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# Biochemical and Electrochemical Perspectives of the Anode of a Microbial Fuel Cell

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Additional information is available at the end of the chapter

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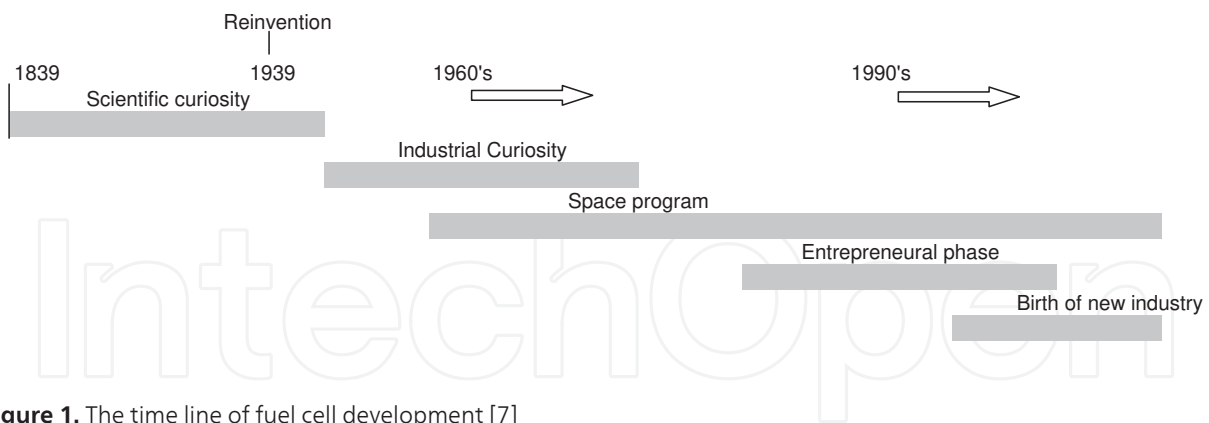
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## 1. Introduction

### 1.1. History of biofuel cell

The statement “Perhaps the most refined fuel cell system today is the human body, a mechanism that catalytically burns food (fuel) in an electrolyte to produce energy, some of which is electrical” highlights the connection between living organisms and electricity [1, 2]. With an experiment conducted using frog leg, Biologist Lugi Galvani in 1780’s proved that electrical energy and biology have a close connection to each other [3]. Michael Cresse Potter, a Botany professor also demonstrated that living organisms can generate voltage and deliver current [4].

The term “fuel cell” has been in use for over a century. Despite some uncertainty about who first fabricated one, credits of designing and experimenting with first fuel cells go to both Sir William Grove (1839) and the Swiss scientist Christian F. Shoenbein (1868). In early 19<sup>th</sup> century, different organisms like bacteria, algae, and yeast were considered for this research. With the advent of space race, considerable attention was given to energy generation from recycled waste which in turn ignited interest in microbial fuel cell research. Later, during the sixties and in the early seventies, fuel cell related research accelerated as a consequence of increase in oil prices and has sustained momentum to date. [5, 6]. The time lines of fuel cell development are shown in the Fig.1.



**Figure 1.** The time line of fuel cell development [7]

## 1.2. Types of fuel cells

Fuel cells could be broadly categorized into abiotic fuel cells of which the fuel cell components do not comprise any biological material and biotic or biological fuel cells which comprises living organisms or biological material (such as enzymes or derivatives). The primary types of abiotic fuel cells grouped according to the electrolyte used are shown in Table 1.

Type	Features
Alkaline fuel cells (AFC)	Uses KOH as the electrolyte and electro-catalysts such as Ni, Ag and metal oxides
Polymer electrolyte membrane fuel cells (PEMFC)	Uses a proton conductive polymer membrane as the electrolyte and Pt as the catalyst
Phosphoric acid fuel cells (PAFC)	Uses concentrated phosphoric acid as the electrolyte and Pt as the catalyst
Molten carbonate fuel cells (MCFC)	Has a combination of alkali metals (Li, K, or Na )
Solid oxide fuel cells (SOFC)	Uses non-porous metal oxide(s) as the electrolyte

**Table 1.** Different types of commonly known inorganic fuel cells [7].

The biological fuel cell (BFC) can be categorized into two main areas:

1. Microbial fuel cells (MFC)
2. Enzymatic fuel cells (EFC)

The biological fuel cells (BFC) use enzymes or microorganisms as catalysts. In a microbial fuel cell, the oxidation reactions that are catalyzed by microbes; alternatively, when the catalyst is an enzyme, the cell is called as an enzymatic fuel cell. While both microorganisms and enzymes catalyze oxidative reactions that takes place at the anode, only enzymes (sometime coupled with inorganic catalysts) are used in the cathode. Biological fuel cells utilize organic substrates (such as sugars and alcohols) and operate at mild temperature environments where biological activity is optimal. For example, the catalyst used in a microbial fuel cell could simply be an

organism like Baker's yeast that feed on simple sugars or an advanced species like *R. ferrireducens* [8-10] that thrive on more complex substrates.

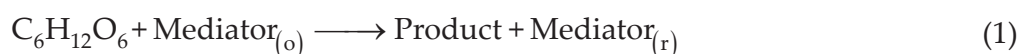
### 1.3. Different categories of microbial fuel cells

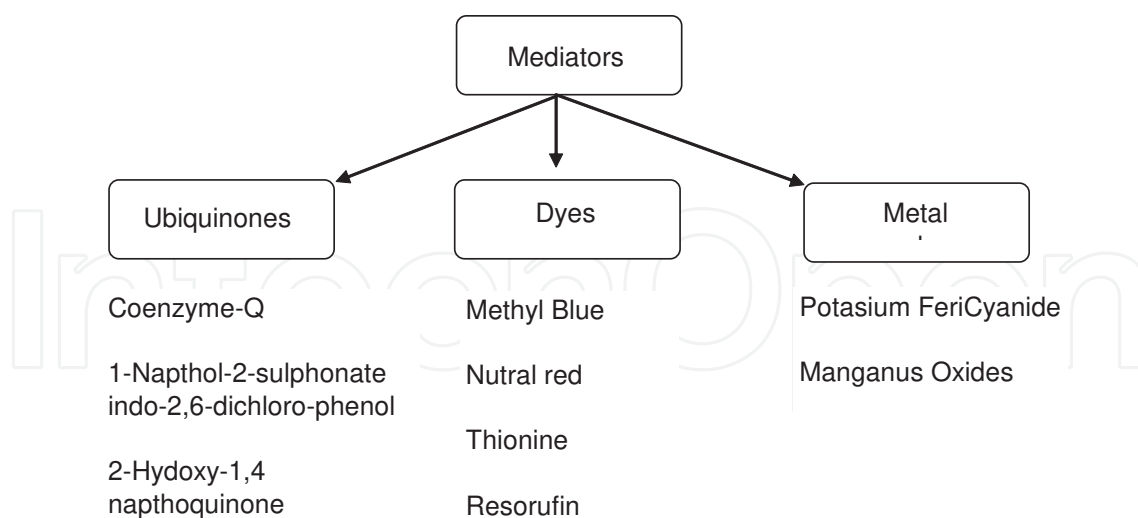
Various types of MFC designs have been developed of which five main categories are common:

1. **Uncoupled bioreactor MFC:** a separate compartment where organisms produce the hydrogen (fuel) and that hydrogen is fed into a hydrogen fuel cell.
2. **Integrated bioreactor MFC:** hydrogen fuel production and the electricity generation both take place in the same chamber.
3. **MFC with mediated electron transfer:** where intermediate molecules shuttle electrons from microbial cells to the electrode.
4. **MFC with direct electron transfer:** where electron transfer to the electrode take place without the presence of any mediator molecules.
5. **Mediator-less and membrane-less microfluidic MFC:** an emerging type of MFC that eliminates use of mediators and cation exchange membrane.

The first two designs use hydrogen that is biologically generated and uses this hydrogen in a PEM-like fuel cell system. Thus, these fuel cells possess similarities with their inorganic counterparts. There are different types of bacteria and algae that generate hydrogen under anaerobic conditions, e.g. *Escherichia coli*, *Enterobacter* and *C. butyricum* that can be used in such reactors. Theoretical output in these fuel cells is in the neighborhood of 10% but in actual environment, the values have been slightly less. Enzyme interactions (such as hydrogenases) with H<sub>2</sub> is one reason for performance reduction. The inhibition of anaerobic hydrogenases by evolving oxygen (during photosynthesis) is another. Contamination of H<sub>2</sub> with other gaseous species such as CO and H<sub>2</sub>S is also considered to contribute toward inefficiencies. The second design mentioned above has similar disadvantages to the first with the only difference being the position of the bioreactor [11, 12]. Such MFCs commonly use Pt as electrode catalysts.

In the third type, an intermediate molecule known as mediator is used for electron transport [13]. The mediator will shuttle electrons between the electrode and redox enzyme in the microorganism [14] following redox cycles as given in Equations 1 & 2. Different mediators that have been utilized in microbial fuel cells are listed in Fig. 2.





**Figure 2.** Different mediators used in biological fuel cells.

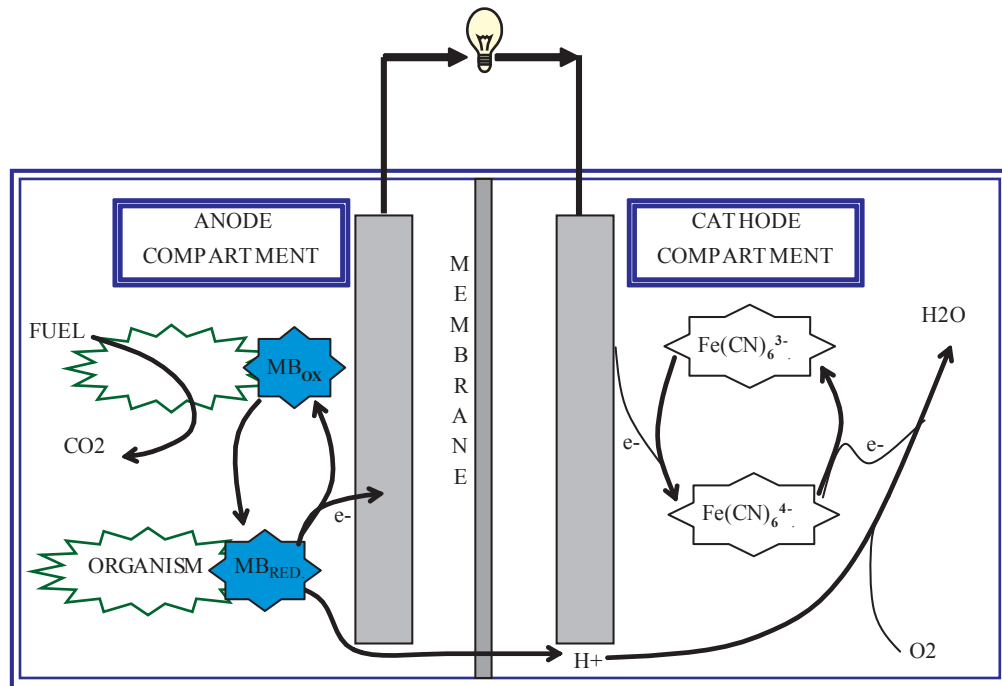
The characteristics that the mediators must possess are well established [15, 16]: 1) The molecules should be able to form redox couples; 2) They should be stable in both, reduced and oxidized form; 3) They should not be biologically degradable; and 4) They should not be toxic to the biological species. However, in practice, mediators have commonly contributed to fuel cell performance issues due to degradation and toxicity to the biological medium [17, 18].

The fourth type of MFCs do not contain any extraneous mediators and in this type, bacteria are believed to communicate directly with the electrode using self-made mediators. Studies supporting this hypothesis have been conducted with iron-reducing bacteria such as *Shewanella putrefaciens*, *Geobacter sulfurreducens*, and *Rhodospirillum rubrum* [19]. The fifth type involves miniaturization of MFC using microfluidic technology which is capable of achieving high energy efficiency and durability. This type enables an advantage over conventional MFCs by eliminating the need for membranes (PEM) as a result of co-laminar flow of fuel and oxidant streams that extemporaneously separate anode and cathode in the cell. Electron transport to the anode can occur using electron mediators, or by direct membrane associated electron transfer, or by purported nanowires produced by certain microbes [20, 21].

## 2. Biochemical and electrochemical phenomena that occur at the electrodes of MFCs

Just like in any other fuel cell, the oxidation reaction in a microbial fuel cell occurs at the anode releasing electrons to and protons at the anode. The concomitant reduction half reaction takes place at the cathode collecting the electrons that travelled through an external circuit and combining them along with the protons with the terminal electron acceptor-which in most

cases is oxygen. In order to keep the fuel from being crossed over to the cathode compartment or oxygen from entering the anode compartment (commonly referred to as cross over reactions), a membrane (commonly referred to as proton exchange membrane or PEM) that is selective only for protons (transport) is generally used. A schematic of the reactions that take place in a microbial fuel cell is depicted in Fig. 3.



**Figure 3.** Schematic diagram of a microbial fuel cell and its operation [22]

### 2.1. Catabolic pathways involved in energy production from microbes

Microorganisms derive energy for living from the free energy produced by fuel oxidation. A part of the free energy produced is retained by the microbes for their catabolic activities and the rest may be utilized to generate electricity. Hence, for sustainable operation of the MFC, it is necessary for the microorganism to balance out the amount of energy consumed for its vitality and that being converted into electrical energy.

There are two metabolic pathways used for energy conversion in microbes namely, respiration and fermentation. In the respiratory or oxidative pathway, the Gibbs free energy is utilized by the microbes for their respiration where the electrons circulate around a respiratory chain and finally exits the microbial cell via membrane-bound electron acceptors.

Under aerobic conditions, oxidation of the fuel glucose follows four distinctive steps [23]: 1) Glycolysis, 2) Krebs Cycle, 3) Electron transport chain, and 4) Oxidative phosphorylation. In glycolysis which is also known as Embden-Mayarhoff-Parnas pathway, the six carbon glucose atom is broken down into two molecules of pyruvate as shown in Equation 3. This takes place in ten successive steps, each of which is catalyzed by a specific enzyme. Pyruvate, the product

of glycolysis goes further through a three stage process where it is finally converted to  $\text{CO}_2$  and more energy in the form of ATP. The total ATP production from glucose in a respiring system is given in the Table 2.

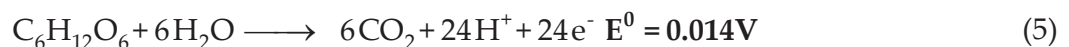
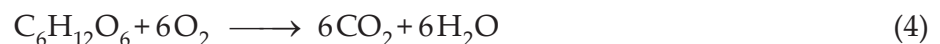


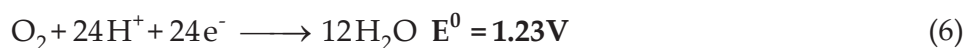
Reaction Sequence	ATP Yield
Glucose $\rightarrow$ Fructose 1,6-diphosphate	-2
2 Trios Phosphate $\rightarrow$ 2,3-phosphoglyceric acid	2
2NAD <sup>+</sup> $\rightarrow$ 2NADH $\rightarrow$ 2NAD <sup>+</sup>	6
2Phosphoenol pyruvic acid $\rightarrow$ 2Pyruvic acid	2
2Pyruvic acid $\rightarrow$ 2Acetyl Co A + 2CO <sub>2</sub>	
2NAD <sup>+</sup> $\rightarrow$ 2NADH $\rightarrow$ 2NAD <sup>+</sup>	6
2Acetyl CoA $\rightarrow$ 4CO <sub>2</sub>	24
<b>Net:</b> C <sub>6</sub> H <sub>12</sub> O <sub>6</sub> + 6O <sub>2</sub> $\rightarrow$ 6CO <sub>2</sub> + 6H <sub>2</sub> O	38

**Table 2.** ATP yield for corresponding biochemical reaction(s)

Even though aerobic respiration is the main form of energy generation in organisms, some can generate energy under anaerobic conditions. Under anaerobic conditions, glucose will be converted to pyruvate as it was under aerobic conditions, but in the next stage instead of the Krebs cycle a different pathway will follow-specific to the type of organism viz. prokaryote/eukaryote. While yeast for instance undergoes alcohol fermentation, some organisms follow the lactic acid fermentation.

In microbial catabolism under anaerobic conditions, carbohydrates are oxidized without the presence of oxygen. This oxidation reaction gets partially completed inside the microbial cellular structure and with the help of co-enzymes, the electrons and protons are transferred outside the cellular membrane. The overall reaction of a fermentative MFC is given in Equation 4. This reaction can be broken down to half reactions as shown by Equation 5&6. Inside the anaerobic compartment of a MFC, the reaction represented in Equation 4 takes place where the electrons released will be transferred to anode and the protons ( $\text{H}^+$ ) travel to the cathode compartment (sometimes via a proton exchange membrane). The released electrons from the anode will go through an external load as shown in Fig.3. and consequently enter the cathode.





Since ATP production is quite low under anaerobic conditions i.e. almost two molecules per one pyruvate molecule, the organisms need rapid processing of NAD<sup>+</sup> and NADH. For the purpose of harvesting H<sup>+</sup> what matters for a MFC is the amount of co-enzyme (NAD<sup>+</sup>) getting involved in the reaction. In that perspective, anaerobic conditions are much favorable for the harvesting of protons when compared with aerobic conditions. Under anaerobic conditions, the rate of NAD<sup>+</sup> to NADH conversion takes place at a significant rate, while under aerobic conditions the already present oxygen in the media reacts with H<sup>+</sup> making it difficult to run the half reaction given by Equation 6.

In a MFC system, the main electron acceptor that is engaged in harboring the electrons released from microbes is a solid anode. The formation of a biofilm on the anode is believed to be driven by the consumption of greater amounts of energy (released by microbial catabolism) by the solid anode when compared to the other electron acceptors. The bridging concepts of electron transfer between microbes and the solid anode is still under speculation and is discussed briefly in the next section.

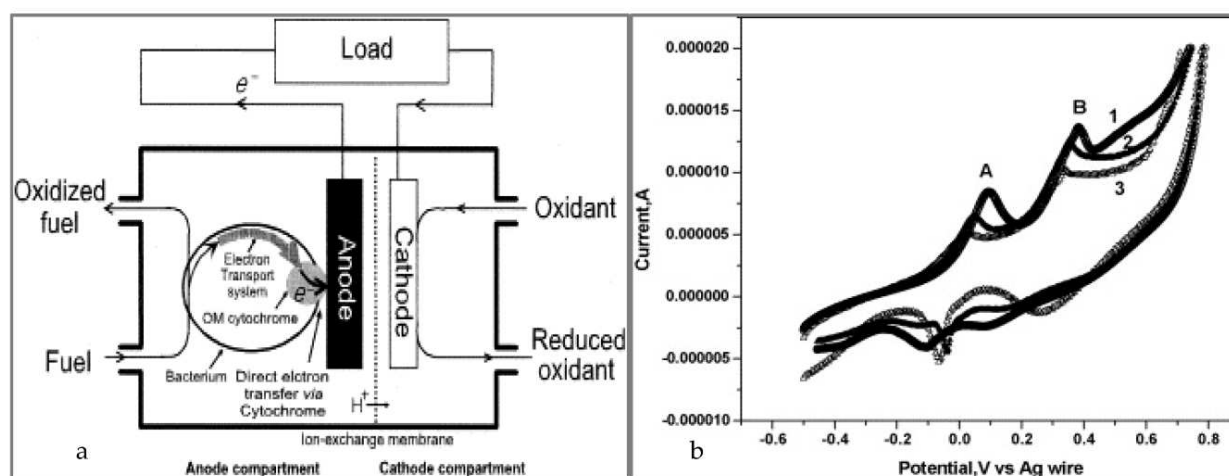
## 2.2. Electron transfer mechanisms

The electron transfer mechanism involves amalgamation of knowledge from electrochemistry, biochemistry and microbiology. Electrons pass on to the electrode of MFC as a result of several irreversible enzymatic reactions and eventually the reversible electrochemical reactions of the electron transport chain [24]. For the reversible interfacial reaction to take place at the anode surface, electrons rely on shuttles such as cytochromes, proteins (such as PQQ), bound or soluble redox mediators to reach at least within 10 Å proximity of the anode [25].

Several electron transfer mechanisms and processes have been proposed including direct electron transfer, indirect electron transfer and shuttle mediated transfer of electrons. Of the various mechanisms proposed, direct contact mechanism indicates formation of monolayer of microbes on the anode surface enabling direct transfer of electrons to the anode via cell membrane or a membrane organelle. Kim et al. [26] observed that certain Fe(III)-reducing bacteria (*Shewanella putrefaciens*) can transfer electrons to the electrode without the aid of synthetic mediators as shown in Fig.4a. Cytochrome, a redox protein perhaps present on the outer membrane of the cell supposedly causes direct electron transfer by its electrochemical activity of reducing water soluble Fe(III). This type of transfer is capable of producing lowest extracellular potential losses due to the negligible gap between microbe and the electrode. However, the electron transfer in this mechanism is limited by the total number of bacteria in direct contact with anode [27]. Also, the c-type cytochrome may not be present in all the Fe(III)-reducing bacteria which further limits the electron transfer to a very few species [28].

Apart from ferric ion reducing bacteria, yeast cells (*Hansenula anomala*) have also been successfully found to enable a direct electrical communication between the cells and anode surface. In this case, electron transfer takes place without any external mediator or any intermediate redox reaction such as Fe(III) reduction, but with the help of redox enzymes,





**Figure 4.** a) Schematic diagram describing direct electron transfer mechanism in MFC [26]; b) CV of yeast biofilm on anode in phosphate buffer at scan rate 50 mV/s. (1) Blank CV; (2 and 3) after successive additions of 0.5 ml each of lactate stock solution [29].

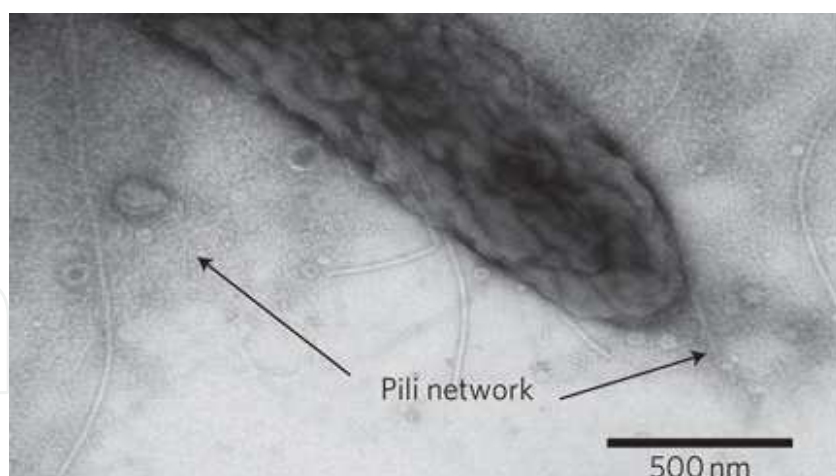
ferricyanide reductase and lactate dehydrogenase that are present on the outer membrane of the yeast cells. As can be seen in Fig.4b., the CV reveals two redox peaks, A and B of the respective redox enzymes that are responsible for the electrochemical activity of the organism. These enzymes get reduced by consuming the electrons that are liberated during the oxidation of the substrate and transfer the electrons to the anode. The CV curves 2 and 3 depict reduction in peak currents upon subsequent addition of substrate indicating direct communication between the enzymes and anode [29].

A recent discovery of interest in the milieu of direct electron transport (DET) mechanism in MFC is the pili growth seen in bacteria (*Geobacter sulfurreducens*). Pili also known as bacterial nanowires, are minute flexible structures made of protein called pillin that help the bacteria to cling to surfaces and distinguish materials in its surroundings. These pili overcome the limitation of the former mechanism by allowing charge transport through multiple layers of biofilms on the anode surface by not only being electrically conductive but also supposedly forming internal networks (see Fig.5.).

The subunit protein, PilA present in pili consists of a cluster of aromatic acids that are believed to play a major role in their conductivity [31, 32]. *Shewanella oneidensis* uses both types of DET mechanisms involving c-type cytochrome and conductive pilus-like nanowires for electron transfer [33, 34]. Conductivity of pili has may be measured using relation (7) given by Malvankar et al. [30]

$$\sigma = G \left( \frac{2a}{gL} \right) \quad (7)$$

where G is the biofilm conductance, L is electrode length, g is thickness of biofilm and a is the non-conducting gap width between two working electrodes (anode).



**Figure 5.** TEM image showing pili formed by the strain KN400 forming an interpenetrating network on the anode biofilm [30]

While there have been positive findings on bacterial nanowires toward long-range electron transfer on anode; further in-depth investigations are required to find precisely what feature of the pili facilitates metal-like conductivity in pili and other microbial species that possess this trait [35].

Although direct contact mechanism is much sought after for electron transfer, extensive research on indirect or mediated electron transfer mechanism indicates that it is a conventional and efficient method for current generation in MFCs [36]. Indirect electron transfer mechanisms can be categorized based on the type of mediator used to wire the microbial catabolism with anode surface. A good mediator displays high membrane permeability, it is adequately soluble, possesses high electron transfer rate, non-toxic to microbial cells and non-biodegradable. In theory, mediators with low redox potentials (such as  $\text{SO}_4^{2-}$ ) are said to be favorable for MFCs. This is because, electrons always flow from the low redox potential (of mediator) to the high redox potential (of electron acceptor or anode). However, mediators with high redox potentials have superior affinity for drawing electrons from the electron carriers in the cell.

Initially artificial redox mediators such as phenazines, phenothiazines, phenoxazines and quinones were used to carry out the electron transfer in the MFC systems. However, there are several disadvantages associated with these mediators that makes their use unsustainable and impractical – low current densities, difficulty in long range electron transfer over thick biofilms, need for regular addition of fresh mediators and toxicity. Hence, it was necessary to employ the intrinsic production of metabolites in microbes for electron transport purposes.

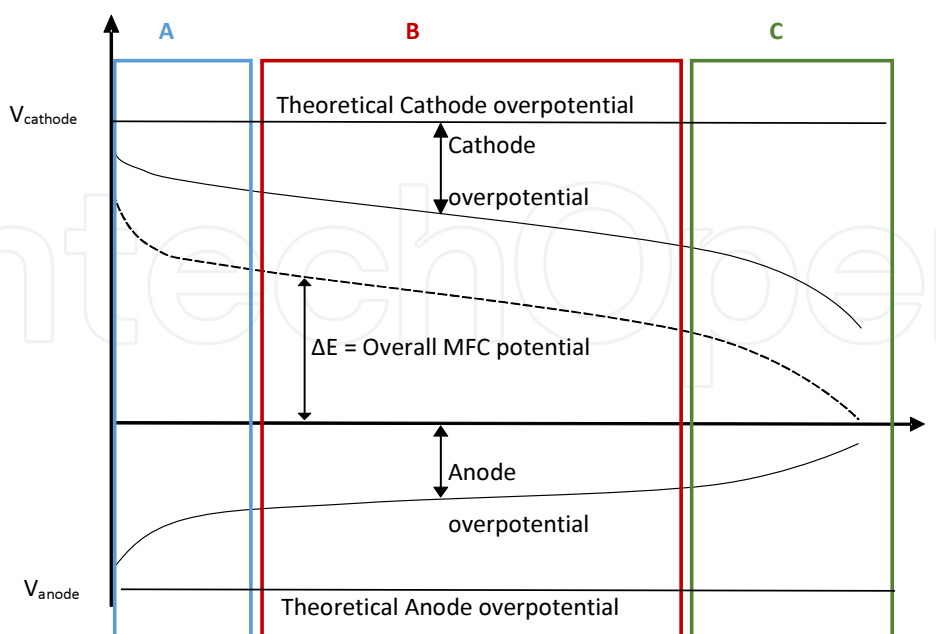
Microorganisms are capable of producing endogenous metabolites using primary and secondary pathways to carryout various biological processes. Primary metabolites such as  $\text{H}_2$  [37] and  $\text{H}_2\text{S}$  [38] that are produced by microbial catabolic oxidation of fuel (anaerobic respiration and fermentation) have also been successfully used as redox mediators. Some secondary metabolites that have been considered for use in MFC applications are phenazine-1-carboxamide, pyocyanine (*Pseudomonas aeruginosa*) [39], neutral red, anthraquinone-2,6-disulfonate (AQDS), thionine, methyl viologen, methyl blue, humic acid [40] and 2-amino-3-

carboxy-1,4-naphthoquinone (ACNQ) (*Bifidobacterium longum*) [41]. These mediators display cyclic redox behavior, which means that a single molecule can be used continuously for electron transfer to and fro the anode or biofilm. This is not only sustainable but also convenient for long range electron transfer in the anodic biofilms resulting in constant and enhanced generation of current.

### 3. Factors that affect performance of microbial fuel cells and potential remedies

#### 3.1. Polarization losses

Theoretically, MFC can attain a maximum cell voltage (emf) of 1.1 V under open circuit conditions. However, in reality it undergoes numerous forms of (polarization) losses and can achieve only 0.8 V [42] in open circuit conditions and around 0.62 V [43] during current generation. Overvoltage which is the difference between the theoretical and measured cell voltage collectively represents the overpotentials of the electrodes as well as the overall ohmic loss of the system [32]. Prominent sources of these overpotentials are the intracellular and extracellular potential losses that occur in the biofilm formed on anode. When the internal resistance is high, there is a significant loss of charge in the system, thus reducing the effective voltage available at the end terminal [7, 44]. Key types of polarization losses (Fig.6.) viz. activation polarization, concentration polarization, ohmic losses and losses due to microbial metabolic activities are discussed in more detail below.



**Figure 6.** The polarization curve for a typical MFC

## Activation losses

Usually, to initiate transport of charges liberated by fuel (electron donor) oxidation to the anode (electron acceptor), an energy barrier must be overcome. The energy barrier comprises of an additional potential known as activation overpotential that is required for transfer of electrons from fuel to microbial shuttles and finally to the anode. Activation losses, represented by region A in Fig.6. is observed as a sharp decrease in MFC voltage (low polarization) at the initial low current densities, but it is steadily overshadowed by ohmic and concentration losses which usually occur at intermediate or high current densities (region B in Fig. 6). Clearly, activation losses occur at both anode and the cathode, and it is important to note that the cathodic overpotentials are much larger than anodic overpotentials. With the increased exchange current density this overpotential can be reduced [7, 44]. The activation losses can be explained from the Tafel equation shown by Equation 8.

$$\Delta V_{\text{act}} = A \times \text{Log}\left(\frac{i}{i_0}\right) \quad (8)$$

where  $\Delta V_{\text{act}}$ : activation overpotential, A: Tafel Slope, i: current density,  $i_0$ : exchange current density.

Some steps that can be taken to minimize the activation losses are:

- **Increasing anode surface area**

Increasing the surface area is a reliable approach to decrease the activation potential as when the surface area is increased the current density gets reduced. This can be done by increasing the electrode surface porosity and roughness.

- **Improving anode-microbe interactions**

In order to decrease the activation losses at the bacteria, it is necessary to improve anode-microbe interactions. Using the correct mediator would eliminate this problem by enhancing electron transfer. As mediators would go inside the cell membrane it can reduce the intracellular activation losses as well. MFC systems employing microorganisms that produce conducting pili have relatively low activation polarization.

- **Increasing the operating temperature**

In an inorganic fuel cell raising the temperature would reduce the activation overpotential but in the microbial fuel cell or in the enzymatic fuel cell it is not possible to increase the temperature unless the bio-reaction section is separate from the anode chamber.

- **Decreasing the activation loss at the electrode surface**

The activation energy at the electrode surface can be decreased by adding catalyst to the electrode. The catalysts that has been widely tested is Pt, which is reported to get polluted by bacterial suspensions. It has been reported that some success has been achieved by coating the

electrode with a conducting layer that shield microbes from direct contact with the catalyst material [45]. Immobilization of catalysts such as neutral red (that also acts as a mediator) and manganese oxide on the electrode surface have shown to increase MFC power output. [15, 46]

The loss of potential due to internal current and crossover of reactants would also be significant if the fuel cell is operating at low current densities. Some electrons will pass through membrane rather than through the external circuit. The membrane is impermeable to the oxygen molecule but certain percentage can be diffused into the anode where it reduces the current that can pass through the external circuit.

### **Ohmic losses**

Ohmic losses depicted as the medium polarization region B in Fig.6. is caused when the flow of charge is hindered as a result of the anodic resistance. The potential drop can be easily represented by ohms law as given in Equation 9 where  $R_i$  represent the total internal resistance and  $I$  is the circuit current.

$$\Delta V = I \times R_i \quad (9)$$

Increasing the conductivity of anode material, minimizing contact resistance and the total travel distance of electrons within the anode helps in limiting the ohmic losses. Use of highly conductive anode materials with 3D architecture (eg. 3D graphite felt electrode) has shown to produce higher current generation by overcoming the ohmic losses [47]. The three dimensional structure not only offers a high surface to volume ratio but also an evident increase in the anode-microbe interaction, thus facilitating higher electron transport [48]. Apart from this, resistance caused by the internal connections in the MFC system and the cation exchange membrane against the ionic flux also contributes to ohmic losses. The anodic (electrical) resistance were reported to be negligible when graphite electrodes were used [7] and the contact resistance can also be significantly low as compared to the ionic resistance. Optimizing the electrode spacing, using a low resistance membrane while improving the conductivity and buffer capacity of the electrolyte (tolerable by the microorganism) are concomitant strategies to improve ion transfer through the membrane [49, 50].

### **Concentration losses**

Imbalance in rate of mass transfer of substrate and products to and from the anode respectively and the total current generated in the system may result in increase in anode potential and decrease in cathode potential or vice versa causing concentration (or mass transport) losses. These losses are most prominent at high current densities due to diffusion-limited mass transfer of fuel to the anode surface. Also, the accumulation of oxidized products and cations in the biofilm may change the redox conditions and alter the metabolic activities of the microbes. Hindrance in cation transport may further cause a pH gradient between the electrodes leading to a significant reduction in the power output. Anode design and operational parameters are contributing factors toward concentration losses represented by the maximum polarization region C in Fig.6.

### **Losses due to fuel scavenging metabolic processes of microorganisms**

Loss of voltage can also occur due to catabolic activities of the microbe while deriving energy from fuel oxidation. As discussed earlier in the chapter, in an effective MFC, the anode potential should be as low as possible to allow attainment of high MFC voltage and adequate catabolic energy gain for the survival of microorganisms. Nevertheless, extremely low anode potential can hinder electron transfer causing fermentation of fuel while producing high energy products, resulting in loss of electrons. Furthermore, this also leads to added electron losses by excessive buildup of anodophilic biomass. A number of factors such as type of microbes, community composition, anode-microbe interaction, rate of fuel degradation by the microbes, number of microbes actively degrading the fuel and mix up of fuel through the electrolyte between the electrodes can affect the microbial metabolic losses.

### **3.2. Microbial interaction with the anode surface**

The electrical performance of a MFC is largely dependent on how well the microorganisms interact with the anode. A prime requirement here is that the biofilm that comprise of the microbes is adhered properly onto the anode.

Microbial adhesion on anode surface can be understood by the notion of surface charges. Most of the micro-organisms are negatively charged by nature and hence, attract positively charged surfaces. So, several (surface) modification techniques have been employed to facilitate this charge attraction process. For example, treating the anode surface with ammonia has been successfully attempted. Ammonia treatment facilitates the negatively charged bacteria to readily attach to the now positively charged anodes. The power output of the treated anode was expectedly much higher than its non-treated counterpart. However, high temperature requirements and, complex conditions and instrumentation have made this process commercially less feasible. In a different instance, treatment of activated carbon felt anodes with nitric acid (acid treatment) for rendering positively charged surfaces has resulted in a 58% increase in power density [51]. Zhou et al. reported that electrochemical oxidation of anode led to the change of the anode properties, such as augmented surface area, reduced internal resistance and anode potential, and therefore aided to the microbial adhesion and electron transfer on the anode surface [52].

#### **Role of anode material and architecture**

Anode material and its architecture directly affects microbial adhesion, electron transfer and fuel oxidation. The noble metal electrodes (Pt, Au, Ag, Pd) have been reported to be less attractive as MFC anodes due to their high cost and weak adhesion of microbes; and other high-performing electrodes (Ni, Cu, Rh, Ir) are being sought as alternatives [53].

Carbon-based anodes have been extensively tested for MFC applications as they exhibit superlative properties such as high conductivity, durability, eco-friendliness and their flexibility to be shaped into various architectures. They can have planar, packed or brush like configuration(s). The conventional electrodes include graphite - rod, felt, plate and fiber brush, carbon - felt, cloth and paper, and reticulated vitreous carbon (RVC) [54, 55]. Logan et al. made an observation that packed and brush design of anodes gave higher power output than the

planar type anodes by increasing the anode specific surface area and consequently the volumetric density of exoelectrogenic bacteria [55]. Additionally, the brush configuration also has high porosity. The fibrils of the brush anode helps the micro-organisms to hold onto the anode structure. Due to its enhanced conductivity and non-corrosive nature, titanium was found to be a suitable core wire on which the carbon or graphite fibers are wound.

Lately, nanomaterials have attracted much attention in various fields due to their unusual yet beneficial structural, chemical and electrical properties. Use of nanomaterial for anodes and electron transport has been reported to augment the performance of MFCs. Fan et al. showed that Au and Pd nanoparticle decorated anodes produced enhanced current densities than that of the control electrodes. They also observed that the anodic performance was significantly affected by not only the chemical composition of nanoparticles but also their size and shape [56]. In 2012, Xu et al. tested Fe nanoparticle-decorated graphite disks which resulted in approximately six-fold higher average current densities than the plain graphite anode. Upon running a whole genome microarray analysis of the gene expression of *Shewanella oneidensis* used in this study, they found out that genes encoding biofilm formation were significantly up-regulated as a response to nanoparticle-decoration [57]. However, it should also be noted that the probable cytotoxicity of certain nanoparticles often limit the possibilities that can be reached using nano-materials for MFC applications.

Coating the anode surface with materials such as carbon nanotubes (CNTs), conductive polymers and nanopolymer composites are other methods used for anode surface modification. Conductive polymer based anodes utilizing polypyrrole [58], poly(3-hydroxy butyrate-co-3-hydroxyvalerate) [59], nanowire networks [60] and nano/-composites of the polymers [61, 62, 63] have been studied and found to significantly increase MFC performance.

Recent studies show that CNTs are promising electrode materials due their high surface area, superior electrical conductivity, chemical inertness, decreased startup time and low internal resistance [64]. Functionalizing the CNTs appropriately, may further enhance electron transport. Mink et al. [65] made observations on a forest type multi-walled carbon nanotubes (MWCNTs) with a nickel silicide contact area that produced current density of 197 mA/m<sup>2</sup> and power density of 392 mW/m<sup>3</sup>. MWCNTs were said to have increased the anode surface-to-volume ratio, which improved the ability of the microbes to couple and transfer electrons to the anode. Nickel silicide were reported to boost the output current by providing a low resistance contact area that allowed efficient shuttling of electrons.

A three dimensional architecture that facilitates augmented growth of the microbes can be achieved by embedding CNTs on carbon cloth and polyester fabric using doping techniques. Also, the CNT coating stimulates active surface interactions with the microbes enabling direct electron transfer thereby giving 68% high power density and 10-fold-lower charge-transfer resistance than the traditional carbon cloth based anodes [48]. Yet another advancement in the three dimensional anodes was the 3D conducting graphene–polyaniline framework that was reported to outperform the planar carbon electrode by additionally providing multiplexed and highly conductive pathways [66]. Wang et al. [67] developed a 3D reduced graphene oxide–nickel foam as an anode for MFC which achieved a remarkable volumetric power density of

661 W/m<sup>3</sup> based on volume of the anode, which is the highest value yet obtained for a MFCs with a pure strain of *S. oneidensis* MR-1.

#### 4. Conclusion

In summary, MFCs have tremendous potential to generate electrical energy from chemical energy present in organic fuels primarily for decentralized stationary power generation applications. However, challenges associated with effective mass and charge transport along with the intricacies associated with making the living organisms interact with the inorganic (electrode) world have hampered this technology coming to technological fruition as yet. Advances in material science coupled with nanotechnology may provide novel tools to effectively harvest, transport and utilize electrical charges generated by MFCs for useful applications in the forthcoming future.

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