

Review Article

**Electric Bacteria: A Review**

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**Abstract:** Electromicrobiology is the field of prokaryotes that can interact with charged electrodes, and use them as electron donors/acceptors. This is done via a method known as extracellular electron transport. EET-capable bacterium can be used for different purposes, water reclamation, small power sources, electrosynthesis and pollution remedy. Research on EET-capable bacterium is in its early stages and most of the applications are in the developmental phase, but the scope for significant contributions is high and moving forward.

**Keywords:** Electric bacteria, *Shewanella*, *Geobacter*, Nanowires, Microbial fuel cell, Bioremediation.

**1. Introduction**

Electron flow is vital for metabolic life for all prokaryotes and eukaryotes through their prokaryote derived mitochondria and chloroplasts. The oxidative reactions that strip insoluble electrons from organic or inorganic substrates and carried to the cell wall by coenzyme (NAD) are well-known mechanisms of electron transport down the cell wall to an available electron acceptor. As the protons accumulate, they establish a proton gradient called the proton motive

force that is used to drive ATP synthesis, flagellar motility and membrane transport. In this process, the soluble protons are pumped across the membrane and conserve available redox energy, create an electrochemical gradient called proton motive force (PMF), which is used to power the synthesis of adenosine triphosphate, transport reactions and power the bacterial flagellum (Fig. 1). Therefore, bacteria are organisms that are electrically powered (Nealson, 2017).

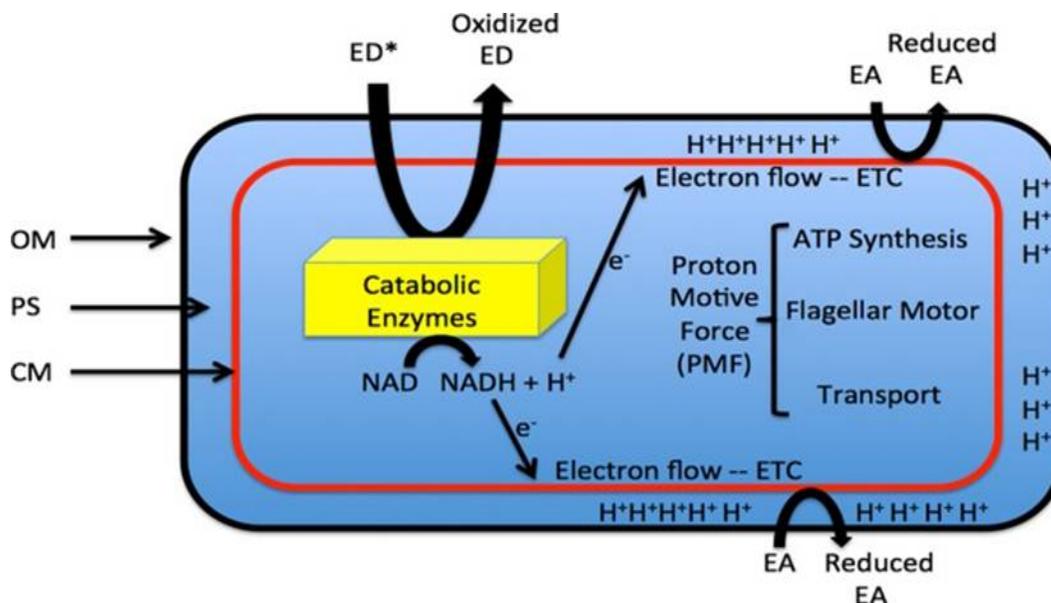


Fig. 1: Energy generation in bacteria (Source: Nealson, 2017).

A microfluidic technique was developed by researchers that can process small bacteria samples quickly and gauge a specific property that has a high correlation with the ability of the bacteria to generate electricity (Fig. 2). This property, known as polarizability, can be used to determine electrochemical activity of bacteria in a safer, more efficient way in comparison with current techniques. This technique may be applied broadly in clean energy generation, bioremediation, and biofuels production (Wang *et al.*, 2019).

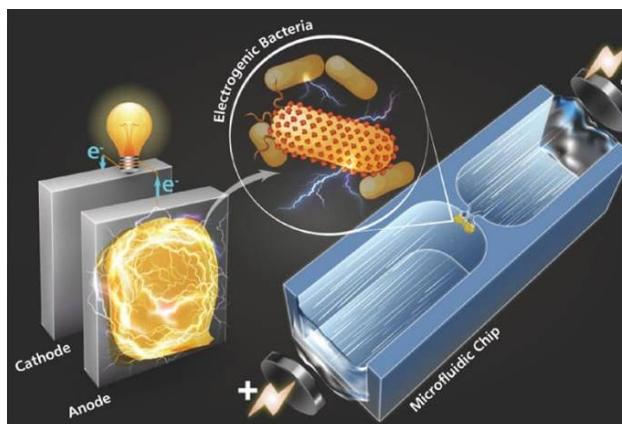


Fig. 2: Microfluidic technique sort bacteria based on their electricity generation capabilities (Credit: Qianru Wang).

## 2. Origin of electric bacteria

An electric bacterium is a type of bacteria that consume and excrete electrons directly at different energy potentials without the need of metabolization of any sugars or other nutrients. There are two known types of electric bacteria are *Shewanella* and *Geobacter* (Figs. 3 & 4). This life form seems to be specially adapted to low oxygen environments. Normal forms of life require an oxygen environment in which the excess of electrons produced is released in the metabolization of sugars. The pathway for releasing electrons is not available in the low oxygen environment. Instead of oxygen, electrical bacteria "breathe" metals, which effectively results in both consume and excretion of electrical charges (Brahic, 2014; Nealson, 1988; Lovley, 1987).

Among several known bacteria that are able to generate electricity, *Geobacter* species are most efficient as today. Discovered in 1987 by Dr. Derek R. Lovley and his coworkers. This organism does not only have the bioremediation ability, but also it can generate electricity.

The ability to exchange electrons is also a fundamental routine property of life that has existed since the very beginning of life. A group of researchers finds electrical life in the deep ocean around hot volcanic vents and formulated a theory that the initial form of life on Earth was based on electrical energy.

Such life forms would have created organic compounds from carbon dioxide through the direct use of geologically generated electrons. This is a simpler process than the use of chemical or light energy, which was previously suggested as the source of energy for life's origin. It is surprising how many different environments, processes and microorganisms have a certain degree of electron exchange. From the deep subterranean and polluted rivers to biogas and biofilms reactors, photosynthetic and metal-cycling bacteria to harmful bacteria in the body (Nielsen, 2019).

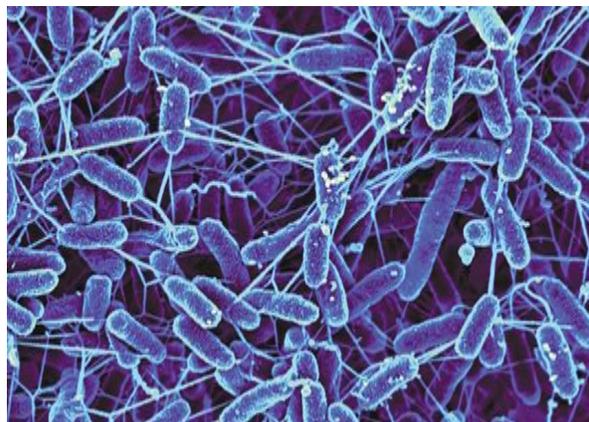


Fig. 3: *Shewanella oneidensis* (Credit: Rizlan Bencheikh and Bruce Arey).

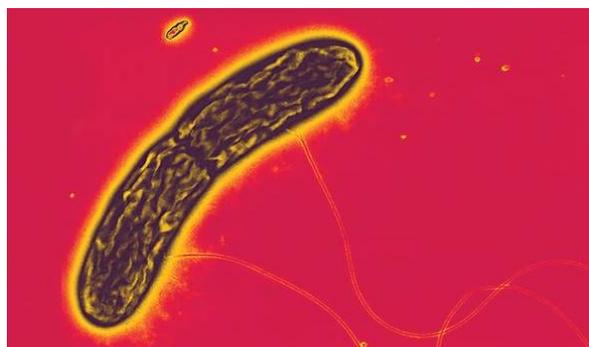


Fig. 4: *Geobacter metallireducens* (Credit: Dr. Derek R. Lovley).

## 3. Electricity produced by gut Bacteria

Some types of bacteria that already found in our gut can generate electricity. Electrogenic bacteria are not something new, they can be found in places far from us, such as the bottom of lakes. But there is no idea so far that bacteria found in decaying mammals or plants, particularly farm animals, generate electricity in a much simpler way. Recently discovered extracellular electron transfer system is a simpler electron transfer chain that has been found in single-cell wall, bacteria classified as gram-positive bacteria and carries electrons as a tiny electrical current. *Listeria monocytogenes*, a bacteria species, which we usually eat, sometimes cause an infection called listeriosis, has

the ability to generate electricity. They transport electrons into the surrounding environments through the cell wall, with the help of flavin molecules. *Listeria monocytogenes* is placed in an electrochemical chamber and generated electrons are captured using a wire or electrode, it is found that these foodborne bacteria generate an electrical current. Possibly the cell structure of *Listeria monocytogenes* and the vitamin-rich ecological niche they are holding facilitates the easiest and most cost-effective approach to transfer electrons out of the cell (Fig. 5).



Fig. 5: *Listeria monocytogenes* (© Amy Cao graphic, UC Berkeley).

There are many facts about why some bacteria produce electricity, like to get rid of electrons made by metabolism, according to a report (University of California, Berkeley). But the main objective is to create energy. But *Listeria monocytogenes* also generates energy in other ways, such as using oxygen. This process of generating electricity is possibly a backup system that they use under certain conditions. For example, it may deploy under low-oxygen gut conditions. The researchers tested mutated bacteria that have missing or changed genes, to identify the genes needed to produce electricity for the bacteria. Those genes in turn code for certain proteins that are key to electricity production. They notice that the system used by these bacteria, a cascade of proteins carrying electrons outside of the bacteria, was simpler than the system used by other electrogenic bacteria living at the lake's bottom. These gram-positive bacteria, their cell wall only has one layer suggests that there's one less hurdle, for the electrons to reach out of the bacteria, but once the electrons come to the outside, where they go is not clear. Typically electrogenic bacteria in their environment, transfer the electrons to minerals like iron or manganese. In the experiment, electrons were flowing into the electrode.

In the gut, different molecules, such as iron, could potentially accept and bind to electrons. Researchers found that flavin, a vitamin B2 variant, is densely populated in the gut is required for survival by these electrogenic bacteria. Later it is also found that bacteria

not only required flavins for survival, but extra, free-floating flavin in the surrounding environment could enhance the electrical activity of the bacteria.

After identifying the genes responsible for electricity generation, different other microbes have been identified that generate electricity, some of them generally exist in the gut (Light *et al.*, 2018; Cahoon & Freitag, 2018).

#### 4. Electric bacteria for Bio-Battery

Efforts are already being made to create batteries or microbial fuel cells, which use bacteria to produce electricity from organic matter, as in waste treatment plants. Because generating electricity from bacteria is much simpler.

In an important step towards bio-battery production, a new study shows bacteria generated electricity when proteins present in their cell membranes come into contact with the mineral surface. For some time, scientists have known that marine bacteria called *Shewanella oneidensis*, found in deep ocean sediments and soil, can generate electrical currents after exposure to heavy metals such as iron and manganese.

As human beings breathe oxygen and use this for energy generation, *Shewanella* bacteria may use minerals such as iron oxide for breathing. Bypassing electrons across their cell membranes, these bacteria are known to produce an electrical current, but it is not well known how this electron transfer from bacteria to mineral takes place. There are two main perspectives: The membrane proteins may directly transfer electrons to the surface of the mineral. Or proteins may be using other molecules to help them to carry electrons across the membrane of the cell.

Showing how membrane proteins generate an electrical current in these bacteria, researchers have developed a bubble-like fatty molecule structure studded with these proteins that mimicked the cell membrane of the bacteria. These bubbles are much easier to study because real bacterial cells are crowded with other structures. This experiment was carried out in an oxygen-free environment because the presence of oxygen can interfere with the chemical reactions. These bubbles contained an electron donor inside, and they were exposed to an iron-containing mineral outside. The speed of the electrical current generated across the membrane is measured by the researchers. This current's speed was very fast and sufficient to suggest that bacteria use this method to generate electrical currents in nature.

Research shows that proteins can touch the mineral surface directly and generate electricity, which means possibly the bacteria lie on the surface of a mineral or metal and conduct electricity through their cell membranes. Understanding how electric current is generated by these bacteria, encourage scientists to

develop bio-batteries for energy storage. On the other hand, the process is reversed and place electricity into the bacteria, such bacteria can be used in making bacteria manufacture useful materials (White *et al.*, 2013).

## 5. Nanowires of electric bacteria

A number of bacteria, especially *Shewanella* and *Geobacter* produced electrically conductive appendages called bacterial nanowires (Figs. 6 & 7). *Geobacter* nanowires are modified pili used to set up connections with terminal electron acceptors. *Geobacter* species transfer electrons to extracellular electron acceptors like Fe(III) oxides using nanowires. Through examining mutants, this function was discovered, pili of which could be attached to the iron, but not reduced it (Reguera *et al.*, 2005).

*Shewanella* nanowires are not pili, but the outer membrane extensions containing outer membrane decaheme cytochromes MtrC and OmcA (Pirbadian *et al.*, 2014). The presence of outer membrane cytochromes and the absence of nanowire conductivity from the MtrC and OmcA-deficient mutant (El-Naggar *et al.*, 2010) directly supports the multistep hopping mechanism for electron transport via the nanowires of *Shewanella* (Pirbadian & El-Naggar, 2012; Polizzi *et al.*, 2012).

Nanowires can ease the long-range transfer of electrons across thick layers of biofilm (Reguera *et al.*, 2006). Connecting with other cells above them, nanowires in anoxic conditions allow bacteria to use oxygen as their terminal electron acceptor. For example, organisms of the *Shewanella* genus were observed to form electrically conductive nanowires in response to electron-acceptor limitation (Gorby *et al.*, 2006). In microbial fuel cells, bacterial nanowires produce electricity by extracellular electron transport to the anode of MFC's (Kodesia *et al.*, 2017). It has been shown that nanowire networks enhance the electricity output of microbial fuel cells with long-range and efficient conductivity (Malvankar *et al.*, 2011). Especially, *Geobacter sulfurreducens* also known as electricigens can create metabolically active biofilms larger than 50 $\mu$ m that helps in converting acetate into electricity (Poddar & Khurana, 2011). Property of *Geobacter sulfurreducens* as microbial nanowires is important for the long-range transfer of electrons through biofilms. Cells at a distance from the anode remain feasible and electrically conductive 'pili' increases biofilm thickness without any reduction in efficiency, sometimes electricity generation increases by 10 times. Transfer of long-range electrons through pili networks allows viable cells to contribute to the flow of electrons that are not directly in contact with the anode. Therefore, in thicker biofilms, increased current production is observed in MFCs (Reguera *et al.*, 2006). Nanowires are coated with metal oxides also

encourages electrical conductivity (Maruthupandy *et al.*, 2017). In addition, these nanowires can carry electrons up to a distance of centimeter-scale. Considering sustainable resources researchers proposed future use of *Geobacter* biofilms as a functional underwater transistors and supercapacitors platform, capable of self-renewing energy (Malvankar & Lovley, 2012).

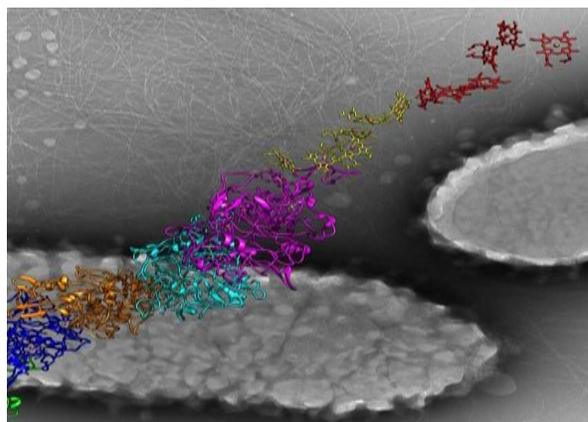


Fig. 6: An atomic model for electricity conducting microbial nanowires (Credit: Edward H. Egelman).

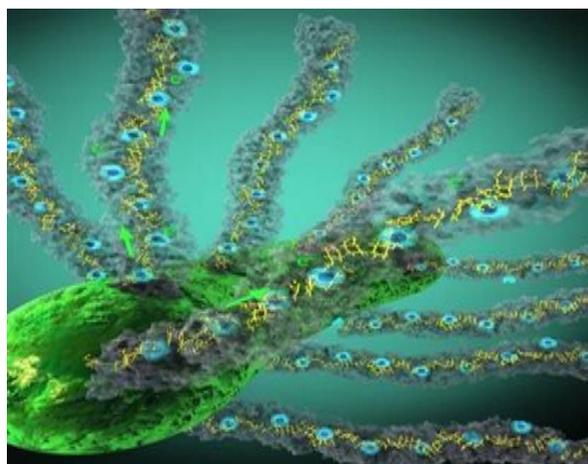


Fig. 7: Nanowires project to facilitate the transfer of electrons from bacteria (Credit: Yale University).

It has been found that bacterial nanowires have significant potential applications in bioenergy and bioremediation fields. Another application significance of bacterial nanowires includes bioelectronics. Transfer of electron between the pili of *Geobacter*, dissimilatory metal-reducing bacteria (DMRB), generates conductivity that drives organic compounds to be converted into electricity in MFC's. Biofilms produced by colonies of *Geobacter* greatly contribute to the overall bioenergy production. They allow electrons to be transferred over a greater distance from the anode via conductive pili. In fact, the output of bioenergy can be further increased by inducing the additional nanowire gene expression. It has been shown that

*Geobacter* strains with increased conductive pili expression produce more conductive biofilms, thus the overall electricity output increases (Sure *et al.*, 2016; Malvankar & Lovley, 2014).

*Shewanella* and *Geobacter* microbial nanowires have also been shown to help in the bioremediation of groundwater contaminated with uranium. To show this, researchers compared and observed uranium concentration removed from piliated and nonpiliated *Geobacter* strains. Through experiments, they concluded that nanowire present strains were more effective in uranium mineralization as compared to nanowire absent mutants (Jiang *et al.*, 2011; Cologgi *et al.*, 2011).

## 6. Microbial fuel cell (MFC) and Microbial desalination cell (MDC)

During the last few years, researchers focus on renewable and alternative energy sources to replace our present fossil fuel dependency. Some of the microorganism's catalytic ability to directly convert chemical energy into electricity from a number of organic substances laid the ground for research on microbial fuel cells (Franks & Nevin, (2010).

A microbial fuel cell (MFC) is a bio-electrochemical device, releases electrons and protons, thereby generate electricity. In MFCs microorganisms or enzymatic catalysis are used to transform chemical energy directly into electrical energy via bioelectrochemical reactions. In MFCs bacteria is used as a catalyst to oxidize a wide range of organic and inorganic matter. Bacteria can donate electrons to the anode and accepted from the cathode (Du *et al.*, 2007). MFCs can be mediated or unmediated. In mediated MFCs, a chemical is used as a mediator such as thionine, methyl viologen, methyl blue, humic acid and neutral red, which transfers electrons in the cell from the bacteria to the anode. In unmediated MFCs, a mediator is not required, the bacteria typically have redox proteins on their outer membrane, such as cytochromes, which are electrochemically active and transfer electrons directly to the anode (Delaney *et al.*, 1984; Badwal *et al.*, 2014; Min *et al.*, 2005).

A typical MFC is built using a bioanode, a biocathode, a cation and an electrical circuit. In Most MFCs, the anode and the cathode compartments are separated by a proton exchange membrane (Figs. 8 & 9). That allows protons to be transferred from the anode to the cathode. The membrane also separates oxygen from bacteria. During oxidation, produced electrons are directly transferred to an electrode. Electron flux is transferred to the cathode. The system's charge balance is balanced by an ionic movement inside of the cell, generally across an ionic membrane. The organic electron donor is used in most MFCs that oxidized and produces CO<sub>2</sub>, protons and electrons. Electron donors such as sulfur compounds or hydrogen were also

identified (Pant *et al.*, 2010). Different electron acceptors are used in MFCs, reduction of oxygen is the most studied method. Other electron acceptors like metal recovery by reduction, nitrate and sulphate reduction have also been studied (Lu *et al.*, 2015). When sugar is consumed by microorganisms in aerobic conditions carbon dioxide and water are produced. When oxygen is not available, microorganisms produce carbon dioxide, hydrogen ions and electrons (Bennetto, 1990), as shown below:

Anodic reaction:



Cathodic reaction:

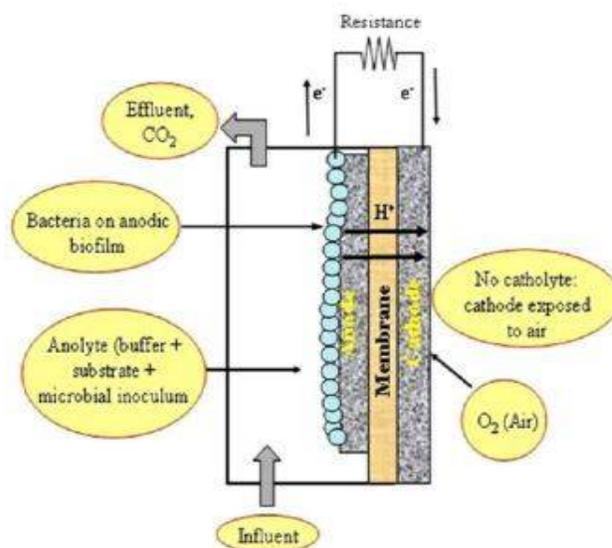


Fig. 8: Schematic diagram of single-chamber MFC (©Pant *et al.*, 2010).

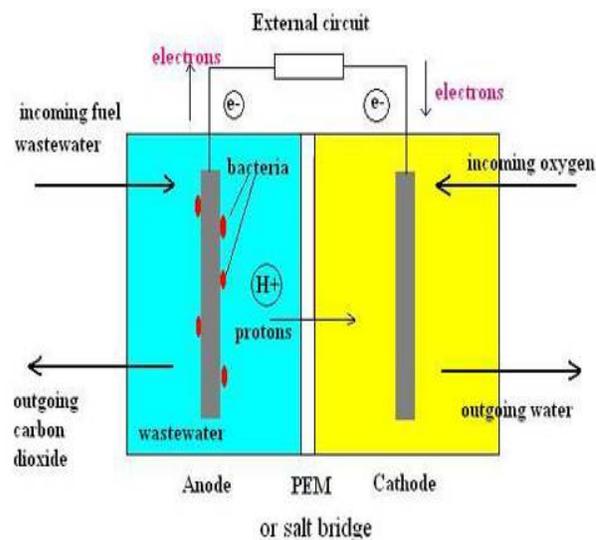


Fig. 9: Schematic diagram of two-chambered MFC.

Microbial Desalination Cell (MDC) is a new technology that is capable of treating wastewater by simultaneously producing an electric current. MDC technology is an extension of the microbial fuel cell process. Due to the great need for free energy and environment-friendly technologies, MDC has received considerable attention for desalination, wastewater treatment and energy production. A unit of MDC consists of an anode, cathode and a pair of ion-selective membranes are inserted between them. The anode and the cathode maintain anaerobic and aerobic conditions respectively. The system can be either used as an independent process or it may be combined with other desalination methods that include reverse osmosis or electrodialysis. Many different MDC modifications have recently been introduced, including stacked MDCs, biocathode MDCs and recirculation MDCs. In the case of electricity production from wastewater, wastewater containing the organic matter enters the anodic side where the available bacteria are proliferated and form a thick cell aggregate known as biofilm (Torres, 2012; Saeed *et al.*, 2015).

## 6.1 Applications of Microbial fuel cell

### 6.1.1 Electricity generation

To use MFCs in electricity generation, the produced electrons must be moved out of the cell's electron transport chain and be deposited on an electrode. This can be done in two ways. Inorganic mediators tap into the electron transport chain and channel electrons produced via lipid membrane and the cell outer membrane and liberate negatively charged electrons at the electrode. Where microorganisms generally require a mediator, they do not have electrochemically active surface proteins for transferring electrons to anode (Strik *et al.*, 2008; Scholz *et al.*, 2003). Alternatively, certain bacteria with pili e.g. *Shewanella putrefaciens* are electrochemically active can be used because they transport electrons directly from the respiratory enzyme to the anode in anaerobic conditions. After all, the difference between free energy and the electrode is greater than that of other available acceptors. The electrons flow to the cathode via a load at which oxygen is easily available to be reduced (Bergel *et al.*, 2005).

In MFC process, the anode is the bacteria recognized terminal electron acceptor in the anodic chamber. Hence the microbial activity depends strongly on the redox potential of the anode (Cheng *et al.*, 2008). Organisms able to generate electrical current are called exoelectrogens. To convert this electric current into usable electricity, exoelectrogen must be placed in a fuel cell. In a solution, the mediator and a microorganism such as yeast are mixed together to which a substrate like glucose is added. This mixture is put in a sealed chamber to avoid oxygen from entering, thus microorganism is forced to start anaerobic

respiration. An electrode which acts as an anode is placed in the solution. In MFCs second chamber there is another solution and electrode (cathode) which is positively charged. This represents the equivalent oxygen sink at the end of the electron transport chain, outside the biological cell. This solution acts as an oxidizing agent that picks up electrons at the cathode.

From the cell, the reduced mediator brings electrons towards the electrode. The mediator is oxidized here because it deposits the electrons. Then they flow to the second electrode across the wire that works as an electron sink. They will move from here to an oxidizing material. The hydrogen ions/protons are also moved from anode to cathode via proton exchange membrane. They will pass through the lower concentration gradient and combine with the oxygen, but to do that they need an electron. The current is formed and the concentration gradient is sustained by the hydrogen (Rashid *et al.*, 2013).

### 6.1.2 Biohydrogen production

Microbial fuel cells can easily be modified to produce hydrogen rather than electricity. This modified system, which was recently proposed, has been considered as an interesting new technology for biohydrogen production from organics and referred to as a bio-electrochemically assisted microbial reactor (BEAMR) process or biocatalyzed electrolysis or electrohydrogenesis. However, the generation of hydrogen from the protons and electrons produced by the anaerobic degradation of a substrate by electrochemically active bacteria in a modified MFC is thermodynamically unfavorable. This thermodynamic barrier can be overcome by using an external potential. In this system, the protons and electrons formed by the anodic reaction migrate and combine at the cathode under anaerobic conditions to form hydrogen.

The potential for the oxidation of acetate (1M) at the anode and the reduction of protons to hydrogen at the cathode are -0.28 and -0.42 V (NHE), respectively (Kim *et al.*, 2008).

### 6.1.3 Wastewater treatment

Some microbes actually grow little electricity-conducting wires, which works like a snorkel, allowing the bacteria to penetrate into the sediment more deeply. To survive, they can use electrochemical cycle where oxygen is not available. These so-called electroactive bacteria purify water up to ten times faster than traditional methods. *Shewanella oneidensis* bacteria are not only useful in cleaning polluted water contain mercury, lead, and iron. It can generate electricity as well. *Shewanella oneidensis* cell membrane contains proteins that conduct electrons and necessary for the respiration of the cells (iMETland project).

The researchers developed the iron-containing molecule DSFO+ with a structure that imitates critical proteins in two mutant *Shewanella* bacteria. It has been

observed that the capacity to produce electricity is not only improved, but DSFO+ also use to replace the function of proteins that occur naturally in mutant bacteria. The ability to change microbial system behavior in such bacteria could be used not only to break down contaminants in wastewater, but this process generates enough electricity to recoup some cost of water treatment (Kirchhofer *et al.*, 2017).

#### 6.1.4 Bioremediation

Bioremediation is a promising method in which microorganisms or their enzymes are used to remove and/or degrade contaminants from the environment. The pathway to the accumulation of these contaminants is adsorption, in which metals are absorbed by microbial cells, this process called biosorption. Microorganism's metabolic ability used to degrade or remove pollutants from the environment provides an economical and safe alternative compared to other physicochemical approaches. For treating heavy metal contamination, bioremediation is the most cost-effective method. Different genetic approaches were developed and used for the optimisation of enzymes, metabolic pathways and organisms of biodegradation relevance (Perpetuo *et al.*, 2011).

In bioremediation, MFCs robustness makes the technology very useful. MFCs are proposed for the cleanup of different types of contamination, from aromatic or substituted organic compounds to heavy metals. During the process of bioremediation, electricity is also produced and therefore the costs are reduced (Cao *et al.*, 2019).

*Geobacter* and *Shewanella* are gram-negative and metal-reducing bacteria. The ability of *Geobacter* and *Shewanella* in bioremediation has been reported in several studies. Include solid waste, groundwater and wastewater, petroleum products (hydrocarbon), soil remediation, uranium remediation, and heavy metal pollution remediation (Rizwan *et al.*, 2014).

*Geobacter* species have the ability to consume contaminants of petroleum and radioactive metals from polluted groundwater by oxidizing these compounds to harmless carbon dioxide. The first *Geobacter* to degrade aromatic compounds was found to be *Geobacter metallireducens*. More specifically, *Geobacter metallireducens* is the only organism that degrades benzene anaerobically in pure culture. *Geobacter* is the main species for coupling the oxidation of organic compounds to the reduction of insoluble metal oxides such as Mn(IV) and Fe(III) in different types of soils and sediments (Anderson *et al.*, 2003; Lovley *et al.*, 2011). *Geobacter* develops electrically conductive pili between itself and the pollutant to degrade the substance. Pili of *Geobacter* have an essential function as protective and catalytic cellular mechanisms for bioremediation of groundwater contaminated by uranium (Cologgi *et al.*, 2011). The findings provide *in situ* field evidence in support that

the uranium can be effectively removed from contaminated groundwater by stimulating subsurface *Geobacter* species activity. *Geobacter* is often the most predominant species in the subsurface environment where extracellular electron transfer is important for bioremediation (Anderson *et al.*, 2003).

*Shewanella* has great metabolic versatility, they are capable to reduce different electron acceptors. Some of these are toxic substances and heavy metals, after being reduced they become less toxic (Dikow, 2011). *Shewanella* uses a number of compounds as electron acceptors including oxygen, iron, manganese, uranium, nitrate, nitrite, etc. During aerobic respiration, it uses oxygen as the terminal electron acceptor however, in anaerobic conditions *Shewanella oneidensis* undertakes respiration through the reduction of alternative terminal electron acceptors such as oxidized metals. The potential of *Shewanella* to decrease the toxicity of contaminated metals and radioactive wastes makes it a useful bioremediation tool (Heidelberg *et al.*, 2002). In particular, *Shewanella oneidensis* strain MR-1 is commonly used to clean contaminated nuclear weapon manufacturing sites.

## 7. Conclusion

Electromicrobiology is typically an emerging biological and microbiological field that has a wider scope for new developments and discoveries with continued growth. There are numerous other potential applications currently undergoing research. However, there is a need for new approaches that can fulfill our sustainable system requirements. The field of electromicrobiology may offer a number of many useful and exciting tools in our search for a sustainable future.

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