribution statement

Argyro Tsipa: Writing – original draft, Visualization, Methodology, Investigation, Conceptualization. **Sebastià Puig:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Luciana Peixoto:** Writing – review & editing, Methodology, Investigation. **Catarina M. Paquete:** Writing – review & editing, Methodology, Investigation, Conceptualization.

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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Data availability

All data have been entered in Table 1 of the manuscript.

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