The ins and outs of microbial-electrode electron transfer reactions

Amit Kumar[a], Huan-Hsuan Hsu[b], Paul Kavanagh[c], Frédéric Barrière[d], Piet N. L. Lens[e&f], Laure Lapinsonnière[d], John H. Lienhard V[a], Uwe Schröder[g], Xiaocheng Jiang [b] and Dónal Leech[f]

[a]Department of Mechanical Engineering, Massachusetts Institute of Technology, 77 Massachusetts Ave, Cambridge, MA, USA.
[b]Department of Biomedical Engineering, Tufts University, Medford, MA, USA.
[c]School of Chemistry and Chemical Engineering, Queen's University Belfast, David Keir Building, Stranmillis Road, Belfast, UK.
[d]Université de Rennes 1, Institut des Sciences Chimiques de Rennes UMR CNRS 6226, Campus de Beaulieu, 35042 Rennes cedex, France.
[e]Unesco-IHE Institute for Water Education, Delft, The Netherlands.
[f]Biomolecular Electronics Research Laboratory, School of Chemistry & Ryan Institute, National University of Ireland Galway, Ireland.
[f]Institute of Environmental and Sustainable Chemistry, Technische Universität Braunschweig, Hagenring 30, 38106 Braunschweig, Germany.

Abstract

Electron transfer between microbes and an electrode — even across long distances — enables the former to make their living by coupling to an electronic circuit. Such a system integrates biological metabolism with artificial electronics, adding to our knowledge of charge transport both in the distinct chemical species as well as, perhaps most importantly, at their interfaces. From a broad standpoint, this understanding may also lead to topical applications of microbial electrochemical technologies. Such systems have shown promise for the generating electricity, as well as biochemical and chemical feedstocks, and with improvement are likely to give rise to viable applications.

Bioelectrochemical systems

A bio-electrochemical system (BES) involves bidirectional electron transfer between biotic and abiotic components, where redox-active microorganisms or bio-macromolecules catalyze the exchange process[1]. A glossary of important terms is provided in Box 1. One widely studied model BES is the microbial fuel cell (MFC), which is similar to a conventional fuel cell in that microorganisms transport electrons to MFC anodes after oxidizing the electron donors, thus generating electrical current toward the cathode[2]. Meanwhile, certain microorganisms are also known for their capability to reduce electron acceptors such as nitrate, perchlorate or metal ions at the cathodes[3]. Alternatively, a BES may involve microbial electrosynthesis (MES), or take the form of a microbial electrolysis cell (MEC), microbial solar cell (MSC), or a plant microbial fuel cell (PMFC), all of which are underpinned by a similar electron transport strategy. These novel and promising electron transport processes bring together fundamental research in microbiology, electrochemistry, environmental engineering, material science and applications in waste remediation and resource recovery, sustainable energy production, and bio-inspired material development. The principles and applications of these different BESs have been comprehensively reviewed by many different groups [4–7].

Enzymatic electrocatalysis was one of the earliest BES models to receive extensive attention, due in no small part in developments in amperometric biosensors and enzymatic fuel cell in the late 20th century[8–13]. This BES model involves electron extraction from specific enzymatic reactions by either direct (tunneling) or indirect (via foreign mediators) transport to a solid-state electrode at which the current is detected. In the absence of a foreign mediator, electron tunneling in enzymes occurs only across distances less than a few nanometers[9]. In most cases, the redox centers of enzymes, including electron relays such as iron-sulfur clusters, are deeply embedded in an insulating protein matrix such that electron transport to a solid-state electrode is limited by this less-than-effective electrical coupling. Strategies to immobilize enzymes on electrodes are necessary to facilitate direct electron transfer for practical applications[14]. Further, the three-dimensional structure of an enzyme is essential to its catalytic activity but is very sensitive to variations in temperature, pH, and the chemical species present[15]. Although many immobilization techniques (enzyme-electrode; enzyme-conductive support-electrode; enzyme-cofactor-mediator complexes-electrodes) do increase durability and efficiency of enzymatic electron transport, the maximum lifespan of the system is only on the scale of hours to a day[10,16]. These limitations have so far restricted the applicability of enzymatic BES to both energy generation and biosensing.

Unlike isolated enzymes, certain microorganisms, usually termed electrochemically active bacteria (EAB), can undergo self-healing to overcome incompatibilities between biological and inorganic interfaces and thereby perform effective, long-term, and long-range electron transport. Extracellular electron transfer (EET) is the key process that links solid state electron donors/acceptors and the microorganisms. When soluble electron acceptors - oxygen in most case - are depleted, EAB transport metabolism-generated electrons to acceptors outside the cell. The concept of EET was first discussed in the early 1900s when Potter[17] and Cohen[18] harvested electricity from microbial metabolisms. In the 1960s, NASA research considering the recycling of human waste to generate electricity during space flights augment interest in the fundamentals of EET[6]. By the early 2000s, several different mechanisms had been proposed by which microorganisms transport electrons to an acceptor either directly or indirectly. The direct EET relies on outer membrane cytochromes or membrane-bound redox enzymes to couple internal metabolism with external charge transport, and generally requires direct contact between the cell membrane and solid-state electron acceptors. Additionally, under acceptor-limited conditions certain EAB also generate conductive pili or pilus-like structures – often referred to as microbial nanowires[19,20] - that serve as alternative electron pathways to extend the direct EET distance and maximize transport efficiency. In the case of indirect EET, some EAB secrete redox-active small molecules such as phenazines, flavins, and quinones[1,21,22] to relay electrons to an outside acceptor such as a solid-state electrode. Ideally, these redox materials can be utilized repeatedly thus been named as "electron shuttles."[1]

While significant progress has been made in understanding and exploiting EET, detailed mechanisms involving protein-protein interactions[23], electron transport inside microbial nanowires[24] and bacterium-solid state material interactions[25] remain poorly defined and actively debated. This Review covers the state-of-the-art in bioelectrochemical systems and EET, also presenting obstacles that limit our comprehensive and unambiguous understanding of BES. Some recent applications of micro- and nanotechnology in single cell measurements are also introduced, with such efforts being expected to inspire and enable us to better design and optimize BES for applications. We first consider mechanisms of microbial-electrode electron transfer with a view to improving electrocatalysis (bioelectricity) and electrosynthesis (biochemical and chemical production). Challenges are identified, as are interdisciplinary research opportunities, which may result in improved yield and efficiency of fuel and chemical production. Lastly, we discuss possible applications that would arise from research into microbial-electrode electron transfer.

Extracellular Electron Transfer at Bioanodes

For EAB, outward EET (electron transfer from microorganisms to extracellular electron acceptor) is the natural process by which microorganisms perform respiration when soluble electron acceptors in the surrounding environment are of limited concentration. Artificial bioelectrochemical systems are often used to harvest energy (e.g. MFC) through microorganisms that perform outward EET. The microorganisms serve as catalysts at the fuel cell anode, with the resulting hybrid thus referred to as a bioanode.

Studies on bioanodes have largely focused on dissimilatory metal reducing bacteria (DMRB), which form colonies on a positively-biased inert carbon or metal electrode surface. The DMRB proliferate by performing metabolism and EET, processes that require bacteria-bacteria and bacteria-electrode connections to be made. These connections can eventually take the form of an electrically conductive biofilm, spanning over 100 μm, that is comprised of cells and extracellular substances. Certain bacteria such as *Shewanella* can even perform long range EET without the physical bacteria-bacteria and bacteria-electrode connections. The mechanisms of extremely long-range electron transport in biology have attracted enormous attention as such processes have typically been limited to distances comparable to the size of molecules (~1 nm)[26]. In the case of EET within DMRB, it is suggested that iron-sulfur proteins and multi-haem proteins such as c-type cytochromes (c-cyts) are key to mediating electron transport across multiple length scales[1,24,27]. Most EET research to date has focused on the prototypical DMRB strains *Geobacter* and *Shewanella*, with the processes being less systematically understood in the case of *Proteus vulgaris, Pseudomonas sp.*, *Klebsiella pneumonia*, *Bacillus subtilis*, and *Corynebacterium sp.* strains[28]. Mechanistic models for EET in *Geobacter* (Figure 1a) and *Shewanella* (Figure 1b) have been proposed to involve three distinct modes. Transport can occur through outer membrane c-cyts and other redox proteins such as multi-copper proteins (OmpB and OmpC), through pilus-like nanowire structures, or through extracellular or self-excreted small-molecule electron shuttles.[21,22,29–31]

Both *Geobacter* and *Shewanella* transport electrons using c-cyts, with *Geobacter sulfurreducens* featuring 111 genes that code for c-cyts. Of these c-cyts, 73 contain two or more haems, with one containing as many as 27 haem groups. Similarly, *Shewanella oneidensis* has 39 genes encoding c-cyts, 14 which have 4 or more haems. The detailed structures of these c-cyts have been discussed in previous reviews[32]. Through the regulation of gene expression, the key c-cyts of both bacteria models in performing EET have been identified and studied. In *Geobacter*, outer membrane EET is effected by a variety of outer membrane c-cyts (OMCs), including OmcB, OmcE, OmcS, OmcZ. Mehta and coworkers suggested that OmcE and OmcS can facilitate ET to the type IV pili (*vide infra*) for long-range electron transport, with OmcB being the intermediary electron carrier from the periplasm to other OMCs[33]. However, they later proposed different mechanisms in which either OmcS and OmcE also directly transfer electrons to the electrodes, or where OmcB participates in iron reduction but not in EET[34]. More recently, combined electrochemical and genetic studies led the same group to conclude that OmcZ is critical to outer membrane EET current[35], while OmcB, aided by OmcS, mediates electron transport from the periplasm to other OMCs. OmcE, in contrast, is now believed not to participate in EET[36].

Genetic engineering of *Shewanella* has uncovered a series of protein-protein interactions in EET, implicating a critical role for c-cyts. First, CymA transports electrons derived from bacterial metabolism to terminal periplasmic reductases. This step is essential for *Shewanella* EET, with deletion of the CymA gene resulting in a 80% decrease in current generation[27]. In turn, the reductases pass electrons to outer membrane proteins such as MTRs and OMCs, which transport electrons to acceptors or shuttles to complete the EET process. Of the outer membrane proteins, MTRC is considered one of the most important for EET, and deletion of MTRC from *Shewanella* can lead to a >90% current decrease[37]. The involvement of each c-cyt in outer membrane EET, and the methods by which such a process is characterized, are now well known[23,27,38,39].

As with typical enzymes, the effective direct EET range of outer membrane c-cyts is limited to the nanometer scale[40]. In order to achieve long-range EET, *Shewanella* self-excretes flavins and quinones to relay electrons (*Geobacter* are unable to deploy these relays)[21,22,40,41]. Hypotheses regarding the EET mechanism either involve flavin-accelerated EET, wherein cytochrome-bound cofactors facilitate EET with c-cyts but not directly with a solid electron acceptor[42], or instead implicate flavin to be the electron shuttle that directly performs EET at the outer membrane[21]. Other than *Shewanella*, *Pseudomonas* strains also perform EET *via* excreted mediators such as phenazines. Interestingly, these mediators can be exploited by different species and result in interspecies electron transfer[43]. Lastly, we note that other natural products such as sulfides and humics can also serve as electron relays for EET[44].

Both *Geobacter*[45] and *Shewanella*[46] may perform direct long range EET by self-assembling their c-cyts into conductive pilus-like structures up to tens of micrometers in length. In *Geobacter*, the microbial nanowires (type IV pili) have been found to connect the inner membrane to the outer electron acceptor. Other proteins, including OMCs, may transport electrons to an acceptor[23] through type IV pili. Such structures are critical to the EET efficiency of biofilms[20], although the underlying mechanisms of charge transport are still under debate[47,48]. For example, a "metallic-like model" was proposed by Malvankar and coworkers[49], who suggest that electrons are transported through π-π stacked aromatics, a motif present in type IV pili as well as synthetic conducting polymers[45,49,50]. The conductivity of type IV pili is dependent both on temperature and gate voltage, as is the case with nanostructured organic semiconductors[45]. However, the electrochemistry of *Geobacter* biofilms is inconsistent with transport according to a “metallic-like model”, and instead supports an electron hopping mechanism. Such as “superexchange model”, which is similar to that proposed from redox polymers, involves electron transport through a series of redox reactions of the discrete redox cofactors (e.g. haems) inside type IV pili[51,52]. Cyclic voltammetry (CV) of *Geobacter* biofilms supports this mechanism, and at low scan rate the sigmoidal voltammogram of the biofilm is consistent with an electrochemical-chemical (EC) mechanism, with electron transfer being coupled with redox cofactors[53,54]. In the absence of electron donors, symmetric current peaks appear in forward and reverse scans, indicating that EET involves charging-discharging (pseudocapacitance) reactions of redox cofactors in the biofilm[36,54–56]. Here, the multiple peaks suggest the presence of multiple cofactors[36,56]. The “superexchange model” is further supported by advanced bio-electrochemical characterizations[57,58] and charge storage measurements[59].

The situation is different with *Shewanella,* in which microbial nanowires – conducting due to the presence of c-cyts - were first observed and electrically characterized using scanning tunneling microscopy in 2006[19]. Indeed, mutants deficient in c-cyts express nanowires that are poorly conducting, an observation supportive of an electron hopping occurring throughout the cytochrome network[24,46,60,61]. A recent study from the group of Pirbadian demonstrated that the *Shewanella* nanowire comprises the outer membrane and periplasmic extensions but not the pilin-based structures, such a result being consistent with an electron-hopping (cyt redox reactions) mechanism[46].

The current density at a bioanode is limited by the natural metabolic rate of DMRB, the rate of cytochrome-based cross-membrane EET, and the ineffective EET within the evolutionally developed electron transport pathways, especially over long distances. Substrate transport into the biofilm is also a limiting factor, with a further problem being proton accumulation during the bioelectrochemical processes. Maximizing current densities requires one to address these limitations, perhaps by exploiting synthetic biology to express specific genes of DMRB regulating production of electron shuttles or electron transfer proteins. For example, the synthetic flavin biosynthesis pathway from *Bacillus subtilis* was expressed in *Shewanella* MR-1, with the mutant *Shewanella* producing 13.2 times more current than the wild type bacteria[62]. Similarly, expression of five riboflavin synthesis genes in *E. coli* BL-21 induces a 9.5-fold increase in EET[63], and overexpression of the NAD synthetase gene in *P. aeruginosa* enhanced current by a factor of at least three[64]. Aside from using biology to our advantage, it is also possible to facilitate cross-membrane EET by using conjugated oligoelectrolytes (COE), water-soluble oligomers with extended π-delocalized regions. Certain COEs spontaneously "insert" into bacterial membranes and align themselves such that electron transfer through the lipid bilayer is promoted. 4,4′-Bis(4′-(*N*,*N*-bis(6″-(*N*,*N*,*N*-trimethylammonium)hexyl)amino)-styryl)stilbene tetraiodide (DSSN+) is a commonly used conjugated oligoelectrolyte in bacterial EET studies and is largely nontoxic to bacteria[65]. Early work suggested that both cytochrome-based direct electron transfer and flavin-based mediated electron transfer in *Shewanella* MR-1 can be promoted by DSSN+[65]. A 25-fold improvement in *E. coli*-based MFC power density is observed when DSSN+ is present[66], although a consensus has not been reached regarding the basis for this this extra current[67]. Another strategy to promote EET involves constructing hybrid electron pathways. Various nanoscale conductors and semiconductors, including carbon nanotubes[68], graphene[69], Fe_2O_3[70] and FeS nanoparticles[71], have been seamlessly integrated with natural biofilms to afford systems with significantly improved EET at both cell-electrode and cell-cell interfaces.

Several milestones in bioanode research are summarized in Box 2. While genetic engineering approaches allow for one to study the effects of selected proteins on EET, such bioanodes can also be studied using scanning electron microscopy (SEM), atomic force microscopy (AFM) and scanning tunneling microscopy (STM). When complemented by surface enhanced Raman scattering, surface enhanced IR, Raman microscopy and UV-visible spectroscopy,[72–74] one can then understand the unique structural, morphological and electrical properties of key EET components such as whole biofilm, outer membrane cytochromes[75], and microbial nanowires. Further, the use of spectroelectrochemistry can narrow down the possible mechanisms by which electrons are transported in bacterial EET.

Developments in micro- and nanotechnologies have provided additional insights into EET operative in controlled microenvironments and across multiple length scales (Figure 2). Li and coworkers characterized a *Geobacter* biofilm cultured in a microfluidic device, the small size of which enabled a faster response to changes in ambient environment changes relative to the bulk biofilm. Such methodology makes possible high-throughput experiments probing the effects of stimuli such as O_2 and anthraquinone disulfide (AQDS) on the activity of *Geobacter* biofilms. In this case short doses of O_2 are of only minor toxicity to *Geobacter*; with AQDS serving as an effective electron shuttle[76]. Similar fabrication strategies have afforded many micro-scale MFC and biosensors[77], including at the single bacterium level. The key historical developments in such work include the *in vivo* measurement of EET current at a single *Shewanella* using infrared optical tweezers and indium tin oxide (ITO) microelectrodes[78]. A single bacterium gives rise to currents, mediated by c-cyts, in the range 15-100 fA. This single cell approach affords data that can be extrapolated to determine the maximum current of a biofilm. Further, one can study electron transport at the single bacterium level, such that the functions of flavin and the nanowires can be better understood. Nanostructured electrodes with controlled cellular interfaces have been designed to unambiguously determine the EET mechanism in *Geobacter* and *Shewanella* at single- and multibacterium levels[79,80]. Real-time monitoring of localized current generation and cell-electrode interactions provide insights that are hard to come by with bulk film experiments. In a single cell experiments, quantized current "steps" become apparent when the cell initially binds the electrode. Concomitant with proliferation, one can also observe a dramatic current increase as cells get closely packed and form into electrically-connected networks.

Overall, these emerging cell measurement techniques are expected to open up new possibilities to probe and regulate electron transport at bioanode interfaces[81] and to elucidate factors determining bioelectrical power extraction. In turn, such information will aid the design of more efficient BESs.

Box 1: A glossary of important terms.
Microbial Electrocatalyst
A microorganism that catalyzes an electrode reaction.
Electrosynthesis
Electrode reaction resulting in intentional generation of a useful chemical product (e.g. hydrogen or butanol).
Microbial Bioelectrochemical System (BES)
A microbial reactor — a fuel cell or electrolysis cell — that utilizes a microbial electrocatalyst.
Electrogenic Microorganism
A microorganism able to catalyze an anodic electrode reaction.
Electrotrophic Microorganism
A microorganism able to catalyze a cathodic electrode reaction.
Extracellular Electron Transport (EET)
The process by which electrons are transported outside the cell by shuttles/wires (e.g. redox proteins, biopolymers, and protein filaments) secreted by microbes. Transport can occur across

distances exceeding 100 µm, such that intracellular metabolic processes (e.g. acetate oxidation or oxygen reduction) can be interfaced with insoluble extracellular electron acceptors or donors (e.g. minerals and electrodes).

Microbial Bioanode

An electrode colonized by microorganisms that catalyze an anodic reaction (e.g. acetate oxidation).

Microbial Biocathode

An electrode colonized by microorganisms that catalyze a cathodic reaction (e.g. nitrate reduction).

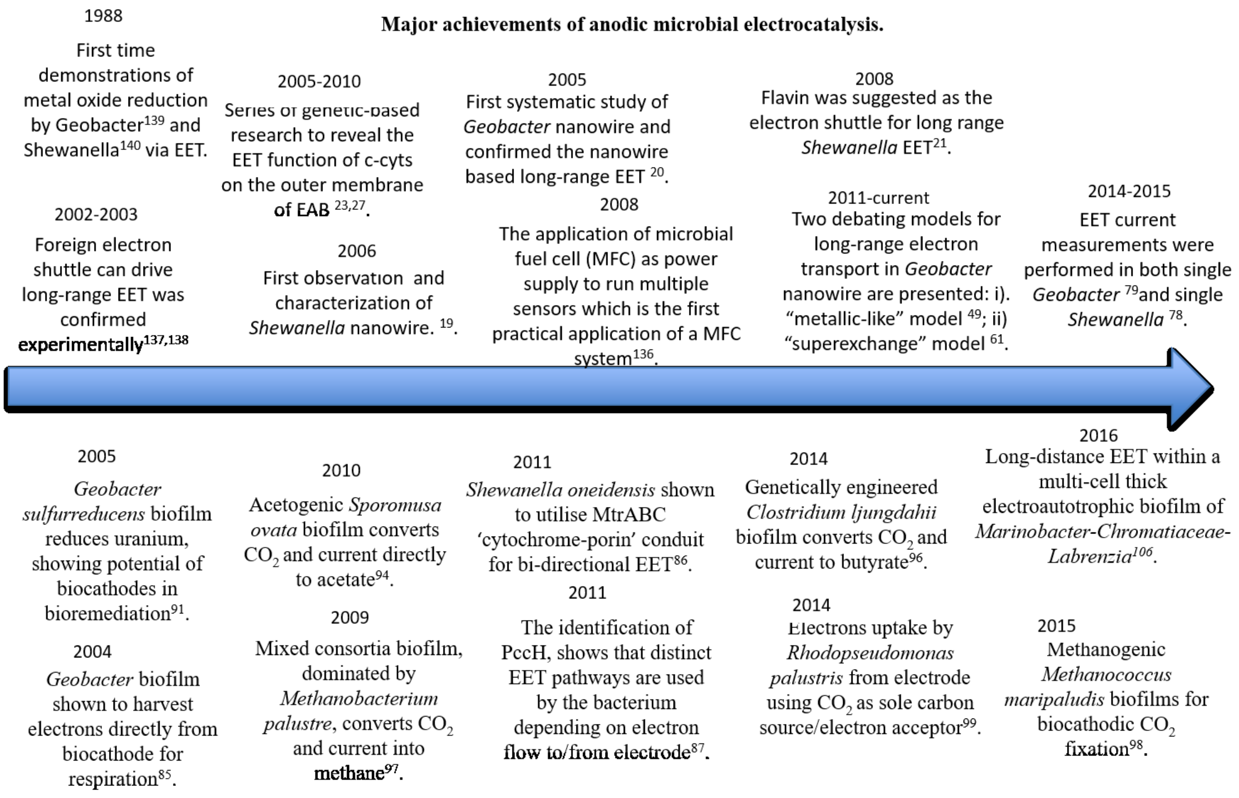
Redox conductivity (incoherent multi-step hopping)

A proposed EET mechanism whereby sequential electron self-exchange between extracellular redox cofactors occurs in a bucket brigade manner, as is the case with redox polymer films on electrodes.

Metallic-like conductivity (coherent conductivity)

A EET mechanism involving transport through delocalized electronic states of extracellular protein filaments ("microbial nanowires"), which function as protein wires with metallic-like conductivity. Such a process is analogous to that occuring in organic semiconductor devices.

Box 2. Major achievements of anodic and cathodic microbial electrocatalysis and electrosynthesis.



Major achievements of anodic microbial electrocatalysis.

1988
First time demonstrations of metal oxide reduction by Geobacter[139] and Shewanella[140] via EET.

2002-2003
Foreign electron shuttle can drive long-range EET was confirmed experimentally[137,138]

2005-2010
Series of genetic-based research to reveal the EET function of c-cyts on the outer membrane of EAB [23,27].

2006
First observation and characterization of *Shewanella* nanowire. [19].

2005
First systematic study of *Geobacter* nanowire and confirmed the nanowire based long-range EET [20].

2008
The application of microbial fuel cell (MFC) as power supply to run multiple sensors which is the first practical application of a MFC system[136].

2008
Flavin was suggested as the electron shuttle for long range *Shewanella* EET[21].

2011-current
Two debating models for long-range electron transport in *Geobacter* nanowire are presented: i). "metallic-like" model [49]; ii) "superexchange" model [61].

2014-2015
EET current measurements were performed in both single *Geobacter* [79]and single *Shewanella* [78].

2005
Geobacter sulfurreducens biofilm reduces uranium, showing potential of biocathodes in bioremediation[91].

2004
Geobacter biofilm shown to harvest electrons directly from biocathode for respiration[85].

2010
Acetogenic *Sporomusa ovata* biofilm converts CO_2 and current directly to acetate[94].

2009
Mixed consortia biofilm, dominated by *Methanobacterium palustre*, converts CO_2 and current into methane[97].

2011
Shewanella oneidensis shown to utilise MtrABC 'cytochrome-porin' conduit for bi-directional EET[86].

2011
The identification of PccH, shows that distinct EET pathways are used by the bacterium depending on electron flow to/from electrode[87].

2014
Genetically engineered *Clostridium ljungdahii* biofilm converts CO_2 and current to butyrate[96].

2014
Electrons uptake by *Rhodopseudomonas palustris* from electrode using CO_2 as sole carbon source/electron acceptor[99].

2016
Long-distance EET within a multi-cell thick electroautotrophic biofilm of *Marinobacter-Chromatiaceae-Labrenzia*[106].

2015
Methanogenic *Methanococcus maripaludis* biofilms for biocathodic CO_2 fixation[98].

Major achievements towards biocathode applied technologies including microbial electrosynthesis microbial electrocatalysis.

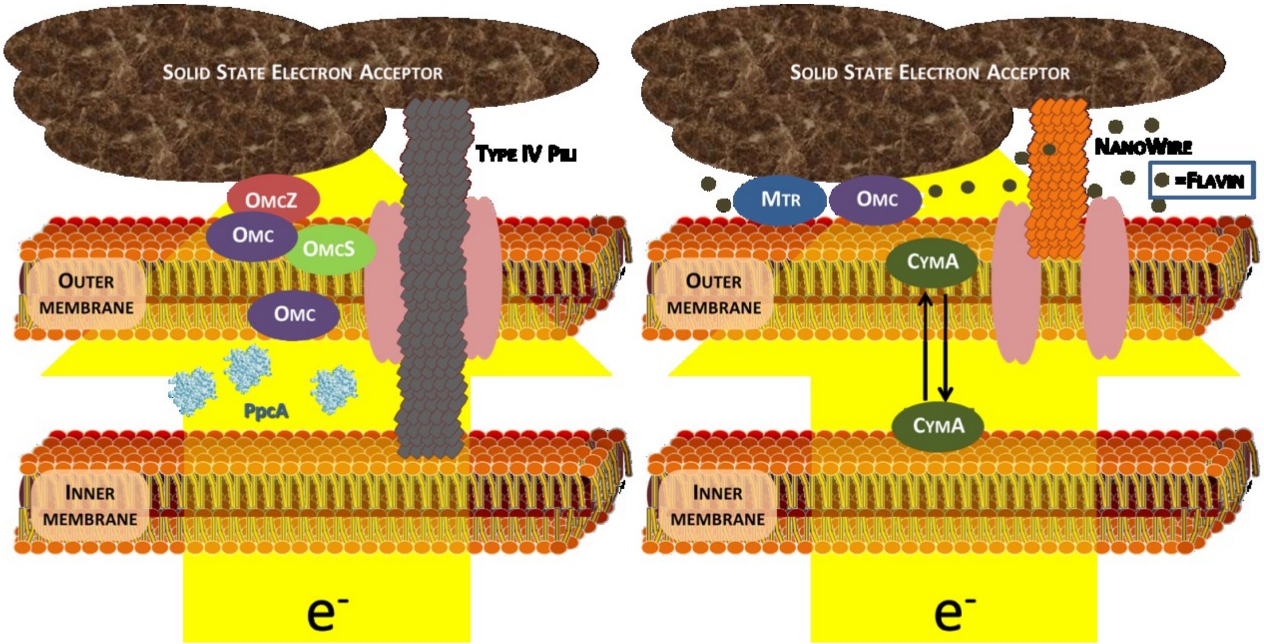


Figure 1. Schemes of EET in *Geobacter* (a) and *Shewanella* (b). In *Geobacter*, type IV Pili can directly transport electron from inner membrane to electron acceptor. OmcZ mainly contributes to the outer membrane EET while other OMCs support the EETs of both type iv Pili and OmcZ. Periplasmic c-type cytochrome (PpcA) which serve as an intermediary electron carrier between electron donors such as acetate, which are metabolized in the cytoplasm, and outer membrane. In *Shewanella* the electron generated on inner membrane is transport by CymA to outer membrane then be transported to electron acceptor by MTRs and OMCs to complete EET. The nanowires are considered as the extension of outer membrane and perform EET by electron hopping. Self-excreted flavin also involved in the EET process as the electron shuttle or cofactors.

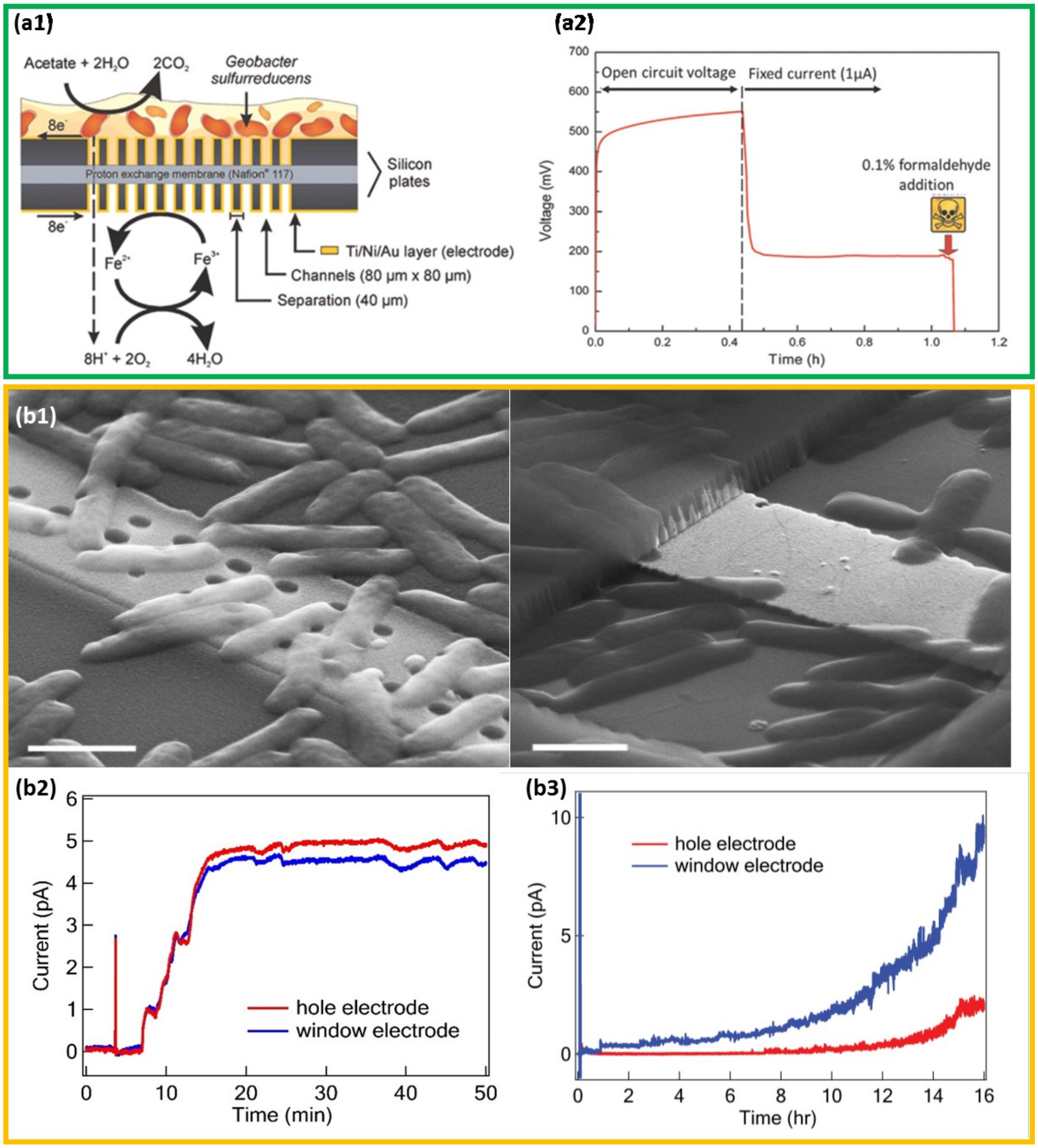


Figure 2 Micro-scale EET studies: (a) The micro-scale Geobacter biofilm and its application in biosensor development: (a1) scheme of micro biofilm and (a2) toxicity (0.1 % formaldehyde) sensing; (b) Probing EET mechanisms of both *Shewanella* and *Geobacter* in microscale; (b1) is the images of bacteria on electrodes with nanoholes and window, respectively (Scale bar, 1 μm); (b2) and (b3) are the simultaneously short-circuit current measurement on electrodes with nanoholes (red) and large window (blue). Reprint with permission[77,80]

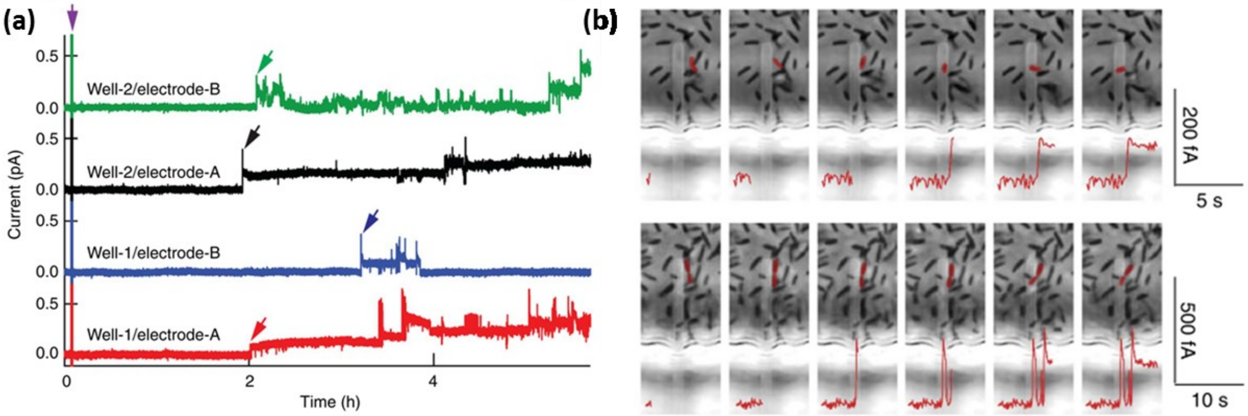


Figure. 3 Microfabricated well and nano electrodes for in situ EET current measurement of single Geobacter: (a) Short-circuit current recording on four selected electrodes, the purple arrow indicates the injection of *Geobacter* into, the red, blue, black and green arrows mark the occurrence of the first current step on each electrode. The current variations are generated by the contact of *Geobacter* (b) Evolution of in situ phase-contrast images and the current variations of Geobacter cells on and not-on contact with the measured micro electrode, EET current of single Geobacter is obtained as 92(±33) and 196(±20) fA. Reprint with permission[79]

Microbial biocathode

Lithotrophs are microbes that metabolise minerals, of which iron is a particularly common example[82]. Metallic iron affords reducing equivalents that are transferrable to electron sinks such as sulfate, a process that allows energy generation in certain microbes[83]. This process, commonly referred to as ‘biocorrosion’, presents a considerable challenge to the maintenance of iron-based installations, such as gas pipelines, located in suboxic sulfur-rich environments[83]. Although a comprehensive understanding of biocorrosion remains elusive, possible mechanisms include microbial consumption of ‘cathodically generated’ H_2 at the metal surface, chemical corrosion by biogenic H_2S, or direct uptake of electrons from the metal[83]. The latter mechanism was proposed for sulfate-reducing *Delsulfobacterium*- and *Methanobacterium*-like microbes that accept electrons from solid iron at a rate unachievable by H_2 scavenging alone[84]. Although a more direct route for electron uptake is thus implied, the complete ET mechanism remains unsolved as the exclusion of H_2 involvement in this process has yet to be verified[83].

At about the same time that biocorrosive 'DET' mechanism was first proposed, *Geobacter sp.* biofilms were demonstrated to accept electrons directly from a solid graphite electrode for respiration[85]. Subsequent *Geobacter sp.*[85] and *Shewanella sp.*[86] pure culture studies showed that both organisms, whilst forming thinner films than their bioanodic counterparts[87], could directly harvest electrons from electrodes. Genomic analysis revealed that a periplasmic monohaem cytochrome, PccH, is essential for electron uptake by *G. sulfurreducens*[87], though gaps remain in the identification of additional proteins required for ET across both membranes. PccP is not required for EET to electrodes, an observation consistent with inward and outward electron flow for *G. sulfurreducens* involving two distinct ET pathways[87]. In contrast, electron flow in both directions in *Shewanella sp.* is mediated by a single OmcA-MtrABC respiratory pathway[86].

The utility of microbial biocathodes in reducing low-value or pollutant species to higher value, or benign products is both economic and environmental in nature[88]. Reduction of nitrates[85], chlorinated solvents[89] and toxic metal ions[90,91] by *Geobacter sp.*[85,89,91] and *Shewanella sp.*[90] biocathodes has highlighted their potential application in bioremediation[92]. However, the microbial biosynthesis applications of heterotrophic *Geobacter sp.* and *Shewanella sp.* are limited by their inability to fix carbon[93]. More useful are autotrophic microbes, which utilise energy from inorganic chemical reactions (chemotrophs) or light (phototrophs) for carbon fixation, can adapt to use an electrode as an electron source for growth (electrotrophs). For example, cathodic biofilms of the acetogenic bacterium *Sporomusa ovate*, were shown to reduce CO_2 with electrons solely from a graphite electrode, affording acetate with >85 % Faradaic efficiency[94]. Other acetogenic electrotrophes that have been identified include various *Sporomusa*[95] and *Clostridium*[99] species and *Mororella thermoacetica*[95], although little is known about the metabolic ET pathways. Conversion of electrons and CO_2 to methane by *Methanobacterium sp.*-dominated biocathodes has also been demonstrated[97]. Although ET was first thought to proceed by DET from the electrode to the biofilm, recent evidence shows that *Methanobacterium sp.* secretes proteins that diffuse to electrode surface, where they catalyse the production of H_2 that is rapidly consumed by the organism[98]. Biocathodes composed of the natural Fe(II)-oxidising prototroph *Rhodopseudomonas palustris* fix CO_2 under both light and dark conditions[99]. The operon *PioABC* codes for an OM porin, a periplasmic cytochrome and Fe-S cluster protein essential for growth of *R. palustris* on an electrode[99]. It is likely that other as-yet unharnessed Fe(II)-oxidising autotrophs may be incorporated into biocathodes for carbon fixation.

Although much progress has been made in microbial electrosynthesis, further improvements in rates and yields will require a deeper understanding of the EET pathways at play. Many microorganisms that induce iron corrosion have also been shown to harvest electrons either directly[100] or indirectly[98] from electrodes. Whilst detrimental to solid iron, such corroding biofilms, if harnessed at an electrode, may sustain rapid formation of value-added products. In addition, mechanistic insights gained from biocorrosion studies may benefit the advancement of microbial electrosynthesis applications, particularly with regard to ET pathways necessary for rapid electron uptake[98]. The conversion of synthesis gas to bulk chemicals and biofuels, mediated by bio-engineered autotrophs[101], might also be catalyzed by electrotrophs, such as the engineered *Clostridium ljungdahlii* that shows activity for butyrate production. Although their study is far from a mature area, the proliferative and self-healing nature of microbial biocathodes is promising when contrasted with the often limited stability of traditional enzyme-decorated cathodes[102]. However, the successful implementation of microbial biocathodes as alternatives to existing technologies will require improvements in substrate scopes, turnover frequencies and product yields.

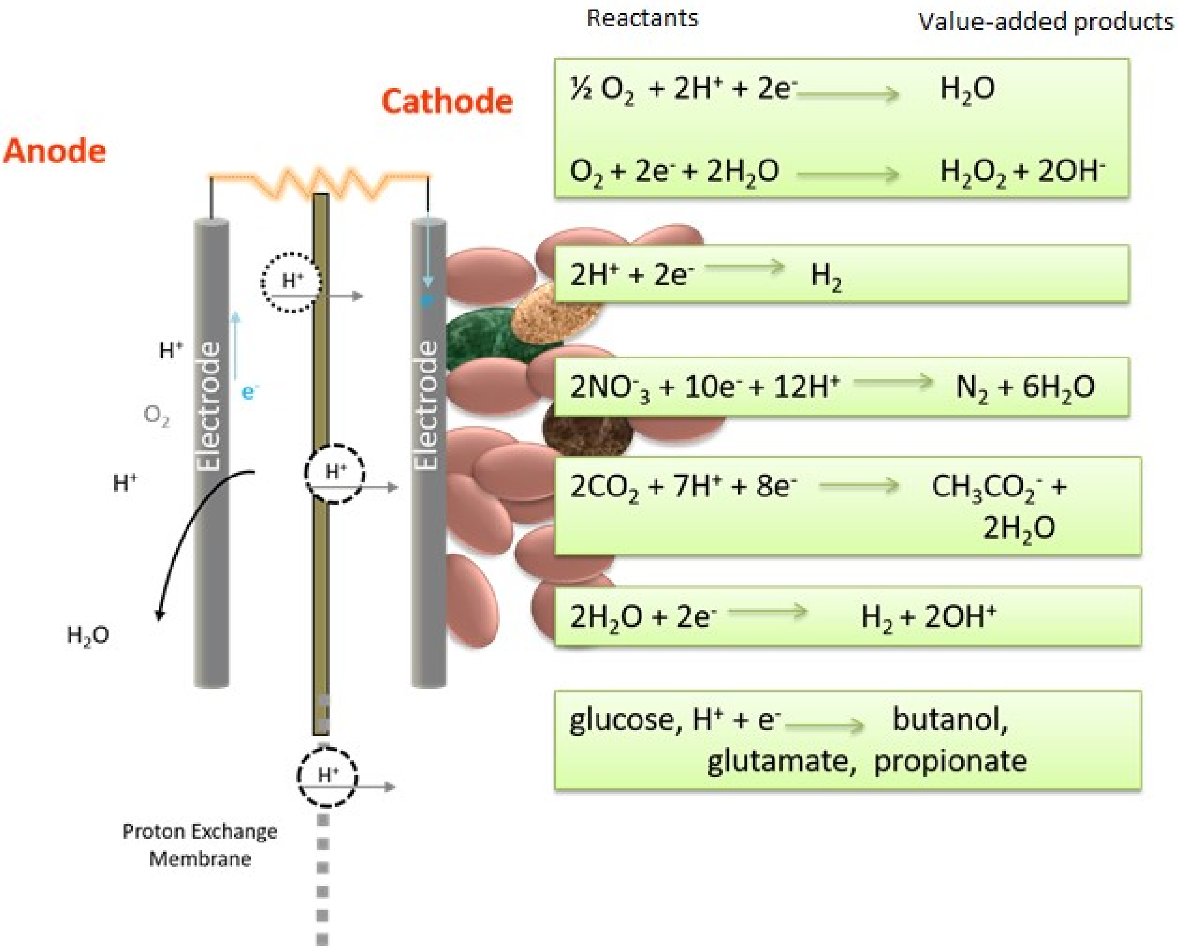


Figure 4. The cathode as an electron donor and the biochemical reactions that lead to the production of products and reactants.

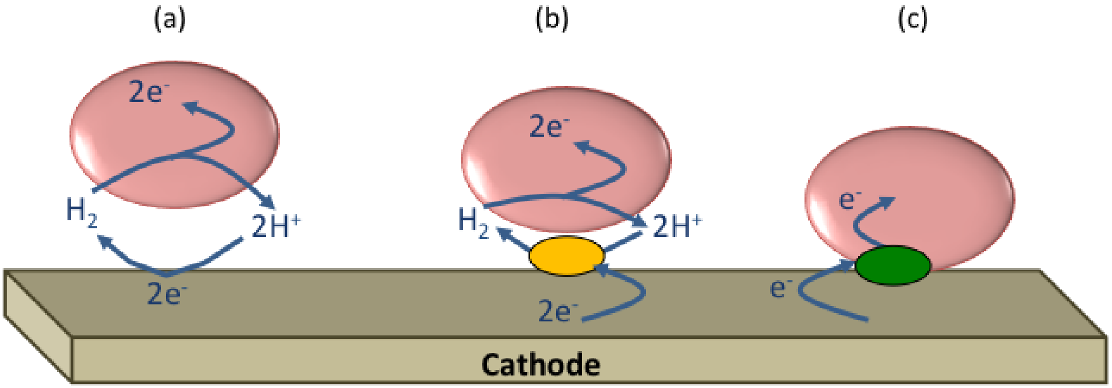


Figure 5. Proposed electron transfer pathways utilised by microbes for extracellular uptake of electrons. Scavenging of cathodically generated H_2 at electrode surface (a), uptake of H_2 generated by secreted redox proteins e.g. hydrogenases (b), and direct uptake of electrons by outer membrane bound redox proteins e.g. cytochromes (c).

Surface chemistry in microbial BES design

Understanding the interdisciplinary chemistry of cell attachment, interconnection, and charge transport at the microbe-electrode interface is necessary to rationally optimize BES technologies. A surface's composition, roughness and charge density, as well as its hydrophobic/hydrophilic and lipophobic/lipophilic nature, can each influence biofilm formation[107]. Furthermore, the structures of surface functional groups can strongly influence electron transfer rates at biofilm-electrode interfaces and can interfere with the natural EET process. Although most electrocatalytic biofilms are grown on unmodified carbon-based electrodes, it is of interest of consider how modifying the electrodes prior to biofilm growth can affect the biofilm-electrode interaction and overall performance. The importance of the nature of the electrode surface with respect to microbial BES applications is further underscored by noting that *Shewanella* will not form an electrocatalytic biofilm on gold.

Pretreating an electrode with an acid or a surface-binding molecule, or instead subjecting it to high temperatures or plasmas, indeed does influence biofilm development and performance. Chemical pretreatment of graphite electrodes, including electrochemical oxidation in sulfuric acid, affects the microbial composition of electroactive biofilms on graphite electrodes imbedded in marine sediment[104]. In another study, it was also noted that an increase in the nitrogen-to-carbon ratio on the surface was favorable for biofilm development and catalytic performance[105]. Although these early results proved difficult to rationalize, we now can more controllably modify electrode surfaces to achieve a predictable outcome. Among the methods for grafting chemical functionalities onto surfaces, the electrochemical reduction of aryldiazoniums has proven useful owing to its versatility, ease of implementation and the control with which one can deposit a certain amount of a functional group. Many such groups can be used, and one can thereby modify the hydrophilic/lipophilic character of the electrode surface, with the induced changes being explicable in terms of electrostatic or wetting concepts in line with the negative charge at the outer-membrane surface[106,107].

Key modifications of microbial anodes can be made by more rationally and controllably tailoring the physico-chemical properties of electrode surfaces[108]. Arguably a more important challenge is the modification of electrodes for electroacatalytic biocathodes[109], which might, for example involve modifying the growth scaffold with carbon nanotubes[110]. While strategies for direct wiring of anodic biofilms to electrodes have been established, the same approach has been less explored for cathodic biofilms. Cathodic biofilms that can be sustained by accepting electrons from an electrode should be grown on electrodes that are carefully and rationally modified. Once more, it is striking to note that the rapid development of bioanodes contrasts the more lightly-studied nature of biocathodes.

An important and tunable factor for promoting biofilm development is surface roughness, with near-atomically flat surfaces generally taking more time to be colonized than those with roughness at least on the order of the average bacterial size (ca. 1 micrometer)[108]. Indeed, highly porous rough electrode materials show significantly improved biomass concentrations (mass of cells and extracellular substances per unit projected/geometric surface area) and current generation compared to smooth and planar electrodes[109,110]. Other factors that can influence biofilm formation on an electrode include the nature, amount and physico-chemical properties of the chemical groups present on the electrode surface. For example, we noted above that pretreatment of graphite electrodes by electrochemical oxidation in sulfuric acid affects the microbial composition of biofilms formed on graphite electrodes in marine sediment[111], and nitrogen-doping of carbon-based electrodes favors biofilm development and electrocatalytic performance[112]. However, neither physical, chemical or biochemical bases for these empirical results, nor the amount and/or the nature of the modifications are precisely known. As is now outlined, by conducting deliberately controlled surface modification, one can develop our knowledge so as to design conditions under which biofilm development and EET are promoted.

One study on the effects of surface modification on BES involves grafting aminophenyl groups onto graphite and subsequently using these modified graphite electrodes as anodes in microbial fuel cells (Figure 6). The aminophenyl-decorated electrode is more rapidly colonized by *Geobacter* than is graphite, and biofilms grown on the former are superior anodic catalysts[113,114]. Such a result can be rationalized in terms of the increased positive charge and hydrophilicity of the electrode surface, and electrodes decorated with carboxylate groups are less readily colonized, presumably because they repel the anionic *Geobacter* surface. Based on these results, it may not come as a surprise that the incorporation of triphenylphosphonium groups on electrode surfaces proved beneficial for dense colonization and electrocatalytic performance. In this case the effect is not only a result of modifying the biofilm/electrode interface but also might involve triphenylphosphonium diffusing into the biofilm. Delocalized lipophilic cations bearing triphenylphosphonium groups are indeed widely incorporated into drugs as they confer aqueous solubility yet can also cross hydrophobic cell or mitochondrial membranes[115]. It is thus likely that the effect of surface chemistry on biofilm performance is a complex combination of electrostatic interaction and lipophilicity. Aside from modifying charge and lipophilicity, there are more specific ways by which one can engineer electrode surfaces. In one example, electrodes decorated with phenylboronic acid groups are quickly colonized in a mixed culture inoculum, with the high activity of the resulting bioanodes rationalized in terms of specific binding to carbohydrates on the outer membrane of cells[116], although subtle interactions of the outer bacterial membrane with the exopolymeric biofilm scaffold may also play a role. Related to lipophilicity, the wettability of an electode is also important, and when indium tin oxide is suitably modified it supports *Shewanella loihica* biofilms that afford high current density. Here it is thought that redox potential shifts of outer membrane-bound cytochrome haem(s) are effected by being in a polar environment that allows for greater currents at a given applied potential[117].

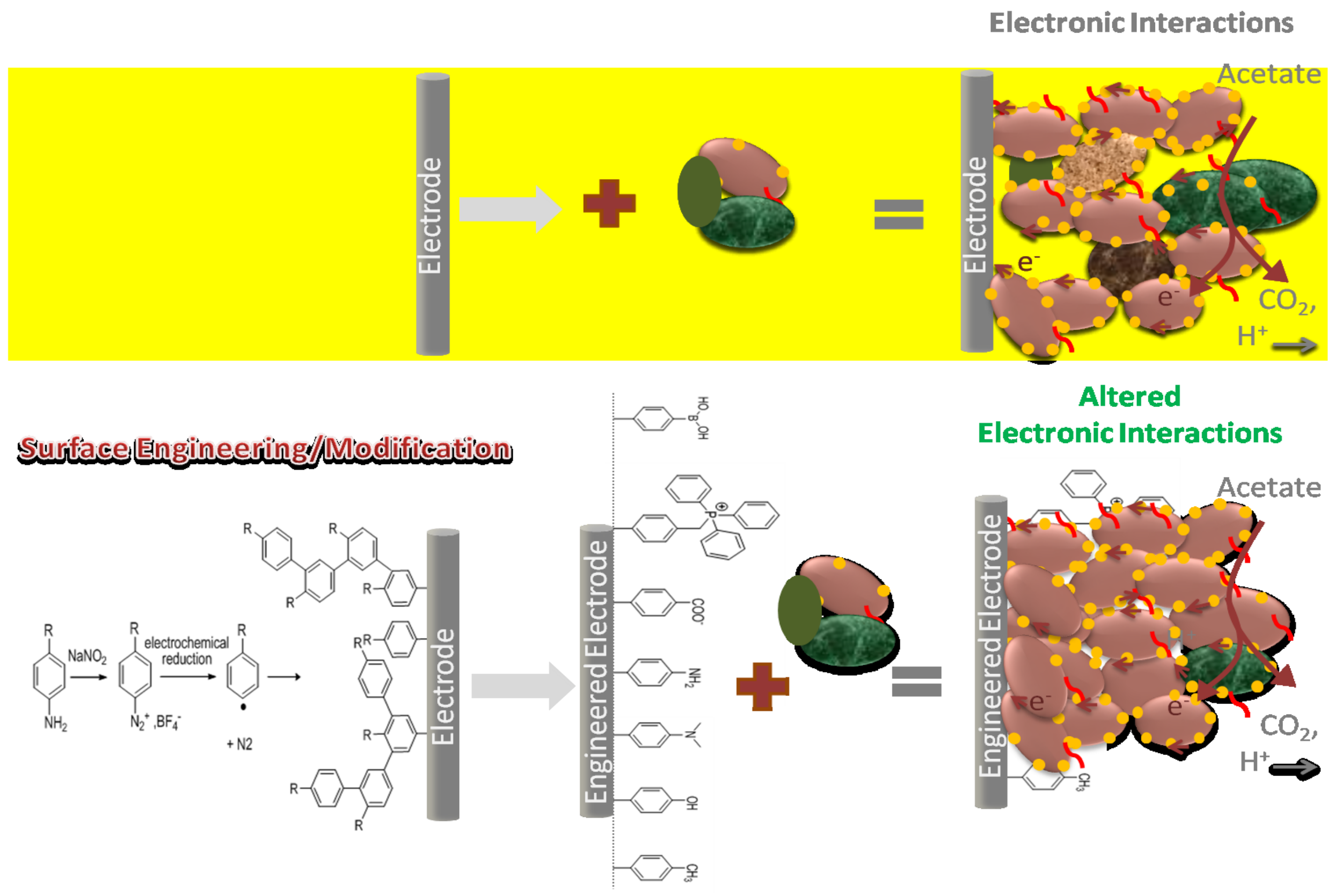


Figure 6. Surface engineering of the microbe-electrode interface alters microbial-electrode interactions for acetate-oxidizing bioanodes. A functional group, R (where R is boronic acid, triphenylphosphine, carboxylate, amine, dimethylamine, hydroxyl or methyl groups from top to bottom on the engineered electrode), is grafted over the electrode surface via *in-situ* diazotization of an arylamine and subsequent electrochemical reduction, providing an engineered electrode with physico-chemical characteristics that can alter microbial-electrode interactions.

The surface modifications discussed thus far involve rather simple and rational treatments whereby cell attachment to an electrode can be promoted. A more promising and less studied approach is to modify an interface specifically to improve electron transfer rates between biofilms and the electrodes. There is a wealth of information on controlling protein-surfaces interactions[118] and on optimizing electron transfer between isolated redox proteins[119]. In systems containing the archetypal haem protein *c*-Cyt, the distance between the haem and electrode of course has a strong influence on ET. Moreover, the orientation of the haems relative to the surface is also key[120,121] - haems parallel to the surface exhibit higher ET rates than those perpendicular to the surface, with ET through axial ligands bound to iron haems apparently being faster than ET through the porphyrin ring[122]. The parallel orientation is favored on a hydrophilic surface whilst a perpendicular orientation is favored on a hydrophobic surface. As a consequence, an enhancement of electrocatalytic biofilm performance is observed on hydrophilic surfaces, underscoring the mechanistic insights that can be gleaned from such studies. In addition to modifying electrode surfaces, one can use redox and/or conducting polymers[123,124] and/or nanomaterials to electrically wire microorganisms to electrodes. Alternatively, one might connect metabolic processes inside cells to electrodes outside cells in a manner analogous to that used to wire redox enzymes to electrode surfaces[107,125]. Such an underutilized approach to engineer microbial BESs may expand the scope of useable microorganisms to encompass strains that are catalytically active but are unable electrically wire themselves to electrodes[126,127].

EET mechanisms may differ depending on whether electrons are entering or leaving a microbe. Despite this, it is encouraging that surface modifications that promote biofilm formation on anodes also tend to benefit biofilm formation on cathodes. For instance, introduction of cationic groups onto carbon cloth electrodes significantly improves formation and performance of *Sporomusa ovate* biocathodes in the electrosynthesis of acetate in a microbial electrolysis cell[128,129]. Similarly, decoration of a surface with carbon nanotubes – known to improve bioanodes – also enhanced mixed consortia biofilm formation and acetate production at the resulting biocathode[130]. This improvement was attributed to more favorable microbial adhesion provided by the carbon nanotube network, and is not simply a result of the increased surface area of the nanotube-decorated electrode relative to the bare electrode.

An important aspect in developing surface engineering for optimizing microbial BES will be clarify the effects of surface modification on the physico-chemical properties of an electrode, as well as the resulting an impact on biofilm development and performance. To this end, fundamental ET studies on immobilized redox proteins will continue to provide mechanistic insights. Approaches to effectively wire microbial layers to the electrode surface through the use of chemical modifications and redox mediators to surfaces also merit investigation, despite the challenges associated with confirming the specific mode(s) of binding involved.

Chemistry considerations of other BES components

It goes without saying that application of microbial half-cells requires them to be partnered with a half-cell of opposite polarity. In the case of microbial bioanodes, a suitable cathodic half-cell is needed to form a working microbial fuel cell or microbial electrolysis cell. The most plentiful (and cheap) oxidant is O_2, a molecule that typically poisons electrocatalytic anaerobes. Therefore, most bioanodes are typically coupled with abiotic O_2-reducing cathodes through an ion exchange membrane as a salt bridge. This component is either separate from or integrated with the cathode to afford an air cathode, with the bioanode being protected from O_2 but not electrically insulated from the cathode. The remaining problems are nontrivial - the conditions under which oxygen reduction to water is favored typically differ to those amenable to electrocatalytic bacteria[131], which demand neutral pH and relatively low temperatures – conditions that favor neither ion transport through membranes nor oxygen reduction. Studies that identify more durable electrocatalytic organisms and/or those that protect existing electrocatalytic organisms under harsher conditions will make BESs significantly more effective. Aside from engineering the bioanode, the development of O_2-reducing cathodes optimized for neutral pH, as well as ion exchange membranes able to operate at lower temperature, also offer research opportunities. The above discussion also applies to microbial biocathodes, which are typically paired with anodes that oxidize water in a separate half-reaction. In this case, an ideal anode is one that affords high currents at low overpotentials and at the physiological conditions required by the biocathode.

Outlook

Bioelectrodes presently afford only modest reaction rates and yields. Improved performance can come only through a more detailed understanding of the composition and spatial organization of all extracellular substances under physiologically relevant conditions. Although Raman microscopy allows one to confirm the presence of redox proteins in *Geobacter* bioanodes at the single cell level[132], the study of BESs would benefit greatly from imaging at the single molecule level. Complementary work would address the identification, isolation and characterization of key EET pathways by making use of genetic mutations, differential proteomic and metabolomic studies, as well as NMR and crystallographic structural determinations of isolated proteins and complexes. Other advanced *in situ* analytical techniques such as conducting-probe atomic force microscopy, electrostatic force microscopy, electrochemical-surface plasmon resonance and electrochemical quartz crystal microbalance, when applied on living biofilms, will help elucidate electron conduction pathways. Approaches to wire microbial layers to electrode surfaces through application of surface modification and/or addition of redox mediators also warrant further study. The development of matrices that encapsulate and protect microbes without inhibiting electrocatalytic activity (including the ability to perform EET) appears attractive in that it may expand conditions under which microbial electrocatalysts can operate. Additionally, there exists a need to identify exoelectrogenic organisms able to operate under conditions also suitable for O_2-reducing cathodes and/or ion transport through separators. In parallel, the development of cathodes and separators that function optimally at mild pHs and temperatures would also be beneficial. Aside from finding the best possible reaction conditions, it is desirable to understand biofilm responses under various system configurations[133–135]. In this regard, further electrochemistry will prove useful[13], as will a greater control and characterization of electrode conductivity, system geometry and hydrodynamics[93, 133, 135].

Anodic electroactive bacteria are typically chemotrophs (mostly chemoorganotrophs), occurring in environments devoid of O_2 or other soluble electron acceptors. EET takes place right under our feet – in soils, sediments and aquatic biofilms – and likely has an important role in biogeochemical processes. Thus, a better understanding of EET may not only lead to improved METs and BESs, but also contribute to our understanding of the cycles occurring on our planet. Appreciating the ins and outs of microbe-electrode electron transfer reactions requires a truly interdisciplinary approach, one that promises improved EET in BES approaches key to many emerging technologies including the generation of energy or chemical feedstocks from waste or renewables.

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Conflict of financial interest statement

We don't have a conflict of financial interest.

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