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Elie Desmond-Le Quéméner[a]*, Roman Moscoviz[b], Nicolas Bernet[a], Andrew Marcus[c]

[a]INRAE, Univ Montpellier, LBE, 102 avenue des Etangs, 11100, Narbonne, France

[b]SUEZ, Centre International de Recherche Sur l'Eau et l'Environnement (CIRSEE), Le Pecq, France

[c] Biodesign Swette Center for Environmental Biotechnology, Arizona State University, Tempe, AZ, USA

*Correspondance: elie.lequemener@inrae.fr (E. Desmond-Le Quéméner).

Abstract

Interspecies electron transfer (IET) is a key phenomenon in anaerobic ecosystems, which is traditionally modelled as hydrogen transfer. Recently discovered alternative mediated IET (MIET) or direct IET (DIET) offer exciting alternative mechanisms of microbial partnerships that could lead to new strategies for the improvement of biotechnologies. Here, we analyze mathematical modelling of DIET and MIET in anaerobic ecosystems. Bioenergetics approaches already enable the evaluation of different energy sharing scenarios between microorganisms and give interesting clues on redox mediators and on possible ways of driving microbial communities relying on IET. The modeling of DIET kinetics however is currently only in its infancy. Recent concepts introduced for the modeling of electroactive biofilms should be further exploited. Recent modeling examples confirms the potential of DIET to increase the IET rates compared to H_2-MIET, but also point out the need for additional characterizations of biological components supporting IET to improve predictions.

Introduction

In anaerobic environments, methane formation and oxidation are the result of the activity of syntrophic communities of archaea and bacteria. A key mechanism in these anaerobic communities is interspecies electron transfer (IET) between the different microorganisms that live in conditions close to thermodynamic equilibrium [1].

Mediated interspecies electron transfer (MIET), *i.e.* transfer of electrons between bacteria and archaea by shuttle components (Figure 1), has been widely studied for many years. In the case of anaerobic digestion (AD), well-known examples of MIET are interspecies hydrogen or formate transfer. Particularly, the discovery of interspecies hydrogen transfer about fifty years ago was a significant breakthrough in the understanding of the anaerobic digestion process and more widely of methanogenic anaerobic communities [2].

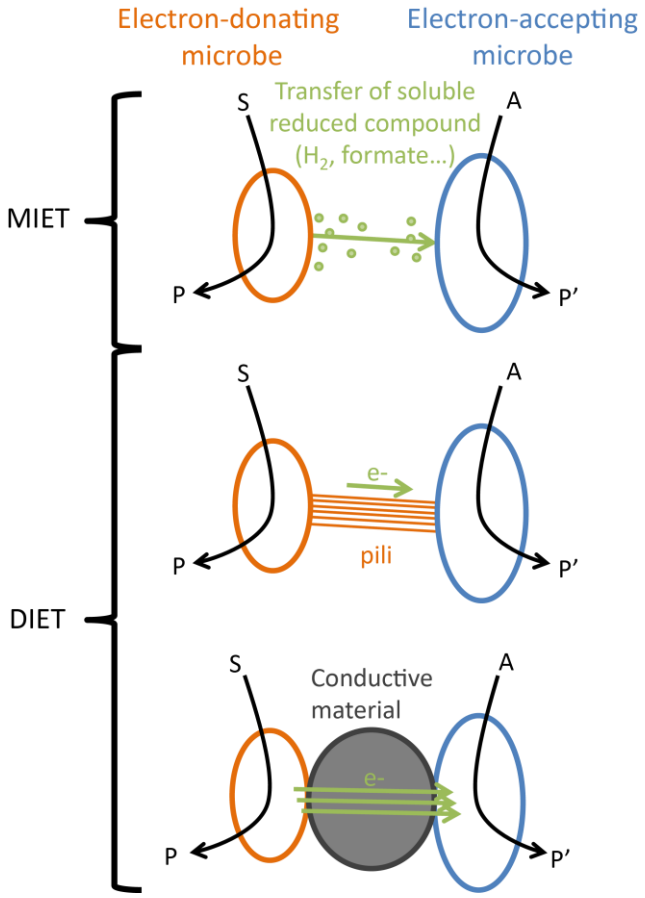

Figure 1: Schematics of mediated and direct interspecies electron transfer using either soluble electron mediators (MIET), pili or conductive materials (DIET). S is the substrate for the electron donating microbe, A is the electron acceptor for the electron accepting microbe, P and P' are products from the metabolism. Adapted from [3].

In the past decades, the development of research on microbial electrochemical technologies in which microorganisms exchange electrons with electrodes led to a deeper understanding on how

microbes transfer electrons [4]. Recently, direct interspecies electron transfer (DIET) was discovered as an alternative IET pathway to MIET in defined cocultures involving a *Geobacter* species as one of the partners [2]. Potential DIET mechanisms include electron transfer through electrically conductive pili, through electrically conductive materials or using proteins associated with outer cell surfaces [2] (Figure 1). As highlighted by D.R. Lovley [5], DIET has a potential to transfer signals and energy faster and more specifically than diffusion. *Methanosaeta* species, which are abundant in diverse methanogenic environments, but also *Methanosarcina barkeri*, have been shown to be capable of DIET through partnering with *Geobacter* species [6,7]. DIET between methanotrophic archaea and bacteria in the case of thermophilic anaerobic oxidation of methane (AOM) using sulfate as the final electron acceptor has also been reported [8]. Recent research indicates that electroactive microbes are highly diverse [9,10] suggesting that DIET might constitute an important mechanism in numerous habitats.

In terms of modeling, MIET and, specifically, interspecies hydrogen transfer is explicitly modeled in the IWA Anaerobic Digestion Model No. 1 (ADM1) [11]. The conversion of small organic molecules such as acetate, propionate, butyrate or ethanol into methane and CO_2 occurs near the thermodynamic equilibrium and thus requires a tight coupling between fermentative and methanogenic metabolisms [12]. H_2-MIET is thus strongly constrained by hydrogen concentrations and plays an important role in AD models that take it into account either using explicit bioenergetics computation [13,14] or by proxy using a kinetic inhibition function (*e.g.* in ADM1) [11,14].

Since the discovery of DIET, its importance to AD and its potential to increase and stabilize performances have been widely studied and it is now considered that DIET, rather than MIET, could be the main mechanism of interspecies electron transfer within complex anaerobic microbial communities [15]. It has been shown that conductive materials such as activated carbon [16] or biochar [17] can act as a conduit for electrons between DIET microbial partners and that the addition of conductive materials in a digester can promote the establishment of DIET [18] and increase stability and performances [19]. However, the mechanisms underlying these effects remain unclear. They could indeed be related to the stimulation of DIET but might as well be due to other properties associated with the biofilms forming on the introduced particles. In that context, the accurate modeling of DIET would give precious information on what can be expected from

77 the stimulation of DIET in AD and on the best ways to stimulate it. Moreover, in the context of
78 AOM or fermentations, an accurate modeling of IET mechanisms would provide clues on possible
79 ways of improving/controlling those processes using approaches such as ecological engineering.

80 The objectives of this mini-review are (i) to show how simple thermodynamic calculations can be
81 used to get insight into IET, (ii) to describe electrochemical and bioelectrochemical equations that
82 can be used to model DIET and (iii) to review the few models explicitly considering DIET and to
83 evaluate the potential advantages of DIET over H_2-MIET for bioprocesses. Finally, we point out
84 the crucial parameters that are currently missing for an adequate modeling of DIET and for a true
85 evaluation of its role in anaerobic ecosystems as well as its potential for biotechnologies.

86

87 **Using thermodynamics to investigate redox mediators supporting IET**

88

89 Thermodynamics and static models based on mass balances are easy to apply, require minimal
90 data and provide deep insights on IET mechanisms. They capture the net energy available for
91 growth and how it is shared between IET partners. Moscoviz *et al*. [20] for example used a mass
92 balance approach to devise possible scenarios such as energetic mutualism, commensalism or
93 parasitism associated with IET. More recently, Gu *et al*. and Liu *et al*. [21,22] analyzed the energy
94 distribution in syntrophic methanogenesis. Here, we would like to demonstrate how simple
95 thermodynamic calculations give insights into the redox mediators supporting IET.

96

97 First, let's illustrate the calculations using the example of syntrophic ethanol oxidation used by Liu
98 *et al*. [22] (Table 1 and Supplementary material 1). Under realistic conditions, the Gibbs free
99 energy for the global conversion of ethanol to methane is about -58.8 kJ/mol ethanol (see Table
100 1). In H_2-MIET, the Gibbs free energy of the reactions and thus the energy distribution depends
101 on H_2 partial pressures ($p(H_2)$). In IET, either mediated by a soluble electron mediator (EM) or
102 through a membrane-bound EM and DIET, the energy distribution depends on the potential of the
103 EM (E_{EM}). These calculations allow deriving the range of $p(H_2)$ and of E_{EM} that make both ethanol
104 oxidation and methanogenesis feasible, *i.e.* that make both associated ΔG negative. In our case,
105 those ranges are $2 \cdot 10^{-6}$ bar < $p(H_2)$ < $2 \cdot 10^{-2}$ bar and -0.41 V *vs* SHE < E_{EM} < -0.25 V *vs* SHE,
106 where SHE stands for standard hydrogen electrode.

107

108 **Table 1: Thermodynamics of ethanol syntrophic oxidation.** ΔG values were calculated for T
109 = 298 K, pH = 7, $p(CH_4) = 10^{-2}$ bar and C = 10^{-2} mM for soluble compounds. Standard Gibbs free

energy values were derived from [23]. ΔG is expressed as a function of hydrogen partial pressure $p(H_2)$ in the case of H_2-MIET and as a function of redox potentials E_{EM} in the case of IET via an electron mediator (EM-IET).

Process	**Type of IET**	**Reaction**	**ΔG (kJ.mol^{-1})**
Ethanol oxidation	H_2-MIET	$\text{ethanol} + H_2O \rightarrow \text{acetate}^- + 2 \cdot H_2 + H^+$	$8.3 + 2 \cdot R \cdot T \cdot \ln(p(H_2))$
	EM-IET	$\text{ethanol} + 4 \cdot EM + H_2O \rightarrow \text{acetate}^- + 4 \cdot EM^- + 5 \cdot H^+$	$-156.8 - 4 \cdot F \cdot E_{EM}$
Methanogenesis	H_2-MIET	$HCO_3^- + 4 \cdot H_2 + H^+ \rightarrow CH_4 + 3 \cdot H_2O$	$-134.1 - 4 \cdot R \cdot T \cdot \ln(p(H_2))$
	EM-IET	$HCO_3^- + 8 \cdot EM^- + 9 \cdot H^+ \rightarrow CH_4 + 8 \cdot EM + 3 \cdot H_2O$	$196.1 + 8 \cdot F \cdot E_{EM}$
Overall process	H_2-MIET or EM-IET	$\text{ethanol} + \frac{1}{2} \cdot HCO_3^- \rightarrow \text{acetate}^- + \frac{1}{2} \cdot H^+ + \frac{1}{2} \cdot CH_4 + \frac{1}{2} \cdot H_2O$	-58.8

The ranges estimated with this first approach are quite large; however, when $p(H_2)$ or E_{EM} is close to the extreme values of these ranges, it means that one organism gets all the energy and the other none. This approach can then be extended to get a more precise evaluation of ranges taking into account realistic growth yields in cocultures [21,22] using the Gibbs energy dissipation method [23]. This method considers the Gibbs energy balance of the metabolism, which consists of the macroscopic equations for catabolism and anabolism:

$$\Delta G_{metabolism} = \Delta G_{anabolism} + \lambda \cdot \Delta G_{catabolism} = -\Delta G_{dissipated} \quad (1)$$

where λ is a coupling parameter indicating how many times the catabolism reaction needs to be carried out to sustain one anabolic reaction. It is worth mentioning that the value for λ is dependent on the formalism used to describe catabolism and anabolism. Yet, once such formalism is defined, a unique λ value can be calculated from experimental growth yields [23].

In Equation 1, the remaining energy generated by catabolism that was not used to support anabolism is considered to be dissipated by cells. Heijnen [23,24] found an empirical relationship to estimate $\Delta G_{dissipated}$ based on the carbon source characteristics only:

$$\Delta G_{dissipated} = 200 + 18 \cdot (6 - N_C)^{1.8} + \exp\left((3.6 + 0.4 \cdot N_C) \cdot (-0.2 - \gamma)^{0.32}\right) \quad (2)$$

where N_C and γ are the carbon length and the oxidation state of the carbon source, respectively.

This relationship was built considering 89 experimental observations encompassing diverse metabolisms (*e.g.* 30 different carbon sources including CO_2, aerobic and anaerobic respiration, fermentation, etc.) and provides $\Delta G_{dissipated}$ estimates with a relative error of about 30%. This empirical correlation can be used to solve Equation 1 for the coupling parameter λ and thus predict growth yields (mean relative error of 19%), as described in detail by Kleerebezem and Van Loosdrecht [23].

When growth yields are experimentally measured, the Gibbs energy dissipation method can be applied individually to each IET partner of a coculture to estimate the redox potential at which electrons are exchanged. The latter potential can be introduced in Equation 1 by using the equivalence between Gibbs energy calculation and redox potentials [20]:

$$\Delta G° = \sum_{i \neq y} \nu_i \cdot G°_f(product_i) - \sum_{j \neq z} \nu_j \cdot G°_f(reactant_i) - \nu_{EM} \cdot F \cdot E°_{EM} \quad (3)$$

where species *y* and *z* are the oxidized or reduced forms of the electron mediator used during IET, $E°_{EM}$ is the standard potential this electron mediator, ν_{EM} the amount of reduced electron mediator produced in the considered equation and F the Faraday constant.

An application of this method is pictured in Figure 2 (calculations provided in Supplementary material 2), where a coculture of *Geobacter sulfurreducens* and *Wolinella succinogenes*, experimentally studied by Cord-Ruwisch *et al.* [25], was analyzed to determine E_{EM}. The dissipation method clearly highlights that H_2-mediated IET (E = -0.22 V *vs* SHE at pH_2 = 0.02 Pa and pH = 7) could not explain the experimental growth yields, as already pointed out in the original paper. Kaden *et al.* later proved that IET in this coculture was dependent on the addition of cystine/cysteine to the medium and concluded that this couple acted as redox mediator [26]. However, the authors calculated that the cystine/cysteine couple during this experiment had a potential of about -200 mV *vs* SHE. Such potential would result in an energy partition between *G. sulfurreducens* and *W. succinogenes* similar to what would have been observed for an H_2-mediated IET and is therefore implausible. Instead, the dissipation method predicts that E_{EM} was likely between +25 and +240 mV *vs* SHE. Thus, it is probable that either (i) the cystine/cysteine

ratio was very high, thus increasing the actual redox potential of the couple, or (ii) a cysteine derivative having a high redox potential acted as electron shuttle rather than cysteine *per se*.

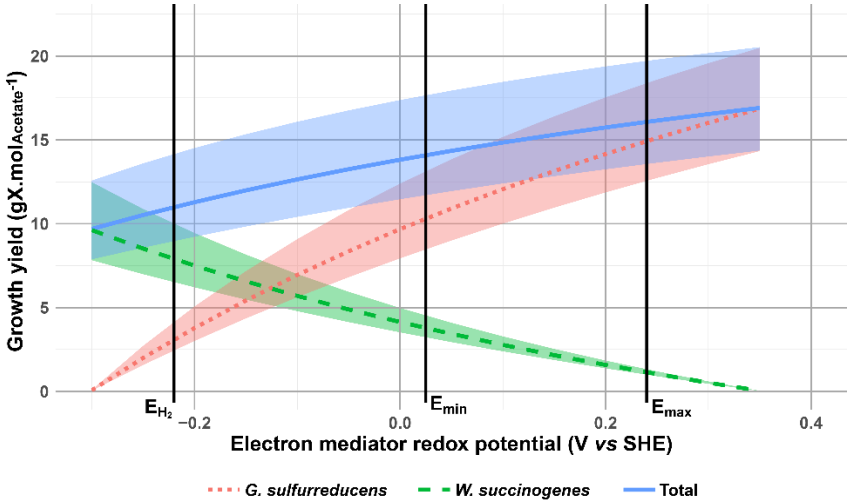

Figure 2: Theoretical growth yield of *Geobacter sulfurreducens* and *Wolinella succinogenes* interacting through IET. The electron donor and acceptor are acetate and nitrate, respectively. Calculations were carried out assuming pH = 7, a temperature of 30°C and a concentration of 10 mM for all soluble species (see Supplementary material 2). For each curve, the transparent area provides the uncertainty related to a 30% relative prediction error for the dissipated energy ($\Delta G_{dissipated}$) [24]. E_{H2} corresponds to the special case were the electron mediator is H_2 and pH_2 = 0.02 Pa (E = - 0.22 V *vs* SHE), as measured in [25]. E_{min} (25 mV) and E_{max} (240 mV) correspond to situations where *G. sulfurreducens* growth yield is 4 to 9 times higher than the one of *W. succinogenes*, as experimentally measured [25]. The total biomass yield predicted for potentials between E_{min} and E_{max} (14.1 to 16.1 ± 3.0 gX.mol$_{Acetate}^{-1}$) is consistent with the value of 18.5 ± 3.2 gX.mol$_{Acetate}^{-1}$ measured experimentally [25].

The dissipation method can also be combined with kinetic models to better estimate growth yields. However, this method suffers from two main limitations: (i) this method implicitly assumes that all the redox gradient available to each species is coupled to energy conservation, which may not be the case in reality [27–29] and (ii) Heijnen's correlation is only valid for non-inhibitory conditions and does not account for cell maintenance or biological regulations that would reduce growth yields [20]. Thus, this method will provide maximum growth yield and is more likely to provide fair electron mediator redox potential for syntrophic rather than non-mutualistic IET [30].

IET kinetics: introducing concepts from electrochemistry

Research in microbial electrochemical systems led to the development of kinetic models of electroactive biofilms on electrodes. Kinetic models link the current generation (the rate of electron exchange) with the electrochemical potential (the energy value of electrons exchanged), which are useful for understanding the energy gained by microbial partners in IET and energy lost to interfacial reactions and mass transfer processes.

As illustrated in Figure 3a), kinetic models consider a combination of processes in electroactive bacteria related to microbial kinetics and extracellular electron transfer (EET). Processes related to microbial kinetics are (i) substrate oxidation and (ii) intracellular electron transfer. Processes related to EET are (iii) extracellular electron transfer through the extracellular matrix and (iv) interfacial electron transfer. As shown in Figure 3b), these steps are analogous to the processes of the electron-donating partner in IET. The energy lost during microbial metabolism and interfacial charge transfers are parts of activation overpotentials. The energy lost to electrical resistance during EET and ion transport in the extracellular matrix are related to Ohmic losses.

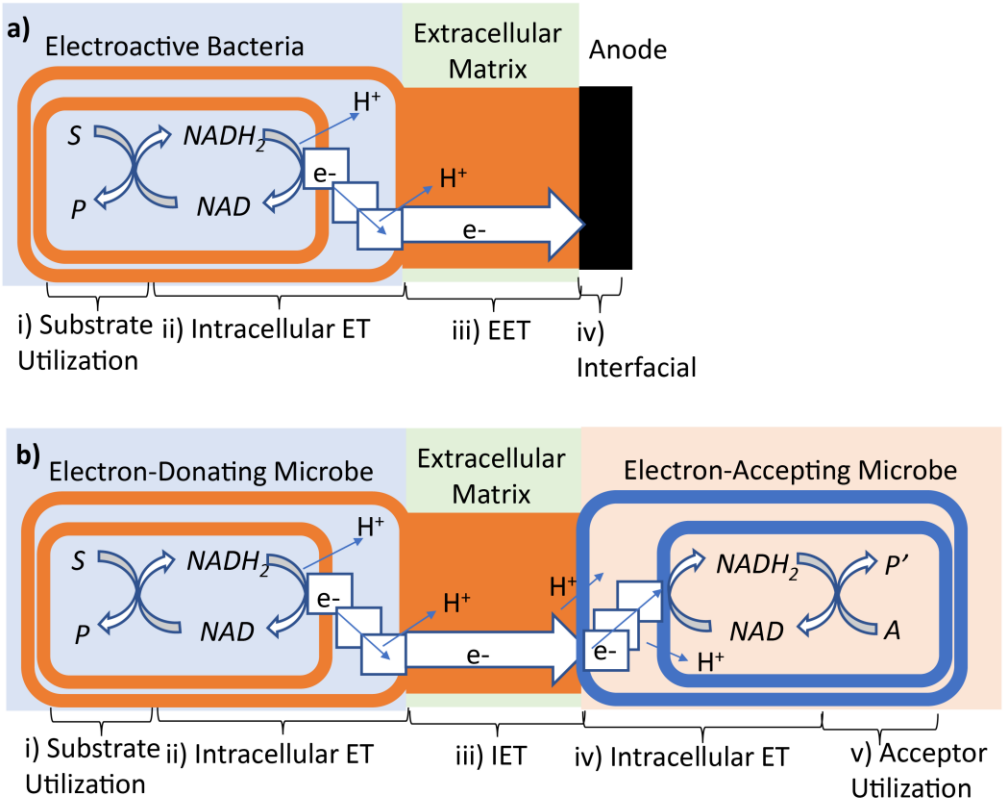

Figure 3: Illustration of processes described in microbial electrochemistry and analogous parts identified in interspecies electron transfer (IET). a) Four processes commonly studied in an electroactive microbe are i) substrate utilization, ii) intracellular electron transfer (ET), iii) extracellular electron transfer (EET), and iv) interfacial electron transfer. b) Analogous processes for IET. The steps for the electron-donating microbe are analogous to those for the electroactive bacteria. For electron-accepting microbe, Step iv) is the intracellular electron transfer reaction which involves internalization of electrons and protons; Step v) is the electron acceptor utilization.

Microbial Kinetics - Two main classes of microbial kinetic models of electroactive bacteria are based on the Nernst-Monod equation and the Butler-Volmer equation (models 1 and 2 in Table 2). The Nernst-Monod equation was derived from the Monod equation by recognizing an analogy between the concentration of the electron acceptor and the activity of electrons [31]. In the Nernst-Monod equation, the potential E represents the characteristic potential of the terminal electron carriers in the cell. The Nernst-Monod equation demonstrates a sigmoidal relationship between the current and potential, which is observed in cyclic voltammograms of many electroactive

bacteria under a low scan rate (v ~ 1 mV/s). Deviations from the sigmoidal relationships are observed under high scan rates (v > 10 mV/s) due to mass transfer limitations (discussed later).

The Butler-Volmer equation is well-established in electrochemistry for describing interfacial reactions. The Butler-Volmer equation assumes that the interfacial electron transfer is reversible and its rate depends on the difference in the electrochemical potential between the anode (E) and electrochemically active species (E^f). Hamelers *et al*. [32] developed an analytical expression describing the interfacial reaction of intracellular mediators by linking the Butler-Volmer equation to the kinetics of substrate utilization. Korth *et al*. [33] expanded the scope of the model by considering a sequence of electron transfer reactions from the substate to NAD/H, intracellular mediators, and extracellular electron acceptor. These models are potentially advantageous for describing the oxidation-reduction states of NAD/H and intracellular cytochromes within the cell [34]. They were also used for the estimation of activation overpotentials in IET [35].

Extracellular Electron Transport – Models for EET describe the rate of electron transfer through the extracellular matrix and the energy dissipated as heat. These models are useful for understanding the extracellular factors limiting the transfer of electrons between microbial partners. The two mechanisms most commonly used to describing EET are metallic-like conduction (MLC) and gradient diffusion (GD) (models 4 and 5 in Table 2). GD is also known as electron-hopping or superexchange mechanism [36]. MLC is proposed to occur in conductive materials produced by microbes (e.g., pili and filaments). MLC is described mathematically using Ohm's law [31,33,37]. In the context of IET, the key parameters limiting conduction are the number of wire-like materials connecting the microbial partners (either pili or filaments) and their conductance. In GD, electrons "hop" across a chain of redox-active compounds, such as cytochromes. A gradient diffusion model has been formulated based on an analogy to conductive polymers [38,39].

For both MLC and GD, the amount of investments in the EET infrastructure is important for sustaining IET. He *et al*. [40] modeled IET using the Monod equation multiplied by a thermodynamic factor (model 3 in Table 2). They found that sustaining DIET in anaerobic methane-oxidizing floc requires approximately 10 pili between microbial partners or 10^{-5} M of extracellular mediators.

Table 2: Summary of model equations for microbial kinetics and EET kinetics that we review. Their potential benefits and drawbacks.

Microbial Kinetics Equations	**Potential Benefits**	**Potential Drawbacks**
1) Nernst-Monod Equation $j = j_m \underbrace{\frac{S}{S+K_S}}_{Monod} \underbrace{\frac{1}{1+\exp\left(-\frac{F}{RT}\left(E-E_{KA}\right)\right)}}_{\text{Nernst-Monod}}$	A simple model with only one parameter, E_{KA}, that accurately captures the behavior of cyclic voltammograms.	The model has not been tested for interspecies electron transfer.
2) Butler-Volmer (BV) $j = j_0 \left\{ \underbrace{\exp\left(\frac{\alpha z F}{RT}\left(E-E^f\right)\right)}_{\text{oxidation}} - \underbrace{\exp\left(\frac{(1-\alpha) z F}{RT}\left(E-E^f\right)\right)}_{\text{reduction}} \right\}$	Models using BV link the intracellular mediator concentration with the rate of electron transfer.	More parameters need to be specified.
3) Jin and Bethke $j = j_0 \underbrace{\frac{S}{S+K}}_{\text{Monod}} \underbrace{\max\left(0.1-\exp\left(-\frac{f_x}{\chi RT}\right)\right)}_{\text{Thermodynamic Factor}}$	Model has been tested with interspecies electron transfer.	Not tested with microbial electrochemistry.
Models for Extracellular Electron Transport	**Potential Benefits**	**Drawbacks**
4) Ohm's law for metallic-like conduction (MLC) $j = -\sigma \frac{dE}{dx}$	A simple model with the biofilm conductivity that	Ohm's law does not explain scan-

	many studies characterize.	rate dependent behaviors
5) Gradient Diffusion (GD) $j_{diff} = \underbrace{-D\frac{dC}{dx}}_{diffusion}$ $j_{mig} = \underbrace{-zDFRTC\frac{dE}{dx}}_{migration}$	Captures some of the non-steady-state behaviors observed in fast-scan voltammetry experiments	none

While the mechanisms for EET is a fascinating research area, the transport of ions can be limiting IET [41]. Models for EET and microbial kinetics can be linked to the Nernst-Planck equations to describe the pH effects on electroactive microbes [42,43].

Kinetics of DIET vs. H_2-MIET

Inefficiencies associated with the IET kinetics can dissipate energy as heat and lower the amount of energy available to be shared between the IET partners. Several notable works have modelled IET kinetics to identify scenarios where DIET and H_2-MIET are non-limiting. In 2014, Cruz Viggi *et al*. [44] introduced theoretical considerations for the comparison of electron flow associated with H_2-MIET vs. conduction-based DIET. Indeed, considering conduction and diffusion equations (models 4 and 5 in Table 2), it is possible to make a rough estimate of rates:

$$diffusion\,rate = A_{cell} \cdot D \cdot \frac{\Delta C}{\Delta x} \cdot n \cdot N_a \quad (4)$$

$$conduction\,rate = A_{cond} \cdot \sigma \cdot \frac{\Delta E}{\Delta x} \cdot \frac{N_a}{F} \quad (5)$$

where the rates are expressed in e-/cell/s, with A_{cell} the surface area of a cell, D the diffusion coefficient of the considered chemical species, $\frac{\Delta C}{\Delta x}$ the concentration gradient of electron carrier

between cells, n the number of electrons per electron carrier, A_{cond} the cross-sectional area of the electron conduit, σ the electrical conductivity of the electron conduit, $\frac{\Delta E}{\Delta x}$ the voltage gradient between cells, N_a the Avogadro constant and F the Faraday constant.

For their calculations, they considered maximal and minimal hydrogen concentrations required for propionate oxidation and methanogenesis respectively, in a similar manner as what is illustrated in Table 1. They then estimated the voltage from the overall reaction of propionate transformation to methane using the Nernst equation. Finally, making few assumptions on the cell shape, magnetite shape and interspecies distance, they made a first rough estimate of the rates using Equations 4 and 5. They came up with a diffusion rate of $2 \cdot 10^{-8}$ nmol H_2/s i.e. $2 \cdot 10^{7}$ e-/cell/s and a conduction rate of $3 \cdot 10^{-5}$ A i.e. $2 \cdot 10^{14}$ e-/cell/s and concluded that DIET had a clear kinetic advantage over MIET.

In 2016, Storck *et al*. [35] proposed a more comprehensive approach with a spatially explicit model of syntrophic associations with either MIET or DIET. For the conduction rate estimation, they refined the electrochemical concepts by accounting for all possible energy losses associated with electron conduction such as activation overpotentials, electrical resistance and ions migration. They estimated a hydrogen diffusion rate of $5 \cdot 10^{3}$ e-/cell/s and a conduction rate of $4 \cdot 10^{4}$ e-/cell/s, quite different from those estimated previously. The discrepancy can be explained by the low conductivity of nanowires compared to magnetite and by concentration and voltage gradients estimated by the spatially explicit approach which are several orders of magnitude lower than the maximal gradient estimated by Cruz Viggi *et al* [44]. They also estimated formate diffusion rate as an alternative mechanism for MIET and found a rate of $3 \cdot 10^{5}$ e-/cell/s showing that similar electron transfer rates for formate-MIET and DIET can be achieved with a slight thermodynamic advantage for DIET. This very thorough model thus clearly showed the importance of taking into account electrochemical phenomena such as activation overpotentials for a correct evaluation of rates in DIET models and paved the way for the mechanistic modeling of DIET.

The last example of IET modeling in a syntrophic association of microbes was recently introduced by He *et al*. [40] for the modeling of AOM. They used a similar approach as the one used by Storck *et al*. [35] and introduced the GD mechanism. Interestingly their conclusion is in line with

those from Storck *et al.* concerning H_2-MIET vs. DIET. Indeed, according to their model the maximal transfer rate associated with H_2-MIET was 10^{-2} fmol CH_4/cell/d i.e. 10^3 e-/cell/s and was considerably lower than those estimated for DIET. DIET rates could indeed reach the highest rates measured in AOM consortia around 10^2 fmol CH_4/cell/d i.e. 10^7 e-/cell/s, but were highly dependent on numerous parameters in the model. They also modeled disulfide transfer as an alternative MIET mechanism and showed that it had similar outcomes as the DIET model with a high range of possible rates depending on parameters.

The recent introduction of electrochemical concepts for the modeling of DIET and comparison with MIET thus already gave interesting clues on the fundamental constraints associated with IET in microbial syntrophic associations. It however also showed that the estimation of true values of biological parameters such as cell-nanowire cofactor electron transfer rates was crucial for accurate predictions [35,40].

Conclusions and perspectives

Studies estimating hydrogen diffusion and electron conduction rates have already given interesting results and confirmed the potential of DIET to increase electron transfer rates in environmental biotechnology. However, they also underscore the importance of experimental studies measuring the physical-chemical properties of biological mechanisms supporting IET. In this regard, experimental approaches such as biocalorimetry have allowed energy capture by microbes to be distinguished from the energy lost to transport and interfacial processes [29]. A comparable approach may be desirable to complement the thermodynamic models for quantitatively understanding the energy gained by each IET partner and the energy lost to electron transfer processes during DIET and MIET. More generally, the experimental study of DIET in bioprocesses requires the development of appropriate characterization methods and strategies. Van Steendam *et al.* [45] recently proposed to combine metaomics, electrochemistry and microscopy techniques to obtain important parameters and data.

Given the potential importance of DIET in AD, the integration of alternative electron transfer mechanisms to H_2-MIET in classical models such as ADM1 would be highly valuable. This may

allow correcting current inconsistencies between commonly used growth yields in ADM1 and energy available for the oxidation of volatile fatty acids [14] and accounting for high rates associated with DIET. Currently only Liu *et al*. [46] have made such a proposal and introduced alternative electron transfer via a pool of redox mediators. It would be interesting to deepen this kind of approach by implementing concepts from microbial electrochemistry (Figure 3 and Table 2).

Beyond AD and syntrophy, IET seems to play an important role in various ecosystems [9,30]. A theoretical framework for the modeling of various IET processes would thus be valuable for many researchers working on mixed microbial communities.

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