Microbes trading electricity in consortia of environmental and biotechnological significance

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1. Abstract

Favorable interspecies associations prevail in natural microbial assemblages. Some of these favorable associations are cometabolic dependent partnerships in which extracellular electrons are exchanged between species. For such electron exchange to occur, the cells must exhibit electroactive interfaces and get involved in direct cell-to-cell contact (**D**irect **I**nterspecies **E**lectron **T**ransfer/**DIET**) or use available conductive mineral grains from their environment (**C**onductive-particle-mediated **I**nterspecies **E**lectron **T**ransfer/**CIET**). This review will highlight recent discoveries and knowledge gaps regarding DIET and CIET interspecies associations in artificial co-cultures and consortia from natural and man-made environments and emphasize approaches to validate DIET and CIET. Additionally, we acknowledge the initiation of a movement towards applying electric syntrophies in biotechnology, bioremediation and geoengineering for natural attenuation of toxic compounds. Next, we have highlighted the urgent research needs that must be met to develop such technologies.

2. Introduction

We live on a microbial planet—a planet where microbes control the distribution of nearly all life's essential elements. Recent estimates place prokaryotes as the second most abundant organisms of all Earth's biomass (bacteria ≈12.7% and archaea ≈1.3% of ≈550 Gt bound-C). Although largely surpassed by plants in terrestrial environments, prokaryotes dominate the subterrestrial (90%) and oceanic realms (70%)[1].

Prokaryotes do not live in isolation and typically establish associations between species or with eukaryotes in the environment. Interactions between microbial species could be favorable, like mutualism, or unfavorable, like competition. Favorable interspecies associations based on cross-feeding prevail in natural microbial assemblages, as shown by a thorough survey of 800 microbial communities [2]. During favorable interspecies associations, prokaryotes synchronize their activity and growth via an array of information exchange strategies like quorum sensing, membrane vesicles, intercellular junctions, or intercellular membrane nanotubes [3]. Remarkably, the exchange of cellular material between species can even implicate the entire cytoplasm leading to hybrid cells that can reproduce – a possible unexplored driver of evolutionary diversification. The later has been recently investigated in a *Clostridium ljungdahlii* and *Clostridium acetobutylicum* co-culture, which exchanged RNA and proteins [4].

Favorable interactions between prokaryotes involve the exchange of cell material (**Fig.1)** including:

1. exchange of small metabolites (e.g., H_2, formate)
2. exchange of electrons (e.g., via shuttles, conductive materials, or native redox-active cell-surface molecules) and
3. exchange of other small molecules and cytoplasmic material (e.g., iron, vitamins, amino acids, antibiotic resistance proteins).

The first two are also known as syntrophy or metabolic cross-feeding - a cooperative interaction in which two species, (A) a syntroph/electron-donating species and (B) an electron-accepting partner, survive environmental conditions that would benefit neither species alone. In environments without soluble electron acceptors, syntrophs carry out energetically unfavorable reactions, like organic matter oxidation, by releasing reducing equivalents outside of the cell as electrons or small metabolites. These are scavenged by the accepting partner and used as electron

donors for their metabolism (**Fig. 1**). Without their partner, syntrophs experience catabolite repression. Without the syntroph, the electron-accepting partner experiences famine. Thus, only together, they prevail.

Syntrophic interactions via diffusible chemicals (H_2 or formate) or mediated by electron shuttle molecules (e.g., cysteine, flavin, quinones) have been described elsewhere [5–7].

In this review, we will focus on "electric" syntrophy, established either by relying on direct cell-to-cell electrical contacts (DIET – Direct Interspecies Electron Transfer) or mediated by electrically conductive materials (CIET – Conductive-particle-mediated Interspecies Electron Transfer) in artificial co-cultures and consortia from natural and manmade environments. A timeline of the discoveries in this research field is highlighted in *Figure 2*. We will highlight methods to validate DIET and CIET in such environments. Finally, we will provide a list of open questions regarding the ecology of electric syntrophies and their role in future technology applications.

3. Direct interspecies electron transfer in artificial co-cultures

DIET between *Geobacter metallireducens* and *Geobacter sulfurreducens*. DIET was first demonstrated in an artificial *Geobacter* co-culture provided with ethanol as the electron donor and fumarate as an electron acceptor [8]. Neither partner could use the ethanol-fumarate energy sources alone. When the ethanol-oxidizing *G. metallireducens* was placed together with the fumarate-reducing *G. sulfurreducens*, a metabolically co-dependent consortium was formed [8].

The partnership did not require enzymes for the metabolism of formate or H_2 [9]. Instead, it required a distinct apparatus for extracellular electron release in the donor strain (*G. metallireducens*) or extracellular electron uptake in the electron-accepting partner (*G. sulfurreducens*) (*Figure 1*). To release electrons, *G. metallireducens* required electrically conductive pili (e-pili) [10,11] and outermembrane multiheme *c*-type cytochromes [10] . Conversely, to accept extracellular electrons, *G. sulfurreducens* did not require e-pili [11], but required an outermembrane multiheme cytochrome (OMC) - OmcS [8] - an OMC, which could self-assemble into electrically conductive cytochrome-chains [12,13].

DIET between *Geobacter metallireducens* and *Methanosarcinales*. In methanogenic environments, syntrophy is the key process in organic matter decomposition [6]. Thus, methanogens were expected to play the role of electron-accepting partners for syntrophs

like *Geobacter metallireducens*. Indeed, DIET was possible between the alcohol-utilizing *G. metallireducens* and *Methanosarcinales* [14–18], including strict non-H_2 consumers [14,16–18]. Instead, *G. metallireducens* could not interact syntrophically with strict H_2 or formate-consuming methanogens [14,17]. It was conceivable that strict acetoclastic methanogens like *Methanosaeta harundinacea,* were only transferring acetate. However, expression analyses coupled with stoichiometry and $^{14}CO_2$-radiolabeling incubations showed that CH_4 was generated from CO_2 and not acetate alone. Additionally, incubations of *G. metallireducens – M. harundinacea* cocultures with long-chain alcohols (e.g., butanol) that cannot split into acetate, but oxidize to their respective long-chain fatty acids (e.g., butyrate), led to DIET-based cocultures, independent of acetate-transfer [18]. All these results confirmed that *Methanosaeta* was exchanging electrons with *G. metallireducens*. Nevertheless, the electron uptake mechanisms in *Methanosaeta* has not been studied and remains enigmatic.

During DIET with *Methanosarcinales, G. metallireducens* required conductive pili [14,15] and outermembrane multiheme *c*-type cytochromes (OMCs) [15]. The process of electron uptake usually involves OMCs in many autotrophs that accept extracellular electrons [19]. Therefore, *Methanosarcinales* were expected to retrieve electrons similarly as the only methanogens with *c*-type cytochromes [20]. However, not all *Methanosarcinales* capable of DIET flaunted multiheme *c*-type cytochromes (MHC) in their genomes [17]. Besides, one *Methanosarcina* (*M. mazei*) which contains a MHC (Mma_0663), did not require it for growth with extracellular electrons from DIET partners or electrodes [17]. Therefore, it appears that *Methanosarcinales* may use unprecedented electron uptake mechanisms that remain profoundly unexplored.

Other DIET co-cultures. The diversity of DIET syntrophic interactions in co-cultures is expanding (Figure 2), beyond typical electroactive species. Typically, effective electrogens [22,23] play the role of electron-donating strains to DIET-accepting partners but not HIET-partners (H_2-based interspecies electron transfer). These electrogens belong to the genera *Geobacter* and *Rhodoferax* [17,21,22] namely, *G. metallireducens*, *G. sulfurreducens* and *G. hydrogenophilus* and *R. ferrireducens*. Contrariwise, non-electrogenic (*G. bemidjiensis*) or poor electrogenic *Geobacter* (*G. bremensis*, G. *uraniireducens*, *G. humireducens*, *G. chapeleii*) could not interact syntrophically with DIET-accepting-partners [22].

However, recent studies appear to challenge the hypothesis that effective electrogens are better DIET-ers [24,25], indicating that DIET relationships may occur between unexpected partners and under unusual conditions. For example, one interaction occurred only under light conditions, between the acetate-oxidizing *G. sulfurreducens* and the CO_2-reducing phototrophic partner *Prosthecochloris aestuarii* [21].

Semenec *el at.* paired a formate-oxidizing *Pseudomonas aeruginosa* with the fumarate-reducing *G. sulfurreducens* as the electron-accepting partner and showed that the interaction was dependent on multiheme cytochromes [25]. Although *P. aeruginosa* is capable of extracellular electron transfer (EET), it does so with the aid of self-secreted phenazine shuttles retained in a network of extracellular DNA [26]. Yet, *Pseudomonas'* phenazines were not required for its interaction with *G. sulfurreducens*. Walker *et al.* showed that the typical H_2-producing syntroph (*Syntrophus aciditrophicus*) – never characterized as an electrogen - harbored e-pili and switched to DIET, when a DIET option was available [24]. This DIET interaction was demonstrated by placing *Syntrophus* with a partner incapable of H_2 and formate uptake - a *G. sulfurreducens,* which lacked a subunit for formate dehydrogenase and one for hydrogenase [24]. Besides, *Syntrophus* is not the only syntrophic bacterium encoding e-pili in its genome, hinting at a potential option for other syntrophs to do DIET [24].

Moreover, DIET does not always correlate with electroactivity in methanogens.

For example, a *Methanosarcina horonobensis* could not use a cathode as the electron donor, but could form DIET consortia with *G. metallireducens* [16].

DIET was also indicated as mode of interaction for a new *Methanobacterium* isolate (and strict formate utilizer) co-cultured with *G. metallireducens* [27]. Astoundingly, the *Methanobacterium-Geobacter* co-culture was independent of *e*-pili. So, it remains to be determined whether and how this *Methanobacterium* receives extracellular electrons.

Mostly, effective electroactive microorganisms interact by DIET. So, what makes "non-electroactive species" capable of DIET? Furthermore, how come that archetypal syntrophs interact by DIET-syntrophy in the absence of a possibility for H_2/formate-transfer? What ecological advantages might they have when switching from H_2/formate-transfer to DIET and vice-versa? These questions remain open to future investigations.

Conductive materials accelerate DIET co-culture metabolism. The metabolism in DIET co-cultures is accelerated by electrically conductive particles (iron-oxide minerals [28] and carbon-based materials [15–17,29–31]. Such cell-particle-cell interactions are not strictly speaking DIET. When minerals mediate the interaction between species (CIET), cells are not in direct contact and genes typically involved in DIET are significantly downregulated. For example, a *Geobacter* co-culture amended with a semi-conductive iron-oxide (magnetite) downregulated the expression of OmcS, which was not required for the mineral-mediated interaction, but it required e-pili [28].

Moreover, conductive materials are sometimes essential for the syntrophy between partners otherwise incapable of DIET, as was the case for an acetate-nitrate fed *Geobacter sulfurreducens – Thiobacillus denitrificans* co-culture, which can only grow together in the presence of iron-oxide minerals [32] or redox active humic substances [33].

Approaches to validate DIET in co-cultures. Precise validation of DIET in artificial co-cultures requires a polyphasic approach (Figure 3). This approach includes: i) determining the potential to form a cross-feeding interaction (with DIET and non-DIET partners) along with ii) the syntrophic consortia's physiology, iii) genomics to document the potential absence of alternative electron transfer strategies, iv) gene expression and v) targeted gene-deletion studies. For example, the incapacity to exchange electrons via H_2/formate was tested with the help of a donor strain incapable of H_2/ formate transfer [14,17]. In instances where the donor strain can oxidize their substrate to H_2/formate [17,22,24], researchers tested first if the donor strain was unsuccessful at establishing co-dependent interactions with H_2/formate-utilizing partners [14,17] and second if it was successful with acceptor strains unable of H_2/formate uptake (naturally or artificially by gene deletion) [9,16,24]. Additional tests are needed to exclude other electron transfer possibilities between species via self-generated shuttles or other redox-active compounds (e.g., flavins or cysteine, respectively). For example, cysteine could be transiently excluded from the media [15], or co-cultures could be spiked with spent cell filtrate, which would significantly stimulate metabolism if rich in shuttles [34].

4. Evidence for DIET-syntrophy in environmental dual-species consortia

Recent investigations indicate that DIET is a relevant electron transfer process in microbial consortia catalyzing the anaerobic oxidation of methane (AOM) and higher gaseous alkanes, both coupled with sulfate-reduction. Sulfate-dependent AOM is a process with broad

climate impact, controlling methane emissions to the atmosphere. AOM-mediating consortia are abundant in various methane-rich habitats [35] while archaea oxidizing higher alkanes appear widespread in hydrocarbon-impacted sediments [36,37].

DIET in anaerobic methane-oxidizing consortia.

Sulfate-dependent AOM consortia consist of anaerobic methanotrophic (ANME) Archaea tightly packed with partner sulfate-reducing bacteria (SRB). Reducing equivalents from methane oxidation are transferred from ANME-Archaea to the partner SRB, which reduces sulfate to sulfide [35]. Two studies indicated that the ANME-SRB interaction is based on DIET [38,39]. As determined by stable isotope assimilation [38] and confirmed by modeling [40], the distribution of metabolically active cells within natural ANME-2−SRB aggregates from cold seeps could only be explained by an interspecies association dependent on electrically conductive conduits between cells, similar to DIET.

Additionally, ANME-2 genomes contain large multiheme cytochromes (MHC) similar to those in electrogens like *Geobacter* [38]. Researchers identified probable electroactive interfaces in cellular membranes and the interstitial space between cells via heme staining [38]. Besides, they identified MHC genes in the genomes of both partners of thermophilic AOM consortia, ANME-1 and HotSeep-1 SRB, enriched from hot seeps [39]. Moreover, HotSeep-1 encoded type IV pili proteins. MHC and pili genes were specifically overexpressed under methane-oxidizing conditions, and nanowire-like structures were observed in consortia's intercellular space, indicating DIET coupling [39].

DIET in anaerobic butane- and ethane-oxidizing consortia. Recently, DIET interactions have been proposed for thermophilic archaea candidate lineages oxidizing butane (*Ca*. Syntrophoarchaeum) or ethane (*Ca*. Ethanoperedens) in consortia with SRB of the HotSeep-1 clade [36,41]. The SRB partners of both *Ca*. Syntrophoarchaeum [41], and *Ca*. Ethanoperedens [36] encode and express MHC or type IV pili, and nanowire-like structures have been observed connecting cells within consortia [41]. A representative of the HotSeep-1 clade (*Ca*. Desulfofervidus auxilii) was enriched without its archaeal partner and shown to be a chemolithoautotrophic H_2-oxidizer [42]. Together with the detection of H_2 in thermophilic AOM cultures [39], this raised the prospect of a hydrogen-based coupling of alkane oxidation to sulfate reduction. However, after specific inhibition of the SRB partner, the H_2 concentrations in AOM and butane-oxidizing consortia were far too low to explain the measured sulfate reduction rates,

leaving DIET as the only reasonable electron transfer mechanism [39,41]. Nevertheless, direct proofs for pili and MHCs being undoubtedly linked to DIET in such consortia is yet to be determined.

Proposed alternative mechanism via zero-valent sulfur (S^0). Chemical imaging of AOM consortia of ANME-2 and *Desulfosarcina*-SRB showed a high abundance of S^0 in the archaeal cells [43]. S^0-abundance was corroborated with physiology experiments and immunolabelling of canonical enzymes and interpreted as interspecies electron transfer mediated by S^0-based (polysulfides) compounds. This model had one major drawback, the reliance on a hypothetical, cryptic sulfate-reduction pathway producing S^0/polysulfides in archaea whose enzymes were never identified. Recently, archaea with high S^0 content have been identified in an ethane-oxidizing culture [44]. Like ANME archaea, the ethane-oxidizing archaea (*Ca*. Argoarchaeum ethanivorans) also depend on partner SRB, but they do not form aggregates and do not exhibit nanowire-like structures. These recent findings revived the idea that in some consortia, alkane-oxidation may be coupled to sulfate reduction via S^0-mediated IET and not by DIET [44].

5. Evidence for CIET-syntrophy in environmental communities

Some syntrophic partners may interact via conductive mineral-chains [45]. Conductive minerals are often present in natural environments (e.g., in coastal sediments, rice paddies, hydrothermal vents)[46–48] and their absence during laboratory incubations severely impacts species distribution and survival [34,49,50].

Interactions dependent on conductive particles (CIET) are more straightforward to investigate than DIET. This is because we can use conductive minerals to specifically enrich CIET-partners from environmental communities where partners may rely on conductive minerals to interact with each other. Under such enrichment conditions, non-syntrophic species fade out. For example, a *Geobacter* - *Methanosarcina* consortium from Baltic Sea sediments required the presence of conductive materials (iron-oxides or activated carbon) to carry out syntrophic acetate oxidation [34]. Without conductive minerals, syntrophic acetate oxidation ceased, and both groups went extinct. Without conductive minerals, a less abundant and metabolically ineffective species took over acetate turnover via acetoclastic methanogenesis [34]. Stable isotope analyses clearly showed that acetate was processed via syntrophic acetate oxidation coupled with CO_2

reductive methanogenesis [34,51], likely relying on the conductive iron-minerals abundant in marine sediments [34,49,50].

6. **Approaches to validate DIET and CIET in natural guilds.**

The application of the polyphasic approach mentioned above (Figure 3) to confirm DIET and CIET in environmental communities is not always possible because some syntrophic partners cannot be separated or genetically manipulated, especially those in obligate syntrophic interactions like the ANME-SRB consortia.

Secondly, significant concerns have been raised at describing "electric"-syntrophy based on metabolism stimulation by conductive materials [52], because conductive materials enhance the metabolism of some methanogens (e.g. carbon nanotubes [53]) independent of being coupled with an "electric"-syntroph.

Besides, investigations of environmental DIET/CIET associations based on the mere presence of the DNA/RNA of "electric"-syntrophs (see references in [17]) are not ideal, since species abundance (e.g., *Geobacter*) or expression of a certain protein does not necessarily mean they perform DIET/CIET in the environment.

With many *Geobacter*-species incapable of establishing syntrophic associations [22] and many uncharacterized potential "electric"-syntrophs out there [49,50], novel investigation strategies are needed. A combination of tools must be employed after case-by-case adjustment to the process and the environmental considered. For example, to demonstrate CIET-dependent interactions we must verify the strict dependence on conductive minerals over non-conductive materials [34,49]. For both DIET and CIET, expression studies can inform on MHC and pili content [54]. However, these cannot inform whether two species are coupled metabolically. For this we need to monitor metabolites to inform on consortias' stoichiometry, and determine species co-occurrence. Plus, specific inhibition of the donating and accepting partners could inform whether the oxidation and reduction processes are co-dependent [34,49,50]. The role of H_2/formate or shuttles/enzymes as interspecies intermediates can be excluded by following their impact on the metabolism of the interspecies association [55]. For example, H_2-additions to a consortium relying on H_2-transfer would block the syntroph's metabolism by feedback inhibition [56]. Or suppose the interaction depends on shuttles/enzymes generated by the consortia. In that case, the shuttles/enzymes in the spent media would facilitate the extracellular electron exchange. Such experiments testing the spent media, are typical when investigating electron

uptake during Fe°-biocorrosion [57,58]. Additionally, we can apply electrochemical methods like cyclic voltammetry to determine the presence of active redox molecules or enzymes in the live/heat-killed spent culture media [59].

DIET and CIET interactions may have specific isotopic signatures, specific microscopic distribution patterns, molecular and elemental signatures (e.g., high metal-content on cell surfaces) compared to H_2/formate IET. None are understood or explored sufficiently. Therefore, innovative methods to simplify the verification of these processes in the environment require immediate attention.

7. Ecological and biotech ramifications of electric syntrophies

It is apparent that cooperative metabolic dependencies greatly influence environmental chemistry and, consequently, impact our health, climate, and industries (Figure 4). Because we lack tools to study DIET and CIET in the environment, our understanding of how interspecies interactions impact environmental processes is in its infancy. Nevertheless, we mentioned studies that showed how both methane production and methane consumption in marine environments appear to be controlled by DIET/CIET interactions, possibly influencing the release of this greenhouse gas in the atmosphere. Therefore, it is imperative to understand better the triggers and controls for these processes of climate relevance.

Biotechnologies dependent on DIET and CIET are budding, with DIET-syntrophs and conductive materials often applied to stimulate industrial processes like anaerobic digestion. Several recent reviews summarized the implications of electric syntrophies in anaerobic digestion and demanded the development of suitable detection methods (extensively reviewed in Refs. [52,60]).

Another role for DIET and CIET is the bioremediation of toxic compounds from industry off streams or already released in the environment. Recent studies investigated the possibility to apply CIET in order to improve the degradation of toxic compounds from the effluents of various industrial processes like: nitrobenzene – found in herbicides, insecticides and pharmaceuticals [61], azo dyes from the textile industry [62], solvents from the printing industry [63], chlorinated compounds (e.g.,[64,65]) generally used as precursors for PVC-production, and petroleum hydrocarbons [66–69].

Geoengineering approaches using CIET to stimulate the attenuation and degradation of contaminants and decontaminate sediments are now under consideration [70]. Two recent studies

showed that the addition of activated carbon stimulated polycyclic aromatic hydrocarbon degradation under anaerobic conditions when CIET was possible, but not under aerobic conditions [71,72]. However, the actual implications of DIET and CIET in environmental decontamination remains to be verified. It is advisable to proceed stepwise because adding conductive minerals to contaminated soils could significantly enhance CIET and methane production, possibly enhancing methane emissions to the atmosphere. Thus, it is paramount that primary tests are carried out to investigate the effect of such materials on communities through the sediment depth and verify the effect on microorganisms along the entire spectrum of electron acceptors.

8. Conclusion

DIET and CIET have been intensely studied in laboratory co-cultures, natural dual-species consortia and enriched environmental consortia. However, methods to easily fingerprint DIET/CIET associations in the environment are lacking. Here we indicate a polyphasic approach to study such associations in environmental samples and call for additional tools to be developed.

The significance of electric associations along other types of interspecies associations in natural processes is ambiguous. Thus, it is imperative to understand the role of "electric" syntrophies in global element cycles, especially in the interplay between the iron and methane cycles. Climate change has led to increased erosion and input of rock and mineral particles in our oceans, possibly enhancing CIET interactions and the release and perhaps consumption of the potent greenhouse gas -methane. Overall, this significantly influences our present-day climate models since we do not comprehend potential novel methane sources and sinks in natural environments. The wastewater and anaerobic digestion industries are now investing resources to determine DIET and CIET implications in speeding up organic matter decomposition. Additionally, geoengineering approaches are being sought considering conductive mineral particle additions to contaminated environments to induce bio-attenuation of pollutants. It is time we, as a scientific community, come together to cover these knowledge gaps.

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Figures and figure legends

Figure 1. Examples of favorable interactions between prokaryotic species based on intercellular material exchange: (a) via diffusible molecules (e.g., H_2 and formate see ref. [9]); (b) via an electron shuttle (e.g., via flavins see ref. [73]); (c) by direct cell-to-cell contacts (e.g., pili [14] and outermembrane c-type cytochromes [15]); (d) via conductive particles (e.g., magnetite [28]). The first four (a-d) are typical interactions based on extracellular electron transfer. However, cells can also transfer larger cellular material by (e) membrane fusion (e.g., between two species of *Clostridium* [4]); (f) vesicles (e.g., interspecies iron delivery [74]) or nanotubes (e.g., interspecies aminoacid transfer to compensate for amioacid auxotrophies [75]).
ED – electron donor; EA – electron acceptor; ES – electron shuttle; ox – oxidized; red – reduced.

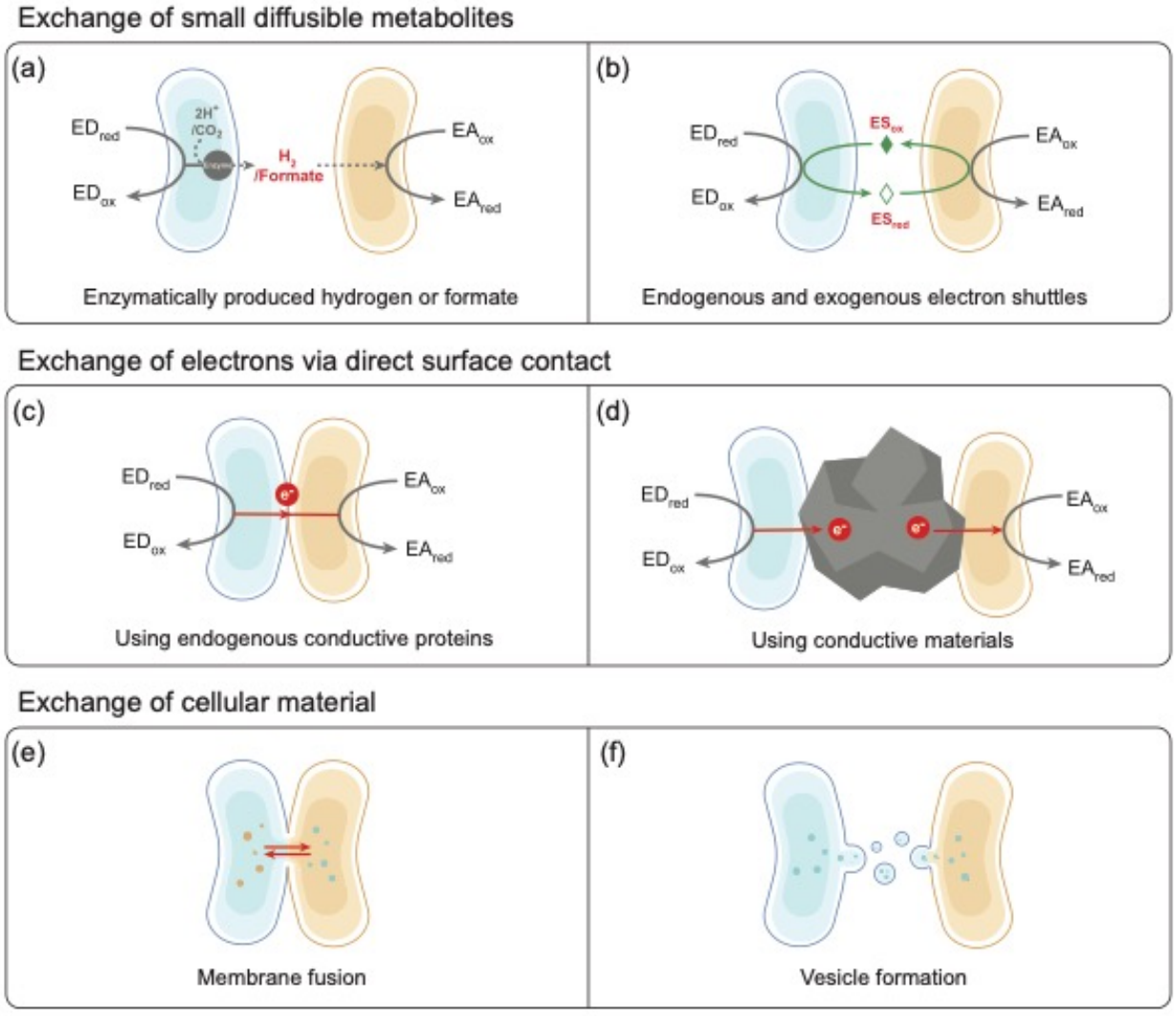


Figure 2. Timeline of discoveries regarding direct interspecies electron transfer (DIET – above the arrow) and conductive mineral mediated interspecies electron transfer (CIET – below the arrow). GM; *Geobacter metallireducens,* GS; *Geobacter sulfurreducens*, GH; *Geobacter hydrogenophilus*, MB; *Methanosarcina barkeri*, MH; *Methanosaeta harundinacea*, PA; *Prosthecochloris aestaurii*, PA*; *Pseudomonas aeruginosa*, PC; *Pelobacter carbinolicus*, RF; *Rhodoferax ferrireducens*, SA; *Syntrophus aciditrophicus*, AOM; anaerobic oxidation of methane, OMC; outer membrane cytochrome, HIT; hydrogen interspecies transfer, NP; nanoparticles, IET; interspecies electron transfer, AQDS; anthraquinone-2,6-disulfonate, EET; extracellular electron transfer, ANME; anaerobic methanotrophic archaea. Additional references not discussed in the manuscript text [76–82].

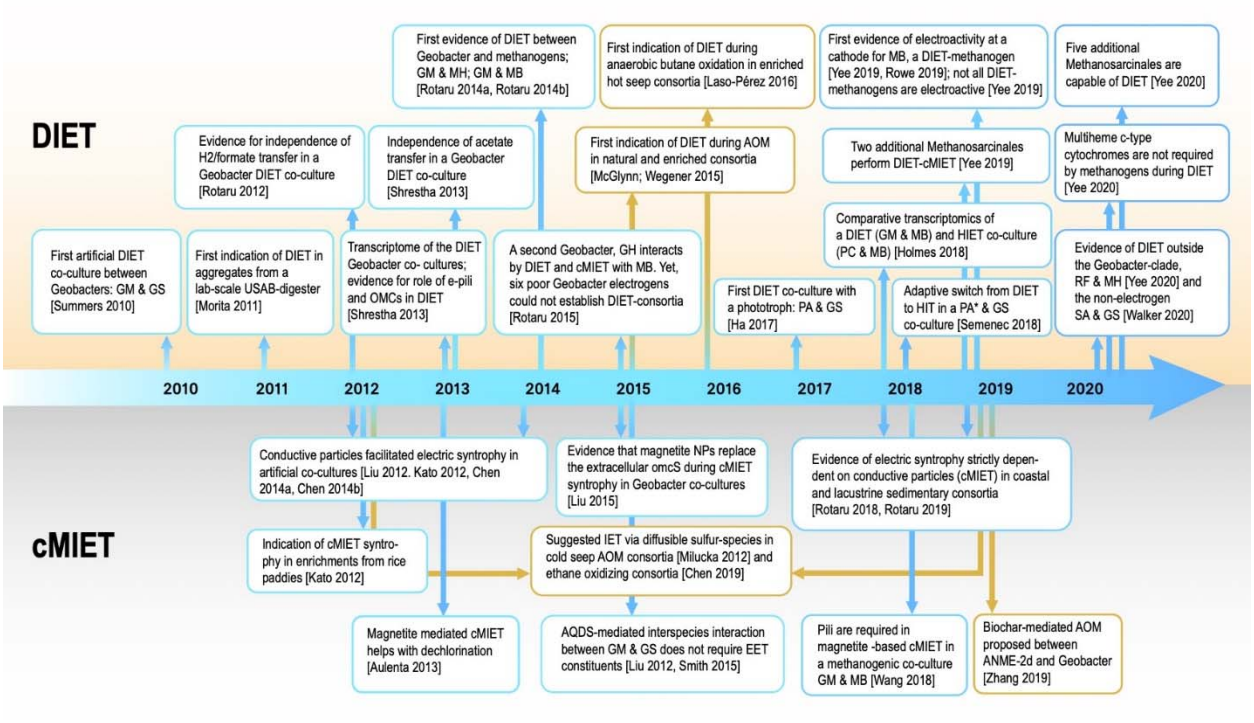


Figure 3. Methods to validate DIET in a co-culture. (a) Establishing cross-feeding interactions to ensure substrate selectivity of each member, (b) Ensuring the incapability of other alternative interspecies electron transfer (e.g. via hydrogen or formate) (c) Monitoring expression profiles of electron transfer conduit proteins, (d) Validating the absence of possible exogenous and endogenous electron shuttles in the culture media and (e) Deletion studies targeting genes involved in extracellular electron transfer (e.g. pili, outer membrane cytochromes).

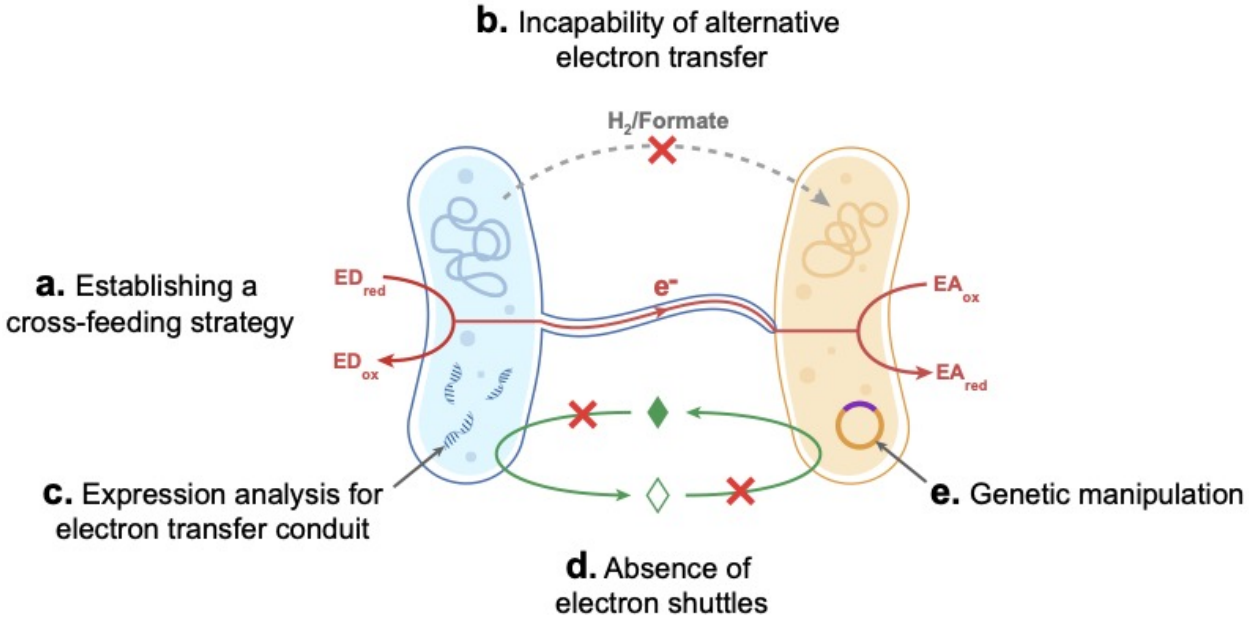
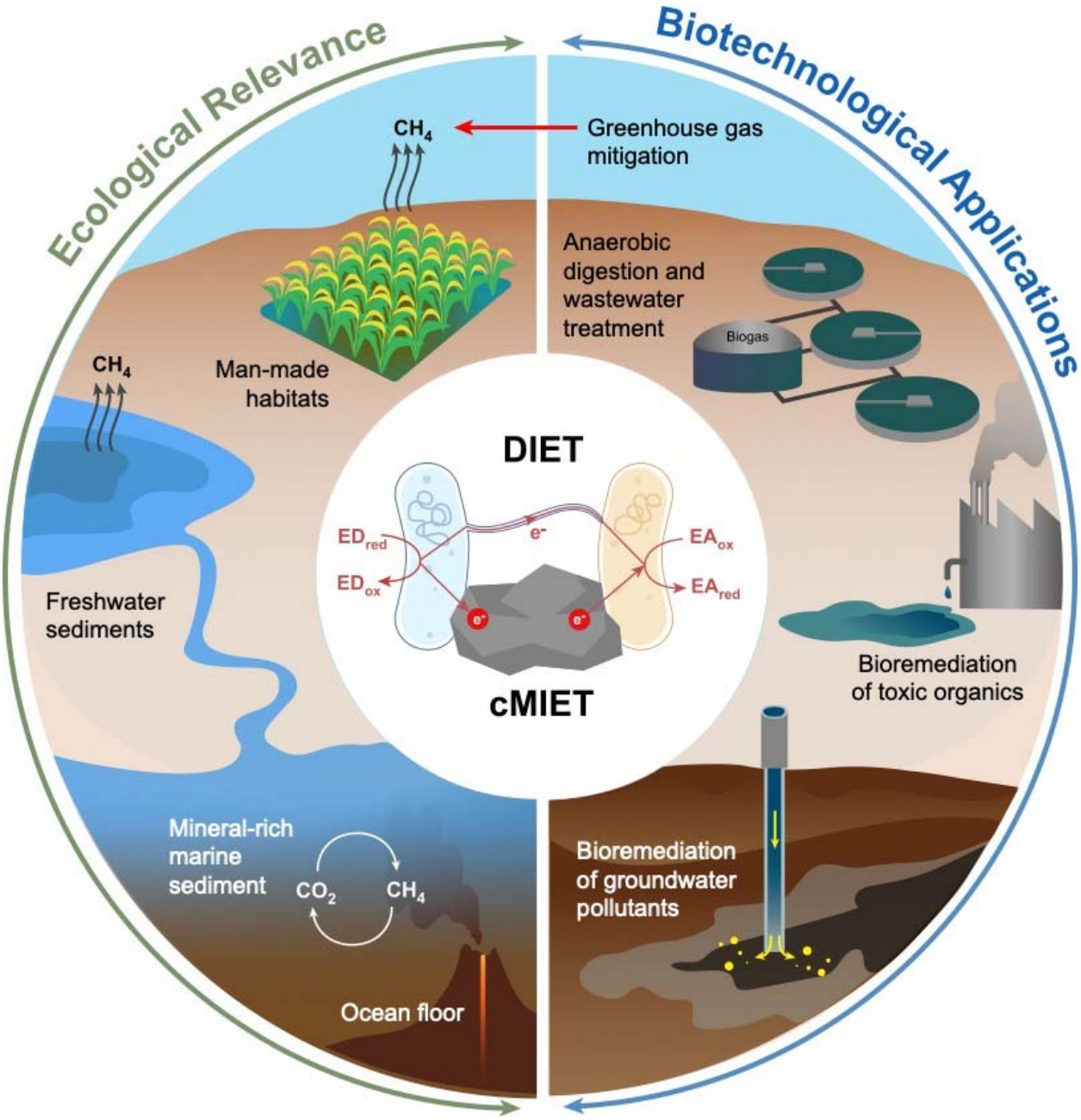


Figure 4. Ecological relevance and potential applications of DIET and CIET interactions.



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