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Article

# Accelerated H<sub>2</sub> Evolution during Microbial Electrosynthesis with *Sporomusa ovata*

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**Abstract:** Microbial electrosynthesis (MES) is a process where bacteria acquire electrons from a cathode to convert CO<sub>2</sub> into multicarbon compounds or methane. In MES with *Sporomusa ovata* as the microbial catalyst, cathode potential has often been used as a benchmark to determine whether electron uptake is hydrogen-dependent. In this study, H<sub>2</sub> was detected by a microsensor in proximity to the cathode. With a sterile fresh medium, H<sub>2</sub> was produced at a potential of −700 mV versus Ag/AgCl, whereas H<sub>2</sub> was detected at −500 mV versus Ag/AgCl with cell-free spent medium from a *S. ovata* culture. Furthermore, H<sub>2</sub> evolution rates were increased with potentials lower than −500 mV in the presence of cell-free spent medium in the cathode chamber. Nickel and cobalt were detected at the cathode surface after exposure to the spent medium, suggesting a possible participation of these catalytic metals in the observed faster hydrogen evolution. The results presented here show that *S. ovata*-induced alterations of the cathodic electrolytes of a MES reactor reduced the electrical energy required for hydrogen evolution. These observations also indicated that, even at higher cathode potentials, at least a part of the electrons coming from the electrode are transferred to *S. ovata* via H<sub>2</sub> during MES.

**Keywords:** industrial biotechnology; electrochemistry; biohydrogen; biocatalysis; process development; bacteria

## 1. Introduction

Reductive bioelectrochemical processes rely on the transfer of electrons from a cathode to a microbial catalyst for the reduction of a substrate with protons coming from an anodic reaction [1,2]. The substrate can be inorganic carbon molecules like CO<sub>2</sub> that will be reduced to multicarbon compounds or CH<sub>4</sub> via microbial electrosynthesis (MES) [3–10]. Organic carbon compounds can also be converted into commodity chemicals via electrofermentation [11,12] or electrorespiration [13].

In reductive bioelectrochemical systems (BES), the electrons are thought to be transferred directly via physical contact between the microbes and the cathode or indirectly via an electron shuttle such as H<sub>2</sub> [14–16]. Experimental evidences suggest that H<sub>2</sub> evolution from a graphite electrode often used in reductive BES starts happening only at potentials below −800 mV vs. Ag/AgCl in batch experiments [17]. Thus, it has been proposed that when the cathode potential is set higher than −800 mV, electrons are transferred via a H<sub>2</sub>-independent mechanism that could possibly involve the direct acquisition of electrons by components of the bacterium cell wall [3,18].

A recent study indicated that in the presence of cell-free spent medium from the electroactive acetogen *Sporomusa sphaeroides*, a significant quantity of H<sub>2</sub> is produced in a BES with a cathode potential set at −710 mV vs. Ag/AgCl [19]. Furthermore, the same study showed that in the presence of cell-free spent medium from the electroactive methanogen *Methanococcus maripaludis*, H<sub>2</sub>, as well as formate, are formed in a BES at higher cathode potential compared to sterile fresh medium control and in sufficient quantities to account for all the methane produced from CO<sub>2</sub>. The authors suggested a novel electron transfer mechanism in which hydrogenases and formate dehydrogenase released in the medium from microbial cells would interact with a cathode set at a potential above −800 mV vs. Ag/AgCl to catalyze the formation of soluble electron shuttles. Alternatively, other groups have proposed that copper, nickel, iron, or vanadium deposited at the surface of a cathode via microbial activity could be responsible for the increase of bioelectrochemical hydrogen production observed at different potentials after exposure of the cathode to microbial catalysts [20–22].

Cathode materials [23], reactor designs [24–27], and operating modes [28,29] are all parameters that can positively affect H<sub>2</sub> evolution. The chemistry of the solution filling the cathodic reactor also has an impact on the relation between the cathode potential and H<sub>2</sub> evolution through changes in H<sub>2</sub> initial concentration [22,30], changes in buffer composition [31–33], and through the presence of weak acids [34,35]. During MES, the microbial catalyst will alter the chemical environment surrounding the cathode by releasing metabolic wastes, products, or diverse cell components or debris in the cathodic solution [19,21,36,37]. To study the possible correlation between microbial alterations and H<sub>2</sub> evolution in a MES system, a hydrogen microsensor was placed in close proximity to the surface of a cathode set at different potentials to measure H<sub>2</sub> evolution in situ in the presence of sterile medium, bacterial culture or cell-free filtrate. *Sporomusa ovata*, one of the best pure culture MES catalysts for the production of acetate from CO<sub>2</sub> [38,39] is used here as a model because of its capacity to perform MES over a large range of cathode potential [3,40–46]. In order to further understand H<sub>2</sub> evolution and electron uptake during MES, other variables were investigated, including the presence of metals at the cathode surface as well as the presence of hydrogenases and other enzymes in the cell-free spent medium.

## 2. Results and Discussion

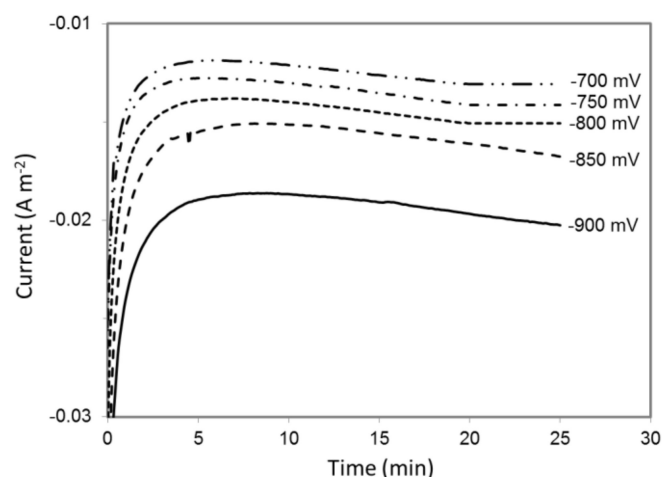
### 2.1. H<sub>2</sub> Evolution in an Abiotic MES Reactor

A hydrogen microsensor with a sensitivity of  $\geq 0.1 \mu\text{M}$  was inserted into an abiotic MES reactor to monitor H<sub>2</sub> evolution with a cathode set at potential ranging from −900 to −400 mV vs. Ag/AgCl (Figure S1). H<sub>2</sub> concentration was measured in close proximity to the cathode surface where microbial catalysts in operating MES reactor [3,38,41] are likely to oxidize large fraction of H<sub>2</sub>, if any is produced, before it can diffuse away in the medium and in the reactor gas phase. The initial evaluation of H<sub>2</sub> evolution was conducted in an abiotic MES reactor. Both cathodic and anodic chambers were filled with sterile 311 medium at pH 6.8, which is the growth medium as well as the electrolyte solution normally used in *S. ovata*-driven MES reactors [3]. Under these experimental conditions, the highest cathode potential at which H<sub>2</sub> evolution was observed was −700 mV vs. Ag/AgCl (Table 1). As expected, more current was drawn at lower potentials versus higher potentials because H<sub>2</sub> evolved faster at lower potentials (Figure 1, Table 1, Figure S2).

**Table 1.** H<sub>2</sub> evolution, current density and electrons recovery in MES reactors with sterile fresh 311 medium <sup>a</sup>.

Potential vs. Ag/AgCl (mV)	H <sub>2</sub> Evolution Rates ( $\mu\text{M min}^{-1}$ )	Current Density ( $\text{mA m}^{-2}$ )	Electrons Recovery in H <sub>2</sub> (%)
−900	$0.088 \pm 0.012$	$-19.8 \pm 2.1$	$87 \pm 1.8$
−850	$0.073 \pm 0.010$	$-16.3 \pm 1.7$	$85 \pm 3.7$
−800	$0.059 \pm 0.008$	$-14.9 \pm 1.8$	$80 \pm 2.1$
−750	$0.052 \pm 0.011$	$-13.7 \pm 1.2$	$79 \pm 9.0$
−700	$0.048 \pm 0.007$	$-12.8 \pm 1.0$	$78 \pm 7.0$
−600	n.d. <sup>b</sup>	n/a <sup>c</sup>	n/a

<sup>a</sup> Each result is the mean and standard deviation of three replicates. <sup>b</sup> Not detected. <sup>c</sup> Not applicable.

**Figure 1.** Current draw in a MES reactor filled with sterile fresh 311 medium at different cathode potentials. Results shown are from a representative example of three replicate.

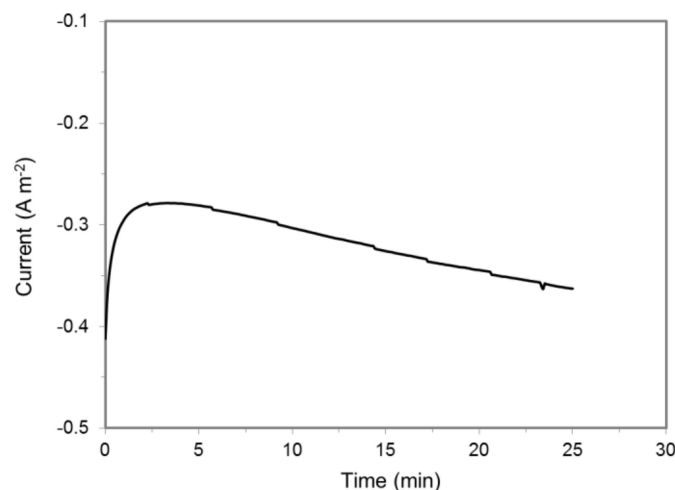
## 2.2. H<sub>2</sub> Evolution in the Presence of a *S. ovata* Cell Suspension

*S. ovata* is an efficient acetogenic microbial catalyst for the production of acetate from CO<sub>2</sub> by MES capable of acquiring electrons from the cathode at potentials as high as −600 mV vs. Ag/AgCl [1,3,41]. To determine the impact of *S. ovata* on H<sub>2</sub> accumulation in a MES system, a cell suspension was added to a cathode chamber equipped with a H<sub>2</sub> microsensor. At the tested potentials higher than −900 mV, no H<sub>2</sub> was detected (Table 2). At −900 mV,  $0.11 \pm 0.02 \mu\text{M min}^{-1}$  ( $n = 3$ ) of H<sub>2</sub> accumulated, and the electron recovery from current to H<sub>2</sub> was only  $8.5 \pm 1.5\%$  (Table 2, Figure 2, Figure S3), indicating that *S. ovata* cells probably quickly consumed most of the H<sub>2</sub> generated at the cathode. In the meantime, ca.  $6.6 \pm 0.2 \mu\text{M}$  of acetate was produced by *S. ovata* cell suspension from CO<sub>2</sub> in the cathode chamber of the MES system over a period of 25 minutes, and the coulombic efficiency was  $75 \pm 3\%$ .

**Table 2.** H<sub>2</sub> evolution, current density and electrons recovery in MES reactors with *S. ovata* cell suspension <sup>a</sup>.

Potential vs. Ag/AgCl (mV)	H <sub>2</sub> Evolution Rates ( $\mu\text{M min}^{-1}$ )	Current Density ( $\text{mA m}^{-2}$ )	Electrons Recovery in H <sub>2</sub> (%)
−900	$0.11 \pm 0.02$	$-317 \pm 32$	$8.5 \pm 1.5$
−850	n.d. <sup>b</sup>	n/a <sup>c</sup>	n/a
−800	n.d.	n/a	n/a
−750	n.d.	n/a	n/a
−700	n.d.	n/a	n/a
−600	n.d.	n/a	n/a

<sup>a</sup> Each result is the mean and standard deviation of three replicates. <sup>b</sup> Not detected. <sup>c</sup> Not applicable.



**Figure 2.** Current draw in a MES reactor containing a *S. ovata* cell suspension at a cathode potential of  $-900$  mV vs. Ag/AgCl. Results shown are from a representative example of three replicates.

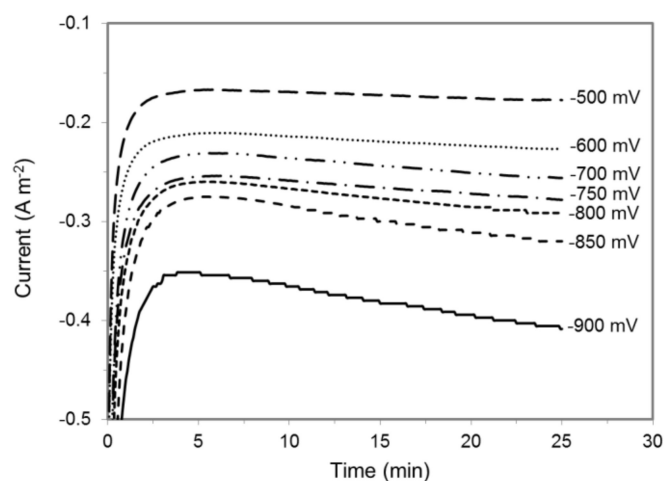
### 2.3. $H_2$ Evolution Shifting in the Presence of a Cell-Free Filtrate from *S. ovata* Culture

To recreate the chemical environment of an operating MES reactor in the absence of the  $H_2$ -oxidizing microbial catalyst, a cell-free filtrate from a *S. ovata* culture grown beforehand for four weeks under electrosynthetic condition was employed as the cathodic solution. Compared to a *S. ovata* cell suspension,  $H_2$  was present in significant quantities at  $-900$  mV vs. Ag/AgCl with simultaneous current draw indicating that the cell-free filtrate was unable to oxidize detectable amount of  $H_2$  (Table 3, Figure 3). Furthermore, higher  $H_2$  evolution rates in the MES reactor were detected with the cell-free filtrate than with the sterile fresh medium at cathode potential ranging from  $-900$  mV to  $-700$  mV vs. Ag/AgCl (Figure 4, Figure S4). In addition, the detectable  $H_2$  evolution in the presence of cell-free filtrate was shifted by  $+200$  mV compared to sterile medium control.  $H_2$  started to accumulate at  $-500$  mV with *S. ovata* cell-free filtrate, whereas  $H_2$  evolution was detected at  $-700$  mV with sterile fresh 311 medium. These results suggested that secreted metabolites, secreted cell components or chemical characteristics of the *S. ovata* cell-free filtrate enabled  $H_2$  evolution at higher cathode potentials and accelerated it. Furthermore, it seems to indicate that electron transfer in MES driven by *S. ovata* at high cathode potentials involved  $H_2$  as an electron shuttle. However, further characterization is required to determine whether all the electrons required for the acetate production by MES at high cathode potentials are transferred via  $H_2$ , or if a significant fraction of the electrons comes from an alternative  $H_2$ -independent route such as direct electron transfer.

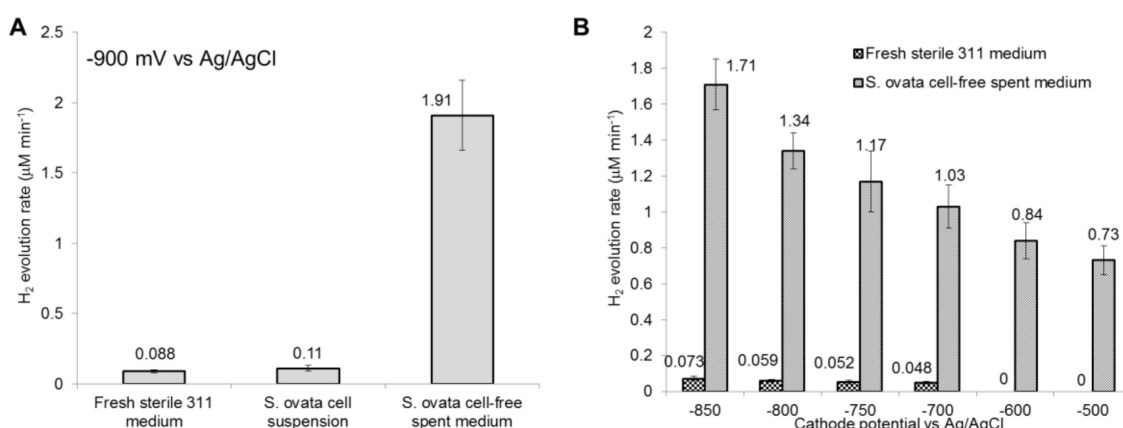
**Table 3.**  $H_2$  evolution, current density and electrons recovery in MES reactors with cell-free spent medium <sup>a</sup>.

Potential vs. Ag/AgCl (mV)	$H_2$ eVolution Rates ( $\mu\text{M min}^{-1}$ )	Current Density ( $\text{mA m}^{-2}$ )	Electrons Recovery in $H_2$ (%)
$-900$	$1.91 \pm 0.25$	$-398 \pm 90$	$87 \pm 8.4$
$-850$	$1.71 \pm 0.14$	$-320 \pm 86$	$89 \pm 8.0$
$-800$	$1.34 \pm 0.10$	$-289 \pm 38$	$85 \pm 7.9$
$-750$	$1.17 \pm 0.17$	$-276 \pm 51$	$80 \pm 3.2$
$-700$	$1.03 \pm 0.12$	$-254 \pm 60$	$75 \pm 3.0$
$-600$	$0.84 \pm 0.10$	$-225 \pm 47$	$74 \pm 5.9$
$-500$	$0.73 \pm 0.08$	$-179 \pm 42$	$77 \pm 9.0$
$-400$	n.d. <sup>b</sup>	n/a <sup>c</sup>	n/a

<sup>a</sup> Each result is the mean and standard deviation of three replicates. <sup>b</sup> Not detected. <sup>c</sup> Not applicable.



**Figure 3.** Current draw in a MES reactor containing cell-free spent medium from an electrosynthetic *S. ovata* culture. Results shown are from a representative example of three replicates.



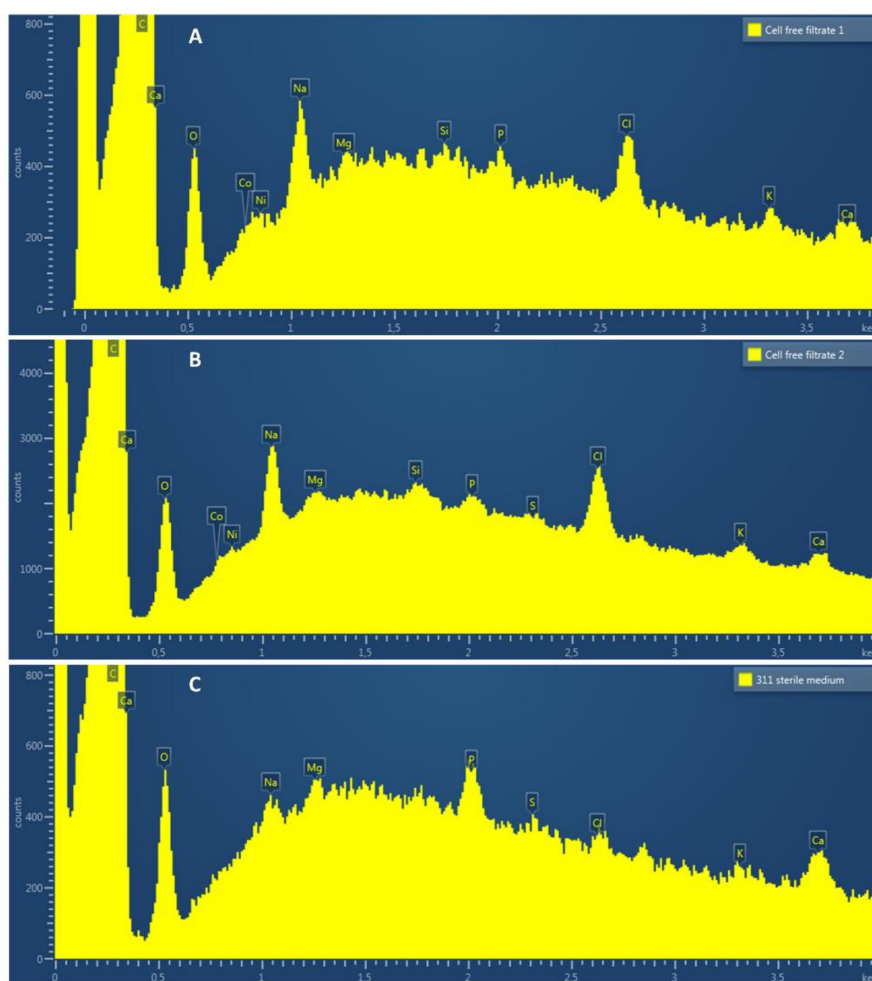
**Figure 4.** H<sub>2</sub> evolution rate with fresh sterile medium, *S. ovata* cell suspension and cell-free spent medium. (A) Cathode potential at  $-900$  mV vs. Ag/AgCl and (B) cathode potentials ranging from  $-850$  to  $-500$  mV. Above  $-500$  mV vs. Ag/AgCl, no H<sub>2</sub> evolution was detected under all tested conditions. No H<sub>2</sub> evolution was detected with *S. ovata* cell suspension above  $-900$  mV vs. Ag/AgCl. Each result is the mean and standard deviation of three replicates.

A possible explanation for the faster H<sub>2</sub> evolution observed here would be the presence of acetic acid in the cell-free spent medium. Weak acids including acetic acid have been shown to have a catalytic effect on H<sub>2</sub> evolution in BES at acidic pH as well as in abiotic electrochemical systems [34,35]. In the cell-free filtrate samples of *S. ovata* tested here, the concentration of acetate/acetic acid varied from 10.4 to 12.4 mM (Figure S5). This was generated by the filtered cells beforehand during the MES process. However, the pH of the cell-free filtrate maintained with a carbonate buffer was measured at ca. 6.8, which suggests that the acetic acid/acetate ratio is unlikely to be high enough to have a significant impact on the electrochemical generation of H<sub>2</sub>.

#### 2.4. Metals at the Surface of the Cathode after Exposure to Cell-Free Spent Medium

Energy-dispersive X-ray spectroscopy (EDS) was employed to examine the presence of elements on the surface of cathode electrodes exposed to electrosynthetic *S. ovata* cell-free spent medium or sterile 311 medium. As expected, C, O, Na, Mg, P, Cl, K and Ca were observed on spectra for each condition (Figure 5). Interestingly, two metal elements, i.e., cobalt and nickel, that may be involved in the acceleration of H<sub>2</sub> evolution, were detected with the electrode samples exposed to cell-free spent medium but not with samples exposed to sterile 311 medium. When subtracting the background, the EDS signal corresponding to cobalt had an X-ray count of ca. 50 and 120 in cell-free spent

medium replicate A and B, respectively. For nickel, the X-ray count was ca. 60 for replicate A and 140 for replicate B. No signal corresponding to nickel or cobalt was detected with sterile 311 medium (Figure 5C).



**Figure 5.** EDS spectra (from 0.0 to 4 KeV) of the cathode electrode surface after exposure to cell-free spent medium from electrosynthetic *S. ovata* (A,B) or to sterile fresh 311 medium (C).

Multiple proteins and enzymes found in acetogens, such as NiFe hydrogenases, acetyl-CoA synthase, CO dehydrogenase, corrinoid iron-sulfur protein and cobalamin-dependent methyltransferases, have metal centers containing nickel or cobalt [47–49]. Evidence suggested that hydrogenases released in the medium by *S. sphaeroides* cells are responsible for improved H<sub>2</sub> evolution from a cathode [19]. In this study, we were unsuccessful at detecting hydrogenase activity in the cell-free spent medium with a methyl-viologen based assay. Additionally, SDS-PAGE and mass spectrometry were applied to examine *S. ovata* cell-free spent medium and no intact hydrogenases were detected (Figure S6). Further research is warranted to establish if functional hydrogenases or other redox enzymes are released by *S. ovata* during MES, and if these enzymes participates actively to electron transfer from the cathode.

Three main visible bands were excised from the SDS-PAGE corresponding to an aldehyde oxidoreductase (Sov\_1c12660), a glutamate synthase (gltB2) and an ll-diaminopimelate aminotransferase (dapL2) (Figure S6). All three are predicted to be cytoplasmic proteins [50], which suggest that microbial cell content was spilled in the MES reactor medium due to lysis. This further increased the chemical complexity of the material surrounding the cathode. The presence of cytoplasmic enzymes in the cell-free spent medium suggests that the detected nickel and cobalt at

the surface of the cathode could come from three sources: intact enzymes, metal centers attached to damaged enzymes and free metal centers detached from apoenzymes. Furthermore, metal centers associated for instance with Sov\_1c12660 (Fe, Mo), as well as with other proteins possibly found in the cell-free spent medium of *S. ovata*, could also interact transiently with the cathode and facilitate hydrogen evolution.

### 3. Materials and Methods

#### 3.1. Bacterium and Growth Conditions

*S. ovata* DSM-2662 [51] was obtained from the Deutsche Sammlung Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). *S. ovata* strains were routinely maintained in the DSMZ-recommended 311 medium at 30 °C and at pH 6.8 with a H<sub>2</sub>:CO<sub>2</sub> (80:20) atmosphere (1.7 atm). Casitone, sodium sulfide, yeast extract, and resazurin were omitted from the 311 medium. For MES experiments, cysteine was also omitted from the 311 medium.

#### 3.2. Preparation of Cell Suspension

Triplicate of 300 mL of anoxic cultures of *S. ovata* were harvested by centrifugation and washed two times with 311 sterile medium before being resuspended in a final volume of 3 mL. H<sub>2</sub>:CO<sub>2</sub>-grown *S. ovata* cells were harvested when the optical density was ca. 0.3 (545 nm). Cell suspensions were then used to inoculate the cathodic chamber of MES reactors containing 250 mL of sterile 311 medium.

#### 3.3. Cell-Free Spent Medium of *S. ovata*

*S. ovata* cultures at an OD<sub>545</sub> of 0.2 catalyzing the conversion of CO<sub>2</sub> to acetate for 4 weeks in a MES reactor with a cathode potential of −900 mV vs. Ag/AgCl/3M KCl were filtered two times with 0.45 µm pore size filters. 250 mL of cell-free spent medium was then injected in the cathodic chamber of a MES reactor.

#### 3.4. MES Reactor and H<sub>2</sub> Evolution

Three-electrode H-cell glass bioreactor (Adams and Chittenden Scientific Glass, Berkeley, CA, USA) systems were used for H<sub>2</sub> evolution experiments during MES. The anode chamber was filled with 250 mL of sterile 311 medium, whereas the cathode chamber contained *S. ovata* cell suspension, *S. ovata* cell-free spent medium or sterile 311 medium. The anode and cathode chambers were separated by a Nafion 115 membrane (DuPont Inc., Wilmington, DE, USA). Graphite plates (type HLM, SGL carbon, Wiesbaden, Germany) with a normalized surface area of 35.5 cm<sup>2</sup> were used as both anode and cathode. The anode and cathode chamber was stirred at 300 rpm and bubbled with N<sub>2</sub>:CO<sub>2</sub> (80:20) gas mixture at a flow rate of 18.5 ± 1.0 mL/min (ADM 2000 Flowmeter, Agilent, Santa Clara, CA, USA). For the data presented here, the stirring and bubbling in cathode chamber was paused for 25 min during H<sub>2</sub> measurement to avoid interference of accuracy measurement. Fixed potentials were applied to the cathode from −900 to −400 mV vs. Ag/AgCl using a multi-potentiostat (CHI 1000C, CH Instrument, Inc., Austin, TX, USA).

H<sub>2</sub> evolution was measured in close proximity to the surface of the graphite cathode of a MES reactor maintained at 25 °C with a hydrogen microsensor (H2-500, Unisense, Aarhus, Denmark). The microsensor with a tip surface area of 0.2 mm<sup>2</sup> was installed in the MES reactor and the distance between the tip of the H<sub>2</sub> microsensor and the cathode surface was ca. 2 mm (Figure S1). Before each experiment, the microsensor was calibrated with a gas mixture containing 7% H<sub>2</sub>. The sensor data was logged continuously every second using a data logger (Microsensor Multimeter, Unisense, Aarhus, Denmark). H<sub>2</sub> evolution was measured for a maximum of 25 min until H<sub>2</sub> concentration curves reached an equilibrium plateau. The rates of H<sub>2</sub> evolution presented in this study are the slopes of H<sub>2</sub> concentration curves before this plateau.



### 3.5. High-Performance Liquid Chromatography (HPLC)

Acetate concentration was measured with an HPLC apparatus equipped with a HPX-87H anion exchange column (Bio-Rad Laboratories Inc., Hercules, CA, USA) at a temperature of 30 °C, with 5 mM H<sub>2</sub>SO<sub>4</sub> as the mobile phase, and a flow rate of 0.6 mL/min.

### 3.6. SDS-PAGE and Mass Spectrometry

Proteins from 220 mL of *S. ovata* cell-free spent medium were concentrated 375 times with Amicon Ultra-15 centrifugal filter devices with a nominal molecular weight limit of 3 kDa (Merck Millipore, Hellerup, Denmark). Protein concentration was measured with a Coomassie Plus Assay kit (ThermoScientific, Hvidovre, Denmark) and 1.5 µg of protein from two cell-free spent medium samples were loaded on a Sodium Dodecyl Sulfate Poly-Acrylamide Gel Electrophoresis (SDS-PAGE, 12.5%). The PageRuler prestained protein ladder (ThermoScientific) was loaded on the same gel to evaluate the molecular weight of protein bands. After protein separation, the SDS-PAGE was stained with the GelCode Blue stain reagent (Life Technologies, Carlsbad, CA, USA). Revealed protein bands were excised and sent to Alphalyse (Odense, Denmark) for protein identification by matrix-assisted laser desorption/ionization-tandem mass spectrometry (MALDI-MS/MS).

### 3.7. Hydrogenase Activity Assay

Hydrogen-evolving hydrogenase activity in the cell-free spent medium from a *S. ovata*-driven MES reactor and in 311 sterile fresh medium was measured in triplicate by monitoring the decrease in the absorbance of dithionite-reduced methyl viologen as described previously [52,53]. Briefly, cell-free spent medium or sterile medium were combined with 0.1M HEPES buffer (pH 8.0) and 100 µM reduced methyl viologen in an anoxic rubber-stoppered cuvette. Change in the absorbance at 604 nm was monitored over time at room temperature. The extinction coefficient of methyl viologen at 604 nm is 13.9 mM<sup>-1</sup> cm<sup>-1</sup>.

### 3.8. Energy-Dispersive X-ray Spectroscopy (EDS)

Duplicate electrode samples were air-dried and examined with a Quanta 200 FEG scanning electron microscope (FEI, Hillsboro, OR, USA). EDS data were collected at an accelerating voltage of 20 kV under high vacuum conditions, with 10 mm working distance and 4.0 spot size.

### 3.9. Equations

The Nernst Equation (1) was used to calculate theoretical cathode potential (E) at which H<sub>2</sub> evolution starts as described in Vincent et al., 2007 [30].

$$E = E^0 + 2.3RT/nF \log\{(aH^+)^2/p(H_2)\} \quad (1)$$

where E<sup>0</sup> is the standard reduction potential for H<sub>2</sub>, R is the gas constant, T is the absolute temperature, n is the number of electrons involved (2 e<sup>-</sup> for H<sub>2</sub> evolution), F is the Faraday constant, aH<sup>+</sup> is the activity of H<sup>+</sup> and p(H<sub>2</sub>) is the H<sub>2</sub> partial pressure.

Henry's law 2 was used to calculate H<sub>2</sub> partial pressure in the MES reactor gas phase.

$$pH_2 = k_H C \quad (2)$$

where k<sub>H</sub> is the Henry's law constant for H<sub>2</sub> and C is the concentration of H<sub>2</sub> in solution.

## 4. Conclusions

This study showed that in a *S. ovata*-driven MES reactor, faster H<sub>2</sub> evolution at higher cathode potential was enabled because the presence of microbial catalyst modified the cathodic solution chemistry. A higher accumulation of H<sub>2</sub> means more reducing power, which should lead to lower

requirements for electrical energy input for microbial CO<sub>2</sub> reduction. The observed acceleration of H<sub>2</sub> evolution could be due to the deposition of cobalt and nickel at the surface of the cathode caused by *S. ovata* catalytic activity. However, given the complexity of chemical species in *S. ovata* cell-free filtrate, it is possible that a more complicated synergistic effect is involved in H<sub>2</sub> evolution during MES with *S. ovata*.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2073-4344/9/2/166/s1>, Figure S1: Experimental setup for H<sub>2</sub> measurement in close proximity to the cathode surface of a MES reactor, Figure S2: H<sub>2</sub> evolution profile over a period of 25 min with fresh sterile medium in the cathode chamber of a MES reactor, Figure S3: H<sub>2</sub> evolution profile over a period of 25 min with *S. ovata* cell suspension in the cathode chamber of a MES reactor, Figure S4: H<sub>2</sub> evolution profile over a period of 25 min with *S. ovata* cell-free spent medium in the cathode chamber of a MES reactor, Figure S5: Evolution of acetate concentration over time in a MES reactor filled with sterile medium or with *S. ovata* cell-free spent medium with a cathode set at a potential of either −600 mV or −900 mV vs. Ag/AgCl, Figure S6: Proteins found in the cell-free spent medium of electrosynthetic *S. ovata*.

**Author Contributions:** P.-L.T. and T.Z. conceived the project and designed the experiments. N.F. assembled and operated MES reactors. N.F. prepared *S. ovata* cell suspension, cell-free spent medium and measured H<sub>2</sub> evolution with a hydrogen microsensor. Energy-dispersive X-ray spectroscopy, protein identification via SDS-PAGE and hydrogenase activity assay were done by P.-L.T. and T.Z. P.-L.T. and T.Z. wrote the manuscript with feedback from N.F.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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