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Review

Let’s chat: Communication between electroactive microorganisms

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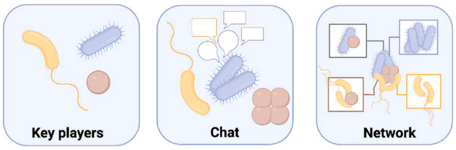
HIGHLIGHTS

- Extracellular microbial electron transfer plays a key role in our environment.
- Cellular communication is fundamental to tuning electroactivity in communities.
- Cell-to-cell interactions may push electroactivity potential applications.
- Genetic engineering are compelling strategies to promote electroactivity.

GRAPHICAL ABSTRACT



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ABSTRACT

Electroactive microorganisms can exchange electrons with other cells or conductive interfaces in their extracellular environment. This property opens the way to a broad range of practical biotechnological applications, from manufacturing sustainable chemicals via electrosynthesis, to bioenergy, bioelectronics or improved, low-energy demanding wastewater treatments. Besides, electroactive microorganisms play key roles in environmental bioremediation, significantly impacting process efficiencies. This review highlights our present knowledge on microbial interactions promoting the communication between electroactive microorganisms in a biofilm on an electrode in bioelectrochemical systems (BES). Furthermore, the immediate knowledge gaps that must be closed to develop novel technologies will also be acknowledged.

1. Introduction

Many phylogenetically divergent microorganisms can exchange electrons with extracellular electrically conductive interfaces, by performing extracellular electron transfer (EET). These interfaces can be conductive minerals like metal oxides common in natural environments, or engineered electrodes in bioelectrochemical systems (BES) (Cahoon and Freitag, 2018; Gupta et al., 2020; Logan et al., 2019; Wang et al., 2019a). The microorganisms that can exchange electrons with electrodes are known as electroactive microorganisms. Up to date, more than 100 organisms have been described as electroactive, being able to transfer (named exoelectrogens; anode reaction) or receive (named electrotrophs; cathode reaction) electrons from an electrode (Logan et al., 2019). These can be Gram-negative or Gram-positive bacteria, archaea or yeasts, being found in numerous environments, including lakes and rivers, sea, sludge, sediments, rice paddy soils, deep subsurface aquifers and even in mammalian gut and oral plaque (Holmes et al., 2004; Logan et al., 2019; Naradasu et al., 2019; Wang et al., 2019b; Yee

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et al., 2020). The research field studying these microorganisms is known as electromicrobiology, which progressed significantly over the last decades given its significance in numerous biotechnological applications, including BES for wastewater treatment, bioremediation, biosensing and bioelectrosynthesis, contributing to tackle numerous societal challenges (Li et al., 2019; Prévoteau et al., 2020; Rotaru et al., 2021; Wang et al., 2020).

Cell-to-cell associations are beneficial in these systems as cells coordinate their growth, activity and movement, leading to the formation of complex and highly organized multicellular and multispecies structures such as biofilms (Sime-Ngando et al., 2019). In biofilms, cells are held together and embedded in a matrix typically composed of carbohydrate polymers, proteins and nucleic acids (Edel et al., 2019). Such extracellular polymeric substances (EPS) play a role in cell-to-cell communication (Chen et al., 2017a; Hammer and Bassler, 2003; Marketon et al., 2003; Tan et al., 2014; Yang et al., 2017), and electron transfer (Xiao et al., 2017; Xiao and Zhao, 2017; Zhuang et al., 2020). Additionally, EPS facilitates the transfer of solutes in the defined extracellular matrix and stabilizes protein complexes that may act as electron conduits through the biofilm (Li et al., 2016).

Electroactive microorganisms can interact using several processes, including:

(i) Quorum sensing via low molecular weight sensor molecules that leads to coordinated gene expression at a specific cell density; and various environmental factors that can also stimulate quorum sensing;
(ii) Outer-membrane vesicles (OMVs) that can mediate cell communication and EET;
(iii) Physical interactions between neighbouring cells via cell surface structures like intercellular membranous nanotubes, type IV pili, surface-bound multiheme *c*-type cytochromes that can also even be stacked into cytochrome- nanowires, facilitating direct cell-to-cell EET;
(iv) Small diffusible metabolites (e.g., hydrogen or formate, flavins) that enable electron transfer between cells without cell-to-cell contact.

In BES, cell interactions are crucial to guarantee efficient electron transfer between the bacteria and electrodes, which is important to define its performance and applicability (Wang et al., 2020). EET to an electrode can occur directly or indirectly. Direct electron transfer occurs when cell-surface proteins (e.g. multiheme cytochromes) or conductive cellular appendages form a conduit for electron transfer between the cell and the electrode. Indirect electron transfer occurs when a soluble electron shuttles is reduced and/or oxidized by the cell (e.g. quinones, flavins, phenazines) mediating electron transfer between the cell and the electrode (Fig. 1) (Costa et al., 2018; White et al., 2016). Besides shuttles, EET can also be facilitated by extracellular enzymes, that catalyse the uptake of electrons from the surface of an electrode or metallic iron and conversion into substrates (e.g., hydrogen, formate) that can then be used by hydrogen-consuming methanogens or acetogens (Deutzmann et al., 2015; Tsurumaru et al., 2018).

This review will focus on cell interactions that enable electroactive organisms to communicate with each other, in a biofilm on an electrode in a BES. We will emphasize the efforts made in the last decades to study cellular interactions involved in biofilm formation and in electron exchange mechanisms, mainly focusing on how these interactions may increase electron transfer in biotechnological applications. This review aims to provide insights into cellular interactions that support electroactive organisms to interact with other organisms and to electrodes, a knowledge gap that is fundamental to start guiding the implementation of emerging applications of BES and develop novel technologies towards a sustainable society.

2. Cell-to-cell communication

2.1. *Quorum sensing in an electroactive biofilm*

Concerted microbial actions like the aggregation in biofilms or the common activation of certain biosynthetic pathways are typically controlled by quorum sensing (QS). Quorum sensing is inter-cellular communication through self-produced small molecules. While Gram-positive bacteria uses modified oligopeptides with variable sequences as QS signals, Gram-negative bacteria use acyl homoserine lactones (AHLs). These molecules are initially produced by the microbial cells through the basal expression of an inducer gene. When cell densities increase, the concentration of these small molecules can reach a certain threshold to activate a corresponding QS regulator (primarily) within the same species (Fig. 2). The activation of this regulator increases the expression of the inducer gene, amplifying the signalling, and it can activate downstream regulatory systems. The most famous QS inducer/regulator pairs are the LuxS/LuxR system from *Vibrio* spp. (Papenfort and Bassler, 2016) and the LasI/LasR and RhlI/RhlR systems from *Pseudomonas aeruginosa* (Lee and Zhang, 2015; Williams and Cámara, 2009). An extensive review of bacterial signalling mechanisms was recently published (Das et al., 2021).

In electroactive microorganisms, QS may influence the electroactivity of the bacteria in two ways: i) it can control the biofilm formation required for direct electron transfer, and ii) it can control the production of redox-active constituents required for indirect electron transfer.

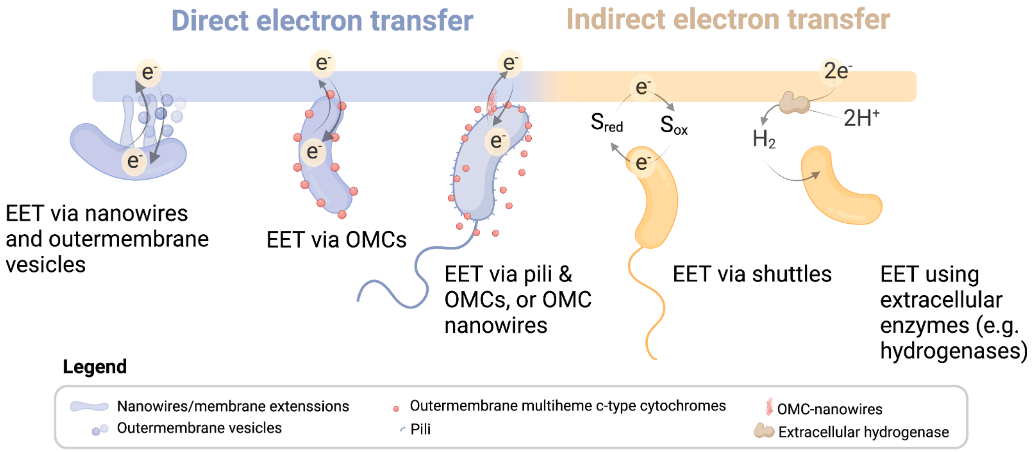

Fig. 1. Extracellular electron transfer mechanisms performed by electroactive bacteria. Electron transfer can occur through a direct process using nanowires, OMVs, pili or cell-surface cytochromes, or by an indirect process using electron shuttles or extracellular enzymes. Created with Biorender.com.

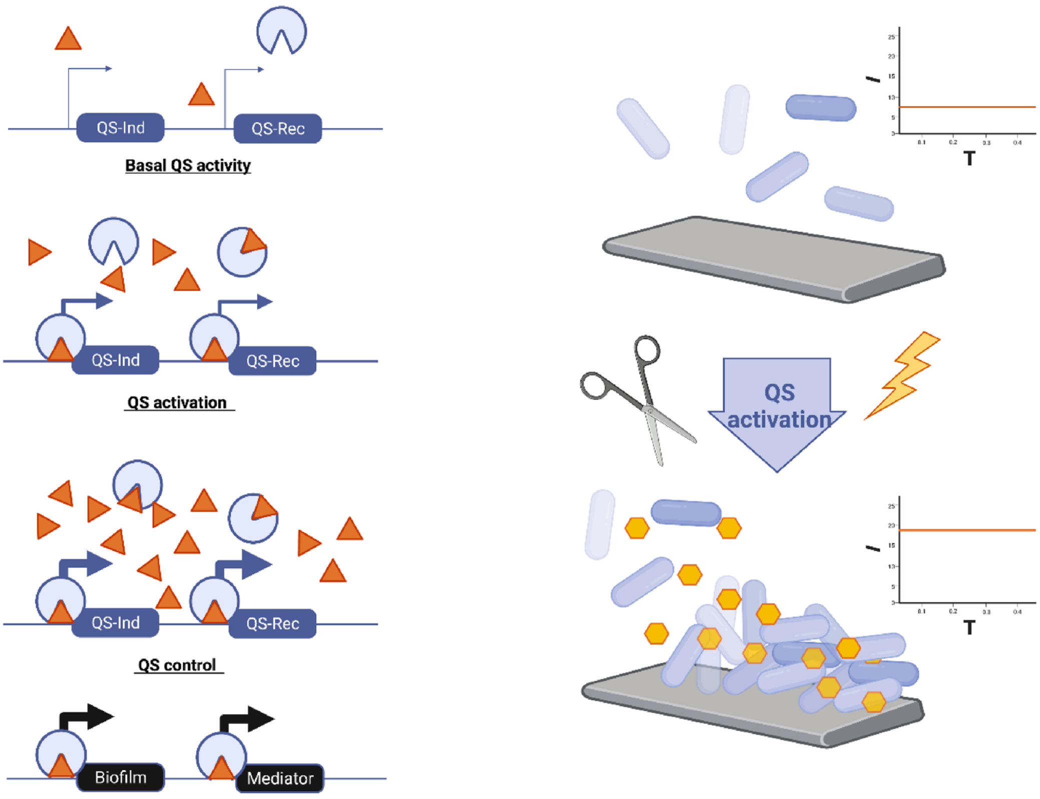

Fig. 2. Quorum sensing mediated electroactive biofilm formation. Left diagram showing three common QS situations according to the presence of stimulus. Top- in the absence of stimulus (or in the event of low cell density) QS is in basal activity and no effect is observed. Middle- Increasing cell density stimulates the production of autoinducer and the QS cascade is activated. Bottom- Stimulated QS signal can activate additional genes promoting biofilm formation. Right- Engineered cells can be tailored for a QS induced biofilm formation, increasing cells densities at the electrode surface, and stimulating electroactivity, which leads to enhanced current production (inserted graphs). Created with Biorender.com.

2.1.1. *Tuning biofilm formation to enhance electroactivity*

One of the most studied fields of community-controlled QS regulation is biofilm formation. Therefore, strategies to promote biofilm formation for electroactive bacteria are arising. A recent review focused on genetic engineering strategies to enhance the production of EPS as the “glue” of a biofilm to enhance the performance of electroactive biofilms in microbial fuel cells (MFCs) (Angelaalincy et al., 2018). For example, exogenous QS signals, such as quinolone, when added to the extremophile *Halanaerobium praevalens*, led to a 95% increase in biofilm mass (Monzon et al., 2016). This biofilm formation increase was responsible for the 30% increase in generated power. A study by Chen *et al.* investigated the activity and influence of QS control mechanisms within undefined mixed culture MFC biofilms in more detail (Chen et al., 2017b). Through biological sensing of the AHL inducer molecules, they confirmed the activity of QS processes within the anodic biofilm, but, due to the low concentrations, the identification of the AHLs was not possible. Interestingly, the addition of three very common AHLs (N-butanoyl-HSL (C4-HSL), N-hexanoyl-HSL (C6-HSL), and N-3-oxo-dodecanoyl-HSL (3OC12-HSL)) resulted in some remarkable improvements in the electroactive biofilm function. The overall biofilm amount, compactness, and ratio of living to dead cells improved compared to the non-stimulated control. They also found an increase in redox activities of the EPS upon AHL stimulation of the biofilm formation. The study did not investigate, which specific microbial groups of the community were responsible for this activation. But interestingly, the abundance of *Geobacter* sp., which often represent the most electroactive members of mixed culture anodic biofilms, increased with QS stimulation (Chen et al., 2017b). For the EET model organism *G. sulfurreducens*, a QS network has not been described, yet. However, the finding of Chen *et al.* confirms an earlier work of this group with *G. soli* (Jing et al., 2019). This study investigated the effect of endogeneous and exogeneous AHLs in biofilm formation and electroactivity of this strain. Indeed, they detected endogeneous AHLs, which improved the relative abundance of outer membrane proteins and enhanced EPS production. The added AHLs also resulted in enhanced activity of the electroactive biofilms (Jing et al., 2019). Edel *et al.* recently showed that intrinsic QS-based control might also be important in the second model organism for direct electron transfer, *Shewanella oneidensis* (Edel et al., 2021). The study revealed that the secreted redox-active riboflavin not only serves as an electron mediator for *S. oneidensis*, but it also stimulates the expression of genes responsible for biofilm formation (especially *speC*) and thicker biofilms, in turn, showed a higher current generation. With the external addition of riboflavin, the threshold for this activation was determined. This concentration was lower than the concentration of flavins found in the biofilm matrix of unstimulated cells, indicating that the stimulation of biofilm formation is self-regulated by cell density-based riboflavin secretion (Edel et al., 2021).

All so far mentioned studies showed QS enhancement of anodic electroactive biofilms, where electrons are discharged to the electrode. Chavert *et al.* showed that it might also play an important role in electroactive biofilm development for cathodic electron uptake processes (Chavert et al., 2017). The pre-colonization of *Acidithiobacillus ferrooxidans* on ferrous iron or sulfur-doped carbon felt electrodes was strongly improved with the external addition of a mixture of AHL QS inducers, yielding stronger and much denser biofilms. When inserted in an electrochemical set-up, these electrodes also yielded higher-performing biocathodes with current densities about twice as high as the non-stimulated control (Chavert et al., 2017).

2.1.2. *Stimulating mediated electron transfer through quorum sensing*

One of the model organisms for mediated electron transfer is *P. aeruginosa*, which employs phenazine redox mediators for EET (Rabaey et al., 2005; Venkataraman et al., 2011, Venkataraman et al., 2010). The production of these phenazine redox mediators is controlled by both cell density-based QS regulation and environmental factors such as substrate, nutrient (iron or phosphate) or oxygen availability (Venkataraman et al., 2010). Thereby, a hierarchical regulatory network of four levels is involved in modulating phenazine expression. On the top two levels, the QS inducers (3OC12-HSL and C4-HSL) regulate the expression of the regulators LasR and RhlR, respectively. Thereby, the first system upregulates the second and the third regulatory layer, the 2-heptyl-3,4-dihydroxyquinoline (=PQS) regulon (Higgins et al., 2018). But also environmental factors have regulatory input on the second and third regulon, e.g., through the two-component system GacS/GacA (Kay et al., 2006; Wang et al., 2013a). RhlR and the PQS regulon then directly address the phenazine synthetic genes *phzA-G* (for the synthesis of phenazine-1-carboxylic acid) (Higgins et al., 2018) and further modify enzymes to generate phenazine derivatives such as pyocyanin. Finally, the fourth layer of regulation directly involves phenazines, which could act as transcription factors to regulate genes related to phenazine export, such as the *mexGHI-ompD* operon (Dietrich et al., 2006; Price-Whelan et al., 2006).

Several studies have shown that the modulation of the QS regulation can be applied to steer the electroactivity of *P. aeruginosa* through the changes in phenazine redox mediator production. Venkataraman *et al.* showed that synthetic modulation of environmental factors, such as oxygen or iron availability, influence the electroactivity of *P. aeruginosa* (Venkataraman et al., 2010). But also, the interaction in microbial communities influences this complex regulation of redox mediator production. It was shown that certain metabolites of microbial interaction partners could stimulate phenazine synthesis and even modulate the composition of produced phenazine derivatives (Venkataraman et al., 2011). Berger *et al.* showed that spontaneous or synthetic genetic modifications of the Las QS system (here in a LasR knockout) could boost phenazine production and subsequent electroactivity (Berger and Rosenbaum, 2017). In a more complex setting, Yang *et al.* evaluated the effect of a lack of endogenous AHL production (in a LasI/RhlI knockout) and found reduced electroactivity, which could be compensated by specific AHL addition (Yang et al., 2018). This shows the essential function of QS activation of biofilm and phenazine related gene expression. Surprisingly, the same study found a contrasting result for the activity of the PQS regulatory network: a PQS deficient strain showed enhanced electroactivity, while a PQS overexpression strain showed no change in current production (Yang et al., 2018). This result corresponds to an earlier study, which modulated the PQS activity (Wang et al., 2013b). Since the PQS regulon is less active under anaerobic conditions, Yang *et al.* synthetically overexpressed the PqsE effector in a PQS negative background strain, leading to higher phenazine production and a five-fold enhanced current generation under anaerobic conditions. Another recent study investigated the knockout of RpoS, a global sigma factor, which is in tight interaction with the QS cascade and controls many QS-controlled genes in the stationary growth phase, and also found an enhanced formation of biofilm and redox-active compounds (Yu et al., 2018).

2.1.3. Synthetic quorum sensing networks to steer electroactivity

In a remarkable recent study, Li *et al.* took QS control of electroactivity to an entirely new level (Li et al., 2020). They engineered a population-state decision system to control and enhance the electroactivity of the model organism *S. oneidensis*. Through the integration of a synthetic LuxI/LuxR QS system to measure cell density as the decision-making unit and decision implementation via Lux-controlled gene expression of genes responsible for *Shewanella* electroactivity (e.g., the outer membrane cytochrome complex and riboflavin synthesis), they successfully reallocated cellular energy towards EET once a specific cell density was reached. Furthermore, they confirmed the broad implication of the increased electroactivity by measuring almost five-fold enhanced electric current output, boosted reduction activity towards WO_3, and accelerated reduction of methyl orange, a model azo-dye pollutant (Li et al., 2020). This type of intelligent reprogramming of biocatalysts for environmental biotechnology and bioremediation might inspire completely new approaches to these challenging tasks.

All the examples above, dealing with both direct and indirect electron transfer in bacteria, show that genetic engineering approaches can be a powerful strategy to promote electroactivity. However, while this may become a very effective and successful strategy for confined BES, it may be circumvented for open environmental applications, such as bioremediation, due to concerns regarding the uncontrolled spreading of genetically modified microorganisms. For environmental applications, knowledge about the cell's natural or environmental modulation of QS activity may be beneficial in steering microbial community functions.

2.2. Outer-membrane vesicles of electroactive bacteria

Microbes produce and release membrane-bound materials, often designated as OMVs (Schwechheimer and Kuehn, 2015). OMVs range in size from 100 to 300 nm, consist of lipids from cellular membranes, and contain several biomolecules, including proteins (membrane-bound, periplasmic and cytoplasmic), DNA, RNA and organic compounds (Jan 2017). These vesicles have been linked to many physiological processes, including interbacterial communication. The presence of DNA in bacterial OMVs provides one of the mechanisms of horizontal gene transfer, which has been essential for prokaryotic evolution and transfer of antibiotic resistance genes or virulence genes within bacteria (Perez-Cruz et al., 2013). OMVs are also suitable for delivering hydrophobic signal molecules between cells (Avila-Calderón et al., 2021) or transfer of cellular metabolites including nutrients (Schwechheimer and Kuehn, 2015). In *S. vesiculosa* HM13, the protein P49 was shown to be a major cargo on OMVs, although the physiological function of this protein remains to be elucidated (Kamasaka et al., 2020). The OMVs are likely involved in EET since they incorporate redox active centers like multiheme *c*-type cytochromes (Chong et al., 2019; Gorby et al., 2008). It was also demonstrated that OMVs produced by *Geobacter sulfurreducens* during its natural growth cycle are redox-active due to the presence of multiheme cytochromes, promoting EET from microbial cells to an electrode (Guo et al., 2020). OMVs were shown to be part of bacterial biofilms, promoting biofilm formation (Schooling and Beveridge, 2006; Seike et al., 2021; Toyofuku et al., 2012). For example, in *Aeromonas*, proteins localized on the surface of OMVs were proposed to act on specific receptors of this strain and promote biofilm formation (Seike et al., 2021). In *S. oneidensis*, Pirbadian *et al.* observed that OMVs were formed under anaerobic conditions when cells had to respire a solid electron acceptor. This bacterium formed extracellular membrane extensions that extend many times the length of the cell, as chains of vesicles or as membrane tubes (Pirbadian et al., 2014).

The importance of OMVs for cellular functions and extracellular interactions has been explored over the past 20 years, however it remains to be determined whether we could apply this property to enhance electroactivity in bacteria and increase its applicability in biotechnological processes.

3. Cell-to-cell interactions between species

3.1. Microbial interspecies interactions

Microbial interspecies interactions, especially cross-feeding interactions or syntrophym, are crucial in bioremediation, wastewater treatment and biosensing (Rotaru et al., 2021). Typically, syntrophic associations are dependent on the transfer of electrons from a syntrophic bacterium oxidizing an organic food substrate to a partner methanogenic archaeon reducing the only available electron acceptor – carbon dioxide. Only together, the syntrophic partners can oxidize the organic substrate at the expense of CO_2 respiration. The electron exchange between syntrophic partners (Fig. 3) occurs typically either via diffusible intermediates (e.g., H_2, formate; Fig. 3A) or by direct electron exchange between physically connected cells (Fig. 3B) (Rotaru et al., 2021; Shrestha and Rotaru, 2014). Physical cell-to-cell connections are required to transfer electrons directly via intrinsic biotic (Direct Interspecies Electron Transfer / DIET) or extrinsic abiotic constituents (Conductive particle mediated Interspecies Electron Transfer / CIET; Fig. 3C).

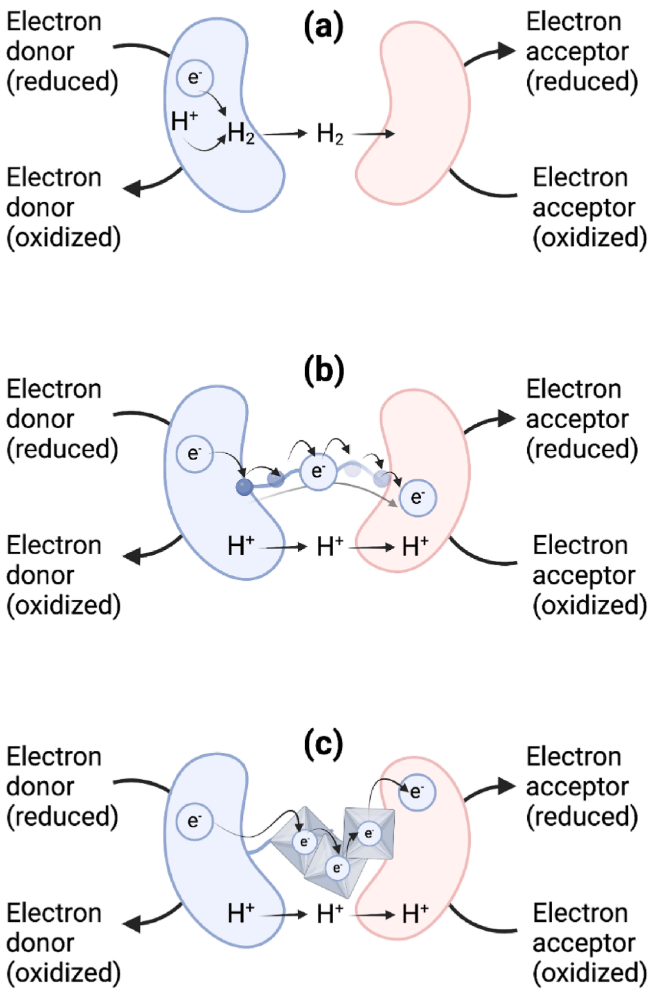

Fig. 3. Electron exchange between syntrophic partners occurs typically either via: a) diffusible intermediates, b) direct electron exchange between physically connected cells and c) Conductive particles. Created with Biorender.com.

3.1.1. *Direct interspecies electron transfer (DIET)*

During DIET, electrons are transferred via intrinsic biotic constituents on the cell surface, such as outer-membrane multiheme *c*-type cytochromes and/or electrically conductive type IV pili (Holmes et al., 2021; Rotaru et al., 2014b, 2014a; Summers et al., 2010). DIET interactions have been documented and thoroughly investigated between different species of the genus *Geobacter* (Summers et al., 2010), well known for their EET properties (Lovley, 2011a), between *Geobacter* and methanogens of the order *Methanosarcinales* (Holmes et al., 2021; Rotaru et al., 2014a, 2014b; Yee et al., 2019; Yee and Rotaru, 2020) and between *Geobacter* and phototrophs (Liu et al., 2021). However, only highly effective electrogenic *Geobacter* could interact via DIET (Rotaru et al., 2015). Outside of the *Geobacter* genus, other possible DIET syntrophs were *Rhodoferax* (Yee and Rotaru, 2020) and under certain circumstances (e.g., absence of an H_2-transfer option) *Syntrophus* (Walker et al., 2020).

Typical DIET-partners like *Geobacter* and *Methanosarcinales* coexist in many natural and man-made environments. For example, *Geobacter* and *Methanothrix* dominant communities have been found in lab-scale anaerobic reactors (Morita et al., 2011), industrial-scale anaerobic digesters (Shrestha et al., 2014) and rice paddies (Holmes et al., 2017). Expression studies suggested that *Geobacter* and *Methanothrix* were in a possible DIET relationship in these environments (Holmes et al., 2017; Morita et al., 2011; Rotaru et al., 2014b).

3.1.2. *Conductive particle mediated interspecies electron transfer (CIET)*

Abiotic conductors artificially added to a synthetic DIET-consortia stimulated the interactions considerably (Chen et al., 2014b, 2014a; Liu et al., 2012; Rotaru et al., 2014a). For instance, the addition of nanosized conductive iron oxide (e.g. magnetite) to a synthetic *Geobacter* co-culture, led to the expression downregulation of an extracellular multiheme *c*-type cytochrome, OmcS. In a typical *Geobacter*, OmcS outlines the entire length of electroactive pili. In consortia amended with nanosized magnetite, magnetite outlined the entire length of the electroactive pili (Liu et al., 2015). Conductive particles like magnetite may replace outer membrane *c*-type cytochromes. Hence, magnetite might reduce the energy investment required to build own EET cytochromes.

In the last years, the use of conductive carbon-based materials and minerals (such as magnetite) serving as conduits between the electron-donating and the electron-accepting microbes have been studied. Hassanein *et al.* recently reviewed the effect of the type and size of conductive nanoparticles on improving microbial processes within anaerobic digestions systems and correspondingly the biogas output (Hassanein et al., 2021). Inline, Cruz-Viggi *et al.* showed that supplementation of magnetite particles to a methanogenic sludge enhanced the methane production rate up to 33% from a key intermediate in the anaerobic digestion (propionate) (Cruz Viggi et al., 2014). The same authors demonstrated that cells supplemented with magnetite exhibited a substantially higher yield of acetate production relative to unamended controls in microbial electrosynthesis (Cruz-Viggi et al., 2020).

Spiking with conductive particles to promote CIET has been expansively used in recent years, especially to treat wastewater streams (Gahlot et al., 2020), bioremediate toxic compounds (Aulenta et al., 2021; Rotaru et al., 2021). However, the exact mechanisms used by microorganisms interacting with each other via a conductive mineral surface have been barely addressed, remaining an active topic of research.

3.2. *Enhancing cell-to-cell interaction*

Cell-to-cell communication can boost EET in mixed cultures. For example, in the synthetic microbial consortium composed by *S. oneidensis* MR-1 and *Bacillus subtilis* RH33, the high concentration of riboflavin produced by the Gram-positive bacterium *B. subtilis* RH33 could be used by *S. oneidensis* MR-1 to boost bioelectricity generation (Liu et al., 2017). On another study, metabolites produced by *Pseudomonas* sp. CMR12a, that includes phenazine and biosurfactants, increased the EET capabilities of the Gram-positive bacterium *Brevibacillus* sp. PTH1 (Pham et al., 2008). In this study it was demonstrated that the phenazine only improved the electrochemical activity of *Brevibacillus* if the surfactant rhamnolipids was present, proposing that it was the surfactant that improved the solubility and availability of the phenazine by the Gram-positive bacterium increasing EET.

The addition of specific microbial strains to a bacterial community to enhance microbial electroactivity is not new, and have been used as a strategy to improve microbial elecroactivity. For example, the addition of *Shewanella haliotis* ATCC 49,138 (Raghavulu et al., 2012) and of *P. aeruginosa* (Raghavulu et al., 2013) onto an anodic native microflora was shown to enhance the electrogenic activity of MFCs. In these studies, the augmented MFC presented a stable and higher electrogenic activity throughout the operation given the synergistic interaction in the microbial community, when compared with the fuel cell operated with non-augmented native anodic consortia (Raghavulu et al., 2013). On another study, an increased in electricity generation was observed in a corn stover fed single-chamber MFC when the native mixed consortia was supplemented with a consortium developed to perform saccharification with corn stover at high rate (Wang et al., 2009). Furthermore, power output of co-cultures with Gram-positive *E. faecium* and Gram-negative organisms, increased by 30–70% relative to the single cultures (Read 2010). Rosenbaum et al. (2011) demonstrated that while in a MFC operated with *S. oneidensis*, only lactate could be used as an electron donor source for current production, in the presence *of Lactococcus lactis*, the co-culture was also able to convert glucose into current (Rosenbaum et al., 2011). The electrogenic properties observed for *B. cereus* were used to enhance the power generation of a MFC (Islam et al., 2017). It was demonstrated that *B. cereus* contains electrogenic and anti-methanogetic properties, that promotes the formation of an electroactive biofilm in an anaerobic sludge, and supress methanogenesis. Recently the potential benefits of a combined cultivation of *G. sulfurreducens* and *S. oneidensis* were evaluated, showing that the mixed culture perform better in BES as opposed to the individual pure cultures. It was demonstrated that the planktonic presence of *S. oneidensis* have a positive effect on *G. sulfurreducens*, allowing this organism to form thicker biofilms at the anode surface, which was accompanied with an increased current density (Engel et al., 2019).

All these studies demonstrate that cell-to-cell communication achieved by bioaugmentation not only helps to design interactive microbial communities for specifically targeted biotechnological processes, but also favour synergistic or mutualism relationships that increases microbial electroactivity. Although further studies need to be performed to evaluate how cell interactions improve EET, this strategy seems promising to improve specific BES applications.

4. Future perspectives

Electroactive microorganisms play key roles in the global cycling of carbon and nutrients (i.e. nitrogen and phosphorus), the remediation of environmental contaminants, and various bioenergy strategies (Lovley, 2011b). The potential networks for the dialogues between these microorganisms can be widely used. For instance, the discovery of DIET represented a significant breakthrough in the understanding of anaerobic digestion systems for bioenergy production treating organic waste streams (Rotaru et al., 2012; Summers et al., 2010). Since then, research efforts have been focused on enhancing biogas production, reducing the start-up times, and promoting stability in anaerobic digestion systems (Lovley, 2012; Shrestha and Rotaru, 2016). In microbial electrosynthesis or electro-fermentation, the electron exchange between syntrophic partners occurs typically via diffusible intermediates (mainly H_2) (Chandrasekhar et al., 2021; Puig et al., 2017; Rovira-Alsina et al., 2021), with higher production rates and product titers in respect to direct electron transfer systems (Bian et al., 2020; Dessì et al., 2021).

Electro bioremediation is also a significant niche for electroactive microorganisms (Wang et al., 2020). However, the relevance of dialogues between electroactive microorganisms has been only marginally explored. Aulenta *et al.* showed the potential of anaerobic petroleum hydrocarbon-degrading microbial communities to carry out specific syntrophic and/or cooperative metabolisms of major environmental relevance (Aulenta et al., 2021). Electrobioremediation of petroleum hydrocarbons-contaminated soils was comprehensively analysed in a recent review by (Tucci et al., 2021). Similarly, Vilajeliu-Pons *et al.* characterized a diverse community that integrated members of different functional groups involved in the nitrogen cycle in microbial electricity-driven anoxic ammonium removal systems (Vilajeliu-Pons et al., 2018).

Microbial aggregation could overcome limitations found in electroactive microorganisms which suffer from limited microbial adherence and growth. Novel concepts such as microbial electrochemical fluidized bed reactors or capacitive granules are flowering, enhancing the biological treatment of contaminated water (Caizán-Juanarena et al., 2020; Tejedor-Sanz et al., 2018). Such systems are composed of electroactive microorganisms and electroconductive particles (i.e. activate carbon) for treating wastewater. These are promising sustainable applications of microbial electrochemical technologies.

The underlying driving force to balance metabolic processes likely is strongly interlinked with intraspecies and interspecies communication. Yet, how "energy spill" mechanisms (e.g., electron trade-off mechanisms due to imbalanced metabolism) affect other partners in the consortium has not been widely explored but opens up a myriad of possibilities with potential applications to engineered systems. If this would be a true (one word) electric-dialogue or an advantage market for non-expected commensals would depend on the specificity of the electron transfer mechanisms. We believe that this level of microbial interaction is so essential to electroactive reactions that understanding their language or at least recognizing the mechanisms of microbial communication will be key for any fundamental boosts of electroactivity for new BES applications, especially in complex environmental settings. Sustainable decentralized energy generation but also powerful *in-situ* bioremediation are central environmental tasks of the next decade. Electroactive microorganisms provide key strategic features to tackle these tasks. But we have to move from the lab bench to the complex environmental mixed culture setting and start listening to their interactions.

Although numerous strategies have been applied to improve electron transfer of electroactive for biotechnological applications, most of them relies on engineering modifications, material engineering approaches and biofilm enhancement (reviewed recently in (Angelaalincy et al., 2018; Chiranjeevi and Patil, 2020; Wang et al., 2022; Zhao et al., 2021)), and only a few studies have been dedicated to cell-to-cell communication. Relying on the natural capabilities of microbes to facilitate and enhance EET processes seems to be a promising approach to enhance microbial electroactivity. This however still requires significant investigation to unravel the communication processes by which electroactive organisms chat with each other.

5. Conclusions

The chit-chat between electroactive microorganism has a major environmental relevance. Most of the knowledge gained sofar about EET came from studies of model organisms giving a very narrow mechanistic view on electroactivity. Realizing that these actions are not singular processes but are embedded within a complex process of intra- and interspecies communication and interaction, will be key to utilizing electroactivity in complex environmental settings. A deeper understanding of complex QS regulatory networks is a crucial task for promoting the electroactivity of diverse microbial mixed cultures. Further research should elucidate the exact mechanisms of cell-to-cell interactions in mixed cultures.

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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