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CORRESPONDENCE

Thermodynamic constraints on the window of opportunity for direct interspecies electron transfer (DIET)

Syntrophy plays a pivotal role in methanogenic bioreactors, where methanogens depend on other organisms to furnish the substrates they live off, while many of the feeder organisms in turn depend on methanogens as acceptors of the protons and electrons they produce (Schink, 1997; Zhu et al., 2020). Bryant et al. (1967) were the first to describe this obligate interaction and famously invoked thermodynamics to rationalize its implications. Based on the tenet that the protons and electrons produced by the syntrophs combine to form H_2, interspecies hydrogen transfer (IHT) (Ianotti et al., 1973) was subsequently considered the main mechanism underpinning syntrophy, with thermodynamics constraining the range of hydrogen levels at which both the hydrogen producing and the hydrogen consuming reaction would be exergonic ($\Delta G < 0$) (Dolfing, 1987; Dolfing et al., 2008; Schink, 1997).

The discovery of direct interspecies electron transfer (DIET) (Lovley, 2017) and its importance in methanogenic bioreactors (Lovley & Holmes, 2022) adds a new dimension to the window of opportunity paradigm as its asks for an additional step in the calculations. For DIET, calculating the range of reduction potentials at which both the electron producing and the electron consuming partner organisms can obtain energy starts with working out the Gibbs free energy change (ΔG) for the electron-producing and energy-consuming reactions. The additional step is to convert the outcomes to reduction potentials via the equality $\Delta G = nF\ \Delta E$ (Dolfing, 2015).

DIET- and IHT-based windows of opportunity are conceptually similar. In DIET-based syntrophy, electron activity is the master variable; in IHT-based syntrophy, H_2 activities set the window. However, there is a subtle difference between the two. H_2 levels around the cells are essentially uniform, that is everywhere at its cell envelope a cell experiences the same H_2 level. In DIET, on the other hand, reduction potentials at the cell envelope may well be more variable. Electron-producing organisms release electrons at different levels depending on where in their metabolism the electrons are generated. The reduction potential of these electrons are different from the averaged value stipulated in the above equality. Thus, the DIET window of opportunity is currently poorly constrained.

The above discussion begs the question whether pili in a given DIET-based syntrophy (Lovley, 2017; Lovley & Holmes, 2022) all operate at the same reduction potential or have individually distinct potentials connecting specific outlets at the electron-producing organisms with specific inlets at the electron-accepting organisms. The alternative would be a cloud-like random network of pili all poised at essentially the same reduction potential. Tailor-made measuring nano-sized devices more sophisticated than the ones currently available are needed to tackle those questions and the thermodynamic framework underlying them.

The current methodology does not yet operate at that level of detail. To study the effects of poised electrodes on the flow of electrons in an ethanol-fed DIET-based syntrophic culture of *Geobacter metallireducens* and *Methanosarcina barkeri*, Yee et al. (2024) for example use cm-sized electrodes. As to be expected, as the electrode potential was set at −700 mV, id est well outside the window of opportunity for ethanol-based methanogenesis, the authors observe a detrimental effect of electrochemical stimulation on the consortium, including disruption of interspecies electron transfer and induction of H_2 accumulation. Unfortunately, the thermodynamics invoked to analyse and rationalize the findings was flawed. The authors state correctly that ethanol to methane has a standard Gibbs change of −91.6 kJ/mol. Using Hanselmann's free energy and enthalpy values gives a minor change to −94.5 kJ/mol at 37°C (Dolfing, 2015; Hanselmann, 1991). Yet, somehow the authors estimate values of −445 and −263 kJ for their reactors. Similarly, their figure 3 indicates a huge impact of the acetate concentration on the energy change for acetoclastic methanogenesis. However, a 10-fold change would translate in an effect of 5.9 kJ/mol at 37°C (5.7 at 25°C). The changes in Yee et al. (2024) figure 3 are much larger.

In future endeavours, it will be interesting to evaluate to what extent poising electrodes at a voltage within the DIET window of opportunity affects the activities of and electron fluxes between the syntrophs and the

methanogens in DIET-based communities. It is tempting to speculate that this approach may even allow calibration of the window of opportunity based on in situ measurements. The interdigitated electrode array (IDA) described by Snider et al. (2012) could be instrumental in such work.

AUTHOR CONTRIBUTIONS

Jan Dolfing: Conceptualization; writing – original draft.

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CONFLICT OF INTEREST STATEMENT

The author declares no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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