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1 **Modeling of interspecies electron transfer in anaerobic microbial communities**

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3

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10

11 **Abstract**

12 Interspecies electron transfer (IET) is a key phenomenon in anaerobic ecosystems, which is
13 traditionally modelled as hydrogen transfer. Recently discovered alternative mediated IET (MIET)
14 or direct IET (DIET) offer exciting alternative mechanisms of microbial partnerships that could
15 lead to new strategies for the improvement of biotechnologies. Here, we analyze mathematical
16 modelling of DIET and MIET in anaerobic ecosystems. Bioenergetics approaches already enable
17 the evaluation of different energy sharing scenarios between microorganisms and give interesting
18 clues on redox mediators and on possible ways of driving microbial communities relying on IET.
19 The modeling of DIET kinetics however is currently only in its infancy. Recent concepts introduced
20 for the modeling of electroactive biofilms should be further exploited. Recent modeling examples
21 confirms the potential of DIET to increase the IET rates compared to H₂-MIET, but also point out
22 the need for additional characterizations of biological components supporting IET to improve
23 predictions.

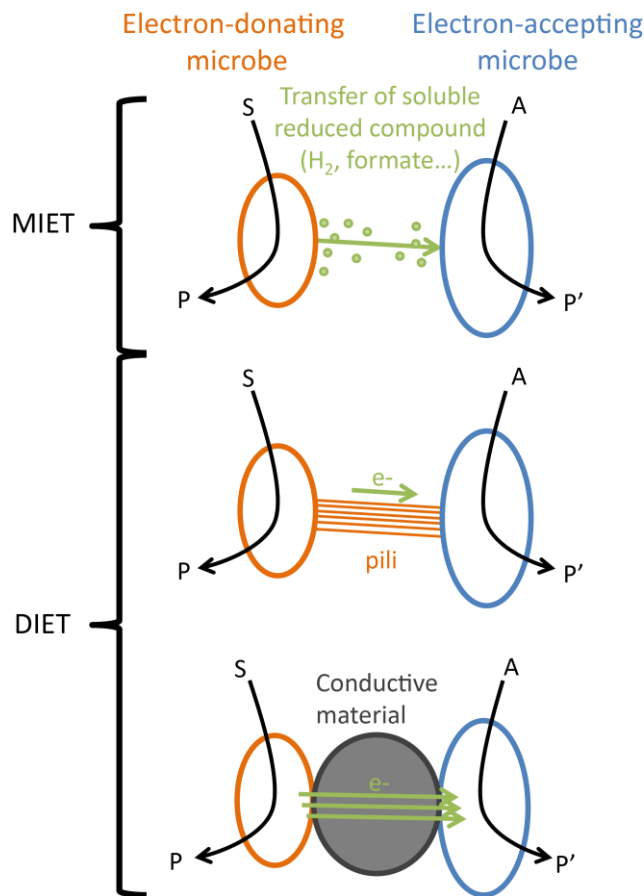
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25 **Introduction**

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

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

36



37

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

42

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

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

57

58 In terms of modeling, MIET and, specifically, interspecies hydrogen transfer is explicitly modeled
59 in the IWA Anaerobic Digestion Model No. 1 (ADM1) [11]. The conversion of small organic
60 molecules such as acetate, propionate, butyrate or ethanol into methane and CO₂ occurs near
61 the thermodynamic equilibrium and thus requires a tight coupling between fermentative and
62 methanogenic metabolisms [12]. H₂-MIET is thus strongly constrained by hydrogen
63 concentrations and plays an important role in AD models that take it into account either using
64 explicit bioenergetics computation [13,14] or by proxy using a kinetic inhibition function (e.g. in
65 ADM1) [11,14].

66

67 Since the discovery of DIET, its importance to AD and its potential to increase and stabilize
68 performances have been widely studied and it is now considered that DIET, rather than MIET,
69 could be the main mechanism of interspecies electron transfer within complex anaerobic microbial
70 communities [15]. It has been shown that conductive materials such as activated carbon [16] or
71 biochar [17] can act as a conduit for electrons between DIET microbial partners and that the
72 addition of conductive materials in a digester can promote the establishment of DIET [18] and
73 increase stability and performances [19]. However, the mechanisms underlying these effects
74 remain unclear. They could indeed be related to the stimulation of DIET but might as well be due
75 to other properties associated with the biofilms forming on the introduced particles. In that context,
76 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 H₂-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 H₂-MIET, the Gibbs free energy of the reactions and thus the energy distribution depends
101 on H₂ partial pressures ($p(\text{H}_2)$). 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 $p(\text{H}_2)$ 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 $2 \cdot 10^{-6} \text{ bar} < p(\text{H}_2) < 2 \cdot 10^{-2} \text{ bar}$ and $-0.41 \text{ V vs SHE} < E_{\text{EM}} < -0.25 \text{ V vs SHE}$,
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, $p(\text{CH}_4) = 10^{-2} \text{ bar}$ and $C = 10^{-2} \text{ mM}$ for soluble compounds. Standard Gibbs free

110 energy values were derived from [23]. ΔG is expressed as a function of hydrogen partial pressure
 111 $p(\text{H}_2)$ in the case of H_2 -MIET and as a function of redox potentials E_{EM} in the case of IET via an
 112 electron mediator (EM-IET).

Process	Type of IET	Reaction	ΔG (kJ.mol ⁻¹)
Ethanol oxidation	H_2 -MIET	$\text{ethanol} + \text{H}_2\text{O} \rightarrow \text{acetate}^- + 2 \cdot \text{H}_2 + \text{H}^+$	$8.3 + 2 \cdot R \cdot T \cdot \ln(p(\text{H}_2))$
	EM-IET	$\text{ethanol} + 4 \cdot \text{EM} + \text{H}_2\text{O} \rightarrow \text{acetate}^- + 4 \cdot \text{EM}^- + 5 \cdot \text{H}^+$	$-156.8 - 4 \cdot F \cdot E_{\text{EM}}$
Methanogenesis	H_2 -MIET	$\text{HCO}_3^- + 4 \cdot \text{H}_2 + \text{H}^+ \rightarrow \text{CH}_4 + 3 \cdot \text{H}_2\text{O}$	$-134.1 - 4 \cdot R \cdot T \cdot \ln(p(\text{H}_2))$
	EM-IET	$\text{HCO}_3^- + 8 \cdot \text{EM}^- + 9 \cdot \text{H}^+ \rightarrow \text{CH}_4 + 8 \cdot \text{EM} + 3 \cdot \text{H}_2\text{O}$	$196.1 + 8 \cdot F \cdot E_{\text{EM}}$
Overall process	H_2 -MIET or EM-IET	$\text{ethanol} + \frac{1}{2} \cdot \text{HCO}_3^- \rightarrow \text{acetate}^- + \frac{1}{2} \cdot \text{H}^+ + \frac{1}{2} \cdot \text{CH}_4 + \frac{1}{2} \cdot \text{H}_2\text{O}$	-58.8

113
 114
 115 The ranges estimated with this first approach are quite large; however, when $p(\text{H}_2)$ or E_{EM} is close
 116 to the extreme values of these ranges, it means that one organism gets all the energy and the
 117 other none. This approach can then be extended to get a more precise evaluation of ranges taking
 118 into account realistic growth yields in cocultures [21,22] using the Gibbs energy dissipation
 119 method [23]. This method considers the Gibbs energy balance of the metabolism, which consists
 120 of the macroscopic equations for catabolism and anabolism:

121
 122
$$\Delta G_{\text{metabolism}} = \Delta G_{\text{anabolism}} + \lambda \cdot \Delta G_{\text{catabolism}} = -\Delta G_{\text{dissipated}} \quad (1)$$

123
 124 where λ is a coupling parameter indicating how many times the catabolism reaction needs to be
 125 carried out to sustain one anabolic reaction. It is worth mentioning that the value for λ is dependent
 126 on the formalism used to describe catabolism and anabolism. Yet, once such formalism is defined,
 127 a unique λ value can be calculated from experimental growth yields [23].

128
 129 In Equation 1, the remaining energy generated by catabolism that was not used to support
 130 anabolism is considered to be dissipated by cells. Heijnen [23,24] found an empirical relationship
 131 to estimate $\Delta G_{\text{dissipated}}$ based on the carbon source characteristics only:

132
 133
$$\Delta G_{\text{dissipated}} = 200 + 18 \cdot (6 - N_C)^{1.8} + \exp((3.6 + 0.4 \cdot N_C) \cdot (-0.2 - \gamma)^{0.32}) \quad (2)$$

134

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

136

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

143

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

148

$$149 \Delta G^\circ = \sum_{i \neq y} \nu_i \cdot G^\circ_f(\text{product}_i) - \sum_{j \neq z} \nu_j \cdot G^\circ_f(\text{reactant}_j) - \nu_{EM} \cdot F \cdot E^\circ_{EM} \quad (3)$$

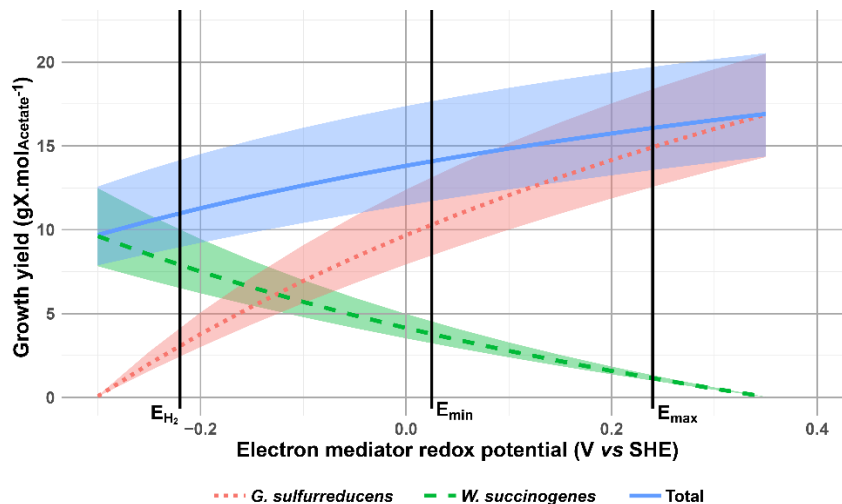
150

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

154

155 An application of this method is pictured in Figure 2 (calculations provided in Supplementary
156 material 2), where a coculture of *Geobacter sulfurreducens* and *Wolinella succinogenes*,
157 experimentally studied by Cord-Ruwisch *et al.* [25], was analyzed to determine E_{EM} . The
158 dissipation method clearly highlights that H_2 -mediated IET ($E = -0.22$ V vs SHE at $\text{pH}_2 = 0.02$ Pa
159 and $\text{pH} = 7$) could not explain the experimental growth yields, as already pointed out in the original
160 paper. Kaden *et al.* later proved that IET in this coculture was dependent on the addition of
161 cystine/cysteine to the medium and concluded that this couple acted as redox mediator [26].
162 However, the authors calculated that the cystine/cysteine couple during this experiment had a
163 potential of about -200 mV vs SHE. Such potential would result in an energy partition between *G.*
164 *sulfurreducens* and *W. succinogenes* similar to what would have been observed for an H_2 -
165 mediated IET and is therefore implausible. Instead, the dissipation method predicts that E_{EM} was
166 likely between +25 and +240 mV vs SHE. Thus, it is probable that either (i) the cystine/cysteine

167 ratio was very high, thus increasing the actual redox potential of the couple, or (ii) a cysteine
168 derivative having a high redox potential acted as electron shuttle rather than cysteine *per se*.
169
170



171
172 **Figure 2: Theoretical growth yield of *Geobacter sulfurreducens* and *Wolinella***
173 ***succinogenes* interacting through IET.** The electron donor and acceptor are acetate and nitrate,
174 respectively. Calculations were carried out assuming pH = 7, a temperature of 30°C and a
175 concentration of 10 mM for all soluble species (see Supplementary material 2). For each curve,
176 the transparent area provides the uncertainty related to a 30% relative prediction error for the
177 dissipated energy ($\Delta G_{\text{dissipated}}$) [24]. E_{H₂} corresponds to the special case where the electron
178 mediator is H₂ and p_{H₂} = 0.02 Pa (E = - 0.22 V vs SHE), as measured in [25]. E_{min} (25 mV) and
179 E_{max} (240 mV) correspond to situations where *G. sulfurreducens* growth yield is 4 to 9 times higher
180 than the one of *W. succinogenes*, as experimentally measured [25]. The total biomass yield
181 predicted for potentials between E_{min} and E_{max} (14.1 to 16.1 ± 3.0 gX.mol_{Acetate}⁻¹) is consistent with
182 the value of 18.5 ± 3.2 gX.mol_{Acetate}⁻¹ measured experimentally [25].

183
184 The dissipation method can also be combined with kinetic models to better estimate growth yields.
185 However, this method suffers from two main limitations: (i) this method implicitly assumes that all
186 the redox gradient available to each species is coupled to energy conservation, which may not be
187 the case in reality [27–29] and (ii) Heijnen’s correlation is only valid for non-inhibitory conditions
188 and does not account for cell maintenance or biological regulations that would reduce growth
189 yields [20]. Thus, this method will provide maximum growth yield and is more likely to provide fair
190 electron mediator redox potential for syntrophic rather than non-mutualistic IET [30].

191

192 **IET kinetics: introducing concepts from electrochemistry**

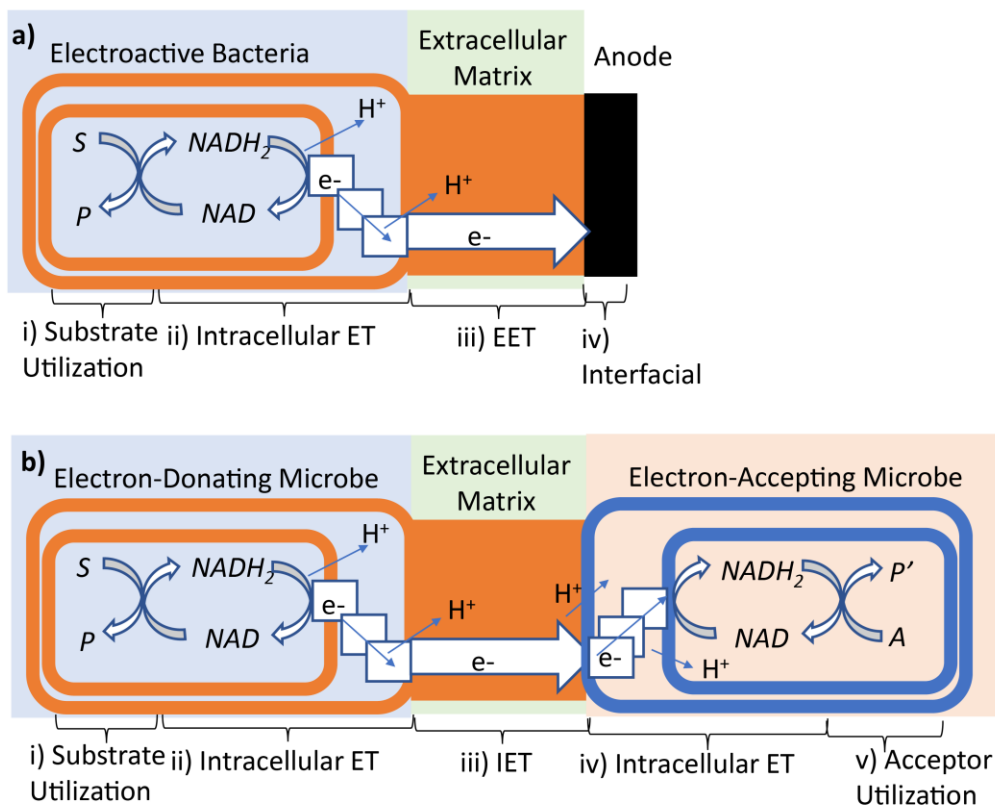
193

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

199

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

208



209
 210 **Figure 3: Illustration of processes described in microbial electrochemistry and analogous**
 211 **parts identified in interspecies electron transfer (IET).** a) Four processes commonly studied
 212 in an electroactive microbe are i) substrate utilization, ii) intracellular electron transfer (ET), iii)
 213 extracellular electron transfer (EET), and iv) interfacial electron transfer. b) Analogous processes
 214 for IET. The steps for the electron-donating microbe are analogous to those for the electroactive
 215 bacteria. For electron-accepting microbe, Step iv) is the intracellular electron transfer reaction
 216 which involves internalization of electrons and protons; Step v) is the electron acceptor utilization.

217
 218 *Microbial Kinetics* - Two main classes of microbial kinetic models of electroactive bacteria are
 219 based on the Nernst-Monod equation and the Butler-Volmer equation (models 1 and 2 in Table
 220 2). The Nernst-Monod equation was derived from the Monod equation by recognizing an analogy
 221 between the concentration of the electron acceptor and the activity of electrons [31]. In the Nernst-
 222 Monod equation, the potential E represents the characteristic potential of the terminal electron
 223 carriers in the cell. The Nernst-Monod equation demonstrates a sigmoidal relationship between
 224 the current and potential, which is observed in cyclic voltammograms of many electroactive

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

227
228 The Butler-Volmer equation is well-established in electrochemistry for describing interfacial
229 reactions. The Butler-Volmer equation assumes that the interfacial electron transfer is reversible
230 and its rate depends on the difference in the electrochemical potential between the anode (E) and
231 electrochemically active species (E^{\ominus}). Hamelers *et al.* [32] developed an analytical expression
232 describing the interfacial reaction of intracellular mediators by linking the Butler-Volmer equation
233 to the kinetics of substrate utilization. Korth *et al.* [33] expanded the scope of the model by
234 considering a sequence of electron transfer reactions from the substrate to NAD/H, intracellular
235 mediators, and extracellular electron acceptor. These models are potentially advantageous for
236 describing the oxidation-reduction states of NAD/H and intracellular cytochromes within the cell
237 [34]. They were also used for the estimation of activation overpotentials in IET [35].

238
239 *Extracellular Electron Transport* – Models for EET describe the rate of electron transfer through
240 the extracellular matrix and the energy dissipated as heat. These models are useful for
241 understanding the extracellular factors limiting the transfer of electrons between microbial
242 partners. The two mechanisms most commonly used to describing EET are metallic-like
243 conduction (MLC) and gradient diffusion (GD) (models 4 and 5 in Table 2). GD is also known as
244 electron-hopping or superexchange mechanism [36]. MLC is proposed to occur in conductive
245 materials produced by microbes (e.g., pili and filaments). MLC is described mathematically using
246 Ohm's law [31,33,37]. In the context of IET, the key parameters limiting conduction are the number
247 of wire-like materials connecting the microbial partners (either pili or filaments) and their
248 conductance. In GD, electrons “hop” across a chain of redox-active compounds, such as
249 cytochromes. A gradient diffusion model has been formulated based on an analogy to conductive
250 polymers [38,39].

251
252 For both MLC and GD, the amount of investments in the EET infrastructure is important for
253 sustaining IET. He *et al.* [40] modeled IET using the Monod equation multiplied by a
254 thermodynamic factor (model 3 in Table 2). They found that sustaining DIET in anaerobic
255 methane-oxidizing floc requires approximately 10 pili between microbial partners or 10^{-5} M of
256 extracellular mediators.

257

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

Microbial Kinetics Equations	Potential Benefits	Potential Drawbacks
1) Nernst-Monod Equation $j = j_m \underbrace{\frac{S}{S + K_s}}_{\text{Monod}} \underbrace{\frac{1}{1 + \exp\left(-\frac{F}{RT}(E - E_{KA})\right)}}_{\text{Nernst-Monod}}$	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) $j = j_0 \left\{ \underbrace{\exp\left(\frac{\alpha z F}{RT}(E - E^f)\right)}_{\text{oxidation}} - \underbrace{\exp\left(\frac{(1-\alpha) z F}{RT}(E - E^f)\right)}_{\text{reduction}} \right\}$	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) $j_{diff} = -D \underbrace{\frac{dC}{dx}}_{diffusion}$ $j_{mig} = -zDFRTC \underbrace{\frac{dE}{dx}}_{migration}$	Captures some of the non- steady-state behaviors observed in fast- scan voltammetry experiments	none

260

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

264

265 **Kinetics of DIET vs. H₂-MIET**

266

267 Inefficiencies associated with the IET kinetics can dissipate energy as heat and lower the amount
 268 of energy available to be shared between the IET partners. Several notable works have modelled
 269 IET kinetics to identify scenarios where DIET and H₂-MIET are non-limiting. In 2014, Cruz Viggi
 270 *et al.* [44] introduced theoretical considerations for the comparison of electron flow associated
 271 with H₂-MIET vs. conduction-based DIET. Indeed, considering conduction and diffusion equations
 272 (models 4 and 5 in Table 2), it is possible to make a rough estimate of rates:

273

274 $diffusion\ rate = A_{cell} \cdot D \cdot \frac{\Delta C}{\Delta x} \cdot n \cdot N_a$ (4)

275 $conduction\ rate = A_{cond} \cdot \sigma \cdot \frac{\Delta E}{\Delta x} \cdot \frac{N_a}{F}$ (5)

276

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

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

282

283 For their calculations, they considered maximal and minimal hydrogen concentrations required
284 for propionate oxidation and methanogenesis respectively, in a similar manner as what is
285 illustrated in Table 1. They then estimated the voltage from the overall reaction of propionate
286 transformation to methane using the Nernst equation. Finally, making few assumptions on the cell
287 shape, magnetite shape and interspecies distance, they made a first rough estimate of the rates
288 using Equations 4 and 5. They came up with a diffusion rate of $2 \cdot 10^{-8}$ nmol H₂/s i.e. $2 \cdot 10^7$ e-/cell/s
289 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
290 advantage over MIET.

291

292 In 2016, Storck *et al.* [35] proposed a more comprehensive approach with a spatially explicit model
293 of syntrophic associations with either MIET or DIET. For the conduction rate estimation, they
294 refined the electrochemical concepts by accounting for all possible energy losses associated with
295 electron conduction such as activation overpotentials, electrical resistance and ions migration.
296 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,
297 quite different from those estimated previously. The discrepancy can be explained by the low
298 conductivity of nanowires compared to magnetite and by concentration and voltage gradients
299 estimated by the spatially explicit approach which are several orders of magnitude lower than the
300 maximal gradient estimated by Cruz Viggi *et al.* [44]. They also estimated formate diffusion rate
301 as an alternative mechanism for MIET and found a rate of $3 \cdot 10^5$ e-/cell/s showing that similar
302 electron transfer rates for formate-MIET and DIET can be achieved with a slight thermodynamic
303 advantage for DIET. This very thorough model thus clearly showed the importance of taking into
304 account electrochemical phenomena such as activation overpotentials for a correct evaluation of
305 rates in DIET models and paved the way for the mechanistic modeling of DIET.

306

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

310 those from Storck *et al.* concerning H₂-MIET vs. DIET. Indeed, according to their model the
311 maximal transfer rate associated with H₂-MIET was 10⁻² fmol CH₄/cell/d i.e. 10³ e-/cell/s and was
312 considerably lower than those estimated for DIET. DIET rates could indeed reach the highest
313 rates measured in AOM consortia around 10² fmol CH₄/cell/d i.e. 10⁷ e-/cell/s, but were highly
314 dependent on numerous parameters in the model. They also modeled disulfide transfer as an
315 alternative MIET mechanism and showed that it had similar outcomes as the DIET model with a
316 high range of possible rates depending on parameters.

317

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

323

324 **Conclusions and perspectives**

325

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

338

339 Given the potential importance of DIET in AD, the integration of alternative electron transfer
340 mechanisms to H₂-MIET in classical models such as ADM1 would be highly valuable. This may

341 allow correcting current inconsistencies between commonly used growth yields in ADM1 and
342 energy available for the oxidation of volatile fatty acids [14] and accounting for high rates
343 associated with DIET. Currently only Liu *et al.* [46] have made such a proposal and introduced
344 alternative electron transfer via a pool of redox mediators. It would be interesting to deepen this
345 kind of approach by implementing concepts from microbial electrochemistry (Figure 3 and Table
346 2).

347
348 Beyond AD and syntrophy, IET seems to play an important role in various ecosystems [9,30]. A
349 theoretical framework for the modeling of various IET processes would thus be valuable for many
350 researchers working on mixed microbial communities.

351

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353

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359

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