Environ. Sci. Technol. **2008,** *42,* 2445–2450

Whole Cell Electrochemistry of Electricity-Producing Microorganisms Evidence an Adaptation for Optimal Exocellular Electron Transport

JUAN PABLO BUSALMEN,*,† ABRAHAM ESTEVE-NUÑEZ,‡ AND JUAN MIGUEL FELIU†

Instituto de Electroquímica, Universidad de Alicante. Apartado de correos 99, 03080, Alicante, Spain, Centro de Astrobiología (CSIC/INTA), Ctra. de Torrejón a Ajalvir, Km 4. 28850 Torrejón de Ardóz, Spain

Received October 11, 2007. Revised manuscript received January 9, 2008. Accepted January 11, 2008.

The mechanism(s) by which electricity-producing microorganisms interact with an electrode is poorly understood. Outer membrane cytochromes and conductive pili are being considered as possible players, but the available information does not concur to a consensus mechanism yet. In this work we demonstrate that *Geobacter sulfurreducens* cells are able to change the way in which they exchange electrons with an electrode as a response to changes in the applied electrode potential. After several hours of polarization at 0.1 V Ag/AgCl—KCl (saturated), the voltammetric signature of the attached cells showed a single redox pair with a formal redox potential of about −0.08 V as calculated from chronopotentiometric analysis. A similar signal was obtained from cells adapted to 0.4 V. However, new redox couples were detected after conditioning at 0.6 V. A large oxidation process beyond 0.5 V transferring a higher current than that obtained at 0.1 V was found to be associated with two reduction waves at 0.23 and 0.50 V. The apparent equilibrium potential of these new processes was estimated to be at about 0.48 V from programmed current potentiometric results. Importantly, when polarization was lowered again to 0.1 V for 18 additional hours, the signals obtained at 0.6 V were found to greatly diminish in amplitude, whereas those previously found at the lower conditioning potential were recovered. Results clearly show the reversibility of cell adaptation to the electrode potential and point to the polarization potential as a key variable to optimize energy production from an electricity producing population.

Introduction

A microbial fuel cell (MFC) is a device through which chemical energy can be converted to electricity by coupling the biocatalytic activity of bacterial cells to the electrochemical reduction of an oxidant (*1*). In the general implementation, a solid state resistor is used to connect two electrodes located at both sides of an aerobic/anaerobic interface, in which biologically reduced compounds in a bacterial culture are oxidized at the anaerobic side, while oxygen or other oxidant is reduced in the aerobic one (*2*). Although MFCs have been known for several decades, interest on this technology took off very recently from the discovery of bacteria that are able to produce electricity by the transport of the electrons coming from their central metabolic pathways to an electrode without the requirement of any electron transfer mediator (*3*). The power output of the so-called "mediator-less" MFCs is much higher than that provided by their "mediated" predecessors and promises to be even higher as the research on these devices advances (*2, 4*).

The most efficient electricity-producing microorganisms are Fe(III)-reducing bacteria (*4*). This microbial capacity is probably related to their natural ability to transfer electrons onto solid Fe(III) and Mn(IV) oxides. The mechanisms behind these processes are not completely understood, but intensive microbiological research has led to the identification of some molecular elements that participate in the electron transport. For instance, although their exact role in the electron pathway is not completely clear, external cytochromes as OmcS in *Geobacter sulfurreducens* (*5*) or mtrA, mtrB, OmcA/mtrC, and cymA in *Shewanella oneidensis* (*6*) have been found necessary for optimal electricity production (*7, 8*).

A kind of conductive appendages called pili have also been proposed to participate as "nanowires" in the electrical connection to Fe(III) oxides (*9, 10*). Indeed, they have been found to contribute as a structuring network in electricity-generating *Geobacter*'s biofilms (*11*). Unfortunately, the way in which these pili would mediate the electron transport still remains obscure (*12*).

Among the Fe(III)-reducing bacteria, the most intensive research has primary been performed on *G. sulfurreducens*, a member of the family Geobacteraceae that was the first reported microorganism able to conserve energy for growth by oxidizing organic compounds, with an electrode serving as sole electron acceptor (*8*). For this reason *G. sulfurreducens* was selected to perform the present work.

The mechanism involved in the microbial electron transport to external solid acceptors (oxides and electrodes) is essentially electrochemical, but only a few reports on the use of electrochemistry methods for analyzing the bacteria/electrode interface have been presented (*13–16*). In this way, the aim of this work is to analyze the electrochemical activity of *G. sulfurreducens* cells attached to graphite electrodes. We have investigated how the polarization potential influences on the bacterial electron transfer mechanism. Using cyclic voltammetry, the bacterial redox couples, predominantly active at various potentials, were detected. Chronopotentiometric measurements were performed to estimate the formal redox potential of the couples. Finally, by applying sequential polarization at different potentials the reversibility of the bacterial response was explored.

Experimental Section

Electrochemical Measurements. Experiments were performed using graphite electrodes (AGKSP, ultra carbon, MI) constructed from bars of 0.3 cm in diameter and 10 cm in length, on which a gold wire was fixed for the electric contact. Several electrodes were partially immersed (3.8 cm^2 of geometric area) on one side of a two-compartment glass device separated by a Nafion membrane. Electrodes were connected in parallel and used as the working electrode of a three electrodes electrochemical cell in which a graphite felt and a Ag/AgCl—KCl saturated (sat.) electrode were positioned on the other side of the device as the counter and reference electrode, respectively. All potentials are reported

* Corresponding author e-mail: jbusalmen@ua.es.
† Universidad de Alicante.
‡ Centro de Astrobiología.

10.1021/es702569y CCC: $40.75
Published on Web 02/27/2008

as relatives to an Ag/AgCl- KCl sat. electrode ($E_{vs\ NHE} = 0.197$ V). The working electrodes side was gently purged with a N_2/CO_2 (80:20) gas mixture and filled with a bacterial culture as described below. The auxiliary electrodes side was filled with a solution composed by 2.5 g l^{-1} sodium bicarbonate and 2.2 g l^{-1} KCl in PureLab Ultra (Elga-Vivendi) water.

Graphite electrodes were polarized at the selected potential using a Voltalab 10 potentiostat (Radiometer Analytical, Lyon, France), and the evolution of current was recorded over time. Experiments were performed at room temperature (i.e., 24 ± 2 °C), and cell cultures were stirred at low rate using a magnetic bar.

After the exposure to the bacterial culture, the working electrodes were transferred one at a time to another two-compartment cell in which a complete electrochemical analysis was performed. In this case, the reference electrode was positioned on the anaerobic side. The electrolyte was a sodium bicarbonate solution purged by the N_2/CO_2 mixture as previously described. Cyclic voltammetry was performed scanning the potential between −0.7 and 0.8 V starting positively from −0.3 V. The scan rate was 0.01 V s^{-1} unless otherwise indicated. Chronopotentiometry was performed measuring the potential evolution over time while applying a 5×10^{-6} A current unless otherwise indicated. All the experiments were performed with an μAutolab III potentiostat (Eco Chemie, The Netherlands). From chronopotentiometric results the formal redox potential of the acting redox pair was estimated as the quarter wave potential ($E_{\tau/4}$) of the transition time (τ), being τ the time after the application of the current for the potential to shift toward more positive values due to exhaustion of the reduced form in the redox pair (*17*).

Culture of Microorganisms. All the bioelectrochemical assays were performed using *G. sulfurreducens*. Cells were anaerobically cultured in chemostats as previously described (*18*). Acetate was used as the carbon source, and the electron donor under conditions in which the electron acceptor fumarate was the growth-limiting factor. Steady-state cells were directly and anaerobically transferred from the chemostat to the working side of the electrochemical cell to perform the electrodes exposure.

All the electrochemical experiments were sequentially performed immediately after the transference of electrodes to the electrochemical cell.

Scanning Electron Microscopy (SEM) of Adsorbed Bacteria. After the exposure to the bacterial culture, sample pieces of graphite electrodes containing the adsorbed bacteria were fixed in glutaraldehyde 2.5% during 15 min, dehydrated by immersion in an alcoholic series (40, 60, 80, and 100% ethanol in ultrapure water), air-dried, and sputtered with gold for the observation by SEM. Samples were observed in a JEOL JSM-5600LV scanning electron microscope.

Results

Microbial Electricity Production Is Potential-Dependent. In order to gain insight into the way in which electrons coming from the bacterial metabolism are transferred to a solid electrode, *G. sulfurreducens* cells grown in a chemostat with the electron acceptor fumarate as limiting factor were used as the anolyte in the electrochemical cell. Graphite bar electrodes were immersed in the culture and a chronoamperometric measurement was performed for 18 h of continuous polarization at 0.1 or 0.6 V. Experiments were also performed in the absence of bacteria as the nonbiotic control. During the experiments in the presence of bacteria the current continuously increased with no appearance of lag phase (Figure 1). In contrast, in absence of bacteria a slow current decay, presumably due to surface passivation, was observed. The current production (after 6 h of polarization) rate, normalized to the initial value, was 0.095 h^{-1} when

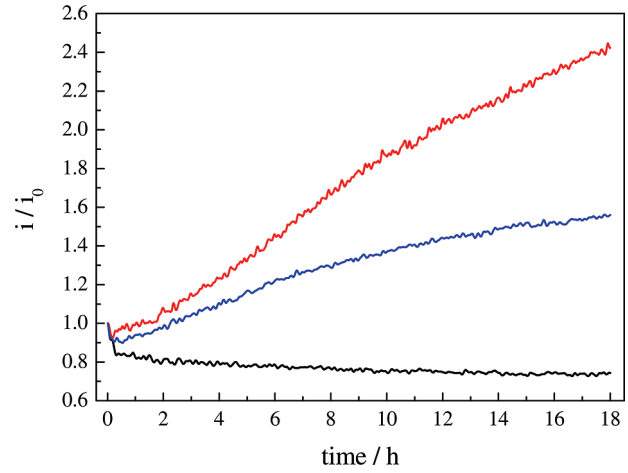

FIGURE 1. Normalized current density obtained from graphite electrodes polarized at 0.1 V (blue line) and 0.6 V (red line) into a fumarate-limited culture of *G. sulfurreducens*. Values from a nonpolarized electrode (black line) are included for comparison.

the electrode was polarized at 0.6 V, doubling the rate measured with electrodes polarized at 0.1 V (0.045 h^{-1}). SEM observations confirmed the presence of a cell layer firmly attached to the electrode surfaces (Figure 2). An almost complete layer was observed on electrodes polarized to 0.6 V (Figure 2a), whereas the amount of cells on electrodes polarized at 0.1 V was comparatively lower (Figure 2b). Only a few cells were found to be randomly distributed on the surface of electrodes that remained nonconnected (Figure 2c).

Electrochemical Analysis of the Redox Elements in *G. sulfurreducens*. The presence of external electron-transfer elements in attached cells was explored by cyclic voltammetry and chronopotentiometry. Polarized electrodes bearing the bacteria were first analyzed by cyclic voltammetry to determine the presence of redox elements in the biological population. The analysis of electrodes polarized at 0.1 V showed a redox pair that evolved in consecutive cycles (Figure 3a). The peak potential of the oxidation process changed from about −0.15 V in the first voltammetric cycle to a stable value of 0.02 V in the 10th cycle. This signal was coupled to a reduction process whose initial peak potential was about −0.60 V and reached a value of −0.36 V after ten cycles. The entire voltammogram became stable after 10 cycles.

A completely different voltammetric profile was observed on electrodes that were polarized at 0.6 V (Figure 3b). During the very first anodic excursion two different oxidation processes were observed at about 0.39 and 0.70 V, respectively, but in the following cycles only the last process remained. As observed previously the voltammogram was evolving until reaching a well defined profile that included a main oxidation peak at about 0.65 V with a poorly defined shoulder at lower potentials, and two clearly separated reduction waves at 0.50 and 0.23 V (Figure 3b). The low potential redox pair previously found after conditioning at 0.1 V was also present in the voltammogram (red arrow in Figure 3b), but the peak current density was 1 order of magnitude lower than that previously measured at the lower polarization. It is important to note that other redox pairs were neither detected during reported experiments nor those performed at intermediate polarization (0.4 V) (Figure S1 in the Supporting Information).

Nonpolarized electrodes were also exposed to the bacterial culture as controls for the potential-related microbial activity. Cyclic voltammetry over such electrodes gave a featureless profile in the whole potential range of interest (Figure S2 in the Supporting Information). Only a low amplitude redox

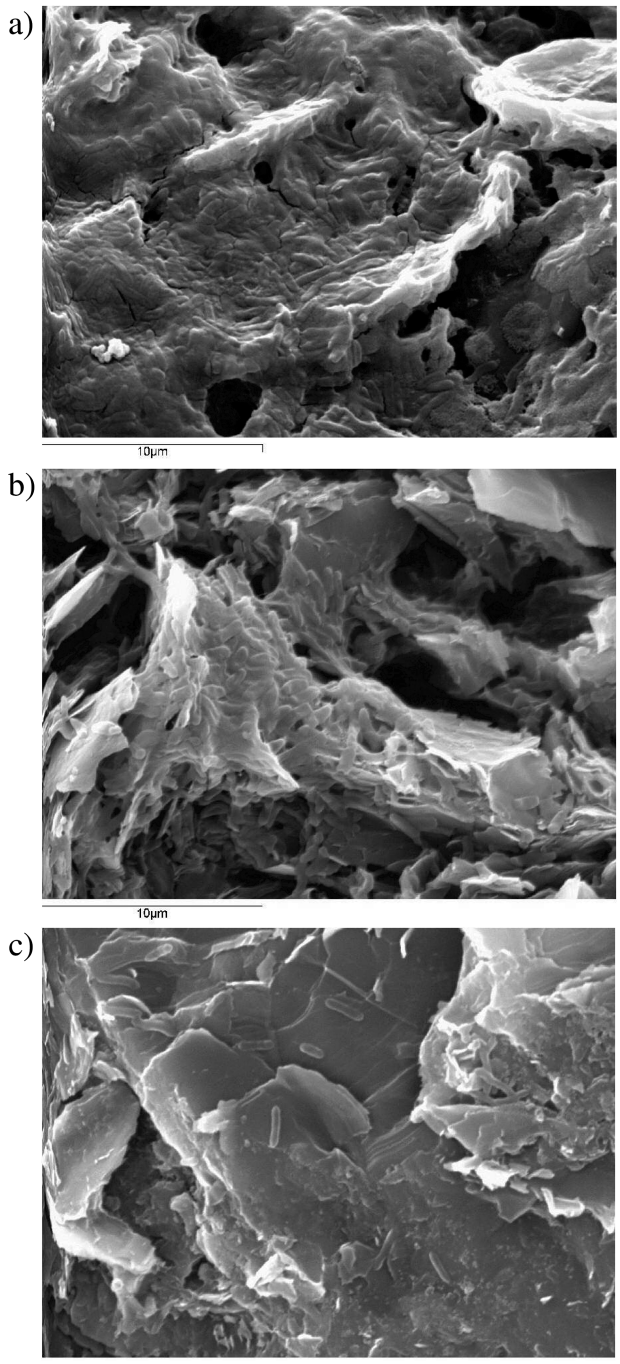

FIGURE 2. Scanning electron microscopy images of graphite electrodes after 18 h of continuous polarization at (a) 0.6 V, (b) 0.1 V, and (c) the open circuit potential, in a fumarate-limited culture of *G. sulfurreducens*.

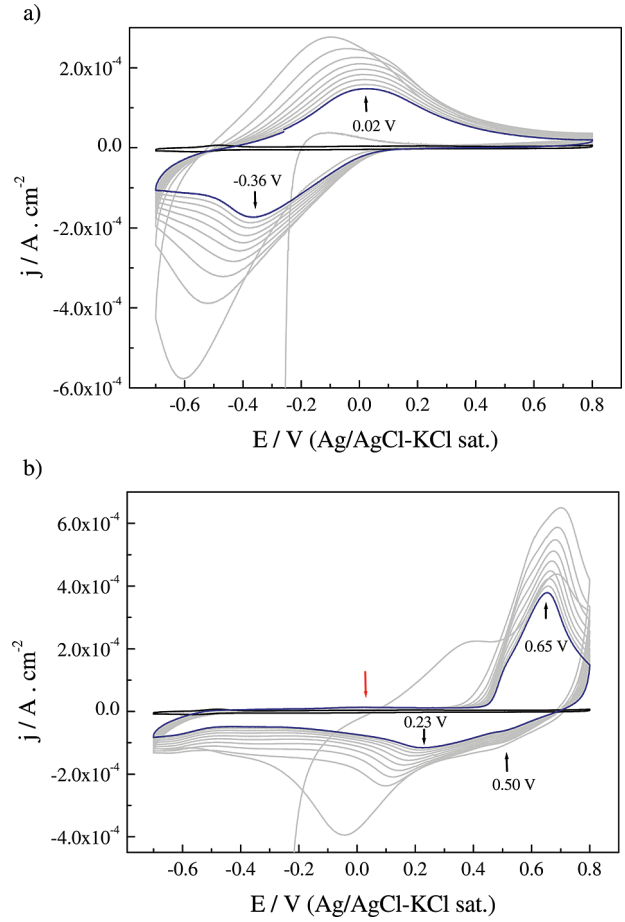

FIGURE 3. Cyclic voltammograms obtained on graphite electrodes after the exposure by 18 h to a fumarate-limited culture of *G. sulfurreducens* under continuous polarization at (a) 0.1 V and (b) 0.6 V. The estimated peak potential of redox processes is indicated. The red arrow indicates the position of a very low amplitude peak at 0.02 V. Gray lines: cycles one to nine. Blue line: cycle 10. Black line: cycle 10 of a control experiment performed at the open circuit potential.

couple was detected at very negative potentials with an estimated half-cell potential ($E_{1/2}$) of −0.50 V (Figure S2). As in the previous cases, the first voltammetric cycle was different that those recorded in the following cycles but the stationary state was readily attained (Figure S2).

Further analysis of external redox elements was performed by constant current chonopotentiometry. In this method the potential of the electrode moves to values characteristic of the acting couple and varies with time following the change of the oxidized/reduced ratio caused by the imposed current (*17*). When a positive current density of 1.3×10^{-6} A cm^{-2} was applied to the system, the redox couple of cells adapted to 0.1 V was able to sustain the electrode potential at negative values for at least 500 s (Figure 4). Then, the potential increased rapidly reaching the cutoff value of 0.80 V beyond 1000 s (Figure 4). The transition time for this process was ca. 740 s. Assuming a reversible electron transfer process (*17*) the $E_{\tau/4}$ would be −0.08 V.

Potentiometric measurements performed on electrodes bearing cells conditioned to 0.6 V showed a scarcely defined inflection point near −0.03 V, but reached a plateau at about 0.44 V that persisted even after doubling the applied current (dotted line in Figure 4). In a programmed current potentiometry, the pair was found to sustain a current density up to 1×10^{-5} A cm^{-2} (data not shown).

The potential of nonconnected electrodes, on the other hand, rapidly grew to values higher than 0.60 V reaching the cutoff potential after 1500 s. Two poorly defined inflection points were observed at about −0.50 and 0.20 V probably related to the couples shown in Figure S2.

Reversibility of the Biological Response to the Polarization. In order to investigate the biological response of electrodes-attached bacteria to changes in polarization, a set of bacteria-colonized electrodes polarized during 18 h at 0.6 V were shifted to 0.1 V and maintained at this potential for 18 additional hours. As shown in Figure 5 the voltammogram of these electrodes (blue line) was dominated by signals at potentials close to that previously observed in Figure 3a, e.g., after conditioning at 0.1 V. Interestingly, the signal corresponding to the response to 0.6 V still persisted at high potentials although appeared with much lower amplitude

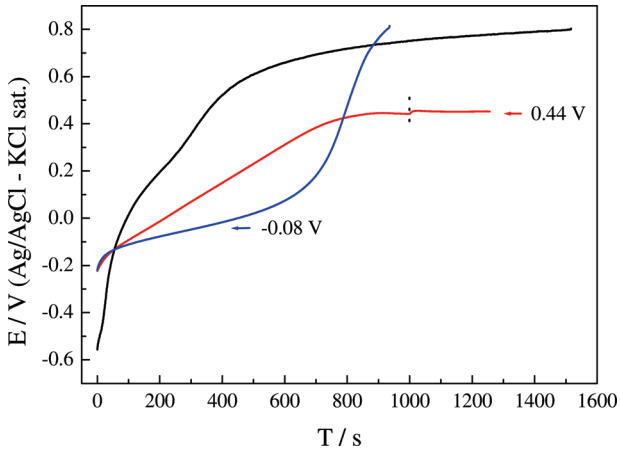

FIGURE 4. Potential evolution during chronopotentiometric measurements performed on graphite electrodes after the exposure by 18 h to a fumarate-limited culture of *G. sulfurreducens* under continuous polarization at 0.1 V (blue line) and 0.6 V (red line) or without polarization (black line). The dotted line indicates the time at with galvanostatic current was doubled. The quarter wave potential of redox processes is indicated.

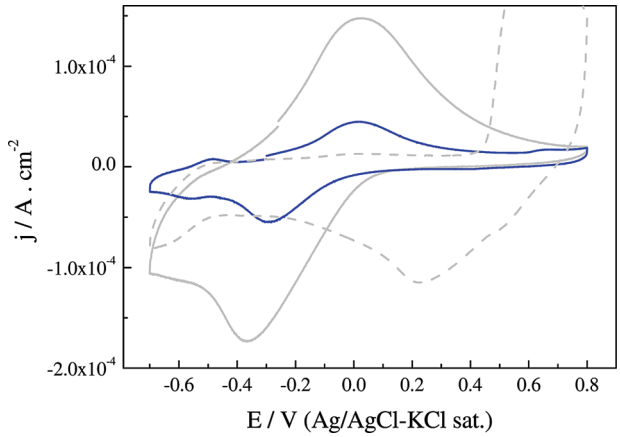

FIGURE 5. Cyclic voltammogram obtained on a graphite electrode after two 18 h steps of continuous polarization at 0.6 V (first step) and 0.1 V (second step) in a fumarate-limited culture of *G. sulfurreducens* (blue line). Voltammograms obtained after single step experiments at 0.6 V (grey dotted line) and 0.1 V (grey line) are included for comparison.

(Figure 5) (stationary data for single polarization experiments at 0.1 and 0.6 V were included for comparison).

Discussion

Microbial fuel cells (MFCs) have recently emerged as a new and promising technology to convert organic compounds into electricity by means of Fe(III)-reducing bacteria as *G. sulfurreducens* that function as biological catalysts (*4*). The results described here provide the first insight into the *Geobacter*–electrode interface using classical electrochemical tools. We have reported the bacterial response to applied potential regarding electrode attachment, current production, and redox signal profiling. These results demonstrate that *G. sulfurreducens* conveniently adapt their exocellular electron transfer elements to the potential applied to the electrodes and open new lines of investigation for the optimization of current production.

The Biological Adaptation to the Potential. It is widely accepted that bacterial adhesion to a solid surface depends on hydrophobicity and charge of the interacting surfaces (*19, 20*). The surface charge of a polarized graphite electrode, determined by the magnitude and sign of the applied potential as compared to the potential of zero charge (PZC) of the material, is known to be increasingly positive from 0.1 to 0.6 V (*21*). It also determines an increasing favorable interaction for the adhesion of negatively charged bacterial cells (*22*) and could help to explain the results presented in Figure 2, where the amount of bacterial cells on electrodes was clearly higher at the highest applied polarization. The increased population found at 0.6 V produced current at a higher density (Figure 1), which could be a direct consequence of the increased number of attached cells, or can also be related to an increased cell capacity for transferring electrons when the electrode polarization becomes high. Actually, it has been previously reported that *G. sulfurreducens* increases the electron transfer rate (3-fold) when the cells are conserving energy from Fe(III)-reduction (formal potential = 0.30V (vs NHE)) compared to fumarate-reduction (formal potential = 0.05V (vs NHE)) (*23*).

As shown in Figure 3, the voltammetric fingerprint of *G. sulfurreducens* adhered to a graphite electrode dramatically changed depending on the applied potential, suggesting the occurrence of two potential domains that produce different bacterial responses. An almost reversible redox couple (Figure 3a) with a formal potential of about −0.08 V (Figure 4) was produced in the low potential region, whereas a more energetic signal (Figure 3b) with a higher formal potential (Figure 4) was the dominant feature when the applied polarization was 0.6 V. It could give support to the idea of an increased cell's electron transport capacity at high potential and adds to the discussion about the selection of the most appropriate anode potential when implementing a MFC. For instance, the anodic polarization reached by coupling sediment-buried electrodes with the oxygen reduction reaction in sediment fuel cells (i.e., 0.0 V) (*24*) is only slightly positive to the lower formal potential of cells (−0.08 V) (Figure 4), thus not exploiting cells oxidation at the highest rate. Polarization at around the oxidation peak potential or higher would be preferable in clear accordance to previously reported results (*25*). Results presented here give support to these previous findings and suggest their direct connection to changes in the bacterial electron transport pathway.

The Physiological Role of the Electrochemical Signals. Taking into account that a crucial point in enzyme electrochemistry is the good electronic coupling between the active site and the electrode surface (*26*), the direct electrochemical detection of redox signals in Figure 3 clearly shows that some elements of the cell surface are close enough to the electrode to undergo the electron exchange. Indeed, the detection of very distinct redox elements, depending on the applied potential, demonstrates that cells have alternative pathways to exchange electrons. It also suggests that they were able to sense either the potential difference across the interface or, alternatively, some variation in the surface chemistry of graphite to develop a differential response. The last became especially evident when cells at 0.6 V polarized-electrodes drastically reorganized their signal profile when polarization was shifted to 0.1 V (Figure 5).

Differential expression of redox elements used by this organism has been previously reported in cells growing on different electron acceptors (*5, 7, 27*), which points to the existence of a recognition mechanism of the electron acceptor in these cells that still remains unknown. In this regard, Childers et al. (2002) (*28*) have shown that *G. metallireducens* is chemotactic toward Fe(II) and Mn(IV) when grown on solid oxides, evidencing the recognition of the acceptor at a distance through the sensing of its reduction products. Although it could be extrapolated to other electron acceptors, in the particular case of electrodes no gradients of a reduced or an oxidized form of the acceptor may exist, thus suggesting that recognition may occur only after random encounter

and physicochemical interaction. Bespalov et al. (1996) showed that *Escherichia coli* is able to sense a redox gradient in the environment. The interaction of putative acceptors with hydrogenases in the electron transport chain modify the electron flux in this bacterium triggering a redox-tactic behavior through which the cells can find a defined position in a redox gradient (*29*). Indeed, the authors showed that the stronger the affinity of a molecule to divert electrons from the electron transport system (i.e., its formal potential), the more potent it is as an effector for a behavioral response. Based on this, it can be inferred that a similar mechanism can be used by *G. sulfurreducens* to recognize acceptors (including electrodes) with different redox potentials. We propose that, depending on the applied potential, a polarized electrode can mimic the presence of different electron acceptors, thus inducing the up-regulation of specific electron pathways. Specificity in the developed response would be determined by the oxidative capacity of the electrode that could activate pathways of higher redox potential as the polarization increases. Due to the higher potential energy available at the interface at 0.6 V the electron flow can be expected to be faster and the current production increased in clear connection with results in Figure 1. A higher potential pathway would also be more energetically productive leading to a faster population growth and a widespread surface coverage, which could explain the results in Figure 2.

In this context, the signal found after the 0.1 V polarization is thought to be due to an external reductase able to interact with electron acceptors of intermediate potential, as iron and manganese oxides (*30, 31*), whereas the high potential signal could be related to the outstanding ability of this bacterium to reduce high potential acceptors as vanadates (*32*).

The Electron Pathway to Electrodes. The natural selectivity that proteins exhibit for their biological or natural reaction partners is expected to extend to protein-electrode interactions (*26*). Consequently, not only the type of electron transport element but also the way in which it interacts with the surface is expected to be controlled by bacteria to improve the electrons disposal.

If the signal reported in Figure 3a corresponds to an external iron reductase, a low enough formal potential is expected for the protein to be able to reduce iron oxides. Values ranging from −0.12 to −0.32 V have been reported for redox potential of the amorphous oxide ferrihydrite (*30*), whereas lower values have been informed for goethite and magnetite (*31*). As shown in Figure 3a, the voltammogram of cells adapted to 0.1 V gradually changed with the cycles. Calculations from these data shown that the peak to peak potential difference of the redox pair decrease toward a more reversible communication with the electrode (Figure 6a), while its $E_{1/2}$ value changes from −0.37 V during the first voltammetric cycle to a stable value of about −0.23 V after 10 cycles (Figure 6b). Such a negative starting value is thought to correspond to the approximated formal potential of the intact protein and it is in clear accordance with the requirements for an iron reductase. Upon potential cycling during voltammetry on the other hand, a conformational transition to a more favorable configuration for the electron exchange with the surface is thought to occur. It has been shown that conformational transitions involved in the electron transport from Cyt C to an electrode are modulated by the electric field at the interface and that this modulation depends on the position of the redox protein on the electrode (*33*). Based on these observations we hypothesize that the position of the electron transport elements at the bacteria/electrode interface would change from a physiological configuration self-organized by bacteria after adsorption, to a new one induced by potential cycling.

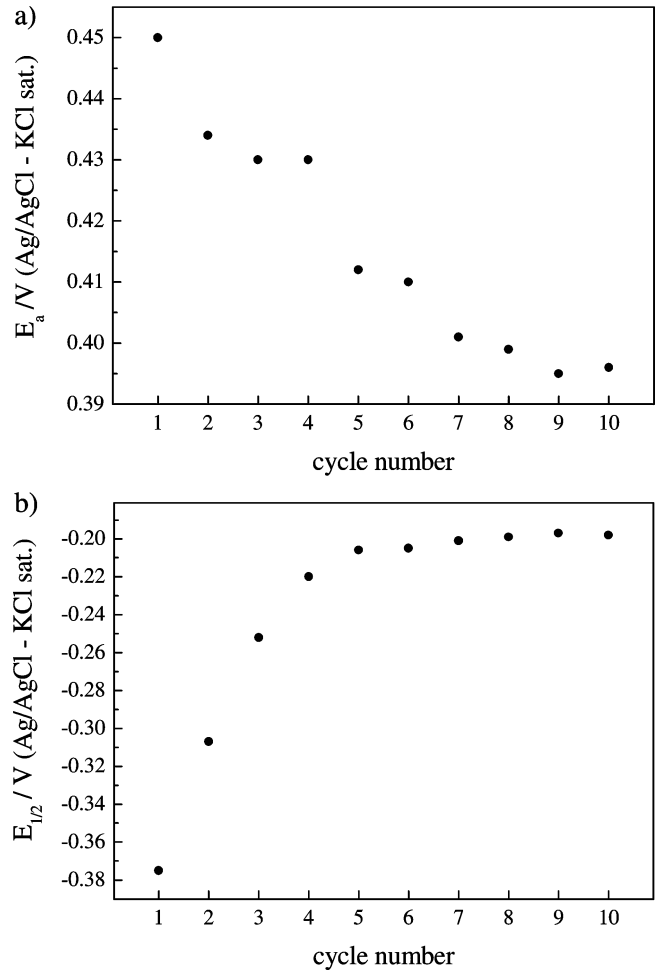

FIGURE 6. Evolution of the redox process peak to peak potential (E_a) (panel a) and estimated formal potential ($E_{1/2}$) (panel b), with the cycles of the voltammetric analysis presented in Figure 3a performed on a graphite electrode bearing 0.1 V adapted cells.

The results presented in this work provide the first electrochemical insight into the mechanism by which *G. sulfurreducens* transfers electrons onto an electrode. The in vivo voltammetric analysis demonstrated that cells conveniently adapt the electron transport pathway to the electrode, to take advantage of the stronger oxidizing power at high polarization. Indeed, it allowed the determination of the potential domains for the cells redox processes, providing important information for the implementation of *G. sulfurreducens*-based microbial fuel cells.

Acknowledgments

The financial support to J.P.B. from the Economic Union through a Marie Curie International Incoming fellowship (contract no.: MIF1-CT-2006-021347) is greatly acknowledged. Partial support from the Ministerio de Educación y Ciencia (Spain) through project CTQ2006-04071/BQU is also acknowledged. J.P.B. is a researcher from the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina. Valuable help from Virginia Souza with SEM is greatly acknowledged.

Supporting Information Available

Voltammogrames of a graphite electrode after 18 h of exposure to a fumarate-limited culture of *G. sulfurreducens* at both, 0.4 V (Figure S1) and the open circuit potential (Figure

S2). This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Park, D. H.; Zeikus, J. G. Electricity generation in microbial fuel cells using neutral red as an electronophore. *Appl. Environ. Microbiol.* **2000**, *66* (4), 1292–7.

(2) Logan, B. E.; Hamelers, B.; Rozendal, R.; Schroder, U.; Keller, J.; Freguia, S.; Aelterman, P.; Verstraete, W.; Rabaey, K. Microbial fuel cells: Methodology and technology. *Environ. Sci. Technol.* **2006**, *40* (17), 5181–5192.

(3) Lovley, D. R. Bug juice: Harvesting electricity with microorganisms. *Nat. Rev. Microbiol.* **2006**, *4* (7), 497–508.

(4) Lovley, D. R. Microbial fuel cells: Novel microbial physiologies and engineering approaches. *Curr. Opin. Biotechnol.* **2006**, *17* (3), 327.

(5) Mehta, T.; Coppi, M. V.; Childers, S. E.; Lovley, D. R. Outer membrane c-type cytochromes required for Fe(III) and Mn(IV) oxide reduction in *Geobacter sulfurreducens*. *Appl. Environ. Microbiol.* **2005**, *71* (12), 8634–41.

(6) Bretschger, O. An exploration of current production and metal oxide reduction by *Shewanella oneidensis* MR-1 wild type and mutants. *Appl. Environ. Microbiol.* **2007**.

(7) Holmes, D. E.; Chaudhuri, S. K.; Nevin, K. P.; Mehta, T.; Methe, B. A.; Liu, A.; Ward, J. E.; Woodard, T. L.; Webster, J.; Lovley, D. R. Microarray and genetic analysis of electron transfer to electrodes in *Geobacter sulfurreducens*. *Environ. Microbiol.* **2006**, *8* (10), 1805–1815.

(8) Bond, D. R.; Lovley, D. R. Electricity production by *Geobacter sulfurreducens* attached to electrodes. *Appl. Environ. Microbiol.* **2003**, *69* (3), 1548–55.

(9) Reguera, G.; McCarthy, K. D.; Mehta, T.; Nicoll, J. S.; Tuominen, M. T.; Lovley, D. R. Extracellular electron transfer via microbial nanowires. *Nature* **2005**, *435* (7045), 1098–101.

(10) Gorby, Y. A. Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. *PNAS* **2006**, *103* (30), 11358–11363.

(11) Reguera, G.; Nevin, K. P.; Nicoll, J. S.; Covalla, S. F.; Woodard, T. L.; Lovley, D. R. Biofilm and nanowire production leads to increased current in *Geobacter sulfurreducens* fuel cells. *Appl. Environ. Microbiol.* **2006**, *72* (11), 7345–7348.

(12) Shi, L.; Squier, T. C.; Zachara, J. M.; Fredrickson, J. K. Respiration of metal (hydr)oxides by *Shewanella* and *Geobacter*: A key role for multihaem c-type cytochromes. *Mol. Microbiol.* **2007**, *65* (1), 12–20.

(13) Kim, H. J.; Park, H. S.; Hyun, M. S.; Chang, I. S.; Kim, M.; Kim, B. H. A mediator-less microbial fuel cell using a metal reducing bacterium, *Shewanella putrefaciens*. *Enz. Microb. Technol.* **2002**, *30* (2), 145–152.

(14) Liu, H.; Cheng, S.; Logan, B. E. Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. *Environ. Sci. Technol.* **2005**, *39* (2), 658–662.

(15) Rabaey, K.; Rodriguez, J.; Blackall, L. L.; Keller, J.; Gross, P.; Batstone, D.; Verstraete, W.; Nealson, K. H. Microbial ecology meets electrochemistry: electricity-driven and driving communities. *ISME J* **2007**, *1* (1), 9–18.

(16) Rabaey, K.; Ossieur, W.; Verhaege, M.; Verstraete, W. Continuous microbial fuel cells convert carbohydrates to electricity. *Water Sci. Technol.* **2005**, *52* (1–2), 515–523.

(17) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods: Fundamental and Applications*. 2nd. ed.; John Wiley & Sons: Toronto, 1980.

(18) Esteve-Nunez, A.; Rothermich, M.; Sharma, M.; Lovley, D. Growth of *Geobacter sulfurreducens* under nutrient-limiting conditions in continuous culture. *Environ. Microbiol.* **2005**, *7* (5), 641–648.

(19) Busalmen, J. P.; de Sanchez, S. R. Adhesion of *Pseudomonas fluorescens* (ATCC 17552) to nonpolarized and polarized thin films of gold. *Appl. Environ. Microbiol.* **2001**, *67* (7), 3188–94.

(20) Busscher, H. J.; Weerkamp, A. H. Specific and non-specific interactions in bacterial adhesion to solid substrata. *FEMS Microbiol. Lett.* **1987**, *46* (2), 165–173.

(21) Barisci, J. N.; Wallace, G. G.; Chattopadhyay, D.; Papadimitrakopoulos, F.; Baughman, R. H. Electrochemical properties of single-wall carbon nanotube electrodes. *J. Electrochem. Soc.* **2003**, *150* (9), E409–E415.

(22) Busalmen, J. P.; de Sanchez, S. R. Electrochemical polarization-induced changes in the growth of individual cells and biofilms of *Pseudomonas fluorescens* (ATCC 17552). *Appl. Environ. Microbiol.* **2005**, *71* (10), 6235–6240.

(23) Esteve-Nunez, A.; Nunez, C.; Lovley, D. R. Preferential reduction of Fe(III) over fumarate by *Geobacter sulfurreducens*. *J. Bacteriol.* **2004**, *186* (9), 2897–9.

(24) Ryckelynck, N.; Stecher, H. A.; Reimers, C. E. Understanding the anodic mechanism of a seafloor fuel cell: Interactions between geochemistry and microbial activity. *Biogeochem.* **2005**, *76* (1), 113–139.

(25) Finkelstein, D. A.; Tender, L. M.; Zeikus, J. G. Effect of electrode potential on electrode-reducing microbiota. *Environ. Sci. Technol.* **2006**, *40* (22), 6990–6995.

(26) Armstrong, F. Voltammetry of proteins. In *Bioelectrochemistry*, 1st. ed.; Wilson, G., Ed.; Wiley-VCH Verlag GmbH: Weinheim, 2002; Vol. 9, pp 11–29.

(27) Ding, Y.-H. R. The proteome of dissimilatory metal-reducing microorganism *Geobacter sulfurreducens* under various growth conditions. *BBA—Proteins Proteom.* **2006**, *1764* (7), 1198–1206.

(28) Childers, S. E.; Ciufo, S.; Lovley, D. R. *Geobacter metallireducens* accesses insoluble Fe(III) oxide by chemotaxis. *Nature* **2002**, *416* (6882), 767–769.

(29) Bespalov, V. A.; Zhulin, I. B.; Taylor, B. L. Behavioral responses of *Escherichia coli* to changes in redox potential. *PNAS* **1996**, *93* (19), 10084–10089.

(30) Thamdrup, B.; Rossello-Mora, R.; Amann, R. Microbial manganese and sulfate reduction in Black Sea shelf sediments. *Appl. Environ. Microbiol.* **2000**, *66* (7), 2888–97.

(31) Weber, K. A.; Achenbach, L. A.; Coates, J. D. Microorganisms pumping iron: anaerobic microbial iron oxidation and reduction. *Nat. Rev. Microbiol.* **2006**, *4* (10), 752–64.

(32) Ortiz-Bernad, I.; Anderson, R. T.; Vrionis, H. A.; Lovley, D. R. Vanadium respiration by *Geobacter metallireducens*: novel strategy for in situ removal of vanadium from groundwater. *Appl. Environ. Microbiol.* **2004**, *70* (5), 3091–5.

(33) Murgida, D. H.; Hildebrandt, P. Electron-transfer processes of cytochrome C at interfaces. New insights by surface-enhanced resonance raman spectroscopy. *Acc. Chem. Res.* **2004**, *37* (11), 854–861.

ES702569Y