



Applications of Microbial Fuel Cell Technology and Strategies to Boost Bioreactor Performance

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ABSTRACT

Renewable energy technologies are developing day by day. One of the novel renewable energy technologies is microbial fuel cells (MFC). MFCs are eco-friendly as it uses electroactive bacteria (exoelectrogens) to generate electricity by using organic and inorganic waste from the wastewater. Electricity generation and wastewater treatment are its primary applications. The construction of MFC consists of the electrodes which may be modified by using nanoparticles (gold and iron oxide) or pre-treatment methods (sonication and autoclave sterilization). This technology is further studied for the detection and reduction of toxic heavy metals in wastewater. The MFCs are also modified into microbial electrolysis cells to make biofuel such as hydrogen. The present review is based on the applications of the MFC, key challenges, and modification strategies.

INTRODUCTION

These days the non-renewable energy resources are depleting rapidly which is causing environmental pollution. This problem can be solved by converting organic/inorganic waste energy into chemical energy or other forms of energy (Alipanahi et al. 2019). Several purification methods along with anaerobic fermentation are used to produce hydrogen and methane biofuels. Their sustainability depends upon the methods of renewable energy technologies (Bhowmick et al. 2019). The MFC has intrigued the researchers because it can generate bioenergy and treat wastewater simultaneously. It uses the metabolism of the microbes for energy generation and treatment of wastewater. It is also being used for bioremediation (Chandrasekhar et al. 2018).

MFCs are bioelectrochemical cells having an anode chamber (containing microorganisms and anolyte under anaerobic conditions) and a cathode chamber (containing an electron acceptor and a catalyst under aerobic/anaerobic conditions), connected by the PEM (proton exchange membrane) e.g., Nafion. The microorganisms at the anode chamber are called exoelectrogens which act as a biocatalyst.

The electrons are produced at the anode and transferred to the cathode through an electrical connection. The electrons react with protons and oxygen to release water (Cui et al. 2019). The exoelectrogen regulates the electrons in their outer membranes by themselves at the anode and transfers them to the cathode where they are reduced by the electron acceptors (oxygen). The most common use of MFC technology is electricity production. The electrons at the anode are produced by oxidation of organic matter which initiates a low redox potential and reduction at the electron acceptor of the cathode creates a high redox potential. The potential difference directs the electrons from the anode to the cathode to generate electricity (Das 2018).

The MFCs are constructed in different ways. Some use the pure culture (e.g., *G. sulfurreducens* and *Shewanella oneidensis*) and others use mixed culture (primary wastewater) for the electric current generation (Das & Ghangrekar 2020). The anode chamber is modified with nanoparticles or microbial genes to enhance the MFC performances. For instance, N-doped carbon nanoparticles are used which raises the power density to three times. On the other hand, *S. oneidensis* MR-1 with a flavin biosynthesis pathway of

B. subtilis raised the power output to 13 times (Delord et al. 2017). This technology operates on the biodegradation of the organic compounds of the wastewater. Hydrogen is produced by the modification of MFC into microbial electrolysis cells (MEC). The voltage is supplied by MFC to the MEC for hydrogen production. In this review article, applications of MFCs and improvement techniques are analyzed critically. The applications of the MFC include electricity production, bioremediation, treatment of wastewater, biosensors, and production of hydrogen (Do et al. 2020).

THE MOLECULAR MACHINERY OF EXOELECTROGENS

The features that make the MFC technology prominent as the non-renewable technology are the use of molecular machinery of bacteria (biomolecules). These molecules transport the electrons between the anode and the cathode. For this purpose, the molecular machinery of *Geobacter* spp. and *Shewanella* spp. are widely studied. Extracellular electron transfer (EET) mechanisms have two types i.e. mediated electron transfer (MET) and direct electron transfer (DET) mechanisms are observed in these bacteria (ElMekawy et al. 2018). The most analyzed exoelectrogen in *G. sulfurreducens*. It creates a layer of biofilm with riboflavin secretion on the surface of the electrode and uses it as a substrate. With the help of the MET mechanism, the riboflavin reacts with c-type cytochromes (OM c-Cysts) in the outer membrane

to transport electrons to the surface of the electrode (Ezziat et al. 2019). *Shewanella oneidensis* also act as an exoelectrogen. It has nanowires-like structures extended from the periplasmic space and multiheme cytochromes in the outer membrane containing vesicles. This bacterium secretes riboflavin (RF) and flavin mononucleotide (FMN) which causes the reduction of many-electron acceptors. RF and FMN are the cofactors for OmcA and MtrC, respectively. The complexes of secretion and cofactor drive the transfer of electrons between electrodes (Flimban et al. 2019).

MFCS FOR ELECTRICITY GENERATION

The MFC is mainly employed to produce electricity. The modification in the electrodes using metal catalysts can be done to increase the current density. In the first step, the exoelectrogens adapt to the anode surface to create a biofilm of 30 μm -50 μm thickness. The biofilm formation is further enhanced by the adhesins and components of extracellular matrix assembly and by cytochromes. The performance of MFC is amplified by using proper bacterial culture and substrate (Gajda et al. 2018a).

The electrons are transported between both electrode surfaces through an electrical connection where they bind with protons and an electron acceptor. Water produces with an oxygen electron acceptor at the cathode with a voltage of 0.805 V. The rate at which oxygen is reduced is increased by the platinum catalyst. For reducing oxygen, microorganisms

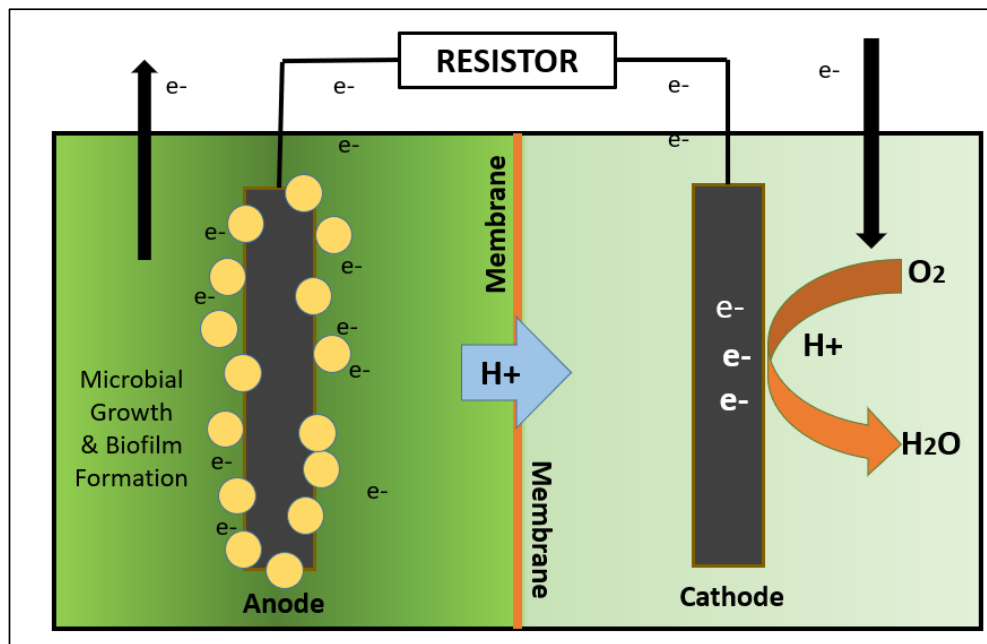


Fig. 1: In a double-chambered MFC, microbial growth takes place on the anode, as microbes consume organic matter, they donate electrons, which then travel the electrical circuit, and the reaction is completed at the cathode (Bose et al. 2018a).

can also be used and ferricyanide and potassium permanganate can act as electron acceptors (Gajda et al. 2018b). Fig. 1 shows some features of double-chambered MFC; for such systems, it is important to maintain the proper functioning via the selection of exoelectrogens, substrate, and electron acceptors. The community structure of microbes is affected by substrate type. For instance, *Geobacter* and *Shewanella* spp. only use acetate for electricity generation (Gajda et al. 2018).

Electrodes are modified with metal catalyst/nanoparticles/chemicals to raise the outputs. The cathode is also modified to alternate the expensive platinum catalyst with a cheaper one having the same properties. The research showed that these modifications lessen the internal resistance and start-up time of the system. The anode is modified with nitrogen-doped electrodes, heat-treated electrodes or gold nanoparticles, graphene, and carbon nanotubes to enhance the output performance, e.g. CNT-gold-titania nanocomposites ameliorate the MFC performance (Goswami & Mishra 2018).

The EET mechanism is boosted by using nitrogen-doped carbon nanoparticles coated on carbon cloth electrodes. *Shewanella oneidensis* MR-1 is inoculated in the two-chamber MFC. It increases the rate of electron transfer and power density by the absorption of flavins secreted by the organism. The studies show that the anode is sprinkled with the CNT powder which enhances the *G. sulfurreducens* biofilm

growth to decrease the internal resistance and the start-up time (Ivars-Barceló et al. 2018). The reduced start-up time promotes adherence of bacteria to the electrode. The carbon nanotube anode in the double-chambered MFC increases the power density up to 4 times as compared to the plain carbon cloth anode. It is suggested that ferric oxide shows a great affinity for c-type cytochromes in the outer membrane of *Shewanella*. The modification of electrodes with iron oxide increases bacterial growth and enhances the EET mechanism and metabolism of the biofilm (Jadhav et al. 2017).

MFCs FOR WASTEWATER TREATMENT

In wastewater treatment, disposal of waste and recycling of polluted water with precautions are done. The industries and urban areas release polluted wastewater which can be treated the MFC technology in single-chambered MFCs as shown in Fig. 2. The wastewater has several compounds which are used as a substrate for bacterial growth. The chocolate industry or palm oil mill effluent (POME) releases wastewater which at as the biocatalysts for the oxidation of the substrate. This water is used as catholyte and electron acceptors. The following portion of this article deals with the efficiency of MFCs in the treatment of wastewater (Jatoi et al. 2021).

SPW is used to estimate the MFC efficiency for wastewater treatment. After operating MFC for 140 days, 98%

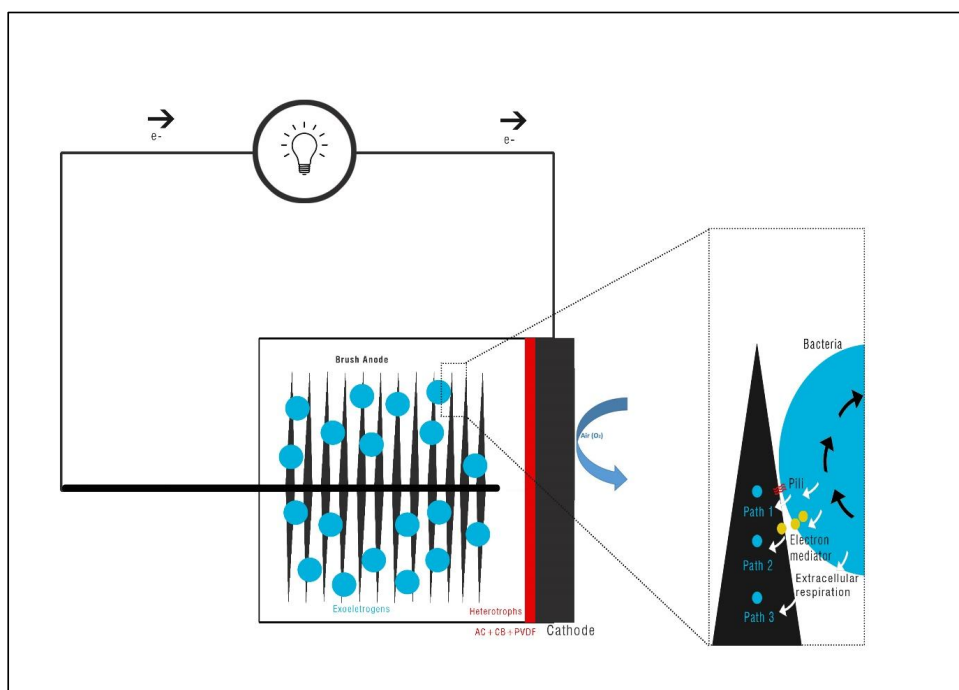


Fig. 2: Schematics of single chambered MFC, in such a system brush anodes, are used for microbial growth with air cathodes, having one side facing wastewater in the system and the other side exposed to the outside air (Bose et al. 2019b).

COD and 91% ammonia-nitrogen were removed. Likewise, a double-chambered MFC treated chocolate. It removed 75% COD, 65% BOD, and 68% total solid in batch mode. Lignocellulosic biomass cannot be removed by conventional techniques. Huang and Logan produced electricity in single-chambered MFC (sMFC) by using paper recycling wastewater (Jiang et al. 2018). After operating for 3 weeks, 76% COD and 96% cellulose were removed. This showed the degradation of lignocelluloses into smaller sugars by the bacterial community (Kaur et al. 2020).

The treatment of brewery wastewater was also performed by MFC. It had 5000 mg.L^{-1} COD and many sugars. These sugars donate electrons to MFC. For instance, the operation of air cathode sMFC at different concentrations of wastewater in a fed-batch removed COD differently. The low COD was removed from wastewater with less COD value. 58% and 98% COD were removed from 84 mg.L^{-1} and 1600 mg.L^{-1} concentration, respectively (Khan et al. 2017). Likewise, sMFC at 600 mg.L^{-1} - 660 mg.L^{-1} COD concentrations removed 43- 46% COD after 2.13 hours of hydraulic resistance

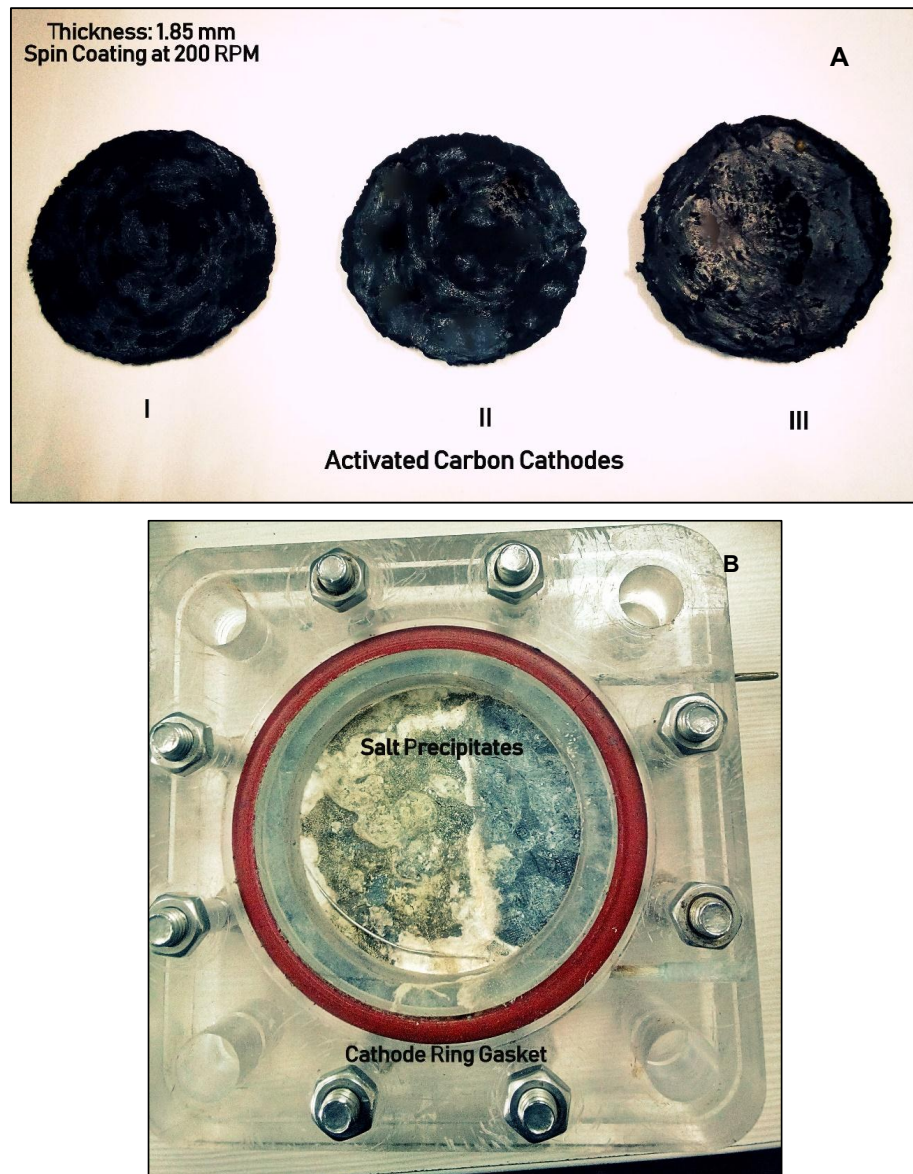


Fig. 3: (A) MFC Cathodes prepare from Activated carbon by a single-step phase inversion method (B) Fouling of MFC cathodes after fifteen full cycles (Adopted from Bose et al. 2018c).

in continuous mode. Ahn and Logan worked on air-cathode sMFC to check the effect of two different temperatures on MFC i.e. ambient temperature ($23 \pm 3^\circ\text{C}$) and mesophilic temperature ($30 \pm 1^\circ\text{C}$). It showed that the mesophilic temperature increased the percentage of COD removal and its removal rate. The efficiency of fed-batch MFC to remove COD is 2.5 times higher than the continuous mode (Khudzari et al. 2018).

The toxicity and COD value are very high in the wastewaters of mills. For instance, 16000 mg.L^{-1} COD and ca. 86 mg.L^{-1} cyanide were observed in cassava mill effluent. Palm oil industries release palm oil mill effluent (POME) which has a COD value of 50000 mg.L^{-1} and a BOD value of 25000 mg.L^{-1} . POME was treated by Cheng et al. in an up-flow membrane-less MFC (UML-MFC) coupling MFC and UASB (up-flow anaerobic sludge blanket) reactors. It removed 96% and 94%, of COD and nitrogen, respectively (Kodali et al. 2018).

The power density of MFC is raised by using wastewater with a high COD value. But, when the concentration of substrate is high, it causes electrode fouling, leading to restriction and accumulation of salts and precipitates as shown in Fig. 3. And the cathode has fewer protons available. Due to this fouling, wastewaters dilutions are used to regulate the performance of the MFCs. In addition, some pre-treatment methods can enhance the performance of the MFCs (Kumar et al. 2018a). For instance, the methanogens, using organic compounds of wastewater to generate methane, are killed by autoclaving. A 5% power density increase was suggested by autoclaving. On the other hand, sonication was used to treat raw wastewater. It increased the power density up to 16% and COD removal up to 5%. Furthermore, COD can be removed from wastewater by stirring technique. Although these pre-treatment techniques increase energy generation, they cannot be used for upscaling (Kumar et al. 2018b).

Domestic wastewater is highly biodegradable and can produce energy from degradation. Therefore, it is used as a substrate in MFC to construct public toilets and to produce electricity. This is an eco-friendly construction. For instance, an air-cathode sMFC (system for struvite extraction) was employed for the treatment of human urine and extraction of struvite (natural fertilizer), simultaneously (Kumar et al. 2019a). The anaerobic sludge was added to the anode. The substrate i.e. human urine had yeast extract and tryptone, 0.5%, and 1%, respectively. At the first level, the power gain was 14.32 W.m^{-3} then in the third level decreased to 11.76 W.m^{-3} . Further, the modification technique was used to increase electricity production and struvite purification. The sea salts were inoculated along with the substrate (urine) raised the power output, COD removal, and struvite extraction by

10%, 16-18%, and 21-94%, respectively. Now, human feces are also being used for electricity production. For instance, fermented human feces wastewater was inoculated in the two-chamber MFC. After operating MFC for 190 h, it generated 70.8 mW.m^{-2} power density and reduced 78% COD (Kumar et al. 2019b).

In the first five months, the MFC operation removed 75% COD which lasted for a year. Its performance can be enhanced up to 94% COD removal by using 10 external resistors. A 90-L MFC was manufactured by Dong et al. It produced energy itself for 180 days of operation by treating brewery wastewater. It was observed that diluted wastewater showed ~87% whereas real wastewater showed 85% COD removal. By all mentioned scaling up processes, the performance of MFC technology can be improved by treating wastewater and increasing electricity production (Liu et al. 2020).

MFCs FOR BIOREMEDIATION OF SPECIFIC CONTAMINATIONS

The electrons are generated by exoelectrogens in the anode of MFC, which are transferred to the cathode where reduction occurs at the electron acceptor. The rate of reduction is ameliorated by a catalyst. The electron acceptors used in MFC have features such as high redox potential, faster kinetics, inexpensive, and easily available. For instance, the commonly used electron acceptor of MFC is oxygen. But different toxic elements either organic or inorganic can be employed as the electron acceptor as shown in Fig. 4. Such systems are called sediment-Microbial fuel cells or s-MFCs. In cathode, they are reduced to less toxic forms. For instance, metal ions, perchlorate, nitrobenzene, azo dyes, and nitrates are being used as electron acceptors (Mathuriya & Pant 2019).

Many heavy metals are used as the electrodes of MFCs for their removal and reduction to non-toxic forms. When the heavy metal is used at the anode, its certain concentration is inoculated to the anolyte and the electron acceptor of the cathode is made up of heavy metal with high redox potential. Several mechanisms are being employed in the MFC for the removal of toxic heavy metals. One of the mechanisms is biosorption (microprecipitation, complexation, chelation, and ion exchange) which separate the toxic heavy metal in the MFCs (Mian et al. 2019). This process is carried out with the help of the biomolecules (polysaccharides, proteins, and lipids) containing certain functional groups (amine, sulfate, carboxylate, hydroxyl, and phosphate) present in the anolyte. In addition, sulfide was removed by the processes such as oxidation, volatilization, and electrode adsorption (Neethu et al. 2019). Cadmium (Cd) and zinc (Zn) was removed by an air-cathode sMFC with efficiencies of 90% and 97%,

respectively. Furthermore, vanadium (V) was used as the electron acceptor at the cathode in a dual-chamber MFC for its toxic removal. After operating for 10 days, the fuel cell removed ca. 70% V at ca. 970 mW.m⁻² power density.

The textile industries use several dyes used to color the fabric. Every year, these fabric dyes produce a huge amount of wastewater containing toxic elements, recalcitrant, and carcinogenic compounds. These are compounds are polluting the environment heavily; hence, this wastewater should be treated before release. It is an eco-friendly replacement for the production of electricity and wastewater treatment. The microorganisms in MFC are used for the reduction of the dye by decolorization technique. This decolorization is carried out in the anaerobic conditions at the anode. For instance, the congo red dye split into the intermediates (aromatic amines) by breaking the azo bond. Further degradation takes place at the cathode abiotically (Palanisamy et al. 2019).

After operating for a single day, the congo red dye was decolorized 98% with the help of bioelectrodes of sMFC. The electrons at the anode are produced by the microorganisms and protons by PEM. The electrons are transferred to the cathode where they react with protons to degrade the azo bond. The aromatic amines are formed as decolorized products of degradation. In the MFC, the bioremediation of pentachloroethene (PCE) and trichloroethene (TCE) is done by using *Geobacter lovleyi* (dechlorinating microorganisms) and graphite electrodes. The optimum conditions for the degradation were 50°C and pH 6 for 0.52 mg.L-h⁻¹ and 0.36 mg.L-h⁻¹. Moreover, a soluble U(VI) was reduced to an insoluble form by *Geobacter* species by using c-type cytochromes of the outer membrane. Likewise, this same process also occurs in *S. oneidensis* using c-type cytochromes. The reduction of chromium to a less toxic form i.e. Cr (VI) to Cr (III) is done at anaerobic biocathodes in MFCs to pro-

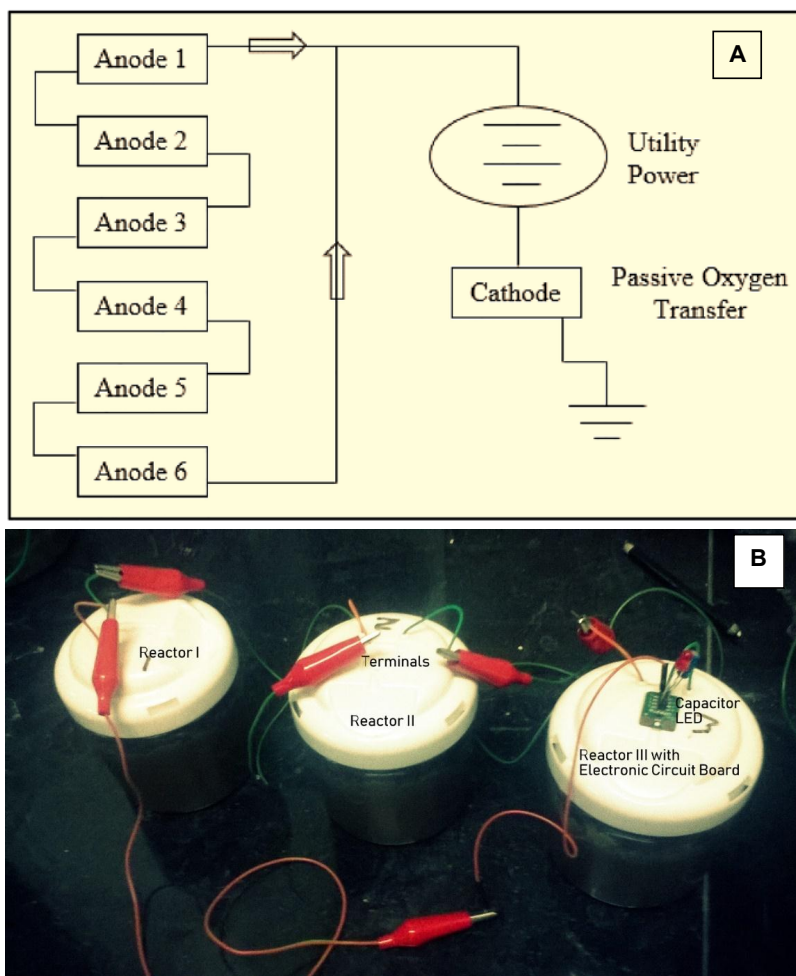


Fig. 4: (A) Schematic representation of s-MFC arrangement with multiple anodes and a single cathode (B) s-MFC reactors with contaminated soil sample connected to an electric circuit for power generation through bioremediation (Adopted from Bose et al. 2021d).

duce electricity (Qi et al. 2018). $\text{Cr}(\text{OH})_3$ also precipitates during this process which raised the rate of reduction to $19.7 \text{ mg}\cdot\text{L}^{-1}$. The rate of reduction is increased in the aerobic condition at the cathode when *Shewanella oneidensis* MR-1 is a biocatalyst and lactate is a biomolecule. A recent 16S rRNA study suggested that a strain similar to *Klebsiella sp.* is able of removing 99.5% cyanide and ca. 88% COD from cyanide-containing wastewater (Rikame et al. 2018).

MFCs AS BIOSENSORS

The MFC is now used as a biosensor for the detection of organic matter and toxic pollutants in the wastewaters. Usually, the transducers are used for the conversion of the energy of organic matter to the signals in the biosensors but MFC does not require transducers because MFC itself plays the role of a transducer. These biosensors use exoelectrogens which generate the signals in the anode (Bose et al. 2020e). The material of electrodes is used as transducers. The time span for the use of elements in the biosensor is enhanced by the biofilm formation of exoelectrogens (Sayed & Abm delkareem 2017).

The toxins are inoculated at the anode which changes the output voltage. The altered voltage is measured and considered as a detection signal. For instance, chromium is added to the anode which decreases the voltage because it causes growth inhibition of the exoelectrogens. Alternatively, acetate is added to the anode which increases the voltage because the growth of exoelectrogens is increased (Trapero et al. 2017). These sensors work on two mechanisms, i.e. flow-through and flow-by electrodes. The first mechanism circulates the water sample through the pores in the electrode, whereas the second mechanism moves the water sample parallel to the surface of the electrode. The MFC biosensor working in a flow-through mode enhances its sensitivity 40 folds by improving the ionic diffusion (Türk et al. 2018).

For the detection of toxicity, the MFC-based biosensors work in turn-off mode. The metabolism of the exoelectrogens is reduced because of the toxin concentration added to the anolyte which leads to the power output change. After detection, this toxicity is calculated by comparing the toxin concentration and electric signal output. Consequently, change in current (I) and inhibition ratio (IR) is measured. IR (inhibition ratio) is the amplitude of the output signal. The bioanodes are suggested to affect the toxic agent sensitivity in MFC biosensors. But both electrodes are modified to decrease the response time and amplify the capacity of detection. For instance, the potentiostat can be used to maintain the sensitivity of the MFC biosensor by optimizing the potential at the anode. The cathode working also influences the amplitude and the output signal precision.

MICROBIAL ELECTROLYSIS CELLS FOR HYDROGEN PRODUCTION

A microbial electrolysis cell (MEC) is used for hydrogen gas production by using electricity. The MEC works on the same principle as MFC (Bose et al. 2020f). The electrons are produced at the anode by the exoelectrogens which react with protons and this reduction releases hydrogen gas. The voltage of 0.2 to 0.8 V is used for this reduction. MFC can generate such low voltage. The electrodes in MEC are made up of the same material as MFC (Wu et al. 2020). The platinum catalyst at the cathode can maintain the potential and catalyze hydrogen generation. All these reactions occur in an anaerobic environment which is favorable for methanogen growth.

The methane production contaminates the hydrogen gas and decreases the output. MEC produces more hydrogen than fermentation. $0.05\text{--}1.05 \text{ mol}\cdot\text{H}_2\cdot\text{mol}\cdot\text{glycerol}^{-1}$ hydrogen is produced by fermentation and $3.9 \text{ mol}\cdot\text{H}_2\cdot\text{mol}\cdot\text{glycerol}^{-1}$ by MEC. Sometimes, a single MFC generates an OCV of 0.5 V only because of the internal resistance, utilization of energy by bacteria, and high potential at the electrode. Hatzell et al. (2014) overcame this drawback by arranging a capacitor in a series configuration in the circuit which stops the voltage from reversing. This setup raises the hydrogen generation to 2.3 folds (Zhang et al. 2019).

The performance of MECs is limited by hydrogen utilization by methanogens (Regassa et al. 2021). To overcome this problem, the cathode is treated with oxygen or UV radiation which causes the inhibition of the methanogens. The cathode can be exposed to air which decreases 3.4% methane to 1%. The use of ultraviolet radiation confirmed 91% hydrogen concentration in the MEC. The antibiotics such as neomycin sulfate, 2-bromoethane sulfonate, 2-chloroethane sulfonate, and 8-aza-hypoxanthine also causes the inhibition of methanogens.

OUTLOOK

MFCs are used as an eco-friendly technology for energy generation and wastewater treatment simultaneously. Organisms such as bacteria and algae have the potential for waste bioremediation (Arora et al. 2021). Different types of wastewaters can be used in MFC with bacteria, either as pure culture or mixed culture. Similar to how probiotics represent the larger domain of the bacteria along with the well-being of human beings (Iqbal et al. 2021), MFCs can utilize these microbes to reduce waste without increasing the carbon burden on the environment. The output performance of MFC is improved by reducing internal resistance, utilizing nanoparticles and genetically modified microorganisms, inoculating pre-treated

cultures, and lessening the start-up time. For instance, carbon paper anode coated with graphene nanocomposites reduces the start-up time and enhances the density of current up to 1800 mA.cm⁻². This electricity is utilized to run electrical equipment. The MFC and anaerobic fermentation is used together to increase the removal efficiency of COD.

MFCs potentially reduce heavy metals. Heavy metals are used as anode and electron acceptors of the cathode. The biomolecules are used as anolytes and remove heavy metals up to 99.5%. The MFCs are also used as BOD/COD biosensors for the detection of toxicity in wastewater. The voltage change detects the presence of toxins. The sensitivity of an MFC biosensor is enhanced by using flow-through mode. MFC is altered to MEC to generate hydrogen. MECs' performance is limited by the hydrogen utilization by methanogens. The antibiotics and ultraviolet radiation treatment cause inhibition of those methanogens.

This technology is applied for many purposes, but still faces many challenges like maintaining the system for upscaling and providing inexpensive electrode elements. Another hurdle is choosing an electron acceptor for the cathode. Oxygen is mostly utilized as an electron acceptor but it can affect the metabolic activity of anaerobic bacteria. Platinum is widely employed for reducing oxygen, but it is a costly element. In addition, the reaction of platinum with the chemicals in the wastewater makes platinum poisonous, therefore, the platinum must be replaced with a better alternate for upscaling of MFCs.

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