Electrochemical communication between microbial cells and electrodes via osmium redox systems

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Abstract
Electrochemical communication between micro-organisms and electrodes is the integral and fundamental part of BESSs (bioelectrochemical systems). The immobilization of bacterial cells on the electrode and ensuring efficient electron transfer to the electrode via a mediator are decisive features of mediated electrochemical biosensors. Notably, mediator-based systems are essential to extract electrons from the non-exoelectrogens, a major group of microbes in Nature. The advantage of using polymeric mediators over diffusible mediators led to the design of osmium redox polymers. Their successful use in enzyme-based biosensors and BFCs (biofuel cells) paved the way for exploring their use in microbial BESSs. The present mini-review focuses on osmium-bound redox systems used to date in microbial BESS and their role in shuttling electrons from viable microbial cells to electrodes.

Microbe–electrode interface
The ability of micro-organisms to transfer electrons to electrodes was first reported by Potter in 1911 [1]. After several years of negligence (with one exception [2]), research attempts during the 1980s brought this interesting technology to the public’s attention [3,4]. However, this obscure field of research was later revived during the end of the 20th Century mainly because of growing concerns over the exhausting fossil fuel reserves, their detrimental effect on the environment and the demand for new sustainable energy, and attracted considerable attention thereafter [4]. The breakthroughs in natural EET (extracellular electron transfer) abilities of mineral-respiring bacteria (referred to as exoelectrogens) provided a foundation for the development of BESSs (bioelectrochemical systems) such as MFCs (microbial fuel cells). MFCs operate to produce sustainable energy by diverting bioconvertible energy to electricity directly by using an anode as an insoluble electron acceptor in place of natural acceptors. In addition to electricity generation, the anode of an MFC can be used to offset the voltage required for electrical-current-driven chemical production at the cathode, referred to as microbial electrocatalysis, expanding the application range of these systems [5]. Furthermore, microbe–electrode interactions have been exploited extensively for application as electrochemical microbial sensors [6]. Regardless of the bioelectrochemical device, ET (electron transfer) from microbes to the electrode is the most imperative and critical task that defines the theoretical limit of the energy conversion [7]. To facilitate cell–electrode communication, electrons produced during respiration exit the bacterial cell membrane either by direct physical transfer of reduced compounds or via electron hopping across the membrane using membrane-bound redox proteins [8]. In the present mini-review, we confine ourselves to discussing recent reports on EET from micro-organisms to electrodes via addition of synthetic osmium-based redox complexes and their applicability to BESSs.

Microbial EET
Microbial EET to electron acceptors other than oxygen is necessary for anaerobes, where mainly minerals containing iron and manganese oxides are reduced. By linking microbial metabolism to electrodes via EET, electrical current can be generated or consumed in BESSs [9,10]. The anodic ET is based on exploitation of the necessity of living cells to dispose electrons liberated during oxidative substrate degradation. The proposed mechanisms involved in microbial ET to electrodes are illustrated in Figure 1. These are mainly classified as DET (direct electron transfer) and MET (mediated electron transfer) [7].

In DET, electrons can be transferred directly to the electrode proposed to be either via cell membrane-bound cytochromes [11,12] or via electrically conductive pilus, referred to as bacterial nanowires [13–15] (as depicted in Figure 1A). DET takes place by close physical contact of the bacterial cell or any organelle of the membrane with the electrode and without involvement of a soluble redox species. The participation of cytochromes localized in the outer membrane in DET has been reported for several
Proposed mechanisms of ET from micro-organisms to the electrode

(A) DET via cell membrane-bound cytochromes and electrically conductive pili (nanowires). (B) MET via microbial or exogenous redox mediators. $M_{ox}$ and $M_{red}$ indicates mediator in oxidized and reduced state respectively.

iron-reducing bacteria, including *Shewanella putrefaciens* [16], *Rhodoferax ferrireducens* [17,18] and several *Geobacter* species [12,17,19]. Additionally, it has been suggested that, for some bacteria such as *Geobacter sulfurreducens* and *Shewanella oneidensis* MR-1, conductive pili allow them to use a distant electron acceptor [13,15,20]. Although DET has been demonstrated for some microbes, it has limitations in terms of coulombic efficiency (the proportion of ET) and the rate of ET (i.e. the current generation) [7]. In practice, few micro-organisms are able to transfer electrons directly to the electrode. In addition, organisms possessing DET capability, such as *Geobacter* and *Shewanella* strains, are unable to utilize complex substrates and metabolize only low-molecular-mass organic acids and alcohols that consequently limits their large-scale applications in BESs [21]. Nevertheless, at the moment, DET-based BESs are the first choice for most researchers for exploration and exploitation of microbe–electrode interactions.

MET is an EET mechanism that occurs via the involvement of redox mediators (Figure 1B). These mediators can be artificial or metabolites produced by the micro-organisms [7]. Remarkably, redox mediators produced by one bacterium can also be used by other bacteria to reach the electrode [8]. Mediators are, in general, electron shuttles that can penetrate the cell membrane, gain electrons from the electron carrier within the cell, leave the cell in a reduced state and ultimately transfer electrons to the electrode. Mediators are essential for microbes that are unable to transfer electrons from the central metabolism to the outside of the cell. The advantage of using mediators in amperometric biosensors is the possibility of measurements at lower overpotential that minimizes interfering reactions contributing to the response signal and therefore increases selectivity. The EET mechanisms in microbe–electrode-based systems have been well documented in several review articles [7,22,23]. In general, most redox mediators are toxic to the bacterial cells at higher concentrations. Therefore their concentration is kept in the micromolar range, which can constrain the overall BES performance. In addition, it is not feasible to use diffusible artificial mediators in most BESs because of requirements to continuously add them to a system. Furthermore, most freely diffusing mediators cannot compete efficiently with natural electron acceptors. To overcome problems associated with the use of diffusible mediators in enzymatic biosensors, ET to electrodes can be established by ‘wiring’ the enzyme with polycationic, water-soluble and highly flexible redox polymers bound to the electrode [24,25]. The communication between the enzyme, such as GOx (glucose oxidase) and an electrode was improved further using osmium-based redox polymers, since they possess long-term redox stability and suitable redox potential to wire GOx [25]. The use of polymeric redox mediators has proved to be convenient because of the synthetic flexibility, opening up the possibility of manipulating the formal potential, the hydrophilicity/hydrophobicity and their electron-shuttling properties [26–28]. Therefore the exploration of various osmium redox polymers has attracted increasing attention in enzyme-based BFCs and biosensors [29–34].

Development of microbial BESs and whole-cell biosensors may thus be possible through exploiting versatile bacteria that are non-exoelectrogens, but may be wired by redox complexes. Here, the term ‘wiring’ implies that the observed current from electrochemical communication between microbe and electrode comes from the redox species incorporated in the cell membrane rather than soluble redox-active cell exudates [35].

**Osmium redox polymers**

Biofilms on electrodes containing redox polymers can transform electrically insulating co-immobilized redox proteins into redox-conducting systems [26] and establish effective ET
Table 1 | List of different micro-organisms ‘wired’ electrochemically with electrodes via various osmium redox systems

<table>
<thead>
<tr>
<th>BES/device</th>
<th>Micro-organism</th>
<th>Type of osmium system</th>
<th>Redox potential ($E^\circ$) compared with the Ag/AgCl electrode (0.1 M KCl) (V)</th>
<th>Working electrode</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFC/microbial sensor</td>
<td>G. oxydens ATCC 621</td>
<td>I</td>
<td>0.140</td>
<td>Gold</td>
<td>[39]</td>
</tr>
<tr>
<td>Microbial sensor</td>
<td>Ps. putida ATCC 126633</td>
<td>I</td>
<td>0.140</td>
<td>Gold</td>
<td>[40]</td>
</tr>
<tr>
<td></td>
<td>Ps. fluorescens DSM 6521</td>
<td>II</td>
<td>−0.195</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbial sensor</td>
<td>Ps. putida DSMZ 50026</td>
<td>I</td>
<td>0.140</td>
<td>CNTs and carbon paste</td>
<td>[41]</td>
</tr>
<tr>
<td>MFC/microbial sensor</td>
<td>E. coli JM109 (WT)</td>
<td>I</td>
<td>0.140</td>
<td>Graphite</td>
<td>[42]</td>
</tr>
<tr>
<td></td>
<td>E. coli JM109/pBSD 1300*</td>
<td>II</td>
<td>−0.195</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. coli JM109/pLUV 1900‡</td>
<td>I</td>
<td>0.140</td>
<td>Graphite and gold</td>
<td>[43]</td>
</tr>
<tr>
<td>MFC/microbial sensor</td>
<td>B. subtilis (WT)</td>
<td>I</td>
<td>0.140</td>
<td>Graphite and gold</td>
<td>[44]</td>
</tr>
<tr>
<td></td>
<td>B. subtilis 36G18-pBSD-1200¶</td>
<td>II</td>
<td>−0.195</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MFC</td>
<td>R. capsulatus ATCC 17015 (WT)</td>
<td>III</td>
<td>0.132</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. capsulatus 37b4 (capsule-lacking strain)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbial biosensor</td>
<td>P. vulgaris NCT 4175</td>
<td>IV</td>
<td>0.200</td>
<td>PPF-SWCNTs assembled on carbon surface</td>
<td>[35]</td>
</tr>
<tr>
<td>MFC</td>
<td>H. polymorpha 356 (WT)</td>
<td>V</td>
<td>0.121</td>
<td>Graphite</td>
<td>[45]</td>
</tr>
<tr>
<td></td>
<td>H. polymorpha tr1§</td>
<td>VI</td>
<td>0.061</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MFC</td>
<td>S. cerevisiae NCTC 10716</td>
<td>VII</td>
<td>0.041</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV</td>
<td>0.200</td>
<td>PPF-SWCNTs assembled on carbon surface</td>
<td>[46]</td>
</tr>
</tbody>
</table>

*Strain overproducing the membrane anchor domain of B. subtilis SQR succinate-quinone reductase.
†Strain overproducing cytochrome $c_{550}$ from B. subtilis.
¶Strain overproducing succinate-quinone oxidoreductase (respiratory complex II).
§Genetically modified strain that overexpress FCb2.

from buried redox protein centres to electrodes. Electrostatic interactions between osmium redox polymers and the protein channel leading to the redox centre of Gox reduces the ET distance and triggers bioelectrocatalytic oxidation of glucose [36]. The redox polymers can be designed to be water-soluble, simplifying the steps required to coat electrodes with films of polymer and redox enzymes, and ensuring that the films are sufficiently hydrated to permit efficient ET throughout a three-dimensional network permitting incorporation of a large number of enzyme molecules [26]. The combination of redox polymers and enzymes with a cross-linker leads to the formation of redox hydrogels that can wire enzyme redox centres irrespective of their spatial orientation, as well as forming multilayers on electrodes that result in much higher current responses compared with monolayers [37]. Such redox hydrogels are used in amperometric biosensors for measurement of analyte (enzyme substrate) concentrations and as enzymatic BFC anodes to increase the current density. Readers are referred to a review [37] for detailed information about redox hydrogels. Details on synthesis of osmium redox polymers are described in [28,34,37,38]. This successful application in enzymatic biosensors and BFCs has raised interest in their possible usage for wiring micro-organisms to electrodes over the last decade.

Wiring of microbial cells to the electrode via osmium redox systems

Table 1 summarizes details of osmium redox systems used to date for wiring micro-organisms in bioelectrochemical devices. To the best of our knowledge, the first application of an osmium system for wiring intact bacterial cells
was reported by Vostiar et al. [39]. They demonstrated efficient electrochemical communication between Gram-negative *Glucobacter oxydans* and gold electrodes with the aid of osmium redox system I having high redox potential and short side chain (see Table 1 for details). *G. oxydans* was chosen because of its known ability to produce periplasmic membrane-bound PQQ (pyrroloquinoline quinone)-containing enzymes that can efficiently oxidize a wide variety of substrates. The efficient wiring was attributed to ET between PQQ dehydrogenases and the osmium redox polymer. Thereafter, Timur et al. [40] investigated two different polymers based on system I and system II, with a lower redox potential and the longer side chain introducing increased flexibility for motion of the redox complex, to wire *Pseudomonas putida* and *Pseudomonas fluorescens* with electrodes. They found that wiring of bacterial cells with system II showed much higher substrate (catechol, phenol and glucose) sensitivity compared with that of system I. This was attributed to the good contact between the longer tethered redox complex in system II and respiratory enzymes in the *Pseudomonas* strains. A more efficient electrochemical response was subsequently reported by using CNT (carbon nanotube)-modified carbon paste electrodes containing system I to form a *Ps. putida*-based biosensor for phenol detection [41].

In relation to the improvement of bioelectrocatalytic current generation, Allerov et al. [42] investigated the use of two different cytochrome-enriched strains of the model bacterium *Escherichia coli*. Neither of the strains showed any detectable electrochemical response on electrodes because the periplasmic space and outer membrane together offer an insulating thickness of 15 nm, hampering ET out of the bacterial cell. However, in the presence of osmium redox systems, electrochemical communication between cells and electrode was facilitated. Among two different redox systems, system II showed a better current response, again postulated to be because of greater flexibility of motion of the redox complex, enhancing accessibility to the active site of redox enzymes in the inner membrane. Previously, it was assumed that it would be difficult for osmium redox systems to permeate the thick cell wall of Gram-positive bacteria, which consists of a peptidoglycan layer of a thickness of approximately 35 nm. Surprisingly, Coman et al. [43] revealed that the Gram-positive bacterium *Bacillus subtilis* can communicate with electrodes via both osmium redox system I and II. This was ascribed to the fact that the polyanionic properties of cell wall components, e.g. peptidoglycan and teichoic acids, contribute to electrostatic interactions with polycationic redox polymer systems resulting in electrochemical communication. Among two strains investigated by Coman et al. [43], the strain overproducing SQR (succinate:quinone reductase) showed better performance with succinate than the wild-type strain. Recently, Rawson et al. [35] reported that single-walled CNTs functionalized with an osmium complex (system IV) can electrochemically communicate with *Proteus vulgaris* [35], simplifying the electrochemical approach to microbial sensor development by excluding the need for soluble mediators, while providing a whole-cell biosensor system for ethanol, sodium azide and ampicillin with long-term stability. Lately, we have wired the metabolically versatile purple bacterium *Rhodobacter capsulatus* (wild-type and capsule-lacking strain) with system III to examine the applicability to BFCs and photobioelectrochemical devices [44]. The wild-type cells embedded in the osmium polymer matrix showed a greater ability to produce a significant and stable current due to succinate oxidation, compared with the capsule-lacking strain, demonstrating that the bacterial lipopolysaccharide improves the stability of the redox polymer matrix layer on the electrode.

The idea of employing yeasts as a substitute to bacteria in BESs is surfacing, since they are robust and generally non-pathogenic. With regard to their capability to metabolize a wide range of substrates and high growth rates, Shkil et al. [45] reported the electrochemical communication of *Hansenula polymorpha* with a graphite electrode, by wiring with osmium redox systems V, VI and VII. Here, genetically modified yeast cells that overexpress FCb2 (flavocytochrome *b*) generated more significant current with the aid of these osmium redox systems compared with that observed for the wild-type cells, highlighting that a plasma membrane redox system (FCb2) is crucial for ET. Rawson et al. [46] recently examined ET from *Saccharomyces cerevisiae* on carbon electrodes that had been modified to introduce an osmium redox system (system IV) [46]. In this case, a stable surface-confined thin layer of osmium complex was introduced to a smooth electrode surface to facilitate ET directly from the external surface of the cell wall to the redox system, opening up queries on the capability of yeasts to ‘shed’ electrons to external acceptors. All of these reports highlighted that the conductive properties of osmium redox complexes promote a good electrochemical communication between electron-offering systems in the microbes and the electrode (as illustrated in Figure 2). However, the mechanisms of electron shuttling from the microbial cells to electrode via osmium polymers have not yet been thoroughly investigated and are still unclear. Predominantly, the electrical wiring of micro-organisms with electrodes is proposed to be due to the strong electrostatic interactions between the negatively charged bacterial cells and the positively charged osmium complexes bound to the polymer matrix at electrodes (Figure 2A). The close contact of the osmium-bound complexes to the cell wall or cell membrane of microbes and immobilization of the microbial cells inside the polymer matrix (Figures 2B and 2C) for electron shuttling to the electrode are proposed to be essential for efficient electrochemical communication.

In summary, studies of interactions between osmium redox systems and micro-organisms open new possibilities for basic bioelectrochemical studies, for reagentless biosensing and perhaps for the construction of more robust and improved performance MFCs. On the basis of published reports, we anticipate that the use of osmium systems (or similar redox polymers) as electron shuttles can enable the possibility of exploration of a wide range of microbial catalysts.
Figure 2 | Proposed schemes for EET from micro-organisms to electrode via osmium redox polymers
The ET occurs via sequential electrical conduit starting from the microbial cell to external osmium redox centres embedded in the polymer backbone and finally towards the electrode surface (A). Based on [44]. (B and C) Exemplary schemes that show electrical wiring of Gram-positive bacterial cell [43] and yeast cell [45] respectively to the electrode via osmium complexes.

(in particular the non-exoelectrogens) for applications as both bioanodes and biocathodes in BESs. With further investigation, we assume that the use of osmium-system-modified electrodes may simplify the design and the operational use of microbial biosensors, particularly for on-site applications. For such applications, the long-term stability of films of these systems on electrodes is among the major concerns. In addition, the relative amount of osmium complex within the system used to modify electrodes is a crucial aspect, as any excess amount might hamper the transfer of electrons from the microbes to the osmium centres. Furthermore, the introduction of cross-linkers such as poly(ethylene glycol) diglycidyl ether or methods to anchor the osmium systems to pre-treated electrodes might help to overcome stability concerns [35,46]. We have recently explored the electrode surface modification with osmium polymer and observed a boost in current generation with the well-known exoelectrogen S. oneidensis MR-1 at graphite electrodes using osmium system III [47]. Several advantages associated with these systems, such as ease of immobilization, high synthetic flexibility, excellent redox conductivity, possibility of forming multilayer scaffolds and strong electrostatic interactions with microbial cell surfaces, makes their use promising and opens a new horizon in microbial BES research.

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