

An overview on biological concept of microbial fuel cells

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Abstract—*The microorganisms such as bacteria act as biological catalysts in microbial fuel cells (MFCs). These microorganisms oxidize the organic matter at the anode and transfer the electrons exogenously to the electrode surface (anode surface) without any need of artificial mediators. Such microorganisms have been referred as exoelectrogens. They form conductive biofilms on the electrode surface, metabolize the substrates into electrons, protons and carbon dioxide. The exoelectrogens produce some redox proteins such as c-type cytochromes and pili for direct electron transfer, and some electron shuttles e.g., pyocyanin for mediated electron transfer.*

Index Terms— *Microbial fuel cell (MFC), exoelectrogens; electricity production; c-type cytochromes (c-Cyts); microbial nanowires).*

I. INTRODUCTION

Microbial fuel cells (MFCs) have long been considered an attractive mean for converting various carbohydrate wastes directly into electricity using electrogenic bacterial cells in the anode compartment. Most MFCs have been operated using anaerobic or facultative aerobic bacteria which oxidize various substrates including glucose, sewage sludge and petroleum hydrocarbon [1]. Power production by MFCs varies with bacterial cell species, specific substrate concentration, cathode catalysts and the MFC configuration [2]. Typically, MFCs which were operated with a mixture of bacterial cells produced higher specific power than MFCs operated by a monoculture in the anode compartment [1]. Knowledge and understanding of the anode biofilm components, morphology formation steps and electron transfer mechanism may lead to better biofilm conductivity in MFC. General principle of a MFC is given in Fig.1.

The world got the first glimpse more than a century ago that bacteria could generate electricity in a MFC

with the publication of Potter in 1911. However, it is only recently in last two decades MFC technology is in the limelight of research for bioelectricity production, achieved waxed power outputs. MFC is a promising technology for harvesting energy that can be advantageously combined with various applications, such as bioremediation, sensors and powering electronic monitoring devices. It is because of the emergence of exoelectrogens since they produce such proteins or molecules that can transfer the electrons exogenously directly to the electrode, deploying the use of toxic and expensive artificial electron shuttles in MFCs. Several phylogenetically diverse microbes (mostly bacteria) have been reported to generate electricity in mediatorless MFCs. Five classes of Proteobacteria, Firmicutes and Acidobacteria phyla; some microalgae, yeast and fungi have shown current generation in the technology. The prevalent bacterial species known to produce electricity in MFCs include dissimilatory iron reducing *Geobacter* spp., *Shewanella* spp., *Rhodoferrax ferrireducens*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Clostridium butyricum* and *Enterococcus gallinarum*. Alternatively, microalgae have been used as a substrate or biocathode in MFC. Microalgae biomass contains high level of proteins (32%) and carbohydrates (51%) which are readily degradable by the exoelectrogens to produce electricity [3]. Velasquez-Orta et al. obtained power density of 277 W/m³ in MFC using *Chlorella vulgaris* (microalgae) powder as a substrate [4]. However, the electron transfer mechanisms and the proteins or molecules involved are known only in limited exoelectrogens, particularly in *Geobacter sulfurreducens* and *Shewanella oneidensis*. *G. sulfurreducens*, a Gram-negative bacterium can oxidize acetate completely into protons and electrons, and can reduce minerals like Fe (III) oxides effectively. It forms highly thick biofilms (more than 50 μ m) on electrode surfaces. In its monolayer biofilms, it transfers the electron to the electrode surface directly through outer membrane c-

Cyts (the pivotal c-Cyts is OmcZ) or via secreting riboflavin that interacts with c-Cyts to shuttle the electrons out of the cell [5]. In thick multilayer biofilms, *G. sulfurreducens* produces conductive proteinaceous pili that are made of PilA monomer units, encoded by the gene *pilA*. Multiple lines of evidence have confirmed the role of pili in electron transfer mechanisms and are also essential in electroactive biofilm formation [6]. Some proteins other than the outer membrane c-Cyts, the outer-membrane multicopper proteins OmpB or OmpC are also required for Fe (III) oxide reduction [6]. The deletion of *ompC* gene inhibited the reduction of insoluble Fe (III) oxides, indicating its importance in optimal Fe (III) oxides reduction by *G. sulfurreducens* [7]. The other widely studied exoelectrogen, *S. oneidensis* can reduce variety of substrates and can transfer the electrons exogenously via outer membrane c-Cyts containing MtrCAB complex [4]. The exoelectrogen secretes two types of flavins i.e. riboflavin and flavin mononucleotide. Both flavins interact with c-Cyts and these flavin- c-Cyts complexes help in hopping the electrons across the membrane [5]. An oxygenic phototrophic cyanobacterium, *Synechocystis sp.* produces nanowires, has been discovered to generate electricity in MFC. Another exoelectrogen i.e. *Aeromonas hydrophila* produces nanowires (type IV pili), are essential for electron transfers to the electrodes and biofilm formation.

In this article, the authors endeavor to elucidate the physiological and molecular concepts of microbial mechanisms that are advantageous to the MFC technology. Further, the article reviews the different mechanisms of electron transfer from microorganisms to electrode and vice-versa, followed by the explanation of high current producing microorganisms used for electricity production at anoxic and oxic compartments of MFC.

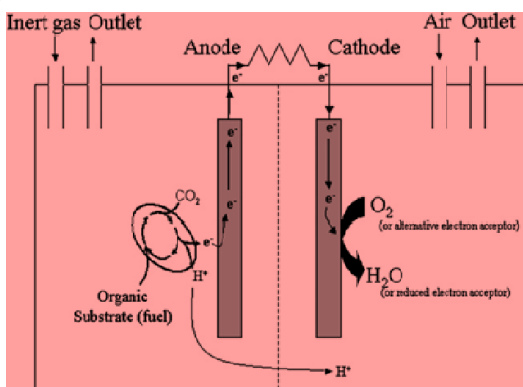


Fig. 1 Principle of a Microbial Fuel Cell.

II. MICROBIAL METABOLISM

Bacteria live in biofilms to a great degree. Bacterial cells embedded in a complex, self-produced polymeric matrix, attached to biotic or abiotic surfaces, referred as a biofilm. Biofilm can be formed by a single bacterial species (pure culture biofilm) or by multiple bacterial species (mixed culture biofilm). In MFCs, it is highly advantageous to produce electroactive biofilms to generate electricity more efficiently. It's blatant from the previous studies that the bacteria incompetent to form biofilms on the electrode are unable to generate substantial current densities in MFCs. However, the bacteria competent to form thick biofilms on the anode generate higher current densities in rival to bacteria adept to form thin biofilms. For example, confocal microscopy revealed that *Geobacter sulfurreducens* forms highly structured and thick multilayer biofilms ($>50\mu m$) and *Therminocola ferriacetica*, a Gram-positive bacteria form thick biofilms ($\sim 38\mu m$), generated a sustained current density $7-8 Am^{-2}$ [8]. While, *Therminocola potens* and *Clostridium ljungdahlii* form monolayer biofilms, produced comparatively lower current densities [9].

Diverse group of exoelectrogens have been experimented in MFCs for electricity generation, bioremediation and other manifold applications. Besides, sundry nutrients (acetate, glucose, starch, sucrose, ethanol, lactate and xylose etc.) and wastewaters (beer brewery wastewater, chocolate industry wastewater, swine wastewater, paper recycling wastewater and protein-rich wastewater etc.) from various sources have been used as substrate for microbial growth in MFC technology [10]. Despite the availability of wide range of substrates and exoelectrogens, only limited and specific exoelectrogens are known to produce electricity in MFCs. Exoelectrogens from various categories such as Gram-positive bacteria, Gram-negative bacteria, yeast, cyanobacteria, algae and even fungi have already been utilized in different kinds of MFCs. The organisms are substantially efficient for electricity generation that can completely oxidize complex organic substrates into their respective components in the anodic chamber. But, a particular exoelectrogen can oxidize specific substrates or a specific type of substrate for its growth and energy production. Moreover, depending on the type of substrate every exoelectrogen has different pathways and genes, enzymes or proteins for its degradation or oxidation. Therefore, selection of a suitable bacterial consortia and preferred substrate

determine the output of MFC. For example, a MFC fed with aerobic-anaerobic sludge inoculum and glucose, when operated for three months increased the bacterial substrate to electricity conversion rates by seven fold [11]. It is also important to get an idea about microbial metabolic pathways regulating electrons and protons flow prior to rate the electricity generation in MFC. In MFC, organic substrates containing carbohydrates, lipids and proteins serve as electron donors for redox reactions at the anode to produce energy. These complex organic molecules further undergo through glycolysis and other respective processes to yield acetyl Co-A, which then participate in citric acid cycle (also known as TCA cycle or Krebs's cycle) (see Fig. 2).

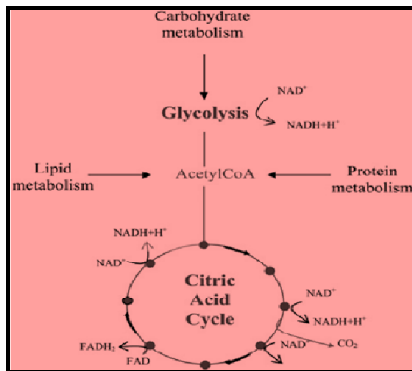


Fig. 2 Reduction of NAD^+ and FAD to their electron carrier forms (NADH and FADH_2) through the Citric Acid Cycle (also known as tricarboxylic acid cycle or TCA cycle).

One complete turn of the cycle converts three equivalents of nicotinamide adenine dinucleotide (NAD^+) into three equivalents of reduced NADH ; one flavin adenosine dinucleotide (FAD) reduces to FADH_2 and CO_2 is released as by-product. These metabolic pathways (glycolysis and Krebs's cycle) occur in cytoplasm in both prokaryotes (bacteria) and eukaryotes (yeast). NADH and FADH_2 acts as electron carriers, which then transfer their electrons to electron transport chain (ETC) to produce energy carrier molecule, adenosine triphosphate (ATP). In bacteria respiratory reaction occurs in the cell membrane (constituting outer cell membrane, inner cell membrane and periplasm), the machinery containing all the proteins or enzymes required for the electron transfers (the basis of MFC). While in yeast, ETC resides on the inner mitochondrial membrane. The ETC typically contains four intermediary proteins NADH dehydrogenase, ubiquinone, coenzyme Q and

cytochromes (however, these intermediary proteins may vary with species). The electrons are passed through these proteins to the final electron acceptor and the protons (reduced) are pumped out of the cell, in the anode which is then transferred to the cathode through PEM. Prior to the prominence that bacteria can facilitate electron transfer, chemical mediators were utilized to catalyse electron transfer from inside the bacterial cell to the anode surface. These mediators react with ETC components and get reduced, release out of the cell and transfer their electrons to the anode.

III. ELECTRON TRANSFER MECHANISM

In MFCs, the bacterial transfer of electrons from the substrates to electrodes is mainly through three ways (Fig.3). The mechanism of electron transfer may be of short range direct electron transfer (via c-type cytochromes), electron transfer via electron shuttles secreted by exoelectrogens or by long range electron transfer (through pili).

Short range direct electron transfer

Diverse exoelectrogens are known to mediate electrons from inside the cells to electrode surfaces via direct electron transfer mechanism. But, *Geobacter sulfurreducens* has been studied most extensively to comprehend the mechanisms for direct electron transfer. *G. sulfurreducens* contains the enzymes for the central metabolism to anaerobically oxidize carbon (effectively acetate) completely to carbon dioxide and water and can transfer electrons to a variety of electron acceptors including metal ions, elemental sulphur and fumarate [12]. Earlier studies revealed that *G. sulfurreducens* does not use shuttles to reduce the electrodes but utilizes alternative electron transfer pathways; takes electrons generated from central metabolism in the cytoplasm and transfer them by direct contact to extracellular electron acceptors, such as Fe (III) oxides. The genetic studies of *G. sulfurreducens* genome unveiled the presence of unprecedented number of c-Cyts containing heme groups in their motifs, exposed on the outer surface of cell [7, 13]. The profusion of cytochromes is an advantageous characteristic for the organism that ameliorates electron transport and suggests the flexibility and redundancy in the electron transfer networks which further assists the reduction of diverse metal ions in natural environments. The alternative electron transport components include dehydrogenases,

quinones, iron-sulfur proteins, and b-type cytochromes. The electron transport proteins reside in the periplasm or on outer membrane of *G. sulfurreducens* that transport the electrons through the plasma membrane to outside of the cell. Besides, many studies including gene deletions demonstrated that c-Cyts transfer electrons to diverse extracellular electron acceptors in vitro as well as in vivo [61, 62]. Furthermore, the studies of *G. sulfurreducens* biofilms evinced that OmcZ (outer membrane c-type cytochrome Z) acts as the electrochemical gate between cells adhered on the electrode and the electrode surface, as some of the cytochromes were present in close proximity of electrode surface for direct electron transfer. The immunogold labelling of *G. sulfurreducens* biofilms validated the accumulation of profuse OmcZ at biofilm and anode interface [7]. Besides, deleting the gene for OmcZ dramatically increases the resistance of electron exchange between the anode and the biofilm. Therefore, all these results confirmed the vital role of OmcZ in direct electron transfers. Multiple lines of evidence suggest that OmcZ is the most important cytochrome in high current producing biofilms, is a octaheme hydrophobic protein occurs in two forms; one large form (OmcZL) with a molecular mass of ca. 50 kDa and a short form (OmcZS) having a molecular mass of ca. 30 kDa [7]. OmcZS is a cleaved product of OmcZL and subcellular-localization studies suggested that OmcZS is the predominant extracellular form of OmcZ. Moreover, redox titration analysis revealed that heme groups in OmcZ cover a large reduction potential range (-420 to -60 mV), with midpoint reduction potential of ca. -220 mV (versus the standard hydrogen electrode) [14]. Cyclic voltammetry of biofilms of wild type and mutant *G. sulfurreducens* strains grown on graphite cloth anodes acting as electron acceptors with acetate as the electron donor evinced that OmcZ participates in homogeneous electron transfer (through the biofilm bulk) while OmcB mediates heterogeneous electron transfer (across the biofilm/electrode interface) and OmcS plays a secondary role in homogenous electron transfer while OmcE is salient in Fe (III) oxide reduction [15]. Deletion of the gene encoding OmcF, a monoheme outer membrane c-type cytochrome, substantially decreased the current production [66]. Further, the results suggested that OmcF is required for optimal current production, not because OmcF is directly involved in extracellular electron transfer but OmcF is required for the appropriate transcription of other genes that either directly or indirectly is involved in electricity production [16].

Electron transfer via electron shuttles secreted by exoelectrogens

Many exoelectrogens including Gram-positive and Gram-negative bacteria, such as *Geothrix fermentans*, *Shewanella oneidensis*, *Pseudomonas aeruginosa*, *Lactococcus lactis* secrete soluble electron shuttles to further the electron transfer to electrodes. The first evidence of an organism synthesizing a soluble compound that promotes electrode reduction came with the publication of Bond *et al.*; suggested that *G. fermentans* releases a soluble electron shuttle which promotes reduction of Fe (III) oxides [17]. *G. fermentans* when grown with Fe (III) but, not with fumarate as electron acceptor; secreted two different soluble redox-active electron shuttles with separate redox potentials [70]; first was riboflavin at redox potential of -0.2 V and the other, still unknown at redox potential of 0.3 V. Earlier, *P. aeruginosa* strain KRP1 isolated from MFC; produced pyocyanin and phenazine-1-carboxamide.

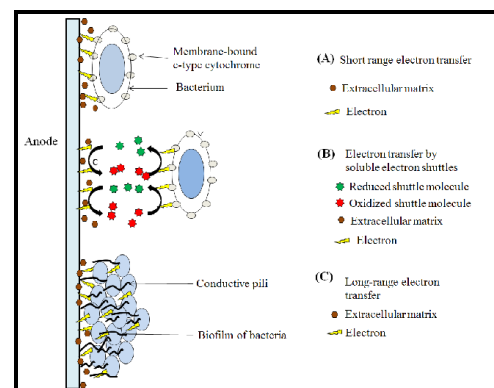


Fig. 3 Different mechanisms of electron transfers
(A) Short-range electron transfer by microorganisms through c-type cytochromes associated with the outer cell membrane. (B) Electron transfer via soluble electron shuttles. An oxidized shuttle molecule is reduced by the redox-active proteins at the outer cell surface which further donates its electron to the electrode. (C) Long-range electron transfer. The electrons from the distant and other cells are transported to the electrode through conductive biofilm via proteinaceous electrically conductive pili [7].

A mutant strain of *P. aeruginosa* KRP1, deficient in the synthesis of pyocyanin and phenazine-1-carboxamide, achieved only 5% power output as compared to wild type's strains [18]. Further, the study demonstrated that pyocyanin promote substantial

electron transfer, not only used by *P. aeruginosa* but also by other bacterial species as well.

Long range electron transfer

In monolayer biofilms, most of cells remain in proximity of electrode surface contribute for generation of current densities. Alternatively, in multiple layer biofilms, only few cells can access the electrode surface, then, how the cells in biofilms distant from the electrode surface transfer electrons to the electrode surface; has been discussed in this section. Long-range electron transfer is mediated by dense network of pili with metallic like conductivity produced by the microorganism, responsible for the conductive biofilms of high current production. Though, diverse exoelectrogens are known to produce pili but only *Shewanella sp.* [19] and *Geobacter sp.* [20] are competent to produce conductive pili that account for electricity production.

The role of conductive pili in long range electron transfer in biofilms was demonstrated earlier in *Geobacter sulfurreducens*, and the study revealed that these electronic networks contributed for more than 10-fold increase in electricity production [21]. *G. sulfurreducens* pili are type IV pili composed by the monomers of PilA protein and are similar to that of other type IV pili [20,5]. Type IV pili are small structural proteins of molecular weight ca. 7 to 20 kDa, 10-20 μm long and 3-5 μm broad with a conserved N-terminal domain forming α -helix with a transmembrane domain and a protein-protein interaction domain that allows monomers to interact with each other to generate a hydrophobic filament core [22]. Moreover, C terminus of PilA contains a conserved sequence of aromatic amino acids (Trp, Phe, Tyr, His, and Met) responsible for overlapping of pi-pi orbitals in the pili structure and consequently for metal like conductivity; lacks in non-conductive biofilms [23]. The function of PilA is directly regulated by PilR which functions as an RpoN-dependent enhancer binding protein. Further, the study revealed that a strain deficient in *pilR* gene showed waned insoluble Fe (III) reduction as well as soluble Fe (III) reduction [24]. *G. sulfurreducens* strain Aro-5 was demonstrated for substitution of alanine amino acid for each of the five aromatic amino acids in C-terminus with a graphite anode serving as the electron acceptor for Fe (III) reduction. The strain expressed pili with multiheme *c*-Cyts OmcS, was incompetent for extracellular electron transfer and Fe (III) reduction and consequently showed extensively diminished

conductivity in rival to wild type strain [25]. Reguera *et al.* demonstrated, *pilA* -deficient mutant of *G. sulfurreducens* was incompetent to reduce Fe (III) oxides, indicating that pili of *G. sulfurreducens* are pivotal for Fe (III) oxide reduction. Further, atomic force microscopy revealed that the pili were highly conductive, transferring electrons from the cell surface to the surface of Fe (III) oxides and hence, serves as microbial nanowires [26]. The hypothesis that cytochromes are associated with *G. sulfurreducens* pili and serve a key role in electron transfer along with pili was ruled out with the publication of Malvankar *et al.* [27, 20]; the study unveiled that conductivity of *G. sulfurreducens* nanowires don't attribute to cytochromes because the spacing between cytochrome-to-cytochrome was ca. 200 times greater than required for electron hopping. It was further clarified by Liu *et al.*, demonstrated a *G. sulfurreducens* strain PA, *pilA* gene was replaced with *pilA* gene of *Pseudomonas aeruginosa* PAO1, expressed the pili subunits and *c*-type cytochrome OmcS similar to control strain.. Further, the results suggested that *c*-type cytochrome OmcS on pili don't confer for the conductivity of pili [28].

The exoelectrogen studied for 'microbial nanowires' so far beyond *Geobacteraceae* family is *Shewanella oneidensis*. Naggar *et al.* by using conducting probe atomic force microscopy technique provided the evidence that *S. oneidensis* MR-1 nanowires are conductive in nature. Further, the study demonstrated that mutants deficient in genes for *c*-type decaheme cytochromes MtrC and OmcA produced nonconductive nanowires [29]. Electronic transport characteristics of *S. oneidensis* MR-1 nanowires was further studied and exhibited p-type, tunable electronic behaviour with a field-effect mobility [30]. A multistep hopping mechanism has been proposed for extracellular charge transfer in *S. oneidensis* MR-1 biofilms, suggesting that redox components are associated with each other at less than 1 nm distance forming a chain along extracellular appendages, responsible for electron hopping or electron tunnelling [29,31]. However, the actual organization of cytochromes on *S. oneidensis* MR-1 nanowires and their exact role in electron transfer mechanism is yet to be clarified.

Direct interspecies electron transfer

It is quite evident from the earlier genetic and transcriptomic studies that during the syntrophic growth, *G. metallireducens* and *G.*