

# Optimal Set Anode Potentials Vary in Bioelectrochemical Systems

RACHEL C. WAGNER, DOUGLAS F. CALL, AND  
BRUCE E. LOGAN\*

Department of Civil and Environmental Engineering, 212 Sackett Building, The  
Pennsylvania State University, University Park, Pennsylvania 16802

Received March 30, 2010. Revised manuscript received June 19, 2010. Accepted  
July 1, 2010.

In bioelectrochemical systems (BESs), the anode potential can be set to a fixed voltage using a potentiostat, but there is no accepted method for defining an optimal potential. Microbes can theoretically gain more energy by reducing a terminal electron acceptor with a more positive potential, for example oxygen compared to nitrate. Therefore, more positive anode potentials should allow microbes to gain more energy per electron transferred than a lower potential, but this can only occur if the microbe has metabolic pathways capable of capturing the available energy. Our review of the literature shows that there is a general trend of improved performance using more positive potentials, but there are several notable cases where biofilm growth and current generation improved or only occurred at more negative potentials. This suggests that even with diverse microbial communities, it is primarily the potential of the terminal respiratory proteins used by certain exoelectrogenic bacteria, and to a lesser extent the anode potential, that determines the optimal growth conditions in the reactor. Our analysis suggests that additional bioelectrochemical investigations of both pure and mixed cultures, over a wide range of potentials, are needed to better understand how to set and evaluate optimal anode potentials for improving BES performance.

## Introduction

Microorganisms respire and capture energy for the production of ATP through the oxidation of organic and inorganic matter and reduction of a terminal electron acceptor. ATP is generated by the proton motive force, which results from protons that are pumped outside the inner cell membrane as electrons are transferred from a reduced to an oxidized compound via the electron transport chain during respiration. Thus, there is the possibility of generating more energy using electrons acceptors with higher potentials, but only if the microorganism can utilize the additional energy through the pumping of additional protons across the membrane.

The maximum amount of energy that can be captured from the oxidation and reduction of two chemical species can be calculated from the Gibbs free energy as

$$\Delta G^{\circ} = -nF\Delta E_0' \quad (1)$$

where  $\Delta G^{\circ}$  is the Gibbs free energy at standard biological conditions ( $T = 25\text{ }^{\circ}\text{C}$ ,  $\text{pH} = 7$ ),  $n$  is the number of electrons

transferred,  $F$  is Faradays constant ( $96,485\text{ C/mol e}^{-}$ ), and  $\Delta E_0'$  is the difference in the potentials between the electron donor and the electron acceptor for a particular chemical reaction. Larger values of  $\Delta E_0'$ , therefore, could provide more energy for the cell. For example, using hydrogen as an electron donor and assuming equimolar concentrations of the terminal electron acceptor, *Paracoccus denitrificans* can obtain more energy by reducing oxygen than by reducing nitrate, due to the greater energy available from reduction of oxygen. Capture of this additional energy could result in increased biomass yields, depending on the metabolic efficiency of this microbe under these conditions. Thermodynamic potentials cannot be used to predict growth rates of microbes, as this is determined by kinetics, but in general aerobes usually grow faster than anaerobic microorganisms. In addition to the different redox potentials of electron acceptors, the energetic requirements of a cell vary depending upon whether an internal electron acceptor (such as fumarate) or an external electron acceptor (such as insoluble Fe(III) or an electrode) is available (1). With an internal electron acceptor, protons are consumed within the cell during reduction of the electron acceptor, whereas with an external electron acceptor, protons produced from substrate oxidation must be transported out of the cytoplasm to avoid acidifying the cytoplasm. This proton transport, required by use of an external electron acceptor, is an energetically intensive process that Mahadevan et al. suggested (2) could have significantly decreased the growth yield of *G. sulfurreducens* on the external electron acceptor.

In a bioelectrochemical system (BES), the anode can be used as a terminal electron acceptor for microorganisms. Microbes must have a mechanism, provided through direct contact or a chemical mediator, for transferring electrons outside of the cell to the anode (3). In order for the reaction to be thermodynamically favorable, the anode must have a higher (more positive) potential than either the terminal protein in the cell's electron transport chain or the mediator that is used. In theory (eq 1), a more positive anode potential will allow the cell to capture more energy, but only if the cell is capable of capturing this energy by pumping additional protons across its inner membrane. Thus, the microbe must possess terminal respiratory proteins that can use this additional potential provided by the anode. For example, if the anode of an acetate fed ( $E_0'_{\text{donor}} = -0.3\text{ V}$ ) BES is set to a highly positive value (e.g.,  $E_0'_{\text{anode}} = +0.4\text{ V}$ ) and a microbe can only adjust its respiratory enzymes to a lower potential (e.g.,  $E_0'_{\text{enzyme}} = -0.05\text{ V}$ , as suggested by ref 4), then the microbe will only be able to capture part of the total available free energy. The additional free energy between the terminal respiratory enzyme and anode potential is wasted.

\* Corresponding author phone: (814)863-7908; e-mail: blogan@psu.edu.

**TABLE 1. Applied Potential Studies Comparing Set Anode Potentials<sup>a</sup>**

set potentials (V vs SHE)	inoculum	reference electrode <sup>b</sup>	working electrode	results <sup>c</sup>	ref
-0.2, 0, +0.2	MFC suspension	Ag/AgCl	graphite granule	<u>most active biomass at -0.2 V</u> ; highest current at maximum power at 0 V; maximum power at 0 V; similar start-up at all voltages	(28)
-0.36, -0.16, +0.04, +0.44, +0.64	<i>D. desulfuricans</i>	SCE	graphite plate; stainless steel	<u>current obtained only at -0.16 V</u>	(37)
-0.26, -0.16, -0.06, +0.04, +0.14, +0.24	<i>G. sulfurreducens</i>	SCE	polished graphite blocks	at constant biomass, -0.16 and -0.26 V had lower current; similar CVs between biofilms grown at -0.16 V and +0.24 V suggesting limited ability to adjust terminal reductase to different voltages	(4)
-0.15, -0.09, +0.02, +0.37	domestic WW	Ag/AgCl (0.27 V vs SHE)	graphite rods (multiple)	<u>at -0.15 and -0.09 V, obtained higher current, and faster start-up, than at other voltages; lower potentials produced a thicker biofilm dominated by <i>G. sulfurreducens</i></u>	(23)
-0.16, 0, +0.4, floating potential	<i>G. sulfurreducens</i>	SCE (0.242 V vs SHE)	carbon paper	higher current, faster start-up, greater biomass for 0 V and +0.4 V; lower use of possible metabolic energy gain at +0.4 V suggesting an upper limit of the terminal reductase for <i>G. sulfurreducens</i> between 0 V and +0.4 V	(17)
+0.4, floating potential	domestic WW	Ag/AgCl (0.197 V vs SHE)	graphite plate	faster startup using poised vs nonpoised anode potentials	(20)
0, +0.2, +0.35, +0.5	<i>S. oneidensis</i>	Ag/AgCl	graphite plate	higher current at +0.5 V	(21)
+0.11, +0.21, +0.31, +0.51	<i>G. sulfurreducens</i>	Ag/AgCl (0.31 V vs SHE)	stainless steel	current obtained only at +0.51 V	(16)
+0.54, +0.74, +0.94	garden compost	SCE	dimensionally stable anodes (DSA)	<u>higher current, faster biofilm development at +0.54 V within one chamber; higher current at +0.94 V when in separate reactors, but this result was inconsistent across replicates; current at +0.74 V &gt; +0.34 V &gt; +0.64 V in one experiment; +0.74 V selected as best potential</u>	(18)
+0.3, +0.8	<i>G. sulfurreducens</i>	Ag/AgCl (0.197 V vs SHE)	graphite plate	higher current, and faster start-up, at +0.8 V; +0.8 produced a thicker biofilm	(15)
+0.14, +0.30, +0.82	marine sediment	Ag/AgCl	graphite rod	higher current, faster substrate oxidation at +0.82 V than other voltages	(11)

<sup>a</sup> Many studies used a single applied potential and did not compare different potentials (24, 35, 38–48). Three additional studies not reviewed here suggest improved performance at more positive potentials (49–51). <sup>b</sup> The value in parentheses is the value reported in the article. No value means that the reference electrode value compared to the SHE was not reported. <sup>c</sup> Results in which a lower anode potential improved performance are underlined.

Setting the anode potential in a BES has allowed researchers to study the electrochemical capabilities of microbes that can transfer electrons to an anode (referred to as exoelectrogens or anode-reducing bacteria (3)). Optimal anode potentials, defined here as those producing high current densities and more rapid start-up times, have not been established. Known exoelectrogens are widely dispersed among many different genera, and factors that can affect an optimal anode potential are diverse, for example: differing redox potentials of the various cytochromes; different mechanisms for transferring electrons from the cell to the anode; and variability in the effectiveness of electron transfer from cells to different anodes materials (for example carbon versus stainless steel). A review of 28 studies (Table 1) reveals that in 50% of these studies, a single potential was used to set the anode for experimentation. When different anode potentials are compared, 71% (10) of these 14 comparison studies show improved performance (i.e., faster start-up or higher current density) at higher potentials, 14% (2) showed mixed results, and 14% (2) show improved performance at lower potentials.

We review here the literature in terms of the effects of anode potentials on start-up time, maximum current den-

sities, and biomass production. Our results show that optimal electrode conditions for mixed communities cannot be set *a priori* and at present must be individually determined; for pure cultures, electrochemical techniques can assist in determining the optimal anode potential.

**Setting Anode Potentials.** Accurately setting an anode potential requires the use of a potentiostat in conjunction with a reference electrode (5). Commonly used reference electrodes include silver/silver chloride (Ag/AgCl) and saturated calomel electrodes (SCE). The standard value of the saturated Ag/AgCl reference is +0.197 V ( $T = 25\text{ }^{\circ}\text{C}$ ) versus the standard hydrogen electrode (SHE) (6). Under unsaturated conditions, the potentials are slightly different (e.g., +0.209 V in 3 M KCl). The SCE reference has a standard value of +0.244 V ( $T = 25\text{ }^{\circ}\text{C}$ ) under saturated conditions, but this value is also dependent on the electrolyte concentration. The actual potential of a reference electrode depends on experimental conditions, such as temperature and solution chemistry, and it can be determined by calibrating the reference electrode using Zobell's solution (7). At the end of an experiment, the reference electrode should be checked for accuracy, as chemicals in solution, such as ammonia and

sulfide, can form precipitates on the electrode, causing variability relative to the standard values reported above (8). The reference electrode should be placed as close as possible to the working electrode, with the location fixed throughout the experiment, to minimize potential loss through the solution and limit variability. Placing a reference electrode 0.6 cm from the anode, for example, resulted in a drift of 0.042 V between the reference potential and the anode potential (9).

Set anode potentials in BESs typically range from  $-0.2$  V to  $+0.8$  V vs SHE at pH 7, with the anode potential more positive than the calculated potential of the substrate. Anode potentials are often set to mimic typical terminal electron acceptors found in natural environments, such as insoluble iron (ferrihydrite/ $\text{Fe}^{2+}$ ,  $-0.100$  to  $+0.100$  V) (10) and oxygen ( $\text{O}_2/\text{H}_2\text{O}$ ,  $+0.818$  V) (11). Hydrogen can theoretically form at potentials more negative than  $-0.414$  V, and oxygen can be produced at potentials more positive than  $+0.818$  V; however, all electrode materials have overpotentials, and the actual potentials at which these reactions occur can be determined using cyclic voltammetry on abiotic controls. Electrochemical hydrogen or oxygen production should be avoided as it may lead to the growth of nonexoelectrogenic bacteria and interfere with interpretation of results. In addition, hydrogen peroxide can be produced at a theoretical potential of  $+0.28$  V when oxygen is present, which has been shown at the cathode of several BES (12–14). Although potentiostats provide the most accurate method for setting anode potentials, they can be a cost-prohibitive lab item, and an alternative method for setting an anode potential using a power supply unit is described by Bond (8).

All voltages are given here with respect to the SHE, pH = 7. In cases where the voltage was not reported vs SHE, Ag/AgCl voltages were approximated by adding  $+0.2$  V and SCE voltages by adding  $+0.24$  V to the reported voltage. We recommend that researchers working on BESs maintain consistency across the discipline by always reporting voltages adjusted to SHE and ensure accuracy of given values by always checking their reference electrodes as mentioned above using Zobell's solution. Differences in potential can be described as "more negative" and "more positive" than one another or as "lower" and "higher", with higher always indicating a more positive potential.

#### Results Supporting the Use of Higher Anode Potentials.

Studies in which better performance is seen at higher potentials suggest that more positive potentials provided more free energy to the microorganisms, although in many cases the range of potentials examined was limited. In one study, for example, *G. sulfurreducens* produced a higher current, and more quickly, at  $+0.8$  V than at the lower potential of  $+0.3$  V (15), but no other potentials were examined. The anode with the more positive potential also produced a thicker biofilm. Dumas et al. (16) also examined the effect of different potentials on current production by *G. sulfurreducens*, but they used stainless steel electrodes rather than graphite, which prevents a direct comparison. Current was only generated at the highest set potential of  $+0.51$  V, but similar to the Busalmen study (15) all the other potentials examined were fairly high ( $+0.31$ ,  $+0.21$ , and  $+0.11$  V).

Examining a wider range of potentials, Wei et al. (17) compared current and biomass production of *G. sulfurreducens* growing on anodes set to  $-0.16$  V,  $0$  V, and  $+0.4$  V. They observed faster start-up times, higher current generation, and greater biomass production for the anode poised at  $0$  V versus  $-0.16$  V; the anode set to  $+0.4$  V showed similar current and biomass production to the one poised at  $0$  V, suggesting an upper limit to the amount of energy that could be captured by the bacteria, regardless of an increase in available potential. They concluded that the key to high current production was faster and thicker biofilm develop-

ment, which could be achieved by using higher anode potentials ( $0$  and  $+0.4$  V).

In addition to the pure culture studies mentioned above, Finkelstein et al. (11) showed improved performance with a mixed culture at higher set potentials than lower potentials. Three different potentials ( $+0.142$  V,  $+0.303$  V, and  $+0.818$  V) were applied to anodes submerged in marine sediment. The highest current and fastest substrate oxidation rate was produced by the anode with the most positive set potential. A mixed culture from garden compost also produced higher current at higher set anode potential ( $+0.94$  V vs  $+0.74$  V and  $+0.64$  V, in separate reactors) (18).

Setting the anode potential at more positive voltages has a clear rationale based on providing the possibility for more energy for the microorganisms, but it creates conditions which are quite different than those that develop in most BESs. When microbial fuel cells are operated, the anode potentials measured during peak voltage generation are often quite negative, with typical values around  $-0.20$  to  $-0.28$  V for mixed cultures oxidizing acetate (19). The exact value is dependent on the substrate used, the cathodic reaction, the external resistor, and the current density. The potential of a nonpoised anode becomes more negative over time (until it reaches a steady-state potential) as an exoelectrogenic biofilm develops (5), whereas a poised anode is controlled externally (e.g., by a potentiostat) at the same potential throughout the duration of the experiment, regardless of biofilm activity. There are few studies comparing current densities achieved using a nonpoised (and therefore variable potential) anode to those produced by a set (and therefore stable) potential. In one such study, Wang et al. (20) used a mixed culture (domestic wastewater and anaerobic sludge). The anode of one reactor was set at  $+0.4$  V, while the potential in the anode of the second reactor was not fixed. The nonpoised anode potential decreased from approximately  $+0.49$  to  $-0.16$  V over 11 batch cycles (52 days). The final cell voltage of the nonpoised reactor (measured across a  $1000 \Omega$  resistor) was the same as that achieved with the poised-anode reactor (disconnected and measured across a  $1000 \Omega$  resistor). However, the poised-anode reactor produced a reproducible current in fewer cycles (6 batch cycles, 30 days), showing that setting the anode potential can result in more rapid reactor acclimation for maximum power. The experiments were not continued past day 60, so longer-term stability of the nonpoised anode was not tested. Faster start-up time using an anode poised at  $+0.4$  V versus a nonpoised anode was also shown with *G. sulfurreducens* (2 and 3.5 days, respectively, to reach the same current) (17). Reducing the length of time it takes an anode to reach a stable maximum voltage is important when considering the use of BESs for practical applications. Wang et al. (20) showed that poisoning an anode reduced start-up time, and in a study using *S. oneidensis*, Cho and Ellington (21) showed that the most positive potential had the shortest start-up time ( $+0.5$  V vs  $+0.35$  V,  $+0.2$  and  $0$  V).

In the studies mentioned in this section (10 of the 28 studies reviewed here), there appears to be a clear trend of improved performance with higher potentials. These observations of improved performance at higher potentials do not consider many studies where relatively low potentials were used. Thus, it is clear that more positive potentials can be used to study microorganisms on the anodes in BESs, but comparisons with a broader range of potentials - including those much closer to the potential of the substrate - are necessary for a complete understanding of BES performance.

#### Results Supporting the Use of Lower Anode Potentials.

The low anode potential developed by a nonpoised MFC is typically only slightly more positive than the  $E_0'$  for the half cell reaction for oxidation of typical substrates, such as  $-0.30$  V for acetate and  $-0.43$  V for glucose (22). This suggests that

setting a more negative potential will create conditions most similar to those in a BES, but using a single potential does not allow us to probe the capabilities of the pure or mixed culture at other electrode potentials.

In order to see what potentials might produce the most optimum conditions for growth in BESs, Torres et al. (23) examined biofilm growth and current generation in a two-chamber BES containing four anodes set at different potentials using a single reference electrode. The anode chamber was inoculated with a mixture of return activated sludge and anaerobic digester sludge. The two anodes set at negative potentials ( $-0.15$  and  $-0.09$  V) generated current sooner, reached higher current densities, and developed visually thicker biofilms than the anodes set at positive potentials ( $+0.02$  V and  $+0.37$  V). These results suggest that in a single competitive environment for acetate, exoelectrogenic microorganisms were most successful (relative to growth and current production) using relatively low anode potentials. The biofilm that developed at  $-0.15$  V was dominated by *Geobacter* (97% similarity to *G. sulfurreducens*), a known exoelectrogen that produces high current densities in BESs (24–27). In contrast, the community at the anode set at  $+0.37$  V was much more diverse and generated very little current. The authors surmised that nonexoelectrogens, and/or mediator-producing exoelectrogenic microbes, may have colonized the  $+0.37$  V anode first, interfering with the ability of *Geobacter* to make direct contact with this anode.

Setting multiple electrodes to different potentials within the same chamber creates conditions that may not exist when only one anode is used per reactor. For example, electrodes placed at different distances from the reference electrode in the same chamber may create small but significant variation from the intended applied potential. Biofilms growing on one electrode could produce mediators at one potential that affect growth and current production of biofilms on electrodes at other potentials. The use of electrodes at different potentials in the same chamber produces surfaces with different charges, promoting negatively charged particle (e.g., bacterial) migration to the more positively charged electrodes. Only one study has examined BESs using both single and multiple electrodes per anode chamber (18). Three dimensionally stable anode (DSA) electrodes were placed into a single reactor, set at three different (positive) potentials of  $+0.54$ ,  $+0.74$ , and  $+0.94$  V, and inoculated with garden compost. In direct contrast to their anodes in separate reactors, average current densities in the single reactor/multiple anodes experiment were highest ( $129 \text{ mA m}^{-2}$ ) for the electrodes set at the lowest applied potential ( $+0.54$  V), and current densities decreased at the higher potentials. In an experiment with three electrodes placed in a single chamber, all at  $+0.74$  V, each electrode produced the same current. However, the current from an electrode in this reactor, with all three electrodes at  $+0.74$  V, was lower than that obtained at the same potential in the presence of two electrodes set at different potentials. The different results for anodes placed in separate reactors compared to a single reactor suggest that the presence of other electrodes set at different potentials within a reactor affected the results. Electrode materials and potentials used by Torres et al. (23) and Parot et al. (18) were also different, and for these reasons further studies are necessary to determine if it is acceptable to use multiple electrodes with different set potentials in the same chamber in BES tests.

Other studies comparing high and low anode potentials show mixed results, in terms of power or current, with different set potentials. For example, Aelterman et al. (28) concluded that the optimal anode potential was 0 V, compared to  $-0.2$  V and  $+0.2$  V, for a mixed culture obtained from a long-running MFC anode. The conclusion that 0 V was optimal was based on maximum attainable power as

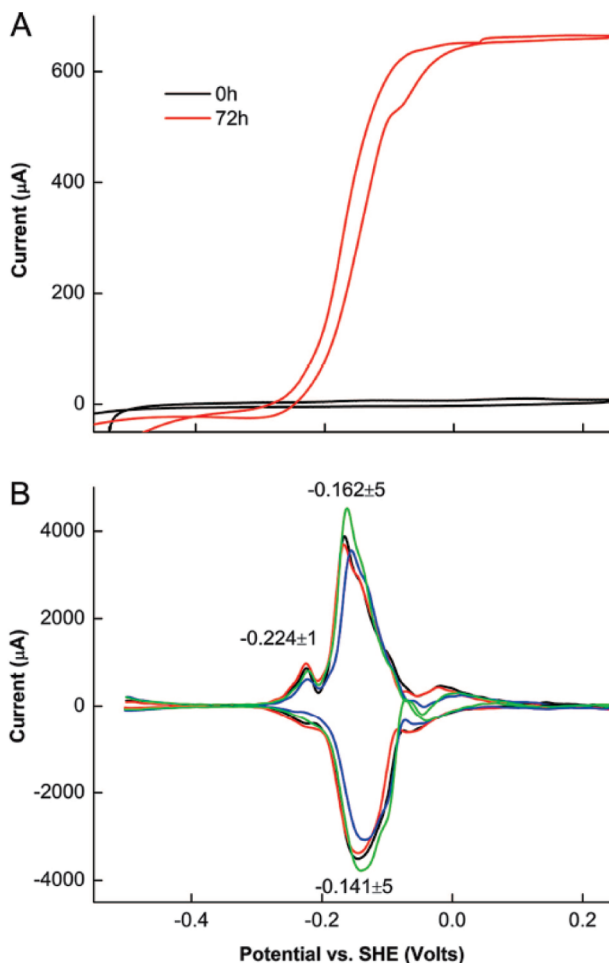
well as the highest current production at maximum power, although the lowest set potential ( $-0.2$  V) reached its maximum power most quickly. The lowest amount of biomass was produced on the electrode at the lowest potential ( $-0.2$  V). However, even though there was less biomass on the electrode set to  $-0.2$  V, this biofilm was the most active, producing considerably more current per unit of biomass than the biofilms on the electrodes set at higher potentials. This suggests that different potentials may be necessary to encourage different biofilm characteristics: first a potential to encourage biofilm growth on the electrode and then a potential to increase the current production.

Setting a highly negative anode potential over long periods has been shown to result in modification of a pure culture by increasing the amount of current that can be produced. Yi et al. (29) set an anode at  $-0.2$  V and obtained a strain of *G. sulfurreducens* capable of current densities over five times larger than the wild type strain, showing that the negative anode potential acted as a selective pressure for the evolution of a more efficient strain. Identical experiments at positive anode potentials were not performed, and it is unknown if different anode potentials can also be used to apply this selective pressure.

Although microbes can theoretically gain more energy from a higher potential terminal electron acceptor, the studies described in this section suggest a more complex picture. Four out of the 28 studies reviewed here suggest that microbes can produce higher current densities and thicker biofilms at low potentials and reinforce that further study is necessary to understand microbial activity across a broad range of applied potentials.

**Electrochemical Tests To Examine the Conditions of Exoelectrogenic Behavior.** The use of electrochemical techniques, such as cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS), may help to identify the reasons for these various findings with different set potentials and microorganisms. For example, there is now evidence using CV and EIS techniques that *G. sulfurreducens* can discharge electrons at more than one redox potential, allowing this microbe to function well at more than one set potential. CV scans of *G. sulfurreducens* biofilms set at two different potentials ( $+0.8$  V and  $+0.3$  V) showed two different sets of redox peaks (15). When set at  $+0.3$  V, there was an oxidation peak at  $+0.22$  V and a reduction peak at  $-0.16$  V. However, biofilms set at  $+0.8$  V showed a different response, with an oxidation peak at  $+0.85$  V and a reduction peak at  $+0.43$  V. The locations of these peaks were dependent on acclimation to a specific potential and were not permanent. Switching the biofilm from the high set potential to low potential produced peaks similar to those in the biofilm originally acclimated at the lower potential. Conversely, Wei et al. (17) obtained identical redox peaks for *G. sulfurreducens* using CV for anodes set to  $-0.16$ , 0, and  $+0.4$  V as well as a nonpoised anode. The peaks for the electrodes set to  $+0.4$  and 0 V were slightly larger in magnitude than the other anodes, which was consistent with the higher potentials producing more biomass than the lower potential. Marsili et al. (4) found very similar CV results between a *G. sulfurreducens* biofilm grown at  $-0.16$  V and  $+0.24$  V, but using differential pulse voltammetry to compare the two potentials, two differences in the peaks were evident.

EIS of a *G. sulfurreducens* biofilm showed that electron transfer could be altered by the choice of set potential (30). Charge transfer resistance decreased by over 100-fold, resulting in an increase in the electron transfer rate, using an electrode set at  $-0.16$  V, compared to one set at  $+0.042$  V. Setting the potential even lower, at  $-0.26$  V, also increased the charge transfer resistance by more than 10 times that at  $-0.16$  V. A lower charge transfer resistance improves the kinetics of electron transfer, allowing for improved power densities in an MFC. A



**FIGURE 1.** CV (A) and first derivative of CV (B) of *G. sulfurreducens*. Reprinted with permission from ref 30. Copyright 2008, American Society for Microbiology.

first-derivative analysis of a CV scan of this microorganism (set potential conditions during growth at +0.24 V; Figure 1) showed a large inflection point at  $-0.16$  V, characteristic of a single rate-limiting reaction indicative of the redox potential of the electron transfer protein. There were also secondary (but substantially smaller) peaks at  $-0.02$  and  $-0.22$  V, indicating the presence of additional electron transfer proteins. These results suggest the presence of multiple different redox pathways, with the more optimal one at  $-0.16$  V.

This optimal potential is consistent with other studies that show an optimal potential for an anodic biofilm in the range of  $-0.15$  to  $-0.2$  V. For example, Torres et al. (23) showed better performance of a mixed culture dominated by *G. sulfurreducens* at  $-0.15$  V, compared to three more positive potentials. A potential of  $-0.16$  V was reached in the nonpoised electrode experiment by Wang et al. (20), and  $-0.2$  V was the potential with the most active biomass obtained by Aelterman et al. (28). Other microbes such as *S. oneidensis* show different responses to CV (31) compared to *G. sulfurreducens*. This likely results from the various types of mechanisms used for electron transfer, which can include for *Shewanella* electron shuttles (32), direct contact (33), and nanowires (34), while *Geobacter* employs direct contact (24), different types of nanowires (35), and recently it has been shown that cytochromes can be localized on these nanowires (36). Thus, they use different terminal respiratory proteins and can have different pathways for electron transfer, resulting in much different performance in terms of power production in MFCs (3). Electrochemical techniques can help to determine the optimal potential for an anodic biofilm

with these and other microbes. Furthermore, insight into the reasons for the optimal set potentials could be obtained by using CV and EIS to examine biofilms for midpoint potentials and redox peaks.

**Outlook.** Desirable characteristics for a microbial fuel cell, such as high current density, high power, and fast start-up, may require setting the anode potential to grow a biofilm most capable of achieving these traits. Our survey of the literature shows that no one set potential will always yield the best results, suggesting that the outcome of a set potential experiment is dependent on culture conditions, electrode materials, and inoculum. The potential to best promote bacterial activity could be set relatively high, which would encourage electron pathways that allow for fast growth and a high energy gain for the cells. Alternatively, a more negative potential may be most useful to produce a high current density, improving the desired output for a functioning BES. Whether a single set potential should be used, or the potential may need to be changed after the biofilm grows during start-up, is not known and should continue to be examined. Furthermore, the effect of setting an anode potential, compared to allowing a mixed community to evolve a potential at a fixed resistance, has not been well studied. Additional comparisons are needed to understand if an initially poised anode will result in improved current production once the anode potential is no longer set. Electrochemical tests, community analyses, and further study of the response of both pure and mixed cultures to set potentials and different resistances will improve our understanding of the behavior of microbial communities in various redox environments and different types of BESs.

### Acknowledgments

The authors thank Dr. Matthew D. Merrill for his help in reference electrode calibration and analysis and Dr. Justin Tokash for assistance with electrochemical analyses. This material is based upon work supported under National Science Foundation Graduate Research Fellowships (R.C.W. and D.F.C.), the National Water Research Institute Ronald B. Linsky Fellowship (D.F.C.), and award KUS-II-003-13 by King Abdullah University of Science and Technology (KAUST).

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