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Editors' Choice—Review—Exploration of Computational Approaches for Understanding Microbial Electrochemical Systems: Opportunities and Future Directions

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Microbial electrochemical systems offer valuable opportunities in the field of electrochemistry for a wide range of applications and fundamental insights. Applications include renewable power generation, electrosynthesis, and sensing, and provide a critical platform for understanding fundamental electrochemical processes between biotic and abiotic components. However, despite several research efforts, the fundamental electron transfer mechanisms inherent to microbial bioelectrochemical systems remain poorly understood, limiting their full potential and applications. This lack of fundamental understanding stems from both the conceptual and experimental complexity of microbial electrochemical systems. In this context, the possibility of multi-disciplinary research utilizing computational methods provides a powerful tool for this field. Herein, we critically review how computational studies and methods employed to study microbial electrochemical systems in multiple dimensions can be used to clarify the different factors governing microbial electrochemical systems. This discussion addresses how the combination of various techniques can enhance fundamental understanding, providing scientists with tools for the rational design of improved systems and opening exciting new research opportunities.

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The need for renewable energy sources to replace environmentally taxing energy production, as well as for more sustainable synthetic and biosensing approaches, is creating a major focus on microbial electrochemistry. Pairing microorganisms with an electrode has been studied extensively in the last decade due to the benefits of long-term stability and the ability to generate electricity from renewable biomass in a carbon-neutral process.[1,2] Microbial electrochemistry has become a key foundation for a wide range of technologies with applications in energy generation,[3] electrosynthesis,[4–8] and biosensing.[9–16] These technologies rely on microorganisms that are capable of transferring electrons to and/or from an electrode and are limited by, among other factors, the poor fundamental understanding of the (extracellular) electron transfer (EET) process.[17] In fact, electrons obtained from microbial metabolism of reduced substrates (i.e., glucose, acetate, malate, etc.) must be transferred outside the cellular membrane, which constitutes an electrical insulator for the transfer of electrons to the electrode.[18] Understanding this process is extremely complex due to the various components active in intracellular electron transfer steps, as well as the sophisticated metabolisms to accomplish electron transport to the electrode. Furthermore, the variety of microorganisms capable of this feat and the diversity among their EET processes make this task even more complicated. Past inherent and natural paths of extracellular electron transfer, engineering approaches to establish electrochemical communication between bacterial cells and electrodes have also been studied.[2,18–24]

The use of computational and mathematical modeling has been shown to advance the field of microbial electrochemistry by tackling the complexity of these systems through transport modeling, advanced molecular modeling and correlations, and genomic scale computational methods.[25–28] A considerable number of modeling frameworks have been focused on the microbial fuel cell (MFC) technology due to the need for optimization and parameterization of the large number of variables driving MFC systems. MFC modeling techniques have been greatly explored and recently reviewed.[26] Several parameters included in these models have the ability to shed light on the fundamental electron transfer of these microbial systems, extending far beyond their applicability in microbial fuel cells to all microbial electrochemical systems. Furthermore, there has been an emergence of using bioinformatic techniques to study microbial electrochemical systems as well. Bioinformatic resources such as metagenomic profiling and transcriptomic studies have been on the rise due to the decrease in cost and increase in resources available to perform computationally expensive analysis.[29] Additionally, advanced computational techniques that have been applied in theoretical and computational chemistry have proven effective in bioelectrochemical studies for obtaining detailed chemical and structural information.[30]

The success of discovering mechanisms of electron transport in electroactivity will likely arise from a combination of all types of modeling supporting and affirming experimental microbial electrochemical studies. Therefore, this work aims to critically review and discuss the applicability of three types of modeling to develop a framework for studying electron transport in microbial systems, using electrochemistry paired with computational or mathematical modeling to enhance and deepen the understanding of these systems.

This review is organized by groups of computational methods, discussing the scientific advancements that lead to the current modeling frameworks and future directions for the field of microbial electrochemistry. The main computational methods discussed are summarized in Fig. 1 and are as follows: i) general mathematical modeling utilizing fundamental electrochemical and biological concepts and equations to predict aspects of electroactive biofilms (critical to microbial electrochemical systems); ii) bioinformatic analysis using both genetic and transcriptomic characterization of single and multi-species bacterial communities for investigation of genetic signatures and transcript expression profiles presented in microbial electrochemical systems; and iii) quantum mechanical methods, which have been applied recently to microbial electrochemical systems.

Understanding future computational research directions in microbial electrochemistry requires a brief discussion and summary of known information in microbial electron transfer. Due to the complexity of the studied system, readers are referred to a recent comprehensive review and terminology classification of microbial electrochemical systems.[31] Briefly, when considering electron transport in a microbial electrochemical system, there are three main

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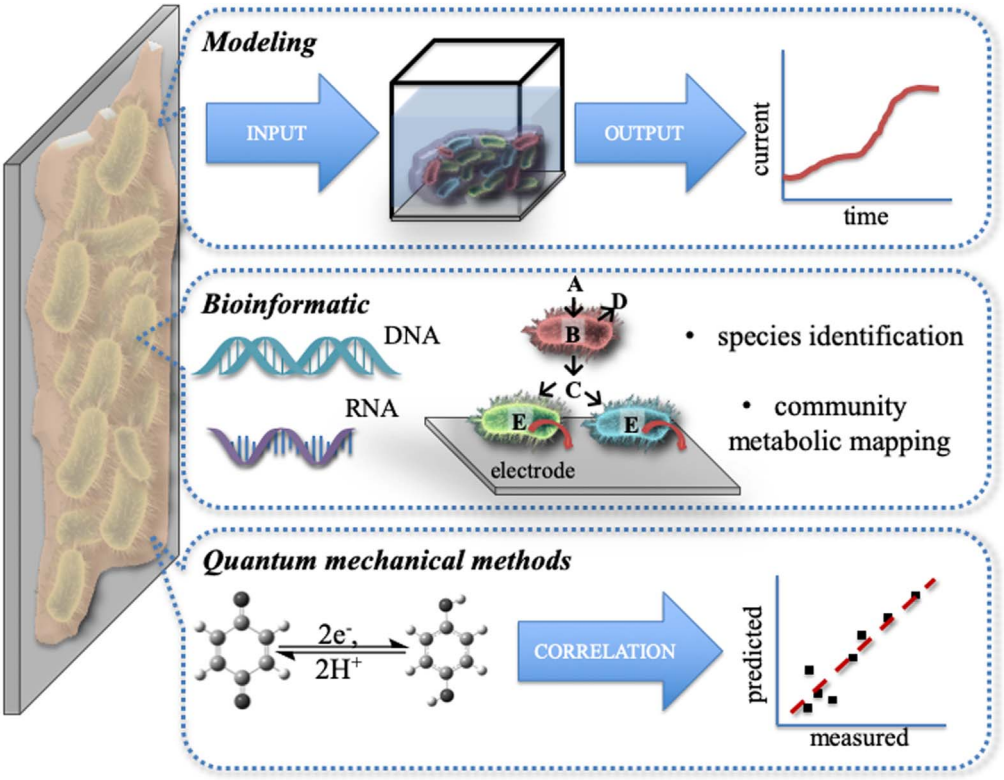

Figure 1. Modeling, bioinformatic, and quantum mechanical approaches for studying microbial electrochemical systems.

conditions considered: direct electron transfer (DET), mediated electron transfer (MET), and interspecies electron transfer (IET), which are summarized in Fig. 2.

The most well-studied microorganisms for microbial electron transfer are members of the *Geobacter* and *Shewanella* species, specifically strains *Geobacter sulfurreducens* and *Shewanella oneidensis* MR-1.[32] Direct electron transfer is seen in both species involving multi-heme c-type cytochromes on the outer membrane capable of electron transfer to electrodes. Furthermore, *Geobacter* species have been reported to accomplish long-distance electron transfer to the electrode surface by means of conductive pili appendages, filaments extending from the outer membrane of the bacterial cell showing metal-like conductivity.[33–35] *Shewanella* species are also observed to be capable of mediated electron transfer through secretion of flavins that act as an electron shuttle between bacterial cells and the electrode surface.[36] Although these electron transfer mechanisms have been characterized, there have been many reports of electroactive bacteria, termed electrogens (or electricogens), well-summarized and taxonomically classified in a recent review.[37] The challenge of developing a universal understanding of microbial electron transfer is particularly difficult due to the variety of mechanisms present with drastic differences even among strains in the same species.[38] With the vast number of studies and research groups aiming to solve these problems, computational and modeling approaches provide additional insights, supplementing experimental techniques. Turning to the methods discussed in this critical review offers a great opportunity to advance the knowledge of microbial electrochemical systems and further their applicability for future real-world applications.

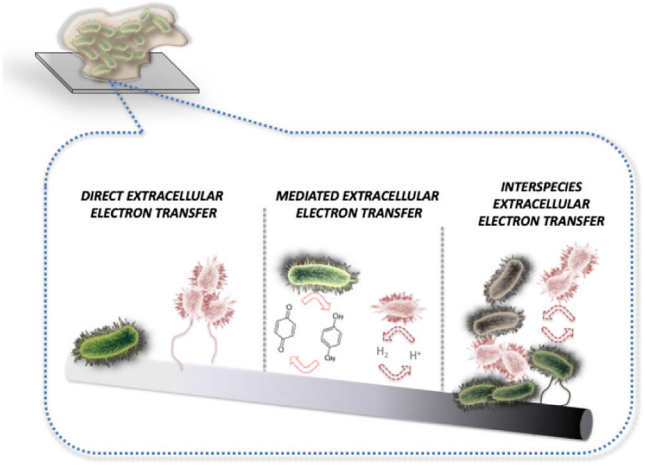

Figure 2. Extracellular electron transfer mechanisms utilized by electroactive bacteria.

Modeling Electroactive Biofilms

A biofilm is a consortium of (different) microorganisms where the bacterial cells assemble together while surrounding themselves with extracellular polymeric substances that protect them from the external environment.[39] Besides being involved in the biogeochemical cycle of several elements, biofilm formation is at the basis of the development of electroactive bioanodes. These are biotic/abiotic interfaces capable of establishing an effective extracellular electron transfer process between living bacterial cells and the electrode surface,[34,40] as well as having improved tolerance to environmental stress parameters (i.e., temperature and salinity, presence of inhibiting/toxic compounds, etc.).[41] As a result, understanding electroactive biofilm development on the electrode surface, the redox reactions taking place, and the influence of biofilm composition over suspended cells on bioelectrocatalysis is of utmost importance for the rational design of bioelectrochemical systems. This is a complicated task, as biological, (electro)chemical, and physical factors all play a role in electro-active biofilm development and behavior (i.e., current generation over time), thus requiring a multidisciplinary approach to achieve a deep understanding of the process. This includes unveiling the role of several physico-chemical

parameters (i.e. substrate concentration, polarization potential, mass transport, electrode material, roughness) and biological aspects such as cell metabolism and growth conditions. Excellent localized studies employing microelectrodes and in situ imaging techniques aimed to clarify micro/macro characteristic bio-chemical processes (i.e., pH profiles, substrate concentrations, etc.) in biofilms have been reported.[15,42–45] The coupling of computational models and different experimental pieces of evidence have provided an outstanding contribution to further improve the understanding and provide the theoretical basis of biofilm electrochemistry. Identification of the key components and parameters representing these systems is critical to formulate electro-active biofilm models, and to later develop mathematical relationships describing the processes affecting them. Below, we critically discuss the journey that has led from the first attempt to model bioelectrochemical systems, considering both biological and chemical processes, to the most recent reports. It is worth mentioning that not only electroactive bioanodes can be the limiting factor in bioelectrochemical systems (such as the MFC, or microbial electrolysis cells), but also the reactions taking place at the cathode electrode, reactor architecture, electrolyte conductivity, etc. We refer the readers interested in modeling of entire bioelectrochemical systems to studies dedicated to these aspects, which are important for reactor design and scale-up of these technologies.[46–49] This section is focused on the modeling of electroactive biofilms for bioanode development.

From modeling electroactive suspended cells to biofilms.—In a 1995 pioneering study, Zhang and Halme reported a model where biochemical and electrochemical processes were combined to describe the electrochemical performance of a microbial fuel cell with suspended cells based on substrate concentration, reducing intermediates (i.e., NADH), exogenous redox mediators (i.e., 2-hydroxy-1,4-naphthoquinone), and polarization of the electrode.[50] The biological processes were modeled through Monod-type equations and several assumptions were defined, including assuming that all mass transport processes are fast enough to consider the concentration of all reactants in the bulk solution equal to those in the bacterial cells or on the electrode surface. The Monod equation is widely utilized to describe specific growth rate as a function of substrate concentration:

$$\mu = \mu_{\max}\left(\frac{S}{K_s + S}\right) \quad [1]$$

where μ is the specific growth rate, $\mu_{\max}$ is the maximum specific growth rate, S is the substrate concentration, and K_s is the affinity constant in the substrate uptake kinetics (i.e., substrate concentration at half $\mu_{\max}$). This equation has been modified by Lawrence and McCarty to describe the effects of substrate concentration on the rate at which a microbial concentration removes the target substrate:

$$\frac{dS}{dt} = -\frac{q_{\max} SX}{K_s + S} \quad [2]$$

where X is the microbial concentration, and $q_{\max}$ is the maximum substrate removal rate.[51] The Nernst equation was utilized to describe the potential of the MFC depending on the concentration of reduced and oxidized mediator (2-hydroxy-1,4-naphthoquinone in their study):

$$E = E^{*} + \left(\frac{RT}{2F}\right)\ln\left(\frac{[HNQH_2]}{[HNQ]}\right) \quad [3]$$

where E^{*} was assumed as 0.7 V based on the MFC system investigated by the authors. R is the ideal gas constant ($R = 8.314$ $CVK^{-1}mol^{-1}$), F is the Faraday constant (96485 $Cmol^{-1}$), and T is the absolute temperature. *[HNQ]* and *[HNQH_2]* are the concentrations of 2-hydroxy-1,4-naphthoquinone in the oxidized and reduced form (mol L^{-1}).

While the model of Zhang and Halme allowed a relatively good description of bioelectrochemical systems with suspended cells, it was not until 2007 that the first model of a biofilm-based bioelectrochemical system was reported by Picioreanu and co-authors.[52] Specifically, the authors reported a model for biofilms of bacteria species in the presence of soluble redox mediators, undergoing the following reaction at the electrode surface (Eq. 4):

$$M_{red} \leftrightarrow M_{ox} + 2H^{+} + 2e^{-} \quad [4]$$

with M_{red} and M_{ox} indicating the reduced and oxidized forms of the mediator, respectively. The Butler-Volmer equation was utilized to represent the current density obtained for the electrochemical oxidation of the redox mediator.

$$i = FAk^{0}[C_O(0,t)e^{-\alpha f(E-E^{o'})} - C_R(0,t)e^{(1-\alpha)f(E-E^{o'})}] \quad [5]$$

Where i is the current density, F is the Faraday constant, A is the area of the electrode, k^0 is the standard rate constant, $C_O(0,t)$ and $C_R(0,t)$ are the concentrations of the oxidized and reduced species at the electrode surface at time t, α is the transfer coefficient, $f = F/RT$ with R being the ideal gas constant and T the temperature, $E–E^{o'}$ is the applied overpotential from the standard potential of the redox couple utilized to mediate the electron transfer.

They introduced a model for the biofilm/bulk liquid where the Gibbs free energy needed to build biomass (biofilm growth) is calculated from the anaerobic catabolism of acetate, following the approach of microbial growth bioenergetics presented by Heijnen.[53] The elemental formula utilized for biomass was:

$$CH_{1.8}O_{0.5}N_{0.2} \quad [6]$$

Furthermore, mass balances for solutes and biomass components are described for biofilms that continuously develop over time adapting biofilm model equations previously reported.[54] The compartments and the sub-domains of the computational model are reported in Fig. 3.

It is worth underlining that multidimensional models (2d and 3d) were reported,[52] which are of particular importance for the case of biofilms composed of mixed bacterial consortia, since the different species compete for the colonization of the electrode surface. The modeling framework of Picioreanu et al. allowed for the correlation of bioelectrochemical performance to biofilm thickness, cell distribution in the biofilm, presence of different bacterial species, and substrate concentration. This provided a good description of the current produced from electro-active biofilms in the presence of soluble redox mediators. When the model was utilized for electroactive biofilms performing extracellular electron transfer in the absence of redox mediators (i.e., the case of *Geobacter* biofilms performing electron transfer by direct contact between bacteria and electrode surface) fitting of experimental results was not completely possible using this model.

Bioelectrochemical studies revealed that the biofilm matrix of *Geobacter sulfurreducens* is conductive several micrometers deep, thanks to both conductive pili, named "nanowires," and c-type cytochromes.[45,55–57] Accordingly, with the aim to develop a mathematical model for this type of biofilm matrix, Marcus et al. reported a dynamic, one-dimensional model for the biofilm-anode that consisted of both active and inactive biomass.[58] The model insinuates that the electrons obtained from the oxidation of the substrate (electron donor) are electrically conducted from the bacteria to the anode passing through the biofilm matrix having a conductivity k_{bio} (mS cm^{-1}). The transfer of electrons from the bacteria to the conductive biofilm matrix is assumed to be fast and reversible. Accordingly, the ET at the interface is described as a Nernstian system, and a Nernst-Monod equation is utilized to describe the rate of substrate oxidation. The Nernst-Monod model is a modification of the Monod model, where the electrode is considered as the final electron acceptor. The electron conduction through the conductive biofilm matrix to the anode is described

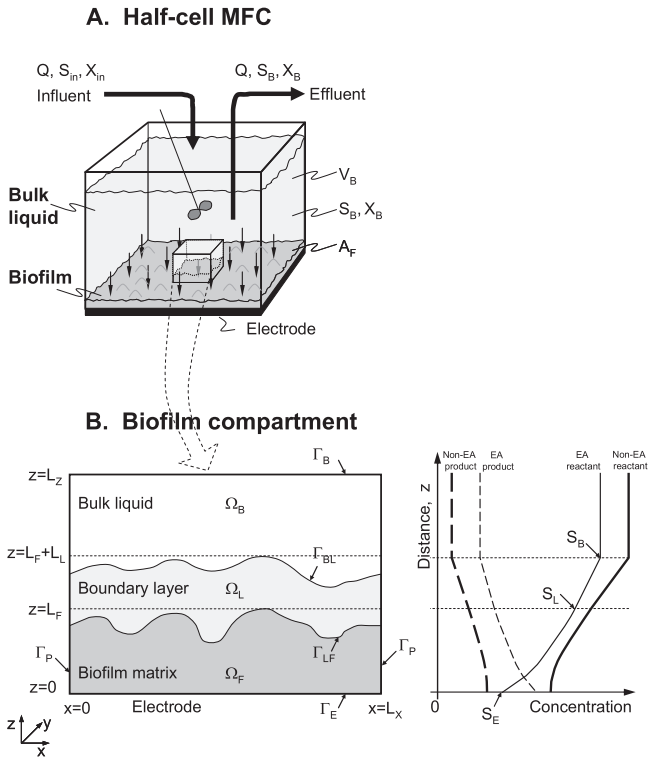

Figure 3. (a) Large scale model including ideally mixed bulk liquid and biofilm developed on the electrode surface. Q = coulombic charge, S = soluble components, X = biomass, V_B = bulk liquid volume, A_F = anode surface area. (b) Zoomed in view of the small scale biofilm model containing three subdomains: i. a zone Ω_B connected to the bulk liquid where complete mixing is obtained; ii. a mass transfer boundary layer Ω_L, and iii. Ω_F representing the biofilm matrix. Averaged solute concentration profiles for (non-)electroactive reactant and (non-)electroactive product are represented along the x direction as a function of distance z from the electrode surface. Reproduced with permission from C. Picioreanu, I.M. Head, K.P. Katuri, M.C.M. van Loosdrecht, K. Scott. A computational model for biofilm-based microbial fuel cells. Water Res. 2007, 41, 2921–2940.[52] Copyright 2007 Elsevier.

based on an electron balance, for which the steady-state is:

$$0 = \frac{\delta j}{\delta z} + \frac{F\gamma_1}{\tau} fe^0 X_{f,a} q + \frac{F\gamma_2}{\tau} X_{f,a} r_{res} \quad [7]$$

where j is the current density (mA cm^{-2}), z is the position in the z-axis perpendicular to the anode surface where the biofilm grows, F is the Faraday constant, γ_1 is the electron equivalence of the utilized electron donor (mmol-e$^-$ mmol-Electron Donor^{-1}), γ_2 is the electron equivalence of active biomass (mmol-e$^-$ mg-VolatileSolids^{-1}), τ is a time conversion (86,400 s day^{-1}), fe° is the fraction of electrons obtained from the electron donor that is used for energy generation to support synthesis, $X_{f,a}$ is the density of active biomass (mg-VolatileSolidscm^{-3}), q is the specific rate of electron donor utilization (mmol-electron donor mg-VolatileSolids^{-1} day^{-1}), and r_{res} is the specific rate of endogenous respiration (day^{-1}). Accordingly, the first term on the right-hand side describes the change in current density in the conductive biofilm matrix. The second term indicates the electrons generated from the oxidation of the electron donor, while the third term indicates the self-oxidation of biomass (endogenous respiration).

The matrix is treated as a porous solid conductor according to Ohm's law:

$$0 = k_{bio} \frac{\delta \eta}{\delta z} + j \quad [8]$$

where η is the local potential (V). Accordingly, a gradient in the local potential is required to drive the current density based on Eq. 8.

The authors showed that biofilm conductivity influences the fluxes of electron donor and current. Moreover, the model revealed that the type of limitation in the biofilms is the electrical potential at low k_{bio} (10^{-5} mScm^{-1}), while at high k_{bio} (10^{-3} mScm^{-1}) is the electron donor mass transfer resistance that limits biofilm performance. Interestingly, the model shows that as k_{bio} increases, the active biomass away from the electrode surface also increases.

When developing this model, the authors utilized different assumptions, including a negligible pH change in the biofilm matrix, the movement of ions in the biofilm with no ohmic loss, and that the conductive biofilm matrix is the only electron acceptor for the generated electrons.

Proton transfer in electroactive biofilms models.—It is important to note that the electrons obtained at the anode from the oxidation of the substrate are transferred to the electrode, with the concomitant release of protons into the biofilm/solution, as indicated by the following reaction for acetate oxidation half-reaction:

$$CH_3COO^- + 4H_2O \rightarrow 2H_2CO_3 + 7H^+ + 8e^- \quad [9]$$

Accordingly, in the anodic biofilm, the oxidation half-reaction becomes the "overall reaction" and transport of protons through the biofilm from the anode to the cathode, where they are consumed for the oxygen reduction reaction, plays a critical role in the bioelectrochemical performance of electroactive biofilms. Later studies focused on the development of computational models addressing this key property.

First, Picioreanu et al. expanded their model reported in 2007 by utilizing Nernst-Planck fluxes of ions to calculate pH together with an ionic charge balance for biofilms where redox mediator (thionine in their study) diffuses freely.[59] Thus, ions move in the potential gradient obtained by imposing electroneutrality in the biofilm domain. Moreover, mass transport by convection and liquid flow over biofilm and electrode surface was considered, with a macroscale perspective of the bulk liquid anolyte and a microscale consideration of the biofilm on the electrode. Figure 4 shows a schematic representation of the model domains.

The model revealed that for the case of a single-species electroactive biofilm growing on a planar electrode, the rate of proton transfer to the cathode through the proton exchange membrane does not generally influence current production and substrate consumption as long as the solution has good buffering capability (i.e., 100 mM bicarbonate buffer). A buffered solution prevents drastic pH changes in the biofilm by combining with H^+ based on the following equation (for a carbonate buffered system):

$$HCO_3^- + H^+ \leftrightarrow H_2CO_3 \quad [10]$$

with a pH maintained near the acid dissociation constant pK_a (i.e., approximately 6.3 for HCO_3^-).

On the contrary, proton transfer becomes significant with poor buffering, resulting in low current and incomplete substrate consumption (i.e., $k_{m,H} = 10^{-6}$ ms^{-1}, and 2 mM bicarbonate buffer). The spatial distribution of solution pH for the latter case is shown in Fig. 5. The generated protons are transferred outside the biofilm by diffusion (driven by concentration gradients) and electromigration (driven by the potential gradient formed as a result of the electroneutrality condition imposed to the system). Diffusion and electromigration fluxes were comparable in the studied biofilm system ($N_{H+,D} = 4.10^{-9}$ mol m^{-2} s^{-1} and $N_{H+,M} = 3.10^{-9}$ mol m^{-2} s^{-1} respectively), and negligible in the bulk liquid where convection dominates ($N_{H+,C} = 5.10^{-7}$ mol m^{-2} s^{-1}).

In addition to the presented case of a single species biofilm developed on a planar electrode, the model was also applied to the study of biofilm growing on a porous electrode having a mixed microbial community (MMC). It was possible to determine the competition for substrate and anode colonization by electroactive and fermentative bacteria, and the resulting effects on current

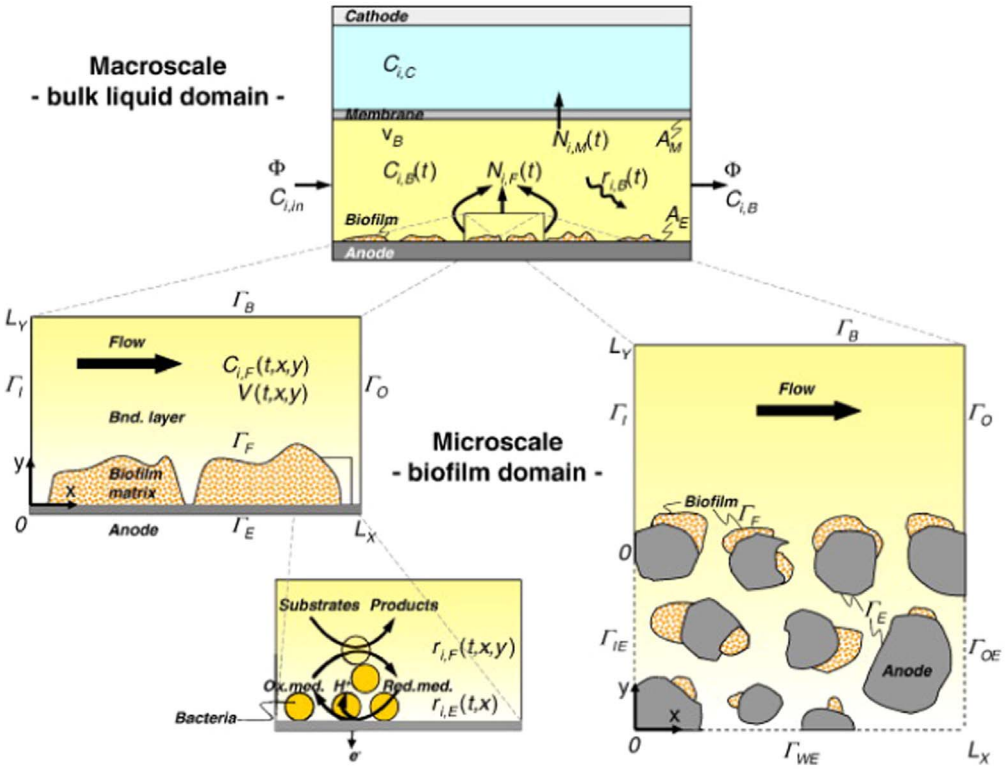

Figure 4. Top scheme shows the bulk liquid domain with V_B being the anolyte volume, A_E the area of the electrode, $C_{i,B}$ is the concentration of each soluble component. The fluxes exchanged with the exterior (Φ), with the biofilm ($N_{i,F}$), with the cathode via the membrane ($N_{i,M}$), and the reaction rates in the bulk liquid ($r_{i,B}$) are considered. The three bottom schemes show the biofilm domain on a planar (left) or a porous electrode (right). Two dimensional distribution of time dependent concentrations $C_{i,F}$, potential V and liquid velocity u are calculated. Boundaries conditions are applied on the biofilm domain boundaries (inlet Γ_I, outlet Γ_O, electrode Γ_E, bulk Γ_B, and biofilm surface Γ_F). The bottom central scheme shows the smallest scale with a set of biological and acid-base volume-based reactions ($r_{i,F}$) coupled to a set of electrochemical surface-based reactions ($r_{i,E}$). Reproduced with permission from C. Picioreanu, M.C.M. van Loosdrecht, T.P. Curtis, K. Scott. Model based evaluation of the effect of pH and electrode geometry on microbial fuel cell performance. Bioelectrochem. 2010, 78, 8–24.[59] Copyright 2010 Elsevier.

density. Accordingly, the reported model provided extremely interesting insights about the different roles of mass transport limitation and pH inhibition for planar or porous electrodes, and the possible approaches to decrease their influence on the performance of the electroactive biofilm.

Other outstanding works focusing on the role of pH in electroactive biofilms were conducted by Marcus et al. where an advanced modeling platform was developed which they named Proton Condition in BIOFILM (PCBIOFILM).[60] The work expanded a previous model developed to couple slow microbial reactions in biofilms to the fast aqueous acid/base and complexation reactions

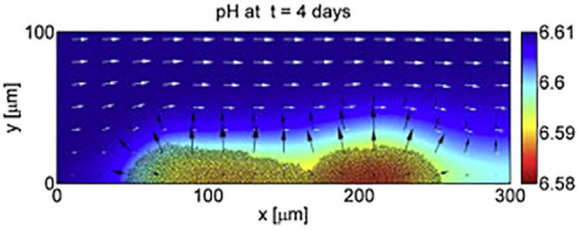

Figure 5. Surface plot of pH for a system with $k_{m,H} = 10^{-6}$ m s^{-1}, and 2 mM bicarbonate buffer. Arrow plots are shown of H^+ diffusion (black) and convection (white, 100 times scaled down) flux vectors. Black dots indicate bacterial cells attached to the electrode surface. Adapted with permission from C. Picioreanu, M. C. M. van Loosdrecht, T. P. Curtis, K. Scott. Model based evaluation of the effect of pH and electrode geometry on microbial fuel cell performance. Bioelectrochem. 2010, 78, 8–24.[59] Copyright 2010 Elsevier.

first developed by VanBriesen and Rittmann.[61] Compared to the model of Picioreanu et al. where electroneutrality condition is utilized, proton condition was used as the mass balance of H^+ allowing for the integration of complexation and other microbiologically driven reactions. Accordingly, in the model of Marcus et al. diffusion is considered as the transport mechanism, and a diffusion operator employing Fick's law is utilized to individually assign diffusivity to H^+ and its conjugates (i.e., CH_3COOH, and HCO_3^-) having large differences in diffusivity. An inhibition function derived by Park et al. for ammonium and nitrite-oxidizing bacteria[62] was adopted in this system. The model allowed correction for the higher current density obtained in carbonate buffered microbial fuel cells over phosphate-buffered systems, thanks to the higher pH inside the biofilm. This resulted from higher diffusion coefficients in water for the carbonate system, leading to a more rapid transport of the weak acid (i.e. H_2CO_3) outside the biofilm and the conjugate base inside the biofilm. The authors further expanded the PCBIOFILM model in a following study to solve the Nernst-Plank equation and included the impact of migration (i.e. considering also electroneutrality), on the electroactive anodic biofilm.[63] The net flux of an ion i was given by:

$$j_{ion,i} = j_{diff,i} + j_{mig,i} = -D_i \frac{ds_i}{dx} + z_i s_i D_i E^* \qquad [11]$$

where $j_{ion,i}$, $j_{diff,i}$, and $j_{mig,i}$ are the net, diffusion, and migration flux of the ion i, respectively. D_i, s_i, and z_i are the diffusion coefficient, the concentration, and the charge of the ion i, respectively. x is the spatial coordinate and E^* a modified electric field, which is proportional to the electric field E:

$$E^{*}=\frac{F}{RT}E \quad [12]$$

with R as the ideal gas constant ($R = 8.314\ \mathrm{CVK^{-1}mol^{-1}}$), F as the Faraday constant, and T as the absolute temperature. This improved PCBIOFILM model eliminated the assumption that ionic current does not limit the electroactive anodic biofilm.

An important aspect to be pointed out when comparing the previously discussed models developed by Picioreanu et al. and Marcus et al. for studying proton transfer in electroactive biofilms is that the different electron transfer processes considered affected the resulting pH gradient. Specifically, in the biofilm studied by Picioreanu et al., the electron transfer in the biofilm is based on the electron shuttling between diffusible redox mediators, resulting in maximum current densities below 1 $\mathrm{Am^{-2}}$, and a limited pH gradient obtained in the biofilm.[59] Conversely, Marcus et al. focused on biofilms where electron transfer is obtained by electron conduction within the biofilm matrix (i.e.; the entire biofilm matrix is composed of conductive materials, such as redox proteins or nanowires, and the electron conduction is driven by a gradient of electrical potential), with current densities up to one order of magnitude higher, and a more significant pH drop taking place in the biofilm (more than 1 pH unit).[60,63] This emphasizes the importance of carefully defining the modeled phenomena as they can significantly affect the obtained results.

Exploring extracellular electron transfer mechanisms through modeling.—Within the effort toward understanding how biofilm metabolism, composition, and mechanism of extracellular electron transfer within the biofilms and with the electrode surfaces influence the performance of an electroactive anodic biofilm, Korth et al. developed a modeling platform for *Geobacter* biofilms.[64] Specifically, the model included the intracellular electron transfer from reduction equivalent NADH (Eq. 14) (obtained from acetate oxidation, Eq. 13) to c-type outer surface cytochromes (i.e., located in the outer membrane and exposed to the external environment) of *Geobacter*, which then transfer electrons to the conductive biofilm matrix, where a metal-like conductivity (microbial nanowires)[65] is assumed (Eq. 15).

$$Ac^{-} + 4H_2O + 4NAD^{+} \rightarrow 4NADH + 2HCO_3^{-} + 5H^{+} \qquad r_{bio} \quad [13]$$

$$NADH + H^{+} + 2R \underset{k_{r,m}}{\overset{k_{f,m}}{\rightleftharpoons}} NAD^{+} + 2RH \qquad r_m \quad [14]$$

$$RH \underset{k_{r,e}}{\overset{k_{f,e}}{\rightleftharpoons}} R + e^{-} + H^{+} \qquad r_e \quad [15]$$

where Ac^{-} indicates acetate, R and RH are the oxidized and reduced forms of the c-type cytochromes, respectively, and the following r_{bio}, r_m, and r_e, indicate the reaction rates for Eqs. 13–15, respectively.

The Butler-Volmer equation was utilized for modeling EET to the conductive matrix (r_e), and the nanowires of the conductive matrix pass electrons among bacterial cells and ultimately to the electrode surface, making Ohm's law an accurate representation of the electrical conduction. It follows that the electron transfer in the biofilm matrix is driven by an electric field, and no gradients of the ratio of oxidized and reduced cytochromes arise. The model showed that only a particularly slow heterogeneous electron transfer rate at the electrode surface could lead to the accumulation of reduced cytochromes at this surface, evolving a redox gradient. This points out that other bottlenecks limit the current production in *Geobacter* based biofilms. Regarding the conductivity of the biofilm, only semiconductor-like values significantly affected the current response. Thicker biofilms allow a higher current density but shift the formal potential to more positive values due to the buildup of ohmic resistance in the biofilm. Furthermore, the model indicated that cytochrome concentration is the most sensitive parameter affecting electrochemical performance during biofilm growth. Interestingly, the modeled concentrations showed a significant difference between biofilms studied at constant potential and by performing cyclic voltammetry, with a 100 fold decrease for voltammetric conditions. This difference suggested the possibility of a significant amount of cytochromes acting solely as an electron pool while not being immediately involved in the EET process. It is important to note, however, that the model described by Korth et al. was constructed using only one species of c-type cytochromes undergoing a 1 electron/1 proton transfer, and more than 100 different types of cytochromes have been reported for *Geobacterceae*.[66] Some of these cytochromes possess more than one electron binding heme groups, and could then present different electron transfer mechanisms. The ability to utilize bioinformatics tools to identify and study the involvement of these cytochromes in the EET is further discussed in the bioinformatics section.

Hamelers et al. developed a Butler-Volmer-Monod model where the biochemical conversion is described by enzyme kinetics and the irreversible biofilm-electrode electron transfer is described by the Butler-Volmer electron transfer kinetics.[67] This model was later utilized by Rousseau et al. to describe how salinity affects electron transfer kinetics in high-salinity-tolerant bioanodes.[68] Other models that dealt with the specific case of simulating voltammetric studies to identify rate-determining steps in biofilm metabolism and EET process were reported by Strycharz et al.,[69] Peng et al. which considered also intracellular electron transfer mechanisms and could describe substrate inhibition,[70] and Rousseau et al.[71] The latter study specifically focused on designing a theoretical model for analyzing transient cyclic voltammetry responses (in comparison to stationary cyclic voltammetry where the potential scan rate is sufficiently low ($\sim\leqslant 1\ \mathrm{mV\ s^{-1}}$) to ensure that metabolic reactions, mass transfer, and electron transfer steps balance each other) of electroactive biofilms both under non-turnover and turnover (catalytic) conditions. In electroanalysis, when plotting the current peaks (J_{peak}) obtained in the cyclic voltammetry vs the scan rate (ν), it is known that diffusion-limited processes give a J_{peak} vs ν^{α} relationship with α equal to 0.5. Conversely, for a monolayer of adsorbed redox species α is equal to 1. However, experimental studies on electroactive biofilms reported various intermediate values for α. The model developed by Rousseau et al. assumed the electron transfer rate of the electrode to be fast enough to ensure Nernstian equilibrium, and provides a theoretical support for explaining the large variety of values obtained for α, with the advantage of also considering metabolic reactions for CVs performed in catalytic conditions.

Rimboud et al. performed a very interesting study utilizing bioanodes formed from fermented sludges having multiple redox systems present.[72] Employing a multi-system Nernst-Michaelis-Menten model allowed determining the contribution of each redox mediator to the final current density, resulting in a better fit of the experimental voltammetric results. The study underlines the importance of determining the nature of the redox systems present in the biofilm developing on the electrode.

It has been shown that a transient current is obtained when, after an initial polarization, a bioanode of *Geobacter* cells is left at the open circuit potential for a certain period of time before polarizing the electrode again.[73] Bonanni et al. developed a model specifically dedicated to determining rate constants for the kinetics of the steps involved upon applying the new polarization.[74] Specifically, the current produced by the discharge of electrons accumulated in the cytochromes present in the interior of the cell, at the biofilm matrix, and the cytochromes at the biofilm–electrode interface could be modeled and distinguished from the current resulting from metabolic activity. The model provides interesting insights on the amount of cytochromes that are present in the biofilm matrix compared to the

intracellular ones, their different role in terms of charge storage, and allows determining rate determining steps upon a new electrode polarization.

Focusing on the possibility of mixed EET mechanisms, Renslow et al. designed a model to study biofilms where the microbial community can simultaneously utilize both diffusion and conduction for long-range EET.[75] This is of particular interest for *Shewanella* biofilms, where both mechanisms play a role,[76,77] to investigate their combination and interaction, including understanding if one mechanism dominates under specific conditions. The model utilized the Nernst-Monod equation and considers both isolated and interacting dual EET mechanisms, meaning that for an isolated dual EET biofilm can utilize both mechanisms but they are isolated from each other, while in interacting dual EET the conductive biofilm matrix can accept/donate electrons from/to soluble mediators. The model provides interesting insights into the contribution of both mechanisms, showing that in biofilms with low conductivity, dual EET provides a metabolic advantage over biofilms showing only one EET pathway. Furthermore, the interaction of both EET mechanisms enhances biofilm activity. However, simulation of CVs and square-wave voltammetry through the developed model did not allow for the deconvolution of the percent contribution of each EET, calling for the development of more sophisticated models.

Hydrodynamic effects on electroactive biofilms.—When considering the models previously discussed, it is important to consider that the hydrodynamic effects on biofilm formation and structure, as well as the detailed influence of electrode roughness and geometry on the early formation of the electroactive biofilms, have not been directly included. Electrodes with higher roughness or improved micro structuring can facilitate cell adhesion to the surface, while potentially introducing mass transport limitations. Focusing on these topics, Champigneux et al. studied the influence of surface topography, experimentally controlled at the nano/micro scale, on the early phase of electroactive biofilm formation and developed a model to theoretically analyze the results.[78] Flat gold electrodes with nano-roughness from 0.8 to 4.5 nm, as well as electrodes with the same roughness (4.5 nm) but having micro-pillars (500 μm in height) with different spacing, were utilized for the development of *Geobacter* biofilms under constant polarization. The indicated values for the electrode roughness refer to the arithmetic mean roughness value, which is defined as the average of the absolute deviation of the roughness irregularities from a mean line.[79] Epifluorescence pictures of the biofilm developing on electrodes with a 125 μm spacing for the pillars are reported in Fig. 6.

The model allowed for the calculation of the concentration of acetate and HCO_3^-, as well as pH profiles inside the biofilm that developed on the different electrodes, where mass transport was dominated by diffusion. The model revealed that acetate always reached the bottom of the biofilms, even for the electrodes with the smallest spacing between arrays, indicating that acetate diffusion was not a significant source of limitation. A more significant diffusion limitation was revealed for HCO_3^- for 100 μm-spaced pillars compared to the 200 μm-spaced structure, resulting in a significant pH gradient. The insights obtained from the model, including the acidification taking place along the micro-pillars, provided a theoretical explanation for the lower current density obtained for biofilms formed on micro-pillar supports compared to flat electrodes having the same roughness. In a later study, the authors further expanded their model to include the effects of porous surfaces on the early-formation of electroactive biofilms.[80] Accordingly, careful modeling of electroactive biofilms on rough and porous electrode surfaces provides the possibility to rationally develop electrodes for maximum cell colonization while minimizing mass transport limitations.

A summary of the equations discussed in this section are given with their relevant microbial phenomena in Table I.

Bioinformatics for Microbial Electrochemical Systems

Bioinformatic tools enable analysis of genomic traits (genomics) in deoxy-ribose nucleic acid (DNA), and ribose nucleic acid (RNA) transcription rates (transcriptomics) in microbial electrochemical systems, providing useful information about microbes from these nucleic acids. Genomic and transcriptomic studies reveal genetic identity (species identification), predicted gene coding sequences, and gene and transcript expression quantification that can advance electron transfer mechanism knowledge in bacterial colonies. DNA analysis may reveal which microorganisms are populating an electroactive biofilm and RNA analysis elucidates differential gene and transcript expression in varying conditions. Additionally, with the rapid advancement of both the genetic sequencing and bioinformatics fields in the past decade, the methodologies to study microbial electrochemical systems have greatly expanded.

16 s rRNA gene sequencing.—Bioinformatic analysis was first shown to be useful for the microbial electrochemical community through 16 s ribosomal RNA (rRNA) gene sequencing for the identification of species present in electroactive microbial systems.[5] 16 s rRNA gene sequencing takes advantage of the highly conserved 16 s rRNA gene introns (coding for the 16s rRNA subunit of the ribosome) among bacterial species allowing for universal primer design and amplifies the entire gene region. The gene for the 16s rRNA subunit also includes variable exon regions, which vary among species and are not encoded to the final 16s rRNA product, useful for species identification. Any characterization of a new electroactive species is commonly first analyzed through 16s rRNA gene sequencing and identifies such microorganisms.[81,82] Identification of electroactive species has occurred across bacteria and archaea resulting in diverse classification among many species.[37] Indexing the species phylogenetics and characteristics electroactive traits such as mediated or direct electron transfer, and cathodic or anodic respiring capabilities can be seen in Fig. 7 revealing the deep genetic diversity in microbial electrochemical systems.[37]

16s rRNA gene sequencing is a powerful tool for microbial electrochemical system analysis due to its ability to analyze large mixed microbial cultures at once for multi-species identification. An example of this point was seen by 16s rRNA gene sequencing analysis to determine the microbial composition dependence in a waste-water fed-batch MFC system under fuel cell operation (MFC), a set-potential (SP) under oxidizing conditions, and open circuit potential conditions (OCP) as shown in Fig. 8.[83] The results indicate that even though the waste-water eluent primary clarifier (PC) contained no significant populations of *Deltaproteobacteria*, the MFC, SP, and OC operation conditions resulted in the anodic biofilm colonization of *Deltaproteobacteria* phylum. Further, the identities of *Deltaproteobacteria* varied based on applied electrochemical conditions with a higher abundance of *Geobacterceae* family species under MFC and SP operation.[83]

16s rRNA gene sequencing has excelled the understanding of not only diverse microbial systems capable of EET, but also changes in the composition of microbial communities induced by common environmental factors. Several 16s rRNA gene studies were able to identify changes in the members of microbial communities dependent on salt, potential, and inoculum sources.[84–87] Additionally, through 16s rRNA gene sequencing, the finding of a strong presence of members of the *Geobacterceae* family in many experimental conditions[84] further promoted the intense study of this microorganism.[66]

For investigating electron transfer mechanisms, 16s rRNA gene sequencing falls short, since it only provides information about the speciation. However, it allowed for the early identification of key microbial players important for extracellular electron transfer, which became the model organisms for the microbial electrochemistry field. 16s rRNA gene sequencing analysis can also be limited and bias to genomes with a lower GC content in their chromosomes due to the

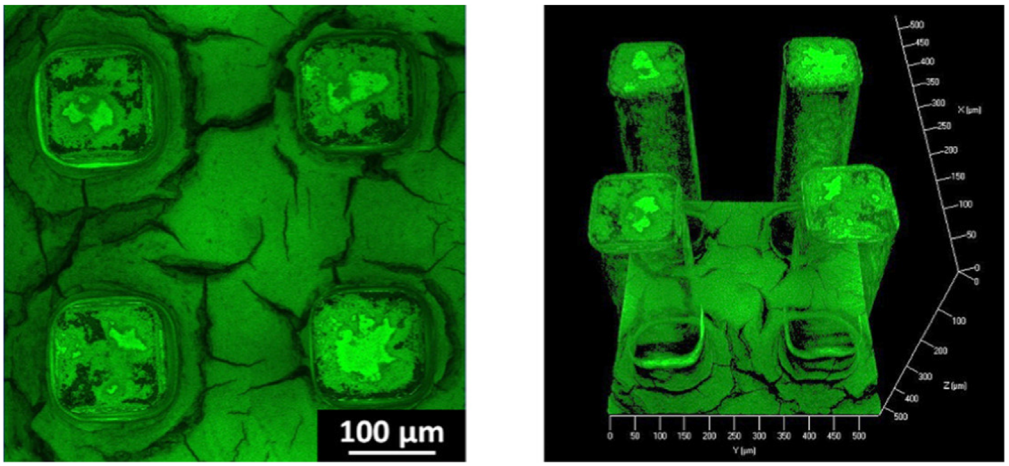

Figure 6. Epifluorescence top view and 3-D reconstruction of micro-pillar pattered gold electrodes (125 μm spacing for the pillars) showing biofilm coverage ratios above 90%. Adapted with permission from P. Champigneux, C. Renault-Sentenac, D. Bourrier, C. Rossi, M.-L. Delia, A. Bergel. Effect of surface nano/micro-structuring on the early formation of microbial anodes with *Geobacter sulfurreducens*: Experimental and theoretical approaches. Bioelectrochemistry. 2018, 121, 191–200.[78] Copyright 2018 Elsevier.

difficulty of annealing the PCR primers required for amplification of the 16s rRNA gene region in GC rich microorganisms.[38] These limitations indicate that 16s rRNA gene sequencing is useful for early quantification of electroactive species, but other genomic techniques are needed for complimenting its analysis.

Whole genome sequencing.—Whole genome analysis goes beyond 16s rRNA gene taxonomic identification with the characterization of the entire genome of a microorganism. Genome sequencing has become widely available recently due to the great advances in next-generation sequencing making sequencing bacterial genomes fast, affordable, and powerful for the field of microbial electrochemistry.[88] Additionally to genetic information, genome sequences can be fed into bioinformatics programs to annotate the genome. Functional genome annotation assigns functions to sequences in the genome including protein-coding sequences, RNA coding sequences, transcriptional start sites, and genome portions of unknown function. Genome annotation predicts the protein-coding sequences by identifying a start codon in the gene sequence and then computationally translating each 3 nucleotide codon thereafter to its resulting amino acid residue. The sequence of amino acid residues can then be compared to existing protein sequence databases for assignment of the probable function. Using this resource, it is possible to predict from the genome sequence if a microbe could suggest electroactivity due to the presence of certain genes, such as outer membrane cytochromes. Commonly gene sequencing studies for electroactive microbial communities are done with metagenomics enabling genome sequencing of many strains at once, as discussed below. However, pure strain genomics has been completed for model electroactive species, as well as for other pure culture studies of electroactive microorganisms. Members of the *Geobacterceae* family, *G. sulfurreducens* and *G. metallireducens* were early studies of this nature and revealed genetic footprints of electroactivity. *G. sulfurreducens* genome showed 111 coding sequences matching c-type cytochromes highlighting the extraordinary electroactive capability of this microorganism.[66] Further analyzing gene sequences allows for identification of common motifs, such as a heme-binding motif, to further differentiate coding sequences such as c-type cytochromes. For example, of the 111 c-type cytochrome coding sequences, 73 contain two or more heme-binding motifs, and one coding sequence contains 27 heme-binding motifs.[66] Genetic analysis of *G. metallireducens* revealed 91 c-type cytochrome coding sequences with 65 contain homologous sequences to c-type cytochromes of *G. sulfurreducens*.[89] Similarly, the genome of another electroactive model microorganism *Shewanella oneidensis* MR-1 has been sequenced, revealing the presence of 39 coding sequences for c-type cytochromes, 32 of which are novel to *Shewanella oneidensis* MR-1.[90] Additional to hints to electron transfer capabilities by presence of c-type cytochromes, genome sequencing can also show methods of carbon metabolism and pollutant metabolisms in *Geobacter* species and *Shewanella oneidensis* MR-1, respectively.[66,89,90] Apart from the genome sequences of model electroactive species, other electroactive species genomes have been sequenced for similar analysis such as the species *Proteus*, shown to be important in MFC power generation.[91] Moving from pure culture studies, recent advances in next generation sequencing and bioinformatic analysis has led to the field of meta-omics. Meta-omics focuses on genetic and transcriptomic of a mixed microbial community, useful for analyzing electroactive communities without the requirement of isolating individual strains.

Metagenomics allows studying of an entire microbial ecosystem and characterization of their genome sequence at the same time. Since the genome sequencing is done through next-generation techniques, the genome reads can be quantified and give quantitative information about the microbial communities in the electroactive community.[92] This allows for not only identification of bacterial species in EABs but also the ability to show microbial ratios important for EABs and which microorganisms dominate such communities. Previously metagenomic studies have been used, and recently reviewed,[25] to gain knowledge on which organisms are responsible for current production in MFC applications, as well as metatranscriptomic data, has been analyzed to determine differential gene expression with and without applied potentials. Microbial diversity has recently been found to be potential-dependent among δ-Proteobacterial populations, composed of *Geobacterceae* family, most common under more oxidative (positive) potential conditions, agreeing with the previously discussed 16s rRNA gene sequencing study results.[38]

It must be noted that genomic studies have one large drawback since they only report on the information present in the genome, which may not be representative of the microbial behavior. Transcriptomic analysis complements genomic analysis well, since the presence of expressed messenger RNA (mRNA) transcripts confirms that the genes are not only present, but are actively being utilized and expressed by the microorganisms.

Transcriptomic analysis.—By using techniques for the quantification of mRNA transcripts, gene expression analysis can show the relevant genes for EET or the genes effected by EET stimuli. For transcriptional analysis, the RNA is isolated from the microbial culture (pure or mixed) and used in techniques such as the quantitative polymerase chain reaction (qPCR), microarray, and

Table I. Important phenomena in microbial electrochemical systems and their relevant equations used in computational and mathematical modeling studies.

Phenomena	Equations	References
Substrate consumption kinetics	1) Monod-type [a)] 2) Michaelis-Menten 3) Double Michaelis-Menten 4) Ping-pong mechanism	1) Zhang and Halme 1995[50] Picioreanu et al. 2007[52] Marcus et al. 2007[58] Marcus et al. 2011[60] Marcus et al. 2010[63] Picioreanu et al. 2010[59] Hamelers et al. 2011[67] Renslow et al. 2013[75] 2) Strycharz et al. 2011[69] Bonanni et al. 2012[74] 3) Korth et al. 2015[64] 4) Peng et al. 2013[70]
H^+ transport and pH in biofilm	1) Nernst-Plank fluxes of ions 2) Fick's Law of diffusion 3) Fick's Law + Nernst-Plank 4) Transport numbers + diffusion	1) Picioreanu et al. 2010[59] 2) Marcus et al. 2011[60] 3) Marcus 2010[63] 4) Champigneux et al. 2018[78] Champigneux et al. 2019[80]
Electron transfer	1) Fick's Law (for diffusible redox mediators) 2) Ohm's Law (for nanowires and conductive biofilm matrix) 3) Fick's Law + Ohm's Law (for biofilms with both a conductive matrix and diffusible redox mediators)	1) Picioreanu *et al.* 2007[52] Picioreanu et al. 2010[59] Strycharz et al. 2011[69] Rousseau et al. 2014[71] 2) Marcus et al. 2007[58] Korth et al. 2015[64] 3) Renslow et al. 2013[75]
Extracellular electron transfer from cytochromes to conductive matrix	Butler-Volmer	Korth et al. 2015[64]
Electrode kinetics	1) Nernst 2) Nernst-Monod 3) Butler-Volmer 4) Butler-Volmer-Monod 5) Nernst-ping-pong 6) Multi-system Nernst-Michaelis-Menten (for modeling cyclic voltammetries of biofilms with multiple redox systems)	1) Zhang and Halme 1995[50] Rousseau et al. 2014[71] 2) Marcus et al. 2007[58] Marcus et al. 2011[60] Marcus et al. 2010[63] Champigneux et al. 2019[80] 3) Picioreanu et al. 2007[52] Picioreanu et al. 2010[59] Strycharz et al. 2011[69] Bonanni et al. 2012[74] Renslow et al. 2013[75] Korth et al. 2015[64] 4) Hamelers et al. 2011[67] 5) Peng et al. 2013[70] 6) Rimboud et al. 2015[72]

a) Monod-type equations include: Monod, Nernst-Monod, and Butler-Volmer-Monod where the apparent Monod constant is dependent on anode potential.

RNA-sequencing to quantify gene expression for genes of interest. Methods of qPCR and microarrays require the marker gene of interest's sequence for experimental design and expression analysis. Recently RNA-sequencing has been emerging in this field due to the advances in next-generation sequencing technologies allowing for low cost, wealth of information obtained, and no requirement of prior knowledge of gene sequences of interest. Since RNA-sequencing is a complete analysis of all RNA in a system, RNA-sequencing also allows for the discovery of novel RNAs and their expression values that may have not been identified in previous studies.

For the model organisms *G. sulfurreducens*, gene expression analysis was used to show a different expression profile for respiratory pathways based on electrode potential.[93–95] Furthermore, gene expression can also be analyzed across different species. Comparing *G. sulfurreducens* and *G. soli* under different solubility of electron acceptors revealed differential expression of c-type cytochrome genes *omcE* and *omcN*. Specifically, even though both organisms have these genes, they are transcriptionally expressed at different quantities in the same conditions.[96] In addition to aiding in determining transcriptomic changes due to an applied potential in microorganisms, RNA-sequencing has also been recently used to elucidate changes in electrocatalytic performance between bacterial cells. In a study of the purple bacterium *Rhodobacter capsulatus*, it was observed that photo-bioelectrocatalysis increased after cells underwent saline adaptation. RNA-sequencing analysis between the saline adapted and non-adapted bacterial cells revealed changes in the gene expression in the photosynthetic electron transport chain and nitrogen metabolism of the bacteria, explaining the difference in the observed current response.[97] RNA-sequencing also excels at detecting non-mRNAs expression levels. Small RNAs (sRNAs) have been a subject of recent research as

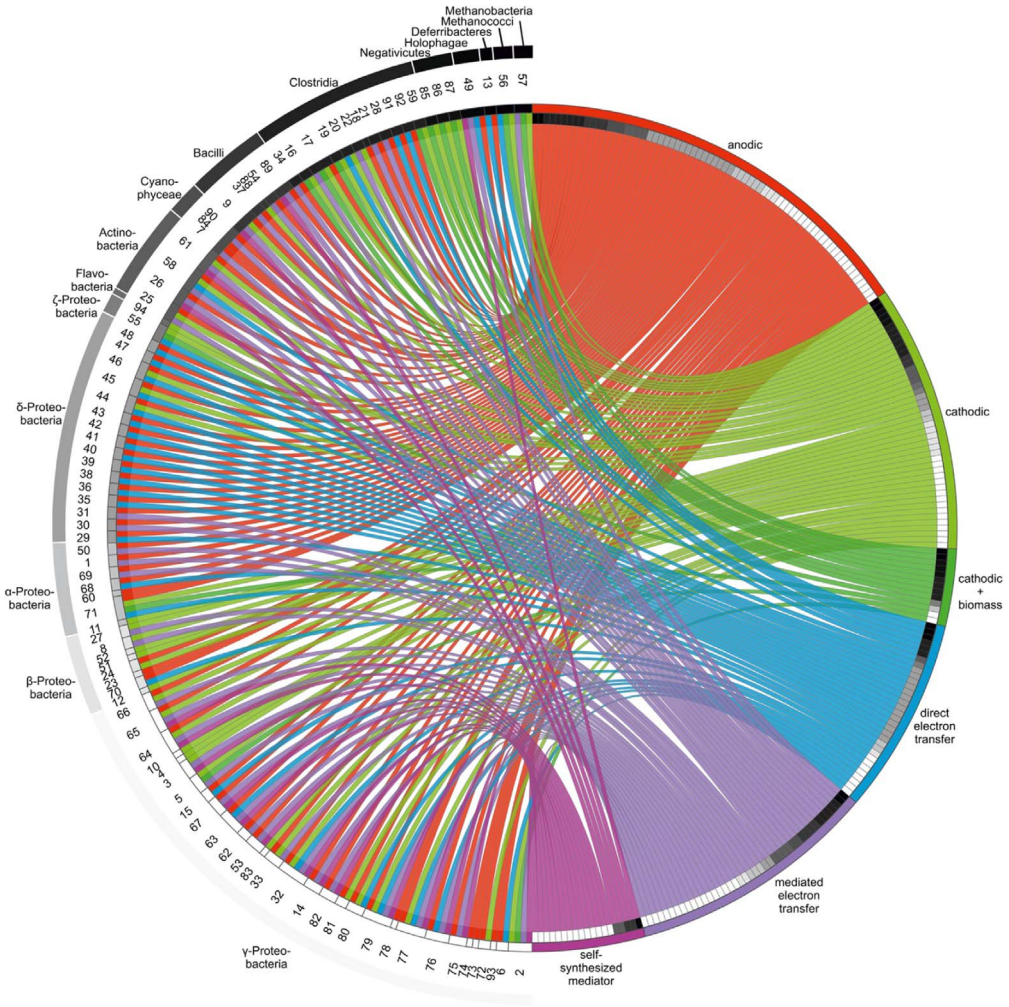

Figure 7. Electron transfer characteristics among electroactive species. Numbers represent references that the electroactive species were first identified from the following review paper. Reproduced with permission from C. Koch, F. Harnisch. Is there a Specific Ecological Niche for Electroactive Microorganisms? ChemElectroChem. 2016, 3, 1282–1295.[37] Copyright (2016) Wiley.

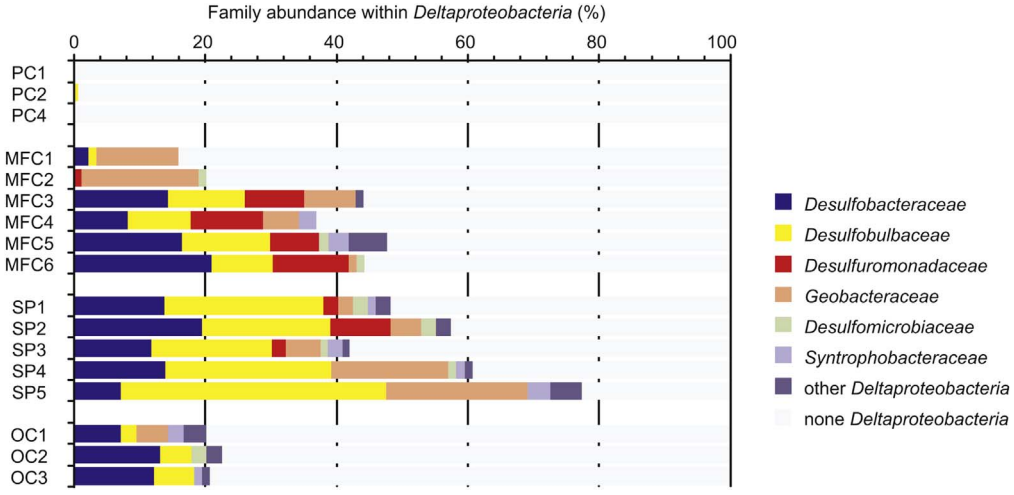

Figure 8. Analysis of genetic composition of microbial communities in phylum class *Deltaproteobacteria* in a waste-water fed MFC. Microbial communities were analyzed from a MFC under 3 conditions: MFC operation (MFC), set-potential (SP), and open-circuit potential (OCP). The MFC anodic biofilm was also exposed to a primary clarifier (PC) during different stages of operation. The numbers represent time-point analysis for each condition with low numbers[1–4] meaning early analysis and large numbers[5,6] representing analysis after 400 days operation. Adapted with permission from S. Ishii, S. Suzuki, T.M. Norden-Krichmar, A. Wu, Y. Yamanaka, K.H. Nealson, O. Bretschger. Identifying the microbial communities and operational conditions for optimized wastewater treatment in microbial fuel cells. Water Res. 2013, 47, 7120–7130.[83] Copyright (2013) Elsevier.

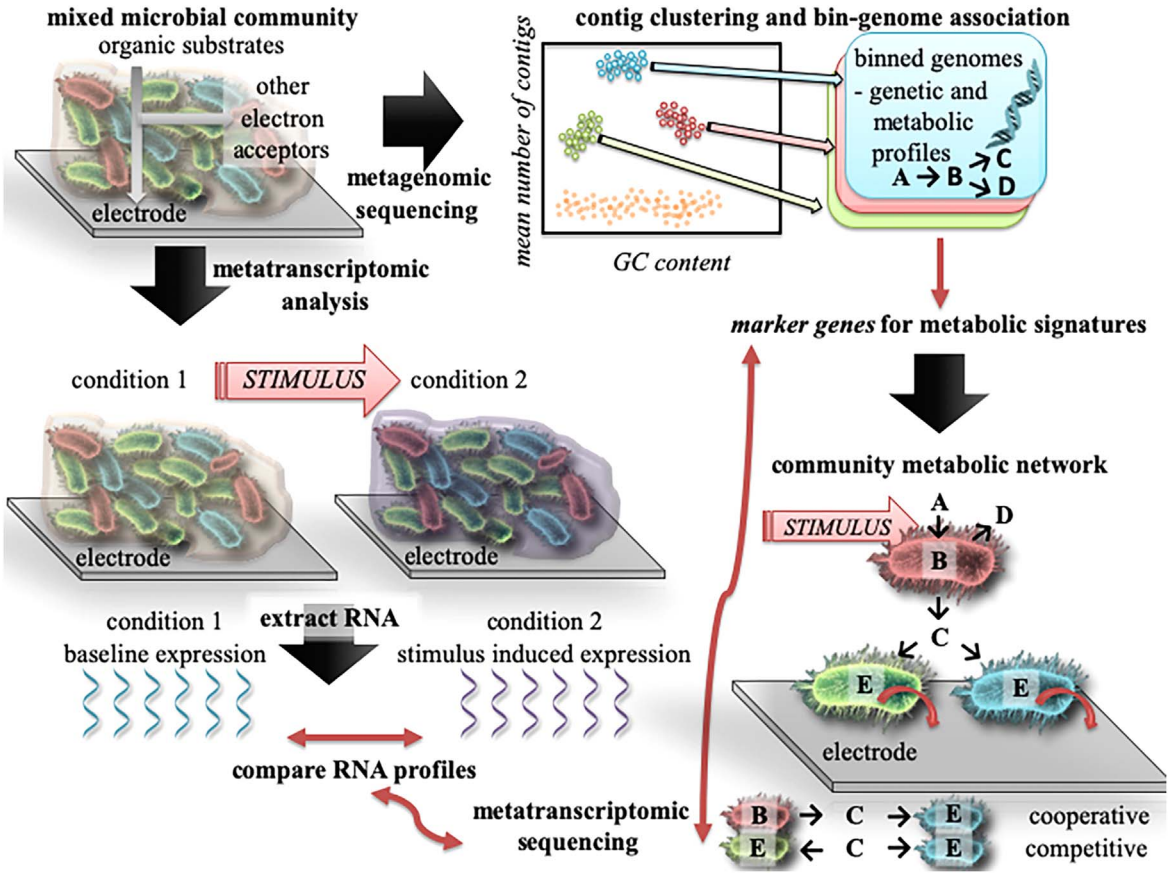

Figure 9. Meta-omics workflow for characterization of a microbial metabolic community and network. First, an electroactive community undergoes metagenomic analysis (top left to top-right) for identification of species present in the microbial mixture. The identification and separation of species in a microbial co-culture is achieved using genome binning, which sorts sequenced DNA contiguous sequences (contigs) based on the abundance and GC content. Contigs that have high abundance and are clustered around the same GC content are then binned as individual genomes and can be annotated for their genetic and metabolic profiles. Then by using this information a microbial network can be formed (top right to bottom right) by analyzing and combining similar metabolic pathways to determine if species would act together to metabolize a complex substrate (cooperatively) or compete to metabolize the same substrate (competitively) in the microbial community. Further by including metatranscriptomics, gene and transcript expression can be quantified for the entire microbial community (top left to bottom). The microbial community is first exposed to a given stimulus to generate condition 1 (without stimulus) and condition 2 (with stimulus) which will then undergo RNA sequencing to generate transcript read counts. These can then be aligned to the marker gene of interest to generate a quantitative metabolic heatmap of expression to determine the effects of the given stimuli on the microbial community network.

their function has remained somewhat elusive. They have been shown to regulate gene expression at various levels,[98] and may play a role in outer-membrane protein regulation.[99] Interestingly sRNA expression was also dependent on the electron acceptor in *G. soli* experiments pointing to the importance of sRNAs for electroactivity and exciting new areas for research with RNA-sequencing.[96]

Similarly to metagenomics, metatranscriptomics can analyze the gene or transcript expression from a multi-species microbial culture sample to determine differential expression in various conditions. One condition often examined for the investigation of electron transfer mechanisms is the effect of potential conditions on a microbial species present in an electrogenic community. Commonly metagenomics and metatranscriptomics are combined, as summarized in a workflow in Fig. 9. This workflow allows for the characterization of species in an electroactive biofilm through metagenomics and the identification of metabolic marker genes or other genes of interest. Following, using metatranscriptomics, the electroactive community can be exposed to a given stimulus (temperature change, potential change, etc.), and expression of the marker gene or gene of interest can be tracked throughout the stimulus.

Metatranscriptomics was first used to investigate extracellular electron transfer by tracking differential expression profiles across a potential gradient. Metatranscriptomics is greatly improved by first performing metagenomics to assemble and bin all genomes present in an electroactive community, and then by performing metatranscriptomics to align the RNA reads to the recently drafted metagenome for the system. This is advantageous due to the possibility of discovering novel species in electroactive communities, as well as aligning the RNA reads to the exact metagenome present in the system instead of attempting to align it to the expected species.[100] This approach was successfully used to investigate a marine sediment electroactive microbial community and identified new species of sulphur-reducing bacteria that were differentially expressed genes homologous to c-type cytochrome genes *omcX* and *omcS*, known to be related to EET in *Geobacter*, in response to EET stimuli.[100]

Additionally, metatranscriptomic analysis can aid in the investigation of interspecies electrochemical communication by deviating between interspecies electron transfer (IET) and hydrogen interspecies transfer, where hydrogen is acting as an electron donor. Differential gene signatures have been determined to show a presence of over-expression of pilus-related cytochrome *omcS* during DIET between *G. sulfurreducens* and *G. metallireducens*, while under the regulation of genes related to acetate metabolism is a signature of hydrogen interspecies transfer, due to the repression of acetate metabolism by H_2, seen between *G. sulfurreducens* and *G. metallireducens*.[101]

Metatranscriptomics has the advantage to be applied to analyze any known genes. In recent work, a variety of genes relating to electron transfer, and metabolic markers were examined to determine the effect of potential. Metatranscriptomics benefits from examining a

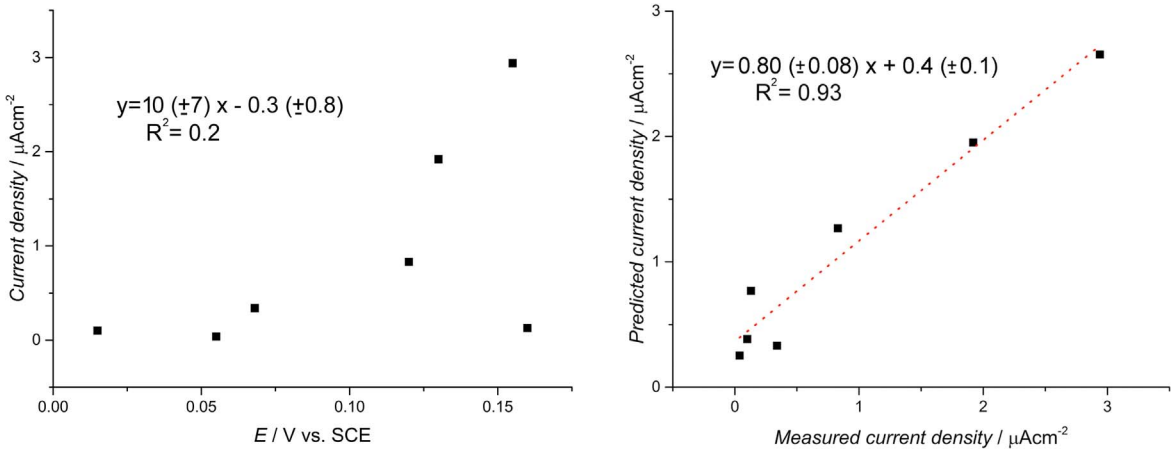

Figure 10. Redox potential current density correlation for different quinone-based mediators (left), measured current density and predicted current density (based on the single electron proton decoupled reduction of quinone based mediators) correlation from purple photosynthetic bacteria under illumination (right). Adapted from M. Grattieri, Z. Rhodes, D.P. Hickey, K. Beaver, S.D. Minteer. Understanding Biophotocurrent Generation in Photosynthetic Purple Bacteria. ACS Catal. 2019, 9, 867–873.[104] Copyright (2019) American Chemical Society.

multi-species culture and show that with given stimuli, the transcriptional responses varied greatly among similar *Geobacter/Pelobacter* species.[38] The varying transcriptional expression responses among species in electroactive communities show the diversity of electron transfer mechanisms and regulation. The upmost importance for clarifying additional species and their mechanisms can greatly aid the advancement of microbial electrochemistry as well as the microbial interactions, which can be studied by meta-omic approaches.

Quantum Mechanical Methods Applied to Microbial Electrochemical Systems

Given the complexity of microbial electrochemistry, calculating properties of entire systems using simulations involving quantum mechanical methods (QMMs) represent a computational challenge even with current technology. However, computations of isolated components of bioelectrochemical systems are feasible and currently have precedent in the literature.[30,102–104] From computing coordination of redox mediators to anode materials to inferring reduction mechanisms based on potentials and other properties, quantum mechanical calculations are applicable to the understanding of microbial electrochemical systems with the judicious application and can supplement and guide other modeling and experimental techniques.

Beginning with the pioneering study of Moore et al. in 2001,[105] QMMs, specifically density functional theory (DFT), found application when used to elucidate the structure of pores in cathodes comprised of granular activated carbon materials. This provided meaningful insights necessary for investigating the properties of the oxygen reduction reaction (ORR) for an air-breathing cathode in wastewater treatment.[106] Subsequent studies employed similar techniques to study the ORR, addressing the use of disparate cathode materials including platinum and pyrolyzed carbon.[107–110]

Research groups concurrently began calculating interaction energies of natural microbial redox mediators with anodes used in MFCs. For example, You et al. used DFT to calculate absorption energies of porphyrin iron to graphitic, pyridinic, and pyrrolic nitrogen centers in respective anodes to understand and explain possible mechanisms of EET in the studied system.[111]

Most recently, DFT has been applied to understand electron transfer mechanisms of both oxidation of analytes for microbial sensing applications and reduction pathways of extracellular mediators. In these cases, structural minima of compounds or interactions are computed, and parameters are extracted which suggest mechanistic details based on low energy structures. Based on DFT computations combined with Raman spectral shifts, Liu et al. found that electrons were passed in their system through a native extracellular redox mediator to an immobilized mediator on the electrode through a conformational shift in the tethered mediator.[112]

Finally, Grattieri et al. used DFT computed parameters to explore mechanistic details of a bio-photoanode (i.e. a biotic/abiotic electrode where photosynthetic microorganisms are utilized as the biocatalyst) as well as optimization of current enhancement based on quantitative structure-property relationship modeling (QSPR), a technique commonly used in drug discovery.[104] Mediator structures were computed, and current enhancement was found to correlate well to the one-electron, proton-decoupled reduction of the quinones. The results provided useful insights in the EET process of photosynthetic bacteria, suggesting that properties of mediators partitioned into cell membranes must be considered when choosing an appropriate electron carrier for the system. Furthermore, they provided evidence of electron transfer to the soluble redox mediator taking place at the active redox site of the photosynthetic electron transport chain, where the one-electron proton-decoupled process is expected to happen.

Examining the current state of QMMs applied to microbial systems shows that most studies come from research groups with expertise in both electro-microbiology and computational approaches applied to separate research challenges. Computational techniques applied to microbial systems creates an inherently interdisciplinary area of study spanning a large gap between microbial chemistry and theoretical chemistry, making collaboration between groups unlikely given unfamiliarity with research problems and potential resources from each field. This makes understanding the capabilities of QMMs important for solving microbial electrochemical problems that are frequently otherwise particularly challenging to be studied.

QMMs allow access to study structures and states that are difficult to observe through experimental means. This makes probing reaction mechanisms possible, as well as understanding properties of given systems. An example includes the understanding of the reduction of an unnatural redox mediator. Typically, reduction/oxidation potentials of mediators are experimentally determined and suggested to correlate in different ways to the performance of the mediator. As shown by Grattieri et al.,[104] this is not always a good single descriptor of mediator performance in a microbial electrochemical system. It was shown, however, that the proton-decoupled, single-electron reduction of the mediators correlated well to their performance, not only allowing for system optimization but also giving possible mechanistic details (quinone reduction of this kind occurs in aprotic media suggesting understanding of mediator characteristics in vivo is most important when choosing a suitable mediator) shown in Fig. 10. The one-electron reduction potential is not observable in the microbial electrochemical system without changing experimental parameters and possibly to a solvent which would result in loss of microbial viability.

Other computable components of microbial systems include not only mediators, but electrodes, substrates, products, and transition materials.[113] These structures and energies, as well as interactions between components, are knowable through QMMs. Parameters that may be computed for these include HOMO/LUMO energies, IR stretches, bond lengths, orbital occupancies, and so forth. Parameters may be computed and correlated based on the hypothesis to electrochemical performance. Transition states of mediators, as well as substrates, may be calculated as well to yield insights into likely reaction mechanisms to suggest conformational changes and rate-limiting steps. Some of these same parameters are computable for electrode materials, using different computational packages.

QMMs can be applied to microbial systems as shown, however, given the general complexity of such systems, computations have only been used to draw conclusions and supplement experimental studies for simple molecules and basic interactions. Resource-intensive computational techniques such as QMMs and others may be applied to larger components of such systems but at large computational cost. Thus, they are better suited as supplemental techniques using today's technology. Computations can yield important results for optimization and understanding of microbial systems that are generally extremely challenging through direct experimental observations, as the deconvolution of minute interactions between small molecules can be difficult in such complex systems. These results may be used in modeling microbial systems, but QMMs is far from an appropriate choice for simulating entire systems.

Combining Computational Techniques

Novel techniques for studying extracellular electron transfer in microbial electrochemical systems are being discovered and utilized to elucidate their fundamental processes and relationships. Each of the computational methods of mathematical modeling, bioinformatic approaches, and quantum mechanical calculations offer their own insights into the electron transfer mechanisms of electroactive bacteria, and the overall performance of a microbial electrochemical system. Specifically, mathematical modeling can be utilized to determine kinetic parameters of electroactive biofilms and guide through the rational design of bioanodes and BES geometry for specific applications. Bioinformatic approaches can show genetic signatures that suggest electron transfer mechanisms may be present and confirm through transcriptomic evidence if gene and transcript expression levels of key genes involved in the electron transport pathways correlate with bioelectrochemical performance. Quantum mechanical calculations can provide important fundamental information about components of microbial electrochemical systems which can be used in modeling. These components, such as mediators, substrates, electrode materials, and so forth can be computed and aid in the exploration of mechanisms and final results. However, the complexity of entire systems prevents the independent use of any discussed technique to solve for system properties, thus promoting the combination of these computational approaches to solve for properties of microbial systems. Below, possible future research directions are presented:

i) mathematical models used for the study of microbial electrochemical systems would greatly benefit by including results from bioinformatics and QMMs. For the case of models discussed above considering contributions of substrate consumption and metabolic flux, transcriptional analysis could provide critical insights into which metabolisms of the microbial strains are active under the given experimental conditions. This would be particularly helpful for microbial species presenting multiple respiratory pathways, such as *G. sulfurreducens*. The information obtained by bioinformatic studies could be utilized when defining model parameters to adjust and separate important microbial respiratory pathways, paving the way for a better description of kinetics and electrochemical characteristics of the system. Similarly, QMMs can reveal important chemical parameters, such as electron density and electrochemical potential of small molecules that can aid development of the Nernst-Monod type models discussed.

ii) models investigating the impacts of extracellular electron transfer on microbial fuel cell bioanode performance can also incorporate both bioinformatics and QMMs. When models investigate outer-membrane cytochromes present in *Geobacter* and *Shewanella* species, bioinformatic analysis could shed light on which specific cytochromes are at play, greatly advancing the model and subsequent understanding of the system under investigation. In this context, it should be noted that the models discussed herein describe modeling cytochromes with 1 heme-binding motif; however, genomic characterization of *Geobacter* (discussed earlier) shows that of the 111 cytochrome coding sequences, 73 coding sequences contain two or more heme-binding motifs with 1 coding sequence containing 27 heme-binding motifs. Future models could perform transcriptional analysis determining which cytochrome(s) is active in their system and adjust models accordingly. Further, important insights could be obtained from QMMs, such as providing binding analysis that can be used to infer heme-binding energetics in cytochromes and relate binding characteristics to resulting electrochemical performance.

iii) models considering multiple extracellular electron transfer pathways, such as in *Shewanella oneidensis* MR-1, could also advance by including considerations resulting from bioinformatics and QMMs. Transcriptional analysis of *Shewanella oneidensis* MR-1 would reveal differences in genetic expression of both DET and MET pathways based on experimental conditions. The expression profiles can then be added to the modeling efforts to expand equations therefore accounting for the contributions of each mechanism based on the modeled conditions. QMMs could provide the electrochemical characteristics for both mechanisms and compare with the electrochemical performance to suggest which mechanism is more likely responsible for the observed behaviors.

In conclusion, mathematical modeling has generally contributed to explaining kinetics of chemical and biological phenomena governing microbial electrochemical systems as a whole, whereas bioinformatics and QMMs offer information on the genetics, transcriptomics, and molecular characteristics of these systems. The combination of these techniques will greatly aid microbial electrochemistry for future work enabling clarification of electron transfer mechanisms. A novel demonstration of this combination identified vast metabolic pathways using meta-omic approaches with subsequent modeling of flux and electron transfer to evaluate a syntrophic community of *G. sulfurreducens* and *G. metallireducens*.[114] Future studies utilizing this type of architecture will enhance microbial electrochemical knowledge especially focusing on diversification of electron transfer mechanisms.

It should also be noted that the computational techniques discussed in this review extend beyond the scope of microbial electrochemical systems. There are reports of using other cellular systems, such as human white blood cells, for whole-cell biofuel cell applications.[115–117] The methods discussed for microbial fuel cell computational studies could benefit the study of substrate dynamics, cell organization, and other similar cellular phenomena in whole mammalian cell applications. Additionally studying extracellular electron transfer of human osteosarcoma cells by interactions with a redox polymer has very recently been reported,[118] and the insights from methods studying microbial extracellular electron transfer could expand knowledge on this phenomena in human and other mammalian cell lines.

Conclusion

Various computational methods commonly used in the chemistry and biology fields prove to be useful in studying microbial electrochemical systems. Their implementation open for further

advancements in the field of microbial electrochemical systems, both from a fundamental and applicative point of view. This review aimed to summarize how computational approaches have been used to specifically study electron transfer mechanisms of bacteria. Achieving a deeper understanding of the EET processes and the influence of external components is paramount for improving and demonstrating the applicability of bioelectrochemical systems. Future microbial electrochemical studies would benefit from pairing with computational methods to further understand the complicated metabolic and bioelectrochemical processes of these systems.

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