Novel Outlook in Microbial Ecology: Nonmutualistic Interspecies Electron Transfer

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Abstract

Recent advances in microbial electrochemical technologies have revealed the existence of numerous and highly diverse microorganisms able to exchange electrons with electrodes. This diversity could reflect the capacity of microorganisms to release and/or retrieve electrons with each other in natural environments. So far, this interspecies electron transfer has been studied with a special focus on syntrophy and was successfully demonstrated for several couples of species. In this article, we argue that electron exchange between microbes exists beyond syntrophy or mutualism and could also promote competitive and even parasitic behaviour. Based on three interesting case studies identified from the literature, we also highlighted that such non-mutualistic interactions could be widespread and of particular significance for the survival of pathogens or the shaping of complex microbial communities.

Keywords

Electromicrobiology, Electroactive microorganisms, Extracellular electron transfer, Ecological interactions, Interspecies energy coupling

Glossary

Conductive nanowires: pili or extension of the outer membrane which is electrically conductive and can be used by microorganisms to physically reach distant terminal electron acceptors.
Electro-fermentation: fermentation process in which polarized electrodes are employed as a driving tool.
Electron shuttles: soluble redox-active compounds (e.g. H_2, flavins) which can be used to reach distant terminal electron acceptors.
Extracellular electron transfer (EET): mechanism that allows electron transfer from a microorganism to an extracellular electron acceptor, or from an extracellular electron donor to a microorganism.
Interspecies electron transfer (IET): mechanism that allows electron transfer between different species of microbes. Transfer can be either direct or mediated by electron shuttles.
Microbial electrochemical technologies (MET): electrochemical processes in which at least one reaction is catalysed by microorganisms.
Standard Hydrogen Electrode (SHE): theoretical redox reference which corresponds to the H^+/H_2 couple under standard conditions (i.e. 25°C and 1 atm).
Terminal electron acceptor (TEA): last electron acceptor of an electron transport chain.

A diversity of microorganisms swapping electrons

Interspecies electron transfer (IET, see Glossary) is essential for the efficient functioning of many microbial communities under anoxic conditions as it is the basis of energy coupling between microbial species [1]. A well-documented example of mutualistic IET is the reducing equivalent exchange through H_2/formate existing between syntrophic fatty acids oxidizers and methanogens in anaerobic digestion [2]. Over the past decades, the development of **microbial electrochemical technologies** (METs) such as microbial fuel cells and microbial electrolysis cells have broadened our understanding of **extracellular electron transfer** (EET) as well as IET mechanisms [3,4]. Indeed, METs have made possible to quantitatively measure and characterize EET between so called electroactive microorganisms and electrodes, the latter acting as artificial electron acceptor or donor. Efforts to improve METs performances have steered the basic research towards mechanistic aspects of EET. In particular, *Shewanella oneidensis* and *Geobacter sulfurreducens* have been extensively studied for their ability to efficiently use anodes as electrons acceptors, leading to the discovery of new direct (*e.g.* through **conductive nanowires** or cell contact) and mediated (*e.g.* through secreted flavins, quinones, phenazines) EET pathways. For more detailed description of EET pathways, readers are invited to read excellent and recent reviews [5–8].

Although there is no consensus on the criteria used to classify microorganisms as electroactive [9], a first simplistic definition could be that electroactive species are those able to exchange electrons with abiotic electrodes. Following this definition, it has been shown that such electroactive species are highly diverse, either in terms of phylogeny, habitat or metabolism, and are not restricted to a specific ecological niche [10–12]. In a review published in 2016, Koch and Harnisch had already identified 94 microbial species from the literature as being able to interact either with anodes, cathodes or both [10], and many more microbial species are expected to be discovered in the future since EET ability can easily be evaluated with METs. These identified electroactive species belong to many different phyla encompassing the three domains of life (Bacteria, Archaea and Eukarya) such as *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Deferribacteres*, *Firmicutes*, *Proteobacteria*, *Euryarchaeota, Ascomycota and Chlorophyta* [10,12,13]. Such diversity raises the question of why electroactivity is so phylogenetically dispersed and widespread over a wide range of environments even though electrodes *per se* are not present in natural environments? Possible natural electrode analogues could be solid minerals such as iron oxides since they are widespread on earth as iron represents the fourth most abundant element on the Earth's crust. For instance, Fe(III) oxide is a common electron acceptor for iron-reducing bacteria such as bacteria belonging to the *Geobacter* genus. However, Rotaru *et al.* (2015) have shown that the effectiveness for Fe(III) reduction was poorly linked to the ability for current production among eight *Geobacter* species. Interestingly, the best current producers in this study were found to be the *Geobacter* species able to perform direct IET (DIET) with *Methanosarcina barkeri* [14]. This study might be a hint that microorganisms could be plausible anode equivalents (*i.e.* electron sink) in natural environments and that respiration or electron dissipation through IET might constitute a widespread natural process. Indeed, experimental evidences that Fe(III) oxide can serve as electron acceptor have overlooked that microbes can indeed provide even more frequent and widespread electron acceptors in the environment.

The range of opportunities for electron sharing reflects simply a struggle for life

Not considering the well-known mutualistic H_2/formate IET (see Box 1), only few co-cultures experiments have been carried out so far to demonstrate electron exchange between microorganisms, either directly (*i.e.* through physical contacts) or indirectly (*i.e.* through soluble **electron shuttles**). The most studied electron-donating microorganisms are *Geobacter* species while various microbial partners act as electron sinks, such as *Wolinella succinogenes*, *Thiobacillus denitrificans*, *Methanotrix harundinacea*, *Methanosarcina barkeri, Methanosarcina horonobensis, Geobacter sulfurreducens* or *Prosthecochloris aestaurii* (see Table 1). In those experiments, *Geobacter* species were grown with their appropriate electron donor but no soluble electron acceptor (except, in some cases, an electron shuttle) and reciprocally the electron accepting microbe was grown with its favourite electron acceptor but no soluble electron donor. This experimental design can only promote syntrophy, an "obligately mutualistic metabolism" in which both partners cooperate on a metabolic level for the benefice of the two [15]. Thus, it is logical that most of studies focusing on IET have concluded that it promotes syntrophy because they were designed on purpose. However, there is no particular reason for IET-based interactions to be restrained to syntrophy or mutualism and other ecological interactions could be stimulated in co-cultures, if at least one of the two species was not dependent on the other.

Table 1. Defined co-cultures demonstrating non-H_2/formate dependant interspecies electron transfer

Electron donating microorganism	Electron donor	Extracellular electron carrier	Electron accepting microorganism	Terminal electron acceptor	Ref
Geobacter metallireducens	Acetate	AQDS	*Wolinella succinogenes*	Fumarate	[37]
	Ethanol	AQDS or DIET	*Geobacter sulfurreducens*	Fumarate	[38–40]
		DIET	*Methanosarcina barkeri*	CO_2	[41,42]
		DIET	*Methanosarcina horonobensis*	CO_2	[41]
		DIET	*Methanotrix harundinacea*	CO_2	[43]
Geobacter hydrogenophilus	Ethanol	putative DIET	*Methanosarcina barkeri*	CO_2	[14]
Geobacter sulfurreducens	Acetate	conductive minerals	*Thiobacillus denitrificans*	Nitrate	[44]
		unknown or Cysteine	*Wolinella succinogenes*	Nitrate	[45–47]
		unknown	*Desulfovibrio desulfuricans*	Nitrate	[45]
		DIET	*Prosthecochloris aestaurii*	CO_2	[48]
		unknown	*Clostridium pasteurianum*	Glycerol	[22]
Pseudomonas aeruginosa	Formate	DIET/H_2	*Geobacter sulfurreducens*	Fumarate	[49]

AQDS: Anthraquinone-2,6-disulfonate; DIET: Direct interspecies electron transfer

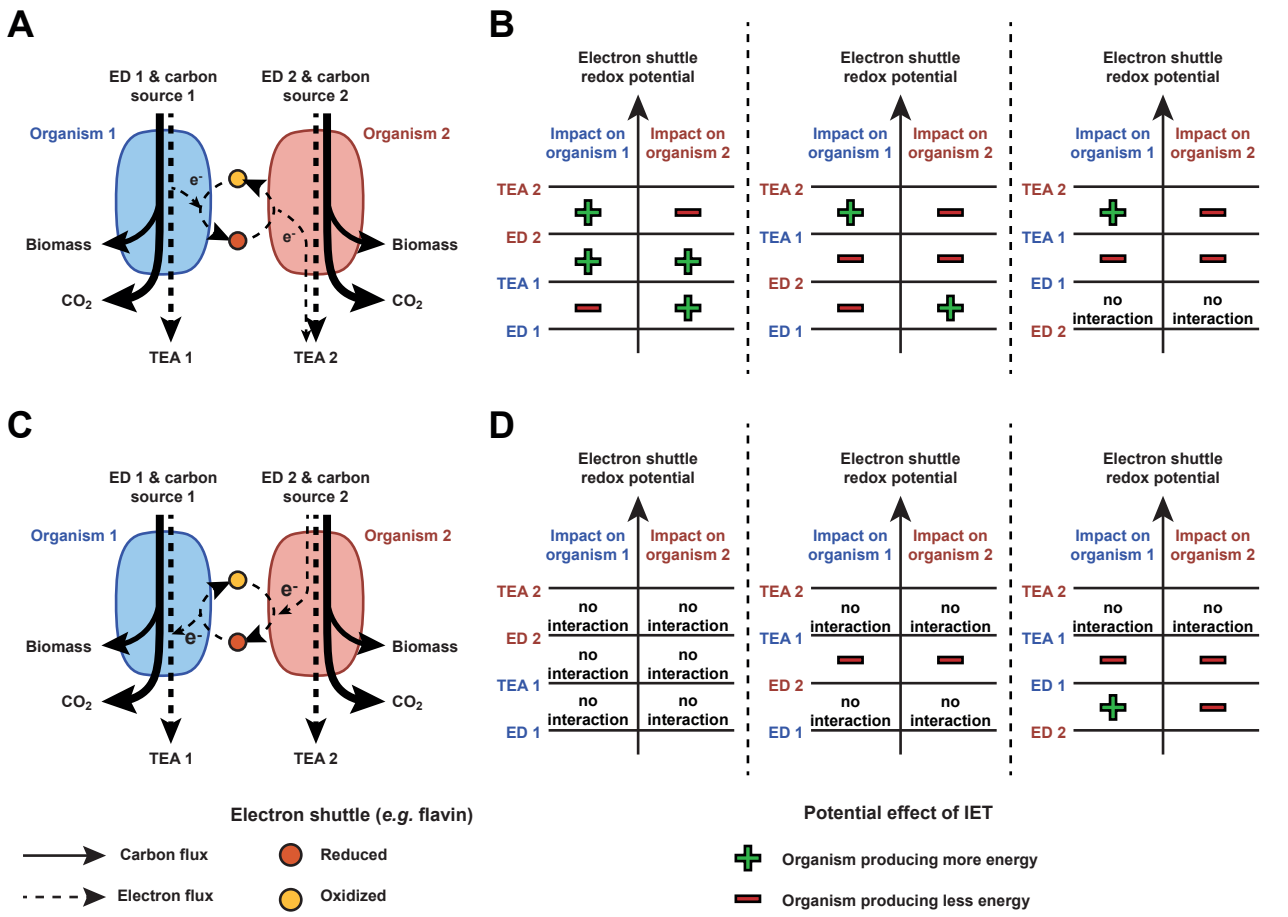

Figure 1. Effect of IET between two heterotrophs able of respiration on their energic metabolism. Without loss of generality, organism 2 is arbitrarily considered to have the highest TEA redox potential. (A-B) Organism 2 is the electron-accepting organism. (C-D) Organism 2 is the electron-donating organism. Potential effects of IET are determined under the assumption that both organisms 1 and 2 have access to their respective carbon source, electron donor and electron acceptor. Moreover, phenomena such as overpotentials and electron bifurcation are not considered and all interactions presented in (B) and (D) correspond to "energetic mutualism", as defined in Moscoviz *et al.* (2017) [20]. IET between the two species is represented as mediated (MIET) by an electron shuttle but could as well be direct (DIET). ED: Electron donor; TEA: Terminal electron acceptor.

To better picture the diversity of situations beyond mutualistic IET, a simplified analysis is provided in Figure 1 (Key Figure). It is focused on the energetic metabolism of two heterotrophs having access to their respective electron donors, electron acceptors and carbon sources while interacting trough IET. This analysis highlights the type of interaction between the two organisms, depending directly on the relative redox potentials of their pairs of electron donors and electron acceptors, as well as the redox potential at which electrons are exchanged. It also indicates that, mutualism can only occur under very specific and restrictive conditions while most situations would lead to parasitic behaviours (see Figure 1B-D). Moreover, the organism having the higher redox potential **terminal electron acceptor** (TEA) is more likely to be the electron-accepting organism, due to the natural flow of

electrons from lower to higher redox potentials (see Figure 1D). In addition to this theoretical analysis, it is possible to define more general prerequisites for non-mutualistic IET, as follows:

(i) The existence of a negatively impacted organism implies that this organism must be capable of producing energy independently from IET. Otherwise, this organism would depend on the IET partner for its energetic metabolism and therefore benefit from IET.
(ii) An electron-accepting organism can be negatively impacted by IET only in case of TEA shortage or if the electron transport chain is congested. Otherwise, extra electrons coming from the electron-donating organism could hitchhike the electron transport chain of the electron-accepting organism without harm.
(iii) Conversely, an electron-donating organism can be negatively impacted by IET only if the electron donor is limiting or the electron transport chain is congested.
(iv) An IET partner can grow better only if it is able to exploit the new redox gradient offered by IET. That is to say, IET for this organism must be coupled to ATP production, directly (*e.g.* using proton translocation) or indirectly (*e.g.* metabolic shift).

Naturally, these considerations would need to be adapted for autotrophic or fermentative organisms. To better illustrate in which circumstances these conditions could be achieved, but also to propose avenues for further research, a careful reading of the literature was carried out seeking for experimental results which could be reinterpreted in the light of non-mutualistic IET.

Experimental hints for non-mutualistic IET

A first observation supporting the existence of such interaction is the decreased growth yield of several fermentative species grown in contact with a cathode (*i.e.* cathodic **electro-fermentation** [16]). In such a process, extracellular electrons are supplied to fermentative species by a cathode using either mediators, such as cobalt-based complexes [17,18], or through direct electron transfer [19]. The primary aim of such process is to shift fermentation patterns toward more reduced products. However, it was observed as a side effect, and for several species such as *Propionibacterium freudenreichii* [17], *Clostridium autoethanogenum* [18] or *Clostridium pasteurianum* [19], reduced growth yields between -14 and -41 % when compared to control fermentations without electrode. A thermodynamical analysis of these electro-fermentation experiments revealed that two main factors could explain these reduced growth yields [20]: (i) less energy produced explained by direct contribution of reducing equivalents to fermentation metabolism (*e.g.* shift from Eq. 5 to Eq. 9; Table II from Box 1), (ii) a shift in metabolism due to biological regulations triggered by EET that would end up with a lower ATP yield for the catabolic reaction (*e.g.* shift from Eq. 5 to Eq. 7; Table II from Box 1) and/or with a higher energy dissipation in the cell linked with maintenance, additional energy expenditure in new pathways, etc. Moreover, a recent electro-fermentation experiment has provided an example showing how an electron flow can be forced into *Escherichia coli* [21]. In this experiment, electrons are provided by a cathode to neutral red, a low potential synthetic phenazine (E°' = -325 mV *vs* **SHE** [21]). Reduced neutral red can passively cross cell membranes and was found to reduce menaquinone in the inner membrane. Redox homeostasis for the menaquinone pool could be maintained if a TEA (*e.g.* nitrate) was present in the environment. Otherwise, menaquinol build-up would trigger the *arcB* redox-sensing cascade, resulting in altered metabolite profiles. Taken together, these results evidenced that some fermentative organisms can serve as potential electron sinks in natural environments, regardless of whether accepting extracellular electrons are beneficial or detrimental to their growth. To investigate

if the same behaviour could be triggered by IET without the need of an electrode, a co-culture of *G. sulfurreducens* and *C. pasteurianum* was recently carried out by our team [22]. In this experiment, *Geobacter* was supplied with acetate as electron donor but no soluble electron acceptor while *Clostridium* could ferment glycerol. As a result, a significant growth of *Geobacter* was measured in parallel with a reduced growth yield and a fermentation shift of *Clostridium,* similar to what was obtained during electro-fermentation [19] (see Figure 1). Additional controls confirmed that no electron acceptor in the co-culture medium or within *Clostridium* fermentation end-products could sustain *Geobacter* growth. This result indicates that *Geobacter* could use electron carriers from *Clostridium* as TEA to sustain its growth, although additional experiments are required to characterize the exact molecular mechanisms involved in this interaction.

Another hint of non-mutualistic interaction based on IET is the production of phenazines by *Pseudomonas aeruginosa*, a facultative anaerobe with limited fermentative capacities. Phenazines such as pyocyanin, phenazine-1-carboxylic acid or phenazine-1-carboxamide are redox-active compounds which have been extensively studied and play multiple roles in *P. aeruginosa* metabolism [23]. In particular, under oxygen and nitrate limitations (the normal TEA for its respiration), it has been shown that *P. aeruginosa* could improve its viability by secreting and using phenazines as carrier of EET to reach a distant oxygen gradient [24] or an anode [25]. Indeed, although *P. aeruginosa* cannot ferment glucose, it is able to oxidize glucose to acetate while reducing phenazines to maintain intracellular redox homeostasis and to produce energy for its survival [26]. Besides, some phenazines such as pyocyanin are known for their broad-spectrum antibiotic properties related to their redox activity [23,27]. Pyocyanin is a soluble compound with a relatively high redox potential ($E^{\circ\prime}$ = -32 mV vs SHE [28]), which can freely cross cell membranes [26]. Pyocyanin antibiotic activity is often associated with oxidative stress due to its high reactivity with O_2 [23,29]. However, few studies have demonstrated that antibiotic effect could also be observed under anaerobiosis [27,30] and highlighted other mechanisms such as electron transport chain shortcut (*i.e.* aerobic and anaerobic respiration inhibition) and inhibition of active transport of solutes across cell membrane [27]. In particular, an inhibitory effect of pyocyanin on bacterial growth was observed for species such as *Bacillus lichenformis* and *E. coli* under fermentative conditions [27]. These results suggest that pyocyanin can interact with a wide variety of microorganisms and intracellular redox active species, independently from the presence of oxygen or active electron transport chain. Thus, it seems plausible that under shortage in electron acceptor, *P. aeruginosa* could use pyocyanin for its survival extension to indirectly respire intracellular redox species of surrounding cells, including bacteria but also host cells in case of human infection. Since accepting electron coming from pyocyanin is detrimental for the metabolism of a wide array of organisms, such an interaction can be regarded as an IET-mediated facultative parasitism.

Finally, another potential case of non-mutualistic IET was recently found in gut microbiome where Light *et al*. (2018) showed that *Listeria monocytogenes*, a fermentative microbe and severe food-borne pathogen, was capable of EET through the reduction of exogenous flavins (*i.e.* mediated EET) using a newly discovered electron transport pathway [31]. Interestingly, the authors demonstrated that flavin-based respiration granted this species a better persistence capacity in the gut. The gut constitutes an interesting habitat where microbial species compete in a plug-flow system, with the fittest species that will survive and with the least adapted species that will gradually decline in abundance or even be washed-out from the system. Indeed, they observed that the abundance of a EET-deficient mutant was decreased six-fold during the intestinal colonization of mice when compared to the wild-type strain, even though fermentation substrates were available in the

environment [31]. One explanation for this observation could be that glucose oxidation coupled to flavin reduction is energetically more favourable than glucose fermentation (see Eq. 8 in Table II from Box 1). However, this advantage cannot self-sustain, but still relies on the oxidation of flavins in interaction with a TEA. So far, this TEA remains unknown and it has been hypothesized that it could be oxygen, nitrate or ferric iron [32,33]. However, these TEA may be scarce in a highly competitive environment such as the mammalian gut. Alternatively, these reduced flavins could also be oxidized by other surrounding bacteria, as hypothesized by Cahoon and Freitag (2018), or by the epithelium of the host. In those cases, thermodynamics predicts that fermentative bacteria or epithelial cells accepting electrons from these flavins would be negatively impacted regarding the energy yield of their catabolism (see Box 1) [20]. Bacteria capable of flavin respiration in the gut would therefore benefit both from an additional energy supply (*e.g.* flavin respiration) and from a chemical warfare (*e.g.* decreased growth rate of competing bacteria), which would constitute a competitive advantage offered by EET.

Significance of non-mutualistic IET for microbial ecology and biotechnology

Although these theoretical considerations and examples are not formal demonstration of IET, they still highlight that non-mutualistic IET is a plausible phenomenon and deserves more in-depth investigation. In the cases of *G. sulfurreducens* and *P. aeruginosa*, the interaction could be an illustration of facultative parasitism as the two species would not rely exclusively on their parasitic activity. In the absence of usual TEA (*i.e.* metals for *G. sulfurreducens* and O_2/nitrate for *P. aeruginosa*), their parasitic behaviour would constitute a survival strategy. Indeed, under prolonged TEA limitation, *G. sulfurreducens* represses anabolism and increases its number of cytochromes [34], while *P. aeruginosa* resorts to the costly synthesis of phenazines [35]. On the contrary, regarding *L. monocytogenes*, EET is probably not involved in survival because, unlike *G. sulfurreducens* or *P. aeruginosa*, this fermentative species does not exclusively rely on external electron acceptors for its growth. In this case, flavin respiration would constitute a valid growth strategy, granting a competitive advantage over fermentative species for abundant resources in the gut environment such as sugars.

Accordingly, investigating non-mutualistic IET would open new perspectives regarding the persistence of pathogens during human infection, the microbial community dynamics in the gut, but also the understanding of microbial interactions in diverse natural and synthetic environments. Indeed, many microorganisms other than *P. aeruginosa* are known phenazine producers and can be found in various environments such as the rhizosphere or in contact with mammals [23,35]. Similarly, Light *et al.* (2018) found that orthologues of the genes responsible for EET in *L. monocytogenes* were present in hundreds of species in the Firmicutes phylum, including many lactic acid bacteria of industrial interest [31]. From a biotechnological point of view, bioaugmentation or selection of microbes able of parasitic behaviour through IET could also lead to interesting improvements of industrial fermentations. For instance, it could be possible to design a fermentation process involving a fermentative species and a parasite able to consume undesired fermentation by-products as electron donor (see Figure 2A). The parasite would produce CO_2 while electrons would flow back to the fermentative species. In such process, less carbon would be diverted into bacterial biomass, more reduced products of interest would be produced, and a first *in-situ* product purification would occur, reducing costly downstream processing steps. The addition of redox mediators in open mixed-culture

processes could also be a strategy to favour species of interest within a complex microbial community (see Figure 2B).

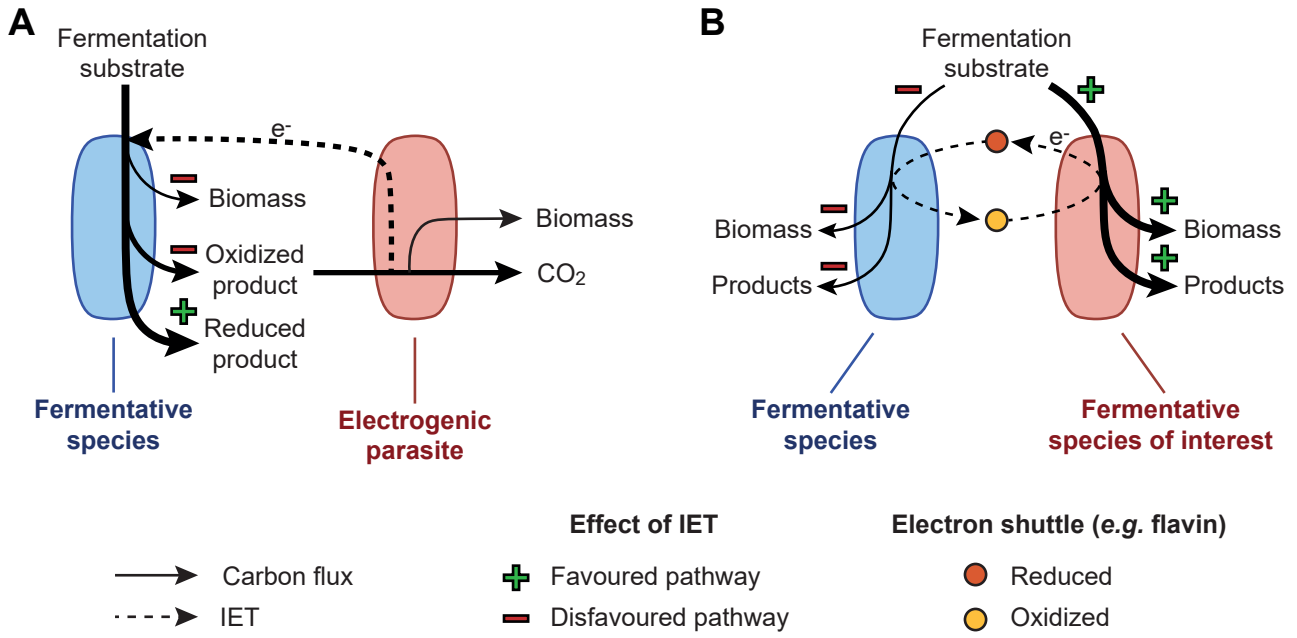

Figure 2. Possible fermentation processes benefiting from non-mutualistic interspecies electron transfer (IET). (A) Electron recycling: A fermentative species serves as terminal electron acceptor for a parasite able to oxidize fermentation by-products. A reduced product is then produced at a higher yield and selectivity in co-culture when compared to a pure culture. (B) Increased competitiveness: A fermentative species of interest having an extracellular electron transfer pathway is favoured by the addition of a redox shuttle (*e.g.* flavins). Other species competing for the fermentation substrate are disfavoured when accepting the electrons released by the species of interest, leading to the predominance of the latter.

Concluding remarks

Significant research efforts are still required to better characterize the potential cases of non-mutualistic IET (see Outstanding Questions). Such interactions may have a strong impact on microbial community structures while being extremely difficult to detect in diverse bacterial populations. Expanding our understanding of electron-accepting mechanisms [4,21,36] could help developing new bioprocesses as well as molecular markers of IET (*e.g.* specific genes, flavins), thus making possible the quantitative study of this phenomenon in complex microbial communities. This research is multi-disciplinary by essence and would require the combined efforts of researchers from diverse fields such as bioelectrochemistry, environmental microbiology, molecular biology or medical microbiology.

References

1 Lovley, D.R. (2017) Happy together: microbial communities that hook up to swap electrons. *ISME J.* 11, 327–336

2 Sieber, J.R. *et al.* (2012) Genomic insights into syntrophy: the paradigm for anaerobic metabolic cooperation. *Annu. Rev. Microbiol.* 66, 429–452

3 Costa, N.L. *et al.* (2018) Electron transfer process in microbial electrochemical technologies: The role of cell-surface exposed conductive proteins. *Bioresour. Technol.* 255, 308–317

4 Choi, O. and Sang, B.-I. (2016) Extracellular electron transfer from cathode to microbes: application for biofuel production. *Biotechnol. Biofuels* 9, 11

5 Pankratova, G. *et al.* (2019) Extracellular electron transfer features of Gram-positive bacteria. *Anal. Chim. Acta* 1076, 32–47

6 Liu, X. *et al.* (2018) Microbial electrocatalysis: Redox mediators responsible for extracellular electron transfer. *Biotechnol. Adv.* 36, 1815–1827

7 Reguera, G. (2018) Harnessing the power of microbial nanowires. *Microb. Biotechnol.* 11, 979–994

8 Kumar, A. *et al.* (2017) The ins and outs of microorganism–electrode electron transfer reactions. *Nat. Rev. Chem.* 1, 0024

9 Koch, C. and Harnisch, F. (2016) What is the essence of microbial electroactivity? *Front. Microbiol.* 7, 1890

10 Koch, C. and Harnisch, F. (2016) Is there a specific ecological niche for electroactive microorganisms? *ChemElectroChem* 3, 1282–1295

11 Doyle, L.E. and Marsili, E. (2018) Weak electricigens: A new avenue for bioelectrochemical research. *Bioresour. Technol.* 258, 354–364

12 Logan, B.E. *et al.* (2019) Electroactive microorganisms in bioelectrochemical systems. *Nat. Rev. Microbiol.* 17, 307–319

13 McCormick, A.J. *et al.* (2015) Biophotovoltaics: oxygenic photosynthetic organisms in the world of bioelectrochemical systems. *Energy Environ. Sci.* 8, 1092–1109

14 Rotaru, A.-E. *et al.* (2015) Link between capacity for current production and syntrophic growth in *Geobacter* species. *Front. Microbiol.* 6, 744

15 Morris, B.E.L. *et al.* (2013) Microbial syntrophy: interaction for the common good. *FEMS Microbiol. Rev.* 37, 384–406

16 Moscoviz, R. *et al.* (2016) Electro-fermentation: How to drive fermentation using electrochemical systems. *Trends Biotechnol.* 34, 856–865

17 Emde, R. and Schink, B. (1990) Enhanced propionate formation by *Propionibacterium freudenreichii subsp. freudenreichii* in a three-electrode amperometric culture system. *Appl. Environ. Microbiol.* 56, 2771–2776

18 Kracke, F. *et al.* (2016) Redox dependent metabolic shift in *Clostridium autoethanogenum* by extracellular electron supply. *Biotechnol. Biofuels* 9,

19 Choi, O. *et al.* (2014) Electricity-driven metabolic shift through direct electron uptake by electroactive heterotroph *Clostridium pasteurianum*. *Sci. Rep.* 4, 6961

20 Moscoviz, R. *et al.* (2017) Revealing extracellular electron transfer mediated parasitism: energetic considerations. *Sci. Rep.* 7, 7766

21 Harrington, T.D. *et al.* (2015) The mechanism of neutral red-mediated microbial electrosynthesis in *Escherichia coli*: menaquinone reduction. *Bioresour. Technol.* 192, 689–695

22 Moscoviz, R. *et al.* (2017) Cooperative growth of *Geobacter sulfurreducens* and *Clostridium pasteurianum* with subsequent metabolic shift in glycerol fermentation. *Sci. Rep.* 7, 44334

23 Pierson, L.S. and Pierson, E.A. (2010) Metabolism and function of phenazines in bacteria: impacts on the behavior of bacteria in the environment and biotechnological processes. *Appl. Microbiol. Biotechnol.* 86, 1659–1670
24 Price-Whelan, A. *et al.* (2007) Pyocyanin alters redox homeostasis and carbon flux through central metabolic pathways in *Pseudomonas aeruginosa* PA14. *J. Bacteriol.* 189, 6372–6381
25 Wang, Y. *et al.* (2010) Endogenous phenazine antibiotics promote anaerobic survival of *Pseudomonas aeruginosa* via extracellular electron transfer. *J. Bacteriol.* 192, 365–369
26 Glasser, N.R. *et al.* (2014) Phenazine redox cycling enhances anaerobic survival in *Pseudomonas aeruginosa* by facilitating generation of ATP and a proton-motive force. *Mol. Microbiol.* 92, 399–412
27 Baron, S.S. *et al.* (1989) Molecular mechanism of the antimicrobial action of pyocyanin. *Curr. Microbiol.* 18, 223–230
28 Kracke, F. *et al.* (2015) Microbial electron transport and energy conservation - the foundation for optimizing bioelectrochemical systems. *Front. Microbiol.* 6, 575
29 Perry, E.K. and Newman, D.K. (2019) The transcription factors ActR and SoxR differentially affect the phenazine tolerance of *Agrobacterium tumefaciens*. *Mol. Microbiol.* 112, 199–218
30 Baron, S.S. and Rowe, J.J. (1981) Antibiotic action of pyocyanin. *Antimicrob. Agents Chemother.* 20, 814–820
31 Light, S.H. *et al.* (2018) A flavin-based extracellular electron transfer mechanism in diverse Gram-positive bacteria. *Nature* 562, 140–144
32 Saunders, S.H. and Newman, D.K. (2018) Extracellular electron transfer transcends microbe-mineral interactions. *Cell Host Microbe* 24, 611–613
33 Cahoon, L.A. and Freitag, N.E. (2018) The electrifying energy of gut microbes. *Nature* 562, 43
34 Bansal, R. *et al.* (2013) Survival during long-term starvation: Global proteomics analysis of *Geobacter sulfurreducens* under prolonged electron-acceptor limitation. *J. Proteome Res.* 12, 4316–4326
35 Price-Whelan, A. *et al.* (2006) Rethinking “secondary” metabolism: physiological roles for phenazine antibiotics. *Nat. Chem. Biol.* 2, 71–78
36 Holmes, D.E. *et al.* (2018) Electron and proton flux for carbon dioxide reduction in *Methanosarcina barkeri* during direct interspecies electron transfer. *Front. Microbiol.* 9, 3109
37 Lovley, D.R. *et al.* (1999) Humics as an electron donor for anaerobic respiration. *Environ. Microbiol.* 1, 89–98
38 Summers, Z.M. *et al.* (2010) Direct exchange of electrons within aggregates of an evolved syntrophic coculture of anaerobic bacteria. *Science* 330, 1413–1415
39 Smith, J.A. *et al.* (2015) Syntrophic growth via quinone-mediated interspecies electron transfer. *Front. Microbiol.* 6,
40 Liu, F. *et al.* (2012) Promoting direct interspecies electron transfer with activated carbon. *Energy Environ. Sci.* 5, 8982
41 Yee, M.O. *et al.* (2019) Extracellular electron uptake by two *Methanosarcina* species. *Front. Energy Res.* 7, 29
42 Rotaru, A.-E. *et al.* (2014) Direct interspecies electron transfer between *Geobacter metallireducens* and *Methanosarcina barkeri*. *Appl. Environ. Microbiol.* 80, 4599–4605
43 Rotaru, A.-E. *et al.* (2014) A new model for electron flow during anaerobic digestion: direct interspecies electron transfer to *Methanosaeta* for the reduction of carbon dioxide to methane. *Energy Environ. Sci.* 7, 408–415

44 Kato, S. *et al.* (2012) Microbial interspecies electron transfer via electric currents through conductive minerals. *Proc. Natl. Acad. Sci.* 109, 10042–10046
45 Cord-Ruwisch, R. *et al.* (1998) Growth of *Geobacter sulfurreducens* with acetate in syntrophic cooperation with hydrogen-oxidizing anaerobic partners. *Appl. Environ. Microbiol.* 64, 2232–2236
46 Galushko, A.S. and Schink, B. (2000) Oxidation of acetate through reactions of the citric acid cycle by *Geobacter sulfurreducens* in pure culture and in syntrophic coculture. *Arch. Microbiol.* 174, 314–321
47 Kaden, J. *et al.* (2002) Cysteine-mediated electron transfer in syntrophic acetate oxidation by cocultures of *Geobacter sulfurreducens* and *Wolinella succinogenes*. *Arch. Microbiol.* 178, 53–58
48 Ha, P.T. *et al.* (2017) Syntrophic anaerobic photosynthesis via direct interspecies electron transfer. *Nat. Commun.* 8, 13924
49 Semenec, L. *et al.* (2018) Deciphering the electric code of *Geobacter sulfurreducens* in cocultures with *Pseudomonas aeruginosa* via SWATH-MS proteomics. *Bioelectrochemistry Amst. Neth.* 119, 150–160
50 Kleerebezem, R. and Van Loosdrecht, M.C.M. (2010) A Generalized Method for Thermodynamic State Analysis of Environmental Systems. *Crit. Rev. Environ. Sci. Technol.* 40, 1–54

Box 1: Thermodynamics of mediated IET

Syntrophic oxidation of volatile fatty acids such as butyrate, propionate or acetate is an essential process of the anaerobic degradation of organic matter [2]. During this process, fatty acids fermenting bacteria cooperate with hydrogenotrophic methanogenic archaea for the conversion of fatty acids to methane. The small amount of energy available in the chemical reactions forces the microorganisms to keep the shuttling intermediate (H_2 or formate) concentration low to ensure an efficient cooperation. For example, butyrate oxidation to acetate and hydrogen is thermodynamically unfavourable with a Gibbs free energy change of 48.2 $kJ.mol^{-1}$ under standard conditions corrected for T = 298 K and pH = 7, but becomes favourable when $[H_2]_{aq}$ concentration is low (see Table I).

Conversely, the anaerobic fermentation of sugars such as glucose is highly thermodynamically favourable (see equations 3-7 in Table II). Flavin respiration coupled to glucose oxidation represents an even more favourable pathway as shown by the calculation of its Gibbs free energy change (equation 8 in Table II). However, the Gibbs free energy change of this reaction depends on the flavin redox state and decreases as flavin mononucleotide (FMN) is consumed and $FMNH_2$ accumulates. It reaches values close to those calculated for fermentations when $[FMNH_2]$ / [FMN] = 300. Flavin respiration thus becomes less favourable under conditions where $FMNH_2$ accumulates and its oxidation back to FMN is mandatory to maintain high energy yields. An example of potential $FMNH_2$ recycling pathway coupled with glucose consumption and propionate production is given by equation 9 in Table II. While this recycling reaction is highly favourable, it remains less energetic than the fermentation producing propionate and acetate from glucose (about 10% less energy, see equation 5 in Table II). Thus, from a competition point of view, microorganisms carrying out this alternative fermentation pathway coupled with $FMNH_2$ oxidation would likely have a decreased growth yield and be rapidly outcompeted by flavin reducers for glucose consumption. Equation 10 and 11

illustrate the switch from glucose oxidation to $FMNH_2$ oxidation for epithelial cells respiring O_2. This switch in substrate would negatively impact the energy yield (calculated here for 6 mol of O_2) but would still allow the production of a large amount of energy for the cell.

Table I: Thermodynamics of butyrate syntrophic oxidation.

Reaction	$\Delta G°'$ ($kJ.mol^{-1}$)*	ΔG ($kJ.mol^{-1}$)**	Eq.
$butyrate^- + 2\ H_2O \rightarrow 2\ acetate^- + 2\ H_2 + H^+$	48.2	-25.9	1
$4\ H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3\ H_2O$	-135.5	-21.4	2

* $\Delta G°'$ was calculated for T = 298 K and pH = 7.

** ΔG was calculated for T = 298 K, pH = 7, $[H_2]_{aq} = 10^{-5}$ $mol.L^{-1}$ and C = 10^{-3} $mol.L^{-1}$ for other compounds. Gibbs free energy values were derived from [50].

Table II: Thermodynamics of various types of glucose fermentations or of redox reactions with flavin or oxygen.

Reaction	$\Delta G°'$ ($kJ.mol^{-1}$)*	ΔG ($kJ.mol^{-1}$)**	Eq.
$glucose + 2\ H_2O \rightarrow butyrate^- + 2\ HCO_3^- + 2\ H_2 + 3\ H^+$	-254.6	-345.9	3
$glucose \rightarrow 2\ lactate^- + 2\ H^+$	-196.8	-214.0	4
$glucose \rightarrow 4/3\ propionate^- + 2/3\ acetate^- + 2/3\ HCO_3^- + 8/3\ H^+$	-308.3	-336.8	5
$glucose + 4\ H_2O \rightarrow 2\ acetate^- + 2\ HCO_3^- + 4\ H_2 + 4\ H^+$	-206.4	-371.8	6
$glucose + 2\ H_2O \rightarrow 2\ ethanol + 2\ HCO_3^- + 2\ H^+$	-225.7	-277.0	7
$glucose + 12\ FMN + 12\ H_2O \rightarrow 6\ HCO_3^- + 12\ FMNH_2 + 6\ H^+$	-455.9	-541.4	8
$glucose + 2\ FMNH_2 \rightarrow 2\ propionate^- + 2\ FMN + 2\ H_2O + 2\ H^+$	-282.8	-300.0	9
$glucose + 6\ O_2 \rightarrow 6\ HCO_3^- + 6\ H^+$	-2843.8	-2807.4	10
$12\ FMNH_2 + 6\ O_2 \rightarrow 12\ FMN + 12\ H_2O$	-2387.9	-2265.9	11

* $\Delta G°'$ was calculated for T = 298 K and pH = 7.

** ΔG was calculated for T = 298 K, pH = 7, $[H_2]_{aq} = 10^{-5}$ $mol.L^{-1}$, $[O_2]_{aq} = 2.7 \cdot 10^{-4}$ $mol.L^{-1}$ and C = 10^{-3} $mol.L^{-1}$ for other compounds. Gibbs free energy values were derived from [6] and [50].