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Microscale gradients and their role in electron-transfer mechanisms in biofilms

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Abstract

The chemical and electrochemical gradients in biofilms play a critical role in electron-transfer processes between cells and a solid electron acceptor. Most of the time, electron-transfer processes have been investigated in the bulk phase, for a biofilm electrode or for an isolated component of a biofilm. Currently, the knowledge of chemical and electrochemical gradients in living biofilms respiring on a solid surface is limited. We believe the chemical and electrochemical gradients are critical for explaining electron-transfer mechanisms. The bulk conditions, an isolated part of a biofilm or a single cell cannot be used to explain electron-transfer mechanisms in biofilm systems. In addition, microscale gradients explain how the reactor configuration plays a critical role in electron-transfer processes.

Keywords

biofilm; electrode; electron transfer; microscale gradient; mineral; solid-phase electron acceptor

Biofilms and electron-transfer processes

Bacteria require electron acceptors to remove electron equivalents during electron donor consumption. Usually, the electron acceptor is a soluble compound; however, bacteria can colonize surfaces to form biofilms on solid electron acceptors [1]. More recently, several bacteria have been shown to colonize solid electrodes in electrochemical systems and transfer electrons [2,3]. To transfer electrons to a solid-phase electron acceptor such as a mineral or electrode, the biofilm must have a transport system that transfers electrons from inside the cell to outside the cell. This has been postulated to occur by direct electron transfer from the bacteria or through electron-transfer mediators [4,5]. Regardless of the electron-transfer mechanism, electron transfer from the top of the biofilm to the bottom will result in chemical and electrochemical gradients. Measuring these microscale gradients inside the biofilm is an important step in understanding electron-transfer mechanisms. In the present mini-review, we use the term electron transfer to describe extracellular electron transfer from cells in a biofilm to a solid electrode acting as an electron acceptor. We cover the fundamental reasons for these microscale gradients and discuss their importance. We

also cover how the reactor configurations used to study these microscale gradients can have a significant impact on the proposed electron-transfer mechanisms. Our goal is to highlight the role that microscale electron-transfer processes may play in improving the electron-transfer efficiency of these biofilms respiring on electrodes.

Microscale gradients

The biofilms that have been studied in the literature had thicknesses ranging from a few microns (single cells) to several hundreds of microns (complex structures). For biofilms that utilize electron-transfer mechanisms to access solid electron acceptors, the thickness of the biofilm is important. For example, the diffusion of nutrients or redox mediators could be hindered by the biofilm matrix [6]. Renslow et al. [7] measured the *in situ* effective diffusivity of water inside *Shewanella oneidensis* MR-1 biofilms using NMR. They found that thick biofilms showed a relative effective diffusivity as low as 50%. The relative effective diffusivity also correlated with biofilm density. Thus we expect that, for dense biofilms, chemical gradients are significant. A 50% relative effective diffusivity tells us how much longer it takes a molecule to diffuse through a finite thickness. This can be calculated using a simple Fickian model: for one-dimensional semi-infinite linear diffusion, the diffusion layer thickness, δ , is $(2D_t)^{-1/2}$ [8]. In other words, if the diffusion is much slower than the rate of microbial activity, significant chemical gradients will occur inside the biofilm. However, simplified assumptions of Fickian diffusion through a heterogeneous biofilm may not be accurate. This underlying physical description of the biofilm is significant since many models to date have assumed a constant diffusivity inside biofilms [9,10]. A mathematical treatment of porous electrodes in microbial fuel cells was given by Picioreanu et al. [11], who showed that the porous structure restricted access when there was no advective flux. Thus the concept that nutrients can reach the bottom of the biofilm even under excess bulk concentrations may not be correct [12].

One of the important consequences of the reduced ability of compounds to diffuse has been shown to be related to buffers. Torres et al. [13] showed that current was dependent on buffer strength. Without access of the buffer to the bottom of the biofilm, the pH inside the biofilm is expected to decrease. This is because protons are generated along with electrons during metabolism. Whereas electrons move towards the electrode surface, protons move towards the bulk solution (Figure 1). Measurements of pH in single-chambered and dual-chambered *Geobacter sulfurreducens* biofilm reactors have been shown to reach values of 6.4 and 6.5 respectively (see Figure 3). We showed that these pH profiles are a consequence of proton flux inside the biofilm [14]. Franks et al. [15] made similar measurements using pH-sensitive dyes that measured a pH of 6.2 at the bottom of a *G. sulfurreducens* biofilm. However, at lower currents, we measured little to no pH changes inside a *S. oneidensis* MR-1 biofilm, suggesting that mass transport only begins to play a role when electron flux reaches a critical value [16]. Another interesting facet is the measurement of the redox-Bohr effect in *G. sulfurreducens* cytochromes by Morgado et al. [17]. The redox-Bohr effect couples the hopping of electrons with the protonation of specific sites in a protein, potentially linking the pH inside the biofilm to electron transfer. It could be that pH is limited by this mechanism inside *G. sulfurreducens* biofilms well before metabolism is inhibited at pH values less than 6 [18]. Understanding the microscale development of pH

inside biofilms transferring electrons will allow us to distinguish how pH plays a critical role in electron transfer in biofilms.

Electron flux through biofilms

Similar to the flux of protons, the flux of electrons inside the biofilm (Figure 1) is expected to cause a distribution of potential inside the biofilm dependent on the resistance across endogenous mediators or conductive pili, or both. The local biofilm potential can be understood as a fall in potential across the biofilm thickness, z , with a certain resistance (Figure 2). The resistance has been recently measured by Malvankar et al. [19] as ranging from 200 to 8000 Ω in *G. sulfurreducens* strains, depending on the culturing technique. Interestingly, they report that the current density and resistance across the measured thickness of the biofilm do not follow Ohm's law, suggesting that mass transport plays a critical role at high current densities. It should be noted that, similar to diffusivity, which is heterogeneous throughout a biofilm, conductivity may also be heterogeneous, as noted by their use of a constant phase element in their equivalent circuit.

The origination of conductivity in biofilms remains a question, as models of both 'metallic-like' conductivity and 'redox-polymer-like' conductivity have been proposed. There is strong evidence for both models; however, they disagree on the nature of the conductivity. Metallic-like conductivity refers to an overlapping of π bonds creating a path for electrons to travel, similar to metals [20]. Redox-polymer-like conductivity refers to electrons hopping across a chain of polymers with discrete redox sites [21]. Conductive pili have been measured in *G. sulfurreducens* biofilms [20]. Cytochromes have been implicated as redox mediators in electron transfer where the redox status of cytochromes was measured as a function of electrode potential [22]. However, there has been no direct measurement of biofilm potential to date. Recently, we have offered a way to make such a measurement based on polarizing a graphite microelectrode tip inside a *G. sulfurreducens* biofilm [14]. The biofilm potential was determined to be entirely dependent on whether current was allowed to pass through the graphite microelectrode tip before measurement. This suggests that electron transfer inside *G. sulfurreducens* biofilms is compartmentalized to bound redox mediators inaccessible to potentiometric sensors.

In order to provide a comprehensive picture of electron transfer in biofilms, the transfer of both soluble and bound electrons must be assessed. Although the transfer of bound electrons has been studied through conductivity measurements, the transfer of soluble electrons has not been studied extensively. One of the ways to measure current passed by soluble redox mediators is to measure the redox potential inside the biofilm (Figure 2). Microelectrodes are often used to measure redox profiles in biofilms [23]. Redox potential tends to increase towards oxidizing surfaces and decrease towards reducing surfaces [16]. If electrons are transferred via accessible redox mediators, a redox profile increasing towards the surface is expected. We measured the redox potential inside *G. sulfurreducens* biofilms during electrode respiration and found that its shape depended on the reactor configuration used to grow the biofilms (Figure 3). We used a three-electrode configuration in which the biofilm, counter and reference electrodes were immersed in the same solution (single-chambered) and a microbial fuel cell configuration (dual-chambered) in which the counter and reference

electrodes were placed in the cathodic compartment while the biofilm electrode was in the anode compartment. Although the pH profiles followed the same trend inside the biofilms, the redox potential increased inside the biofilm in the single-chambered system and decreased inside the biofilm in the dual-chambered system. This is a clear indication that the counter electrode in the system affected the result [14]. The migration of a cathodic reactant, hydrogen, was shown to be the cause (results not shown). Interestingly, we also observed that hydrogen produced at the cathode was consumed by the biofilms in the anode. Whether or not hydrogen plays a role in facilitating specific types of electron-transfer mechanisms different from acetate metabolism remains to be observed. However, this cannot be measured directly unless we use microscale tools to quantify chemical gradients in biofilms.

Practical implications of microscale gradients in biofilms to electron-transfer mechanism

Considering that aspects of the mass transport and conductivity in current biofilm models have been measured, there may be an optimum biofilm thickness that can be engineered to be the operational thickness in biofilm-based systems. The mass transport may have more to do with the density inside the biofilm that restricts nutrients from reaching the bottom of the biofilm or protons from diffusing out of the biofilm. Perhaps both the biofilm density and conductivity can be optimized by altering the growth conditions of the biofilm, making it possible to engineer highly specific types of biofilms. Future optimizations of biofilms depend on further refinement of our understanding of the relationship among electrode potential, biofilm potential and biofilm conductivity. In particular, the source of conductivity of these biofilms needs to be addressed further. Measurements must be made at the microscale inside the biofilm because biofilm processes are interfacial phenomena. Flux calculations for the inside of a biofilm are far more informative than bulk-scale measurements or experimental data generated in batch systems. We expect that the limitations in electron-transfer processes between biofilms and solid electrodes can be explored using microscale techniques.

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Figure 1. biofilm cartoon

Shown are (1) the flux of nutrients, (2) the flux of protons and (3) the flux of electrons inside a biofilm of finite thickness, z .

Figure 2. Definition of potentials

The potential of the electrode with biofilm against a reference electrode is called the biofilm electrode potential. The potential of a microelectrode with a platinum tip is called the redox potential. A microelectrode with a carbon fibre tip in the biofilm passing current connects the tip electronically to the biofilm. The potential of this microelectrode under no-current conditions after the biofilm is electronically connected to the microelectrode tip is called the local biofilm potential (LBP).

Figure 3. pH and redox potential profiles in *G. sulfurreducens* biofilms under electrode-respiring conditions

In the single-chambered biofilm reactor, redox potential increased towards the biofilm (left). In the dual-chambered biofilm reactor separated by a cation exchange membrane, redox potential decreased towards the biofilm (right). The pH profiles in the two reactor set-ups were similar.