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Excess surface area in bioelectrochemical systems causes ion transport limitations

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Abstract

We investigated ion transport limitations on 3D graphite felt electrodes by growing *Geobacter sulfurreducens* biofilms with advection to eliminate external mass transfer limitations. We characterized ion transport limitations by: 1) showing that serially increasing NaCl concentration up to 200 mM increased current linearly up to a total of +273% vs. 0 mM NaCl under advective conditions, 2) growing the biofilm with a starting concentration of 200 mM NaCl, which led to a maximum current increase of 400% vs. current generation without NaCl, and 3) showing that uncolonized surface area remained even after steady-state current was reached. After accounting for iR effects, we confirmed that the excess surface area existed despite a non-zero overpotential at the electrode surface. The fact that the biofilm was constrained from colonizing and producing further current under these conditions confirmed the biofilms under study here were ion transport-limited. Our work demonstrates that the use of high surface area electrodes may not increase current density when the system design allows ion transport limitations to become dominant.

Keywords

Geobacter sulfurreducens; biofilm; electron transfer; bioelectrochemical system; ion transport

Introduction

Bioelectrochemical systems (BESs) have possible applications in wastewater treatment, power generation, biofuel and biochemical production (Logan et al., 2006; Nevin et al., 2010). The commercial viability of BESs depends on the ability of these devices to achieve high current densities. To date, high current densities (based on geometric or projected surface area) have largely been observed in systems employing 3-dimensional (3D) electrodes. Recent examples include 30 A/m² (Chen et al., 2011a), 24.5 A/m² (Katuri et al., 2011), 390 A/m² (Chen et al., 2012) and 85 A/m² (Rousseau et al., 2013). Literature reviews comparing the various BES electrode geometries have revealed that 3D electrodes have only

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been marginally successful in increasing current density when compared to the substantial increase in specific surface area that they supply (2-3-fold higher current densities vs. 10-10,000-fold increases in surface area densities (Gnana kumar et al., 2013; Logan et al., 2006; Nevin et al., 2010; Wei et al., 2011). This low return of current upon increased surface area suggests that these reactors are not limited by the anode surface area, which results in excess surface area. We define excess surface area as the amount of exposed surface area on an electrode that is not contributing to the overall current density as the system reaches maximum current. Having excess surface area implies that some other factor is preventing the biofilm from colonizing the remaining surface area. One possible explanation for such behavior is that the ion flux between the working and counter electrodes cannot sustain more biofilm. Consequently, it is possible that the BES is ion transport limited. The literature has several examples that appear to demonstrate this phenomenon. For example, experiments in which fibers were cut from brush electrodes without a drop in current (Hutchinson, 2011), experiments where scanning electron microscopy (SEM) images of biofilms from reactors at steady-state-current showed limited and spatially heterogeneous surface coverage (Katari et al., 2011; Zhang et al., 2013), and experiments where power outputs in microbial fuel cells increased by adding NaCl and reducing the electrode spacing (Cheng et al., 2006; Liu et al., 2005).

Typically a BES consists of an anode (working electrode), reference and cathode (counter electrode), with the three major reaction/transport processes that control current: I) the anode reaction, II) the cathode reaction, and III) ion transport. The flux of electrons into the anode from acetate oxidation, the flux of electrons out of the cathode toward proton reduction, and the flux of ions in solution between the electrodes must be equivalent. When steps I or II are limiting, several variables contribute to the maximum flux of charge, including: heterogeneous reaction rate, reactant/product mass transport, overpotential, biofilm metabolism, etc. However, when the heterogeneous reaction rate is very fast at the electrode surface, as in the case of a *Geobacter* biofilm (Strycharz et al., 2011) growing on an electrode, the flux of ions between the electrodes may become the rate-limiting-step. This ion-transport limited regime is characterized by linear, or ohmic, polarization curves (Stern 1957). These systems do not reach the limiting current plateaus (Chen et al., 2011a; Katari et al., 2011). The current in ion-transport-limited systems is described by the Nernst-Planck equation:

$$J_j = -D_j \nabla C_j - \frac{z_j F}{RT} D_j C_j \nabla \phi + C_j \mathbf{v} \quad \text{Eq 1}$$

where J is ion flux ($\text{mol m}^{-2} \text{s}^{-1}$), j is an index for each ionic species, C is ion concentration (mol m^{-3}), D is diffusion coefficient ($\text{m}^2 \text{s}^{-1}$), z is the valence of each ionic species (unitless), F is the Faraday constant (C/mol), R is universal gas constant ($\text{V C K}^{-1} \text{mol}^{-1}$), T is temperature (K), $\nabla \phi$ is electric field strength (V m^{-1}), which is a function of the inter-electrode distance, cell potential, and ion concentration and speciation, and $\mathbf{v}$ is advective velocity (m s^{-1}). In practice, ion flux can be enhanced by increasing the electric field strength, advective velocity, or ion concentration. Most importantly, ion concentration affects all three terms in Eq 1 and, therefore, we chose to increase ion concentration in this work.

We note that large current densities are generally required before ion flux begins to limit the current production in BESs. Large current densities also cause a measureable voltage drop between the working electrode and reference electrode (Oelßner et al., 2006) that affects the working electrode potential. Essentially, the increasing current reduces the effective working electrode potential, which is described as:

$$\begin{aligned} E_{effective} &= E_{applied} - iR_u \\ E_{effective} &\geq E_{onset} \end{aligned} \quad \text{Eq 2}$$

where $E_{effective}$ is the effective working electrode potential (V), $E_{applied}$ is the working electrode potential set by the potentiostat (V), i is current (A), R_u is the uncompensated resistance (Ω), and E_{onset} is the working electrode potential where the onset of current occurs (V). Typically, E_{onset} is approximated by the open circuit potential of the electrode. The difference between $E_{effective}$ and E_{onset} can be approximated as an effective overpotential. An iR limited system exists when $E_{effective}$ and E_{onset} are equivalent or when the effective overpotential becomes zero. Thus, there are possibly two limiting factors at large current densities and when using high surface area electrodes: one factor limits the total current (ion-transport limitation) and the other the working electrode potential (iR limitation).

The goal of this study was to investigate ion transport limitations in BESs while accounting for possible iR limitations. A potentiostat-controlled bioelectrochemical reactor with minimized external mass transfer limitations was used to investigate ion transport limitations using *Geobacter sulfurreducens* as the source of anodic current. High surface area 3D porous graphite felt electrode material was used for the working electrodes. Anodic current was monitored as bulk sodium chloride (NaCl) concentrations under both advective flow and stagnant conditions were increased. Biofilms were also grown under increased NaCl concentrations. We compared our results to biofilms grown on flat, rotating disk electrodes (RDEs) as a control. Finally, we tested whether the addition of a buffering agent, sodium bicarbonate (NaHCO_3), could increase the current in the same manner as NaCl.

Materials and Methods

Bioelectrochemical reactor

Bioelectrochemical reactors were constructed using 100 mL liquid volume (per chamber) Adams and Chittenden microbial fuel cells (www.adamschittenden.com) with modified high performance liquid chromatography (HPLC) bottle caps (VICI-JOUR - JR-S-11003) containing bulkhead hose-barb fittings (Eldon James - BH-1428-2-2NK) to allow for extra ports (Figure 1). The anodes consisted of cylindrical coupons of graphite felt with a radius of 1.1 cm and a thickness of 0.6 cm (HP Materials Solutions, Inc.). Graphite felt has about $61,600 \text{ m}^2/\text{m}^3$, which is roughly 120 times greater surface area compared to a planar electrode of the same size. The counter electrode consisted of a large rectangular piece of the same graphite felt ($7 \text{ cm} \times 5 \text{ cm} \times 0.6 \text{ cm}$). The anode was inserted into a polycarbonate sheath so that only one face was exposed. The sheath contained a hose barb on the side opposite the flow entry face, which was connected with a tube to a bulkhead hose barb in the bottle cap of the anode chamber (Figure 1). A 2.5 cm magnetic stir bar at 150 rpm was used

to mix the reactors. The reactors and Norprene tubing (Masterflex - HV-06404-16) were autoclaved, cooled, and filled with anaerobic, sterile medium prior to inoculation. Reactors were set up in a manifold assembly in a water bath set to 30 °C (Figure 1). Although the reactors had two chambers, they were operated as one-chamber cells without a membrane, as ideally mixed three-electrode systems. Advection of the media was induced by recycling media through the anode at 6 mL/min, into the counter electrode chamber, and through the counter electrode. This facilitated acetate delivery to the biofilm inside the anode, while assuring completely mixed conditions in the reactors. Varying the recycle rate above this value did not affect the current, which confirmed that mass transport was not limiting the maximum current. During no advection conditions, the recycle and mixing were stopped. A Ag/AgCl reference electrode was aseptically inserted into the counter electrode chamber. The anode (working), counter, and reference electrodes were connected to one channel of a six-channel potentiostat developed previously (Renslow et al., 2011). The reactors were inoculated in batch mode, with the anode polarized to +400 mV_{Ag/AgCl}, which acted as the sole terminal electron acceptor.

Media and cultures

G. sulfurreducens strain PCA (ATCC 51573) was cultured in anaerobic culture vials without shaking, using the Hungate technique (Miller and Wolin, 1974) in a medium composed of the following: potassium chloride, 0.38 g/L; ammonium chloride, 0.2 g/L; sodium phosphate monobasic, 0.069 g/L; calcium chloride, 0.04 g/L; magnesium sulfate heptahydrate, 0.2 g/L; sodium carbonate, 2 g/L; Wolfe's vitamin solution 100×, 10 mL/L; modified Wolfe's mineral solution 100×, 10 mL/L. The electron donor and acceptor were 20 mM sodium acetate and 40 mM fumarate (inoculum only), respectively. Reactor inocula were prepared in 100 mL liquid volume anaerobic culture vials inoculated with laboratory stock (3% inoculum) and grown until a visible pink biofilm had accumulated on the bottom of the vials. Each vial was centrifuged in two 50 mL centrifuge tubes for 8 minutes at 5,000 g and then concentrated to 25 mL with fumarate-free medium (4× cell concentration), from which 10 mL were used to inoculate the reactors (9 vol% inoculum).

NaCl acclimation

G. sulfurreducens was cultured in NaCl concentrations which were increased sequentially in increments of 25 mM in order both to acclimate the cells to the reactor NaCl concentrations and to determine their tolerance to the salt. Acclimated cultures were used to inoculate reactors containing medium of the same salt concentration. The maximum NaCl concentration investigated in the reactors was 200 mM because at this concentration in fumarate-grown inoculum vials, the planktonic growth rate slowed substantially and the cells began to form flocs instead of a typical biofilm.

Electron microscopy and analytical methods

After the necessary data for each experiment had been collected, the anode was removed, cut in half, and prepared for scanning electron microscopy to investigate biofilm surface coverage. The electrodes were prepared and imaged using the procedure described previously (Renslow et al., 2013). Cyclic voltammetry was performed at a scan rate of 1

mV/s from -700 to $+400$ mV_{Ag/AgCl}. Acetate measurements were performed using high performance liquid chromatography (Agilent Technologies, Santa Clara).

Experimental procedures

Steady-state chronoamperometry with increasing NaCl concentration—The effect of NaCl concentration on the current was studied after the current reached a steady state in a reactor during chronoamperometry. The reactors were inoculated and allowed to reach a steady-state current. At 67 hours post-inoculation, the reactor was serially injected with 1 mL of a concentrated NaCl solution, corresponding to an increased NaCl concentration in the reactor of ~ 25 mM. The current was allowed to stabilize before the next concentration increase, approximately one hour later.

Startup under different NaCl concentrations—In addition to the sequentially increasing NaCl concentration experiments, reactors were set up with different starting NaCl concentrations in the medium prior to inoculation. The reactor medium NaCl concentrations used were 0, 100, and 200 mM NaCl, which were inoculated according to the above method with the appropriate salt-acclimated cultures (at least two passages) and chronoamperometry data and cyclic voltammetry data were collected.

Growth of biofilms on a rotating disk electrode—The details of procedures are described in the literature (Babauta and Beyenal, 2014). Briefly, we used a rotating disk electrode as a flat surface to grow the biofilms. By increasing the rotation rate, we minimized the external mass transfer limitations so that we could compare the results of a 2D electrode with 3D electrodes. We previously used rotating disk electrodes to determine the effect of mass transport limitations for acetate, and similarly use them here to study the mass transport limitations of NaCl as it applies to current generation by the biofilm.

Electrochemical impedance spectroscopy—A Gamry Series G300™ potentiostat (Gamry Instruments, Warminster, PA, USA) was used to obtain impedance spectra. The impedance spectra for potassium ferri/ferro-cyanide were obtained in the same bioelectrochemical reactor except that a 200 mL solution of 100 mM KCl, 100 mM potassium ferricyanide, 100 mM potassium ferrocyanide replaced the growth medium. The E_{DC} , E_{AC} , and frequency range for the potassium ferri/ferro-cyanide impedance spectra were 226 mV_{Ag/AgCl} (i.e. open circuit potential), 5 mV and 25 kHz to 1 Hz, respectively. The impedance spectra for the *G. sulfurreducens* biofilm grown on the RDE was obtained following the same procedures outlined in (Babauta and Beyenal, 2014). The E_{DC} , E_{AC} , and frequency range for the biofilm impedance spectra were -340 mV_{Ag/AgCl}, 5 mV and 100 kHz to 0.2 Hz, respectively.

Results and Discussion

Effect of NaCl concentration increase on mature, steady-state current-producing biofilms

Figure 2 shows the change in biofilm steady-state current with sequential NaCl concentration increases over a ~ 12 -hour period. Each concentrated NaCl injection resulted in an increase of ~ 25 mM NaCl in the reactor medium. At 67 hours post-inoculation in 0

mM NaCl reactor medium, the steady-state current output was 4.5 mA. The first five increases (up to 125 mM NaCl) caused an increase in current of 1.1 mA per event to a maximum total current of 10 mA. The sixth, seventh, and eighth concentration increases resulted in increases of 0.9, 1.0, and 1.4 mA, respectively. The final steady-state current of 12.3 mA in the reactor with an ending NaCl concentration of 200 mM represents a 273% increase in current output vs. the 0 mM NaCl steady-state current. Each increase in NaCl concentration caused an immediate increase in the maximum current output by the *G. sulfurreducens* biofilms, with each subsequent steady-state current reached in less than 45 seconds. There was no gradual increase in current as is normally observed during attachment and growth of *G. sulfurreducens* biofilms on electrodes, but rather an instantaneous increase upon NaCl concentration increase followed by a new steady-state current. This immediate increase could not have been caused by increased cell growth. The strong linear relationship between steady-state current and added NaCl concentration was the initial evidence confirming that the biofilm was operating under ion transport limitations. All of the current coming from the anode was generated by attached cells, which suggests that the biomass was in excess for current production and that the average cell respiration rate was restrained.

Steady-state currents under different ionic strengths and advection conditions

To investigate the effect of different NaCl concentrations on current output from inoculation, pre-acclimated cultures were used to inoculate reactors containing 0, 100, or 200 mM NaCl. The 0, 100, and 200 mM NaCl reactors reached steady-state currents of 3.62, 8.70, and 13.2 mA, respectively (Figure 3A). Figure 3B shows a plot of steady-state current values with respect to starting NaCl concentration. Suspending all advection caused the current from each reactor to drop to 0.65-0.80 mA, regardless of the NaCl concentration. The current density under this condition, normalized by the 2D entry face surface area of the graphite felt electrode, was $\sim 1.7 \text{ A/m}^2$, in close agreement with the current densities of *G. sulfurreducens* in other flat-geometry graphite reactors without advection (Bond and Lovley, 2003; Strycharz et al., 2011). Because current did not increase under the higher NaCl conditions in the absence of advection, we concluded that the reduced current supplied by the biofilm was due to a diffusion limitation on acetate for the internally colonized biofilm. In contrast, under advective conditions, the current displayed a linear response to NaCl concentration as predicted by the Nernst-Planck equation, demonstrating that the system was under ion transport limitations, and that the current-producing surface area inside the electrodes was in excess of the current carrying capacity of the electrolyte when acetate was well-supplied from advection. An additional observation from Figure 3A is that higher NaCl concentration resulted in an increase in time to reach a steady-state current and current growth was slightly inhibited during initial biofilm formation. The time it took to reach a steady state was 6, 16, and 26 hours for the 0, 100, and 200 mM NaCl reactors, respectively. From this result, we can infer that the current increases observed during the NaCl addition experiment (Figure 2) are not due to changes in biofilm attachment, but rather are tied directly to ion transport, which limits the average respiration rate of the biofilm cells on the electrode.

Electrochemical analysis of biofilms at steady-state current

To further understand the effect of ion transport limitations on the presented *G. sulfurreducens* biofilms, cyclic voltammograms were obtained for the pre-acclimated *G. sulfurreducens* reactors. Figure 4A shows slow-scan (1 mV/s) CVs of reactors with 0, 100, and 200 mM NaCl concentrations taken after a steady-state current was reached. Below a polarization potential of -480 ± 5 mV_{Ag/AgCl}, all CVs overlapped and showed non-faradaic background currents between $-25 \mu\text{A}$ and $-130 \mu\text{A}$. From this polarization potential to +400 mV_{Ag/AgCl}, current increased linearly for all reactors. Linear regression in the potential region from -480 to $+400$ mV_{Ag/AgCl} yielded slopes of 4.3, 10.5, and 16.4 mA/V for 0, 100, and 200 mM NaCl, respectively. The linear current-voltage relationships demonstrate ohmic behavior and are indicative of the uncompensated resistance between the working and reference electrodes (uncompensated resistances were measured as 233, 73, and 48 Ω for 0, 100, and 200 mM NaCl, respectively).

Post-experiment iR-compensated CVs are presented in the supplementary information along with a supplemental discussion (Figure S1).

To test the effect of NaCl addition on the current produced by *G. sulfurreducens* grown under anode reaction-limited conditions, we grew biofilms on a rotating disk electrode. The RDE provided a flat electrode surface, which did not have excess surface area, and also allowed us to control mass transfer by rotating the electrode, similar to advection in the 3D electrodes. Biofilm images of the RDE showed complete coverage of cells on the surface (Babauta and Beyenal, 2014). At steady current production, an increase of 37 μA was observed, or an ~36% gain in steady-state current, when the biofilms were grown at 200 mM NaCl compared to when they were grown at 0 mM NaCl. Figure 2B shows the increased current production visualized as the limiting current of cyclic voltammograms for the two NaCl conditions at a rotation speed of 530 RPM. The addition of NaCl did not alter the sigmoid shape of the cyclic voltammograms. Although the 200 mM NaCl condition increased the steady-state current, the increase was significantly lower when compared to the NaCl conditions in Figures 2 and 3A (a 36% increase vs. a 273% increase and 400% increase, respectively). This observation is central to the concept of scale-up in BESs, as the response of the biofilm system to salt concentration depends strongly on the current-producing surface area. The relationship between surface area and steady-state current is non-linear over large surface area (or large current) ranges, which has been shown previously for microbial fuel cells (Dewan et al., 2008). It is for this reason that stacking will likely be the most efficient method of scaling BESs, which circumvents the central issue observed here and in the literature (Chen et al., 2011b; Ieropoulos et al., 2008).

The role of uncompensated resistance and iR

The CVs in Figures 4A and 4B yield important information on the role of the uncompensated resistance in our BESs. As stated earlier, large current densities such as that shown in Figure 4A (as opposed to Figure 4B) can cause an iR drop that reduces the effective working electrode potential (Eq 2) in a potentiostat-controlled system. Therefore, it was important to differentiate between ion transport limitations and iR limitations. In the case of a constant polarization potential, it is simple to compensate post-experiment for iR

by utilizing the slopes estimated from the CVs in Figure 4A. Figure 5 shows that in all cases, iR drop causes the effective working electrode potential to decrease towards the onset potential ($-480 \text{ mV}_{\text{Ag/AgCl}}$) where current generation begins. At steady state, the difference between onset potential and the effective working electrode potential for 0 mM, 100 mM, and 200 mM NaCl are 60, 240, and 240 mV, respectively. Because the differences remained non-zero throughout the growth of the biofilms and neither acetate nor colonizable surface area was preventing the current from increasing higher than the steady state value, we concluded that the biofilms in all three cases were ion transport-limited. Despite an iR drop of 420 mV in the 0 mM NaCl condition, the applied potential was still capable of driving additional current until an effective overpotential of zero was reached. Further verification is obtained by realizing that the effective working electrode potential in both the 100 mM and 200 mM cases converged to a value of $-240 \text{ mV}_{\text{Ag/AgCl}}$, which corresponded to the limiting current plateau observed in Figure 4B. The convergence to the limiting current potential suggested that at this point, the biofilm was restrained from passing additional current. Otherwise, the biofilm would have reduced the effective working electrode potential to that observed in the 0 mM NaCl condition and ultimately to the onset potential. Therefore, the current generated in the 100 mM and 200 mM condition was not limited by iR drop and instead by the rate of ion transport to the counter electrode. It is from this observation that the critical nature of *excess surface area* is reached. When high surface area electrodes are used in BESs as the current collectors, excess surface area can be an indicator of ion transport limited current.

One of the assumptions we have used in this work was that the slope of the CVs in Figure 4A are representative of the uncompensated resistance. To demonstrate this in the same electrochemical system, we used potassium ferri/ferro-cyanide to drive a current up to 30 mA which was twice the max current in our 200 mM NaCl biofilm. We used electrochemical impedance spectroscopy to differentiate between the multiple resistances occurring within the electrochemical cell. These are the uncompensated resistance, the charge transfer resistance (R_{ct}), and the diffusion impedance. Figure 6A shows that these values were 3, 0.56, and 0.1Ω , respectively for a given frequency range of 25 kHz to 1 Hz. Therefore, the total resistance we expect for the given electrochemical cell is 3.66Ω . Figure 6B shows the corresponding CV for this system where the sweep rate was increased from 0.5 mV/s to 10 V/s. As the sweep rate was increased, the slopes converged to a value of 3.60Ω , which corresponded to $R_u + R_{\text{ct}}$. Interestingly, this analysis exposed the origin of the linear behavior of CVs taken in excess surface area electrodes. First, in Figure 4B, the slope of the CV centered on the half-wave potential of the CV was 1288Ω , which was greater than the R_u value of 120Ω shown in Figure 6C. Compare this to the slope in Figure 6B of 3.6Ω and the corresponding R_u of 3.0Ω shown in Figure 6A. Thus, the linear behavior observed in our CVs is attributed to the fact that R_u is greater than the R_{ct} of the biofilm. When R_{ct} is larger than R_u , we observe the typical CV for *G. sulfurreducens* biofilms as shown in Figure 4B. We make the distinction here that the ratio of R_{ct} to R_u dictates the shape of CVs in BESs, that is, whether ohmic CVs or faradaic CVs are obtained. Although previously it has been stated that these are caused by “ohmic losses”, “internal losses”, or any variant therein, these descriptions are misleading as the true cause for the linear

behavior of CVs can be determined from an understanding and the measurement of R_{ct} and R_u .

Confirmation of Excess Surface Area

For our system, excess surface area was qualitatively confirmed by SEM images of electrodes that were harvested and fixed just after reaching steady-state current (Figure 7). Micrographs of the bottom, top, and cross-sectional faces of the anodes were taken from 0 mM and 200 mM NaCl reactors just after steady-state current was reached. These micrographs show sporadic monolayer electrode surface coverage with a plethora of uncolonized graphite felt strands, similar to that reported in another high surface area system (Katuri et al., 2011). Individually attached cells were observed throughout each electrode. Unfortunately, the spatiotemporal monitoring of preference for vertical vs. lateral growth on the fibers within the electrode is impossible to determine for this electrode geometry via microscopic techniques. However, we observed that most biomass accumulated near the center of the entry face. This could be due to increased rates of initial attachment after inoculation, or local electrochemical or hydrodynamic effects. We should note that in the literature, *G. sulfurreducens* biofilm structure is mostly observed to be homogeneous when the cells are grown on small, flat electrodes (Renslow et al., 2013). However, in this study we observed heterogeneous structures. This difference outlines the effect of 3D electrodes, ion transport limitations and consequent excess surface area on electrochemically active biofilm structure, which has not been considered in the previous literature.

Ion Transport and pH Buffers

In addition to demonstrating that the reactors were under ion transport limitations, the data also show that pH was not limiting the current output, since NaCl is not part of a buffer system and could not significantly affect the pH. However, the addition of a buffering agent, NaHCO_3 , also increased the current in the same manner as NaCl (data not shown), due to the fact that it acts as an ionic conductor as well as a weak base/buffering agent. This is in contrast to previous work which showed that the addition of buffering capacity increased current density but the addition of salt did not (Torres et al., 2008). This is a good indicator that the other system was pH, and not ion transport limited and demonstrates that the rate-limiting step in BESs can be heavily influenced by reactor architecture and hydrodynamics. It also demonstrates how the design parameter of excess surface area can act as a buffer against pH limitations to metabolism in BESs.

Conclusions

The use of 3D, high surface area advective electrodes can lead to ion transport limitations, inhibiting the maximum current production of a BES. Serially increasing bulk NaCl concentration in our BES increased current linearly up to a total of +273% under advective conditions. A starting concentration of 200 mM NaCl prior to inoculation increased the eventual maximum current output by up to 400%. For comparison, the addition of 200 mM NaCl prior to inoculation resulted in only a 36% increase on a rotating disk electrode where ion transport was not limiting, but advection was still present. We observed that most of the surface area available for colonization by the biofilm remained un-colonized after steady-

state current was reached, implying that the biofilm was constrained from colonizing and increasing current production. The excess surface area is therefore an indicator of ion transport limitations in BES and could be used to diagnose this limitation in addition to measuring the relative magnitudes of the uncompensated resistance and overall charge transfer resistance operating at the anodes or working electrodes. Our work demonstrates that the use of high surface area electrodes will not increase current density in BES when the BES reactor design allows ion transport limitations to become dominant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Figure 1. Electrode and reactor assembly. *Top Left:* Sheath, graphite felt coupon, and titanium wire before assembly. *Top Right:* Electrode-sheath assembly. *Bottom Right:* Electrode assembly attached to bulkhead fitting in bottle cap. *Bottom Left:* Manifold reactor assembly of Adams and Chittenden microbial fuel cells in a water bath.

Figure 2.

Change in current production with sequential +25 mM NaCl concentration changes in a potentiostat-controlled *Geobacter sulfurreducens* BES. During the time 12 h to 65 h, the current remained at a steady state (~4.5 mA). New steady-state currents formed within 45 seconds after each NaCl concentration increase. The final steady-state current of 12.3 mA, corresponding to a final NaCl concentration of 200 mM, represents a 273% increase in current output over the original 0 mM NaCl steady-state current.

Figure 3.

A) Plot of current development for pre-acclimated *G. sulfurreducens* cultures in reactors with media containing 0, 100, and 200 mM NaCl. Reactors were inoculated at time = 0 h. Final steady-state current was linearly proportional to NaCl concentration: 3.62, 8.70, and 13.2 mA for 0, 100, and 200 mM, respectively. However, higher NaCl concentration resulted in longer times to reach steady state. B) Plot of steady-state current values with respect to reactor NaCl concentration, with and without advection through the electrode. Under advective conditions, a linear relationship between NaCl concentration and steady-state current was observed. Without advection through the electrode, no relationship was observed because the limiting current did not surpass the current-carrying capacity of the electrolyte.

Figure 4.

A) Cyclic voltammograms (1 mV/s scan rate) of pre-acclimated *G. sulfurreducens* reactors with 0, 100, and 200 mM NaCl taken after steady-state current was reached. The linear current-voltage relationships demonstrate ohmic behavior and are indicative of the uncompensated resistance of the solution. The CVs did not reach a maximum limiting current in the tested potential window. The current produced in the 200 mM NaCl reactor at +400 mV_{Ag/AgCl} (14 mA) represents an ~400% increase over the current draw from the 0 mM NaCl reactor (3.8 mA). B) CVs (1 mV/s) generated by a rotating disk electrode spinning at 530 rpm, with 0 and 200 mM NaCl. The character of the CV did not change with the addition of NaCl, but limiting current increased by 36%.

Figure 5.
Plot of effective working electrode potential vs. time for 0, 100, and 200 NaCl salt conditions that correspond to Figure 3A.

Figure 6.

A) Nyquist plot of potassium ferri/ferro-cyanide in the electrochemical system used in this study showing the uncompensated resistance, charge transfer resistance, and diffusion impedance. B) The corresponding CV for the potassium ferri/ferro-cyanide system in A). C) Nyquist plot of the same *G. sulfurreducens* biofilm used to obtain the CV in Figure 4B. The relative magnitudes of uncompensated resistance and charge transfer resistance determine whether ohmic CVs or faradaic CVs are obtained.

Figure 7. Scanning electron micrographs of the 3D carbon felt anodes harvested from a *G. sulfurreducens* BES just after steady-state current was reached. Images are from biofilms grown under both the 0 mM (bottom row) and 200 mM (top row) NaCl conditions, and were obtained from the entry face, the cross-sectional cut face, and the exit face in reference to the flow-direction through the 3D electrodes. In every image, biofilm coverage is low, with a sporadic monolayer and ample electrode surface area uncolonized, in contrast to most other reported *G. sulfurreducens* biofilms harvested after steady-state current.