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Mohamad Nasir Mohamad Ibrahim, Asim Ali Yaqoob and Akil Ahmad

Chapter 11

Influence of micro-organisms on the electrochemical process of microbial fuel cells

Rizwana Kausar and Faiz Ur Rahman

Micro-organisms act as a biological catalyst in microbial fuel cells (MFCs) to generate electrical energy by oxidizing an organic substrate. Biofilm formation is the key characteristic of micro-organisms, which has a profound influence on the MFCs' performance. Extracellular polysaccharides affect greatly the formation, development, and regulation of biofilms and quorum sensing in micro-organisms. The use of biofilm-producing bacteria and consortia versus pure cultures shows the production of high-density current in MFCs. The microbial metabolism is another important factor in MFC electrogenesis. Micro-organisms with various ways of transferring extracellular electrons (the basis of electricity generation in MFCs) without utilizing artificial means are of great importance in improving the MFC performance. The presence of specific proteins and genes in micro-organisms is related to the various mechanisms for the transfer of electrons. Similarly, the choice of the substrate, such as a coastal lagoon, anaerobic sludge of wastewater, rice paddy soil, etc, is also shown to have an impact on the amount of energy production by MFCs. This chapter discusses the formation, development, and regulation of biofilm and the factors influencing it. It also discusses the impact of microbial metabolism in electrogenesis, and various processes of extracellular electron transfer in MFCs which can influence the bioelectrochemical process in MFCs.

11.1 Introduction

Microbial fuel cells (MFCs) use micro-organisms as biological catalysts to generate electrical energy by oxidizing an organic substrate. Protons and electrons are released from the organic substrate as a result of oxidation by the micro-organisms. An electrical current is generated when micro-organisms take these free electrons and transfer them to an anode which is then transferred to a cathode, hence

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generating an electrical current [1]. This electron transfer is carried out by micro-organisms by producing electron shuttles [2], electron mediators [3], or numerous filaments, also called microbial nanowires [4]. Simultaneously, at the cathode protons combine with an electron acceptor such as oxygen and electrons and produce water [1].

MFC technology is a sustainable way of producing bioelectricity as it offers the availability and flexibility of using diverse groups of micro-organisms which can utilize a vast range of organic waste. MFC technology may be helpful not just for producing bioenergy to satisfy rising global energy demands in an era of limited non-renewable energy resources, but also for organic waste biodegradation [5]. As a consequence, it is a promising energy harvesting technology with a wide range of applications, including bioremediation, sensors, and powering electronic monitoring equipment [6]. The basics of MFC design and operation are well understood at this time, but technical issues, particularly microbiological ones, are still under investigation (figure 11.1). MFCs have not yet achieved extremely high power densities, limiting their real-world application. The design and microbiological composition of these devices may be improved further. The most significant microbiological factors that influence an MFC's efficiency are biofilm formation, bacterial metabolism, and bacterial electron transfer.

It has been found that a range of micro-organisms (mostly bacteria) from different taxonomic groups may generate electricity without the need for a mediator in MFCs. Electrical current has been seen in five phyla of Proteobacteria, Firmicutes, and Acidobacteria, as well as using microalgae, yeasts, and fungi as the substrate or to assist the cathode or anode in MFCs. There is not a specific nomenclature at present, and the micro-organisms that assist exogenously in transporting of electrons from the anode with no need of an artificial mediator are known by different names [7]. Some of the terms used by different authors for

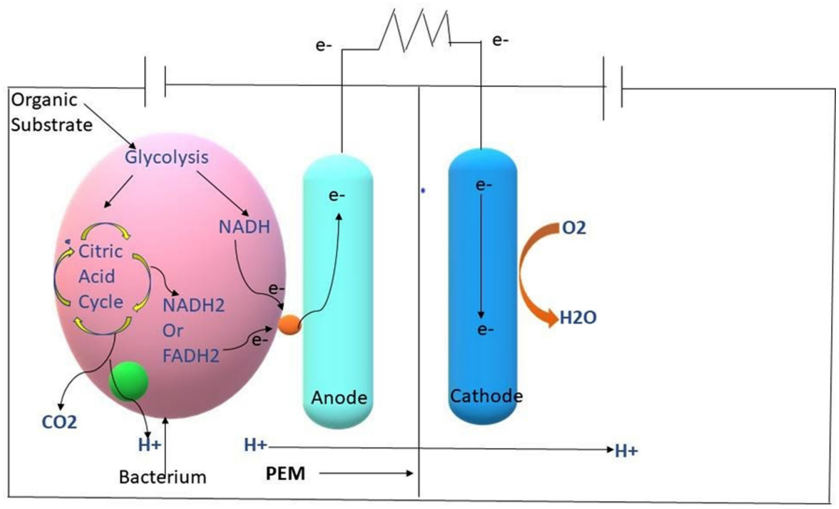

Figure 11.1. Overall principles of microbial fuel cells.

these micro-organisms are anode-respiring bacteria, exoelectrogens, electricigens, electrogenic micro-organisms, anodophiles, and electrochemically active bacteria. For example, based on their functions in MFCs, iron-reducing and sulphate-reducing bacteria may be called iron reducers and sulphate reducers [7]. Electrode reducers provide electrons to the electrode (anode) in MFCs, while electrode oxidizers remove electrons away from the electrodes. The most common bacterial species that generate electricity in MFCs are *Geobacter* spp., *Shewanella* spp., *Clostridium butyricum*, *Rhodoferax ferrireducens*, *Aeromonas hydrophila*, and *Pseudomonas aeruginosa* [8–14]. Microalgae have been used as biocathodes or substrates in MFCs [15]. Excellent current is produced in an MFC by *Hansenula anomala* and *Saccharomyces cerevisiae* [16]. However, it does not seem that the utilization of yeasts for MFC based energy generation has been investigated extensively. *Synechocystis* sp., a phototrophic oxygenic cyanobacterium, can produce electricity in MFCs and nanowires [17]. The micro-organisms that can transfer electrons to the anode because of complete oxidation of organic compounds at a faster rate may make a significant contribution to power generation. The increase in current density has been observed when micro-organisms in a biofilm on an anode directly transfer electrons between themselves and the anode. The micro-organisms in a mixed culture biofilm can produce current densities higher than a biofilm of pure cultures [18]. For example, a pure culture of *Brevibacillus* sp. in an MFC produced less electricity compared to when it was grown with *Pseudomonas* sp. [19]. Micro-organisms with a capacity for dissimilatory metal reduction may produce power efficiently in a mediatorless MFC. Such bacteria can interact directly with cytochromes in their outer membrane or excrete electron shuttles to transfer electrons. After that, micro-organisms were also found to produce microbial nanowires (protein appendages) which help in electron transfer. *Pelotomaculum thermopropionicum* was found to have an electrically conductive appendage connected to *Methanothermobacter thermautotrophics*, allowing interspecies electron transfer [9]. Quorum sensing is another important mechanism of communication inside microbial biofilm. It is carried out by sensing chemicals such as fatty acyl homoserine lactones [20]. Pyocyanin is a signalling molecule generated by *P. aeruginosa* that works not only as an electron shuttle but also regulates the transcription of the quorum sensing genes [21].

This chapter discusses the influence of microbial mechanisms that benefit MFC technology, such as the formation and development of biofilm by micro-organisms, the factors influencing the biofilm development, microbial metabolism and electrogenesis, various types of electron transfer between micro-organisms and electrodes, and a description of micro-organisms suitable for electricity generation.

11.2 Microbial influence on MFCs via the production of biofilm

The essential function of the biofilm and its developmental phases on the anode surface are particularly significant in the MFC system for energy recovery. Bacteria prefer to live in a biofilm, which is a polymeric matrix (containing proteins, lipids, carbohydrates, and other components) produced by bacteria adhering to a surface.

Producing electroactive biofilms in MFCs is important for producing energy more efficiently. Biofilm formation is affected by several variables, including the electrode material, the substrates, the microbes used in the MFC, and the operational settings of the MFCs. Biofilm formation is also affected by the morphological and physiological features of the electrode surface. Micro-organisms prefer to adhere to hydrophobic surfaces rather than hydrophilic surfaces [22]. Biofilm is formed on the electrode surface because of cell-to-cell communication among microbial communities and secretion of extracellular polysaccharides (EPS) [23]. The immobilized bacteria in the biofilms on the surface of electrodes bridge the way for electron transfer and its interaction with electrodes in MFCs [24, 25]. The shorter the distance for electrons to reach the electron acceptor, the higher will be the efficiency of electron transfer [26]. The response of MFCs is significantly affected by the development of biofilm on the anode as a surface attachment, biofilm maturation, substrate diffusion, and electron transport occur in this zone [22, 27]. Therefore, the important factor of the electrochemical reaction of an MFC is a biofilm that is developed on the electrode. Several spectroscopy methods are used to study the biofilm's microbial community on the anode, for example Raman spectroscopy [28] and crystal violet assay [29], and microscopic methods such as confocal laser scanning microscopy [30], scanning electron microscopy [31], and atomic force microscopy [32]. All these spectroscopic and microscopic techniques were employed exclusively to evaluate the developed biofilm. However, the performance of MFCs is influenced by several restricting factors that can be identified by the time taken for biofilm development on the anode and the correlation with the electrochemical reaction taking place there [33].

According to earlier studies, bacteria that are unable to develop biofilms on the electrode are not capable of producing considerable current intensities in MFCs, while dense microbial biofilm formation at the anode produces steady and higher current densities. Thus dense biofilm-producing bacteria have more electricity generating potential in MFCs compared to bacteria that develop thin biofilms. Confocal imaging results showed that generally gram-positive bacteria develop thicker biofilms (~38 μm), producing a constant current density of 7–8 Am^2 [34], whereas gram-negative bacteria that construct monolayer biofilms produced significantly lower current densities [35].

11.2.1 The formation of biofilm

The formation of biofilms is caused by transferring micro-organisms to a surface, then adhering them on that surface (in MFCs, on the cathode or anode), establishing microcolonies, then developing biofilms [36]. Bacterial cells produce adhesins, which connect and encapsulate the bacteria in a biofilm made up of carbohydrates (polysaccharides), nucleic acids, and proteins [37]. The ability of electroactive biofilms to accept terminal electrons produced by metabolism and transfer them to electrode surfaces is their distinctive feature [38]. For biofilm development in *Shewanella* spp. and *Geobacter* spp., the c-type cytochromes are found to be significantly important [8, 9], while type IV pili protein, which is made up of PilA

monomers, is mainly important for the formation of conductive biofilms in *Aeromonas* spp. and *Geobacter* spp. [14, 39]. Biofilm growth and consequently current generation were decreased in *Geobacter sulfurreducens* mutants in the *pilA* and *omcZ* genes, indicating a role for the protein pili and c-type cytochromes in biofilm development [40]. The oxidation–reduction active compounds, such as flavins in *Shewanella* spp., help in exocellular transport of electrons in biofilm [41]. With the help of flagellar movement, bacteria are transferred to the surface in *P. aeruginosa* biofilms. Type IV pili stimulate cellular aggregation and microcolony growth, and a maturation phase involving cell-to-cell communication leads to the production of mushroom-shaped biofilms [42–44]. Quorum sensing is an interaction and coordination process that enables bacterial populations to transmit and organize their actions. In *P. aeruginosa* and other bacteria, quorum sensing controls the expression of genes related to the biofilm and is crucial for the formation of biofilm [45, 46].

Pure culture biofilms generate lower power densities, while mixed culture biofilms provide greater power densities. An MFC of mixed culture generated approximately 20% more energy than a similar MFC using pure culture [47]. The role of non-exoelectrogens (micro-organisms that cannot generate electric current when cultivated in pure cultures) in power generation, however, remains unclear. Bacterial cells in monolayer biofilms remain close to the anodic surface and directly transfer electrons with the help of c-type cytochromes or electron shuttles to the anode. Pili have been found to help in electron transport from distant cells to the anode surface in thick multilayer biofilms [48]. Oxygen reduction on the cathode with the help of micro-organisms is a focus of research to make a cathodic biofilm as for anodic biofilms. Unlike anodic biofilms, power generation in cathodic biofilms has been shown to decrease as thickness increases [49].

11.2.2 The role of extracellular polysaccharides in biofilm regulation and formation

As a result of the development of an internal layer of inactive or dead cells in the biofilm matrix, the density of the biofilm that develops under natural conditions increases with culture age, but it is not wholly electrochemically active [50]. As a result, a method that encourages living or active micro-organisms to build more electroactive biofilms is seen as a potential way to improve MFC power performance. The major biofilm components and their function in the development of biofilm must be realized to accomplish this. Over time, our understanding of the complexity of microbial biofilms has increased [51]. Biofilms are described as a 'city of micro-organisms', with extracellular polysaccharides (EPSs) serving as the 'home of the biofilm cells', in a metaphorical depiction. The EPSs affect the porosity, charge, water content, density, hydrophobicity, absorption characteristics, and mechanical stability of cells in biofilms, dictating the immediate circumstances of life [51]. The organisms are discovered embedded in biopolymers. The density of the biofilm is proportional to the number of EPSs secreted by the organism. A denser biofilm may hold more organisms than a lighter biofilm [52]. As a result, as the age of the organisms increases, so does the density of the biofilm. As previously stated,

biofilm development on the anode is critical in MFCs. The electrochemical performance of a biofilm, on the other hand, is controlled by the physical and temporal positions of the living and dead cells and not by its density within the biofilm [50]. The fast decrease in charge transmission resistance in the presence of fast increasing living cells has been shown in studies of the electroactive bacterium *G. sulfurreducens*. However, when dead cells collect in the inner layer of biofilms, the electrochemical system faces a significant diffusion resistance over time. In such situations, it is assumed that living cells on the outer surface of biofilms help in active electron transfer and, instead of the density of the biofilms, impact the system's high current generation [50]. Apart from EPSs, the biofilm contains a significant number of polysaccharides, as well as minor amounts of glycoproteins, proteins, glycolipids, a small number of nucleotides, and, in rare cases, metals [53], all of which affect the biofilm's physical and physiological outlook. The biofilm matrix, on the other hand, is mostly made up of EPSs. At least three polysaccharides with an active role have been found in biofilm development among the common bacterial EPSs. They are the polysaccharides alginate, Pel, and Psl. Extracellular polysaccharides generated by *Psl* genes are known as Psl polysaccharides. Psl polysaccharides perform a critical role in *P. aeruginosa* biofilm formation [54, 55]. The *Psl* loci include 15 co-transcribed genes, 11 of which are involved in the formation of Psl-dependent biofilms [56–58]. Preliminary attachment, permanent attachment, microcolony development, biofilm maturing, and biofilm spreading are the five sequential stages in the creation of biofilm. As a result, enhanced biofilm development by living or functionally active micro-organisms in an MFC might be attributed to the over-expression of these genes. The function of such polysaccharides during biofilm formation affects the external mobility of succeeding cells [59]. Overproduction of Psl polysaccharides has been shown to increase organism to organism contact and adhesion, facilitating the initial and most important stage in biofilm development [60, 61]. The Psl polysaccharide adheres strongly to the bacterial cell wall in a helical shape, facilitating strong intercellular connections and therefore acting as a skeleton in biofilm matrix development [61]. They are discovered connected to the perimeter of three-dimensionally constructed colonies during biofilm development, giving structural assistance and allowing biofilm distribution at the ends [60]. Extracellular polysaccharides play a critical function in the development, adhesion properties, stability, structural integrity, and longevity of biofilms, and therefore may be used as critical components in biofilm construction procedures by increasing the copy number of these EPS making genes.

11.3 External factors influencing biofilms

Although EPSs are important in biofilm development, it is also affected by external environmental variables and gene expression processes that lead to the establishment of biofilms in particular cells [62]. The development of biofilms, as well as the outer and extracellular elements of the organisms, is influenced by physical and environmental variables. To be more precise, the destiny of biofilm development is determined by exopolysaccharides (EPS) and lipopolysaccharides (LPSs) as well

as quorum sensing (QS) signalling molecules [63]. External components that affect the release of EPS, QS signalling molecules, different stress molecules such as heavy metals [64], salinity [65], pH [66], nutrient starvation [53], pathogen invasion, nutrient depletion, water current in moving water bodies, and growth substrates are all identified to influence biofilms. Genetic engineering of QS signalling molecules and EPS, which are the important contributors in biofilm development, can prevail over the other environmental effects involved in biofilm formation.

11.4 The influence of quorum sensing signalling molecules on biofilm

The bacterial community interacts via cell-to-cell interactions, allowing them to coordinate their collective activity. This necessitates the discharge of autoinducers from the cells, which then ultimately produce quorum sensing. It was found that adding 100 nM quinolone type signalling molecules increased the biofilm mass of *Halanaerobium* prevalence by 95%, resulting in a 30% increase in power capacity [67]. The signalling molecules, Quinolones, that belong to the LuxR proteins family are encoded by the *hmqF* genes in *Halanaerobium* species [68]. Although the complete stimulation of biofilm formation in *P. aeruginosa* has been studied [69], nothing has been reported on the impact of autoinducers produced by other bacteria in activating QS in *Halanaerobium* sp. [67].

11.5 The influence of extracellular polysaccharides and additional physical parameters on biofilms

Separately from the QS signalling molecules, LPSs and EPSs have also been found to play a key role in biofilm development. Bacterial production of c-di-GMP produced by *diguanylate cyclases* (DGC) at elevated levels promotes the synthesis of matrix exopolysaccharides such as Pel and Alginate, which contributes to the development of biofilms. Among the three major polysaccharides, alginate, Pel, and Psl, the increased release of Psl in non-pilated organisms helps in the formation of solid surface-associated biofilms [60], whereas in gram-negative bacteria, the production of Pel, a glucose-rich extracellular matrix, helps in the formation of biofilms for non-pilated organisms [61, 70]. Furthermore, a dual-functional enzyme produced by the *algC* gene of *P. aeruginosa* is essential for the production of four polysaccharides, alginate, Pel, Psl, and LPS, all of which influence the development of biofilms [55]. Almost all the genes that generate any of the aforementioned polysaccharides contribute to the development of biofilms in an organism [55].

11.6 Bioelectrogenesis and microbial metabolism

In MFCs several micro-organisms have been tested for energy production, bio-remediation, and a variety of other uses. In MFC technology different substrates have been used to grow micro-organisms, for example acetate, ethanol, glucose, lactate, sucrose, starch, and xylose, among other nutrients and wastewater such as chocolate industry wastewater, beer brewery wastewater, swine wastewater, protein-rich wastewater, and paper recycling wastewater, among others [71]. Regardless of

the availability of a broad variety of carbon sources and micro-organisms, only a few microbes have been identified as producing electricity in MFCs. Exoelectrogens from a number of sources have been investigated in MFCs, including gram-negative bacteria, gram-positive bacteria, yeast, algae, cyanobacteria, and even fungus. The complex organic materials are completely oxidized in the anodic chamber into simple components by those micro-organisms, making them very efficient for power production. However, for development and energy generation, a certain exoelectrogen may oxidize specific substrates or types of substrates. Furthermore, each exoelectrogen has distinct genes, proteins, enzymes or pathways for breakdown or oxidation depending on the kind of substrate. Hence, the electricity production by MFCs is determined by the choice of bacterial consortia and preferred substrate. When an MFC was supplied with an inoculum of aerobic–anaerobic sludge along with glucose as a substrate for 3 months, the substrate to power conversion rates for the bacteria rose seven-fold [72].

Organic compounds including lipids, carbohydrates, and proteins are used as electron donors in MFCs to generate energy via redox processes at the anode. These complicated chemical compounds are subsequently converted to acetyl Co-A via glycolysis and other mechanisms, which are ultimately used in the citric acid cycle. From three nicotinamide adenine dinucleotide (NAD+), three equivalents of reduced NADH are produced in a single step of the citric acid cycle. CO_2 is created as a by-product when one flavin adenosine dinucleotide (FAD) is converted to FADH2. Both prokaryotes (bacteria) and eukaryotes (humans) have cytoplasmic metabolic pathways (the Krebs cycle and glycolysis). The electron carriers NADH and FADH2 transmit their electrons to the electron transport chain (ETC) to form adenosine triphosphate (ATP), an energy carrier molecule. The respiratory response takes place in the cell membrane of a bacterium (which includes the outer cell membrane, periplasm, and inner cell membrane), which houses the system that allows electron exchanges to take place. This electron exchange is the basis of MFCs. In yeast the ETC is found on the inner mitochondrial membrane. Cytochromes, ubiquinone, NADH dehydrogenase, and coenzyme Q are the four intermediate proteins found in the ETC (however, different species may have different intermediary proteins). The electrons are transported to the terminal electron acceptor by these proteins, while reduced protons are pushed out of the cell and given to the cathode by the PEM. Chemical facilitators were employed to catalyse the movement of electrons from the bacteria to the anode before it was discovered that bacteria can aid in electron transfer [73].

These mediators decrease when they come into contact with ETC components, then electrons are transferred to the anode after they exit the cell. Furthermore, depending on the anode capacity, the bacteria's metabolism may transition to fermentative metabolism from oxidative phosphorylation (metabolism). Bacteria adapt to oxidative metabolism as electron acceptors such as sulphate, nitrate, etc, are present, when the anode potential is low and electron acceptors receive and deposit the electrons. Bacteria prefer to use the fermentation metabolism when there are no electron acceptors available. One-third of the electrons generated during the fermentation of glucose may be utilized to generate electricity, while the extra electrons

remain in the fermentation by-products, that can be anaerobically oxidized further to generate electricity in MFCs. For example *Geobacter* sp. carried out anaerobic oxidation of fermentation by-products [48, 72]. Several bacteria (*Enterococcus* sp., *Clostridium* sp.) have been injected anaerobically in MFCs to harvest fermentation products in addition to power production [74]. *Clostridium* sp. is a highly effective hydrogen generator in MFCs, just as *Geobacter* sp. is a very effective exoelectrogen [75, 76]. Certainly, as compared to pure cultures, a mixed culture biofilm in MFCs has shown higher power capabilities. This might be due to metabolic relationships between bacteria present in biofilms, however, the topic requires further exploration and experimental proof. The anode's potential is crucial in determining the bacterial metabolism. The bacteria are influenced by a negative anode potential to supply electrons via more reduced complexes [77]. Consequently, the output power and energy recovery in MFC increases due to lower energy absorption of the bacteria. A higher power potential of 45 mA m^2 at 0.6 V, compared to 15 mA m^2 at 0.2 V, was produced at negative anode potentials by a mixed culture of sulphate-reducing bacteria [78]. The potential of the cathode has also been found to enhance MFC performance. The time for start-up was lowered from 26 days to 19 days when at a constant −300 V cathodic capacity, the power potential was increased from 4.1 W m^{-3} to 6.4 W m^{-3} in a Cr (VI) reduction MFC [79].

11.7 Electron transfer by micro-organisms in MFCs

Electricity generation in MFCs requires the movement of electrons or transfer of electrons. Electrodes in MFCs are immoveable so the micro-organisms influence this transport of electrons. Electron transport primarily occurs between micro-organisms and electrodes in two directions: the first is from the micro-organisms to the electrode (at the anode), and the second is when a biocathode is used to catalyse oxygen reduction and electrons move from the electrode to the micro-organisms (at the cathode).

11.7.1 Electron transfer from micro-organisms to electrodes

Micro-organisms transfer electrons to an electrode through three different processes. First, micro-organisms contain redox-active proteins, known as cytochromes, present on their cell surfaces, which are involved in short-range electron transfer. Second, micro-organisms secrete certain soluble molecules such as flavins and pyocyanin which act as electron shuttles and aid electron transport. Third, micro-organisms have specialised micro-structures called micropili on the cell membrane which facilitate the long-range transport of electrons and are thus known as conductive pili (figure 11.2).

11.7.1.1 Cytochromes allow direct electron transfer

To better understand the processes of direct electron transfer, the bacteria *G. sulfurreducens* has been researched widely. The complete oxidation of carbon to water and carbon oxide is done anaerobically by a key metabolic enzyme present in *G. sulfurreducens*, this may involve the transfer of electrons to other electron

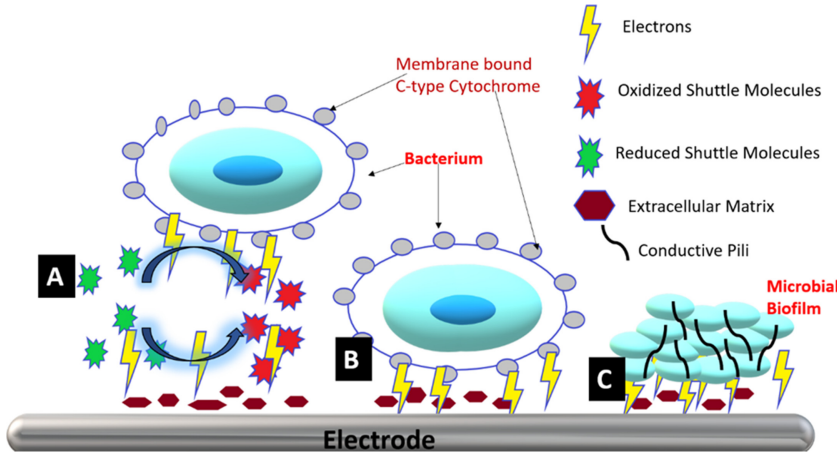

Figure 11.2. Electron transport in MFCs: (a) through micro-organism-secreted electron shuttles; (b) direct transfer through cytochromes; and (c) through microbial nanowires.

acceptors [80]. The *G. sulfurreducens* genome has also been found to have c-type cytochromes with motifs with heme groups. These motifs are involved in exposing c-type cytochromes to the cell's outer surface [40, 81]. The abundance of cytochromes in the organism is a beneficial trait that improves electron transport across the cell/electrode contact. *G. sulfurreducens* has electron transport proteins and substances in the periplasm and on the outer membrane. These additional substances or proteins include iron-sulphur proteins, quinones, and b-type cytochromes. Furthermore, c-type cytochromes have been shown to transport electrons to a variety of extracellular electron acceptors both in *in vitro* and *in vivo* conditions [40, 81, 82]. The OmcZ has a vital role in direct electron transfer, and the presence of OmcZ at the biofilm–anode interface enabling electron transfer was verified by immunogold labelling of *G. sulfurreducens* biofilms. However, between the anode and biofilm the electron transmission is hindered by the OmcZ mutant strain [40]. In 2009 Nevin *et al* examined the expression of genes in *G. sulfurreducens* biofilm cells, which are cultured on several electron acceptors. They compared the gene expression between the cells which are grown on graphite with fumarate and on simple graphite [83]. According to microarray research, C-type cytochrome encoded genes are *omcB*, *omcE*, *omcS*, *omcT*, and *omcZ*. In current harvesting cells, the cytochromes *OmcE* and *OmcZ* were found abundantly and *OmcS* was rarely present. Furthermore, cells lacking omcZ were unable to produce current or form biofilms. It demonstrates the significance of the cytochrome in electron transport. Those cells which were lacking in other genes has no effect on current generation or biofilm creation [40, 84, 85]. According to abundant evidence, in biofilms producing higher current, OmcZ appears to be the most significant cytochrome. It is a hydrophobic protein with an octaheme group and exists in two structures, OmcZ L is long and other OmcZ S is short. The latter is the principal form of OmcZ [40]. OmcZ is thought to facilitate electron transport across the biofilm, whereas OmcB is thought to mediate electron transfer across the biofilm–electrode contact in

G. sulfurreducens biofilms. In electron transport via the biofilm, the cytochromes OmcS and OmcE play a minor function [39]. The OmcF mutant strain of *G. sulfurreducens* was found to have a low current density [86]. Furthermore, the data revealed that the OmcF is involved in the electron transfer process either directly or indirectly, meaning that the OmcF is vital in the generation of electricity [86].

In *G. sulfurreducens*, the multicopper proteins OmpB and OmpC of the outer membrane are essential for the reduction of Fe (III) oxide in addition to the c-type cytochromes of the outer membrane [87, 88]. However, this would be an interesting topic of future research, how these multicopper proteins affect the production of electricity in MFCs, as it is still unknown. In *Desulfovibrio alaskensis* G20, a sulphate-reducing bacterium, electron flow components are being studied. A new model for electron transfer has been revealed, also the type I tetraheme cytochrome c 3 (TpI c 3) and transmembrane complexes (QrcA) play a key role in electron transfer across the cell membrane for sulphate reduction [89]. The direct electron transfer mechanism has also been investigated in gram-positive organisms of the genus *Thermincola potens*. During *T. potens* growth on hydrous ferric oxides, the expression of numerous multiheme c-type cytochromes (MHCs) on the cell surface is demonstrated by surface-enhanced Raman spectroscopy. The findings showed new evidence for the involvement of cytochromes associated with the cell wall and MHC in electron transport across the gram-positive bacterium's cell envelope [35]. More knowledge regarding the proteins or genes involved in direct electron transfer, as well as genetic modification, may lead to improvements in MFC output and efficiency.

11.7.1.2 Electron transfer through micro-organism secreted electron shuttles

The secretion of soluble electron shuttles allows electron transport to electron acceptors or electrodes that are soluble or insoluble. Some bacteria, such as *Shewanella oneidensis*, *P. aeruginosa*, and *Geothrix fermentans*, have been identified as mediating electron secreting soluble electron shuttles. *G. fermentans* secretes an electron shuttle that aids in Fe (III) oxide reduction [8]. To reduce Fe (III), *G. fermentans* generates two soluble redox-active electron shuttles. One is riboflavin, at a redox potential of 0.2 V, and the other is unknown, at a redox potential of 0.3 V [87]. *P. aeruginosa* produces important electron transport molecules such as phenazine-1-carboxamide and pyrocyanin. A *P. aeruginosa* mutant strain, lacking in the generation of phenazine-1-carboxamide and pyrocyanin, produced just 5% of the power output of wild-type cultures [90]. Furthermore, research found that pyocyanin, which is utilized by *P. aeruginosa* as well as other bacteria, stimulates significant electron transport [90, 91]. Exocellular electron transfer efficiency as well as power output are increased when the *phzM* (methyltransferase-encoding) gene is overexpressed as it increased pyocyanin production by 1.6-fold in *P. aeruginosa-phzM*-inoculated MFCs [92]. In conjunction with anoxic growth, *Shewanella* species produce riboflavin and flavin mononucleotides as external electron shuttles for the reduction of Fe (III) oxides [93, 94]. *S. loihica* PV-4 strain cultivated on graphite electrode, the amount of quinone derivatives and riboflavin was found to be increased in the cell-free supernatant, resulting in a higher anodic current density

of 90 A cm^{-2} [95, 96]. In *S. oneidensis*, Kotloski and Gralnick (2013) found a flavin adenine dinucleotide transporter that exports flavin electron shuttles, which aids in electron transfer to intractable substrates [97]. OmcA and Decaheme c-type cytochromes MtrC are found on the external surface of *S. oneidensis* MR-1 cells; they are a portion of a multiprotein complex that aids in electron hopping across the cell membrane [89]. During biofilm development, the cytochrome OmcA is also involved in the adhesion of bacteria to the electrode surface [93]. In *S. oneidensis* MtrCAB, a mediated and direct exocellular electron transport complex was found. This MtrCAB was inserted into *Escherichia coli* utilising more flexible induction technique [89, 90]. The *E. coli* strains exhibited poor cell development, poor control of MtrCAB expression, and the data demonstrated that strains with better cell growth and fewer morphological defects provided the highest current densities, not those with more MtrC and MtrA expression [99]. *Lactococcus lactis* generates membrane-associated quinones with different colours that aids in the electron transfer towards external electron acceptors such as Cu (II) and Fe (III) [100]. The bacteria use soluble redox mediators, such as 2-amino-3-dicarboxy-1,4 naphthoquinone, to transfer electrons towards the anode [101]. In MFCs the strain of *Klebsiella pneumoniae* (L17), generates a recycling electron shuttle called 2,6-di-tert-butyl-pbenzoquinon, which transfers electrons from the cathode to the anode [102, 103].

11.7.1.3 Microbial nanowires for electron transfer

Long-range electron transmission is facilitated by the micro-organism's dense network of conductive pili, which are responsible for conductive biofilms with significant current generation. Although many microbes can produce pili, only *Shewanella* sp. [105] and *Geobacter* sp. [39, 104] can produce conductive pili. In long-range electron transport in biofilms, the function of conductive pili is well-studied in *G. sulfurreducens* and these electronic networks are found to cause a tenfold increase in power output [48]. Pili derived from *G. sulfurreducens* are type IV pili composed of PilA monomers [106]. Type IV pili are small structural proteins with a conserved N-terminal domain that forms an α-helix, a transmembrane domain, and a protein–protein interaction domain that have a molecular weight of 7–20 kDa, a length of 10–20 m, and a width of 3–5 m [88]. Furthermore, the PilA C terminus includes aromatic amino acids with a conserved sequence (Tyr, His, Trp, Met, and Phe), which contributes to the pi–pi orbitals overlapping in the pili structure, and hence causing a metal-like conductivity, which is absent in non-conductive biofilms [107]. PilR, which is a RpoN-dependent enhancer-binding protein, directly controls the function of PilA. Moreover, Juárez *et al* (2009) discovered that a strain lacking in the pilR gene exhibited decreased reduction of insoluble Fe (III) and soluble Fe (III) [108]. The hypothesis of Malvankar *et al* (2011) was ruled out, that cytochromes and pili of *G. sulfurreducens* are coupled and thus cytochromes play a role in transport of electron along with pili [39]. The conductivity by nanowires of *G. sulfurreducens* cannot be ascribed to cytochromes since the cytochrome-to-cytochrome distance was nearly 200 times greater than that required for electrification. *G. sulfurreducens* strain PA with the *pilA* gene substituted with the *pilA* gene of *P. aeruginosa* PAO1 produced equivalent pili subunits and

c-type cytochrome OmcS to the control strain, but had lower current output and Fe (III) oxide reduction. Furthermore, pili conductivity is not conferred by the presence of c-type cytochrome OmcS [71, 85]. Research suggests that magnetite may help microbial extracellular electron transport; in Fe (III) oxide reduction, an OmcS-deficient strain is compensated by magnetite for extracellular electron transfers [71]. *S. oneidensis* MR-1 nanowires were shown to be conducive using the study of probe atomic force microscopy and gene deletion of *OmcA* and *MtrC* [17]. Further investigation of the electronic transport properties of *S. oneidensis* MR-1 nanowires revealed p-type, adjustable electronic behaviour with field-effect mobility [109]. For effective electron transfer and energy distribution, a multistep hopping mechanism in the methanogen *Methanothermobacter thermautotrophicus* was used [9]. Direct interspecies electron transfer (DIET) has been observed in anaerobic digester aggregates of *Methanosaeta harundinacea* and *G. metallireducens* [110]. It has been proposed that granular-activated carbon (GAC) stimulates DIET between bacteria and methanogens [71]. The function of pili and related c-type cytochrome in DIET is simulated by GAC [71]. The contribution of DIET in energy generation and its molecular mechanism are poorly known, necessitating a thorough study into the subject.

11.7.1.4 Transfer of electrons from the electrode to micro-organisms

In MFC technology, several micro-organisms have been used as biocathodes but little is known about how electrons go from the electrode to bacteria, although micro-organisms receive electrons from the cathode in a different way than they give electrons to the anode (figure 11.3). *Geobacter* species accepting electrons directly from an electrode was the first proof in this regard [111]. In the aerated cathode, *S. oneidensis* MR-1 produces a molecule riboflavin, which acts as an electron shuttle mediator for transferring electrons to Cr (VI) [112]. Pure cultures of *Acinetobacter calcoaceticus* and *S. putrefaciens* excrete a redox molecule that is comparable to pyrroloquinoline quinone (PQQ) and uses outer membrane-bound redox chemicals for extracellular electron transfer [101]. Acidophile bacteria, *Acidithiobacillus ferrooxidans*, were utilized as a biocathode to show that the outer membrane-bound cytochrome c (Cyc2) is linked to the microbial-catalyzed O_2 reduction [113]. Cyt 572, a unique membrane protein, having helical structure, isolated acidophilic micro-organisms demonstrated the capability to oxidise Fe (II) [114], although it is still unclear if the protein is involved in electron transport processes. Using cyclic voltammetry, researchers discovered an undiscovered redox-active chemical produced by *P. aeruginosa* that is contributing to electron transport from the electrode to specific azo bonds, resulting in azo dye decolourization [115]. During the dichlorination of pentachlorophenol (PCP) in MFC, a bio-cathodic microbial population dominated by Proteobacteria, Bacteroidetes, and Firmicutes transferred electrons directly, since the characterization of the medium by cyclic voltammetry revealed no redox mediator produced by the bacteria [71]. The detailed chemical process by which any microbe accepts electrons from electrodes is not completely discovered and may be considered a future feature.

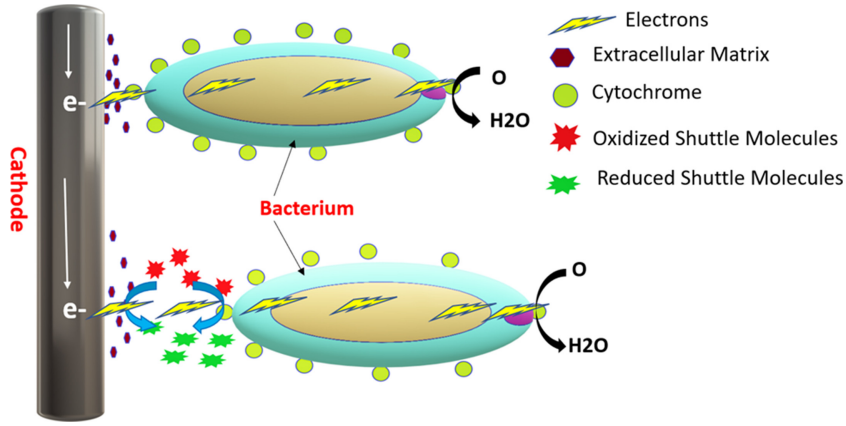

Figure 11.3. Electron transfers from the electrode to micro-organisms: direct transfer through membrane-bound c-type cytochromes and indirect transfer through electron shuttles.

11.8 Micro-organisms with higher power generating potential in MFCs

Unless the MFC design, operating circumstances, chemical solutions, and nutrients used in the process are same, the power density generated by one microbe, such as *Geobacter* sp., cannot be compared to that produced by another species, such as *Shewanella* sp. Until now, a variety of micro-organisms used in various MFCs have generated electrical energy under a variety of conditions. In MFC technology, several novel bacteria have recently been found, and this chapter only covers the most frequent micro-organisms researched (in the cathode and anode) for achieving higher power densities [7]. Tables 11.1 and 11.2 list micro-organisms with unknown and known natural electron mediators, respectively.

11.9 Future recommendations

The uses of MFC technology are still limited to within the four walls of the laboratory. To put it another way, the technology has not yet reached the point of commercialization. Only *Shewanella* spp. and *Geobacter* spp. are well-studied regarding the formation of biofilms and the transfer of electrons from exoelectrogens to electrodes, thus research on other microbes is necessary to understand and utilise their influence on MFCs. Additionally, genetic modification may enhance the exocellular electron transport rates, causing more efficient output by MFCs. It is suggested that conductive pili-producing bacteria be found, albeit such microbes can produce greater power densities. The processes of electron transmission from electrodes to microbes remains unknown. The cathode compartment will be dominated by micro-organisms that can receive electrons from the electrode. OmpB and OmpC, two outer membrane multicopper proteins, have been shown to play a significant part in Fe (III) oxide reduction, although more research into their roles in electron transport pathways is required.

Table 11.1. Micro-organisms that possess well-characterized electron transfer intermediates.

Micro-organisms	Molecules involved in electron transfers	Power density/current density	References
Thermincola ferriacetica	Anthraquinone 2, 6 disulfonate	12,000 mA m^{-2}	[34]
Pseudomonas aeruginosa	Phenazine-1-carboxamide Pyocyanin	4310 mW m^{-2}	[117]
Geobacter sulfurreducens	Type IV pili c-type cytochrome Z	3147 mA m^{-2}	[40]
Shewanella oneidensis	Flavins, riboflavin	3000 mW m^{-2}	[116]
Rhodopseudomonas palustris	c-type cytochromes	2720 mW m^{-2}	[120]
Desulfovibrio desulfuricans	Tetraheme cytochrome C_3	233 mA m^{-2}	[119]
Klebsiella pneumonia	2, 6-di-tert-butyl-p-benzoquinone	199 mA m^{-2}	[102]
Geobacter metallireducens	c-type cytochromes OmcB and OmcE	40 mW m^{-2}	[121]
Shewanella putrefaciens	c-type cytochromes MtrC and OmcA	4.92 W m^{-3}	[118]
Desulfovibrio alaskensis	Transmembrane complexes (QrcA) Tetraheme cytochrome C 3		[89]

mW m^{-2} = units of surface power density, W m^{-3} = volume power density, mA m^{-2} = current density.

Table 11.2. Power density exhibited by micro-organisms utilising unknown electron mediators.

Power density/current density	Micro-organisms	References
	Bacteria	
3390 mA m^{-2}	*Escherichia coli*	[122]
2625 mW m^{-3}	*Ochrobactrum* sp.	[123]
282 mA m^{-2}	*Saccharomyces cerevisiae*	[124]
205 mA m^{-2}	*Citrobacter* sp.	[125]
85 mW m^{-2}	*Lysinibacillus sphaericus*	[126]
	Algae	
2485 mW m^{-3}	*Chlorella vulgaris*	[127]
1926 mW m^{-2}	*Scenedesmus*	[128]
320 mW m^{-3}	*Coriolus versicolor*	[129]
14 mW m^{-2}	*Cyanobacteria* 1	[130]
10 mW m^{-3}	*Arthrospira maxima*	[131]

mW m^2 = units of surface power density, W m^3 = volume power density, mA m^2 = current density.

11.10 Conclusions

Micro-organisms serve as the powerhouses of MFCs, and those that can produce conductive biofilms are particularly important. Specific proteins produced by the bacteria, including c-type cytochromes, pili, and QS, are essential for the development of conductive biofilms. Moreover, the external polysaccharide greatly influences the biofilms, which are the key component in MFCs. In addition, a growing body of evidence indicates that the c-type cytochromes OmcB and OmcZ have a great influence on electron transport processes. *Shewanella* spp. and *Geobacter* spp. are capable of long-distance electron transport via pili. *Shewanella* spp. use flavins to transport electrons to the electrodes, whereas *Pseudomonas* spp. secrete pyocyanin. With riboflavin as an electron mediator, the exoelectrogen is demonstrated to receive electrons from electrodes. Furthermore, the transfer of electrons from the electrode to bacterial cells is mediated by two unique cytochromes, Cyt 572 and Cyt 579. Biocathodes have reduced the cost of the technology. MFC has emerged as the sole technology capable of producing renewable energy and a variety of simultaneous additional uses.

References

[1] Shewa W A, Chaganti S R and Lalman J A 2013 Electricity generation and biofilm formation in microbial fuel cells using plate anodes constructed from various grades of graphite *J. Green Eng.* **4** 13–32

[2] Mevers E, Su L, Pishchany G, Baruch M, Cornejo J, Hobert E, Dimise E, Ajo-Franklin C M and Clardy J 2019 An elusive electron shuttle from a facultative anaerobe *Elife* **8** e48054

[3] Liu X, Shi L and Gu J D 2018 Microbial electrocatalysis: redox mediators responsible for extracellular electron transfer *Biotechnol. Adv.* **36** 1815–27

[4] Costa N L, Clarke T A, Philipp L A, Gescher J, Louro R O and Paquete C M 2018 Electron transfer process in microbial electrochemical technologies: the role of cell-surface exposed conductive proteins *Bioresour. Technol.* **255** 308–17
[5] Ancona V, Caracciolo A B, Borello D, Ferrara V, Grenni P and Pietrelli A 2020 Microbial fuel cell: an energy harvesting technique for environmental remediation *Int. J. Env. Impacts* **3** 168–79
[6] Kumar S S, Kumar V, Kumar R, Malyan S K and Pugazhendhi A 2019 Microbial fuel cells as a sustainable platform technology for bioenergy, biosensing, environmental monitoring, and other low power device applications *Fuel* **255** 115682
[7] Kumar R, Singh L and Wahid Z A 2015 Role of microorganisms in microbial fuel cells for bioelectricity production *Microbial Factories* (New Delhi: Springer) pp 135–54
[8] Bond D R and Lovley D R 2003 Electricity production by *Geobacter sulfurreducens* attached to electrodes *Appl. Env. Microbiol.* **69** 1548–55
[9] Gorby Y A *et al* 2006 Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms *Proc. Natl Acad. Sci.* **103** 11358–63
[10] Chaudhuri S K and Lovley D R 2003 Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells *Nat. Biotechnol.* **21** 1229–32
[11] Jayapriya J and Ramamurthy V 2012 Use of non-native phenazines to improve the performance of *Pseudomonas aeruginosa* MTCC 2474 catalysed fuel cells *Bioresour. Technol.* **124** 23–8
[12] Chisti Y 2007 Biodiesel from microalgae *Biotechnol. Adv.* **25** 294–306
[13] Park D H and Zeikus J 1999 Utilization of electrically reduced neutral red by *Actinobacillus succinogenes*: physiological function of neutral red in membrane-driven fumarate reduction and energy conservation *J. Bacteriol.* **181** 2403–10
[14] Pham C A, Jung S J, Phung N T, Lee J, Chang I S, Kim B H, Yi H and Chun J 2003 A novel electrochemically active and Fe (III)-reducing bacterium phylogenetically related to *Aeromonas hydrophila*, isolated from a microbial fuel cell *FEMS Microbiol. Lett.* **223** 129–34
[15] Mekuto L, Olowolafe A V, Huberts R, Dyantyi N, Pandit S and Nomngongo P 2020 Microalgae as a biocathode and feedstock in anode chamber for a selfsustainable microbial fuel cell technology: a review *S. Afr. J. Chem. Eng.* **31** 7–16
[16] Prasad D, Arun S, Murugesan M, Padmanaban S, Satyanarayanan R S, Berchmans S and Yegnaraman V 2007 Direct electron transfer with yeast cells and construction of a mediatorless microbial fuel cell *Biosens. Bioelectron.* **22** 2604–10
[17] El-Naggar M Y, Wanger G, Leung K M, Yuzvinsky T D, Southam G, Yang J, Lau W M, Nealson K H and Gorby Y A 2010 Electrical transport along bacterial nanowires from *Shewanella oneidensis* MR-1 *Proc. Natl Acad. Sci.* **107** 18127–31
[18] Dumas C, Basseguy R and Bergel A 2008 Electrochemical activity of *Geobacter sulfurreducens* biofilms on stainless steel anodes *Electrochim. Acta* **53** 5235–41
[19] Boon N, Aelterman P, Clauwaert P, De Schamphelaire L, Vanhaecke L, De Maeyer K, Höfte M, Verstraete W and Rabaey K 2008 Metabolites produced by *Pseudomonas* sp. enable a gram-positive bacterium to achieve extracellular electron transfer *Appl. Microbiol. Biotechnol.* **77** 1119–29
[20] Schaefer A L *et al* 2008 A new class of homoserine lactone quorum-sensing signals *Nature* **454** 595–9
[21] Venkataraman A, Rosenbaum M, Arends J B, Halitschke R and Angenent L T 2010 Quorum sensing regulates electric current generation of *Pseudomonas aeruginosa* PA14 in bioelectrochemical systems *Electrochem. Commun.* **12** 459–62

[22] Patil S A, Hägerhäll C and Gorton L 2012 Electron transfer mechanisms between microorganisms and electrodes in bioelectrochemical systems *Bioanal. Rev.* **4** 159–92
[23] Rollefson J B, Stephen C S, Tien M and Bond D R 2011 Identification of an extracellular polysaccharide network essential for cytochrome anchoring and biofilm formation in *Geobacter sulfurreducens J. Bacteriol.* **193** 1023–33
[24] Arulmani S R B, Ganamuthu H L, Ashokkumar V, Govindarajan G, Kandasamy S and Zhang H 2020 Biofilm formation and electrochemical metabolic activity of *Ochrobactrum* Sp JSRB-1 and *Cupriavidus* Sp JSRB-2 for energy production *Env. Technol. Innov.* **20** 101145
[25] Cecconet D, Sabba F, Devecseri M, Callegari A and Capodaglio A G 2020 *In situ* groundwater remediation with bioelectrochemical systems: a critical review and future perspectives *Env. Int.* **137** 105550
[26] Angelaalincy M J, Navanietha Krishnaraj R, Shakambari G, Ashokkumar B, Kathiresan S and Varalakshmi P 2018 Biofilm engineering approaches for improving the performance of microbial fuel cells and bioelectrochemical systems *Front. Energy Res.* **6** 63
[27] Bashir S, Mulvaney S P, Houf W, Villanueva L, Wang Z, Buck G and Liu J L 2021 Microbial fuel cells: design and evaluation of catalysts and device *Advances in Sustainable Energy* (Cham: Springer) pp 681–764
[28] Krige A, Sjöblom M, Ramser K, Christakopoulos P and Rova U 2019 On-line Raman spectroscopic study of cytochromes' redox state of biofilms in microbial fuel cells *Molecules* **24** 646
[29] Steidl R J, Lampa-Pastirk S and Reguera G 2016 Mechanistic stratification in electroactive biofilms of *Geobacter sulfurreducens* mediated by pilus nanowires *Nat. Commun.* **7** 1–11
[30] Kleine D, Chodorski J, Mitra S, Schlegel C, Huttenlochner K, Müller-Renno C, Mukherjee J, Ziegler C and Ulber R 2019 Monitoring of biofilms grown on differentially structured metallic surfaces using confocal laser scanning microscopy *Eng. Life Sci.* **19** 513–21
[31] Nandy A, Sharma M, Venkatesan S V, Taylor N, Gieg L and Thangadurai V 2019 Comparative evaluation of coated and non-coated carbon electrodes in a microbial fuel cell for treatment of municipal sludge *Energies* **12** 1034
[32] Kiran R and Patil S A 2019 Microbial electroactive biofilms *Introduction to Biofilm Engineering* (Washington, DC: American Chemical Society) pp 159–86
[33] Arulmani S R B, Jayaraj V and Jebakumar S R 2016 Long-term electricity production from soil electrogenic bacteria and high-content screening of biofilm formation on the electrodes *J. Soils Sediments* **16** 831–41
[34] Parameswaran P, Bry T, Popat S C, Lusk B G, Rittmann B E and Torres C I 2013 Kinetic, electrochemical, and microscopic characterization of the thermophilic, anode-respiring bacterium *Thermincola ferriacetica Env. Sci. Technol.* **47** 4934–40
[35] Wrighton K C, Thrash J C, Melnyk R A, Bigi J P, Byrne-Bailey K G, Remis J P, Schichnes D, Auer M, Chang C J and Coates J D 2011 Evidence for direct electron transfer by a gram-positive bacterium isolated from a microbial fuel cell *Appl. Env. Microbiol.* **77** 7633–9
[36] Arunasri K and Mohan S V 2019 Biofilms: microbial life on the electrode surface *Microbial Electrochemical Technology* (Amsterdam: Elsevier) pp 295–313
[37] Harmsen M, Yang L, Pamp S J and Tolker-Nielsen T 2010 An update on *Pseudomonas aeruginosa* biofilm formation, tolerance, and dispersal *FEMS Immunol. Med. Microbiol.* **59** 253–68
[38] Logan B E, Rossi R and Saikaly P E 2019 Electroactive microorganisms in bioelectrochemical systems *Nat. Rev. Microbiol.* **17** 307–19

[39] Malvankar N S, Tuominen M T and Lovley D R 2012 Lack of cytochrome involvement in long-range electron transport through conductive biofilms and nanowires of *Geobacter sulfurreducens Energy Env. Sci.* **5** 8651–9
[40] Inoue K, Leang C, Franks A E, Woodard T L, Nevin K P and Lovley D R 2011 Specific localization of the c-type cytochrome OmcZ at the anode surface in current-producing biofilms of *Geobacter sulfurreducens Env. Microbiol. Rep.* **3** 211–7
[41] Wang V B *et al* 2014 Comparison of flavins and a conjugated oligoelectrolyte in stimulating extracellular electron transport from *Shewanella oneidensis* MR-1 *Electrochem. Commun.* **41** 55–8
[42] Stoodley P, Sauer K, Davies D G and Costerton J W 2002 Biofilms as complex differentiated communities *Annu. Rev. Microbiol.* **56** 187–209
[43] Merritt J H, Ha D G, Cowles K N, Lu W, Morales D K, Rabinowitz J, Gitai Z and O'Toole G A 2010 Specific control of *Pseudomonas aeruginosa* surface-associated behaviors by two c-di-GMP diguanylate cyclases *MBio* **1** e00183–10
[44] Malone J G, Jaeger T, Manfredi P, Dötsch A, Blanka A, Bos R, Cornelis G R, Häussler S and Jenal U 2012 The YfiBNR signal transduction mechanism reveals novel targets for the evolution of persistent *Pseudomonas aeruginosa* in cystic fibrosis airways *PLoS Pathog.* **8** e1002760
[45] Yong Y C, Yu Y Y, Li C M, Zhong J J and Song H 2011 Bioelectricity enhancement via overexpression of quorum sensing system in *Pseudomonas aeruginosa*-inoculated microbial fuel cells *Biosens. Bioelectron.* **30** 87–92
[46] Christwardana M, Frattini D, Duarte K D, Accardo G and Kwon Y 2019 Carbon felt molecular modification and biofilm augmentation via quorum sensing approach in yeast-based microbial fuel cells *Appl. Energy* **238** 239–48
[47] Watson V J and Logan B E 2010 Power production in MFCs inoculated with *Shewanella oneidensis* MR-1 or mixed cultures *Biotechnol. Bioeng.* **105** 489–98
[48] Reguera G, McCarthy K D, Mehta T, Nicoll J S, Tuominen M T and Lovley D R 2005 Extracellular electron transfer via microbial nanowires *Nature* **435** 1098–101
[49] Behera M, Jana P S and Ghangrekar M M 2010 Performance evaluation of low cost microbial fuel cell fabricated using earthen pot with biotic and abiotic cathode *Bioresour. Technol.* **101** 1183–9
[50] Sun D, Chen J, Huang H, Liu W, Ye Y and Cheng S 2016 The effect of biofilm thickness on electrochemical activity of *Geobacter sulfurreducens Int. J. Hydrogen Energy* **41** 16523–8
[51] Kreft J U and Wimpenny J W 2001 Effect of EPS on biofilm structure and function as revealed by an individual-based model of biofilm growth *Water Sci. Technol.* **43** 135
[52] Flemming H C, Neu T R and Wozniak D J 2007 The EPS matrix: the 'house of biofilm cells' *J. Bacteriol.* **189** 7945–7
[53] Angelaalincy M, Senthilkumar N, Karpagam R, Kumar G G, Ashokkumar B and Varalakshmi P 2017 Enhanced extracellular polysaccharide production and self-sustainable electricity generation for PAMFCs by *Scenedesmus* sp. SB1 *ACS Omega* **2** 3754–65
[54] Colvin K M, Irie Y, Tart C S, Urbano R, Whitney J C, Ryder C, Howell P L, Wozniak D J and Parsek M R 2012 The Pel and Psl polysaccharides provide *Pseudomonas aeruginosa* structural redundancy within the biofilm matrix *Env. Microbiol.* **14** 1913–28
[55] Wei Q and Ma L Z 2013 Biofilm matrix and its regulation in *Pseudomonas aeruginosa Int. J. Mol. Sci.* **14** 20983–1005

[56] Friedman L and Kolter R 2004 Two genetic loci produce distinct carbohydrate-rich structural components of the Pseudomonas aeruginosa biofilm matrix *J. Bacteriol.* **186** 4457–65
[57] Jackson K D, Starkey M, Kremer S, Parsek M R and Wozniak D J 2004 Identification of psl, a locus encoding a potential exopolysaccharide that is essential for Pseudomonas aeruginosa PAO1 biofilm formation *J. Bacteriol.* **186** 4466–75
[58] Matsukawa M and Greenberg E P 2004 Putative exopolysaccharide synthesis genes influence Pseudomonas aeruginosa biofilm development *J. Bacteriol.* **186** 4449–56
[59] Zhang Y, Jiang J, Zhao Q, Gao Y, Wang K, Ding J, Yu H and Yao Y 2017 Accelerating anodic biofilms formation and electron transfer in microbial fuel cells: role of anionic biosurfactants and mechanism *Bioelectrochemistry* **117** 48–56
[60] Ma L, Jackson K D, Landry R M, Parsek M R and Wozniak D J 2006 Analysis of *Pseudomonas aeruginosa* conditional psl variants reveals roles for the psl polysaccharide in adhesion and maintaining biofilm structure postattachment *J. Bacteriol.* **188** 8213–21
[61] Ma L, Conover M, Lu H, Parsek M R, Bayles K and Wozniak D J 2009 Assembly and development of the *Pseudomonas aeruginosa* biofilm matrix *PLoS Pathog.* **5** e1000354
[62] Toyofuku M, Inaba T, Kiyokawa T, Obana N, Yawata Y and Nomura N 2016 Environmental factors that shape biofilm formation *Biosci. Biotechnol. Biochem.* **80** 7–12
[63] Nocelli N, Bogino P C, Banchio E and Giordano W 2016 Roles of extracellular polysaccharides and biofilm formation in heavy metal resistance of rhizobia *Materials* **9** 418
[64] Chen B, Li F, Liu N, Ge F, Xiao H and Yang Y 2015 Role of extracellular polymeric substances from *Chlorella vulgaris* in the removal of ammonium and orthophosphate under the stress of cadmium *Bioresour. Technol.* **190** 299–306
[65] Hong B H, Joe M M, Selvakumar G, Kim K Y, Choi J H and Sa T M 2017 Influence of salinity variations on exocellular polysaccharide production, biofilm formation and flocculation in halotolerant bacteria *J. Environ. Biol.* **38** 657
[66] Christenson L 2011 Algal biofilm production and harvesting system for wastewater treatment with biofuels by-products *MSc Thesis* Utah State University, Logan, UT
[67] Monzon O, Yang Y, Li Q and Alvarez P J 2016 Quorum sensing autoinducers enhance biofilm formation and power production in a hypersaline microbial fuel cell *Biochem. Eng. J.* **109** 222–7
[68] Agarwal A, Kahyaoglu C and Hansen D B 2012 Characterization of HmqF, a protein involved in the biosynthesis of unsaturated quinolones produced by *Burkholderia thailandensis Biochemistry* **51** 1648–57
[69] Diggle S P, Winzer K, Lazdunski A, Williams P and Cámara M 2002 Advancing the quorum in *Pseudomonas aeruginosa*: MvaT and the regulation of N-acylhomoserine lactone production and virulence gene expression *J. Bacteriol.* **184** 2576–86
[70] Vasseur P, Vallet-Gely I, Soscia C, Genin S and Filloux A 2005 The pel genes of the *Pseudomonas aeruginosa* PAK strain are involved at early and late stages of biofilm formation *Microbiology* **151** 985–97
[71] Liu X, Tremblay P L, Malvankar N S, Nevin K P, Lovley D R and Vargas M 2014 A *Geobacter sulfurreducens* strain expressing *Pseudomonas aeruginosa* type IV pili localizes OmcS on pili but is deficient in Fe (III) oxide reduction and current production *Appl. Env. Microbiol.* **80** 1219–24
[72] Rabaey K and Verstraete W 2005 Microbial fuel cells: novel biotechnology for energy generation *Trends Biotechnol.* **23** 291–8

[73] Watmough N J and Frerman F E 2010 The electron transfer flavoprotein: ubiquinone oxidoreductases *Biochim. Biophys. Acta—Bioenerg.* **1797** 1910–6
[74] Mitra R, Balachandar G, Singh V, Sinha P and Das D 2017 Improvement in energy recovery by dark fermentative biohydrogen followed by biobutanol production process using obligate anaerobes *Int. J. Hydrogen Energy* **42** 4880–92
[75] Singh L and Wahid Z A 2015 Enhancement of hydrogen production from palm oil mill effluent via cell immobilisation technique *Int. J. Energy Res.* **39** 215–22
[76] Singh L, Siddiqui M F, Ahmad A, Rahim M H A, Sakinah M and Wahid Z A 2013 Biohydrogen production from palm oil mill effluent using immobilized mixed culture *J. Ind. Eng. Chem.* **19** 659–64
[77] Zakaria B S, Lin L and Dhar B R 2019 Shift of biofilm and suspended bacterial communities with changes in anode potential in a microbial electrolysis cell treating primary sludge *Sci. Total Env.* **689** 691–9
[78] Chou T Y, Whiteley C G and Lee D J 2014 Anodic potential on dual-chambered microbial fuel cell with sulphate reducing bacteria biofilm *Int. J. Hydrogen Energy* **39** 19225–31
[79] Huang L, Chai X, Chen G and Logan B E 2011 Effect of set potential on hexavalent chromium reduction and electricity generation from biocathode microbial fuel cells *Env. Sci. Technol.* **45** 5025–31
[80] Kiely P D, Regan J M and Logan B E 2011 The electric picnic: synergistic requirements for exoelectrogenic microbial communities *Curr. Opin. Biotechnol.* **22** 378–85
[81] Leang C, Qian X, Mester T and Lovley D R 2010 Alignment of the c-type cytochrome OmcS along pili of *Geobacter sulfurreducens Appl. Env. Microbiol.* **76** 4080–4
[82] Richter K, Schicklberger M and Gescher J 2012 Dissimilatory reduction of extracellular electron acceptors in anaerobic respiration *Appl. Env. Microbiol.* **78** 913–21
[83] Nevin K P *et al* 2009 Anode biofilm transcriptomics reveals outer surface components essential for high density current production in *Geobacter sulfurreducens* fuel cells *PLoS One* **4** e5628
[84] Strycharz S M, Glaven R H, Coppi M V, Gannon S M, Perpetua L A, Liu A, Nevin K P and Lovley D R 2011 Gene expression and deletion analysis of mechanisms for electron transfer from electrodes to *Geobacter sulfurreducens Bioelectrochemistry* **80** 142–50
[85] Smith J A, Tremblay P L, Shrestha P M, Snoeyenbos-West O L, Franks A E, Nevin K P and Lovley D R 2014 Going wireless: Fe (III) oxide reduction without pili by *Geobacter sulfurreducens* strain JS-1 *Appl. Env. Microbiol.* **80** 4331–40
[86] Kim B C, Postier B L, DiDonato R J, Chaudhuri S K, Nevin K P and Lovley D R 2008 Insights into genes involved in electricity generation in *Geobacter sulfurreducens* via whole genome microarray analysis of the OmcF-deficient mutant *Bioelectrochemistry* **73** 70–5
[87] Mehta T, Childers S E, Glaven R, Lovley D R and Mester T 2006 A putative multicopper protein secreted by an atypical type II secretion system involved in the reduction of insoluble electron acceptors in *Geobacter sulfurreducens Microbiology* **152** 2257–64
[88] Holmes D E, Mester T, O'Neil R A, Perpetua L A, Larrahondo M J, Glaven R, Sharma M L, Ward J E, Nevin K P and Lovley D R 2008 Genes for two multicopper proteins required for Fe (III) oxide reduction in *Geobacter sulfurreducens* have different expression patterns both in the subsurface and on energy-harvesting electrodes *Microbiology* **154** 1422–35
[89] Keller K L, Rapp-Giles B J, Semkiw E S, Porat I, Brown S D and Wall J D 2014 New model for electron flow for sulfate reduction in *Desulfovibrio alaskensis* G20 *Appl. Env. Microbiol.* **80** 855–68

[90] Baron D, LaBelle E, Coursolle D, Gralnick J A and Bond D R 2009 Electrochemical measurement of electron transfer kinetics by *Shewanella oneidensis* MR-1 *J. Biol. Chem.* **284** 28865–73

[91] Shen H B, Yong X Y, Chen Y L, Liao Z H, Si R W, Zhou J, Wang S Y, Yong Y C, OuYang P K and Zheng T 2014 Enhanced bioelectricity generation by improving pyocyanin production and membrane permeability through sophorolipid addition in *Pseudomonas aeruginosa*-inoculated microbial fuel cells *Bioresour. Technol.* **167** 490–4

[92] Yong X Y *et al* 2014 Enhancement of bioelectricity generation by manipulation of the electron shuttles synthesis pathway in microbial fuel cells *Bioresour. Technol.* **152** 220–4

[93] Coursolle D, Baron D B, Bond D R and Gralnick J A 2010 The Mtr respiratory pathway is essential for reducing flavins and electrodes in *Shewanella oneidensis J. Bacteriol.* **192** 467–74

[94] Von Canstein H, Ogawa J, Shimizu S and Lloyd J R 2008 Secretion of flavins by *Shewanella* species and their role in extracellular electron transfer *Appl. Env. Microbiol.* **74** 615–23

[95] Jain A, Connolly J O, Woolley R, Krishnamurthy S and Marsili E 2013 Extracellular electron transfer mechanism in *Shewanella loihica* PV-4 biofilms formed at indium tin oxide and graphite electrodes *Int. J. Electrochem. Sci.* **8** 1778–93

[96] Marsili E, Baron D B, Shikhare I D, Coursolle D, Gralnick J A and Bond D R 2008 *Shewanella* secretes flavins that mediate extracellular electron transfer *Proc. Natl Acad. Sci.* **105** 3968–73

[97] Kotloski N J and Gralnick J A 2013 Flavin electron shuttles dominate extracellular electron transfer by *Shewanella oneidensis MBio* **4** e00553–12

[98] You J, Deng Y, Chen H, Ye J, Zhang S and Zhao J 2020 Enhancement of gaseous o-xylene degradation in a microbial fuel cell by adding *Shewanella oneidensis* MR-1 *Chemosphere* **252** 126571

[99] Goldbeck C P, Jensen H M, TerAvest M A, Beedle N, Appling Y, Hepler M, Cambray G, Mutalik V, Angenent L T and Ajo-Franklin C M 2013 Tuning promoter strengths for improved synthesis and function of electron conduits in *Escherichia coli ACS Synthet. Biol.* **2** 150–9

[100] Fuller S J, McMillan D G, Renz M B, Schmidt M, Burke I T and Stewart D I 2014 Extracellular electron transport-mediated Fe (III) reduction by a community of alkaliphilic bacteria that use flavins as electron shuttles *Appl. Env. Microbiol.* **80** 128–37

[101] Freguia S, Masuda M, Tsujimura S and Kano K 2009 *Lactococcus lactis* catalyses electricity generation at microbial fuel cell anodes via excretion of a soluble quinone *Bioelectrochemistry* **76** 14–8

[102] Deng L, Li F, Zhou S, Huang D and Ni J 2010 A study of electron-shuttle mechanism in *Klebsiella pneumoniae* based-microbial fuel cells *Chin. Sci. Bull.* **55** 99–104

[103] Torres C I, Marcus A K, Lee H S, Parameswaran P, Krajmalnik-Brown R and Rittmann B E 2010 A kinetic perspective on extracellular electron transfer by anode-respiring bacteria *FEMS Microbiol. Rev.* **34** 3–17

[104] Snider R M, Strycharz-Glaven S M, Tsoi S D, Erickson J S and Tender L M 2012 Long-range electron transport in *Geobacter sulfurreducens* biofilms is redox gradient-driven *Proc. Natl Acad. Sci.* **109** 15467–72

[105] Pirbadian S and El-Naggar M Y 2012 Multistep hopping and extracellular charge transfer in microbial redox chains *Phys. Chem. Chem. Phys.* **14** 13802–8

[106] Richter L V, Sandler S J and Weis R M 2012 Two isoforms of *Geobacter sulfurreducens* PilA have distinct roles in pilus biogenesis, cytochrome localization, extracellular electron transfer, and biofilm formation *J. Bacteriol.* **194** 2551–63

[107] Vargas M, Malvankar N S, Tremblay P L, Leang C, Smith J A, Patel P, Synoeyenbos-West O, Nevin K P and Lovley D R 2013 Aromatic amino acids required for pili conductivity and long-range extracellular electron transport in *Geobacter sulfurreducens MBio* **4** e00105–13

[108] Juárez K, Kim B C, Nevin K, Olvera L, Reguera G, Lovley D R and Methé B A 2009 PilR, a transcriptional regulator for pilin and other genes required for Fe (III) reduction in *Geobacter sulfurreducens J. Mol. Microbiol. Biotechnol.* **16** 146–58

[109] Leung K M, Wanger G, El-Naggar M Y, Gorby Y, Southam G, Lau W M and Yang J 2013 *Shewanella oneidensis* MR-1 bacterial nanowires exhibit p-type, tunable electronic behavior *Nano Lett.* **13** 2407–11

[110] Rotaru A E, Shrestha P M, Liu F, Markovaite B, Chen S, Nevin K P and Lovley D R 2014 Direct interspecies electron transfer between *Geobacter metallireducens* and *Methanosarcina barkeri Appl. Env. Microbiol.* **80** 4599–605

[111] Gregory K B, Bond D R and Lovley D R 2004 Graphite electrodes as electron donors for anaerobic respiration *Env. Microbiol.* **6** 596–604

[112] Xafenias N, Zhang Y and Banks C J 2013 Enhanced performance of hexavalent chromium reducing cathodes in the presence of *Shewanella oneidensis* MR-1 and lactate *Env. Sci. Technol.* **47** 4512–20

[113] Carbajosa S, Malki M, Caillard R, Lopez M F, Palomares F J, Martín-Gago J A, Rodríguez N, Amils R, Fernández V M and De Lacey A L 2010 Electrochemical growth of *Acidithiobacillus* ferrooxidans on a graphite electrode for obtaining a biocathode for direct electrocatalytic reduction of oxygen *Biosens. Bioelectron.* **26** 877–80

[114] Jeans C, Singer S W, Chan C S, VerBerkmoes N C, Shah M, Hettich R L, Banfield J F and Thelen M P 2008 Cytochrome 572 is a conspicuous membrane protein with iron oxidation activity purified directly from a natural acidophilic microbial community *ISME J.* **2** 542–50

[115] Wang Y Z, Wang A J, Zhou A J, Liu W Z, Huang L P, Xu M Y and Tao H C 2014 Electrode as sole electrons donor for enhancing decolorization of azo dye by an isolated *Pseudomonas* sp. WYZ-2 *Bioresour. Technol.* **152** 530–3

[116] Ringeisen B R, Henderson E, Wu P K, Pietron J, Ray R, Little B, Biffinger J C and Jones-Meehan J M 2006 High power density from a miniature microbial fuel cell using *Shewanella oneidensis* DSP10 Environ. Sci. Technol. **40** 2629–34

[117] Rabaey K, Boon N, Siciliano S D, Verhaege M and Verstraete W 2004 Biofuel cells select for microbial consortia that self-mediate electron transfer Appl. Environ. Microbiol. **70** 5373–82

[118] Pandit S, Khilari S, Roy S, Pradhan D and Das D 2014 Improvement of power generation using Shewanella putrefaciens mediated bioanode in a single chambered microbial fuel cell: effect of different anodic operating conditions Bioresour. Technol. **166** 451–7

[119] Kang C S, Eaktasang N, Kwon D Y and Kim H S 2014 Enhanced current production by Desulfovibrio desulfuricans biofilm in a mediator-less microbial fuel cell Bioresour. Technol. **165** 27–30

[120] Cheng S and Regan J M 2008 Electricity generation by Rhodopseudomonas palustris Environ. Sci.Technol. **42** 4146–51

[121] Min B, Cheng S and Logan B E 2005 Thermodynamic analysis of a single chamber microbial fuel cell Water Res. **39** 1675–86

[122] Qiao Y, Li C M, Bao S J, Lu Z and Hong Y 2008 Direct electrochemistry and electrocatalytic mechanism of evolved *Escherichia coli* cells in microbial fuel cells *Chem. Commun.* **11** 1290–2

[123] Li X, Zhong G Z, Qiao Y, Huang J, Hu W H, Wang X G and Li C M 2014 A high-performance xylose microbial fuel cell enabled by *Ochrobactrum sp.* 575 *cells* RSC Adv. **4** 39839–43

[124] Raghavulu S V, Goud R K, Sarma P N and Mohan S V 2011 *Saccharomyces cerevisiae* as anodic biocatalyst for power generation in biofuel cell: influence of redox condition and substrate load *Bioresour. Technol.* **102** 2751–7

[125] Xu S and Liu H 2011 New exoelectrogen *Citrobacter sp*. SX-1 isolated from a microbial fuel cell *J. Appl. Microbiol.* **111** 1108–15

[126] Nandy A, Kumar V and Kundu P P 2013 Utilization of proteinaceous materials for power generation in a mediatorless microbial fuel cell by a new electrogenic bacteria *Lysinibacillus sphaericus* VA5 *Enzyme Microb. Technol.* **53** 339–44

[127] González del Campo A, Cañizares P, Rodrigo M A, Fernández F J and Lobato J 2013 Microbial fuel cell with an algae-assisted cathode: a preliminary assessment *J. Power Sourc.* **242** 638–45

[128] Cui Y, Rashid N, Hu N, Rehman M S U and Han J I 2014 Electricity generation and microalgae cultivation in microbial fuel cell using microalgae-enriched anode and bio-cathode *Energy Conv. Manage.* **79** 674–80

[129] Wu C, Liu X W, Li W W, Sheng G P, Zang G L, Cheng Y Y, Shen N, Yang Y P and Yu H Q 2012 A white-rot fungus is used as a biocathode to improve electricity production of a microbial fuel cell *Appl. Energy* **98** 594–6

[130] Yuan Y, Chen Q, Zhou S, Zhuang L and Hu P 2011 Bioelectricity generation and microcystins removal in a blue-green algae powered microbial fuel cell *J. Hazard. Mater.* **187** 591–5

[131] Inglesby A E, Beatty D A and Fisher A C 2012 *Rhodopseudomonas palustris* purple bacteria fed *Arthrospira maxima cyanobacteria*: demonstration of application in microbial fuel cells *Rsc Adv.* **2** 4829–38