Critical transport rates that limit the performance of microbial electrochemistry technologies

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Abstract

Microbial electrochemistry technologies (METs) take advantage of the connection of microorganisms with electrodes. In the classic case of a microbial anode, the maximization of current density produced is often the goal. But, current production is dependent on many transport processes occurring, which can be rate-limiting. These include the fluxes of electron donor and acceptor, the ionic flux, the acidity and alkalinity fluxes at anode and cathode respectively, the electron transport flux at the biofilm, and the reactant/product crossover flux. Associated with these fluxes are inherent concentration gradients that can affect performance. This critical review provides an analysis on how these transport processes have hindered the development of METs, and how MET designs have evolved as more knowledge of these transport limitations is gained. Finally, suggestions are provided on how to design MET systems taking into consideration critical transport processes that are intimately linked to the current produced.

1. Introduction

The early era of developing microbial electrochemistry technologies (METs) was largely focused on the wastewater-to-energy idea (Liu et al., 2004; Rabaey and Verstrate, 2005). In this scenario, the anode of a microbial fuel cell (MFC) would be fed with municipal wastewater for anode-respiring bacteria (ARB) to generate electrical current from the organic contaminants in the wastewater. These remarkable microorganisms and their capability to carry out anode respiration were discovered in the early 2000s (Reimers et al., 2001; Bond et al., 2002). Current densities higher than 0.1 mA/cm^2 (or 1 A/m^2) were reported soon after their discovery (Bond and Lovley, 2003), with over 1.0 mA/cm^2 reported only a few years later (Cheng et al., 2006a; Torres et al., 2008a; Marsili et al., 2010). Converting 1.0 mA/cm^2 into a chemical oxygen demand (COD) flux results in $> 70 \text{ g COD/m}^2\text{-day}$ converted, much higher than aerobic biofilms ($\sim 20 \text{ g COD/m}^2\text{-day}$) (Casey et al., 1999; Syron and Casey, 2008) or even high-rate methanogenic reactors (up to $68 \text{ g COD/m}^2\text{-day}$) (Cresson et al., 2006; Ghaniyari-Benis et al., 2009) used in wastewater treatment. This means that ARB biofilms consume organic compounds at a faster rate than any other biofilms considered for environmental engineering applications. The hype was obvious, and researchers flocked quickly to this area.

But how could ARB biofilms be faster than aerobic biofilms? The answer lies in transport. As transport is the main topic of this review, this phenomenon is discussed first in bacterial biofilms.

1.1 Transport limitations in biofilms

Biofilms are a conglomerate of cells and extracellular polymeric substances that grow at a solid, often static surface. Serving as a living catalyst, biofilms will continue growing as long as they have access to substrates and nutrients. This access is determined by the diffusion of the compounds (electron donor or acceptor in most cases) into the biofilm. Thus, a biofilm will continue to grow and increase its consumption rate until the limiting substrate concentration reaches zero inside the biofilm, indicating a maximum flux according to Fick's Law; this is known as a "deep biofilm" (Rittmann and McCarty, 2001). This is an important aspect of biofilm growth, in which a steady state is eventually reached that is not limited by microbial catalysis, but rather limited by the transport of reactants/products in and out of the biofilm.

Encountering the famous no-slip condition, biofilms rely mostly on diffusion from the bulk medium to receive the compounds required for growth. This often implies that, under mixed conditions at the bulk medium, biofilms will encounter a diffusive boundary layer of tens to hundreds of microns in which no mixing occurs. Moreover, biofilms can be several hundreds of microns thick, and substrates and nutrients should be transported to the inner parts of the biofilm to maintain life. This diffusive transport within the biofilm occurs at a slower rate due to the tortuous path encountered in a matrix that is ~ 40% cells. A simple calculation using Fick's Law of diffusion shows that for an air saturated bulk liquid at 25 °C (having a concentration of ~ 8 mg O₂/L), the maximum oxygen flux to a biofilm across an equivalent diffusion distance (*l*) = 200 μm would be:

$$J = \frac{D}{l} \Delta C = \frac{1.81 \text{ cm}^2/\text{d}}{0.02 \text{ cm}} \left(8 \times 10^{-6} - 0 \frac{\text{g O}_2}{\text{cm}^3} \right) \left(\frac{10,000 \text{ cm}^2}{1 \text{ m}^2} \right) = 7.24 \text{ g } \frac{\text{COD}}{\text{m}^2\text{-d}}$$

where *J* is the oxygen flux (in g COD/m²-d), *D* is the diffusion coefficient of O₂ in water (in cm²/d) and Δ*C* is the concentration gradient (in g/cm³) in which maximum flux is achieved at a zero concentration at the surface of the biofilm.

Compared to the 70 g COD/m²-d discussed above (by an ARB producing 1 mA/cm²), this example shows how O₂ transport can be a common limitation, leading to a smaller flux in aerobic biofilms. In ARB biofilms, the limitation for the electron acceptor is not apparent, and higher fluxes are possible. Yet, other transport limitations are possible, including electron transport to the anode surface and proton transport out of the biofilm; these will be discussed later.

1.2 METs and transport processes occurring at the same rate

ARB biofilms in METs will encounter similar transport limitations as the ones discussed above. However, an MET has more complex transport linkages. In order to explain this, consider a simple MFC at neutral pH in which acetate is consumed at the anode and oxygen is reduced at the cathode. The half reactions and overall reaction can be written as:

While, each electrode half reaction can be studied separately, the overall reaction shows that the half reactions are coupled: they need to occur at the same rate. Moreover, the transport between anode and cathode, which is required for electroneutrality and acidity/alkalinity equilibration must also occur at the same rate as the anodic and cathodic half reactions. Thus, the critical fluxes in METs are identified below; each flux (cathodic and anodic) should be equal as shown below:

$$j/F = n_{\text{Donor}} * J_{\text{Donor}} = J_{\text{e- transport, anode}} = J_{\text{acidity, an}} = J_{\text{ionic}}/z = n_{\text{Acceptor}} * J_{\text{Acceptor}} = J_{\text{alkalinity, cat}} \quad (4)$$

where j is the current density (mA/cm^2), F is Faraday's constant ($96,485 \text{ C}/\text{mol e}^-$), n_{Donor} and n_{Acceptor} are the number of electrons per mole of electron donor (at the anode) and electron acceptor (at the cathode) respectively, J_{Donor} and J_{Acceptor} are the molar fluxes of the donor and acceptor ($\text{mmol}/\text{cm}^2\text{-s}$), $J_{\text{e- transport, anode}}$ is the electron transport flux from ARB to the anode ($\text{mmol e}^-/\text{cm}^2\text{-s}$), $J_{\text{acidity, an}}$ and $J_{\text{alkalinity, cat}}$ are the acidity and alkalinity fluxes at the anode and cathode respectively ($\text{mmol}/\text{cm}^2\text{-s}$), J_{ionic} is the ionic flux between anode and cathode ($\text{mmol}/\text{cm}^2\text{-s}$), and z is the charge of the ion transported.

The flux model shown above, derived from classic fuel cell modeling efforts (O'Hayre et al., 2009), realizes that many processes must occur simultaneously at the same rate in order to achieve a complete electrochemical reaction, as shown in Figure 1. Since they must occur simultaneously, the transport of all individual species related to such transport must also occur at the same rate. Thus, any of the above transport processes can be rate-limiting in an MET. While certain transport processes could reduce the current produced, others could cause an overpotential, i.e. an operational potential that includes some voltage losses from the theoretical potential. For example, although an ARB biofilm could produce higher current densities, the

power density produced by an MFC may not be increased because its cathode does not have a fast enough transport of O_2 to the catalyst, leading to cathodic concentration overpotential. Or, an ARB biofilm with a high concentration of electron donor might not be able to produce high current densities because the liquid medium has low conductivity that limits ionic transport, and thus ultimately the anode potential available to the ARB.

Figure 1. Fluxes encountered in METs. A single chamber electrochemical cell is used as an example. Blue arrows represent the fluxes that are equal to each other in METs, according to equation 1. J_{leak} is an additional flux that is important in MET operation, but not linked to the overall MET reaction.

In this critical review, transport processes in METs are analyzed to elucidate how they have affected the evolution of METs in this relatively young field. Associated with these transport processes are inherent gradients that affect MET performance; important gradients associated to transport will thus be discussed as well. A chronological approach is taken to discuss how the hurdles encountered in MET research are largely related to transport. Finally, a discussion is given on how researchers have found approaches to avoid transport limitations through efficient MET design and how these designs might evolve in the near future. We also use the classic case of an MFC, where the theoretical voltage that can be produced is 1.1 V, to illustrate how the different transport processes affect the current densities or the voltage efficiencies, and how new designs tackle improving performance.

2. The first hurdle – Ionic transport and Ohmic losses

The classic approach in environmental engineering to improve the performance of, and eventually scale up, any biofilm reactor has been to increase the specific surface area of the biofilm-carrier material, which thus results in higher volumetric rates of substrate consumption (Rusten et al., 1995; Nicolella et al., 2000). In the case of METs, the anode surface area is thus an important parameter that can be increased to achieve higher volumetric rates. The first approach was to make larger anodes that can allow for more ARB biofilm formation and thus higher current densities. One example of such an approach is the brush anode, which is made from carbon fibers of micron-sized diameter connected together with a central wire that acts as a current collector (Logan et al., 2007). While the brush anode has been shown repeatedly to provide high power densities with solutions containing >50 mM phosphate buffer, the performance with real wastewaters is only marginally improved (Hays et al., 2011), in comparison to how much additional area is added in contrast with planar electrodes. The reason behind this is an important transport phenomenon that affects the performance of any electrochemical cell – ion transport.

To maintain charge neutrality, cations need to move to the cathode, or anions to the anode. The flux of ions is governed by the conductivity of the electrolyte, i.e. the medium through which the ions need to travel. In the case of proton exchange membrane (PEM) fuel cells, the only electrolyte in the system is the PEM. The success of PEM fuel cells has depended greatly on the invention of Nafion, an electrolyte that provides very high conductivities (>160 mS/cm) for protons (Okada et al., 1999). In the case of METs, in addition to the membrane, the ions need travel through the liquid medium used to grow the microorganisms. Most wastewater streams have conductivities that are two orders of magnitude lower than Nafion, and thus provide a large resistance to transport of ions, which results in a voltage loss.

The voltage loss that results due to sluggish transport of ions in electrochemical cells is referred to as an Ohmic overpotential (η_{Ohmic}). Note that η_{Ohmic} also includes the overpotential due to resistance to electron transfer through the circuit, but as in most electrochemical cells, in METs too, this fraction is small as long as highly conductive materials are used as electrodes. Thus, only ionic transport is discussed here in the context of η_{Ohmic} .

η_{Ohmic} follows the Ohm's law, and thus can be represented as

$$\eta_{Ohmic} = -\frac{j_{ionic} d}{\kappa} = -\frac{J_{ionic} F d}{z \kappa} \quad (5)$$

where, j_{ionic} is the ionic current density per cross-sectional area (mA/cm^2), d is the distance of ion transport between the anode and the cathode (cm), and κ is the conductivity of the electrolyte (mS/cm). From the equation it is clear that, in addition to the conductivity of the electrolyte, an important parameter is the distance between the anode and the cathode. In the case of METs, where low conductivity solutions have to be used as electrolytes, it is thus of paramount importance that the distance between the anode and the cathode is reduced (See Figure S1). In fact, η_{Ohmic} was noted as one of the key limitations of METs by McCarty et al. (2011) in their feature on developing wastewater treatment technologies that are net energy producers.

Two problems arise when using large surface area anodes in order to increase the total current density. First, the high surface area could increase the effective distance from the cathode resulting in a large η_{Ohmic} . To take one example, if the distance between the anode and the cathode is 1 cm, but a brush anode that is 5 cm in diameter (if placed vertically in relation to the cathode) is used, the effective distance thus becomes larger. Assuming that this results in an effective distance of 3.5 cm between the anode and the cathode, and a wastewater stream that has a conductivity of 3 mS/cm is used as the electrolyte, in absence of a membrane, this leads to, at a current density of 0.5 mA/cm^2 , an η_{Ohmic} of 0.583 V, which is more than half of the theoretical voltage of an MFC!

Second, η_{Ohmic} can impede the growth of ARB in high surface area anodes, resulting in a limiting J_{ionic} . Although the anode has the same electrostatic potential, areas that are farther away from the cathode (or the reference electrode in a 3-electrode system) have a lower solution potential due to the ohmic loss. The consequence is a lower thermodynamic energy for ARB growth. Operating often at less than a 200-mV overpotential, ARB could be easily affected by a relatively small η_{Ohmic} . This is discussed and modeled by Lacroix et al. (2014), who compared anodes placed vertically or horizontally with respect to the cathode. Due to the η_{Ohmic} effect, ARB will grow only at distances closer to the cathode, leaving the rest of the anode surface unutilized. This effect is obviously most prominent in lower-conductivity electrolytes. This effect explains why increasing the anode surface area by 100x does not lead to a 100x increase in current. Instead, MFCs that are operated with high surface area anodes show maximum j_{ionic} (i.e., normalized by the membrane, cathode, or liquid cross sectional area) that are less than 3X higher than the flat-surface anode (Chen et al., 2011). Thus, MET designs cannot be significantly improved by increasing a single flux, as all fluxes discussed in Figure 1 are linked and equal to each other.

The issue of balancing anode surface area with distance between the anode and the cathode was elegantly demonstrated by Hutchinson et al. (2011) and Lanas et al. (2014). Hutchinson et al. performed experiments with brush anodes with progressively smaller areas but with the active area placed close to the cathode. Their study showed, with different electrolyte conductivities, that the power density produced even by removing 80% of the brush anode, remains very similar. Lanas et al. performed a similar experiment where they compared the power density produced in MFCs with three different sizes of brush anodes, but with the same effective distance between the anode and the cathode. As expected, the brush anodes with the larger size provided the highest power density in this case, but when the smaller brush anodes were placed closer to the cathode, this resulted in higher power densities.

In fact, some of the highest performances reported in METs so far have been achieved with designs that limit the distance between the anode and the cathode, while providing somewhat higher surface areas for the anode in comparison to flat surfaces. Fan et al. (2012), used an MFC that consisted of a carbon cloth anode and cathode separated by a non-woven fabric only, and made into a separator electrode assembly, to achieve power densities of $>4 \text{ W/m}^2$, which is amongst the highest shown in any MFCs so far. Jeremiasse et al. (2011) used a microbial electrolysis cell (MEC) with a carbon felt anode placed next to an anion exchange membrane in contact with a nickel foil cathode. This resulted in the highest cathodic hydrogen production rate at a fixed applied voltage of 1 V. In both these examples, the reduction η_{Ohmic} played an important part in achieving a high voltage efficiency and thus overall performance. In a more recent study by Ki et al. (2016), through using short distances between the anode and the cathode, Ohmic overpotential was reduced to $<0.1 \text{ V}$ even at current densities $>1 \text{ mA/cm}^2$, which would represent only a $\sim 10\%$ voltage loss in comparison to the theoretical voltage of 1.1 V in an MFC. Overall, these studies have shown that the most effective design for METs is one where the anode and the cathode are placed as close as possible, and only separated by a non-electrically-conductive separator, to alleviate any ion transport limitations in low conductivity electrolytes. This design does however bring about another transport issue, which is related to leak currents, as discussed further below.

3. The second hurdle – Crossover transport and leak currents in METs

In chemical fuel cells, part of the performance loss occurs as a result of leak current (j_{leak}), which results from parasitic losses, reactant crossover and side reactions. One example is the crossover of hydrogen in PEM fuel cells from the anode to the cathode, which results in lower

current density as a given cell voltage because of a decrease in the cathode voltage. J_{leak} in PEM fuel cells is often reported to be as high as $\sim 20 \text{ mA/cm}^2$ (Niya et al., 2014), although that due to oxygen crossover to the anode is often reported to be an order of magnitude lower (O'Hayre et al., 2009). Even so, if oxygen crossover from the cathode to the anode of a fuel cell causes a leak current of 2 mA/cm^2 , a rate that is higher than the typical current actually produced in MFCs, how is it that MFCs produce any current at all? The answer lies in diffusive transport limitations.

In a chemical fuel cell, such as the PEM fuel cell, chemical species can diffuse from one electrode to the other by diffusion in the through a thin membrane. In the case of MFCs that have liquid electrolytes, any oxygen that is unused at the cathode must now diffuse through the liquid and the diffusion layers at the interface of the liquid with the cathode and/or the anode, to have an effect on the current density produced by the ARB (since ARB can be inhibited by oxygen), or the consumption of the electron donor via oxygen respiration and thus an effect on the Coulombic efficiency – the fraction of the organics removed at the anode that are converted to electrical current. While the decrease in Coulombic efficiency in MFCs, as a result of using air-cathodes is widely documented, the problem could be further exacerbated if there were no diffusive transport limitations for oxygen.

Despite this diffusive limitation for oxygen, Coulombic efficiencies, especially in single-chamber MFCs, with anode and cathode placed closed to each other, have been reported to be very low (Liu et al., 2005; Cheng et al., 2006b; Heilmann and Logan, 2006). A classic example of an approach to overcome this issue of crossover flux (J_{leak}) is the study by Cheng et al. (2006a), who applied multiple coatings of gas diffusion layers made of PTFE and carbon powder on an air-cathode of a single-chamber MFC. The results from this study showed an increase in Coulombic efficiency at a current density of 0.1 mA/cm^2 , from $\sim 10\%$ to $\sim 35\%$, when adding up to 8 layers of the gas diffusion layer, with only moderate improvement beyond four coatings. This is only a modest improvement however because the effect here is on slowing down oxygen diffusion in the gas phase, which occurs at a faster rate than in the liquid phase.

The highest Coulombic efficiencies in MFCs are achieved when a membrane is used to separate the anode and the cathode. This adds an additional diffusional resistance to oxygen transport. Several other alternatives have also been explored to reduce oxygen crossover in MFCs. One such approach is to coat the cathodes on the side facing the anode with a polymer. Watson et al. (2011) tested various ion exchange polymers as coatings for air-cathode, and

showed that the Coulombic efficiency could be increased from ~30% with an uncoated cathode (that still has four gas diffusion layers) to ~60% with select polymer coatings.

A different approach to reduce J_{leak} and thus improve Coulombic efficiencies in MFCs could be the use high substrate loading. By using a high concentration of acetate, along with a high specific surface area of anode (by reducing the anode chamber volume), Fan et al. (2012) achieved a Coulombic efficiency of 74-98%, despite spacing the electrodes close to each other, and only using a cloth as a separator. This effect occurs as a result of limiting substrate consumption via oxygen respiration in the anolyte vs. increasing the rate of anode respiration at the anode. This bodes well for applications of MFCs in treatment of high strength wastewater where losses in Coulombic efficiency would be negligible.

Crossover of reactants or products in METs is not just limited to oxygen in MFCs. Another example of crossover related issue is associated with MECs for hydrogen production. It has been shown that hydrogen crossover from the cathode of MECs to the anode can result in an artificially high Coulombic efficiency due to recycle of hydrogen - an electron donor for ARB - and thus lower hydrogen production rates, and thus high voltage requirement. This was demonstrated by Lee and Rittmann (2010) in a single-chamber MEC where hydrogen accounted for ~62-76% of the current that was produced. An additional issue with hydrogen crossover in MECs is the formation of methane from the activity of hydrogenotrophic methanogens in the anode. This was one of the primary reasons why the pilot-scale tests performed with winery wastewater in an MEC resulted in largely methane production, as opposed to hydrogen production (Cusick et al., 2011).

The issue of J_{leak} thus presents an important challenge. While to avoid large η_{Ohmic} , MET designs should have minimal distance between the anode and the cathode, this can lead to the losses in performance discussed above as a result of transport of reactants or products from one electrode to the other. Such an issue could be alleviated by adding further diffusional barriers, such as the membranes or polymer coatings on electrodes as discussed here.

4. The third hurdle – “proton” transport leads to voltage losses and ARB inhibition

The half reactions of an MET are often presented as producing protons at the anode while consuming protons at the cathode. Under this scenario, protons *must* be transported from anode to cathode. This proton transport across the MET system, depicted in numerous

schematics in literature, is the biggest misconception regarding transport in METs. For practical purposes, proton transport is non-existent in METs.

In PEM fuel cells, with negative charges fixed onto the PEM backbone, protons are the only soluble ionic species. As a consequence, protons are the only charged species that can be transported to satisfy electroneutrality as electrons move from anode to cathode. Thus, protons are mainly transported through migration forces created by the potential gradient between the electrodes. Unfortunately, this is not the case in METs, where neutral pH medium (or wastewater) creates a very different environment in which protons are a negligible fraction of the ionic species present. In this case, many other ions can be transported through migration that are at a much higher concentration; this effective driving force for ions is “wasted” by moving ions such as Na^+ , Cl^- , and K^+ instead of protons (Rozendal et al., 2006). The consequence is a pH gradient, where the anode is acidified and the cathode is alkalified. Diffusion becomes the main driving force to alleviate this pH gradient and transport alkalinity (and/or acidity) between electrodes. The effect of these two gradients that have been extensively documented in literature are briefly discussed below.

4.1 At the cathode

A high cathodic pH leads to a shift in the reaction mechanism of typical reduction reactions. For an MFC and an MEC, the reactions thus are:

Note that protons are not consumed in this high-pH, “proton-free” environment. Instead, an OH^- is produced. In an MET containing a membrane, it is common to find that the cathode chamber pH increases to ~ 12 due to this OH^- production (Rozendal et al., 2007). This high pH is known to cause a Nernstian voltage drop of ~ 60 mV/pH unit, leading to a ~ 300 mV potential loss at the cathode. This is $\sim 25\%$ of the total theoretically available voltage in an MFC! In order to alleviate this pH gradient, many researchers have opted to remove the membrane altogether. While removing the membrane helps reduce costs and the typical anode acidification when using CEMs (Liu and Logan, 2004; Call and Logan, 2008), we have shown it does not alleviate the potential losses (Popat et al., 2012); an MFC without a membrane does not show a 300 mV increase in voltage compared to an MFC with a membrane. The reason for this lies in diffusive transport limitations.

A cathode contains a porous catalyst and a diffusive boundary layer, as depicted in Figure 2. As discussed above, migration forces are not effectively used when multiple ions are present. Thus, diffusion will dominate OH⁻ transport out of the porous catalyst. Using Fick's Law of diffusion, for an MFC that is producing 1 mA/cm² with a small diffusion boundary layer of 100 μm at the cathode, a quick estimation can be made for the OH⁻ concentration at the cathode surface, assuming a neutral pH in the bulk:

$$J = \frac{j}{zF} = \frac{D^{eff}}{\delta} C^* - C_{bulk}$$

$$C^* = \frac{j\delta}{zFD^{eff}} = \frac{1 \frac{mA}{cm^2} * 0.01 cm}{1 * 96485 \frac{C}{mol} * 5.27 \times 10^{-5} \frac{cm^2}{s}} = 0.002 \frac{mmol}{cm^3} \text{ or } pH = 11.3$$

The calculation above shows that a small, 100 μm diffusion boundary layer is enough to create the typical potential loss observed in METs, no membrane required. This is defined as a concentration overpotential, η_{OH^-} in which slow transport leads to a Nernstian potential loss. Comprehensive studies have shown that η_{OH^-} is responsible for up 40% of potential losses in METs (Jeremiasse et al., 2011; Ki et al., 2016).

Figure 2. Schematic of a single-chamber microbial fuel cell cathode. Blue arrows represent the same fluxes presented in Figure 1, now shown with respective concentration gradients of O₂, buffer speciation (HB and B⁻), and pH.

How can η_{OH^-} be avoided? There are no simple approaches yet. Agitating the bulk liquid reduces η_{OH^-} by reducing δ , but only partially (Popat et al., 2014). Having a buffer present in its acid form helps transport OH⁻ by diffusing to the catalyst layer and releasing its proton, while diffusing back to the bulk in its basic form, as shown in Figure 2 (Cord-Ruwisch et al., 2011). However, even high buffer concentrations (e.g. 100 mM phosphate or bicarbonate) are only beneficial at low current densities, as their diffusion coefficient is lower than that of OH⁻. Only, NH₄⁺ has been shown to largely avoid η_{OH^-} at current densities above 1 mA/cm² due to its higher diffusion coefficient and a lower pK_a of 9.24 (Popat et al., 2014). In this case, cathode potentials for oxygen reduction as high as 0.5 V can be achieved. This results in >50% improvement in cathode performance in comparison to unbuffered solutions. The relevant pK_a for bicarbonate and phosphate buffers to have dominant acid species, when the bulk medium starts at pH 7, are 10.3 and 12.2 respectively. But, NH₄⁺ is only available in certain wastewaters and is inhibitory to many microorganisms.

Another approach to reduce η_{OH^-} is the addition of CO₂ at the cathode. This has been tested in various settings with different approaches (Torres et al., 2008b; Fornero et al., 2010; Popat et al., 2012; Ishizaki et al., 2014; Ki et al., 2016). The CO₂ is typically produced at the

anode, being an acid that can be recycled to the cathode to achieve pH neutrality. Up to a 300 mV decrease in overpotential has been shown with CO₂ addition (Ki et al., 2016).

4.2 At the anode

Biofilms at the anode are acidified due to proton production; a proton is typically produced for every electron released, resulting in the highest proton production of any microbial process known. Yet, ARB such as *Geobacter sulfurreducens* are not resistant to acidic conditions, with growth inhibited below pH 5.8. In nature their proton production is neutralized by base released through metal oxide reduction (e.g., hematite to goethite reduction, $\text{Fe}_2\text{O}_3 + 2\text{H}_2\text{O} + 2\text{e}^- \leftrightarrow 2\text{FeOOH} + 2\text{OH}^-$), which might explain why these excessive-proton producers have not developed low-pH resistance. Unlike the cathode, where higher currents can be achieved at the expense of an η_{OH^-} , the effect of the pH gradient in the ARB biofilm results in a limitation of current density due to inhibition.

As protons are produced in the biofilm anode (Equation 1), they must be transported out of the biofilm to avoid acidification. Once again, migration forces do not play an important role in preventing this acidification, accounting for less than 15% of total acid transport in the biofilm (Marcus et al., 2010). Diffusion is thus the main transport mechanism. But, with such a small proton gradient ($\Delta\text{H}^+ < 10 \mu\text{M}$), proton transport is, again, insignificant. In this case, due to the small pH range of ARB, buffers are essential to produce any significant amount of current density. A buffer in its basic form (B^-) will serve as a proton carrier out of the biofilm, as depicted in Figure 3. This buffer transport is defined as the acidity flux ($J_{\text{acidity, an}}$) out of the biofilm in Figures 1 and 3. Note that while basic buffer is needed at the anode (Figure 3), acidic buffer is needed at the cathode (HB, Figure 2) to alleviate the increase in pH. The ideal situation is purposely shown in both cases, in which either HB (for the cathode) or B^- (for the anode) are at high concentration.

Figure 3. Schematic of an MET anode. Blue arrows represent the same fluxes presented in Figure 1, now shown with respective concentration gradients of electron donor, buffer speciation, pH, and anode potential (E_{an}).

Early studies showed the strong dependency that buffer has on the current density produced by ARB, an almost linear correlation due to the linear relationship between flux and concentration in Fick's Law of diffusion (Torres et al., 2008a). pH gradients along the biofilm have been documented in *G. sulfurreducens* biofilms, showing pH values near the anode drop down to the growth limit of this microorganism (Franks et al., 2009; Babauta et al., 2012). Thus, it is expected that *G. sulfurreducens* will be completely inhibited at this pH, limiting the current that can be produced.

On flat electrodes, current densities as high as 1.5 mA/cm² have been observed for *Geobacter* when 100 mM bicarbonate buffer is used (Marcus et al., 2011). When 30 mM bicarbonate is used, a common medium concentration, the maximum current obtained is ~ 0.8 mA/cm² and this is a value commonly observed in literature (Nevin et al., 2008; Marsili et al., 2008b; Yi et al., 2009) and examples discussed below. To this date, no experiment using *Geobacter* on flat planar electrodes have shown higher currents than those reported at high bicarbonate concentrations, indicating that all biofilms studied so far are to some degree

inhibited by pH. This pH inhibition affects the way study other processes in ARB biofilms, such as electron transport (see next section for details).

Rate limitations due to pH gradients also seem to occur in biocathodes. Babauta et al. (2013) shows a significant increase in pH (up to 8.8) in an O₂ reducing biocathode, suggesting an inhibitory condition that could limit biofilm growth. Unlike electrochemical cathodes, biocathodes cannot increase their pH significantly to achieve higher fluxes and current densities. Thus, biocathodes seem to rely on a buffer to transport OH⁻ out of its biofilm. However, no specific studies have confirmed this dependency, to the authors' knowledge.

Recently, our group performed experiments using thermophilic ARB *Thermincola ferriacetica* (Parameswaran et al., 2013). This microorganism has the advantage of faster diffusion coefficients at thermophilic temperatures (~60 °C), which should result in ~190% faster $J_{\text{acidity, an}}$, in comparison to mesophilic temperatures (~30 °C). The results reflect that; *T. ferriacetica* produces 0.8 mA/cm² with only 10 mM bicarbonate buffer (vs. 30 mM for *G. sulfurreducens*). Most interestingly, *T. ferriacetica* reaches a buffer-independent region, suggesting that the pH gradient has been minimized and a different limitation impedes higher current densities (Lusk et al., personal communication). It is possible that this limitation is related to electron transport, but the limited knowledge of the electron-transport pathways in this gram-positive microorganism does not allow us to elaborate further this hypothesis. Nevertheless, the use of thermophilic conditions can be a possible solution to acidity transport limitations.

It is not surprising that MET performance has been directly correlated to buffer concentrations in most studies. High current densities are achieved only with media compositions that have high buffer concentrations. High buffer concentrations have three positive effects on METs: 1) a lower η_{Ohmic} due to an increase in ionic conductivity, 2) a lower $\eta_{\text{OH-}}$ due to enhanced transport at the cathode, and 3) a higher anodic current density due to an alleviated pH inhibition at the biofilm anode. These benefits are clearly observed in many studies. The study by Cheng and Logan (2011) exemplifies the benefit of buffers. Note that the benefit of adding a buffer in this study occurs mostly at the cathode, as $\eta_{\text{OH-}}$ is alleviated. However, lower buffer concentrations, like those present in domestic wastewater (ww) lead to anodic limitations due to biofilm acidification. It is thus, not surprising that many studies using diluted wastewater are not able to achieve high current densities.

Two design approaches are worth noting that decrease the biofilm acidification, increasing current densities. First is to increase the anode surface area in order to decrease the actual surface current density while still achieving high volumetric current densities. This approach can lead to higher current densities per cross-sectional area of the MET (Logan et al., 2007; Chen et al., 2011; Chen et al., 2012; Flexer et al., 2013). However, the high surface area often comes at the expense of a 3D anode structure that increases η_{Ohmic} . η_{Ohmic} can become a main limitation, especially in diluted wastewaters that suffer from low alkalinity limitations, as discussed earlier.

A second approach is to use membrane/electrode assemblies (MEAs) where a porous anode is in close contact with the membrane or separator. MEAs can achieve high current densities due to the close distance between anode and cathode, where protons and OH⁻ are produced and neutralize each other. This approach has led to lab-scale studies with the highest current densities achieved (Fan et al., 2012; Jeremiasse et al., 2011). However, this short distance is known to affect performance in MFCs due to oxygen crossover, as discussed in the section on leak current. In order to prevent crossover from stopping any current production altogether, high concentrations of substrate are often needed (Fan et al., 2012). The use of a membrane can aid in decreasing crossover in this design.

4.3 Electron transport limitations

ARB transport electrons to the anode through several approaches. Earlier studies demonstrated, for example, how *Shewanella oneidensis* can use soluble electron shuttles to produce electrical current (Marsili et al., 2008a). The transport of electrons using this approach can be rate-limiting, as evidenced by lower current densities obtained, while higher shuttle concentrations led to higher current densities (Torres et al., 2010).

In contrast, other ARB are able to use a direct electron transport through a solid extracellular matrix. Through this method, ARB such as *G. sulfurreducens* are able to produce high current densities, indicative of an effective electron transport (Marsili et al., 2010). The specific nature of this solid matrix electron transport is still debated in the microbial electrochemistry field (Malvankar et al., 2011; Strycharz-Glaven et al., 2011; Malvankar et al., 2012b; Strycharz-Glaven and Tender, 2012a), and is not the topic at hand. Instead, this section focuses on specific experimental work that would demonstrate or suggest that electron transport from ARB cells to the anode ($J_{e^- \text{ transport, anode}}$ in Equation 1) can be the rate-limiting step in anode respiration.

Many research efforts have documented a potential gradient in *G. sulfurreducens* biofilms. This potential gradient is mostly evidenced by the shift in the redox state of cytochromes along the biofilm (Liu et al., 2011; Liu and Bond, 2012; Robuschi et al., 2013; Viridis et al., 2014). Similarly, reported conductivities of *G. sulfurreducens* biofilms would suggest a serious electron-transport limitation due to Ohmic resistances along the biofilm (Malvankar et al., 2011; Malvankar et al., 2012a). However, there are limited efforts that have attempted to improve the total current density from this ARB through improving electron-transport components. The main reason for this is the lack of knowledge of what the electron-transport machinery exactly is. Thus, the main efforts so far are based on directed evolution of *G. sulfurreducens*. Mutants KN-400 and CL-1, evolved to improve electron transport, produced 0.7 mA/cm² and 0.25 mA/cm², respectively (Yi et al., 2009; Leang et al., 2013), over their wild-type controls producing ~0.16 mA/cm². Yet, these apparently high current densities still fall below the current density indicative of a buffer transport limitation (0.8 mA/cm²) at the bicarbonate concentration used. Thus, these mutants might be better suited for anode respiration, but still produce current densities that are known to be within a proton/buffer transport limitation range.

Pocaznoi et al. (2012) presented an interesting experiment that highlights important aspects of transport in anode respiration. In their work, thin wires from 1,000 μm down to 25 μm diameter were used as anodes for a mixed ARB enrichment. As expected, the thicker wires of 500-1000 μm produced current densities of $\sim 0.7 \text{ mA/cm}^2$, which are similar to the limitations of *G. sulfurreducens*' buffer transport in flat electrodes. However, as the electrode diameter becomes smaller, there is a shift in the transport lengths between electrons and protons (buffer) since the biofilm thickness becomes similar or larger than the anode thickness (approximated by SEM images). In the thinnest anode, electrons must be transported to a 25 μm diameter anode surface, while the exchange of proton carriers occurs at the biofilm/water interface having a diameter of $\sim 200 \mu\text{m}$. As a consequence, buffer transport is "enhanced" when compared against the small anode surface area. The consequence is an increase in current density: the 25 mm wire anode biofilm was able to produce 6.6 mA/cm^2 . This is the highest current density ever reported by an ARB on a non-porous electrode!

Pocaznoi et al.'s experiments provide an important lesson: ARB can achieve a $J_{e^- \text{ transport, anode}}$ that lead to much higher current densities than what is typically reported. This suggests that typical current densities of up to 1 mA/cm^2 should not be electron-transport limited. However, if Pocaznoi et al.'s current densities are normalized by the reported anode + biofilm surface areas (i.e., the outer biofilm area), the place at which buffer transport occurs, the current densities are very similar ($0.59 \pm 0.1 \text{ mA/cm}^2$) and consistent with a buffer transport limitation. This analysis confirms that transport at the biofilm/liquid interface is indeed the rate-limiting step, and the current densities with this 30 mM bicarbonate buffer medium are consistent with an acidity transport ($J_{\text{acidity, an}}$) limitation.

Despite the fact that there is limited data that shows a correlation between electron transport components and significant changes in current density, there are many studies that document potential gradients and resistances within ARB biofilms. This data is enhanced by several mathematical models that explain how potential gradients can lead to a rate-limiting electron transport (Strycharz-Glaven et al., 2011; Strycharz-Glaven and Tender, 2012b; Snider et al., 2012; Bonanni et al., 2013). It is difficult to alleviate such potential gradients in order to obtain higher current densities and prove that electron transport is actually rate-limiting under relevant conditions. Moreover, there is a possibility that electron and acidity transport are coupled, as a shift in pH causes a shift in the redox response of *G. sulfurreducens*. If a proton is transported along an electron to the anode surface, the pH gradient and consequent Nernstian shift could explain the potential gradients observed through cytochrome redox states. Thus, this possibility of a proton-coupled electron transport should be investigated further in the near future.

5. Feasible METs = efficient transport, a perspective for future designs

Ultimately, the knowledge gained on transport processes in METs should translate into more efficient designs. The research progress has definitely shown important changes to MET architecture as more details about transport limitations are elucidated. Recent designs move away from the idea of larger, bulky anodes that increase η_{Ohmic} and focus on thinner electrodes that still maintain a 3D structure for biofilm growth (Jeremiasse et al., 2011; Fan et al., 2012). Designs have also taken into consideration acidity and alkalinity gradients by either removing the ionic membrane or placing anode and cathode close to each other, therefore minimizing gradients that should cancel each other. Moreover, specific applications studied or envisioned for METs have shifted from diluted municipal wastewater to high strength and high alkalinity wastewaters that can alleviate anodic gradients that can be rate limiting (Zhang et al., 2009; Kaewkannetra et al., 2011; Sevda et al., 2013).

While many of the transport processes discussed are common among different METs, their design is specific towards the application. For example, MECs that produce hydrogen at the cathode often suffer from hydrogen recycle or methanogenesis if they are designed as a single chamber unit (Lee and Rittmann, 2010; Cusick et al., 2011). However, the lack of O_2 at the cathode implies that j_{leak} , while present as a H_2 leak flux, do not impact negatively the ARB biofilm. Thus, recent MEC designs have ion exchange membranes and very short distances between anode and cathode (Jeremiasse et al., 2011; Ki et al., 2016). This approach has also

been tried in MFCs (Fan et al., 2012), but is effective only if the electron donor is at high concentrations in order to consume the oxygen transported from the cathode.

In the future, designs that optimize all fluxes depicted in Figure 1 will result in the best performance. This is only possible if, similar to chemical fuel cells, METs are modular, having many electrochemical cells with short distances between anode and cathode. The specific application can also help take advantage of the MET output, with high strength streams that have a significant buffering capacity aiding in the performance, and at the same time have a non-limiting J_{donor} . These might include waste streams such as food and animal wastes, but could also be solid electron donor, such as cellulosic biomass, in which the liquid stream can be catered towards the MET application.

Throughout the short history of MET development, transport processes have been directly or indirectly a central research theme. From fundamental studies of biofilm conductivities in ARB biofilms to applied studies on MET design, transport is the answer to many of the uncertainties encountered. While specific transport processes are studied, the linkage between all fluxes and their associated gradients is seldom considered in these studies. This review aims to elucidate the importance of this linkage, and the fact many chemical species in METs must be transported *as fast as an electron*.

6. Conclusions

Despite significant laboratory research on METs in recent years, there have been very few successful larger scale trials. This stems from a low performance in large-scale systems that is almost always linked to transport limitations, including ionic transport and acidity/alkalinity transport from the electrodes. These transport processes need to occur at the same rate that microorganisms generate or consume electrical current in METs, and this fundamental understanding about the need for matching transport rates of all species should be considered when designing and operating METs.

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