Linking Bacterial Metabolism to Graphite Cathodes: Electrochemical Insights into the H₂-Producing Capability of Desulfovibrio sp.

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Microbial biocathodes allow converting and storing electricity produced from renewable sources in chemical fuels (e.g., H₂) and are, therefore, attracting considerable attention as alternative catalysts to more expensive and less available noble metals (notably Pt). Microbial biocathodes for H₂ production rely on the ability of hydrogenase possessing microorganisms to catalyze proton reduction, with a solid electrode serving as direct electron donor. This study provides new chemical and electrochemical data on the bioelectrocatalytic activity of Desulfovibrio species. A combination of chronoamperometry, cyclic voltammetry, and impedance spectroscopy tests were used to assess the performance of the H₂-producing microbial biocathode and to shed light on the involved electron transfer mechanisms. Cells attached onto a graphite electrode were found to catalyze H₂ production for cathode potentials more reducing than −900 mV vs. standard hydrogen electrode. The highest obtained H₂ production was 8 mmol L⁻¹ per day, with a Coulombic efficiency close to 100%. The electrochemical performance of the biocathode changed over time probably due to the occurrence of enzyme activation processes induced by extended electrode polarization. Remarkably, H₂ (at least up to 20% v/v) was not found to significantly inhibit its own production.

Introduction

The possibility of converting and storing electrical energy produced from renewable sources (e.g., sunlight, wind, or organic wastes) into chemical fuels such as H₂ is a major scientific and technological challenge that could have, in the future, a remarkable impact on our energy systems.[1–3] In this respect, the discovery that electricity can be directly fed to bacteria that use it to produce reduced (value-added) compounds is attracting considerable attention. The electricity-driven and microbially catalyzed synthesis of (gaseous and liquid) fuels or chemicals is an emerging field of research, which is commonly referred to as “microbial electrosynthesis”.[4–6]

The idea of using electrodes as electron donors for metabolic production is not completely new since it was initially proposed by Park and Zeikus, who demonstrated that fumarate could be reduced to succinate at a high efficiency by providing Actinobacillus succinogenes with electrical current and dissolved neutral red (NR) as an electron shuttle.[7] Similarly, electrically reduced NR was successfully used to stimulate growth and electrode (cathode) to living cells of the species Geobacter mettallireducens attached to a graphite cathode poised at 300 mV vs. the standard hydrogen electrode. The widespread use of Geobacter spp. similar mechanisms mediate the electron transfer in the reverse direction, that is, from electrodes to microbes, which is the basis for microbial electrosynthesis.

In a recent literature review, a key role of hydrogenases in the uptake of electrons in hydrogenophilic bacteria, and therefore also possibly in certain species of Geobacter, has been proposed.[10] This latter hypothesis is consistent with the ability of hydrogenase-possessing microorganisms to catalyze proton reduction, with a solid electrode serving as direct electron donor. This study provides new chemical and electrochemical data on the bioelectrocatalytic activity of Desulfovibrio species. A combination of chronoamperometry, cyclic voltammetry, and impedance spectroscopy tests were used to assess the performance of the H₂-producing microbial biocathode and to shed light on the involved electron transfer mechanisms. Cells attached onto a graphite electrode were found to catalyze H₂ production for cathode potentials more reducing than −900 mV vs. standard hydrogen electrode. The highest obtained H₂ production was 8 mmol L⁻¹ per day, with a Coulombic efficiency close to 100%. The electrochemical performance of the biocathode changed over time probably due to the occurrence of enzyme activation processes induced by extended electrode polarization. Remarkably, H₂ (at least up to 20% v/v) was not found to significantly inhibit its own production.

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**Geobacter sulfurreducens** to catalyze \( \text{H}_2 \) production (from \( \text{H}^+ \) reduction) only at cathode potentials lower than \(-600 \text{ mV} \) (vs. SHE), a value that is far more reducing than that reported for anodic electron transfer and relatively closer to the working potential of hydrogenases.[12] In addition to *Geobacter* spp., few other microorganisms and mixed cultures were found to catalyze \( \text{H}_2 \) production via direct extracellular electron transfer at the cathode of bioelectrochemical systems; these include *Desulfovibrio* species (for cathode potentials lower than \(-700 \text{ mV} \) vs. SHE),[13] a *Desulfotibacterium* - and *Dehalococcoides*-enriched culture (for cathode potentials lower than \(-750 \text{ mV} \) vs. SHE),[14] and a naturally selected mixed culture fed with bicarbonate (for cathode potentials lower than \(-500 \text{ mV} \) vs. SHE).[15, 16] In the majority of the reported cases, however, the underlying electron transfer mechanisms as well as many other fundamental aspects inherent to the biocathode, such as electrocatalytic activity, start-up time, and long-term sustainability, remained largely unknown.

In brief, catalysts are a crucial issue for \( \text{H}_2 \) (as well as for other fuels) production, and there is intense interest in finding alternatives to noble metals. Over the last years, major research efforts have focused on chemical catalysts, and promising results were achieved using stainless steel- or Ni-based materials, and molybdenum disulfide, which were found to be characterized by relatively low overpotentials for hydrogen evolution, low cost, and high chemical stability.[17–21] More recently, microbial biocathodes have also started to attract considerable attention, as they are inexpensive, self-regenerating, and not susceptible to corrosion. Moreover, in addition to \( \text{H}_2 \) production, microbial biocathodes have been used to catalyze the formation of methane,[22, 23] ethanol,[24] and even multicarbon organic compounds.[25]

The aim of this study was to gain a deeper understanding of the ability of *Desulfovibrio* species to uptake electrons from a polarized graphite electrode and to assess the electrocatalytic features of the developed microbial biocathode towards \( \text{H}_2 \) production.

**Results and Discussion**

**Chronoamperometry and \( \text{H}_2 \) production tests**

When the cathode compartment of the bioelectrochemical cell was inoculated with a microbial suspension of *Desulfovibrio paquesii* (*D. paquesii*, pre-grown in serum bottles on lactate and sulfate) and the graphite electrode was poised at \(-900 \text{ mV} \), a reduction current was observed. Initially, the current density was around \(-0.15 \text{ mA cm}^{-2} \), then it gradually increased (as absolute value) up to around \(-0.3 \text{ mA cm}^{-2} \) during the first 8 h of the test. Conversely, a six-fold lower current was measured with the bare graphite electrode under identical conditions but for the absence of *D. paquesii* (Figure 1A). Gas-chromatographic analyses revealed that \( \text{H}_2 \) accumulated in the headspace of the (biotic and abiotic) cells during continuous electrode polarization at \(-900 \text{ mV} \), but at a considerably higher rate in the presence of *D. paquesii* (Figure 1B). During the test, the maximum measured rate of \( \text{H}_2 \) production, in the presence of *D. paquesii*, was 5.1 mmol L\(^{-1}\) per day (corresponding to 0.12 m\(^3\) m\(^{-2}\) per day), whereas it was 0.91 mmol L\(^{-1}\) per day in the abiotic test.

It is worth noting that, in the presence of *D. paquesii*, the electric charge cumulatively transferred during the test (calculated by integrating the electric current over the period of electrode polarization) was nearly completely recovered into \( \text{H}_2 \), whereas a substantially lower recovery (i.e., Coulombic efficiency) of around 40% was observed in the abiotic experiment (Figure 1B). This proves that the presence of the bacterial culture not only increased the rate of \( \text{H}_2 \) formation, but also resulted in a more selective conversion of electric current into \( \text{H}_2 \). Both features are indicative of a bioelectrocatalytic activity of *D. paquesii* towards \( \text{H}_2 \) production, and in turn of the ability of the microorganism to link its metabolism (or at least its enzymatic activity) to the electron flow from the graphite electrode.

When, in parallel experiments, the biocathode was polarized at values less reducing than \(-900 \text{ mV} \) vs. SHE (i.e., \(-500 \text{ and } \text{700 mV} \) vs. SHE), the measured current was very similar to that obtained in the corresponding abiotic controls (data not reported). This is consistent with previous findings indicating that *Desulfovibrio* does not effectively use electrons available at a potential at or above \(-700 \text{ mV} \) vs. SHE.[26]

Hydrogen production tests (at \(-900 \text{ mV} \) vs. SHE) were repeated at different times, starting from the day of inoculation (day 0) to day 20 (Figure 1 refers to the test carried out on day 16). Prior to each test, the headspace of the cell was thoroughly flushed with a \( \text{N}_2/\text{CO}_2 \) (70:30%) gas mixture, 10% of...
the liquid phase was replaced with fresh anaerobic medium, and the pH of the solution was corrected to 7.5. Between the tests, the cell was continuously polarized to $-900 \text{ mV vs. SHE}$. As shown in Figure 2, the rate of $\text{H}_2$ production remained, over time, consistently higher than that measured in abiotic experiments. It is worth noting that abiotic control tests were carried out both in “fresh” anaerobic medium and in a filtered (0.45 µm) liquor (initially containing lactate and sulfate) after $D. \text{paquesii}$ had grown on it. This latter control allowed to verify that soluble by-products of $D. \text{paquesii}$ metabolism, such as sulfide, did not affect or contribute to the observed $\text{H}_2$ production.

The rate of $\text{H}_2$ production (in abiotic experiments) did not show a clear trend over time: It apparently increased during the initial seven days (from 5 to around 8 mmol L$^{-1}$/d per day), and then decreased back to around 5 mmol L$^{-1}$/d per day in the following tests. In the tests carried out on day 0 and 6, the measured Coulombic efficiency of $\text{H}_2$ production was lower than 60%, most probably due to the presence in the liquid phase of some residual sulfate from the original inoculum, which caused some $\text{H}_2$ consumption via hydrogen-dependent sulfate reduction. In all the following tests, the Coulombic efficiency remained stable between 80 and 100%, and no other metabolic products (e.g., methane) were detected in the headspace of the cell.

On day 20, the entire liquid phase in the cathode compartment, surrounding the graphite electrode, was removed and replaced by anaerobic basal medium (not containing vitamins). By doing this, all soluble compounds (possibly released by $D. \text{paquesii}$) as well as planktonic cells were nearly completely removed. Subsequently, a chronoamperometric test, with the electrode again poised at $-900 \text{ mV}$, was carried out to evaluate the effect of medium replacement on the kinetics of $\text{H}_2$ production. As shown in Figure 2, the rate of $\text{H}_2$ production was apparently not affected by the medium replacement, thereby indicating that the observed electrocatalytic activity was ultimately due to cells attached onto the electrode surface.

By combining this latter finding with the observation that a sustained $\text{H}_2$ production was observed already few hours after inoculation of the bacterial suspension to the cathode compartment (e.g., in the test carried out on day 0), it can be concluded that attachment of cells onto the electrode surface occurred, at least initially, via (rapid) physical adsorption, rather than via (slower) biofilm formation. This finding is in agreement with our previous observations that $\text{H}_2$ or CH$_4$ production in bioelectrochemical system is rapidly established after inoculation of carbon-based cathodes with specialized microbial cultures.$^{[14,18]}$

Based on the results of this study, it remains unclear whether hydrogen production was ultimately linked to energy conservation and possibly to microbial growth. An energy-conservation mechanism for $\text{H}_2$-producing microbial bioelectrodes has been recently proposed, although not experimentally demonstrated.$^{[12,27]}$ The possibility for cathodic hydrogen production to be coupled to microbial growth is not only fundamentally, but also practically, of great relevance because it would allow the bio cathode to self-regenerate and accordingly to operate stably for long periods. This important scientific issue warrants further investigations.

### Electrochemical impedance spectroscopy

To gain further insights into the bioelectrocatalytic activity of $D. \text{paquesii}$ towards $\text{H}_2$ production, electrochemical impedance spectroscopy (EIS) tests were performed at $-900 \text{ mV vs. SHE}$. Also in this case, prior to the start of each EIS test, the headspace cell was flushed with a $\text{N}_2/\text{CO}_2$ gas mixture, and the pH value of the solution was adjusted to 7.5. In some cases (day 0, 16, and 20), the tests were performed just before the start of the chronocoulometric tests described in the previous paragraph.

The Nyquist plots recorded at different times after inoculation of the graphite electrode with $D. \text{paquesii}$ are shown in Figure 3 A. For comparison, the Nyquist plot of the (abiotic) graphite electrode in anaerobic medium prior to the inoculation is also shown.

The magnitude of impedance (represented by the diameter of the semicircle in the Nyquist plot) of the inoculated electrode was lower than for the abiotic electrode and steadily decreased over time. To retrieve more quantitative information from EIS, impedance data were analyzed by using the three element equivalent circuit shown in Figure 3 B, which was used previously to describe the electrocatalytic behavior of electroactive biofilms.$^{[29–30]}$ The circuit is composed of an electrolyte resistance ($R_\text{e}$), a charge-transfer resistance ($R_\text{ct}$), and double layer capacitance ($C_\text{dl}$). For this latter, a constant phase element (CPE) was introduced to represent heterogeneities of the electrode/electrolyte interface. Experimental data were fitted to the equivalent circuit to obtain values for $R_\text{ct}$, a parameter that characterizes the electrode/electrolyte interface impedance.
that is inversely proportional to the ease of electron transfer from the electrode.\(^{[28]}\)

As shown in Figure 3B, \(R_{CT}\) of the inoculated electrode was consistently lower than that of the abiotic electrode and steadily decreased over time (from 455 \(\Omega\) on day 0 to 27 \(\Omega\) on day 20). Apparently, the decrease of \(R_{CT}\) occurred between day 0 and 1; successively, the decrease appeared to be more gradual. This finding points again to an initial physical adsorption as the predominant mechanism by which bacterial cells wire up to graphite electrodes. However, the observed decrease of \(R_{CT}\) was not mirrored by a corresponding increase of \(H_2\) production rates. This clearly indicates that factors other than \(R_{CT}\) (e.g., mass transport phenomena) were probably limiting the rate of \(H_2\) production in chronoamperometric tests. Identification of these factors warrants further investigations.

Cyclic voltammetry

Cyclic voltammetry (CV) was used to shed light on the electron transfer mechanism(s) underpinning the electrocatalytic activity of \(D. paquesii\) at graphite electrodes. Figure 4 shows a typical voltammogram of the inoculated electrode, recorded at a scan rate of 10 mVs\(^{-1}\) just before a chronoamperometric test (Figure 4A) and after 48 h of continuous electrode polarization at \(-900\) mV vs. SHE (Figure 4B). For comparative purposes, the voltammogram of an identical abiotic electrode (in anaerobic medium) is also shown (Figure 4C). As expected, the CV of the abiotic electrode did not reveal the occurrence of significant redox processes in the window (i.e., from \(+200\) to \(-1000\) mV vs. SHE) of potentials investigated. Differently, in the presence of \(D. paquesii\), a large cathodic current, which started at \(-750\) mV vs. SHE and most likely corresponded to \(H^+\) reduction to \(H_2\), was observed during the cathodic sweep. Notably, the voltage required to initiate hydrogen production (i.e., \(-750\) mV vs. SHE) was not much lower than that reported, for example, for stainless steel mesh cathodes (i.e., \(-670\) mV vs. SHE)\(^{[19]}\) even though substantially lower than that commonly reported for Pt-based cathodes (i.e., around \(-400\) mV vs. SHE at pH 7); conversely, the observed values of current densities for the \(Desulfovibrio\) biocathode were at least one order of magnitude lower than those commonly reported for abiotic cathodes.\(^{[19]}\) It is possible that higher current densities could be achieved by increasing biomass density at the electrode.

Interestingly, during the anodic sweep of the CV, a small anodic peak centered at \(-570\) mV vs. SHE appeared, which corresponded to \(H_2\) oxidation and confirmed the ability of \(Desulfovibrio\) hydrogenases to operate reversibly.\(^{[31, 32]}\) The increased magnitude of the cathodic current compared to the anodic one is indicative of a substantial catalytic bias of the enzyme, which seems to be more active in producing \(H_2\) than in consuming it. Clearly, the lack in the system of a terminal electron acceptor (e.g., sulfate) acting as a sink for the electrons resulting from \(H_2\) oxidation could explain the lower intensity of the oxidation peak.

The CV of the inoculated electrode was repeated after an extended period (over 48 h) of polarization of the electrode at \(-900\) mV vs. SHE. The shape and intensity of the catalytic current corresponding to \(H_2\) production did not markedly change, it even slightly increased in spite of the presence of over 20\% \(H_2\) in the headspace of the cell, which, in other
cases, has been shown to inhibit its own production. On the other hand, the oxidation peak at $-570 \text{ mV vs. SHE}$ significantly increased and other redox signals appeared, the main one being a couple of broad redox peaks (anodic and cathodic) centered at approximately $-100 \text{ mV vs. SHE}$. The increased intensity of the $H_2$ oxidation peak(s) is probably the result of the increased $H_2$ partial pressure in the headspace of the cell. Conversely, the nature and role of the redox peaks that appeared in the most oxidizing region, as well as the reason why they appeared only after an extended period of polarization, could not be determined in this study. However, their midpoint potential (i.e., around $-100 \text{ mV vs. SHE}$) is at least compatible with $H_2$-producing capability of $Desulfovibrio$ species.

The observed changes in the voltammogram could also be due to a gradual activation of the enzyme(s) in contact with the electrode, induced by the continued polarization of the electrode. This hypothesis could also explain the observed increase of the current, over time, during the chronoamperometric tests (Figure 1A). The possibility of activating (or deactivating) the hydrogenases of $Desulfovibrio$ spp., attached onto a carbon-based electrode, by either electrochemical control or addition of $H_2$ was previously described.

Finally, the discovery that $H_2$ itself did not inhibit its own production is very relevant from a practical point of view as it indicates that it would be possible to produce a gas highly enriched in $H_2$, which is an essential prerequisite for an economically applicable application of the microbial biocathode.

Conclusions

This study presented new chemical and electrochemical insights into the ability of living cells of $Desulfovibrio paquesii$ to accept electrons—in direct manner—from a polarized graphite cathode ($-900 \text{ mV vs. SHE}$) and to use these electrons to catalyze $H_2$ production from $H^+$ reduction. The microbial biocathode stably produced $H_2$ (over 20 days) at rates of 5–8 mmolL$^{-1}$ per day (i.e., around 5–10 times higher than with identical abiotic electrodes) and with Coulombic efficiencies up to nearly 100%. The observed electrocatalytic activity was due to cells in direct contact with the surface of the electrode. Importantly, such a contact was quickly established, probably through physical adsorption mechanisms. Over time, other mechanisms, such as the activation of key enzymes induced by the extended polarization, could also have played a role, as suggested by the gradual decrease of the charge transfer resistance documented by means of electrochemical impedance spectroscopy. In spite of that, however, the observed $H_2$ production did not follow a clear increasing trend over time, suggesting that other factors (e.g., mass transport phenomena), in addition to the charge transfer resistance, were rate limiting. Finally, this study showed that $H_2$ (at least up to 20%) did not inhibit its own production. This finding has an important applicative significance in view of a cost-effective product recovery.

Experimental Section

Strain, medium, and culture conditions

$Desulfovibrio paquesii$ was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). $D. paquesii$ is an hydrogenotrophic, sulfate-reducing bacterium that was isolated from sulfidogenic sludge of a full-scale synthesis-gas-fed bioreactor used to remediate wastewater from a zinc smelter. Hydrogen, formate, pyruvate, lactate, malate, fumarate, succinate, ethanol, and glycerol served as electron donors for sulfate reduction. Organic substrates were incompletely oxidized to acetate. For the purpose of this study, the cells were grown at 25 °C in sealed serum bottles containing 150 mL of anaerobic medium supplemented with lactate (10 mm) and sulfate (5 mm) in a CO$_2$/N$_2$ (30/70% v/v) atmosphere. The medium contained the following components: NH$_4$Cl (0.5 g L$^{-1}$), MgCl$_2$·6H$_2$O (0.1 g L$^{-1}$), K$_2$HPO$_4$ (0.4 g L$^{-1}$), CaCl$_2$·2H$_2$O (0.05 g L$^{-1}$), trace metal solution (10 mL L$^{-1}$) [35] vitamin solution (10 mL L$^{-1}$) [36] and NaHCO$_3$ (15 mL L$^{-1}$, 10% w/v). All solutions were purged for at least 0.5 h with a N$_2$/CO$_2$ (70:30% v/v) gas mixture before use. The pH value of the medium was 7.5. For the bioelectrochemical studies described hereafter, a sample (50 mL) of a stationary-phase culture was anaerobically transferred to the cathode compartment of the bioelectrochemical cell.

Bioelectrochemical cell setup

The bioelectrochemical cell setup used in this study consisted of two gastight borosilicate glass bottles (with a total volume of about 270 mL per bottle) separated by a 3 cm$^2$ cross-sectional area, Nafion 117 proton exchange membrane (PEM). The PEM was boiled successively in H$_2$O$_2$ (3% v/v), deionized (DI) water, then in 0.5 M H$_2$SO$_4$ and DI water each for 2 h, and stored in DI water. The cathode was a graphite rod (6 mm diameter, Sigma Aldrich, Milano, Italy), whereas the anode was a glassy carbon rod (5 mm diameter, HTW GmbH, Germany). The nominal surface area of the cathode (calculated by taking into account only the part of the electrode that was immersed in the liquid phase) was 9.7 cm$^2$. The distance between the anode and the cathode was around 10 cm. A KCl-saturated Ag/AgCl reference electrode (±199 mV vs. standard hydrogen electrode, SHE; Amel S.r.l., Milano, Italy) was also placed in the cathode chamber. The catholyte and anolyte consisted of anaerobic medium, unless indicated otherwise. During the target cathode reaction, that is, H$_2$ evolution, the anode reaction was water oxidation.

Bioelectrochemical experiments

All the electrochemical measurements and experiments were carried out by using a VSP potentiostat (Bio-logic, Clax, France). For chronoamperometric tests, the working electrode (i.e., cathode) was polarized at $-900 \text{ mV vs. SHE}$, and the current was recorded over time. Abiotic (control) as well as biotic chronoamperometric tests were conducted. For the abiotic tests, the cathode compartment contained either “fresh” anaerobic medium (150 mL), or “fresh” (100 mL) medium plus filtered medium (0.45 μm, 50 mL; initially containing lactate and sulfate) after $D. paquesii$ had grown on it. The aim of this latter test was to verify whether by-products of the $D. paquesii$ metabolism could contribute to current generation and/or $H_2$ production. For the biotic tests, the cathode compartment contained anaerobic medium (100 mL) and $D. paquesii$ culture (50 mL). Regardless of the type of test conducted, gaseous
samples were removed from the headspace of the cell at regular intervals (e.g., every 2 h) by using a gastight syringe (Hamilton, Reno, USA) and analyzed by means of gas chromatography for hydrogen and methane, as described in the following section. H₂ production calculations were performed by measuring the increase in concentration in the headspace of the cell. During the tests, the cell was maintained at 25 °C in a water bath under vigorous magnetic stirring to ensure that current generation was not substantially affected by mass transfer phenomena. The cumulative electric energy produced in the frequency range from 100 kHz to 10 mHz. For cyclic voltammetry experiments, the electrode (abiotic or biotic) was also polarized at 100 %. For electrochemical spectroscopy impedance (EIS) measurements, the electrode (abiotic or biotic) was also polarized at −900 mV vs. SHE, and then a potential wave signal of 10 mV was applied in the frequency range from 100 kHz to 10 mHz. For cyclic voltammetry experiments, the electrode potential was varied in the range +200 to −1000 mV vs. SHE at different scan rates (from 10 to 100 mV s⁻¹).

**Analytical methods**

H₂ was analyzed in a 500 μL gaseous sample by a Trace Analytical TA3000R reduction gas detector (RGD, H₂ detection limit was 0.02 ppmv; Trace Analytical, Menlo Park, CA). The H₂ level above the range of the RGD (approximately 50 ppmv) was quantified by using a Varian 3400 gas chromatograph (stainless-steel column packed with molecular sieve (Supelco); He carrier gas at a flow rate of 18 mL min⁻¹; oven temperature 180 °C; thermal-conductivity-detector (TCD) temperature 200 °C). Methane was analyzed by injecting 50 μL of sample headspace (by using a gas-tight Hamilton syringe) into the Varian 3400 gas chromatograph (2 m × 2 mm glass column packed with 60/80 mesh Carbopack B/1 % SP-1000 (Supelco); He carrier gas at a flow rate of 18 mL min⁻¹; oven temperature 50 °C; flame ionization detector (FID) temperature 260 °C).

**Chemicals**

Hydrogen (99.5 ± %) and all the other chemicals were purchased from Sigma-Aldrich (Milano, Italy, except where indicated differently). The chemicals used to prepare the mineral medium were of analytical grade and used as received.

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These are not the final page numbers!
Electrons as pasture for microbes: Feeding microbes with electricity offers new opportunities for storing renewable electrical energy in chemical fuels like hydrogen. By applying a combination of chemical and electrochemical techniques, new insights into the unique capacity of *Desulfovibrio* sp. to accept electrons from a polarized cathode and use them to catalyze the hydrogen production reaction have been obtained.