A kinetic perspective on extracellular electron transfer by anode-respiring bacteria

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Received 6 April 2009; revised 13 July 2009; accepted 4 October 2009. Final version published online 6 November 2009.

DOI:10.1111/j.1574-6976.2009.00191.x

Editor: Ferran Garcia-Pichel

Keywords
microbial fuel cells; microbial electrolysis cells; Nernst–Monod kinetics; electron shuttles; solid conductive matrix; microbial nanowires.

Abstract
In microbial fuel cells and electrolysis cells (MXCs), anode-respiring bacteria (ARB) oxidize organic substrates to produce electrical current. In order to develop an electrical current, ARB must transfer electrons to a solid anode through extracellular electron transfer (EET). ARB use various EET mechanisms to transfer electrons to the anode, including direct contact through outer-membrane proteins, diffusion of soluble electron shuttles, and electron transport through solid components of the extracellular biofilm matrix. In this review, we perform a novel kinetic analysis of each EET mechanism by analyzing the results available in the literature. Our goal is to evaluate how well each EET mechanism can produce a high current density (> 10 A m⁻²) without a large anode potential loss (less than a few hundred millivolts), which are feasibility goals of MXCs. Direct contact of ARB to the anode cannot achieve high current densities due to the limited number of cells that can come in direct contact with the anode. Slow diffusive flux of electron shuttles at commonly observed concentrations limits current generation and results in high potential losses, as has been observed experimentally. Only electron transport through a solid conductive matrix can explain observations of high current densities and low anode potential losses. Thus, a study of the biological components that create a solid conductive matrix is of critical importance for understanding the function of ARB.

Introduction
Most microorganisms use respiration to convert biochemical energy into ATP; this process involves a cascade of reactions through a system of electron-carrier proteins in which electrons are ultimately transferred to the terminal electron acceptor. Most forms of respiration involve a soluble compound (e.g. oxygen, nitrate, and sulfate) as an electron acceptor; however, some microorganisms are able to respire solid electron acceptors (metal oxides, carbon, and metal electrodes) in order to obtain energy. Several mechanisms explain how microorganisms respire using a solid electron acceptor (Hernandez & Newman, 2001; Weber et al., 2006; Rittmann et al., 2008b). Some of these mechanisms involve the use of chelators or siderophores that effectively solubilize the solid electron acceptor and introduce them into the cell (Gralnick & Newman, 2007). Other mechanisms involve extracellular electron transfer (EET), in which microorganisms externalize their electron transport to the surface of the solid electron acceptor.

Researchers have proposed three distinct EET mechanisms, which are depicted in Fig. 1. The first mechanism proposes direct electron transfer between electron carriers in the bacteria and the solid electron acceptor. This mechanism is supported by the presence of outer-membrane (OM) cytochromes that can interact directly with the solid surface to carry out respiration (Myers & Myers, 1992, 2001; Beliaev et al., 2001; Magnuson et al., 2001). Bacteria using this mechanism require direct contact with the solid electron acceptor and, thus, cannot form a biofilm. The second mechanism proposes the presence of a soluble electron shuttle: a compound that carries electrons from the bacteria by diffusive transport to the surface of the metal oxide (or electrode) and is able to react with it, discharging its electrons. Then, this compound, in its oxidized state, diffuses back to the cells, which should be able to use the same compound
repeatedly (hence the name ‘shuttle’). Bacteria are known to produce compounds that act as electron shuttles, including melanin, phenazines, flavins, and quinones (Newman & Kolter, 2000; Turick et al., 2002; Hernandez et al., 2004; von Canstein et al., 2008). The third mechanism proposes a solid component that is part of the extracellular biofilm matrix and is conductive for electron transfer from the bacteria to the solid surface. This mechanism is supported by the recent discovery of the possible role of cellular pili as nanowires (Reguera et al., 2005; Gorby et al., 2006), which are being characterized for their capability to conduct electrons. Other components may also be conductive and contribute in EET, such as extracellular cytochromes or bound electron mediators (Lovley, 2008; Marsili et al., 2008a; Rittmann, 2008).

Currently, researchers have not reached a consensus regarding the conditions under which these EET mechanisms are dominant in natural and engineered systems. Evidence can be found to support more than one EET mechanism in some cases. For example, recent discoveries have shown that *Shewanella oneidensis* is capable of producing $e^{-}$ shuttles (Marsili et al., 2008a; von Canstein et al., 2008) and nanowires (Gorby et al., 2006). It is not obvious under which conditions an EET mechanism would be used and whether more than one mechanism is concurrently utilized by *S. oneidensis* and other bacteria.

The use of EET is of special importance in microbial fuel cells and electrolysis cells (collectively referred to as MXCs). In MXCs, anode-respiring bacteria (ARB) carry out a respiration process in which a solid electrode (the anode) is their electron acceptor. Because most MXC electrodes are solid conductors that can neither be solubilized nor reduced (it only acts as a conductor), ARB can only externalize electrons through EET in order to respire using the anode. To date, ARB include members from diverse phyla, such as Alpha-, Beta-, Gamma-, and Deltaproteobacteria, Firmicutes, Acidobacteria, and a yeast (Logan, 2009). Most of these members are known to utilize solid Fe (III) as an electron acceptor, and they are anaerobic, gram-negative, oligotrophs. Substrate-utilization capabilities of most of these bacteria are limited to simple fermentation products, such as acetate and H$_2$. However, some members can utilize a wider range of substrates, such as propionate, butyrate, lactate, and glucose (Lovley et al., 1993; Holmes et al., 2004; Debabov, 2008).

The result of anode respiration is an electrical current that can be used for various purposes in the cathode of MXCs. As shown in Fig. 2a, the current resulting from ARB respiration can be coupled to the reduction of oxidized compounds (mainly oxygen) to produce electrical power in microbial fuel cells (Kim et al., 2002; Logan et al., 2006). Alternatively, it can serve as an electron source to produce hydrogen gas from water by applying a small amount of voltage in a microbial electrolysis cell, as shown in Fig. 2b (Liu et al., 2005). Both of these cathodic reactions can be performed chemically or through the use of microorganisms that accept electrons from the cathode (Bergel et al., 2005; Rozendal et al., 2008). Microorganisms in the cathode can also be used to reduce oxidized contaminants, such as nitrate and trichloroethylene (Gregory et al., 2004; Aulenta et al., 2009). Combining the enzymatic versatility of microorganisms with the robustness of electrochemical cells, MXCs have the potential to become a key technology in the bioenergy industry (Lovley, 2008; Rittmann, 2008; Rittmann et al., 2008a).

From the point of view of using an MXC as a feasible technology, ARB must be able to perform a twofold task: (1) produce a high current density (see Box 1) that minimizes...
Box 1. Anode-respiring bacteria and high-current densities

Bacteria transferring electrons to an anode are carrying out a respiratory metabolism. Usually, bacteria carrying out respiration respire (or reduce) the terminal electron acceptor (e.g. iron-respiring bacteria reduce Fe^{3+} to Fe^{2+}). In the case of ARBs, the anode is not the terminal electron acceptor, as it transfers the electrons to the cathode, where a variety of compounds can act as a terminal electron acceptor (e.g. O_{2}, H_{2}O, H^{+}). The terminal electron acceptor in the cathode does not control the rate at which bacteria respires in the anode, because it is remote from the bacteria; instead, the anode potential determines their rate of respiration (Torres et al., 2008b). Therefore, the anode potential is the analog to the concentration of a soluble electron acceptor in conventional respiration processes, and respiration occurs only when the anode accepts the electrons. Thus, it is correct and insightful to refer to these bacteria as ARB. We define ARB as any bacteria capable of transferring electrons to an anode, regardless of the EET mechanism used.

Many studies have reported maximum current densities (per anode surface area) by ARB of 10–15 A m^{-2} (Fan et al., 2007; Catal et al., 2008, Torres et al., 2008a, b); these high current densities are desirable in MXCs. However, do these current densities correspond to a high respiration rate by ARB? In order to answer this question, we compare ARB respiration with other biofilm reactors reported in the literature. Table 1 compares the biofilm substrate flux obtained by various researchers using aerobic heterotrophs, nitrifiers, denitrifiers, and methanogenic biofilm reactors. We convert these substrate fluxes into electron fluxes (expressed as A m^{-2}) for comparison among them and with ARB current densities. Aerobic biofilms reactors often encounter dual-substrate limitation (e^{-} donor and acceptor limitation) due to the low O_{2} concentrations found in water saturated with air (~8 mg O_{2} L^{-1} or 0.25 mM). As a result, aerobic heterotrophic and nitrifying biofilms show lower substrate fluxes. Anaerobic denitrifying and methanogenic biofilm reactors often have higher substrate fluxes due to the higher concentrations of e^{-} donor and acceptor. Similarly, aerobic biofilms fed with 100% O_{2} exhibit higher fluxes (up to 8.4 A m^{-2}) (Casey et al., 1999; Syron & Casey, 2008). MXCs have successfully obtained current densities that are higher than or comparable to most biofilm processes. Thus, given that most biofilm technologies operate at biofilm substrate fluxes that are lower than those obtained in MXCs, the current densities obtained by ARB so far must be considered to be high.

A few studies have shown that the maximum current densities produced by ARB are limited by proton transport inside the biofilm (Torres et al., 2008b; Franks et al., 2009). If protons produced as a result of substrate oxidation accumulate inside the biofilm, they decrease the pH and inhibit the ARB. Thus, the maximum current density obtained appears to depend on proton transport rather than factors associated with EET. The highest current densities reported so far are consistent with relatively high buffer concentrations (> 100 mM buffer) (Fan et al., 2007; Logan et al., 2007; Torres et al., 2008b; Xing et al., 2008). Thus, it is possible that higher current densities are achievable in ARB biofilms if better proton transport is achieved.

How to characterize anode potential losses?

Figure 3 depicts the sequential losses of electrical potential that become conceptually important when addressing the function of an ARB biofilm and contribute to the anode potential loss. For our analysis, we consider ARB that are located at a certain distance from the anode surface. The total anode potential loss ($\eta_{anode}$) is defined as the difference between the electron-donor potential and the anode potential ($\eta_{anode} = E_{donor} - E_{anode}$). An energy loss associated with the intracellular potential determines the energy at which electrons are released (potential, $E$) from the OM of the cell, through OM cytochromes, into the EET mechanism ($E_{OM}$). [The term ‘potential’ ($E$) has units of V or J C^{-1}. Given that a coulomb is a unit of charge that is related to the number of electrons (1 mol of electrons = 96 495 C), the ‘potential’ can

<table>
<thead>
<tr>
<th>System</th>
<th>Substrate flux</th>
<th>Equivalent current density (A m^{-2})</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerated heterotrophic reactors</td>
<td>11–20 g O_{2} m^{-2} day^{-1}</td>
<td>1.6–2.8</td>
<td>Zhu et al. (2009); Casey et al. (1999); Brindle et al. (1999); Syron &amp; Casey (2008)</td>
</tr>
<tr>
<td>Nitrifying biofilms</td>
<td>1.2–2.1 g N-NH_{4}^{+} m^{-2} day^{-1}</td>
<td>0.8–1.3</td>
<td>Einter et al. (2007); Nowak (2000); Akker et al. (2008)</td>
</tr>
<tr>
<td>Denitrifying membrane</td>
<td>6.1–8 g NO_{3}^{-} m^{-2} day^{-1}</td>
<td>3.9–5.1</td>
<td>Ergas &amp; Rheinheimer (2004); Hasar (2009)</td>
</tr>
<tr>
<td>Methanogenic reactors</td>
<td>3.5–68 g COD m^{-2} day^{-1}</td>
<td>0.5–9.5</td>
<td>Cresson et al. (2006); Ghaniyari-Benis et al. (2009)</td>
</tr>
<tr>
<td>MXCs</td>
<td>–</td>
<td>Up to 15</td>
<td>Catal et al. (2008); Fan et al. (2007); Torres et al. (2008b)</td>
</tr>
</tbody>
</table>

*Membrane biofilm reactor fed with 100% O_{2} and high substrate concentrations.

1COD, chemical oxygen demand (1 mol e^{-} = 8 g COD).
refer to the energy of electrons. We use ‘energy’ and ‘potential’ interchangeably throughout the manuscript when referring to electrons.] After ARB release the electrons, two additional processes can result in potential losses. The first is the EET mechanism that transports electrons to the anode interface, which will change the electron energy from \( E_{OM} \) to \( E_{interface} \). The second is the reaction occurring at the anode interface, which can decrease the potential from \( E_{interface} \) to \( E_{anode} \). As we explain later, ARB only gain energy from intracellular processes (\( E_{donor} - E_{OM} \)), and the energy loss in extracellular processes (\( E_{OM} - E_{anode} \)) is mostly dissipated as heat. Because MXCs must be operated to manage energy efficiently by maximizing energy conversion to ATP (intracellular processes) and minimizing losses due to extracellular processes (EET, anode interface reaction) that do not yield energy to the cell.

The electrical current produced by ARB can be measured in an MXC with great precision and in real time (Bamford & Compton, 1986; Bard & Faulkner, 2001). Therefore, we can interrogate the kinetic response of ARB in order to study all kinetic processes that could lead to potential losses within an ARB biofilm. A full understanding of ARB kinetics (see Box 2) in an anode will reveal EET kinetics: the rate at which electrons are transferred between ARB and the electrode interface. Are the high current densities observed in the literature achievable with any of the three proposed EET mechanisms? What are the potential losses due to EET required to achieve these high current densities? By understanding ARB kinetics, we can evaluate which EET mechanisms are more suitable for ARB to use in MXCs and which of these mechanisms have limitations that impede us from achieving our goals of obtaining high current densities at a low \( \eta_{anode} \).

In the following section, we discuss intracellular potential losses, which are shared by all EET mechanisms, and its importance in characterizing anode potential losses. Then, we discuss the kinetic processes associated with extracellular potential losses within the context of each EET mechanism.

**Intracellular potential losses (\( E_{donor} - E_{OM} \))**

Two kinetic processes are involved in intracellular potential losses from \( E_{donor} \) to \( E_{OM} \); these processes are common in all respiratory bacteria and are depicted in Fig. 5. First, bacteria oxidize the electron donor, producing intracellular reducing power (Fig. 5, 1) in the form of an electron carrier such as NADH. To generate energy for the cells, the electron carrier is oxidized by transferring its electrons into the membrane-associated proteins that are part of the electron transport chain, which ultimately leads to the external electron acceptor. In the case of ARB, the electrons are transferred to OM proteins (Fig. 5, 2) that initiate the EET process. Recent review papers have discussed the specific proteins and mechanisms involved in transferring electrons to an OM protein (e.g. Weber et al., 2006; Gralnick & Newman, 2007; Lovley, 2008); we refer the reader to these reviews.

**Rate of substrate utilization by ARB**

The rate of substrate utilization in microbial processes is frequently modeled using the Monod relationship (Monod, 1949; McCarty & Mosey, 1991; Pavlostathis & Giraldo-Gomez, 1991; Rittmann & McCarty, 2001). This relationship explicitly describes the rate at which bacteria oxidize the substrate and produce the reduced intracellular electron carrier. When the substrate is rate-limiting for the entire process of metabolism and EET, the current density generated by ARB can be written as Eqn. (1) for a biofilm setting (Torres et al., 2007):

\[
j = j_{max} \frac{S}{K_{s,app} + S}
\]

where \( j \) is the current density obtained by ARB, \( j_{max} \) is the maximum current density of the ARB biofilm, \( S \) is the substrate concentration in the liquid, and \( K_{s,app} \) is the apparent half-saturation substrate concentration in a biofilm. In most MXC experiments, researchers maintain a high concentration of substrate in the reactor to avoid substrate limitation. This is especially true for experiments that focus on electron-transfer kinetics (e.g. Marsili et al., 2008a; Torres et al., 2008a, b). Therefore, our discussion assumes that the substrate is available in excess and the Monod term is saturated (i.e. \( S/[K_{s,app} + S] = 1 \).
Box 2. Voltammetric techniques as a tool to evaluate ARB kinetics

Electrochemists use various voltammetric techniques to characterize electrochemical reactions at an electrode surface. In MXC's, researchers have used several voltammetric techniques to characterize ARB's response to changes in anode potential. These techniques include cyclic voltammetry (CV), low-scan cyclic voltammetry (LSCV), differential pulse voltammetry, and chronocoulometry (Fricke et al., 2008; Marsili et al., 2008b; Torres et al., 2007, 2008b; Richter et al., 2009).

LSCV is a powerful voltammetric technique, as it measures the steady-state response of ARB as a function of the anode potential. In LSCV, the anode potential is scanned within a potential range at a slow enough rate to allow ARB to reach a steady-state metabolic condition for all potentials. To reach this steady state, a typical voltage scan rate is 1 mV s$^{-1}$ or lower (Marsili et al., 2008a, b; Torres et al., 2008a; Richter et al., 2009). Figure 4 shows an example LSCV curve from experiments (Torres et al., 2008a) with a young ARB biofilm producing a maximum current density of $\sim$0.15 A m$^{-2}$. The voltage is scanned back and forth between $-0.33$ and $+0.15$ V vs. the standard hydrogen electrode (SHE) at 1 mV s$^{-1}$. The forward and backward curves are similar to each other, indicating steady-state conditions that are not affected by the scanning direction. $\eta_{\text{anode}}$ can be measured from these curves by calculating the $E_{\text{donor}}$ (for acetate in this case, $E_{\text{acetate}} = -0.285$ V vs. SHE). At $E_{\text{anode}} = E_{\text{donor}}$, ARB cannot gain any energy-transferring electrons to the anode; therefore, $j \sim 0$. As $E_{\text{anode}}$ becomes more positive, the ARB metabolic rate increases (indicated by the increase in $j$) until it saturates. Note that $\eta_{\text{anode}}$ varies depending on the current density obtained. For example, this ARB biofilm requires an $\eta_{\text{anode}} \sim -0.15$ V to generate 0.1 A m$^{-2}$.

**Electrochemists use various voltammetric techniques to characterize electrochemical reactions at an electrode surface.**

**Fig. 5. Schematic of processes involved in intracellular potential losses by heterotrophic ARB.** 1. Substrate utilization – electrons are transferred from the electron donor ($E_{\text{donor}}$) to intracellular reducing power (NADH). 2. Extracellular electron production – the intracellular reducing power ultimately reaches the OM proteins ($E_{\text{OM}}$), resulting in a potential loss.

The rate of reduction of electron shuttles can be modeled by the Monod relationship expressed in terms of the concentration of the soluble shuttle (Bae & Rittmann, 1996). This modeling approach has been used for ARB that use an oxidized electron shuttle as their electron acceptor (Picioreanu et al., 2007). However, because the electron acceptor for ARB is a solid anode, we cannot use the Monod relationship, because we cannot define an anode concentration. Thus, we transform the concentration of an electron acceptor into the anode potential, which is achieved using the Nernst–Monod relationship developed by Marcus et al. (2007) to express the current density in an ARB biofilm:

$$j = j_{\text{max}} \left( \frac{1}{1 + \exp \left( \frac{F}{RT} \left( E - E_{K} \right) \right)} \right)$$

(2)

where $R$ is the ideal gas constant (8.3145 J mol$^{-1}$ K$^{-1}$), $F$ is the Faraday constant (96 485 C mol$^{-1}$ e$^-$, $T$ is the temperature (K), and $E_{K}$ is the potential at which $j = 1/2j_{\text{max}}$ (V). The Nernst–Monod relationship combines the Monod relationship typically used to calculate the rate of electron-acceptor utilization with the Nernst equation for describing the anode potential availability as the electron acceptor for ARB (Marcus et al., 2007). As we discuss later, the Nernst–Monod equation has been successfully used to model ARB’s response to changes in anode potentials (Torres et al., 2008a).

Because it is derived from the Monod relationship, the Nernst–Monod relationship describes irreversible reactions occurring in microbial metabolism when the intracellular...
conditions are at a steady state. Thus, the curve of the Nernst–Monod relationship resembles that of a coupled irreversible reaction (Bard & Faulkner, 2001), similar to that of enzymatic reactions (S + E → SE → E + P, in which E is the enzyme, S is the substrate, and P is the product). Being irreversible, the ARB metabolism modeled with the Nernst–Monod equation has only an oxidation current (i.e. electrons flowing into the anode).

A similar kinetic equation is used in enzyme electrochemistry to model the steady-state catalytic rate of an enzyme/electrode system (Sucheta et al., 1992; Heering et al., 1998; Bard & Faulkner, 2001). The equation, which is derived from the Michaelis–Menten and the Nernst equation, is similar to the Nernst–Monod equation [Eqn. (2)], but instead of an $E_{KA}$ term, it has a half-wave potential $E'_{1/2}$ (Heering et al., 1998). $E'_{1/2}$ is analogous to $E_{KA}$, but in a known enzymatic pathway, $E'_{1/2}$ can be defined based on the kinetic parameters of proteins that are being oxidized or reduced at the anode and mass transfer coefficients of reactants/products (Bard & Faulkner, 2001). Therefore, $E_{KA}$ should be related to the standard reduction potential of proteins associated with respiration. However, given the complex enzymatic processes occurring in an ARB electron-transport chain, it is not practical for us to define $E_{KA}$ in the same manner as $E'_{1/2}$. Thus, $E_{KA}$ in the Nernst–Monod relationship becomes an empirical parameter like $K_S$ for the Monod relationship. Other researchers have successfully modeled ARB’s kinetic response using the model that contains $E'_{1/2}$ (Richter et al., 2009), but their analysis does not define $E'_{1/2}$ as a function of enzymatic potentials. Therefore, their use of this equation is analogous to using the Nernst–Monod equation.

Because enzyme/electrode and bacteria/electrode systems have similar kinetic processes, we can analyze the potential losses in the ARB biofilm (depicted in Fig. 6) in a similar manner as is performed in enzyme electrochemistry. The enzyme shows an electrochemical response that is represented by Eqn. (2), regardless of whether the electron transport is carried out by electron shuttles (Limoges & Savéant, 2003) or by direct electron transfer (Léger & Bertrand, 2008). Slow kinetics for either EET or interface electron transfer will cause the kinetic response to deviate from the sigmoidal curve represented by Eqn. (2). A comprehensive review of these deviations is presented by Léger & Bertrand (2008). Similarly, we use the Nernst–Monod equation as the baseline to distinguish intracellular potential losses from extracellular potential losses due to EET and interface electron transfer.

**Extracellular potential losses**

($E_{OM} - E_{anode}$)

Two kinetic processes are involved in extracellular potential losses. First, electrons are transported from the ARB’s OM proteins (e.g. cytochromes) to the surface of the anode by either electron shuttles (Fig. 6, 3a) or by a solid conductive
matrix (Fig. 6, 3b). This reduces the electron energy from $E_{\text{OM}}$ to $E_{\text{interface}}$. Then, electrons are transferred to the electrode by interface electrode transfer (Fig. 6, 4), reducing the electron energy from $E_{\text{interface}}$ to $E_{\text{anode}}$. Given that these potential losses occur on the outside of the cell, it is unlikely that these losses are associated with ARB energy conservation and growth (Lovley, 2008). The underlying mechanisms involved in these kinetic processes (diffusive transport, conduction, and electrochemical reactions) are known to dissipate energy as heat or as an increase in entropy, thus strengthening our assumption that ARB cannot recover this energy for growth (Bard & Faulkner, 2001; Qian et al., 2002). In order to maximize their growth by capturing as much energy as they can, ARB must minimize extracellular potential losses. We discuss these losses in the context of the various EET mechanisms used by ARB: (1) direct contact, (2) electron shuttles, and (3) solid conductive matrix. Figure 6 summarizes the different kinetic processes that are associated with intracellular and extracellular potential losses in an ARB biofilm. Depending on the EET mechanism used by ARB, different kinetic processes must be considered.

**Direct contact mechanism**

In direct contact, a single layer of ARB colonizes the anode and directly transfers electrons to the electrode from a portion of its OM that is in contact with the anode. Direct contact should represent the lowest extracellular potential loss, because electrons do not need to travel over a significant distance to reach the anode. After electrons have reached the surface of the electrode, an electrochemical reaction must occur that releases these electrons into the conductive anode (Fig. 6, 4). This reaction occurs between an OM protein and the electrode and is probably reversible, given the reversibility of electron transfer observed in cytochromes (dos Santos et al., 1999).

The rate at which a reversible reaction occurs at an electrode interface is often described by the Butler–Volmer equation, written here for anodic current only (Bard & Faulkner, 2001):

$$j = -j_0 \exp \left[ \frac{nF(1 - \alpha)(E_{\text{anode}} - E_{\text{interface}}^0)}{RT} \right]$$  

(3)

where $j_0$ is the exchange current density (A m$^{-2}$), $\alpha$ is the electron-transfer coefficient or the symmetry coefficient for the anodic or the cathodic reaction, $E_{\text{anode}}$ is the anode potential (V), and $E_{\text{interface}}^0$ is the standard potential (V) of the reaction occurring at the anode interface. The Butler–Volmer equation describes the final potential loss for all EET mechanisms; this reaction can occur between a protein and the anode or by a compound (such as an electron shuttle) and the anode. The Butler–Volmer equation is derived from the first-order rate expressions of a reversible reaction occurring at the anode surface (Bard & Faulkner, 2001). Thus, the parameter $j_0$ combines the kinetic rate constants associated with the reaction and the equilibrium concentration of electroactive species. If potential losses are small, the Butler–Volmer equation can be simplified into a first-order equation, thus simplifying its analysis (Bockris et al., 2000; Finkelstein et al., 2006).

Although low extracellular potential losses are possible with direct contact, the total amount of biomass in contact with the anode is surely a limiting factor in achieving a high current density. If ARB need to be in direct contact with the electrode, a monolayer biofilm would be the highest amount of biomass active in EET; this would be equivalent to a biofilm thickness of $L_0 < 2 \mu$m. We calculate the maximum current density achievable by this monolayer biofilm using a simple biofilm model.

In the case of direct transfer, $j_{\text{max}}$ is a function of the active biofilm thickness according to the following equation simplified from Torres et al. (2007):

$$j_{\text{max}} = \gamma_s q_{\text{max}} X_f L_{fa}$$  

(4)

where $\gamma_s$ is a conversion factor from mass of substrate to coulombs, $q_{\text{max}}$ is the maximum specific rate of substrate utilization (mol e$^{-1}$ dwt h$^{-1}$), $X_f$ is the concentration of active biomass in the biofilm (g dwt m$^{-3}$), and $L_{fa}$ is the biofilm thickness active in EET (m). For a monolayer of ARB ($L_{fa} = 2 \mu$m and $X_f = 2.8 \times 10^5$ g dwt m$^{-3}$) to produce 15 A m$^{-2}$, $q_{\text{max}}$ would have to be $\sim 1$ mol e$^{-1}$ g$^{-1}$ dwt h$^{-1}$, more than an order of magnitude higher than reported values of *Escherichia coli* respiring oxygen (18 mmol O$_2$ g$^{-1}$ dwt h$^{-1}$ or 72 mmol e$^{-1}$ g$^{-1}$ dwt h$^{-1}$) (Paalme et al., 1997; Xu et al., 1999). Our previous calculations for ARB biofilms in MXCs estimated $q_{\text{max}} X_f = 4500$ mol e$^{-1}$ m$^{-3}$ h$^{-1}$ (860 mg BOD cm$^{-3}$ day$^{-1}$) (Torres et al., 2008a). Assuming this value and $L_{fa} = 2 \mu$m, the maximum current density obtained would be 0.24 A m$^{-2}$, almost two orders of magnitude smaller than the highest current densities obtained in MXCs. This value is an upper limit that assumes that ARB can transfer all electrons through the small fraction of the OM that is in contact with the electrode. Our calculations are consistent with previous studies performed by Reguera et al. (2006), in which the biofilm thickness was correlated to the current produced by a *Geobacter sulfurreducens* biofilm. In their study, a pilin-deficient mutant was only able to produce a biofilm of $\sim 3 \mu$m thickness, and it produced $\sim 0.16$ A m$^{-2}$. These results, along with our calculations, show that although direct electron transfer is a viable mechanism by ARB, it cannot account for the high current densities obtained recently in MXCs. Therefore, in order to achieve high current densities in MXCs, it is essential that ARB use another EET mechanism that allows them to form a biofilm with multiple layers of cells producing current (as those shown in Fig. 7a).
discuss two EET mechanisms that allow an ARB biofilm by transporting electrons through water (soluble electron shuttles) or through solid components of the extracellular biofilm matrix.

**Soluble electron shuttle mechanism**

Many bacteria are known to produce compounds that can act as electron shuttles between cells and the solid electron acceptor (e.g. metal oxides, electrodes) (Newman & Kolter, 2000; Weber et al., 2006; Lovley, 2008). ARB known to produce electron shuttles in MXCs include members of Shewanella, Pseudomonas, and Escherichia (Rabaey et al., 2005; Marsili et al., 2008a; von Canstein et al., 2008; Zhang et al., 2008). In addition, molecules found in the environment, including humic substances and leaf extracts, can be used by bacteria as electron shuttles (Lovley et al., 1996; Nevin & Lovley, 2002a). The use of electron shuttles allows ARB to be located away from the anode surface and to accumulate more than a monolayer of bacteria. Although shuttles allow more ARB to be active per anode surface area, the distance between ARB and the anode becomes a limiting factor due to diffusion limitations of the electron shuttles (Picioreanu et al., 2007).

A previous modeling study suggested that ionic migration caused by the external potential field can play an important role in the transport and concentration gradient of electron shuttles in an ARB biofilm (Rabaey et al., 2007). Their model calculated the transport of electron shuttles inside the biofilm using the Nernst–Planck equation. According to their results, electron shuttles would tend to accumulate inside the biofilm due to migration forces that attract them to the anode surface. However, their calculation assumed that electron shuttles are the only ions affected by migration. This assumption is erroneous, because all ions present in solution are affected by migration, and their transport due to migration forces is a function of their concentration (Siegrist & Gujer, 1985; Torres et al., 2008b). Given that electron shuttles are often found at μM or nM concentrations (Nevin & Lovley, 2002b; Turick et al., 2002; Marsili et al., 2008a), while many other ions (e.g. sodium, potassium, chloride, phosphate, and bicarbonate) are present in mM concentrations, the transport of electron shuttles due to migration is negligible. Thus, transport of soluble electron shuttles is mainly carried out by diffusion through Fick’s law, shown here modified to reflect current density calculations:

$$j = nF \left( \frac{D_{\text{shuttle}} \Delta C_{\text{shuttle}}}{\Delta z} \right)$$  

(5)

where $D_{\text{shuttle}}$ is the diffusion coefficient of the electron shuttle (m$^2$ s$^{-1}$), $\Delta z$ is the transport distance (m), $\Delta C_{\text{shuttle}}$ is the concentration gradient of either oxidized or reduced shuttle (mol m$^{-3}$), and $nF$ converts from moles to coulombs.

In a biofilm model considering electron shuttles, Eqn. (5) must be coupled with the rate of shuttle oxidation at the anode surface (Picioreanu et al., 2007).

The total current density obtained by ARB using electron shuttles can be limited by the diffusion of electron shuttles according to Eqn. (5). Diffusion coefficients of organic molecules are relatively small, indicating that diffusion is an inherently slow process. Even though we could not find calculated $D_{\text{shuttle}}$ values for any known electron shuttle, if we can assume it is similar to other organic molecules, such as glucose, then $D_{\text{shuttle}} \approx D_{\text{glucose}} = 6.7 \times 10^{-10}$ m$^2$ s$^{-1}$ (Lide, 2006). $\Delta C_{\text{shuttle}}$ is limited by the total concentration of electron shuttles present in the ARB biofilm. Various experiments have shown an observed accumulation of flavins by S. oneidensis of up to ~0.5 μM (Marsili et al., 2008a; von Canstein et al., 2008), while other electron shuttles in mineral-respiration experiments were present in low μM levels (Nevin & Lovley, 2002b; Turick et al., 2002). Using $D_{\text{shuttle}} = 6.7 \times 10^{-10}$ m$^2$ s$^{-1}$, $\Delta C_{\text{shuttle}} = 1$ μM = 1 × 10$^{-3}$ mol m$^{-3}$, and $n = 2$ for the electron shuttle reaction (Kubota & Gorton, 1999; von Canstein et al., 2008), the flux of an electron shuttle across 1 μm of biofilm ($\Delta z = 1$ μm) is only 0.13 A m$^{-2}$. This calculated value is 100 times smaller than observed current densities. In order to
achieve high current densities through electron-shuttle diffusion, the gradient would have to be 100 times larger (∼100 μM per 1 μm of biofilm); thus, shuttle concentrations inside a > 10-μm-deep biofilm would have to be in the mM range. This simple analysis also shows that ARB in suspension cannot produce a considerable current, because the Δξ value would be much larger than 10 μm.

The results obtained by known ARB using electron shuttles are consistent with our analysis. Marsili et al. (2008a) obtained a $j_{\text{max}}$ ~0.16 A m$^{-2}$ using S. oneidensis that produced flavins as electron shuttles; their $j_{\text{max}}$ increased to ~0.25 A m$^{-2}$ when additional riboflavin (0.25 μM) was added. Low current densities were also obtained by different strains Pseudomonas aeruginosa using pyocyanin as an electron shuttle: $j_{\text{max}}$ was < 0.01 A m$^{-2}$ (Rabaey et al., 2005; Pham et al., 2008) and addition of 50 μM pyocyanin only increased $j_{\text{max}}$ to two to three times this value (Rabaey et al., 2005). Similar low current densities (< 0.01 A m$^{-2}$) are obtained with Geothrix fermentans and E. coli using unknown electron shuttles (Bond & Lovley, 2005; Zhang et al., 2008). A mathematical model based on electron shuttles (Picioreanu et al., 2007) also calculated a $j_{\text{max}}$ limitation due to electron shuttle diffusion. Even at an extremely high electron shuttle concentration (1 mM), the calculated $j_{\text{max}}$ was ~0.57 A m$^{-2}$. Thus, experimental and estimated $j_{\text{max}}$ when using electron shuttles, are at least 20 times smaller than the higher values observed in the literature (10–15 A m$^{-2}$; e.g. Fan et al., 2007; Catal et al., 2008; Torres et al., 2008a, b).

Electron-shuttle transport results in an inherent potential loss due to the concentration gradient needed for diffusive transport. The potential loss for an electron-shuttle reaction ($\text{Shuttle}_{\text{ox}} + 2e^- \leftrightarrow \text{Shuttle}_{\text{red}}$) can be calculated by the Nernst equation:

$$E = E^0 - \frac{RT}{2F} \ln \left( \frac{C_{\text{Shuttle}_{\text{red}}}}{C_{\text{Shuttle}_{\text{ox}}}} \right)$$  \hspace{1cm} (6)

where $E$ is the potential at any point in the ARB biofilm (V), and $C_{\text{Shuttle}_{\text{red}}}$ and $C_{\text{Shuttle}_{\text{ox}}}$ are the respective concentrations of the reduced and oxidized electron shuttle (mol m$^{-3}$). In order to maximize $j_{\text{max}}$, the concentration gradient must be maximized, but the gradient causes a potential loss according to Eqn. (6). Assuming that we have a concentration gradient that is 99% of the total shuttle concentration in order to maximize the diffusion rate (i.e. 99% oxidized at the OM and 99% reduced at the anode interface), $E_{\text{OM}} - E_{\text{interface}}$ would be equal to 118 mV. This potential loss is observed in experimental data by Marsili et al. (2008a), as well as in modeling results by Picioreanu et al. (2007), as a deviation from the typical Nernst–Monod curve.

The value for $E^0$ is also an important parameter to characterize the potential loss of electron shuttles, as it determines the point at which the current density approaches zero ($j_0$) by Butler–Volmer kinetics (Fig. 6, 4). ARB using electron shuttles have a wide range of $E^0$, depending on the shuttle used. Riboflavin produced by S. oneidensis has an $E^0 = –0.208$ V vs. SHE (Marsili et al., 2008a), leading to a small thermodynamic loss. On the other hand, pyocyanin produced by P. aeruginosa has an $E^0 = –0.334$ V vs. SHE (Friedheim & Michaelis, 1931; Hernandez & Newman, 2001), leading to a thermodynamic loss of 251 mV when assuming acetate as the donor substrate ($E^0_{\text{acetate}} = –0.285$ V vs. SHE).

Loss of electron shuttles in the effluent of the MXC poses another challenge to its use by ARB. Given that electron shuttles must be soluble in order to ‘shuttle,’ most of them could be lost from MXC systems in the effluent water. Because biofilm systems are often operated at low hydraulic retention times of a few hours or minutes (Gray, 2004), the loss of electron shuttles can be substantial. A few studies showed a decrease in current density when the medium was replaced in batch experiments using ARB that produce electron shuttles (Bond & Lovley, 2005; Marsili et al., 2008a). This can be observed in Fig. 8d, where Marsili et al. (2008a) replaced the reactor medium with a fresh medium in an MXC with S. oneidensis, resulting in a decrease in current density. This effect could be stronger in a continuous system, where the steady loss of electron shuttles in the effluent liquid decreases their concentration in the biofilm significantly. Marsili et al. (2008a) have proposed that electron shuttles can be at higher concentrations inside the biofilm by binding to the electrode surface and biomass. Binding cannot increase the flux of electrons through diffusion, because the attached shuttles cannot diffuse.

**Solid conductive matrix mechanism**

A relatively unexplored electron-transfer mechanism by researchers is the use of a solid conductive matrix by ARB. The possibility that ARB are able to use a solid conductive or a semi-conductive material for electron transfer is mainly supported by recent findings of microbial ‘nanowires’ that appear to be responsible for EET (Reguera et al., 2005, 2006; Gorby et al., 2006) [The term ‘electrical conductor’ is used in engineering as a material (typically a metal) that contains loosely held electrons and is able to transfer electrons with minimal potential losses. Given the characteristics of possible exopolymeric substances (EPS) produced by ARB (organic molecules, some with metal molecules), the solid conductive matrix is likely to act as a semi-conductor ($10^{-8}$ mS cm$^{-1} < \kappa < 10^{3}$ mS cm$^{-1}$) rather than as a conductor. For simplicity, we will continue to use the terms ‘conductive’ instead of ‘semi-conductive.’]. Figure 7b shows a scanning electron micrograph of a biofilm producing a high current density (∼8 A m$^{-2}$) in our experiments (Torres
et al., 2009); putative nanowires are observed to connect bacteria with other sections of the biofilm. It is possible that other solids within the extracellular biofilm matrix are also conductive; the use of loosely bound cytochromes (Lovley, 2008) and bound 'electron shuttles' (Marsili et al., 2008a; Rittmann, 2008) has also been proposed as being responsible for the conductivity of the solid matrix. Unlike soluble electron shuttles, the solid mechanism is not restricted by Fick’s Law, but by the rate at which the solid conductive matrix is able to conduct electrons. Depending on the characteristic of the solid conductive matrix, its conductivity could be modeled using various equations that describe conductors and semi-conductors (Seeger, 1997). We have used Ohm’s Law in our past modeling (Marcus et al., 2007):

\[ j = -\frac{\kappa_{\text{bio}}(E_{\text{OM}} - E_{\text{interface}})}{\Delta z} \]  

where \( \kappa_{\text{bio}} \) is the conductivity of the solid conductive matrix (R \(^{-1}\) L \(^{-1}\)). Thus, \( \kappa_{\text{bio}} \) determines the potential losses \( (E_{\text{OM}} - E_{\text{interface}}) \) associated with \( j \). A high \( \kappa_{\text{bio}} \) can benefit ARB by minimizing \( E_{\text{OM}} - E_{\text{interface}} \) and maximizing \( j \) across the conductive matrix.

So far, no one has directly measured the bulk \( \kappa_{\text{bio}} \) value for the solid matrix of an ARB biofilm; however, a few studies are attempting to characterize the conductivity of the components within the solid matrix that may be conducting the electrons. Reguera et al. (2005) and El-Naggar et al. (2008) measured conductivity values for bacterial nanowires across the width of the nanowires, and efforts are underway to measure their conductivity along their length (M.Y. El-Naggar, pers. commun., N. Hansmeier, pers. commun.). It is also unknown whether nanowires are themselves conductive or whether they serve as a surface for conductive proteins/polymer to attach. Studies suggest that cytochromes (probably attached to nanowires) are somewhat responsible for the conductivity of the solid matrix (Gorby et al., 2006; Busalmen et al., 2008; El-Naggar et al., 2008; Richter et al., 2009). Obtaining nanowire conductivity and the nanowire density inside the ARB biofilm can allow us to calculate \( \kappa_{\text{bio}} \) based solely on bacterial nanowires. This calculation could help us evaluate whether nanowires are capable of transferring the observed high current densities.

Our previous modeling studies (Marcus et al., 2007) estimated that, for a current density of a few A m \(^{-2}\), \( \kappa_{\text{bio}} \) must be \( > 10^{-3} \) mS cm \(^{-1}\) to make EET potential losses negligible. A higher current density would require a higher \( \kappa_{\text{bio}} \). These values are within the range of what is considered a semi-conductor \( (10^{-6} \text{ mS cm}^{-1} < \kappa < 10^{6} \text{ mS cm}^{-1}) \). If
Table 2. Summary of kinetic analysis on the three EET mechanisms known to be used by ARB

<table>
<thead>
<tr>
<th>EET mechanism</th>
<th>Anode potential losses (η&lt;sub&gt;anode&lt;/sub&gt;)</th>
<th>Current density (j&lt;sub&gt;max&lt;/sub&gt;)</th>
</tr>
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<tbody>
<tr>
<td>Direct contact</td>
<td>Minimal EET losses due to the proximity of ARB to the electrode. Thus, η&lt;sub&gt;anode&lt;/sub&gt; is determined mainly by the Nernst–Monod relationship</td>
<td>Low j&lt;sub&gt;max&lt;/sub&gt; due to ARB’s inability to form a large enough biofilm to accumulate enough EET-active biomass. j&lt;sub&gt;max&lt;/sub&gt; should be much lower than 0.3 A m&lt;sup&gt;-2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Electron shuttles</td>
<td>Gradients associated with electron shuttle diffusion can lead to EET losses of &gt; 100 mV. This leads to high deviations from the Nernst–Monod relationship</td>
<td>Diffusion fluxes limit j&lt;sub&gt;max&lt;/sub&gt; especially at low electron-shuttle concentrations. Observed j&lt;sub&gt;max&lt;/sub&gt; for ARB using shuttles are lower than 0.25 A m&lt;sup&gt;-2&lt;/sup&gt;, even after adding extra electron shuttles</td>
</tr>
<tr>
<td>Solid conduction</td>
<td>Low EET losses can be achieved, provided k&lt;sub&gt;bio&lt;/sub&gt; is &gt; 10&lt;sup&gt;-3&lt;/sup&gt; mS cm&lt;sup&gt;-1&lt;/sup&gt;. Recent data suggest that k&lt;sub&gt;bio&lt;/sub&gt; can be as high as 0.5 mS cm&lt;sup&gt;-1&lt;/sup&gt;. Under these conditions, η&lt;sub&gt;anode&lt;/sub&gt; is determined mainly by the Nernst–Monod relationship</td>
<td>If minimal potential losses are achieved with a high k&lt;sub&gt;bio&lt;/sub&gt;, high j&lt;sub&gt;max&lt;/sub&gt; can be achieved. High j&lt;sub&gt;max&lt;/sub&gt; observed in MXCs (&gt; 10 A m&lt;sup&gt;-2&lt;/sup&gt;) suggests that solid conduction is effectively used by ARB</td>
</tr>
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</table>

ARBs are able to maximize k<sub>bio</sub>, their extracellular potential losses would be minimal, and their voltammetric curve would resemble that of the Nernst–Monod relationship. ARB would also be able to produce higher j<sub>max</sub> values, because their current production would be determined by their metabolic activity and not by their EET mechanism.

Recent studies have shown that ARB known to produce a solid conductive matrix can produce high current densities. Dumas et al. (2008) obtained up to 8 A m<sup>-2</sup> using G. sulphurreducens grown on graphite, ARB that are known to produce microbial nanowires (Reguera et al., 2005) and do not produce soluble electron shuttles (Lloyd et al., 1999). Similarly, our previous results showed up to 10 A m<sup>-2</sup> in a mixed culture enriched with G. sulphurreducens (Torres et al., 2008a). These results, as well as others presented in Fig. 8, also show minimal potential losses, indicative of a high k<sub>bio</sub>.

**Perspective of EET mechanisms in MXCs**

We use a kinetic analysis to characterize current densities and anode potential losses in an ARB biofilm. Based on our analysis, we are able to evaluate the proposed EET mechanisms based on our goals for an MXC: low potential loss and high current density. Table 2 summarizes our findings. With direct contact, current density is severely limited due to the very small amount of active ARB on the anode surface. Soluble electron shuttles allow the accumulation of more ARB biomass, but this mechanism also has a current-density limitation arising from the slow diffusion of reduced/oxidized electron shuttles. In order to overcome this limitation, the concentration of electron shuttles would have to be in the mM range, but observed concentrations are in the low μM or nM range. The electron-shuttle mechanism also incurs potential losses associated with the electron-shuttle concentration gradient, which can easily reach > 100 mV, and the soluble shuttles can be washed out in the reactor effluent.

Electron transfer through a solid conductive matrix has characteristics that are more favorable for achieving MXC goals. Unlike electron shuttles, a solid material is not likely to leave the reactor in the liquid effluent. Even more importantly, this mechanism offers the possibility of obtaining high current densities at low anode potential losses, as long as the matrix’s conductivity is high enough. If the EPS produced by ARB has k<sub>bio</sub> greater than about 1 mS cm<sup>-1</sup>, potential losses due to EET can be negligible, thus maximizing the energy used for ARB growth.

Figure 8 shows various examples of LSCVs of ARB biofilms (Fricke et al., 2008; Marsili et al., 2008a,b; Torres et al., 2008a), which are compared with a Nernst–Monod function. The experimental results match the theoretical Nernst–Monod curve almost perfectly for curves in Fig. 8a–c, indicating that most of the anode potential losses are associated with intracellular potential losses, while losses associated with EET or interface electron transfer are minimal. According to our modeling results for the LSCV in Fig. 8a, k<sub>bio</sub> must be higher than ~0.5 mS cm<sup>-1</sup> in order to achieve these low potential losses at high current densities (up to 10 A m<sup>-2</sup>). Similar results were reported by Srikanth et al. (2008), Marsili et al. (2008b) (Fig. 8b), and Fricke et al. (2008) (Fig. 8c) using G. sulphurreducens, an ARB known to produce nanowires. As explained before, the combination of high current densities combined with low extracellular potential losses is only possible if a solid conductive matrix is used for EET. These results not only confirm the presence of a solid conductive matrix, but they also satisfy the requirements of high current densities at low anode potential losses for MXCs.

In contrast, the results in Fig. 8d(Marsili et al., 2008a) are for a system where S. oneidensis was used riboflavin as an electron shuttle. Instead of a saturated sigmoidal curve at 200 mV from j~0, as the Nernst–Monod proposes (Fig. 8a–c), their voltammetric curves begin to saturate only at > 400 mV from j~0 (Fig. 8d). In addition, the current density obtained by S. oneidensis in Fig. 8d (~0.16 A m<sup>-2</sup>) under standard conditions was ~15 times smaller than the current density obtained by G. sulphurreducens in Fig. 8b (~2.6 A m<sup>-2</sup>) (both experiments were performed in similar MXCs) and ~50 times
smaller than the current density in Fig. 8a (8 A m\(^{-2}\)). These results reinforce that only the electron transfer through the solid conductive matrix allows higher current densities.

ARB found in natural settings may not need to have a high current density simultaneously with low potential losses. Box 3 describes the differences between some natural settings and MXCs.

Although the kinetic evidence strongly suggests the need for a solid conductive matrix to achieve high current densities and low anode potentials in MXCs, we have only limited scientific knowledge of the biological components that are responsible for its conductivity. Conductivity measurements of individual nanowires have yet to be related to \(k_{\text{bdc}}\) in order to understand their role in EET. Other proposed contributors to the solid conductive matrix, such as loosely bound cytochromes or bound 'electron shuttle' compounds, have not been researched in detail and remain only hypotheses. Moving MXCs toward commercial applications will create an inherent need to understand the mechanisms utilized by ARB to respire using the anode. Therefore, the study of solid conduction as an EET mechanism is of great importance in our field.

**Box 3. EET mechanisms in natural systems**

Conditions encountered by bacteria in natural systems (e.g. sediments) are, in many cases, dissimilar to those encountered in engineered systems; we have identified three differences between MXCs and natural systems that can affect the preferred EET mechanism used by bacteria. First, MXC researchers run their experiments with excess electron donor, nutrients, and carbon source. Bacteria in natural systems often encounter limitations of these building blocks of life, which leads them to minimize biomass production and have a slower metabolism. Under these conditions, bacteria-reducing metal oxides probably would prefer an EET mechanism that minimizes nutrient and carbon consumption. Direct electron transfer may meet this goal best, as ARB in this case do not need to produce exogenous shuttles or EPS. Solid conduction may imply the highest use of nutrients and carbon, due to the need to produce a continuous solid to transfer the electrons. Second, most MXCs operate with a continuous feed and a small hydraulic retention time, both typical of biofilm processes (Rittmann & McCarty, 2001). Under these conditions, electron shuttles are washed out (Marsili et al., 2008a), leading to increasing energy costs by bacteria to reproduce the lost shuttles. However, sediments often have minimal water flow, which could allow bacteria to maintain a higher concentration of electron shuttles in solution. Third, metal oxides in sediments may be present in small precipitates that can be totally reduced by bacteria in a small period of time. If the solid acceptor is consumed, bacteria need to be able to move to another piece of metal oxide. This phenomenon was observed in Geobacter metabireducens, which increases its motility when grown with an insoluble electron acceptor (Childers et al., 2002). Under these conditions, the production of a solid conductive matrix, which involves the formation of a biofilm, may be inefficient.

**References**


