Set potential regulation reveals additional oxidation peaks of *Geobacter sulfurreducens* anodic biofilms

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**A B S T R A C T**

Higher current densities produced in microbial fuel cells and other bioelectrochemical systems are associated with the presence of various *Geobacter* species. A number of electron transfer components are involved in extracellular electron transfer by the model exoelectrogen, *Geobacter sulfurreducens*. It has previously been shown that 5 main oxidation peaks can be identified in cyclic voltammogram scans. It is shown here that 7 separate oxidation peaks emerged over relatively long periods of time when a larger range of set potentials was used to acclimate electroactive biofilms. The potentials of oxidation peaks obtained with *G. sulfurreducens* biofilms acclimated at 0.60 V (vs. Ag/AgCl) were different from those that developed at −0.46 V, and both of their peaks were different from those obtained for biofilms incubated at −0.30 V, 0 V, and 0.30 V. These results expand the known range of potentials for which *G. sulfurreducens* produces identifiable oxidation peaks that could be important for extracellular electron transfer.

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1. Introduction

Bioelectrochemical systems (BESs), such as microbial fuel cells (MFCs), microbial electrolysis cells (MECs), and microbial desalination cells (MDCs), have attracted increasing interest as new sustainable techniques for producing bioelectricity, biofuels, and other chemical products. In BESs, the anode is the terminal electron acceptor for microorganisms. Theoretically, more positive anode potentials favor the electron transfer from bacteria to the electrode [1–3], and a number of studies [1–8] have demonstrated that the bacterial activity is significantly affected by the anode potential. Many different *Geobacter* species have been identified to be predominant in BESs with high current densities [8,9]. *Geobacter sulfurreducens* is the best known, and most widely studied, exoelectrogenic microorganism in BESs. Genomic analysis of *G. sulfurreducens* has identified 111 coding sequences of c-type cytochromes indicative of heme groups that could potentially be active for cell respiration under different conditions [10].

Microorganisms regulate their extracellular pathways to tailor them to specific terminal electron acceptors [11], usually to enable them to gain more energy from respiration. However, little direct evidence exists for improved energy capture in BESs, although it is clear that some exoelectrogenic microbes can alter respiratory enzymes used at different electrode potentials. By investigating open-circuit potentials of mixed community biofilms acclimated to different set potentials, it was proposed that exoelectrogenic bacteria could self-regulate the redox potentials of their operative terminal reductases to be slightly more negative than the anode potential [4]. Recently, direct evidence was provided for this theory by showing that *G. sulfurreducens* expressed a new redox enzyme after conditioning at 0.6 V, compared to acclimation at 0.1 V or 0.4 V (vs. Ag/AgCl) [12]. Other studies [3,13] have shown that the electron transfer components of *G. sulfurreducens* were not affected by anode potentials ranging from −0.37 V to 0.4 V (vs. Ag/AgCl), based on the locations (potentials) of the oxidation peaks in cyclic voltammograms.

In this study, the influence of anode potential on the electroactivity of *G. sulfurreducens* anodic biofilms was investigated over a wide range of set potentials, varying from −0.46 V to 0.60 V (vs. Ag/AgCl), for biofilms cultured over a relatively long period of time (>20 days). New evidence was obtained for the expression of additional extracellular electron transfer components (EETCs) through the expansion of the set potential range and a longer acclimation period. EETCs are broadly defined here as organic or inorganic compounds used to convey electrons from cells to anodes, and thus they can be cell-associated or dispersed in solution.

2. Experimental

Single chamber MECs were constructed using 5 mL clear glass serum bottles as previously described [14]. The anode was a graphite plate 1 cm × 1.5 cm (GM-10, 0.32 cm thick) that was polished using sandpaper (grit type 400), sonicated to remove debris, cleaned by soaking in 1 N HCl overnight, and rinsed three times in Milli-Q water. The cathode was a piece of stainless steel (SS) mesh (Type 304, mesh size 90 × 90) having the same size. The gap between the anode and the cathode was ~1 cm, and an Ag/AgCl reference electrode was placed between the electrodes. Bottles were sealed using
a thick butyl rubber stopper (20 mm diameter) crimped using an aluminum cap. The electrode wires for anodes (Ti wires) and cathodes (SS wires) as well as the reference electrode passed through the rubber stopper. All potentials reported here are versus Ag/AgCl (+210 mV vs. a standard hydrogen electrode, SHE).

G. sulfurreducens PCA (ATCC 51573) was obtained from a frozen stock (−80 °C) and cultured in ATCC medium 1957 (30 mM bicarbonate buffered medium with 10 mM sodium acetate as the electron donor, and 8 g L−1 sodium fumarate as the electron acceptor). All media were sparged and maintained under a CO2:N2 (20%:80%) atmosphere.

Duplicate MECs were autoclaved, filled with sterile-filtered CO2:N2 (20%:80%) gas, inoculated using 1 mL of the pre-cultured cell suspension in 4 mL of ATCC 1957 growth medium without sodium fumarate, and incubated at five different anode potentials (−0.46 V, −0.30 V, 0 V, 0.30 V, and 0.60 V vs. Ag/AgCl) set using a potentiostat (Uniscan PG580RM) in a temperature controlled room (30 °C). Reactors were operated in fed-batch mode. Solution in MECs was replaced with 5 mL fresh, sterile, and anaerobic growth medium every day by piercing the butyl stopper with a sterile needle and syringe.

Cyclic voltammetry (CV) was conducted on the anode of MECs with the cathode as the counter electrode, and an Ag/AgCl reference electrode. Scans ranged from −0.6 V to either +0.5 V or +0.7 V at a rate of 1 mV/s. First derivative CV (DCV) was obtained from CV data by plotting the slope of each CV point against the anode potential, with the slope calculated by a central difference quotient (\( f = \frac{dI}{dE} \)). All experiments performed in duplicate showed good agreement, so results from only one of the duplicates were presented here.

3. Results and discussion

MECs were incubated over multiple (18 to 26) fed batch cycles at the five different anode potentials until reproducible cycles of current generation were observed (42 days at −0.46 V, versus 20–25 days at other potentials). The maximum current produced in a single cycle increased for set potentials between −0.46 V and 0 V, but no further increase was obtained between 0 V and 0.60 V (Fig. 1). The increase of maximum current with set potential up to 0 V was likely due to increases in biomass at these potentials as shown by others [2,3], which would result from enhanced adhesion, electron transfer rates, or more effective electron transfer processes at higher set potentials. No increase in maximum current above 0 V could be due to a lack of effective electron transfer processes at these potentials, substrate limitations, hindered electron transfer, or insufficient rates of protons transport out of the biofilm. Of these, we were particularly interested in effective electron transfer and whether different electron transfer components were used at these higher potentials.

In order to investigate the influences of anode potentials on the expression of electron transfer components by G. sulfurreducens, we analyzed the biofilms using CV in 10 mM sodium acetate growth media (Fig. 2a). The CV peak currents increased with acclimation anode potentials from −0.46 V to 0 V, but did not further increase above 0 V, consistent with polarization results. DCVs clearly showed that the redox active potential range changed for biofilms incubated at different potentials (Fig. 2b). The biofilm acclimated at −0.46 V exhibited increasing electroactivity over a narrow potential range, from ca. −0.55 V to −0.33 V, while the redox active potential range for the biofilm incubated at 0.60 V was very broad from ca. −0.55 V to 0 V. The biofilms developed at −0.30 V, 0 V, and 0.3 V had similar ranges with activities spanning ca. −0.55 V to −0.22 V. These results showed that the electron transfer components of G. sulfurreducens could be altered by the use of different anode potentials.

To more fully examine the EETCs expressed at different set potentials, CVs were further performed on the anodes of MECs at different scan rates (1 mV/s and 0.5 mV/s) in depleted sodium acetate growth medium (when current in the MECs decreased to be less than 0.1 mA) (Fig. 3). Three oxidation peaks (p1 at −0.48 ± 0.01 V, p2 at −0.42 ± 0.01 V, and p3 at −0.37 ± 0.01 V) were observed on the biofilm acclimated at −0.46 V. There was a previous report of an oxidation peak at −0.515 V, but it appeared electrocatalytically inactive as the current was less than zero in an acetate depleted medium, and no current was generated in a medium with substrate at this potential [15]. In addition, a peak at −0.515 V would be obscured by the other electrocatalytically active peaks observed here. The peak (p1) at the lowest potential of −0.48 V has not previously been reported. Most studies observed one or two
peaks in the potential range from $-0.45$ V to $-0.36$ V. For example, Fricke et al. [15] observed one electrocatalytically active peak with formal potential of $-0.376$ V. Katuri et al. [13] reported two oxidation peaks at $-0.45$ V and $-0.39$ V, and Marsili et al. [16] observed oxidation peaks at $-0.43$ V and $-0.37$ V. The potentials of p2 and p3 were comparable to these reported peaks, and their potentials were close to the midpoint potentials reported for multiheme cytochrome OmcZ ($-0.42$ V), OmcB ($-0.39$ V), and the periplasmic cytochrome C (PpcA, $-0.37$ V) purified from G. sulfurreducens [13,17,18].

When the cells were incubated at $-0.30$ V, peaks p1 and p2 disappeared, the size of peak p3 greatly increased, and two new oxidation peaks appeared: p4 at $-0.30 \pm 0.01$ V, and p5 at $-0.08 \pm 0.01$ V. Based on previous studies, it was expected that the higher electroactivity of G. sulfurreducens at $-0.30$ V would occur due to enhanced expression of PpcA or OmcB, but the appearance of the new EETCs could also have been important. The p4 has been observed in several studies [3,15,19], but the EETC that it represents is still unknown. In a previous study, the potential of a peak similar to that of p5 increased from $-0.15$ V to 0.02 V as the scan times increased [12], and then reached a stable potential of 0.02 V. In addition, there was a corresponding reduction peak at $-0.36$ V in that study, but here there was no evident reduction peak corresponding to p5. This suggests that p5 might represent a different EETC than that previously reported by others [12].

The potentials of the oxidation peaks for biofilms developed at 0 V and $0.30$ V were similar to those for the biofilm acclimated to $-0.30$ V, but peak p5 greatly increased in size for biofilms developed at 0 V and
Results extend the range of EETCs expressed by G. sulfurreducens at 0 V and 0.30 V could be due to the over-expressed EETC represented by peak p5. The similar potentials of the EETCs expressed by the biofilms cultured here at −0.30 V, 0 V and 0.30 V were in agreement with those previously reported [3,13].

The oxidation peaks that appeared on the biofilm acclimated to 0.60 V were quite different from those obtained at other potentials. The oxidation peaks p1, p2, and p5 disappeared, p3 and p4 were greatly decreased in size, and two new peaks appeared: p6 at −0.16 ± 0.01 V, and p7 at 0.58 ± 0.01 V. A peak at the potential of −0.16 V (p6) has also never been previously reported in other studies. It needs to be noted that p6 is likely different from p5. As the scan rate decreased or the electron transfer rate declined, p5 shifted to more negative values with its lowest potential of ca. −0.12 V (Fig. 3b). When p6 appeared, there was a corresponding reduction peak, but there was no corresponding reduction peak for p5. The appearance of a peak for the biofilm developed at 0.60 V at the similar potential of p7 was previously reported, but peaks p3, p4, and p6 were not observed in that study [12]. The acclimation time in this previous study was very short (18 h), compared to 25 days used here, which may have precluded observation of these other peaks in CVs due to insufficient acclimation of the biofilms. Additionally, this longer acclimation time may also have precluded the ability to see an increase in current densities above 0 V as seen previously [12], possibly due to substrate, electron, or proton transport limitations to a fully developed biofilm.

4. Conclusions

Different EETCs were expressed by G. sulfurreducens acclimated at five different anode potentials. Based on CVs and DCVs, the EETCs on biofilms developed at −0.30 V, 0 V, and 0.30 V were similar, but they were all different from those on biofilms incubated at −0.46 V and 0.60 V. Oxidation peaks appeared at −0.46 V and −0.42 V for the biofilm developed at −0.46 V, and at −0.16 V and 0.58 V for that acclimated at 0.60 V. These results extend the range of EETCs expressed by G. sulfurreducens biofilms in response to set anode potentials.

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