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REVIEW ARTICLE

Eco-physiological and interdisciplinary approaches for empowering biobatteries

Ganesh Mahidhara[1] · **Venkata Ramana Chintalapati**[1]

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Abstract Electrogenic bacteria have evolved with a tendency to oxidize various organic matter and donate electrons to terminal electron acceptors. This forms the basis for power generation by microbe-driven fuel cells when these electro-active bacteria interact with electrodes. Depending on an electrode's potential for oxidation of an electron donor at an anode (E_{ano}) compared to the reduction of an electron acceptor at a cathode (E_{cat}), a net positive/negative potential difference (ΔE) will arise. Correspondingly, a positive ΔE will result in power generation (microbial fuel cells, MFCs) while negative ΔE requires power (microbial electrolysis cells). Herein, various factors that reduce power efficiency in MFCs, compared to theoretical calculations and their troubleshooting, are discussed. Furthermore, eco-physiological studies of electrogenic bacteria, in relation to their electron transfer molecular mechanisms when grown in varying electron donor-acceptor ratios are also discussed. Hence, the information with respect to the choice of an electrogen, the type of inocula, and electrode material (depending on the terminal electron acceptor) for the development of novel MFCs is understood. Finally, improvements of anode performance in MFCs, using advances in nanotechnology were explored to generate ideas for enhancing power densities. Altogether, a combinatorial approach in the discovery of electrogenic molecular mechanisms, along with improving the existing electrode material, can significantly enhance the generation of alternative and eco-friendly electricity.

✉ Venkata Ramana Chintalapati
chvrsl@uohyd.ernet.in

[1] Department of Plant Sciences, School of Life Sciences, University of Hyderabad, P.O. Central University, Hyderabad 500 046, India

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Introduction

Generating electricity whilst maintaining a carbon neutral environment is of vital need to overcome the contemporary fuel crises, as well as global warming. One of the ways to reduce the atmospheric pollution problem is by researching and creating renewable bioenergy (Du et al. 2007). Recently, vast quantities of water have been used for electricity generation using conventional power plants, increasing the stress on water bodies and their inbuilt ecosystems. For the efficient treatment of wastewater and its reuse, energy is also required, with substantial amounts of energy is being lost or wasted in the organic matter present in industrial/household waste. For example, the United States uses around 15 GW of power (3 % of its gross power) for the treatment of its organic-rich wastewater (McCarty et al. 2011). However, it was estimated that around 17 GW of power could be efficiently generated from intrinsic energy in animal, domestic, and industrial wastewater, which together constitute ~ 1.5×10^{11} kWh of potential energy (Logan 2004). The aforementioned issues can be addressed by using microbial fuel cells (MFCs)—a promising technology in which wastewater can be treated while simultaneously creating electricity, possibly making it an energy neutral process (Lovley 2006). MFCs use microorganisms as catalysts to convert organic substrates into electricity. These microorganisms are termed electrogens, based on their brilliant ability to transport electrons to insoluble electron acceptors. Basic alignment of an MFC consists of anode and cathode chambers partitioned by a proton exchange membrane (PEM) (Gil et al. 2003). Conventional electrochemical process involves fuel (electrolyte) that provides

electrons and an electron acceptor, which gets oxidized in the process. In an MFC, electrolyte is the organic matter and oxygen (for aerobic bacteria) or other chemicals, such as nitrate, sulfate, and carbon dioxide (for anaerobic bacteria), serve as electron acceptors. In brief, microbes generate electrons and protons in the anodic chamber with the oxidation of organic substrates. Anodes absorb and transport the electrons to the cathode with an external circuit (Fig. 1). After crossing a PEM or a salt bridge, the protons enter the cathodic chamber where they combine with oxygen to form water (Pant et al. 2010).

Why are not all bacteria electrogenic?

Even though about 15,974 bacterial names have been described up to 2015 (http://www.bacterio.net/-number.html), only a few of these are electrogenic. The principal requirement for determining electrogenicity is the capability of the bacteria to transfer electrons to the anode, either by direct or indirect means (given in detailed below). A majority of the efficient electrogens possess naturally evolved appendages such as nanowires. More recently, Pirbadian et al. (2014) in their fascinating study, have demonstrated the molecular basis for the generation of nanowires in *Shewanella oneidensis* MR-1. In this study, nanowires were proven to be the extensions of the outer membrane and periplasm by using techniques such as in vivo fluorescence measurements, immune labeling, and quantitative gene expression analysis. Outer membrane multiheme cytochromes, which are involved in exo-electron transfer (EET)—namely, MtrC and OmcA—were shown to be localized in the nanowires. This finding enhances the curiosity to know further mechanistic and bio-physiological impact of these structures in electron transport. Besides efficient electron transfer to the anode, other factors such as the capacity to metabolize complex organic matter also determine electrogenicity, as shown in Fig. 1. In order to generate an electric current, the anode is set to act as a terminal electron acceptor for the electrogens, in the conventional MFC design. In an MFC, net positive cell voltage arises when oxidation of an electron donor at the anode with lower electrode potential (E_{ano}) is coupled to the reduction of an electron acceptor with a higher electrode potential (E_{cat}) at the cathode ($E_{cat} > E_{ano}$) (Rabaey and Rozendal 2010).

This phenomenon could be explained by observing the glucose to water redox couple (Fig. 1). Standard electrode potential (E') at pH 7 for the oxidation of glucose to HCO_3 measured versus the standard hydrogen electrode (SHE) is −0.41 V; when this is coupled to the reduction of O_2 to water (E' = 0.82 V), power is produced. The reaction is driven forward as the resulting cell voltage (cathode potential *minus* anode potential; 1.23 V) is positive. Conversely, an input of power is required when the oxidation of an electron donor at the anode is coupled to the reduction of an electron acceptor with a lower electrode potential at the cathode ($E_{cat} < E_{ano}$). For example, when acetate/HCO_3 (E' = −0.28 V versus SHE) is coupled to H^+/H_2 (E' = −0.41 V versus SHE) the resulting cell voltage is negative (−0.13 V) (Thauer et al. 1977; Logan 2008). Hence,

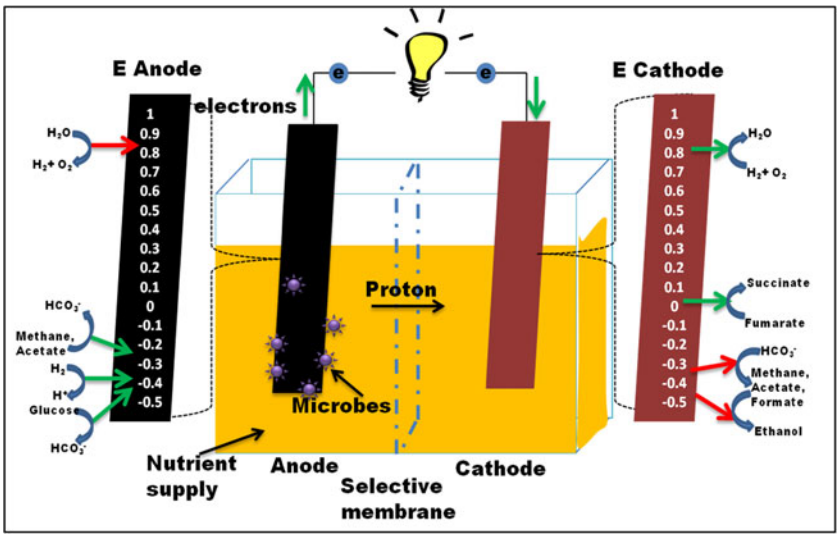


Fig. 1 Basic setup for the microbial fuel cell operation. Electrogens that inhabit the anode compartment (shown in violet) catalyze the oxidation of organic substrates, which generate electrons and protons. The anode (black) absorbs and transports the electrons to the cathode (red) via an external circuit. After crossing a PEM or a salt bridge (dotted square), the protons enter the cathodic chamber where they combine with oxygen to form water. The combination of redox couple represented by the green arrow in cathode and anode chambers, respectively, gives a positive electromotive force (emf) ($E_{emf} = E_{cat} - E_{ano}$), which means the generation of electricity. In order for catalysis to happen for the redox couple represented by the red arrow, an input of energy is needed.

depending on the type of organic compounds oxidized by the electrogenic microorganisms, their complete and/or incomplete degradation in the anode chamber in combination with the chemistry at the cathode chamber governs whether the reaction is driven forward. Microbial electrogenesis is a complex process governed by the presence of appendages for EET, along with having a capacity to degrade complex organic substances. Based on these characteristics, not all the aforesaid bacteria are electrogenic. Two types of bioelectrochemical systems were defined, depending on the reactions taking place at the electrodes: 1) microbial fuel cells when they produce power, and 2) microbial electrolysis cells, which require an input of power (Fig. 1).

Barriers of power efficiency in MFC: deviation from theoretical calculations

Electron transfer (direct or mediated) from bacteria towards the anode is normally hampered by various predicaments, which impact the energy efficiency of the fuel cell. High internal resistance, membranes, pH gradients, limited temperature, complex organic matter degraded by microorganisms, and biofilm kinetics are significant factors that impede the electrogenesis (http://www.microbialfuelcell.org). Three different losses, including mass transfer losses, activation and ohmic losses, are normally possible during MFC operation (Raghavulu et al. 2012). Metabolic activity of the anodic biofilm is vulnerable to the accumulation of oxidized intermediates or protons. This might change the redox conditions and hamper mass transfer of substrate or electron acceptors towards the electrode, subsequently resulting in mass transfer or concentration losses. In other words, concentration losses arise as a result of a large oxidative force of the anode. This situation occurs at lower resistance, where substrate oxidation is faster at the anode, as there is a decreased number of electrons to the anode surface followed by the cathode (Rozendal et al. 2008). Catalyzed oxidation of a compound via electrogenic bacteria occurs at the bacterial surface or internal side, and the subsequent electron transfer to the anode requires activation energy. This kind of situation generally results in a voltage loss or activation overpotential, generally described as activation loss. Activation losses are considered in the lower range of current densities and are crucial (Clauwaert et al. 2007). The possibility to undergo multiple metabolic reactions/pathways simultaneously with mixed culture incurs a high energy resulting in activation losses. This also facilitates electron quenching, which normally arises due to competition between metabolic pathways. Activation losses can be avoided by enhancing effective surface area of the electrode, operating temperature, improving the electrode catalysis, and choice of microorganisms that form enriched biofilms. Resistance possessed by the anode material was supposed to impose ohmic losses in the MFC. Hence, conductivity of the anode material is inversely related to ohmic losses; higher conductivity lowers the ohmic losses (Fan et al. 2008). Similarly, transport of ions across the electrodes is essential for current flow, the resistance that ions experience also contributes to ohmic losses. This can be minimized by using an ideal combination of electrolyte and semi-permeable membranes. As mentioned above, in an MFC, while electrons flow through an external circuit, protons migrate across a partition. Ion exchange membranes, size-selective separators, and salt bridges are three major classes of membranes. Lower bioelectrocatalytic activity and coulombic efficiency were reported (Liu et al. 2005) in the absence of a separator with an increase in oxygen, substrate diffusion causes rapid befouling/inactivation of the cathode. Thus, the separator is useful in keeping the electrodes at a minimum distance to reduce internal resistance and to ensure the efficient and sustainable operation of MFCs (Harnisch and Schröder 2009). The drawbacks, however, include pH variations in electrode chambers, increase in overall internal resistance, and total cost of the setup (Kim et al. 2007). These drawbacks can be answered by recent developments in separator electrode assemblies, together with significant advances in separator materials (Li et al. 2011a, b). For example, bipolar membranes that were formed by mounting cathode exchange membranes and anode exchange membranes were shown to be more efficient in ion exchange between the electrodes and ease the proton transport across them. Hence, cheaper and more efficient separators need to be explored alongside optimizing the separator operating conditions in order to develop mechanically robust separators.

Electrogenesis and mechanisms underlying electrode-electron transfer

Bacteria known as exo-electrogens (termed electrogens in this article) have the ability to transfer electrons outside the cell to insoluble electron acceptors, such as iron and other metal oxides, or to the electrodes in bioelectrochemical systems. Anodophiles, exoelectrogens, electrogenic microorganisms, anode-respiring bacteria, and electrochemically active bacteria were proposed as an alternative terminology to denote microorganisms that can transfer the electrons to fuel cell anodes. Electricigens was the name postulated for the microorganisms that completely

oxidize organic compounds to carbon dioxide with an electrode serving as the sole electron acceptor (Logan 2009). Microbe-electrode exchange is a result of natural selection; electrogenic bacteria have developed effective strategies for extracellular electron exchange over billions of years of evolution. It was suggested that they use insoluble minerals and related natural extracellular electron acceptors or electron donors for the electrogenesis (Yi et al. 2009). Although electrodes are not a part of natural environment, they are more efficient based on the fact that they can provide an ideal platform for long-term electron-mediators, compared to readily depletable individual minerals (Lovley 2012). This phenomenon can be explained by examining *Geobacter* species. In one study, differences between physiology and proteomic profiling of cells growing with Fe (III) oxide vs. soluble Fe (III) citrate as the electron acceptor was studied using accurate mass and time tag technique. It was found that when actively reducing Fe(III) oxides, motile flagella were produced by *Geobacter* species in search of new Fe(III) sources (Ding et al. 2008). On the other hand, when oxidizing the electrodes, *Geobacter* species were found to colonize in thick (>50 μm), metabolically active biofilms (Nevin et al. 2009). Therefore, biofilm forming ability on the surfaces of electrodes gives an advantage compared to individual minerals for the electrogenic bacteria, in order to maintain a superior relationship. Thus, in microbial electricity production, the electrons are transferred to an electrode rather than to a free electron acceptor such as Fe (III). As autotrophs play a central role in the conversion of solar energy to chemical energy, they could be used as an additional means, if incorporated in the chain of events, to produce eco-friendly electricity. Given that cyanobacteria are the first oxygenic photosynthetic microorganisms evolved in ocean beds, prevalent anoxic conditions, and graphitic deposits, there is a general curiosity to know their power efficiency. In this regard, cyanobacterial members such as *Nostoc, Spirulina, Anabaena, Synechocystis* PCC 6803, were investigated for electrogenic activity based on photosynthesis. A two chambered MFC was shown to produce electricity at a range of 6.7 mW/m^3 when used *Synechocystis* PCC 6803 as the electrogen, which is a carbon neutral process (Madiraju et al. 2012). In another attempt, a sediment MFC having acidic fermentation broth of cyanobacteria has produced 7.2 mW/m^3 power density, with a significant chemical oxygen demand (COD) removal efficiency (Zhao et al. 2012). More recently, cyanobacterial *Nostoc* sp. was immobilized on carbon nanotubes (CNT) and used as anode material. This system was found to produce power densities of 3.5 mW/m^3 with the interaction of CNT with photosystem II (Sekar et al. 2014). Other photosynthetic bacteria such as *Rhodopseudomonas* spp. were investigated, and in the anodic chambers of MFCs that were illuminated to the light, an approximate10 % increase in power densities compared to MFCs in dark (Xing et al. 2009). In a similar study, mixotrophic photosynthetic consortium was successfully used as an anolyte to produce electric current with lower emission of CO_2 (Chandra et al. 2012). Taken together, fixation of CO_2 in the ingredients of the anolyte is possible using the photosynthetic microorganisms, making the overall process carbon neutral. Table 1 also summarizes various electrogenic bacteria, their mode of transfer and the type of metal/metal oxide they can reduce.

Two basic means of electron transfer were investigated (Fig. 2a): short-range and long-range electron transfer. These are founded on the modes of transfer of the electrons from bacteria to the terminal electron acceptor. Based on the type of transfer, these modes are again categorized in three different mechanisms.

(i) Artificial electron transport mediators: These electron shuttles accept electrons from electron carriers within the cell and transfer to the electrode surface after crossing cell membranes. Common electron shuttles include thionine, benzylviologen, 2,6-dichlorophenolindophenol, 2-hydroxy-1,4-naphthoquinone, and various phenazines, phenothiazines, phenoxoazines, iron chelates, and neutral red (Lovley 2006). Artificial mediators are used in the case of bacteria such as *Escherichia coli*, *Pseudomonas*, *Proteus*, and *Bacillus* species, since these microorganisms are unable to transfer, resulting electrons from the central metabolic pathways (McKinlay and Zeikus 2004). However, there are more disadvantages in the usage of these artificial mediators, with drawbacks including toxicity of most of the mediators, their use in open systems, additional cost to the MFC systems, and the continuous production of fermentation products (Park and Zeikus 2000).

(ii) Microbial mediators for electron transport: Some microorganisms (including Gram positive and Gram negative) transport electrons to insoluble metal oxides by producing electron shuttles (Rabaey et al. 2007). In one study (Nevin and Lovley 2002), extracellular electron-shuttling compounds released by *Geothrix fermentans* were determined by incorporating poorly crystalline Fe(III) oxide into microporous alginate beads, which prevented contact between *G. fermentans* and the Fe(III) oxide. *G. fermentans* reduced the Fe(III) within the beads, suggesting that one of the compounds that *G. fermentans* releases is an electron-shuttling compound that can transfer electrons from

Table 1 Important electrogenic microorganisms capable of mediating direct electron transfer

S. No	Electrogenic bacteria	Class	Terminal electron acceptor	Mode of transfer	Reference
1.	*Geobacter sulfurreducens*	δ - proteobacteria	Insoluble extracellular deposits of Fe(III) and Mn(IV)	Direct transfer (pili)	(Bond and Lovley 2003)
2.	*Geobacter metallireducens*	δ - proteobacteria	Insoluble extracellular deposits of Fe(III) and Mn(IV)	Direct transfer (pili)	(Min et al. 2005)
3.	*Geobacter anodireducens*	δ - proteobacteria	Soluble or insoluble Fe (III)	Yet to be determined	(Sun et al. 2014)
4.	*Shewanella oneidensis*	γ - proteobacteria	Fe(III) deposits	Endogenous electron mediators	(Ringeisen et al. 2006)
5.	*Desulfovibrio*	δ - proteobacteria	Hydrogen sulphide	Indirect ET through the interaction of reduced metabolic products	(Kang et al. 2014)
6.	*Aeromonas hydrophila*	γ - proteobacteria	Fe(III) deposits	Possibly OMCs	(Pham et al. 2003)
7.	*Anaeromyxobacter dehalogenans*	δ - proteobacteria	Oxidizes Nitrate, U(VI),	Direct electron transfer	(Strycharz et al. 2010)
8.	*Rhodoferax ferrireducens*	β - proteobacteria	Fe(III) deposits	Direct electron transfer	(Chaudhuri and Lovley 2003)
9.	*Rhodopseudomonas palustris*	α - proteobacteria	Fe(III), sulfer and other minerals	Direct electron transfer	(Xing et al. 2008)
10.	*Thermincola* spp.	Fermicutes	Fe(III) deposits	OMC	(Carlson et al. 2012)
11.	*Citrobacter* sp. SX-1	γ - proteobacteria	Fe(III) deposits/ oxygen	Direct electron transfer	(Xu and Liu 2011)

the cell to Fe(III) oxide. More recently, it was found that *G. fermentans* secreted two different soluble redox active electron shuttles with separate redox potentials (−0.2 V and 0.3 V). This study demonstrated the existence of high-potential redox active compounds involved in extracellular electron transfer (Mehta-Kolte and Bond 2012). Functional mechanism for the reduction of Fe (III) oxides by *Shewanella* spp. was found to be linked to the secretion of the electron shuttles. One study has (von Canstein et al. 2008) suggested the existence of flavin mononucleotide (FMN), or riboflavin-5′-phosphate, and riboflavin as the extracellular electron shuttles in a range of *Shewanella* spp. Effects of various electron acceptors, including fumarate, ferrihydrite, Fe(III)-nitrilotriacetic acid, nitrate, and trimethylamine oxide, on the secretion of flavins by *Shewanella oneidensis* MR-1 suggested fluctuation in the levels of riboflavin and FMN among the abovementioned moieties (Wu et al. 2013). Thus, phylogenetically distinct Fe(III)-reducing microorganisms may use significantly different strategies for Fe(III) reduction. Electron shuttles play a principal mode of electron transport, even when cells are in direct contact with the electrode. For example, knockout mutagenic analysis of cytochromes in *S. oneidensis* MR-1 has indicated similarities between direct and mediated electron transport. From this study, it is also evident that a *c*-type cytochrome, Mtr-C (part of multi protein complex) is an important transporter of electrons from periplasm to outside of the cell (Carmona-Martinez et al. 2011). The importance of flavins in extracellular electron transport was recognized, as demonstrated by a previous study. A transposon mutagenesis screen was performed with *S. oneidensis* to identify mutants unable to secrete flavins indicated a defect in the rate of insoluble Fe (III) oxide reduction (Kotloski and Gralnick 2013). Extracellular respiratory pathways consisting of decaheme cytochromes found on the outer surface of the cell, and MtrC in particular, was found to be essential for the reduction of flavins (Coursolle et al. 2010). In total, MtrC is responsible for reduction of flavins at the outer cell surface and the subsequent reduction of electrodes.

(iii) Direct electron transfer using external appendages for long-range interactions: In the case of MFCs, higher current densities were reported with microbial biofilms rather than a single layer of microorganisms around the electrode (Malvankar et al. 2011). This was attributed to the presence of a dense forest of 3–5 nm wide pili spanning between the biofilm and electrode (length ~15

μm). This postulation first came from chemotactic studies on *Geobacter metallireducens* grown on insoluble Fe(III) or Mn(IV) oxides. These experiments suggested that the bacterium produces external appendages with increased gene expression corresponding to the PilA, the structural protein for type IV pili, when it senses the metal oxides (Childers et al. 2002). In yet another interesting study, differences in the proteomic profiles of *Geobacter sulfurreducens* grown on poorly crystalline Fe(III) oxide or on soluble Fe(III) citrate was analyzed in order to identify proteins that might be specifically associated with Fe(III) oxide reduction. A sum of 269 proteins was found to be abundant in insoluble Fe(III), which incorporate 13 *c*-type cytochromes and PilA (Ding et al. 2008). This indicates the importance of pili in the long-range electron transport to the insoluble metal oxides. More recently, two isoforms of conductive pili were found in *G. sulfurreducens* (Richter et al. 2012). The short PilA isoform in intracellular fraction helps in secretion of *c*-type cytochromes and stabilizes the longer isoform, which in turn is required for the formation of pili with the secretion of PilA to the outer cell surface. The existence of conductive pili has been described in other species of electrogenic bacteria. For instance, electrically conductive pilus-like appendages called bacterial nanowires were identified in dissimilatory metal-reducing bacteria *Shewanella oneidensis* MR-1, oxygenic phototrophic cyanobacterium *Synechocystis* PCC 6803, and the thermophilic, fermentative bacterium *Pelotomaculum thermopropionicum* using high throughput electron microscopic techniques (Gorby et al. 2006). Mutational studies have showed that MR-1 Msh pilin proteins were involved in extracellular electron transfer in MFCs enriched with *S. oneidensis* (Fitzgerald et al. 2012). When the structural nanofilament Msh pilin proteins are deleted (ΔmshA-D), *S. oneidensis* retains its ability to generate current through extracellular electron transfer. Nevertheless, no current was generated when Msh pilin protein complex was deleted (ΔmshH-Q) (Fitzgerald et al. 2012). This indicates that pili are not important in extracellular electron transfer in the case of *S. oneidensis,* as it can use nanowires or soluble electron shuttles as alternative means.

In natural environments, graphite deposits were shown to be efficient reservoirs for microbial electron transfer, which is renowned with the geobattery model (Bigalke and Grabner 1997). Since the graphite geobattery deposits served as

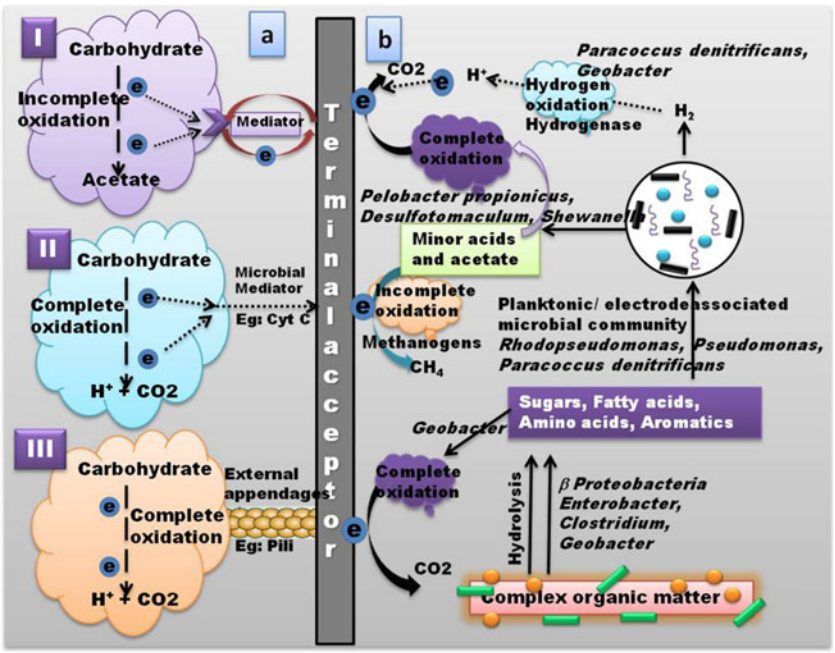


Fig. 2 Broad variety of microbial metabolomics. a) Different modes of electron transport; I using artificial electron transporters such as neutral red; II electron transport by means of bacterial cellular components such as outer membrane cytochromes; III direct electron transport by using external appendages such as flagella and nanowires. b) Schematic illustration of complex organic matter degradation. Planktonic microbial community (for example, *Rhodopseudomonas, Pseudomonas*, *Paracoccus denitrificans*) degrades the hydrolysates of the complex organic matter in to short-chain acids such as acetate with the production of H_2, with a trivial exogenic electron transport. Electrogenic bacteria (*Pelobacter propionicus, Desulfotomaculum, Shewanella, Geobacter*) then can mediate complete degradation of these organic compounds. Bacterial communities such as *Paracoccus denitrificans* and *Geobacter* further mediate the oxidation of hydrogen with the help of hydrogenases

electron reservoirs, over thousands of years, electrogenic bacteria that were evolved in these sediments have a natural tendency to use graphite as their preferred electrode material in MFCs. Future developments in research areas aimed at the biodiversity of electrogenic microbes in natural sediments, particularly focusing on their interaction with electrode-natural analogs, could enlighten the understanding of microbe-electrode interactions.

Microbial eco-physiological studies for escalating bio-electricity

In order to achieve a dual goal of power generation and water treatment, wastewater from household and industrial effluents has been used as inocula for power generations in MFCs. These effluents comprise complex organic moieties with varying composite chemistry. Complete degradation of organic compounds by bacteria is the preferred aspect for increased power densities (Fig. 2b). Eco-physiological studies of electrogenic bacteria significantly improve our understanding on the microbial electron transport and most importantly in the choice of inocula and suitable electrode. Initial research concerning the mechanisms of microorganism–anode interactions were focused on genome-scale investigations based on gene expression and proteomics. These studies were more precisely understood using pure cultures and in most cases 16S rRNA analysis, denaturing gradient gel electrophoresis (DGGE), and fluorescence in situ hybridization (FISH) were chosen as a basis for the determination of the bacterial community that is predominant in metabolizing complex substances into simpler ones with a concurrent electron transfer. The majority of complex waste degradation is mediated by syntrophic interactions between different microbial species (Stams and Plugge 2009), as outlined in Fig. 2b. With intense 16S rRNA sequencing, DGGE, and FISH techniques, it was found that *Thauera* spp. and *Geobacter* spp. were the abundant exoelectrogens that degrade the complex organic matter such as domestic and industrial wastewater to simple sugars and fatty acids (Patil et al. 2009; Kiely et al. 2011). Similarly, *Clostridium* spp. was shown to produce hydrogen, acetate, and ethanol as primary byproducts from cellulose, which were further metabolized by the *Geobacter* spp. (Ren et al. 2007). The abovementioned short chain organic acids such as acetate and fumarate were completely oxidized by *Pelobacter propionicus*, *Geobacter* spp., *Azoarcus* spp., and *Desulfuromonas* spp. with CO_2 as the end product (Chae et al. 2009). Formate dehydrogenase enzyme was shown to be used by *Paracoccus denitrificans* for the generation of hydrogen and CO_2 from formate. The production of hydrogen can also be augmented by using alternative methods such as using *N*-heterocyclic aromatic compounds (Archana et al. 2003). Hydrogen produced in the reaction is further catalyzed by hydrogenases in *Geobacter* spp. and other species to produce electricity (Bond and Lovley 2003). In another study, hydrogenases produced by *Geobacter* spp. were shown to be unique in the δ-subdivision of the *Proteobacteria*, which might be the appealing reason behind the *Geobacter* spp.-mediated hydrogen oxidation (Coppi 2005). Consequently, the syntrophic interactions among the chemotrophs become predominant as it could decrease the energy barriers for the formation of products, with increased forward reaction rates. This is due to an efficient product removal from the other partner of the syntrophic community. Moreover, the models studied using MFCs may or may not work similarly in the natural systems, as electrogens are subjected to an artificial pressure in the case of MFCs compared to their natural habitat. Thus, studies that correlate to the metabolomics of syntrophic interactions that superiorly use the complex matter and their energitics need to be explored. Therefore, discovery of pure cultures that directly mediate the electron transfer to electrodes, with an additional advantage such as models for complete oxidation of organic compounds and the molecular mechanisms is much desired for improvement of power densities in the MFCs.

Electrogenic molecular studies conducted so far were mainly focused in *Geobacter* spp. and *Shewanella* spp., arguably because of the amount of existing literature about high power densities produced by these two microorganisms in MFCs (Lovley et al. 2011). The increased number of publications on discovery of new strains, such as *Geobacter anodireducens,* also makes them interesting candidates to explore electrogenic mechanisms (Sun et al. 2014). In order to understand better the evolution of electron transport in *Geobacteriaceae*, and to help provide foundational data, genomes of six species were compared. The results suggest that enzymes involved in the central metabolic pathway were well conserved, which includes NADH dehydrogenase, ATP synthase, and several TCA cycle enzymes. However, outer membrane cytochromes were poorly conserved in all the strains; additionally, each strain had more abundant and unique cytochromes (Butler et al. 2010). These results suggest that energy generation from acetate is common among the bacterial species, whereas electron transport across the inner to outer membrane is different. Furthermore, metabolic flux analysis was conducted to investigate the metabolic fate of ^{13}C-labelled acetate in *Geobacter sulfurreducens* in response to electron donors (acetate, hydrogen) and acceptors (Fe(III), fumarate), by analyzing proteinogenic aminoacids. The results showed an increase in metabolic flux corresponding to the initiation of gluconeogenesis, oxidative TCA cycle, when fumarate was used as an electron acceptor and acetate as electron donor compared to Fe(III) acting as electron acceptor (Yang et al. 2010). In another interesting study, *G. sulfurreducens* was grown with acetate as an electron donor and fumarate as an electron acceptor and genomic regulation of sigma factor RpoS was analyzed, using microarray. It was shown to

regulate a number of genes such as *c*-type cytochromes and oxidative stress resistance and genes involved in adaptation to nutrient limitation. Interesting similarities were found using proteomics under similar conditions (Núñez et al. 2006). Similarly, RpoN was shown to be an essential sigma factor in a global regulator *G. sulfurreducens* involved in a complex transcriptional network such as pilA, cytochromes and involved in extracellular electron transfer to Fe(III) controlling a variety of cellular processes and central metabolism (Leang et al. 2009). Further studies related to understanding the metabolism of *G. sulfurreducens* using different electron donors and acceptors will guide improvements in strategies or designs of the future MFC configurations. High power densities in *G. sulfurreducens* fuel cells were attributed to the outer surface components analyzed using transcriptomic profiling. MFCs with graphite anodes as electron acceptors encompassing biofilms of ~50 μm thick were compared to their counterparts served with fumarate as the sole electron donor and grown on graphite surface, but disconnected from the cathode (Nevin et al. 2009). Mechanistic factors delineating the lower power efficiency of fumarate grown fuel cells were investigated by transcriptomic analysis compared to wild type biofilms (biofilms grown on graphite anodes). Microarray results showed up regulation in pilA, omcB (outer membrane cytochrome), and omcZ genes and down regulation of omcS and omcT in the wild-type biofilms, compared to the fumarate grown biofilms. Furthermore, deletion mutational analysis revealed the significance of pilA or omcZ, without which, there was a stern decrement in current production. Thus, OmcZ in concert with pili (pilA) were established to be the inbuilt members in long range electron transfer in *G. sulfurreducens* (Fig. 3). In the same study, high power densities were reported to be produced by omcB and omcE deletion mutants (Nevin et al. 2009). Nevertheless, this phenomenon can be attributed to the involvement of different types of electron donor/acceptor moieties and different biofilm kinetics of these MFCs. Some of the results in the above study contradicted previous reports, which had shown that *G. sulfurreducens* deficient in omcS or omcE could no longer reduce Fe(III) oxide (Mehta et al. 2005). Comparing both studies, one can hypothesize that the same organism uses different outer membrane cytochromes for EET depending on the niche and type of electron acceptor. In yet another fascinating study, substantial decrease in current production was observed in the case of *G. sulfurreducens* with a deletion of omcF, encoding a monoheme outer membrane *c*-type cytochrome (Kim et al. 2008). Whole genome microarray analysis revealed modulation of genes involved in metal efflux and/or type I secretion, which are implicated either in electron transport or in the localization of other cytochromes such as OmcS and OmcT to the cell membrane. Thus, OmcF was shown to directly or indirectly influence the current production in *G. sulfurreducens* (Kim et al. 2008). Based on these studies, the possibility for a "switch" used by the bacteria to choose the type of outer membrane cytochromes depending on the conditions offered to it cannot be ruled out. Further studies exposing the electrogenic microorganism to widespread environmental microcosms that engage various electron acceptors may help in drawing better conclusions regarding the usage of extracellular electron donors and the "electrogenic switch".

More recently, metabolomic quantification of key intracellular components in *Shewanella oneidensis* was performed to know the physiological impact of various dilution rates and changing electron donor (Lactate)–acceptor (Fumarate) ratios (Wang et al. 2013). Increase in the ability of the microbe to

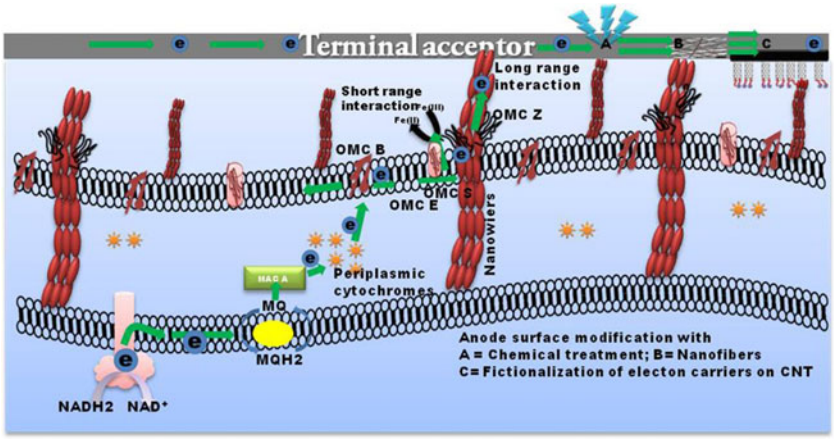


Fig. 3 Mechanism of electron transport by bacterial nanowires and/or outer membrane cytochromes. Microbial biofilms produce electricity by long range electron transport using a dense forest of 15 μm long nanowires. This is made possible in exo-electrogenic bacteria using a symphony of proteins including MAC A, Periplasmic cytochromes, outer membrane cytochromes such as OmcZ and PilA, principle component of nanowires. Other cytochromes such as omcB and omcE, omcS and omcT play an important role in the reduction of metal oxides and especially in the short-range electron transport. As shown in the figure, anode surface modification with chemical treatment, impregnation of synthetic conductive nanowires on the anode surface, and/or functionalization of electron carriers on the vertically aligned carbon nanotubules, improves the electricity generation in MFCs

reduce the model dye (ramazol black B) and an increased cytochrome *c* levels were observed when cells were grown under conditions of electron acceptor limitation. However, yields of extracellular redox mediators (flavins) were found to be unaffected in these conditions. A central metabolic pathway model was constructed using discriminate analysis and a Bayesian network for analyzing key intracellular metabolites and identified key mechanisms involved in controlling the electron transfer (Wang et al. 2013). In an attempt to study the mechanistic differences in electron transport of *S. oneidensis* in aerobic and anaerobic conditions, metabolomic profiles under these conditions were investigated using HPLC and FTIR techniques (Wang et al. 2010). Levels of riboflavin, FMN, and FAD in culture supernatants were quantified using correlations between the cell's FT-IR spectra and partial least-squares regression (PLSR). The results showed that both riboflavin and FMN were found to be dominant electron transporters in both aerobic and anaerobic conditions and those levels are predominant in the case of anaerobic culture conditions. Furthermore, significant alterations in the metabolic fingerprints of the cells grown under diverse culture conditions, growth phases and electron acceptors were observed. In total, these investigations will give significant insight into the eco-physiology of the electrogenic microorganisms under environmentally relevant conditions. For instance, eco-physiology of the electrogenic bacteria can be drawn when they were grown under varying conditions of anaerobic/aerobic atmosphere, electron acceptor limitation, and with soluble or insoluble minerals. Research in finding more potent bugs that are efficient in conversion of organic compounds to electricity opens new vistas for the production of genetically engineered electrogenic bacteria.

The past, present, and future of the electrode material used in MFC

The principle of linking the MFCs in series to increase voltages, such as the case with batteries, is not possible. Nevertheless, wiring the MFCs to the charge capacitors in parallel and then discharging the capacitors in series could result in additive voltages (Kim et al. 2011). On the other hand, superior power densities were observed through a novel idea of enhancing effective surface area of the electrode with the innovative research in electrode material used in MFCs (Zhou et al. 2011). Physico-chemical properties such as surface area, electrical conductivity, and chemical stability of diverse electrode materials vary with their chemical and structural organization. These properties impact microbial attachment, electron transfer, electrode resistance, and the rate of electrode surface reaction of the electrode material. Consequently, choice and development of suitable electrode materials is of great significance in order to optimize and promote the performance of MFCs.

Carbon anode material A graphite rod, graphite fiber brush, carbon cloth, carbon paper, carbon felt, and reticulated vitreous carbon were used as conventional anode materials. Improved versions of carbonaceous anode material include graphite particles and granular activated carbon. An attempt was made to study the efficiencies of electrodes (graphite rod, felt, and foam) and differences in the power density performance of MFCs were observed. The results showed that graphite foam produced approximately threefold increased current density (74 mA/m^2) in comparison to graphite rods or graphite felt electrodes (24 mA/m^2). Thus, with the increment in the geometric surface area (20 x 10^{-3} m^2) graphite foam lodged more biomass and eventually produced more current (Chaudhuri and Lovley 2003). In another study, carbon fiber brushes were made from the twisted cores of two conductive, noncorrosive titanium wires holding carbon fibers. A single chamber air-cathode MFC with a continuous flow, showed current densities as good as 422 mW/m^2(Ahn and Logan 2010) for domestic water inocula. A similar type of setup in the laboratory with nutrient-rich inocula resulted in a mammoth 2400 mW/m^2 power density, being four times greater than when using carbon paper as the anode (600 mW/m^2) (Logan et al. 2007). In another interrelated development, stable operational voltage and the coulombic efficiency were detected to be higher for volumetric anodes constructed with carbon fiber brushes compared to planar anode. Further screening for electrogenic bacterial communities involved in this study have shown 57±4 % of *Geobacter* spp. recovered sequences for the brush and 27±5 % for the planar anode (Vargas et al. 2013), implicating the superior use of anode material with an increased surface area.

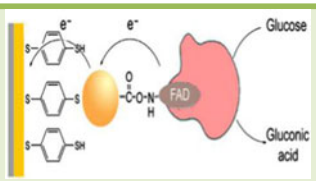
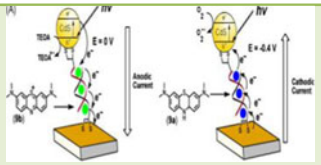
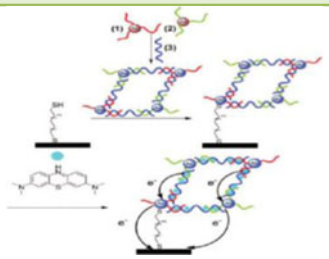
Porous anode material More porous anode material extensively increases the geometric surface area, which in turn necessitates augmentation of electrogenic bacteria per unit volume of the anode chamber. Thus, the efficiency of power density per geometric surface area is directly related to the porosity of anode materials. In this regard, granular and porous carbon materials were evaluated in a number of studies, for their use in electrogenesis. Granular activated carbon (GAC) is an inexpensive material that is generally used in waste removal. In one study, GAC based MFC was constructed for removal of dye and simultaneous production of current from industrial wastewater effluent containing azo dye. The fuel cell produced a power density of 1.7 W/m^3 and efficiently reduced 73 % of dye in 48 h of operation (Kalathil et al. 2011). Jiang and Li (2009) used varying amount of GAC in the anode and observed that when the amount increased from 400 g to 700 g; the power density was improved from 4.2 to 7.2 W/m^3. Increased porosity in the electrode compartment augmented the associated biomass resulting in the enhanced power densities. Graphite particles were also widely tested as an alternative means for increased geometric surface area of the anode.

A graphite-granule anode, tubular air-cathode MFC capable of continuous electricity generation from glucose-based substrates was tested to produce power densities ranging 50.2 W/m^3 (You et al. 2007). Furthermore, Aelterman et al. (2006) have achieved high power densities from various industrial and household wastewater inocula, on the order of 258 $W/m^3$when GAC was used as an electrode material and hexacyanoferrate or acetate as the substrate.

Metal based anode material Smooth surface and close packed crystal structures of metals do not facilitate the adhesion of electrogenic organisms. Thus far, stainless steel, high conductive gold, and titanium were investigated as biocompatible anode materials. *G. sulfurreducens* was found to utilize gold anodes and was capable of producing current similar to graphite anodes (Richter et al. 2008). Pure titanium was found to be an unsuitable anode material; however, surface treatment with metals such as platinum was shown to be as efficient as graphite material (ter Heijne et al. 2008).

Modifications to the electrode material The key idea for modification of the electrode material is to remove impurities of the electrode surface and/or to increase the active area (Logan et al. 2006). Major modifications include ammonia treatment (Cheng and Logan 2007) and chemical modification using techniques such as chemical vapor deposition, carburization, and sintering or soak method (Fig. 3). Alternatively, conductivity and/or functional groups can be added onto the surface for improved sensitivity of the electrode. Modification of an anode by conductive organic polymers is a promising strategy that was implemented in recent MFC studies. One such approach is using polyaniline (PANI) and poly(aniline-co-o-aminophenol) (PAOA) to modify carbon felt anodes and then analyzing their physical and chemical properties along with examination of the anode-associated biodiversity. An increase in power density at 33–35 °C was shown in PANI and PAOA modified anodes 35 % and 18 %, respectively, compared to the control (Li et al. 2011a, b). DGGE analysis showed increased biodiversity on the PANI modified anode surfaces, which may be due to a modified phenolic group. Zhao et al. (2011) have also used the polyaniline nanowire networks (PANI-NNs), hydroxylated, or hydroxylated/aminated PANI NNs to modify anodes. Enhanced electrical outputs of 0.28 mW/cm^2 (per geometric anode area) and 2.9 mA/cm^2 , respectively, were generated for hydroxylated and aminated PANI NNs compared to control. Further modifications of PANI with new co-polymer materials such as poly(aniline-co-o-aminophenol), poly(aniline-co-2,4-diaminophenol), and poly(aniline-1,8-diaminonaphthalene), have shown superior power densities, with a better attachment condition for biofilm (Li et al. 2012). Microbial formation and electrochemical active area in the MFC with a graphite plate, carbon cloth, and graphite felt anodes were increased after modification with poly(3,4-ethylenedioxythiophene) (PEDOT) (Kang et al. 2015). This study also detected that graphite felt anodes synergize with the PEDOT towards increased power density, coulombic efficiency, and chemical oxygen demand. Thus, owing to their conductivity and environmental durability, conductive polymers are gaining attention as potential coating substances on carbon electrodes. In addition, inspirational approaches from nanosensors for water purity testing could be adopted for the development of new generation bio-sensors or electrode material (Table 2). Nanobiotic blends of semiconductor nanoparticles with active biomacromolecules such as enzymes, DNA, or antigens/antibodies could lead to unique configurations of hybrid systems. These systems may well have optoelectronic as well as bio-catalytic properties (Willner et al. 2007). Considering the recent advantages of nanotechnology (Kanwar et al. 2013), various nanotechnology based detection systems have been developed recently, using electrical contacting of redox enzymes via electrodes (Xiao et al. 2003; Gorelik and Voinova 2006). DNA functionalized hydrogels have been employed for detection of Hg^{+2} contaminations in water. One such approach is the generation of smart nanowires exploiting the membrane bound redox proteins of electrogens. These nanowires can be produced by dip pen nanolithography using biocatalytic inks (Salazar et al. 2006). Based on the thin diameter, CNTs have promising insight to be able to enhance the direct electron transfer or redox mediators, which catalyse efficient electron transfer in order to establish electronic communication with redox enzymes serving as intermediates for the electron transfer (Holzinger et al. 2012). More recently, CNT impregnated hydrogels functionalized with multi-enzyme complexes were used as electrode material in the enzymatic biofuel cells (Moehlenbrock et al. 2011). Sequential organization of pentose phosphate pathway enzymes (termed metabolon) in the bioelectrodes served as efficient electrodes compared to their non-crosslinked counterparts (Moehlenbrock et al. 2011). In another fascinating study, multiwalled CNTs functionalized with the carbonyl functional groups were used to construct biocathodes peroxidase deoxyribozyme-hemin complex. When this is paired with a glucose dehydrogenase-modified bioanode, highest power densities in the range of 9.37 ± 2.66 $\mu W/cm^2$ were reported for enzymatic biofuel cells (Zhang et al. 2011). The same principle could be used in the case of microbial fuel cells for enhanced power outputs, as suggested in various recent reports. An attempt was made to study the impacts of diverse CNT types in CNT-based anodes to determine efficiency of wastewater treatment and power generation. Single-walled CNT (SWCNT) with carboxyl group, multi-walled CNT with carboxyl group (MWCNT-COOH), and multi-walled

Table 2 Smart nanomaterials for improved electric current in MFC

S.No	Scheme	Feature	Reference
1		**(FAD)- dependent glucose oxidase with an electrode using 4 nm AuNP as electrical nano connector showed improved electric signal depending on the spacer**	(Xiao et al. 2003)
2	**CNT/MnO2 nanocomposite**	**power density of 120 +/- 1.7 mW/m2 was generated because of the electron transfer enhancement between microbes-electrode and super capacitance of the MnO2.**	(Kalathil et al. 2013)
3		**CdS -semi conductor NP integrated with DNA and methelene blue showed enhanced photocurrents**	(Gill et al. 2005)
4		**Deposition of analyte-induced aggregated, nucleic-acid functionalized Au NPs on thiolated electrode showed enhanced electrochemical sensing after intercalation with methelene blue**	(Willner et al. 2007)
5	**MW CNTs were used as anode for micro sized MFC**	**Endurance of the CNT anode up to 60 days of operation and 19.6 m W/m^2 current density was produced.**	(Mink and Hussain 2012)

CNT with hydroxyl group (MWCNT-OH) were used to fabricate CNT-based anodes. MWCNT-OH showed the highest power density 167 mW/m^2 and voltage compared to all the other combinations (Thepsuparungsikul et al. 2014). Interestingly, in another study, a layer-by-layer self-assembly technique was used to fabricate multi-walled CNT and polyeletrolyte polyethyleneimine on to carbon paper electrode (Roh 2013). Improved electrochemical properties and maximum power density of 480 mW/m^2 indicated the significance of using MWCNT. Vertical alignment of CNTs (VA-CNT) on a substrate could be achieved by chemical vapor deposition method (Van Hooijdonk et al. 2013). Pyrolysis of organometallic precursors is the basis of such a formulation, where the growth of CNT takes place on a template (at a high temperature) such as graphite by vaporization and sublimation of a catalyst precursor. Addition of catalytic functional groups to the VA-CNT can be achieved by carbodiimide chemistry. The introduction of VACNT in MFC bioelectrodes is also helpful for frequent clean-up of agglomerates formed during the electrogenesis process. In recent studies, bioelectrodes were also formed by compression of a CNT/enzyme mixture into pellets and wired using a carbon paste (Zebda et al. 2013). This strategy can overcome limitations of current biofuel cell elements for improved production of eco-friendly electricity.

Conclusions

Evolution of electrogenic bacteria over millions of years in the natural habitat, with exposure to varying concentration of redox mediators, is probably the reason for the involvement of various outer membrane cytochromes during the electron

transfer. Thus, there is a possibility of an "electrogenic switch" involved in these microorganisms, underlying the use of various molecular mechanisms, which in turn related to the availability and/or diverse chemistry of the electron acceptors present in their natural habitat. High throughput metabolomic research on the diversity of electrogenic bacteria, using varying electron donor/acceptor ratios, adds to the understanding of bacterial electron transport. Mechanistic investigations in the molecular mode of electron transport of the electrogenic bacteria gives a detailed idea for using ideal electron acceptors in MFCs. Electrode performance is the biggest limiting factor in the present-day MFCs. Advancements in nanotechnology could be used to improve surface area, alongside cleverly enhancing the electric current, by using smart nanomaterials. Thus, the power densities in MFCs could be amplified with an interdisciplinary approach combining microbiology, chemistry and nanotechnology.

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