Extracellular Electron Uptake by Acetogenic Bacteria: Does H₂ Consumption Favor the H₂ Evolution Reaction on a Cathode or Metallic Iron?

Jo Philips*

Department of Engineering, Aarhus University, Aarhus, Denmark

Some acetogenic bacteria are capable of using solid electron donors, such as a cathode or metallic iron [Fe(0)]. Acetogens using a cathode as electron donor are of interest for novel applications such as microbial electrosynthesis, while microorganisms using Fe(0) as electron donor cause detrimental microbial induced corrosion. The capacity to use solid electron donors strongly differs between acetogenic strains, which likely relates to their extracellular electron transfer (EET) mechanism. Different EET mechanisms have been proposed for acetogenic bacteria, including a direct mechanism and a H₂ dependent indirect mechanism combined with extracellular hydrogenases catalyzing the H₂ evolution reaction on the cathode or Fe(0) surface. Interestingly, low H₂ partial pressures often prevail during acetogenesis with solid electron donors. Hence, an additional mechanism is here proposed: the maintenance of low H₂ partial pressures by microbial H₂ consumption, which thermodynamically favors the H₂ evolution reaction on the cathode or Fe(0) surface. This work elaborates how the H₂ partial pressure affects the H₂ evolution onset potential and the H₂ evolution rate on a cathode, as well as the free energy change of the anoxic corrosion reaction. In addition, the H₂ consumption characteristics, i.e., H₂ threshold (thermodynamic limit for H₂ consumption) and H₂ consumption kinetic parameters, of acetogenic bacteria are reviewed and evidence is discussed for strongly different H₂ consumption characteristics. Different acetogenic strains are thus expected to maintain different H₂ partial pressures on a cathode or Fe(0) surface, while those that maintain lower H₂ partial pressures (lower H₂ threshold, higher H₂ affinity) more strongly increase the H₂ evolution reaction. Consequently, I hypothesize that the different capacities of acetogenic bacteria to use solid electron donors are related to differences in their H₂ consumption characteristics. The focus of this work is on acetogenic bacteria, but similar considerations are likely also relevant for other hydrogenotrophic microorganisms.

Keywords: acetogenesis, extracellular electron transfer mechanisms, energy conservation, ATP gain, Butler-Volmer equation, zero valent iron, biocathode, HER reaction
INTRODUCTION

Acetogenic bacteria are a phylogenetically diverse group of microorganisms that share a unique metabolism for energy conservation and carbon fixation, i.e., the Wood–Ljungdahl pathway (Drake et al., 2008). This pathway reduces the electron acceptor CO₂ with the electron donor H₂ to acetyl-CoA for carbon fixation or to acetate or other organic compounds (e.g., ethanol) for energy conservation:

\[
2 \text{CO}_2 + 4 \text{H}_2 \rightarrow \text{CH}_3\text{COO}^- + \text{H}^+ + 2 \text{H}_2\text{O} \quad \text{(Reaction 1)}
\]

\[
\Delta G^0_{\text{acetogenesis}} = -55.8 \text{kJ} \cdot \text{mol}^{-1}
\]

With \(\Delta G^0_{\text{acetogenesis}}\), the Gibbs free energy change of acetogenesis in standard conditions (pH 0, all concentrations 1 M and all partial pressures 1 atm), in contrast to \(\Delta G^0_{\text{acetogenesis}}\) (~95.6 kJ mol⁻¹) in physiological standard conditions (pH 7).

Intriguingly, some acetogens can use solid electron donors, including a cathode (Nevin et al., 2010, 2011), metallic iron [Fe(0)] (Kato et al., 2015; Philips et al., 2019) and possibly reduced minerals. The use of a cathode as electron donor is of high interest for the development of innovative bioelectrochemical technologies. Microbial electro-osynthesis, for instance, is a promising process for the conversion of excess renewable electricity and CO₂ into biofuels or other valuable organic compounds using acetogenic bacteria as biocatalysts (Rabaey and Rozendal, 2010; Lovley and Nevin, 2013). In contrast, microorganisms using Fe(0) as electron donor cause microbial induced corrosion, resulting in severe damage to steel infrastructure (Enning and Garrelfs, 2014). Finally, the oxidation of reduced minerals by acetogens could have a still unknown impact on global biogeochemical cycles.

Not all tested acetogens are capable of using a cathode or Fe(0) as electron donor (Table 1). The highest electron uptake rates from cathodes have been reported for Sporomusa ovata strains (Nevin et al., 2010; Aryal et al., 2017). In contrast, the well-studied strain Acetobacterium woodii is not capable of withdrawing electrons from cathodes poised at a potential of ~0.4 V vs. Standard Hydrogen Electrode (SHE) (potential slightly more positive than the standard potential for H₂ evolution at pH 7, see calculations below). At more negative cathode potentials (~<−0.6 V vs. SHE), almost all tested acetogenic strains withdraw cathodic electrons, with the exception of Sporomusa aerivorans (Table 1). Acetogenic communities enriched on cathodes (potentials usually ≤~−0.6 V vs. SHE) are often dominated by Acetobacterium species (Table 2). Only one study has performed a metagenome analysis to identify their cathode-dominating acetogen and found a Acetobacterium wieringae strain (Marshall et al., 2017). Interestingly, similar acetogenic genera are found in enrichments using Fe(0) as electron donor (Table 2) and acetogenic strains related to Sporomusa sphaeroides and A. wieringae have been isolated with Fe(0) (Kato et al., 2015; Philips et al., 2019; Table 1). Moreover, a limited to no Fe(0) corrosion enhancement was found for A. woodii (Table 1). Consequently, acetogenic strains likely use a similar mechanism to withdraw extracellular electrons from a cathode as from Fe(0), while not all acetogens have such a mechanism.

This work first reviews the different extracellular electron transfer (EET) mechanisms that have been proposed for acetogenic bacteria. Next, an additional EET mechanism is proposed: the maintenance of low H₂ partial pressures by H₂ consumption, favoring H₂ evolution by the cathode or Fe(0) surface. The H₂ consumption characteristics of acetogens are further described using thermodynamic and kinetic calculations and a literature review. Finally, this work hypothesizes that the

**TABLE 1** Overview of acetogenic strains and their capacity to withdraw electrons from Fe(0) or a cathode.

| Strain                     | Fe(0)       | Cathode                |
|----------------------------|-------------|------------------------|
| Acetobacterium carbinolicum| No (Kato et al., 2019) | n.d.                   |
| Acetobacterium malicum     | Yes (Philips et al., 2019) | n.d.                   |
| Acetobacterium woodii      | Yes (Philips et al., 2019), No (Mand et al., 2014; Kato et al., 2015) | 0.4 V: Yes (Nevin et al., 2011), −0.71 V: Yes (Arends, 2013) |
| Clostridium acetici        | n.d.        | −0.4 V: Yes (Nevin et al., 2011) |
| Clostridium ljungdahliii   | n.d.        | −0.4 V: Yes (Nevin et al., 2011), No (personal communication Miriam Rosenbaum), −0.7 V: Yes (personal communication Miriam Rosenbaum; Bajracharya et al., 2015) |
| Moorella thermoacetica     | n.d.        | −0.3 V: Yes (Faraghiparapari and Zengler, 2017), −0.4 V: Yes (Nevin et al., 2011) |
| Moorella thermoautotrophica| n.d.        | −0.3 V: Yes (Faraghiparapari and Zengler, 2017), −0.4 V: Yes (Yu et al., 2017) |
| Sporomusa acidivorans      | n.d.        | −0.69 V: Yes (Aryal et al., 2017) |
| Sporomusa aerivorans       | n.d.        | −0.69 V: Yes (Aryal et al., 2017) |
| Sporomusa malonica         | n.d.        | −0.69 V: Yes (Aryal et al., 2017) |
| Sporomusa ovata            | No (Kato et al., 2015) | −0.3 V: Yes (Faraghiparapari and Zengler, 2017), −0.4 V: Yes (Nevin et al., 2010), −0.69 V: Yes (Aryal et al., 2017) |
| Sporomusa silvacetica      | n.d.        | −0.4 V: Yes (Nevin et al., 2011) |
| Sporomusa sphaeroides      | Yes (Kato et al., 2015; Philips et al., 2019) | −0.4 V: Yes (Nevin et al., 2011), −0.5 V: Yes (Deutzmann et al., 2015) |
| Thermoanaerobacter kivui   | n.d.        | −0.3 V: No (Faraghiparapari and Zengler, 2017) |

The cathode potential (expressed vs. SHE) at which the electron uptake from a cathode was tested is indicated. n.d., not yet determined.
different capacities of acetogenic bacteria to use solid electron donors are related to differences in their H\textsubscript{2} consumption characteristics. This work mainly focuses on acetogenic bacteria, but similar considerations are likely also valid for other hydrogenotrophic microorganisms.

### EXTRACELLULAR ELECTRON TRANSFER MECHANISMS OF ACETOGENS

An overview of the different EET mechanisms that have been proposed for acetogenic bacteria is shown in Figure 1. Direct EET (Figure 1A) is a mechanism that is well-studied in microorganisms using solid electron acceptors, as for instance *Geobacter* spp. (Philips et al., 2016). Other microorganisms, e.g., *Acidithiobacillus ferrooxidans*, use a direct EET mechanism to withdraw electrons from solid electron donors (Valdes et al., 2008). A direct EET mechanism typically involves outer-membrane bound cytochromes, transporting extracellular electrons from the inside to the outside of the cell or the other way around (Philips et al., 2016). A direct extracellular electron uptake has been proposed for acetogenic bacteria (Nevin et al., 2011; Kato et al., 2015), but clear evidence is still lacking. Moreover, *Moorella* and *Sporomusa* spp. have cytochromes, but most other acetogens have not (Moller et al., 1984; Schuchmann and Müller, 2014).

Some microorganisms excrete redox shuttles to mediate EET (Figure 1B). *Shewanella oneidensis* and *Pseudomonas aeruginosa*, for instance, use respectively flavins and phenazines to mediate the transport of electrons to an anode (Philips et al., 2016). Artificial mediators have been applied to improve the EET of acetogens from cathodes (Song et al., 2011), but acetogens were often not found to excrete redox mediators to mediate EET from Fe(0) (Philips et al., 2019).

Another possibility is an indirect EET mechanism relying on the evolution of H\textsubscript{2} on the cathode or Fe(0). All acetogens are capable of using H\textsubscript{2} as electron donor. In addition, a cathode at a sufficiently low potential (calculated in detail below) generates H\textsubscript{2} through proton reduction:

\[
2 \text{H}^+ + 2 e^- \rightarrow \text{H}_2 \quad (\text{Reaction} \ 2)
\]

Moreover, the presence of Fe(0) in anoxic conditions always leads to H\textsubscript{2} generation through the anoxic corrosion reaction:

\[
\text{Fe}(0) + 2 \text{H}^+ \leftrightarrow \text{Fe}^{2+} + \text{H}_2 \quad (\text{Reaction} \ 3)
\]

Nevertheless, an indirect EET mechanism depending on H\textsubscript{2} has often been disregarded, because the acetate levels in biological treatments are often higher than can be explained by the H\textsubscript{2} levels in abiotic controls. For instance, no H\textsubscript{2} evolution was recorded for an abiotic cathode poised at −0.4 V vs. SHE, while several acetogenic strains consumed current and produced acetate at the same potential (Nevin et al., 2011). Similarly, some acetogens have a higher acetate production rate with Fe(0) as electron donor than can be explained just by chemically generated H\textsubscript{2} (Kato et al., 2015; Philips et al., 2019). Consequently, an H\textsubscript{2}-dependent EET mechanism can only explain the extracellular electron uptake by acetogens, if the microorganisms somehow increase the H\textsubscript{2} evolution on the cathode or Fe(0) surface.

Acetogens could increase H\textsubscript{2} evolution on a cathode or Fe(0) through a syntrophic association with an electrotrophic microorganism, which produces H\textsubscript{2} using a direct EET mechanism (Figure 1C). Such a mechanism was proposed for a cathodic microbial community dominated by *Acetobacterium* and *Desulfovibrio* spp. (Marshall et al., 2017). In addition, acetate production by *A. woodii* on a cathode poised at −0.4 V vs. SHE was facilitated through H\textsubscript{2} generation by strain IS4 (Deutzmann and Spormann, 2017), i.e., a sulfate reducer isolated with Fe(0) as electron donor (Dinh et al., 2004) and possibly using cytochromes for a direct EET (Beece-Vasbender et al., 2015).

Interestingly, some acetogenic strains can increase H\textsubscript{2} evolution on a cathode or Fe(0), also without a syntrophic partner. Deutzmann et al. (2015) found that cell-free spent medium of *S. sphaeroides* increased the H\textsubscript{2} evolution rate on a cathode poised at −0.5 V vs. SHE, while similar results were reported with Fe(0) for *Sporomusa* and *Acetobacterium* strains (Philips et al., 2019). Tremblay et al. (2019) detected H\textsubscript{2} already at a cathode potential of −0.3 V vs. SHE with cell-free spent medium of *S. ovata*, while H\textsubscript{2} could only be detected at −0.5 V vs. SHE in fresh medium. Deutzmann et al. (2015) suggested that spent medium contains extracellular enzymes, such as hydrogenases, that absorb on the cathode or Fe(0) surface and catalyze the H\textsubscript{2} evolution reaction (Figure 1D). For the methanogen *Methanococcus maripaludis*,

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TABLE 2 | (Putative) acetogenic genera in acetogenic enrichments on Fe\textsuperscript{0} or cathodes.

| Solid electron donor | (Putative) acetogenic genera | References |
|----------------------|-------------------------------|------------|
| Fe(0)                | Acetobacterium                | Mand et al., 2014 |
| Fe(0)                | Sporomusa, Clostridium        | Kato et al., 2015 |
| Fe(0)                | Acetobacterium, Sporomusa, Clostridium, Acetoanaerobium | Philips et al., 2019 |
| Cathode (−0.6 V)     | Acetobacterium                | Marshall et al., 2012, 2013; LaBelle et al., 2014 |
| Cathode (−0.7 V)     | Acetobacterium                | Su et al., 2013 |
| Cathode (−0.85 V)    | Acetobacterium                | Jourdin et al., 2016 |
| Cathode (−1.0 V)     | Acetobacterium                | Patil et al., 2015; Arends et al., 2017 |
| Cathode (−1.0 V)     | Acetobacterium, Acetoanaerobium | Xafénias and Mapelli, 2014 |
| Cathode (−0.65 V)    | Acetobacterium                | Saheb-Alam et al., 2018 |

Cathode potentials are expressed vs. SHE.
a heterodisulfide reductase supercomplex was isolated, which catalyzes the reduction of CO₂ to formate at a cathode and Fe(0) surface (Lienemann et al., 2018). In addition, this methanogen excretes a [NiFe] hydrogenase to stimulate the anoxic corrosion reaction (Reaction 3) (Tsurumaru et al., 2018). So far, the H₂ excretes a [NiFe] hydrogenase to stimulate the anoxic corrosion surface (Lienemann et al., 2018). In addition, this methanogen catalyzing components in the spent medium of acetogens have not yet been identified.

The recent evidence discussed above (Deutzmann et al., 2015; Philips et al., 2019; Tremblay et al., 2019), suggests that H₂ plays an important role in the EET mechanism of acetogenic bacteria. Nevertheless, H₂ can often not be detected during acetogenesis with a cathode or Fe(0) as electron donor (Jourdin et al., 2016; Philips et al., 2019). For that reason, this work proposes that the maintenance of low H₂ partial pressures is an additional mechanism by which acetogens favor H₂ evolution on a cathode or Fe(0) surface (Figure 1E). The importance of the H₂ partial pressure for the H₂ evolution reaction on a cathode or Fe(0) is elaborated next.

LOW H₂ PARTIAL PRESSURES FAVOR THE H₂ EVOLUTION REACTION ON A CATHODE AND FE(0)

Effect of the H₂ Partial Pressure on Cathodic H₂ Evolution

The cathode potential below which H₂ evolution (Reaction 2) is thermodynamically favorable, i.e., the H₂ evolution onset potential (E_\text{H⁺/H₂}) (V), is given by the Nernst equation:

\[ E_{\text{H⁺/H₂}} = E^\theta_{\text{H⁺/H₂}} - \frac{R \cdot T}{2 \cdot F} \cdot \ln \left( \frac{p_{\text{H₂}}}{[\text{H⁺}^2]} \right) \]  

(1)

With R the ideal gas constant (8.314 \times 10^{-3} \text{ kJ} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}), T the temperature (K), F the Faraday constant (96.485 \text{ kJ} \cdot \text{V}^{-1}) and p_{\text{H₂}} the H₂ partial pressure (atm) and [H⁺] the proton concentration (M). Equation 1 further neglects activity coefficients, assuming that activities can be approached by concentrations. The H₂ partial pressure (atm) is used throughout this work even for conditions in solution (such as at the cathode or Fe(0) surface), but can be related to the dissolved H₂ concentration using the Henri constant. \( E^\theta_{\text{H⁺/H₂}} \) is the standard potential (pH 0, 1 atm H₂) for H₂ evolution, which is 0 V (i.e., the potential of the SHE), while \( E^\theta_{\text{H⁺/H₂}} \) is −0.414 V (pH 7, 1 atm H₂).

Equation 1 demonstrates that the H₂ evolution onset potential depends on the H₂ partial pressure and the pH at the cathode surface (Figure 2A; Vincent et al., 2007; May et al., 2016). For instance, at a H₂ partial pressure of 50 Pa (5 \times 10^{-4} \text{ atm}), the H₂ evolution onset potential becomes −0.316 V (pH 7), while at pH 5.5 [optimal pH for some acetogens (Liew et al., 2016)], the H₂ evolution onset potential is −0.325 V (1 atm H₂). Consequently, H₂ evolution can thermodynamically be favorable at cathode potentials less negative than −0.4 V vs. SHE, even though this potential is often used in bioelectrochemical studies to avoid H₂ evolution.

The H₂ evolution onset potential, and thus the H₂ partial pressure (Equation 1), also affects the kinetics of the cathodic H₂ evolution (Rheinlander et al., 2014), which can be described by the Butler–Volmer equation (Bard and Faulkner, 2001):

\[ j = j_0 \cdot \left( e^{-\frac{\alpha F}{n F} (E_{\text{electrode}} - E_{\text{H⁺/H₂}})} - e^{1 - \frac{\alpha F}{n F} (E_{\text{electrode}} - E_{\text{H⁺/H₂}})} \right) \]  

(2)

With j the current density (A \cdot \text{cm}^{-2}), j₀ the exchange current density (A \cdot \text{cm}^{-2}), α the transfer coefficient (-) (usually approximated by 0.5) (Bard and Faulkner, 2001) and \( E_{\text{electrode}} \) the potential at which the electrode is poised. The left exponential expresses the cathodic reaction (H⁺ to H₂ reduction), while the right exponential expresses the anodic reaction (H₂ to H⁺ oxidation). When \( E_{\text{electrode}} \) is \( E_{\text{H⁺/H₂}} \), (thermodynamic equilibrium) both the anodic and the cathodic current density become j₀, hence the net current density is zero. Remark that Equation 2 is only valid if mass transfer is not limiting, e.g., when the concentrations at the electrode surface are the same as in the bulk liquid, which is only true at electrode potentials close to \( E_{\text{H⁺/H₂}} \) or, in other words, at very low currents.
The importance of the H₂ evolution onset potential for the current density (equivalent to the H₂ evolution rate) is illustrated in Figure 3A. A less negative \( E(H^+/H_2) \) value, for instance due to a lower H₂ partial pressure, allows cathodic current at less negative potentials.

In addition, the effect the exchange current density \( j_0 \) is illustrated in Figure 3B. The exchange current density inversely relates to the activation overpotential (Bard and Faulkner, 2001), as a smaller exchange current density entails that a more negative electrode potential is needed to enable a substantial cathodic current (Figure 3B). In bioelectrochemical studies, the overpotentials for cathodic H₂ evolution are often high (0.2 V more negative than \( E(H^+/H_2) \)), due to the low reactivity of the often used carbon-based electrode materials. Choosing for more reactive cathode materials (materials with high \( j_0 \)) strongly reduces the activation overpotential of the H₂ evolution reaction (Jeremiasse, 2011). Moreover, cell-free spent medium of S. ovata was also found to decrease the overpotential for cathodic H₂ evolution (Tremblay et al., 2019), likely because it contains hydrogenase enzymes or other components catalyzing the H₂ evolution (Figure 1D).

In summary, Equation 2 demonstrates that the current density (H₂ evolution rate) depends both on the H₂ evolution onset potential (and thus the H₂ partial pressure; thermodynamic effect) and on the exchange current density (kinetic effect, related to the electrode material and catalysis by enzymes), when mass transfer is not limiting.

**Effect of the H₂ Partial Pressure on Anoxic Fe(0) Corrosion**

The H₂ partial pressure also affects the anoxic chemical corrosion reaction, as the Gibbs free energy change of Reaction 3 \( (\Delta G_{anoxic\ corrosion}) \) depends on the H₂ partial pressure and pH at...
the Fe(0) surface (Figure 2B):

\[
\Delta G_{\text{anoxic corrosion}} = \Delta G^0_{\text{anoxic corrosion}} + R \cdot T \cdot \ln \left( \frac{P_{H_2} \cdot [Fe^{2+}]}{[H^+]^2} \right)
\]  

(3)

With \([Fe^{2+}]\) the dissolved Fe\(^{2+}\) concentration (M) and \(\Delta G^0_{\text{anoxic corrosion}}\) the standard Gibbs free energy change (−78.9 kJ · mol\(^{-1}\), pH 0). Consequently, hydrogenotrophic microorganisms can thermodynamically favor H\(_2\) evolution on Fe(0) by maintaining low H\(_2\) partial pressures on the Fe(0) surface. For instance, *A. woodii* maintained a H\(_2\) partial pressure on Fe(0) of 150 Pa (1.5 × 10\(^{-3}\) atm) (Philips et al., 2019), leading to \(\Delta G_{\text{anoxic corrosion}}\) of −32 kJ · mol\(^{-1}\), while this is only −22 kJ · mol\(^{-1}\) in abiotic conditions (0.1 atm or 10.000 Pa H\(_2\); assuming \([Fe^{2+}]\) of 1 mM) (Philips et al., 2019). All other experiments tested in the same study maintained lower H\(_2\) partial pressures on Fe(0) than *A. woodii* [below the detection limit of a TCD detector (40 Pa)] (Philips et al., 2019), thus leading to a \(\Delta G_{\text{anoxic corrosion}}\) value at least as negative as −36 kJ · mol\(^{-1}\).

Also the rate of the anoxic chemical corrosion reaction likely depends on the H\(_2\) partial pressure. In addition, Reaction 3 was found to be catalyzed by hydrogenases (Bryant and Laishley, 1990; Da Silva et al., 2004; Rouvre and Basseguy, 2016). Consequently, the rate of the anoxic corrosion reaction likely depends both on the H\(_2\) partial pressure (thermodynamic effect) and on enzymatic catalysis (kinetic effect), similar as for a cathode.

In general, the H\(_2\) partial pressure at the cathode or Fe(0) surface results from the balance (steady-state) between the H\(_2\) evolution rate on the cathode or Fe(0) and the H\(_2\) consumption rate by the microorganisms. For that reason, the H\(_2\) consumption characteristics of acetogenic bacteria are discussed next.

### H\(_2\) Consumption Characteristics of Acetogenic Bacteria

The consumption of H\(_2\) by any microorganism is described by its H\(_2\) threshold (the thermodynamic limit of H\(_2\) consumption) and its H\(_2\) consumption kinetics. Below, the theoretical H\(_2\) threshold is calculated for acetogens and experimentally determined values for the H\(_2\) threshold and H\(_2\) consumption kinetic parameters of acetogens are reviewed.

#### The Theoretical H\(_2\) Threshold of Acetogens

Microorganisms do not completely consume their substrates due to bioenergetic constraints. The theoretical limit for H\(_2\) consumption by acetogenic bacteria is the H\(_2\) partial pressure at which Reaction 1 reaches its thermodynamic equilibrium:

\[
\Delta G_{\text{acetogenesis}} = \Delta G^0_{\text{acetogenesis}} + R \cdot T \cdot \ln \left( \frac{[CH_3COO^-] \cdot [H^+]}{p_{CO_2} \cdot p_{H_2}} \right) = 0
\]  

(4)

With \([CH_3COO^-]\) and \([H^+]\) respectively the acetate and proton concentrations (M) and \(p_{CO_2}\) and \(p_{H_2}\) the CO\(_2\) and H\(_2\) partial pressures (atm). The minimum H\(_2\) partial pressure at which a reaction is thermodynamically feasible is called the H\(_2\) threshold (\(\theta_{H_2}:\) atm) (Cord-Ruwisch et al., 1988) and can for acetogens be derived as:

\[
\theta_{H_2} = e^{- \left( \frac{\Delta G^0_{\text{acetogenesis}}}{R \cdot T} + \ln \left( \frac{[CH_3COO^-]\cdot[H^+]}{p_{CO_2}} \right) \right)}
\]  

(5)

Using pH 7, a temperature of 298 K, a CO\(_2\) partial pressure of 0.2 atm and an acetate concentration of 2 mM [i.e., relevant physiological conditions for acetogens using a cathode or Fe(0) as electron donor (Nevin et al., 2010; Kato et al., 2015; Aryal et al., 2017; Philips et al., 2019)], the H\(_2\) threshold becomes 3 · 10\(^{-5}\) atm or 3 Pa (30 ppm or 0.003%, assuming 1 atm total pressure). Experimental H\(_2\) thresholds for acetogens are always higher than this value (discussed below). This is likely because Reaction 1 does not account for the coupling of acetogenesis to energy conservation. Indeed, calculations of the Gibbs free energy change at experimentally derived H\(_2\) thresholds found a critical Gibbs free energy change, which was not zero but slightly negative (Seitz et al., 1990). This critical Gibbs free energy change likely reflects the energy needed for the microbial metabolism. Accordingly, Reaction 1 should be written as (Poehlein et al., 2012):

\[
2 CO_2 + 4 H_2 + n ADP + n P_i \rightarrow CH_3COO^- + H^+ + n ATP + 2 H_2O
\]  

(Reaction 4)

With \(n\) the number of ATP molecules gained per molecule of acetate (i.e., the ATP gain). Consequently, the expression for the H\(_2\) threshold becomes:

\[
\theta_{H_2} = e^{- \left( \frac{\Delta G^0_{\text{acetogenesis}}}{R \cdot T} + \ln \left( \frac{[CH_3COO^-]\cdot[H^+]}{p_{CO_2}} \right) \right)}
\]  

(6)

with \(\Delta G_{ADP/ATP}\) the Gibbs free energy change for the phosphorylation of ADP to ATP in physiological conditions (also called the phosphorylation potential). Reported values for \(\Delta G_{ADP/ATP}\) range between 30 and 80 kJ · mol\(^{-1}\) (Thauer et al., 1977; Atkins and De Paula, 2011). For *A. woodii*, a phosphorylation potential of only 32 kJ · mol\(^{-1}\) has been measured (Spahn et al., 2015), but for other acetogens this value is unknown. For the calculation of the H\(_2\) threshold of different acetogens described here, a value for \(\Delta G^0_{ADP/ATP}\) of 50 kJ · mol\(^{-1}\) (Poehlein et al., 2012) was used, in order not to underestimate the H\(_2\) threshold.

Equation 6 shows that the H\(_2\) threshold depends on the ATP gain \(n\), which is maximally 1.9 (\(\Delta G^0_{acetogenesis}\) divided by \(\Delta G_{ADP/ATP}\)), but depends on the energy conservation mechanism of the acetogenic strain (Schuchmann and Müller, 2014).

All acetogenic bacteria share the Wood–Ljungdahl pathway, as the enzymes forming this pathway are highly conserved among acetogens (Schuchmann and Müller, 2014). However, the carbon flow through the Wood–Ljungdahl pathway does not
lead to energy conservation. Acetogens conserve energy using chemiosmotic ion gradient-driven phosphorylation. The cation generating this gradient (Na\(^+\) or H\(^+\)), the energy-conserving module (Rnf or Ech complex), as well as other components (electron bifurcating enzymes) creating the electron flow, differ between acetogenic bacteria (Schuchmann and Müller, 2014). So far, only for few model acetogenic strains the energy conservation machinery is (almost) fully unraveled and the theoretical ATP gain \( n \) has become available (Table 3). This ATP gain ranges between 0.3 for \textit{A. woodii} to 1 for \textit{Clostridium autoethanogenum}. Based on Equation 6, this entails that the H\(_2\) thresholds for these model strains range from 14 to 1160 Pa (including different optimal temperatures and pH) (Table 3). Moreover, based on recently sequenced genomes, it is plausible that a wide variability in the energy conservation mechanism of acetogenic strains exists (Poehlein et al., 2016). In theory, the ATP gain \( n \) could range from 0.15 to 1.9, entailing H\(_2\) thresholds ranging over five order of magnitudes (Figure 4). Consequently, acetogenic bacteria strongly differ in the lowest H\(_2\) partial pressure they can use. Strains with a high H\(_2\) threshold (high ATP gain) obtain high energy by performing acetogenesis, but cannot grow at low H\(_2\) partial pressures. In contrast, strains with a low H\(_2\) threshold (low ATP gain) gain low energy from acetogenesis, but have the advantage of being able to grow at low H\(_2\) partial pressures.

Previously, differences in the H\(_2\) threshold of methanogens were similarly linked to different ATP gains (Thauer et al., 2008). The above analysis, however, only holds as long as the metabolism is coupled to energy generation, as it does not incorporate the possibility that acetogenesis continues decoupled from energy generation (Schuchmann and Müller, 2014). Moreover, the above reactions do not incorporate the consumption of H\(_2\) and CO\(_2\) for biomass formation. Furthermore, different energy conservation strategies could exist in a single strain (Mock et al., 2015) and be expressed depending on the H\(_2\) partial pressure. Consequently, further investigations of the energy conservation mechanisms of acetogenic bacteria will be highly important to better understand their H\(_2\) threshold.

### Experimental H\(_2\) Thresholds of Acetogens

Experimental H\(_2\) thresholds have been reported for several acetogenic strains (Table 4). These values are usually measured as the constant H\(_2\) partial pressure that remains after H\(_2\) depletion (other nutrients not limiting) (Cord-Ruwisch et al., 1988; Poehlein et al., 2012). Reported H\(_2\) thresholds range over two orders of magnitudes (Table 4), but strong value variability was reported even for the same strain, as experimental H\(_2\) thresholds for \textit{A. woodii} instance range from 14 to 250 Pa (Table 4). This variability is likely due to varying experimental conditions, as the H\(_2\) threshold depends in theory on the CO\(_2\) partial pressure, the pH, the total pressure and the temperature (Equations 5 and 6). Conrad and Wetter (1990) and Kotsyurbenko et al. (2001) nicely demonstrated that experimental H\(_2\) thresholds followed the theoretical temperature dependence (Equation 5), as long as the temperature remained in the strain’s optimal temperature range. The effect of the other parameters on the experimental H\(_2\) thresholds has much less been studied and often these parameters are not reported together with the experimental H\(_2\) threshold values. Fortunately, some studies have used the same experimental conditions to determine the H\(_2\) threshold of different acetogenic strains and demonstrated significant differences in experimental H\(_2\) thresholds between strains (Cord-Ruwisch et al., 1988; Leclerc et al., 1997; Le Van et al., 1998).

This work advocates the reporting of experimental H\(_2\) thresholds (as well as of the experimental conditions of the measurements) of H\(_2\) consuming anaerobic microorganisms, as this parameter can easily be determined and forms a highly valuable measure to assess bioenergetics, and possibly also the energy conservation mechanism, of new and already-known strains.

### H\(_2\) Consumption Kinetics of Acetogens

Very limited information on the H\(_2\) consumption kinetics of acetogenic bacteria is available in literature, except for frequently reported doubling times (overview in Bengelsdorf et al., 2018). The kinetic parameters for H\(_2\) consumption were previously reported only for four acetogenic strains (Table 5). These strains strongly differ in their H\(_2\) consumption kinetics, as the maximum cell specific growth rate (\(\mu_{\text{max}}\)) differs one order of magnitude between these strains, while the Monod or half saturation constant (\(K_{H_2}\), i.e., a measure for the affinity of the strain for H\(_2\)), ranges over more than two orders of magnitude. The importance of these different kinetic parameters becomes clear in Figure 5, plotting the cell specific growth rate (\(\mu\)) in function of the H\(_2\) partial pressure. This work advocates the reporting of experimental H\(_2\) thresholds as well as of the experimental conditions of the measurements.)

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**Table 3** Comparison of theoretical H\(_2\) thresholds of three model acetogens.

| Strain                        | Acetobacterium woodii | Clostridium autoethanogenum | Moorella thermoacetica |
|-------------------------------|-----------------------|-----------------------------|------------------------|
| Temperature optimum (°C)      | 25                    | 37                          | 55                     |
| pH optimum                    | 7.0                   | 5.5                         | 7.0                    |
| ATP gain \( n \) (mole ATP/mole acetate)\(^a\) | 0.3\(^b\)               | 1\(^c\)                     | 0.5\(^d\)             |
| Hydrogen threshold (Pa)\(^e\) | 14                    | 1160                        | 51                     |

\(^a\) Only ATP gains for the formation of acetate are considered here, ATP gains for the formation of other products are different (Bertsch and Müller, 2015; Mock et al., 2015). \(^b\) The energy conservation mechanism of \textit{A. woodii} is completely unraveled and the exact ATP gain known (Schuchmann and Müller, 2014). \(^c\) This ATP gain is an assumed value (Mock et al., 2015), as the energy conservation mechanism of \textit{C. autoethanogenum} is not yet completely unraveled. \(^d\) This ATP gain is an assumed value (Schuchmann and Müller, 2014; Basen and Müller, 2017), as the energy conservation mechanism of \textit{M. thermoacetica} is not yet completely unraveled. \(^e\) Calculated using Equation 6 and the ATP gain \( n \) and the pH and temperature given in the table and assuming an acetate concentration of 2 mM and CO\(_2\) partial pressure of 0.2 atm. The effect of the temperature on \(\Delta G_{\text{acetogens}}\) and \(\Delta G_{\text{ADP/ATP}}\) was neglected, as the temperature effect on \(\Delta G_{\text{ADP/ATP}}\) is not known.
FIGURE 4 | H₂ threshold in function of the ATP gain of acetogenesis and the pH. The H₂ threshold was calculated according to Equation 6 assuming an acetate concentration of 2 mM and a CO₂ partial pressure of 0.2 atm. Temperature effects were not included in this graph. The H₂ thresholds for three model acetogens (as in Table 3) are indicated. Remark the logarithmic scale for the H₂ threshold.

TABLE 4 | Experimental H₂ thresholds for different acetogens performing acetogenesis from CO₂ and H₂.

| Strain                        | H₂ threshold (Pa) | Conditions¹ | References                     |
|-------------------------------|-------------------|-------------|--------------------------------|
| Acetitomaculum ruminis       | 384³              | 38°C, 24% CO₂ | Le Van et al., 1998           |
| Acetobacterium bakii          | 8–80              | 4–30°C, 20% CO₂ | Kotsyurbenko et al., 2001     |
| Acetobacterium carbinolicum   | 96⁸               | 28–34°C, 20% CO₂, 1 bar | Cord-Ruwisch et al., 1988 |
| Acetobacterium firmaeum       | 5–20              | 5–25°C, 2 mM acetate, pH 7, 28 kPa CO₂ | Conrad and Wetter, 1990 |
| Acetobacterium limneticum     | 15–80             | 4–30°C, 20% CO₂ | Kotsyurbenko et al., 2001     |
| Acetobacterium paludosum     | 15–150            | 4–30°C, 20% CO₂ | Kotsyurbenko et al., 2001     |
| Acetobacterium psammolitcicum | 53⁸              | 30°C         | Krumholz et al., 1999         |
| Acetobacterium tundae         | 10–100            | 4–30°C, 20% CO₂ | Kotsyurbenko et al., 2001     |
| Acetobacterium woodii          | 250               | 30°C, 20% CO₂ | Poeschl et al., 2012          |
|                               | 14–55             | 15–30°C, 2 mM acetate, pH 7, 28 kPa CO₂ | Conrad and Wetter, 1990 |
|                               | 37⁷               | 30°C, 24% CO₂ | Le Van et al., 1998           |
|                               | 18³               | 30°C, 20% CO₂, 1 atm | Leclerc et al., 1997 |
| Moorella thermoacetica        | 156⁸              | 30°C, 20% CO₂, 1 atm | Leclerc et al., 1997          |
| Sporomusa termidita           | 84⁴              | 28–34°C, 20% CO₂, 1 bar | Cord-Ruwisch et al., 1988     |
|                               | 88⁴              | 30°C, 24% CO₂ | Le Van et al., 1998           |
| Thermoanaerobacter kivui      | 300–600           | 50–60°C, 2 mM acetate, pH 7, 28 kPa CO₂ | Conrad and Wetter, 1990 |
| Treponema primitia            | 50³               | 30°C, 20% CO₂ | Graber and Breznak, 2004      |

¹Conditions are mentioned as far as they are reported in the cited reference. ²H₂ thresholds were converted from ppm values assuming a total pressure of 1 atm. ³The H₂ threshold was converted using a Henri coefficient for H₂ of 7.78 10⁻³ mol m⁻³ kPa⁻¹.

This figure shows that strains with a high μ<sub>max</sub>, such as C. ljungdahlii, have the highest growth rate and thus a competitive advantage at high H₂ partial pressures (> 0.25 atm). In contrast, at intermediate H₂ partial pressures, strains with an intermediate <i>K</i><sub>H₂</sub> value, such as S. termidita, have a competitive advantage, while at very low H₂ partial pressures (< 0.0015 atm), strains with a strong affinity for H₂ (low <i>K</i><sub>H₂</sub>), such as A. woodii, have the highest growth rate.
Possibly, these differences explain why acetogenic *Clostridium* spp. are well suited for gas fermentations (Liew et al., 2016), while *Acetobacterium* and *Sporomusa* species are often found on cathodes or Fe(0), where low H₂ partial pressures prevail (Table 2). Importantly, Figure 5 also shows that the highest growth rates are only obtained at high H₂ partial pressures, possibly impeding the production rates attainable with microbial electrosynthesis in comparison to gas fermentation.

Equation 7 can further be extended to also include the H₂ threshold (Kotsyurbenko et al., 2001):

\[
\mu = \frac{\mu_{\text{max}} \cdot (p_{\text{H}_2} - \theta_{\text{H}_2})}{K_{\text{H}_2} + (p_{\text{H}_2} - \theta_{\text{H}_2})}
\]

This equation was not used for Figure 5, as for none of the strains, all three parameters (\(\mu_{\text{max}}, K_{\text{H}_2}, \theta_{\text{H}_2}\)) are reported.

### DISCUSSION

Several acetogenic bacteria are capable of using solid electron donors (Tables 1, 2), implying that these strains have an extracellular electron uptake mechanism. Different EET mechanisms have been proposed for acetogenic bacteria (Figure 1). Recent evidence suggests that an H₂-dependent indirect EET mechanism is combined with a mechanism to increase the H₂ evolution reaction rate on the cathode or Fe(0) surface (Deutzmann et al., 2015; Jourdin et al., 2016; Tremblay et al., 2019). In addition, low H₂ partial pressures often prevail during acetogenesis with a solid electron donor (< 50 Pa) (Nevin et al., 2010; Deutzmann et al., 2015; Jourdin et al., 2016; Philips et al., 2019). This work explained that the H₂ partial pressure affects the H₂ evolution onset potential (Equation 1 and Figure 2A) and the H₂ evolution rate (current) (Equation 2 and Figure 3A) at a cathode, as well the Gibbs free energy change of the anoxic corrosion reaction (Equation 3 and Figure 2B) and likely also the rate of the corrosion reaction. Consequently, hydrogenotrophic microorganisms could favor the H₂ evolution reaction by maintaining low H₂ partial pressures at the cathode or Fe(0) surface (Figure 1E).

The steady-state H₂ partial pressure at the material surface results from the balance between the H₂ evolution reaction and microbial H₂ consumption. Here, the H₂ consumption characteristics of acetogenic bacteria were reviewed, which suggested that acetogens differ in their H₂ threshold (thermodynamic limit for H₂ consumption) (Figure 4 and Tables 3, 4) and their H₂ consumption kinetics (Figure 5 and Table 5). This entails that different acetogens likely maintain different H₂ partial pressures on the surface of a cathode or Fe(0). Therefore, I hypothesize that acetogens that maintain lower H₂ pressures (strains with a lower H₂ threshold and/or higher H₂ affinity) more strongly increase the H₂ evolution reaction on a cathode or Fe(0). Consequently, the differences in the capacities of acetogenic bacteria to use solid electron donors (Table 1) could be related to differences in their H₂ consumption characteristics.

The lowest theoretical H₂ threshold and highest H₂ affinity (lowest \(K_{\text{H}_2}\)) was reported for *A. woodii* (Tables 3, 5). This contradicts with my hypothesis, as *A. woodii* is not capable

### TABLE 5 | Monod kinetic parameters for H₂ consumption by acetogenic strains with \(K_{\text{H}_2}\) (Pa) the Monod or half saturation constant (i.e., a measure for the affinity of the strains for H₂) and \(\mu_{\text{max}}\) the maximum cell specific growth rate (h⁻¹).

| Strain                  | \(K_{\text{H}_2}\) (Pa) | \(\mu_{\text{max}}\) (h⁻¹) | Temperature (°C) | References          |
|-------------------------|-------------------------|-----------------------------|------------------|---------------------|
| *Acetobacterium woodii* | 94                      | 0.024                       | 30               | Peters et al., 1998 |
| *Acetobacterium bakii*  | 520                     | n.d.*                       | 30               | Kotsyurbenko et al., 2001 |
| *Sporomusa termidita*   | 770*                    | 0.09*                       | 30               | Breznak et al., 1988 |
| *Clostridium ljungdahlii* | 42000                   | 0.195                       | 37               | Mohammadi et al., 2014 |

* A value of 768 mmol·h⁻¹ was reported by Kotsyurbenko et al. (2001), but could not be converted to \(\mu_{\text{max}}\) as no growth yield was reported. *Converted using a Henri coefficient for H₂ of 7.78 \(10^{-3}\) mol·m⁻³·kPa⁻¹. *Converted from the doubling time \(g\) (h) using \(\mu_{\text{max}} = \ln 2\cdot g\).
of withdrawing cathodic electrons at a cathode potential of −0.4 V vs. SHE and does not increase Fe(0) corrosion or just to a limited extent (Table 1). However, information on the H$_2$ consumption characteristics of acetogens is limited to just a few strains (Tables 3–5), not including the acetogenic strains most capable of using a cathode or Fe(0) as electron donor, i.e., S. ovata, S. sphaeroides, and A. wieringae (Kato et al., 2015; Aryal et al., 2017; Marshall et al., 2017; Philips et al., 2019). These and other acetogenic strains could have a lower H$_2$ threshold and/or a lower $K_{H_2}$ than A. woodii (Figure 4). A. woodii maintained a H$_2$ partial pressure on Fe(0) of 150 Pa, while all other strains tested in the same study maintained H$_2$ partial pressures on Fe(0) below the detection limit (40 Pa) (Philips et al., 2019), indicating that the other strains have better H$_2$ consumption characteristics to maintain low H$_2$ partial pressures on the Fe(0) surface than A. woodii. Experimental studies are needed to determine the H$_2$ consumption characteristics (H$_2$ threshold and kinetic parameters) of more acetogenic strains and to investigate if those H$_2$ consumption characteristics relate to the capacity of acetogens to use solid electron donors.

It should be noted that H$_2$ consumption depends on more factors than just the H$_2$ threshold and the H$_2$ consumption kinetic parameters. The number of cells on the surface also affects the H$_2$ consumption rate, thus attachment and biofilm formation properties are important. In addition, several components, such as dissolved Fe(II), and a pH deviating from the optimal pH could inhibit H$_2$ consumption. Future studies assessing also these factors will be important to fundamentally understand the role of H$_2$ consumption in increasing the H$_2$ evolution reaction.

This work suggests that microbial H$_2$ consumption favors cathodic H$_2$ evolution. Interestingly, the increase of the anoxic corrosion reaction by microbial H$_2$ scavenging is a well-known theory, often referred to as "cathodic depolarization", initially proposed in 1934 (von Wollzogen Kühr and van der Vlugt, 1934). This theory was thought to be disproven by studies showing that only microorganisms isolated with Fe(0) as sole electron donor were capable of increasing anoxic corrosion, while strains isolated with H$_2$ as electron donor were not (Dinh et al., 2004; Mori et al., 2010; Uchiyama et al., 2010; Enning and Garrelfs, 2014; Kato et al., 2015). Those studies, however, did not consider that hydrogenotrophic microorganisms can differ strongly in their H$_2$ consumption characteristics, as explained in this work for acetogens. Moreover, it is very likely that enrichments with Fe(0) as electron donor select for strains with a low H$_2$ threshold and high H$_2$ affinity, while isolations with H$_2$ (often high H$_2$ partial pressure) select for strains with a high growth yield and growth rate, but a high H$_2$ threshold and low H$_2$ affinity.

Previous studies demonstrated that acetogens stimulate H$_2$ evolution on a cathode and Fe(0) through the excretion of hydrogenases or other components catalyzing the H$_2$ evolution reaction (Deutzmann et al., 2015; Philips et al., 2019; Tremblay et al., 2019). This work used the Butler–Volmer Equation (Equation 2) to demonstrate that the H$_2$ evolution rate (current) on a cathode depends both on the exchange current density (kinetic effect, related to catalysis by enzymes) (Figure 3B) and the H$_2$ partial pressure (thermodynamic effect) (Figure 3A). Consequently, there are two mechanisms by which hydrogenotrophic microorganisms could increase the H$_2$ evolution rate on a cathode or Fe(0) (Figure 6): (1) catalysis of the H$_2$ evolution reaction by extracellular hydrogenases or other components; and (2) the maintenance of low H$_2$ partial pressures by H$_2$ consumption. These two mechanisms are not mutually exclusive, but likely reinforce each other. The relative importance of each mechanism possibly depends on the reactivity of the material (higher for Fe(0) than for carbon-based cathodes), as well as on the H$_2$ consumption characteristics and enzyme secretion mechanisms of the involved strains.

In addition, more EET mechanism than presented in Figure 1 could exist, while strains could combine different EET mechanisms or adjust their EET mechanism depending on the conditions (for instance cathode potential). Moreover, the presence of cytochromes in the acetogenic Sporomusa and Moorella spp. definitely warrants further investigation of a possible direct EET mechanism.

![Figure 6](image_url)
the EET mechanism of acetogenic bacteria depends on their insights into their EET mechanism. I hypothesize here that the role of microorganisms in those processes requires fundamental impact biogeochemical cycles. A good understanding of the... partial pressures. Methanogens differ in their H\textsubscript{2} threshold and H\textsubscript{2} affinity (Thauer et al., 2015; Tsurumaru et al., 2018), while evidence exist that some methanogenic strains have a direct EET mechanism (Beese-Vasbender et al., 2015a; Rowe et al., 2019; Yee et al., 2019). Also the sulfate reducing IS4 strain likely has a direct EET mechanism (Beese-Vasbender et al., 2015b). Consequently, different strategies to obtain extracellular electrons from solid electron donors probably occur in the microbial world (Figure 1).

Acetogens and other hydrogenotrophic microorganisms capable of using a solid electron donors are of interest for biotechnological applications (e.g., microbial electrosynthesis), while they could also cause microbial induced corrosion and impact biogeochemical cycles. A good understanding of the role of microorganisms in those processes requires fundamental insights into their EET mechanism. I hypothesize here that the EET mechanism of acetogenic bacteria depends on their H\textsubscript{2} consumption characteristics. Hence, assessment of the H\textsubscript{2} consumption characteristics of various acetogenic strains could be valuable to select the optimal strain for microbial electrosynthesis applications. Genetic engineering cannot change the H\textsubscript{2} consumption characteristics as easy as it changes the resulting end-products (Humphreys and Minton, 2018), so target strains should be chosen based on their H\textsubscript{2} consumption characteristics. In addition, a good understanding of strain related differences in the EET mechanism will improve the assessment of microbial influenced corrosion based on microbial community compositions.

CONCLUSION

This work explained that the H\textsubscript{2} partial pressure affects the H\textsubscript{2} evolution reaction on a cathode or Fe(0) surface. This led to the assumption that the maintenance of low H\textsubscript{2} partial pressures by hydrogenotrophic microorganisms is a mechanism to increase the H\textsubscript{2} evolution reaction on a cathode or Fe(0), in addition to the catalysis by extracellular hydrogenases or other components (Figure 6). The H\textsubscript{2} consumption characteristics of acetogenic bacteria were further discussed, which suggested that acetogens differ in their H\textsubscript{2} threshold and H\textsubscript{2} consumption kinetic parameters. Consequently, I hypothesize that the differences in the capacity of acetogens to use a solid electron donors, e.g., cathode and Fe(0), are related to the differences in their H\textsubscript{2} consumption characteristics. The focus here was on acetogenic bacteria, but similar considerations are likely also relevant for other hydrogenotrophic microorganisms capable of using a cathode or Fe(0) as electron donor.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

FUNDING

JP was supported by a Starting Grant of the Aarhus Universitets Forskningsfond.

ACKNOWLEDGMENTS

The initial idea for this work originates from the visit of Prof. Em. Rolf Thauer (Max Planck Institute for Terrestrial Microbiology, Marburg, Germany) to Ghent University (Belgium) in 2015. I also acknowledge Dr. Jan B.A. Arends and Prof. Korneel Rabaey (Ghent University) for their support in developing the ideas of this work. I thank Assis. Prof. Jacopo Catalano (Aarhus University) for his advice on the electrochemical aspects of this work. The reviewers and editor’s comments were very helpful to improve this work.

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Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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